SHIMADZU
Excellence in Science

ASTM D8421 PFAS in water samples: a single laboratory verification

August 3, 2023

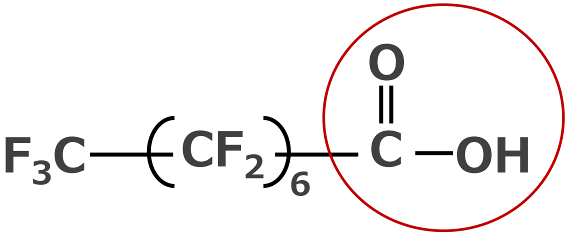
N. Iwasa[1], W. Lipps[2], R. Marfil-Vega[2], J. Nagata[1]

1 - Shimadzu Corporation, Solutions COE

2 - Shimadzu Scientific Instruments, Inc.

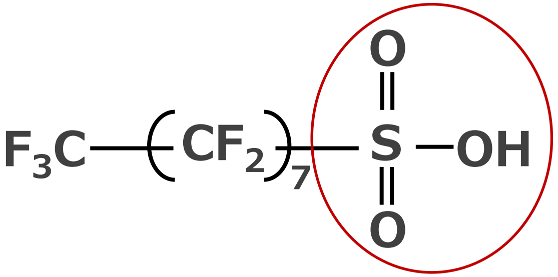
What is PFAS (Per- and Polyfluoroalkyl Substances)

- Perfluoroalkyl and polyfluoroalkyl compounds and their salts.
- Depending on the carbon chain length, functional groups, and structural isomers, more than a few thousand are believed to exist.
- It is used in a wide range of products because of its useful properties such as water repellency and heat resistance.
- While PFAS have useful features, they can pollute surface water, groundwater, soil, and air in various regions around the world, and have adverse effects on human health.
- This has led to a worldwide tightening of regulations on PFAS.



$$F_3C-(CF_2)_6-\overset{\overset{\displaystyle O}{\|}}{C}-OH$$

Perfluorooctanoic acid (PFOA)



$$F_3C-(CF_2)_7-\underset{\underset{\displaystyle O}{\|}}{\overset{\overset{\displaystyle O}{\|}}{S}}-OH$$

Perfluorooctanesulfonic acid (PFOS)

Measurement method of PFAS

	Method									
	EPA					ASTM		Others		
	537 537.1	533	8327	Draft 1633	Draft 1621	D8421-22	D7968-19	ISO 21675	MHLW	MOE
Sample	Drinking water		Ground/ Surface/ Wastewater	Aqueous/ Solid (soil, biosolids, sediment)/ Tissue samples	Aqueous water	Aqueous water (municipal and industrial wastewater, leachate)	Soil/ Sediment/ Sludge	Aqueous water	Drinking water	Aqueous water
Sample preparation	Solid phase extraction	Solid phase extraction	Cosolvation + Direct injection	Solid phase extraction (anionic sorbent) + Clean-up	Carbon collection + CIC	Cosolvation + Direct injection	Solvent extraction + Direct injection	Solid phase extraction (anionic sorbent) + Clean-up	Solid phase extraction (anionic sorbent)	
Target compounds	EPA 537-14 EPA 537.1-18	25	24	40	Adsorbable organic fluorine	44	21	30	PFOS, PFOA	
Applicable LC-MS/MS platforms	LCMS-8045 LCMS-8050	LCMS-8045 LCMS-8050	LCMS-8050 LCMS-8060 LCMS-8060NX	LCMS-8045 LCMS-8050 LCMS-8060 LCMS-8060NX	Combustion equipment and HIC-ESP	LCMS-8050 LCMS-8060 LCMS-8060NX				

ASTM D8421-22

Standard Test Method for Determination
of Per- and Polyfluoroalkyl Substances (PFAS)
in Aqueous Matrices by Co-solvation followed
by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

ASTM D8421-22: Analytes

Short-chain to long-chain PFAS are specified as the compounds to be measured.

e.g.) PFPrA Perfluoropropionic acid $C_3HF_5O_2$

Target 44 per- and polyfluorinated alkyl substances				Surrogate 24 labeled isotopes		
PFTreA	PFDS	NEtFOSAA	PFPrA	13C4-PFBA	D3-NMeFOSAA	13C3-PFBS
PFTriA	PFNS	NMeFOSAA	NFDHA	13C5-PFPeA	D5-NEtFOSAA	13C3-PFHxS
PFDoA	PFOS	PFDoS	PFEESA	13C5-PFHxA	D3-NMeFOSA	
PFUnA	PFHpS	NMeFOSA	PFMPA	13C4-PFHpA	D5-NEtFOSA	
PFDA	PFHxS	NEtFOSA	PFMBA	13C8-PFOA	D7-NMeFOSE	
PFNA	PFPeS	NMeFOSE	3:3 FTCA	13C9-PFNA	D9-NEtFOSE	
PFOA	PFBS	NEtFOSE	5:3 FTCA	13C6-PFDA	13C3-HFPO-DA	
PFHpA	PFOSA	HFPO-DA	7:3 FTCA	13C7-PFUnA	13C2-4:2FTS	
PFHxA	8:2FTS	ADONA	FHUEA	13C2-PFDoA	13C2-6:2FTS	
PFPeA	6:2FTS	9Cl-PF3ONS	FOUEA	13C2-PFTreA	13C2-8:2FTS	
PFBA	4:2FTS	11Cl-PF3OUdS	HQ-115	13C8-PFOSA	13C8-PFOS	

ASTM D8421-22: Sample preparation and analysis

- Sample preparation: Co-solvated by a 1+1 ratio of sample and methanol
- Analysis: Direct injection analysis by Triple-Quadrupole LC/MS/MS

5 mL of aqueous sample

+ Surrogate
+ 5 mL of methanol

Vortex for ~2 min

Filtration to remove particulates

Add acetic acid to all samples and adjust the pH ~4

LC/MS/MS

15 mL polypropylene vial

LCMS-8060NX

Considerations for PFAS analysis by ASTM D8421-22

- Wide range of PFAS, from short to long chain

  ASTM D8421-22 measures short-chain PFAS such as C3.
  ODS columns, commonly used in reversed-phase chromatography, have weak column retention of compounds with short carbon chains, making it difficult to obtain good peak shapes under some conditions.

- PFAS analysis requires high sensitivity.

  Dilution with water is the easiest way to improve the chromatography, but PFAS analysis requires high sensitivity.

Purpose

1. Optimizing analytical conditions for sensitive analysis
    - Column dimensions
    - Co-injection applied
    - Gradient program

2. Confirmation of Analytical Accuracy by ASTM D8421-22

Method optimization

Analytical column dimensions

- Reference column: Shim-pack GIST-HP C18, 2.1 mmi.d. x 50 mmL, 3 µm

  → A) Smaller particle size than the original

  2.1 mmi.d. x 50 mmL, **2 µm**

  B) Longer and wider inner diameter than the original

  3.0 mmi.d. x **100 mmL**, 3 µm

- Standard injection: 25 µl injection

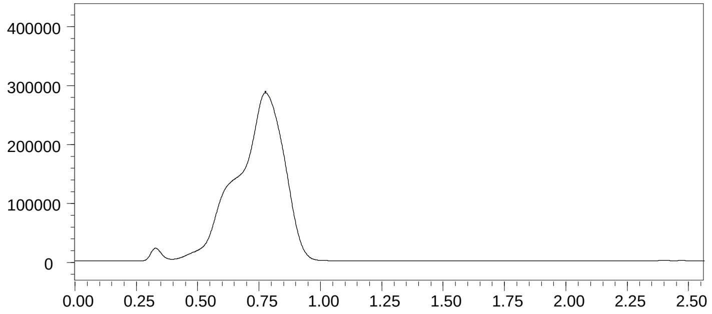
Method optimization: Analytical column

Reference column

Shim-pack GIST-HP C18,
2.1 mmi.d. x 50 mmL, 3 µm

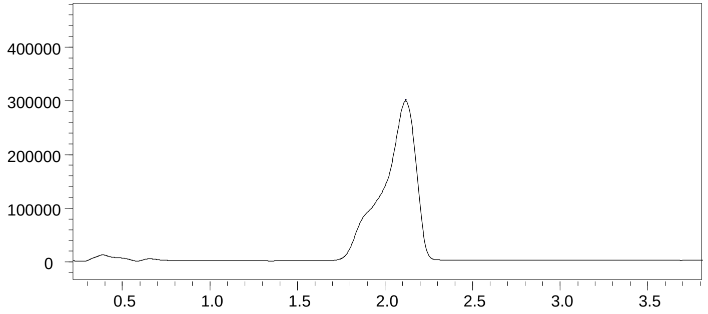
PFPrA ($C_3HF_5O_2$)

m/z 163.00>119.00(-)



PFBA ($C_4HF_7O_2$)

m/z 213.00>169.00(-)



Bad peak shape

Method optimization: Analytical column

Reference column

Shim-pack GIST-HP C18,
2.1 mmi.d. x 50 mmL, 3 µm

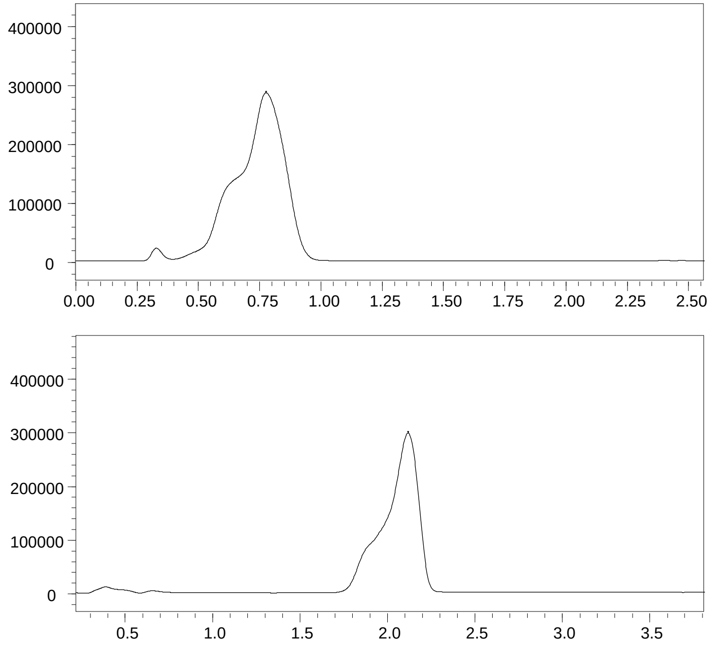
A) Shim-pack GIST-HP C18,
2.1 mmi.d. x 50 mmL, **2 µm**

PFPrA ($C_3HF_5O_2$)

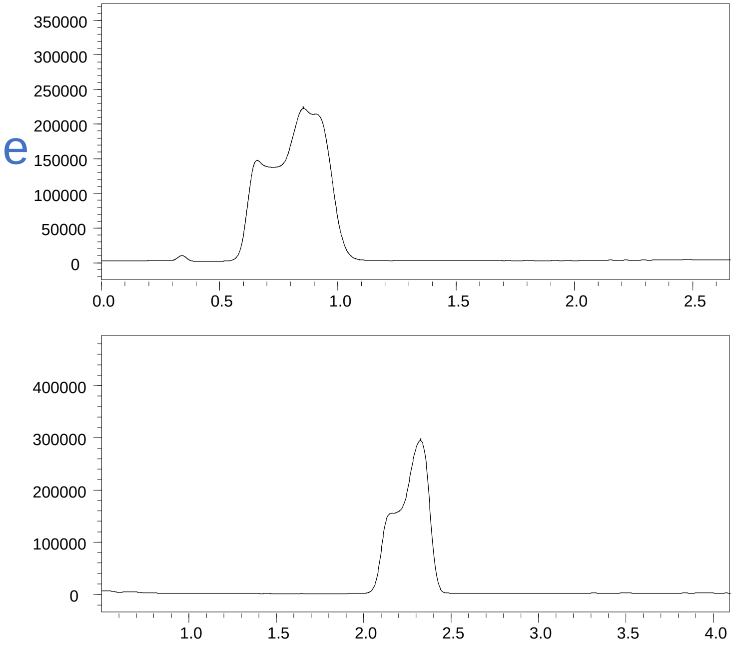
m/z 163.00>119.00(-)

PFBA ($C_4HF_7O_2$)

m/z 213.00>169.00(-)



Smaller particle size than the original



When a column with a small particle size was used, the mixing of the sample solvent and the mobile phase was suppressed, and the peak shape deteriorated due to the suppression of multichannel diffusion.

Method optimization: Analytical column

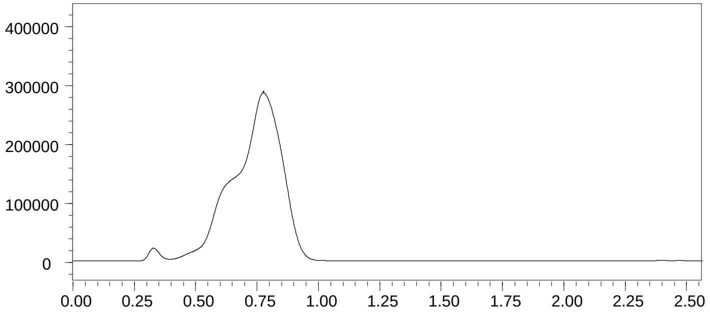
Reference column

Shim-pack GIST-HP C18,
2.1 mmi.d. x 50 mmL, 3 µm

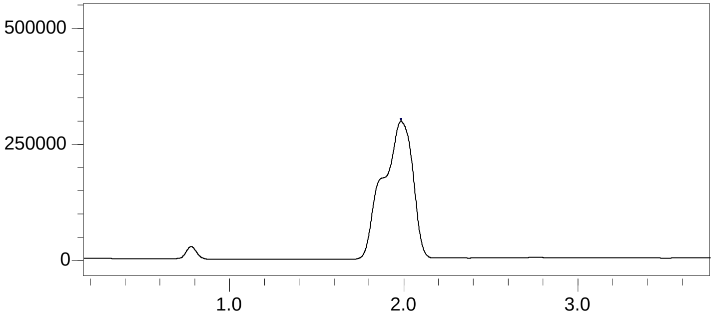
B) Shim-pack GIST-HP C18, **3.0 mmi.d.** x **100 mmL**, 3 µm

PFPrA ($C_3HF_5O_2$)

m/z 163.00>119.00(-)

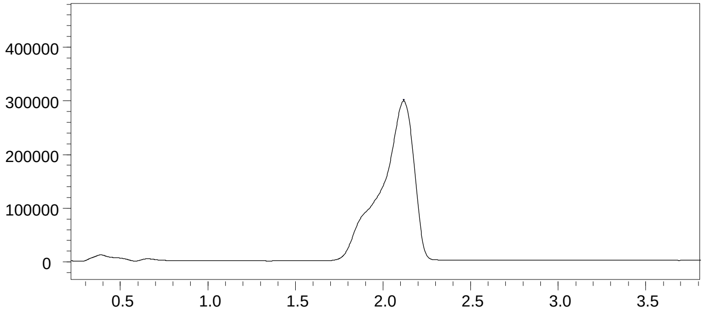


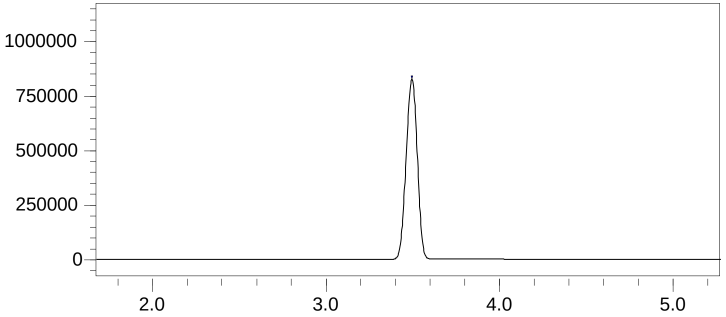
Longer and wider inner diameter than the original



PFBA ($C_4HF_7O_2$)

m/z 213.00>169.00(-)





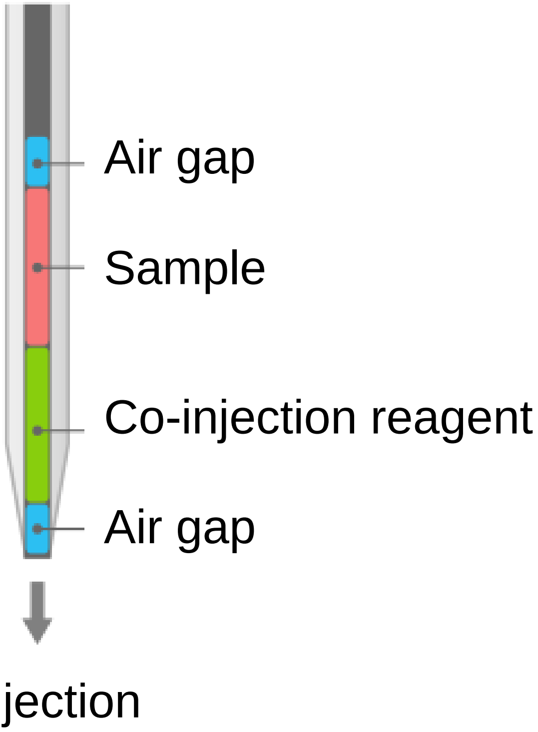
With longer column length and wider inner diameter, axial diffusion improves symmetry at the column exit and improves peak shape.
However, peak shape of PFPrA needs a little more improvement.

Method optimization

Effect of co-injection

- The peak shape is improved by moving the sample solvent closer to the composition of the mobile phase.

- Standard injection: 25 µl injection
  → Co-injection
  25 µL Sample
  ＋
  25 µL Ultra pure water with 0.1 % Acetic acid



Method optimization: Effect of co-injection

Column: Shim-pack GIST-HP C18, 3.0 mmi.d. x 100 mmL, 3 µm

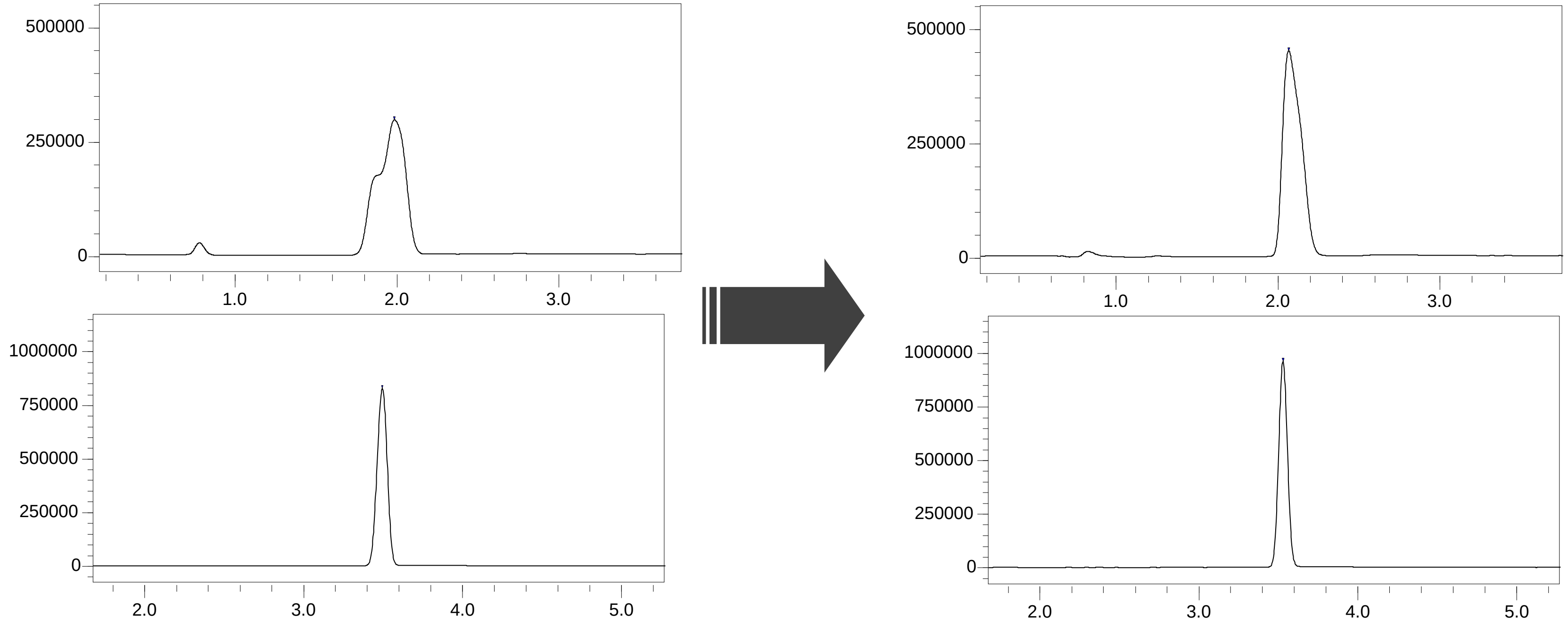
Standard

25 µL Sample

Co-injection

25 µL Sample ＋ 25 µL UPW with 0.1 % AA

(UPA: Ultra pure water, AA: Acetic acid)



PFPrA ($C_3HF_5O_2$)

m/*z* 163.00>119.00(-)

PFBA ($C_4HF_7O_2$)

m/*z* 213.00>169.00(-)

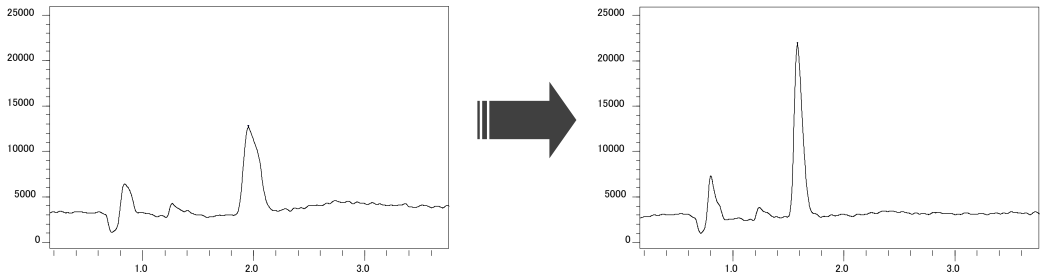
Co-injection with water facilitated column retention and improved peak shape.

Method optimization: Gradient program

- The composition of the mobile phase also affects the peak shape. In particular, the initial mobile phase composition affects the elution of short-chain PFAS.
- Also, it is desirable to separate analytical compounds and impurities from the equipment.

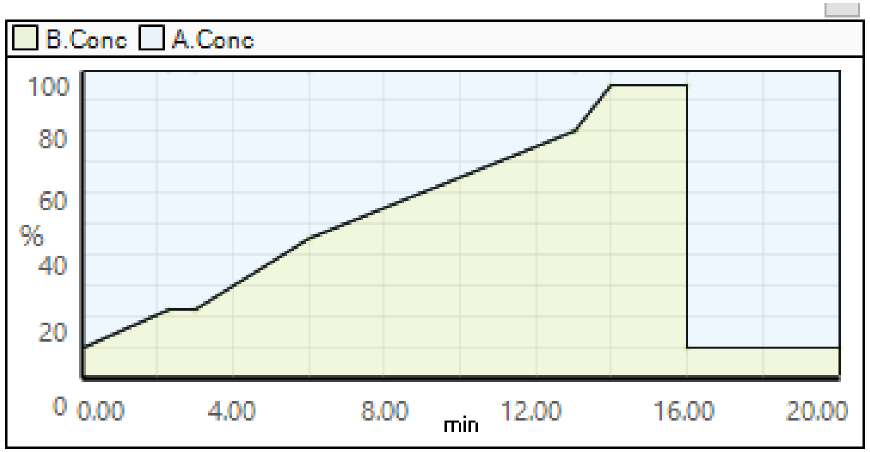
PFPrA ($C_3HF_5O_2$) *m/z* 163.00>119.00(-)

Conc. B 5 % Conc. B 10 %



The peak shape was improved by the difference in mobile phase composition.

Final gradient program



Analysis conditions

	Equipment	LCMS-8060NX
LC	Mobile Phase	A: 2 mmol/L Ammonium Acetate in H2O/ Acetonitrile = 95/5 B: Acetonitrile
	Delay Column	Shim-pack Scepter C18-120 2.1 mmi.d. x 100 mmL, 3 um (P/N: 227-31014-05)
	Analytical Column	Shim-pack GIST-HP C18 3.0 mmi.d. x 100 mmL, 3 um (P/N: 227-30040-04)
	Gradient (%B)	10%(0min)⇒22%(2.3-3.0min) ⇒45%(6.0min)⇒80%(13.0min) ⇒95%(14.0-16min)⇒10%(16.01-20.0min)
	Column Oven Temp.	40 ℃
	Flow rate	0.6 mL/min
	Injection volume	25 µL
	Multiple draw injection program	Co-injection 25 µL Sample → 25 µL 0.1% Acetic acid in H2O
MS	Interface	IonFocus ESI
	Interface Temp.	170℃
	Probe position	+3 mm
	Neblizer gas flow	3 L/min
	Heating gas flow	15 L/min
	Interface Voltage	-0.5 kV (same value for all compounds)
	DL Temp.	200 ℃
	Heatblock Temp.	300 ℃
	Drying gas flow	5 L/min
	Focus bias	-2 kV (same value for all compounds)

Preparation of calibration standards

Reagents used

- PFAS mixture compatible with U.S. EPA Draft Method 1633 (Wellington Laboratories)
- 4 Compounds not measured in EPA1633 were added.
  → FHUEA, FOUEA, HQ-115, PFPrA

Final Concentrations of Calibration Standards (ng/L)

	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7	Cal8	Cal9
PFPeA, PFBA, PFPrA	25	50	100	200	300	400	500	750	1000
All Other PFAS and Surrogates (Exclude PFPeA, PFBA and PFPrA	5	10	20	40	60	80	100	150	200

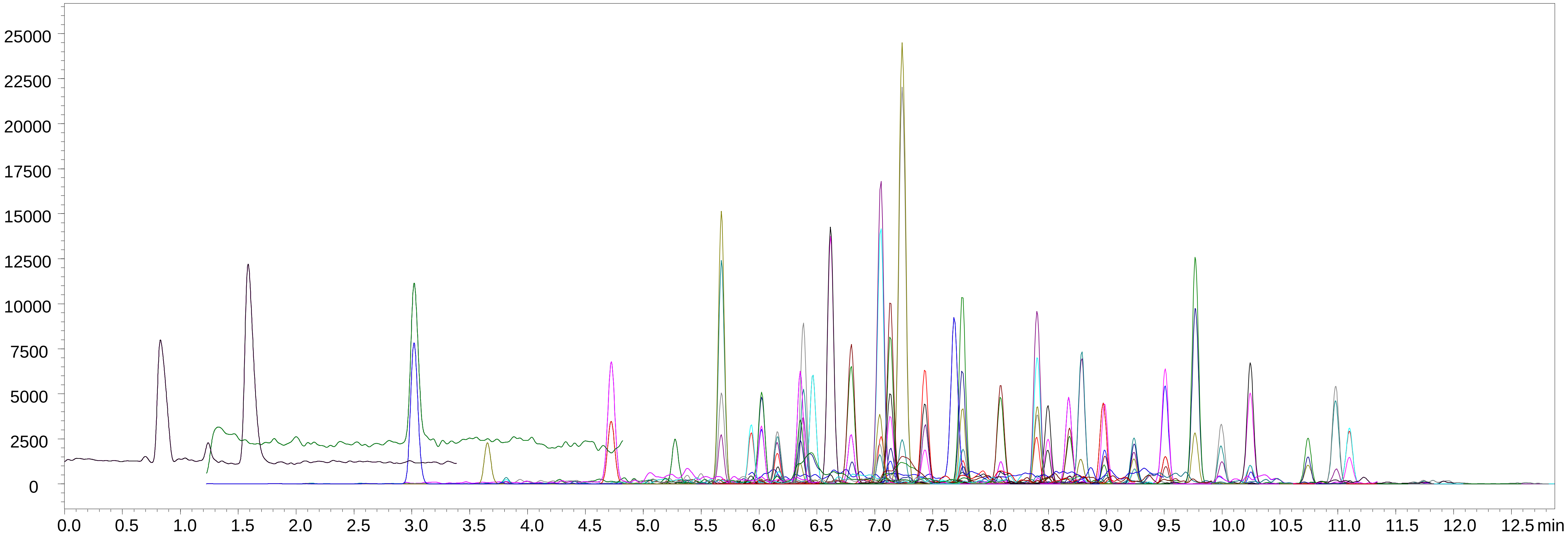
The individual standard solution were prepared in 50:50 (vol: vol) methanol/water with 0.1% acetic acid

Preparation of calibration standards

	Compounds	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8	CS9	CS10	CS11	CS12	CS13	CS14	CS15
Analyte	All analytes unless otherwise noted	1	2.5	5	10	25	40	60	80	100	150	200	250	375	500	800
	PFPeA	2	5	10	20	50	80	120	160	200	300	400	500	750	1000	1600
	PFBA, 4:2-FTS, 6:2-FTS, 8:2-FTS	4	10	20	40	100	160	240	320	400	600	800	1000	1500	2000	3200
	PFPrA, 5:3 FTCA, 7:3 FTCA	5	12.5	25	50	125	200	300	400	500	750	1000	1250	1875	2500	4000
	NMeFOSE, NEtFOSE	10	25	50	100	250	400	600	800	1000	1500	2000	2500	3750	5000	8000
Surrogate	13C9-PFNA, 13C6-PFDA, 13C7-PFUnA, 13C2-PFDoA, 13C2-PFTreA	0.25	0.625	1.25	2.5	6.25	10	15	20	25	37.5	50	62.5	93.75	125	200
	13C5-PFHxA, 13C4-PFHpA, 13C8-PFOA, 13C8-PFOSA, D3-NMeFOSA, D5-NEtFOSA, 13C8-PFOS, 13C3-PFBS, 13C3-PFHxS	0.5	1.25	2.5	5	12.5	20	30	40	50	75	100	125	187.5	250	400
	13C5-PFPeA, 13C2-4:2FTS, 13C2-6:2FTS, 13C2-8:2FTS, D3-NMeFOSAA, D5-NEtFOSAA	1	2.5	5	10	25	40	60	80	100	150	200	250	375	500	800
	13C4-PFBA, 13C3-HFPO-DA	2	5	10	20	50	80	120	160	200	300	400	500	750	1000	1600
	D7-NMeFOSE, D9-NEtFOSE	5	12.5	25	50	125	200	300	400	500	750	1000	1250	1875	2500	4000

Only the calibration points within the method specified range were used.

Measurement results of standard samples

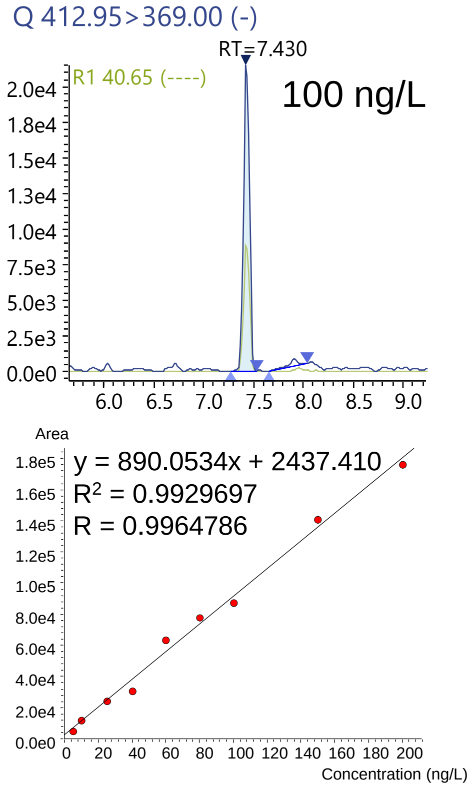


MRM Chromatograms of CS5

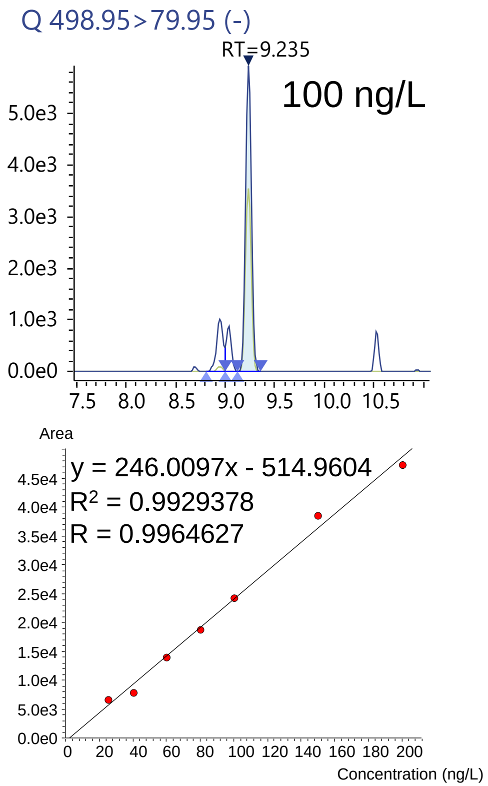
Measurement results of standard samples

Calibration curve and midpoint chromatogram

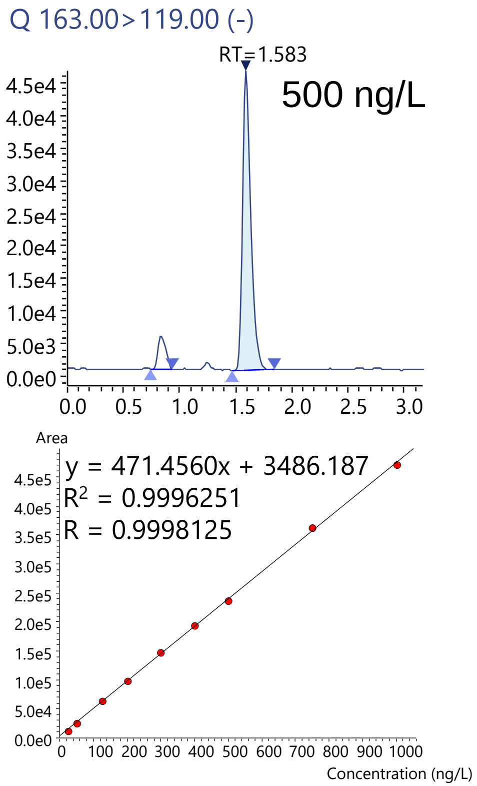
PFOA



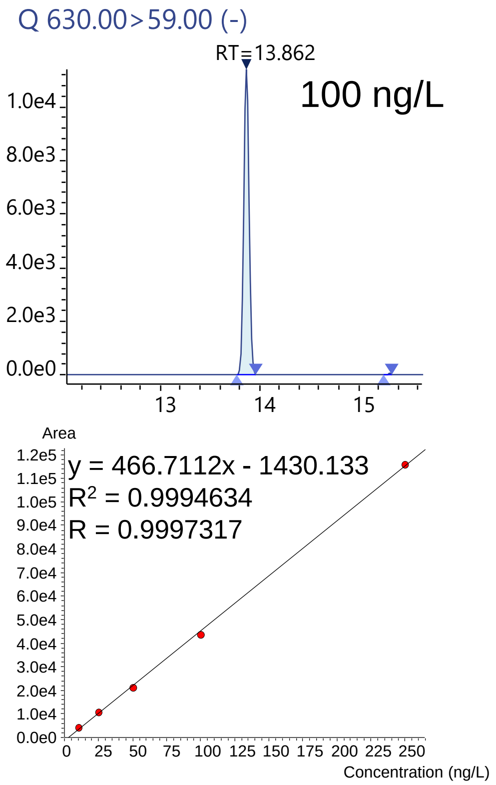
PFOS



PFPrA



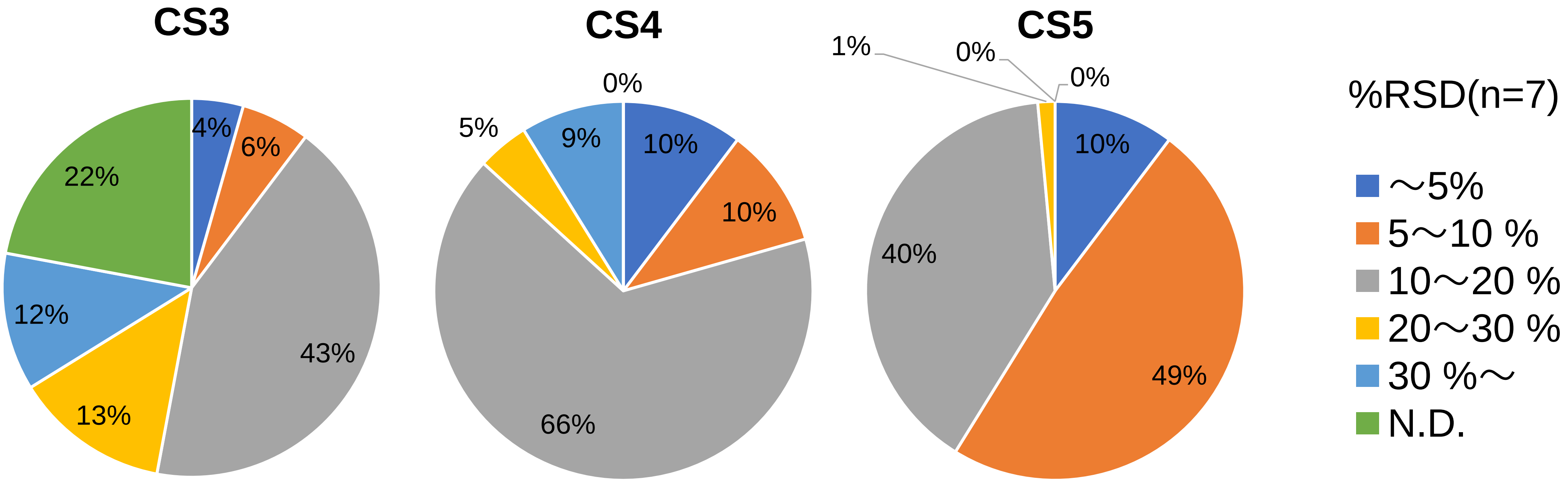
NEtFOSE



Results meet the requirements outlined in the method.

Measurement results of standard samples

Percentage of repeatability at low-concentration standard samples

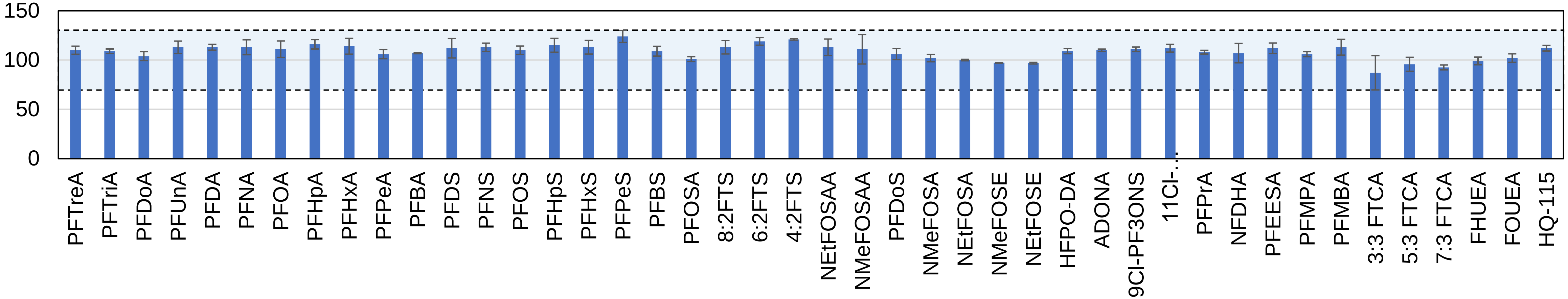


Analyte	5～50 ng/L	10～100 ng/L	25～250 ng/L
Surrogate	1.25～25 ng/L	2.5～50 ng/L	6.25～125 ng/L

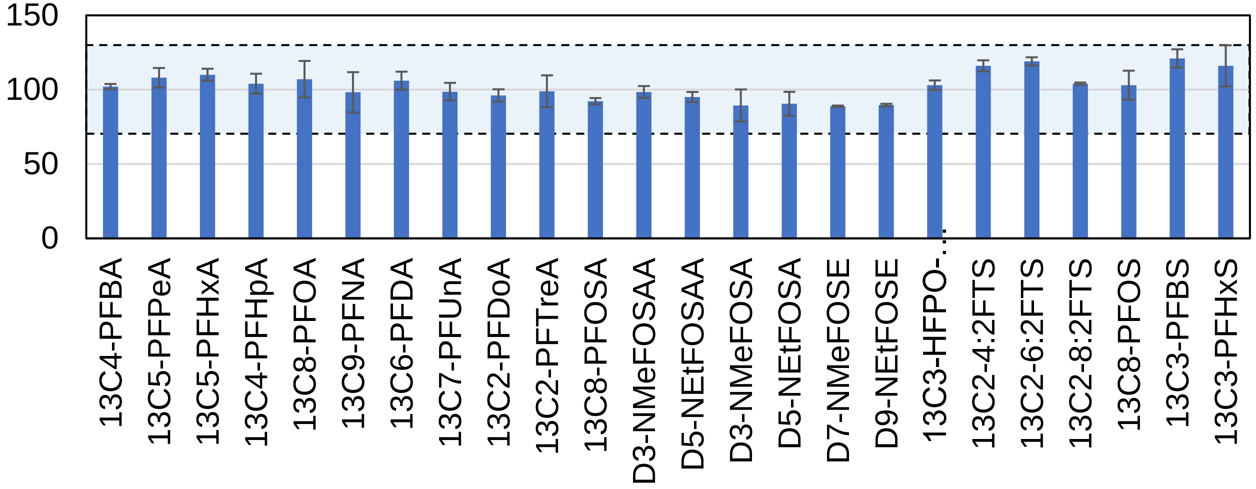
Good repeatability results were obtained at low concentrations for most compounds.

Recovery and Repeatability Study: Reagent Water

<Target>



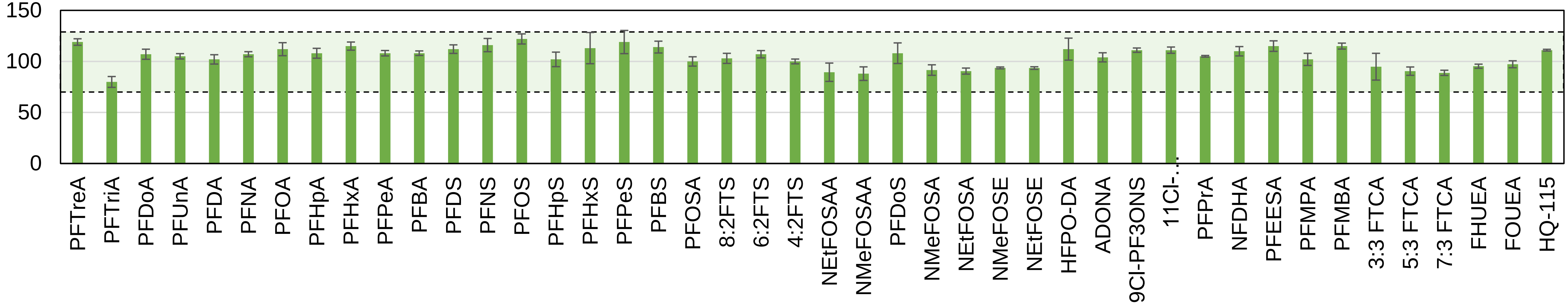
<Surrogate>



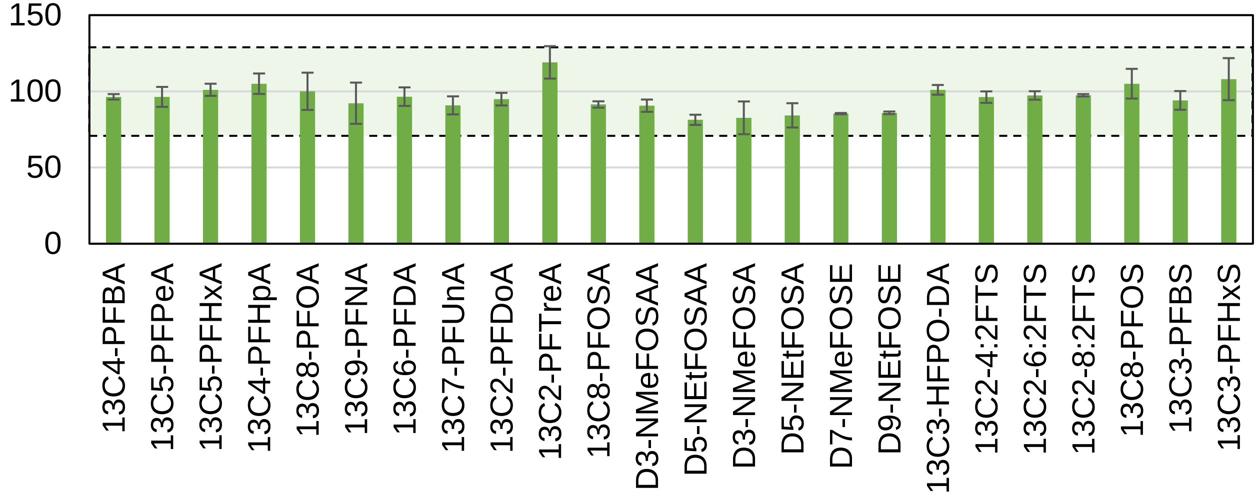
These data are well within the 70 -130 % Recovery and ≤ 30% RSD limits of the method.

Recovery and Repeatability Study: Wastewater

<Target>



<Surrogate>



These data are well within the 70 -130 % Recovery and ≤ 30% RSD limits of the method. MS ionization efficiency is not affected by the sample matrix.

Summary

- ASTM D8421-22 is a simple extraction procedure validated by ASTM for the analysis of PFAS in wastewater samples.
- The LCMS-8060NX easily meets method performance criteria of ASTM D8421-22 for 44 PFAS and 24 surrogates.
- Using the optimized method for ASTM D8421-22 with a column having favorable dimensions, good results can be obtained for short-chain to long-chain PFAS compounds.
- The optimized method does not require any equipment modification, so it is possible to measure EPA draft method 1633 on the same equipment.

Thank you for your attention.