Application Note

Food and Beverage Testing

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Development and Optimization for a Comprehensive LC/MS/MS Method for the Detection of 74 PFAS Compounds

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental and food contaminants linked to adverse health effects. Dietary intake, especially from animal-derived foods, is a major exposure route. To support sensitive detection in complex matrices, an Agilent LC/MS/MS method was developed to target 74 PFAS compounds. The method addresses challenges in mixed standard preparation, bile acid separation, and matrix-related background through injection programming. Optimized for animal extracts, the method is broadly applicable and demonstrates strong sensitivity, with most compounds achieving instrument detection limits (IDLs) below 10 pg/mL (ppt) using an Agilent 6495D Triple Quadrupole LC/MS system.

Introduction

PFAS represent a large and chemically diverse class of synthetic compounds widely used in industrial processes and consumer products due to their exceptional surfactant properties and thermal stability. These compounds are defined by strong carbon-fluorine bonds, which confer resistance to environmental and biological degradation. Consequently, PFAS are persistent, bioaccumulative, and have been detected globally in water, soil, air, and food.[1-3]

Mounting scientific evidence has linked PFAS exposure to a range of adverse health outcomes, including thyroid problems, immune suppression, and increased cancer risk.[4,5] Among various exposure pathways, dietary intake is considered a primary route for the general population, particularly through consumption of animal-derived foods such as meat, eggs, and dairy products[1-3, 6-8], which may accumulate PFAS from contaminated environments.

To support accurate and sensitive detection of PFAS in complex matrices, regulatory agencies have developed targeted analytical methods. The U.S. Environmental Protection Agency (EPA) Method 1633 includes 40 PFAS compounds[9], the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS)[10] has published a method for quantifying 16 PFAS in meat and bovine plasma, and the U.S. Food and Drug Administration (FDA) has published a method for quantifying 30 PFAS in food samples[11] using liquid chromatography-tandem mass spectrometry (LC/MS/MS).

This study presents an optimized LC/MS/MS method capable of detecting 74 PFAS compounds across diverse chemical classes amenable to this technique. Compound transitions and source conditions were developed using the Agilent MassHunter Acquisition Optimizer program. Method development addressed key analytical challenges. These include the instability and cross-contamination risks associated with combining targets into mixed standards, chromatographic optimization to achieve baseline separation of bile acids from PFOS, and the implementation of an injection programming strategy to enhance peak shape and minimize matrix-related background. Although the method was specifically tailored for extracts of animal origin[12], it is broadly applicable to other sample types. IDLs achieved using the 6495D triple quadrupole mass spectrometer are reported, demonstrating the method's sensitivity and robustness.

Experimental

Solutions and standards

Table 1 outlines the compounds included in the study along with their respective suppliers. Formic acid, ammonium acetate, and Optima-LC/MS grade solvents—including water, acetonitrile, isopropyl alcohol, and methanol—were procured from Fisher Scientific (Pittsburgh, PA, USA).

LC/MS/MS conditions

This study was performed using an Agilent 1290 Infinity III LC system consisting of a 1290 Infinity III high-speed pump (G7120A), a 1290 Infinity III Multisampler (G7167B), and an Agilent 1290 Infinity III Multicolumn Thermostat (G7116A). The LC system was modified using an Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006). Injection program and chromatographic separation parameters are detailed in Table 2.

The LC system was coupled to an Agilent 6495D LC/TQ equipped with an Agilent Jet Stream source. All multiple reaction monitoring transitions are provided in Table 1. Compound-specific parameters for the 6495D MRMs were determined using MassHunter Optimizer. The source conditions for the 6495D are shown in Table 3. To address the wide range of PFAS compounds included in the method, source parameters such as temperature and flow rates were fine-tuned using MassHunter Source Optimizer. Data acquisition and analysis were carried out with Agilent MassHunter Workstation software.

IDL determination

Instrument detection limits (IDLs) were determined as discussed in reference 13. Calculation required the repeated injection of seven solvent standards spiked at a concentration 2 to 10x above the expected limit. The standard deviation of these injections was then multiplied by a Student's t-test for a single-tailed 99th percentile t-value. If background contamination is present, this is added to the calculation.

Table 1. Compounds, abbreviations, CAS numbers, group assignment, company purchased, retention times (RT), MRM transtitions, collision energies (CE), and corresponding internal standards for all analytes in LC/MS/MS method. **Note:** Standard iFunnel Voltage was used for all compounds. Compounds with * were purchased as part of a mixture from Wellington.

Compound	Abbreviation	CAS No.	Company	Group	RT (min)	MRM Ions (*m/z*)	CE (V)	Internal Standard
Perfluoro-1-butanesulfonamide	FBSA*	30334-69-1	Wellington	FASA	5.03	298 → 77.9	30	$^{13}C_8$-FOSA
1,1,2,2,3,3,4,4,4-Nonafluoro-N-(2-hydroxyethyl)-1-butanesulfonamide	FBSE	34454-99-4	LGC/TRC	FASA	5.9	342 → 92 342 → 65	25 30	$^{13}C_8$-FOSA
Perfluoro-1-hexanesulfonamide	FHxSA*	41997-13-1	Wellington	FASA	6.94	398 → 77.9 398 → 63.8	60 120	$^{13}C_8$-FOSA
N-[3-(Dimethylamino)propyl] Perfluorohexanesulfonamide	N-AP-FHxSA	50598-28-2	LGC/TRC	FASA	7.19	483 → 169 483 → 119	30 35	$^{13}C_8$-FOSA
Perfluoro-1-octanesulfonamide	FOSA*	754-91-6	Wellington	FASA	8.6	498 → 477.9 498 → 77.9	30 35	$^{13}C_8$-FOSA
N-methyl perfluorooctanesulfonamide	N-MeFOSA	31506-32-8	AccuStandard	FASA	9.7	512 → 218.9 512 → 168.9	30 30	d_3-NMeFOSA
N-ethyl perfluorooctanesulfonamide	N-EtFOSA	4151-50-2	AccuStandard	FASA	10.09	526 → 218.9 526 → 168.9	30 30	d_5-NEtFOSA
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA*	2355-31-9	Wellington	FASAA	8.12	570 → 482.9 570 → 419	15 25	d_3-NMeFOSAA
N-ethylperluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA*	2991-50-6	Wellington	FASAA	8.44	584 → 525.9 584 → 418.9	20 25	d_5-NEtFOSAA
3-Perfluoropropyl propanoic acid	3:3 FTCA	356-02-5	AccuStandard	FTCA	4.06	241 → 117 241 → 63	40 20	$^{13}C_2$-FHEA
2H,2H,3H,3H-Perfluorooctanoic acid	5:3 FTCA	914637-49-3	AccuStandard	FTCA	5.55	342 → 238 342 → 218	13 25	$^{13}C_2$-FHEA
2H,2H-Perfluorooctanoic acid	6:2 FTCA	53826-12-3	AccuStandard	FTCA	5.6	377 → 313 377 → 63	8 8	$^{13}C_2$-FHEA
3-Perfluoroheptyl propanoic acid	7:3 FTCA	812-70-4	AccuStandard	FTCA	7.29	441 → 267 441 → 62.9	35 10	$^{13}C_2$-FOEA
Perfluoro-octylethanoic acid	8:2 FTCA	27854-31-5	LGC/TRC	FTCA	7.3	477 → 392.9 477 → 62.9	16 11	$^{13}C_2$-FOEA
2H,2H-Perfluorododecanoic acid	10:2 FTCA	53826-13-4	AccuStandard	FTCA	8.67	577 → 493 577 → 63	13 10	$^{13}C_2$-FDEA
Hexafluoroamylene glycol/2,2,3,3,4,4-Hexafluoro-1,5-pentanediol	HFAG	376-90-9	SCB	FTOH	3.5	211 → 171 211 → 131	20 20	$^{13}C_4$-PFBA
Sodium 1H, 1H, 2H, 2H-perfluoro-1-hexanesulfonate	4:2 FTSA*	27619-93-8	Wellington	FTSA	4.55	327 → 306.9 327 → 80.9	25 35	$^{13}C_2$-4:2FTSA
Sodium 1H, 1H, 2H, 2H-perluoro-1-octanesulfonate	6:2 FTSA*	27619-94-9	Wellington	FTSA	6.12	427 → 406.9 427 → 80.9	30 35	$^{13}C_2$-6:2FTSA
Sodium 1H, 1H, 2H, 2H-perfluoro-1-decanesulfonate	8:2 FTSA*	27619-96-1	Wellington	FTSA	7.77	527 → 506.9 527 → 80.9	30 40	$^{13}C_2$-8:2FTSA
1H, 1H, 2H, 2H-perfluorododecanesulphonic acid	10:2 FTSA	108026-35-3	AccuStandard	FTSA	8.95	627 → 606.9 627 → 80.9	33 38	$^{13}C_2$-8:2FTSA
2H-Perfluoro-2-octenoic acid	FHUEA	70887-88-6	AccuStandard	FTUCA	5.54	357 → 243 357 → 92.9	41 48	$^{13}C_2$-FHUEA
2H-Perfluoro-2-decenoic acid	FOUEA	70887-84-2	AccuStandard	FTUCA	7.24	457 → 393 457 → 343	8 46	$^{13}C_2$-FOUEA
2H-Perfluoro-2-dodecenoic acid	FDUEA	70887-94-4	LGC/TRC	FTUCA	8.63	557 → 492.9 557 → 242.9	11 41	$^{13}C_2$-FDUEA
1-Propanaminium, N,N-dimethyl-N-oxide-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl] amino]-, hydroxide	Capstone A	80475-32-7	LGC/TRC	Other	7.41	527 → 221.9 527 → 63.9	87 87	$^{13}C_8$-PFOA
1-Propanaminium, N-(carboxymethyl)-N,N-dimethyl-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl] amino]-, hydroxide	Capstone B	34455-29-3	LGC/TRC	Other	7.03	569 → 223 569 → 63.9	15 112	$^{13}C_8$-PFOA
Mono[2-(perfluorooctyl)ethyl] phosphate	8:2 PAP	57678-03-2	LGC/TRC	PAP	7.6	543 → 97 543 → 79	18 105	$^{13}C_4$-6:2diPAP

Compound	Abbreviation	CAS No.	Company	Group	RT (min)	MRM Ions (*m/z*)	CE (V)	Internal Standard
Sodium bis(1H,1H,2H,2H-perfluorooctyl) phosphate	6:2 diPAP	407582-79-0	AccuStandard	PAP	9.72	789 → 442.9 789 → 78.9	25 120	$^{13}C_4$-6:2diPAP
Sodium bis(1H, 1H, 2H, 2H-perfluorodecyl) phosphate	8:2 diPAP	678-41-1	AccuStandard	PAP	10.93	988.9 → 543 988.9 → 97	23 36	$^{13}C_4$-8:2diPAP
Bis(2-perfluorooctylsulfonyl-N-ethylaminoethyl) phosphate	diSAmPAP	2965-52-8	LGC/TRC	PAP	11.45	1203 → 649.9 1203 → 525.9	41 46	$^{13}C_4$-8:2diPAP
Perfluoro-n-butanoic acid	PFBA*	375-22-4	Wellington	PFCA	3.45	213 → 168.9	5	$^{13}C_4$-PFBA
5-H-Octafluoropentanoic acid	5H PFPeA	376-72-7	LGC/TRC	H-PFCA	3.56	245 → 201 254 → 181	8 8	$^{13}C_5$-PFPeA
Perfluoro-n-pentanoic acid	PFPeA*	2706-90-3	Wellington	PFCA	4.01	263 → 218.9	5	$^{13}C_5$-PFPeA
Perfluoro-n-hexanoic acid	PFHxA*	307-24-4	Wellington	PFCA	4.61	313 → 268.9 313 → 118.9	5 15	$^{13}C_5$-PFHxA
Perfluoro-n-heptanoic acid	PFHpA*	375-85-9	Wellington	PFCA	5.35	363 → 318.9 363 → 168.9	5 15	$^{13}C_4$-PFHpA
Perfluoro-n-octanoic	PFOA*	335-67-1	Wellington	PFCA	6.18	413 → 368.9 413 → 168.9	5 15	$^{13}C_8$-PFOA
Perfluoro-n-nonanoic acid	PFNA*	375-95-1	Wellington	PFCA	7	463 → 418.9 463 → 218.9	5 15	$^{13}C_9$-PFNA
Perfluoro-n-decanoic acid	PFDA*	335-76-2	Wellington	PFCA	7.77	513 → 219 513 → 169	15 20	$^{13}C_6$-PFDA
Perfluoro-n-undecanoic acid	PFUdA*	2058-94-8	Wellington	PFCA	8.41	563 → 518.9 563 → 268.9	5 15	$^{13}C_7$-PFUdA
Perfluoro-n-dodecanoic acid	PFDoA*	307-55-1	Wellington	PFCA	8.93	613 → 568.9 613 → 168.9	5 30	$^{13}C_2$-PFDoA
Perfluoro-n-tridecanoic acid	PFTrA*	72629-94-8	Wellington	PFCA	9.36	663 → 618.9 663 → 168.9	10 25	$^{13}C_2$-PFDoA
Perfluoro-n-tetradecanoic acid	PFTeA*	376-06-7	Wellington	PFCA	9.77	713 → 668.9 713 → 168.9	15 30	$^{13}C_2$-PFTeA
Perfluoropentadecanoic acid	PFPeDA	141074-63-7	Chiron	PFCA	10.17	763 → 718.9 763 → 168.7	20 47	$^{13}C_2$-PFTeA
Perfluoro-n-hexadecanoic acid	PFHxDA	67905-19-5	Sigma	PFCA	10.54	813 → 768.9 813 → 218.9	20 30	$^{13}C_2$-PFTeA
Perfluoro-n-octadeconoic acid	PFODA	16517-11-6	AccuStandard	PFCA	11.22	913 → 868.9 913 → 168.9	15 60	$^{13}C_2$-PFTeA
Nonafluoropentanamide	NFPA	13485-61-5	Sigma	Other	5.22	262 → 42	8	$^{13}C_4$-PFHpA
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA*	13252-13-6	Wellington	PFECA	4.82	285 → 184.9 285 → 168.9	15 5	$^{13}C_3$-HFPO-DA
Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA*	2250081-67-3	Wellington	PFECA	5.46	377 → 250.9 377 → 84.9	5 30	$^{13}C_4$-PFHpA
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	AccuStandard	PFECA	4.5	295 → 200.9 295 → 84.9	5 18	$^{13}C_5$-PFHxA
Perfluoro(4-methoxybutanoic) acid	PFMBA	863090-89-5	AccuStandard	PFECA	4.18	279 → 84.9	15	$^{13}C_5$-PFHxA
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	AccuStandard	PFECA	3.71	229 → 84.9	15	$^{13}C_5$-PFPeA
Perfluoro-3,6,9-trioxatridecanoic acid/2,2-difluoro-2-[1,1,2,2-tetrafluoro-2-[1,1,2,2-tetrafluoro-2-(1,1,2,2,3,3,4,4,4-nonafluorobutoxy)ethoxy]ethoxy] acetic acid	PFTODA	330562-41-9	Sigma	PFECA	8.33	561 → 466.8 561 → 234.9	15 35	$^{13}C_7$-PFUdA
2,2'-((Perfluoroethane-1,2-diyl)bis(oxy))bis(2,2-difluoroethanol)	PFDOD	129301-42-4	Sigma	PFEOH	4.68	293 → 172.9 293 → 152.9	15 20	$^{13}C_5$-PFHxA
Perfluoro(2-ethoxyethane)sulphonic acid	PFEESA	113507-82-7	AccuStandard	PFESA	4.33	315 → 134.9 315 → 68.9	30 60	$^{13}C_3$-PFBS
Perfluoro-3,6-dioxa-4-methyl-7-octene-1-sulfonic acid Sodium Salt	Nafion Byproduct	29311-67-9	LGC/TRC	PFESA	5.9	443 → 263 443 → 146.9	20 30	$^{13}C_3$-PFHxS
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl PF3OUdS*	73606-19-6 (F-53B)	Wellington	PFESA	7.47	531 → 350.9 531 → 82.9	30 30	$^{13}C_8$-PFOS

Compound	Abbreviation	CAS No.	Company	Group	RT (min)	MRM Ions (*m/z*)	CE (V)	Internal Standard
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl_PF3OUdS*	83329-89-9	Wellington	PFESA	8.7	633 → 452.9 633 → 450.9	30 30	$^{13}C_8$-PFOS
Perfluorohexylphosphonic acid	PFHxPA	40143-76-8	LGC/TRC	PFPA	4.1	399 → 78.9 399 → 62.8	40 55	$^{13}C_5$-PFHxA
Perfluorooctylphosphoic acid	PFOPA	40143-78-0	LGC/TRC	PFPA	5.48	499 → 78.9 499 → 62.8	41 58	$^{13}C_8$-PFOA
Perfluorodecylphosphonic acid	PFDPA	52299-26-0	LGC/TRC	PFPA	7.23	599 → 78.9 599 → 62.8	46 60	$^{13}C_6$-PFDA
Bis(perfluorohexyl)phosphinic acid	6:6 PFPiA	40143-77-9	LGC/TRC	PFPiA	9.11	701 → 401 701 → 82	63 120	$^{13}C_4$-6:2diPAP
(Heptadecafluorooctyl)(tridecafluorohexyl)-phosphinic acid	6:8 PFPiA	610800-34-5	LGC/TRC	PFPiA	9.8	801 → 500.8 801 → 400.9	63 63	$^{13}C_4$-6:2diPAP
Bis(heptadecafluorooctyl)phosphinic acid	8:8 PFPiA	40143-79-1	LGC/TRC	PFPiA	10.44	901 → 501 901 → 63	67 120	$^{13}C_4$-8:2diPAP
Perfluoroethanesulfonic acid	PFEtS	354-88-1	LGC/TRC	PFSA	2.12	199 → 98.9 199 → 79.9	33 33	$^{13}C_3$-PFBS
Perfluoropropanesulfonic acid Sodium Salt	PFPrS	423-41-6	LGC/TRC	PFSA	3.67	249 → 98.9 249 → 79.9	31 36	$^{13}C_3$-PFBS
Potassium perfluoro-1-butanesulfonate	PFBS*	29420-49-3	Wellington	PFSA	4.1	299 → 98.9 299 → 79.9	30 35	$^{13}C_3$-PFBS
Sodium perfluoro-1-pentanesulfonate	PFPeS*	630402-22-1	Wellington	PFSA	4.68	349 → 98.9 349 → 79.9	35 45	$^{13}C_3$-PFHxS
Potassium perfluorohexanesulfonate	PFHxS*	82382-12-5	Wellington	PFSA	5.4	399 → 98.9 399 → 79.9	45 50	$^{13}C_3$-PFHxS
Sodium perfluoro-1-heptanesulfonate	PFHpS*	21934-50-9	Wellington	PFSA	6.2	449 → 98.9 449 → 79.9	45 50	$^{13}C_3$-PFHxS
Postassium perfluorooctanesulfonate	PFOS*	2795-39-3	Wellington	PFSA	7.01	499 → 98.9 499 → 79.9	45 60	$^{13}C_8$-PFOS
Sodium perfluoro-1-nonanesulfonate	PFNS*	98789-57-2	Wellington	PFSA	7.76	549 → 98.9 549 → 79.9	50 60	$^{13}C_8$-PFOS
Sodium perfluoro-1-decanesulfonate	PFDS*	2806-15-7	Wellington	PFSA	8.4	599 → 98.9 599 → 79.9	60 60	$^{13}C_8$-PFOS
Perfluoroundecane sulfonic acid	PFUnDS	749786-16-1	Wellington	PFSA	8.9	649 → 79.9 649 → 79.9	56 56	$^{13}C_8$-PFOS
Perfluorododecane sulfonic acid	PFDoS	79780-39-5	Wellington	PFSA	9.32	699 → 99 699 → 80	120 80	$^{13}C_8$-PFOS
Perfluorotridecane sulfonic acid	PFTrDS	791563-89-8	Wellington	PFSA	9.71	749 → 99 749 → 80	63 120	$^{13}C_8$-PFOS
Perfluoro-1-($^{13}C_8$)octanesulfonamide	13C8-FOSA*	N/A	Wellington	FASA	8.6	506 → 77.9	40	
N-Methyl-d3-perfluoro-1-octanesulfonamide	d3-NMeFOSA	N/A	Wellington	FASA	9.7	515 → 168.9	30	
N-Ethyl-d5-perfluoro-1-octanesulfonamide	d5-NEtFOSA	N/A	Wellington	FASA	10.09	531 → 168.9	30	
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	d3-NMeFOSAA*	N/A	Wellington	FASAA	8.11	573 → 482.9	15	
N-ethyl-d5-perluoro-1-octanesulfonamidoacetic acid	d5-NEtFOSAA*	N/A	Wellington	FASAA	8.44	589 → 482	25	
2-Perfluorohexyl(1,2-$^{13}C_2$)ethanoic acid	13C2-FHEA	N/A	Wellington	FTCA	5.6	379 → 294	8	
2-Perfluorooctyl(1,2-$^{13}C_2$)ethanoic acid	13C2-FOEA	N/A	Wellington	FTCA	7.3	479 → 393.9	16	
2-Perfluorodecyl(1,2-$^{13}C_2$)ethanoic acid	13C2-FDEA	N/A	Wellington	FTCA	8.67	579 → 494	13	
Sodium 1H, 1H, 2H, 2H-perfluoro(1,2-$^{13}C_2$) hexanesulfonate	13C2-4:2FTSA*	N/A	Wellington	FTSA	4.55	329 → 308.9	25	
Sodium 1H, 1H, 2H, 2H-perluoro(1,2-$^{13}C_2$) octanesulfonate	13C2-6:2FTSA*	N/A	Wellington	FTSA	6.12	429 → 408.9	30	
Sodium 1H, 1H, 2H, 2H-perfluoro(1,2-$^{13}C_2$) decanesulfonate	13C2-8:2FTSA*	N/A	Wellington	FTSA	7.77	529 → 508.9	30	
2H-Perfluoro-2-(1,2-$^{13}C_2$)octenoic acid	13C2-FHUEA	N/A	Wellington	FTUCA	5.54	359 → 244	41	
2H-Perfluoro-2-(1,2-$^{13}C_2$)decenoic acid	13C2-FOUEA	N/A	Wellington	FTUCA	7.24	459 → 394	8	
2H-Perfluoro-2-(1,2-$^{13}C_2$)dodecenoic acid	13C2-FDUEA	N/A	Wellington	FTUCA	8.63	559 → 493.9	11	
Sodium bis[1H,1H,2H,2H-(1,2-$^{13}C_2$)perfluorooctyl] phosphate	13C4-6:2diPAP	N/A	Wellington	PAP	9.72	793 → 445	25	

Compound	Abbreviation	CAS No.	Company	Group	RT (min)	MRM Ions (*m/z*)	CE (V)	Internal Standard
Sodium bis[1H,1H,2H,2H-(1,2-$^{13}C_2$)perfluorodecyl] phosphate	13C4-8:2diPAP	N/A	Wellington	PAP	10.93	992.9 → 545	23	
Perfluoro-n-($^{13}C_4$)butanoic acid	13C4-PFBA*	N/A	Wellington	PFCA	3.47	217 → 171.9	5	
Perfluoro-n-($^{13}C_5$)pentanoic acid	13C5-PFPeA*	N/A	Wellington	PFCA	4.01	268 → 222.9	5	
Perfluoro-n-(1,2,3,4,6-$^{13}C_5$)hexanoic acid	13C5-PFHxA*	N/A	Wellington	PFCA	4.61	318 → 272.9	5	
Perfluoro-n-(1,2,3,4-$^{13}C_4$)heptanoic acid	13C4-PFHpA*	N/A	Wellington	PFCA	5.34	367 → 321.9	5	
Perfluoro-n-($^{13}C_8$)octanoic	13C8-PFOA*	N/A	Wellington	PFCA	6.17	421 → 375.9	5	
Perfluoro-n-($^{13}C_9$)nonanoic acid	13C9-PFNA*	N/A	Wellington	PFCA	6.99	472 → 427	5	
Perfluoro-n-(1,2,3,4,5,6-$^{13}C_6$)decanoic acid	13C6-PFDA*	N/A	Wellington	PFCA	7.77	519 → 474	5	
Perfluoro-n-(1,2,3,4,5,6,7-$^{13}C_7$)undecanoic acid	13C7-PFUdA*	N/A	Wellington	PFCA	8.42	570 → 524.9	5	
Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	13C2-PFDoA*	N/A	Wellington	PFCA	8.93	615 → 569.9	15	
Perfluoro-n-(1,2-$^{13}C_2$)tetradecanoic acid	13C2-PFTeA*	N/A	Wellington	PFCA	9.77	715 → 669.9	15	
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) ($^{13}C_3$)propanoic acid	13C3-HFPO-DA	N/A	Wellington	PFECA	4.82	287 → 168.9	5	
Sodium perfluoro-1-(2,3,4-$^{13}C_3$)butanesulfonate	13C3-PFBS*	N/A	Wellington	PFSA	4.1	302 → 79.9	60	
Sodium perfluoro-1-(1,2,3-$^{13}C_3$)hexanesulfonate	13C3-PFHxS*	N/A	Wellington	PFSA	5.39	402 → 79.9	60	
Sodium perfluoro-1-($^{13}C_8$)octanesulfonate	13C8-PFOS*	N/A	Wellington	PFSA	7.01	507 → 79.8	55	

Table 2. LC conditions.

Parameter	Value
Mobile Phase A	95:5 Water:methanol 2 mM ammonium acetate
Mobile Phase B	Methanol + 2 mM ammonium acetate
Delay Column	Agilent Infinity PFC Delay Column, 4.6 × 30 mm
Guard Column	Agilent ZORBAX Eclipse Plus, 2.1 × 5 mm, 1.8 µm
Analytical Column	Agilent ZORBAX Exclipse Plus C18 RRHD, 2.1 × 100 mm, 1.8 µm
Injection Volume	5 µL
Injection Program	Draw 10 µL of 1% formic acid in water, followed by 5 µL of sample, and 1 µL of air, prior to injection
Column Temperature	50 °C
Gradient	Time (min) %B 1 0 2 50 6 70 7.5 80 12.5 100 14.5 100 15 0 18 0

Table 3. Source settings.

Parameter	Setting
Ionization Mode	Negative
Gas Temperature	220 °C
Sheath Gas Temperature	340 °C
Gas Flow	17 L/min
Nebulizer Pressure	20 psi
Sheath Gas flow	10 L/min
Capillary Voltage	2,500 V
Nozzle Voltage	0 V

Results and discussion

IDLs

IDLs were calculated for all PFAS compounds and are summarized in Table 4. Notably, 70% of the compounds exhibited IDLs below10 pg/mL on-column, indicating strong instrument sensitivity and robust performance across most analytes.

Certain PFAS classes, including FTCAs, PFPAs, and PAPs, exhibited higher IDLs, primarily due to poor ionization efficiency—an issue previously reported in other LC/MS/MS methods.[14,15] Compounds such as Capstone A/B and NFPA, which contain amino or amide functional groups, also demonstrated low ionization, suggesting that negative ion mode at elevated source temperatures may not be optimal for these analytes. Additionally, tailing was observed for both PFPAs and 8:2 PAP, likely due to their di-anionic nature and increased interaction with the column.[15]

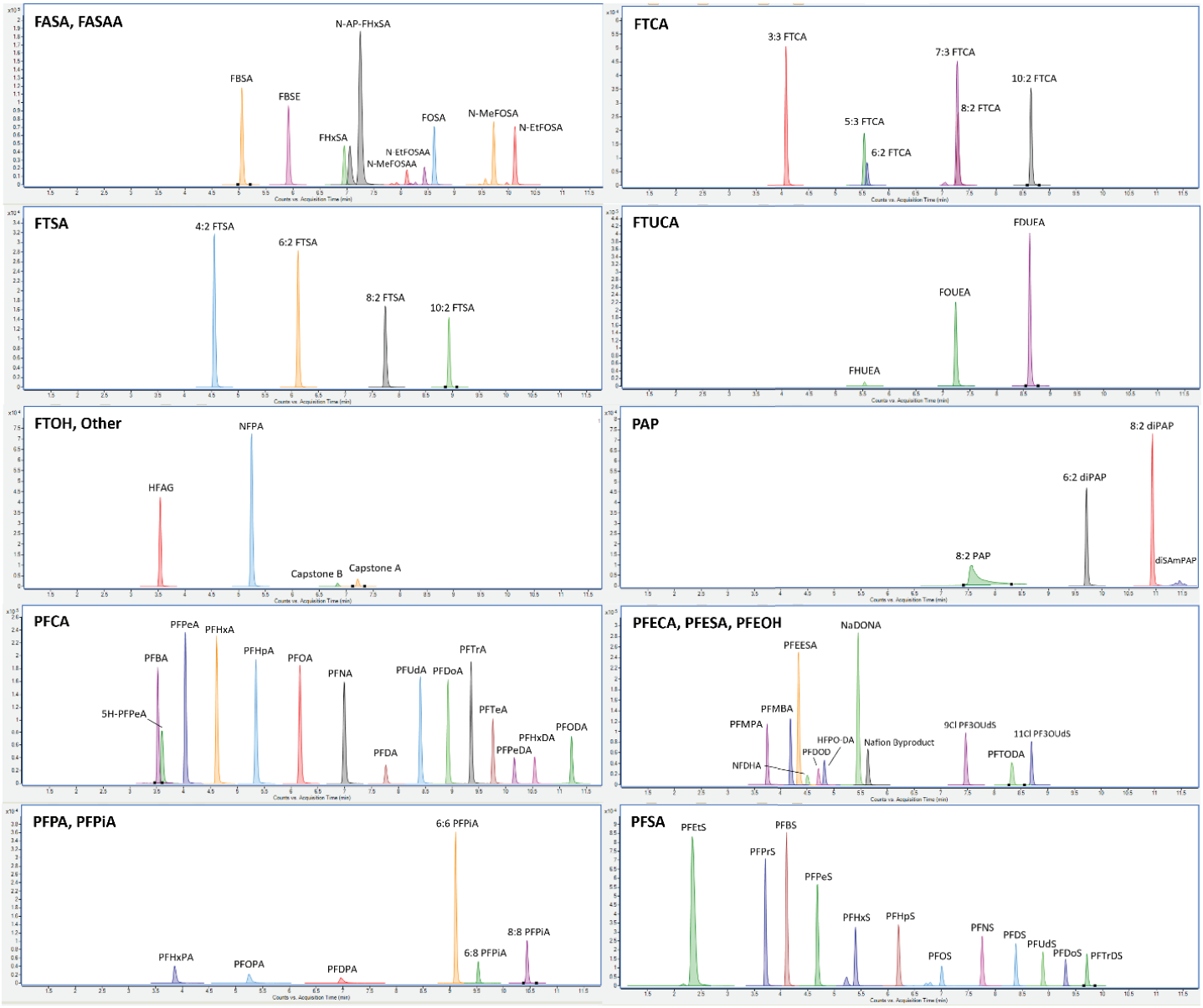

Figure 1. Chromatogram of continuous calibration verification (CCV) standard in 50:50 methanol:water at the mid spiking level (Table 1) for various PFAS groups.

Poor peak shape was particularly evident for diSAmPAP (Figure 1), which produced multiple peaks within a 0.3-minute window. This behavior is likely attributable to its large molecular weight (1,204 amu) and multiple charge centers. Despite efforts, the analyte could not be resolved into a single peak. Instead, spectral summation integration was applied to integrate the entire elution window, which remained consistent across multiple spiking levels.

Calibration curves were constructed with linear ranges spanning 0.01 to 200 ng/mL, based on calculated IDLs. All analytes achieved correlation coefficients (R^2) greater than 0.992 (Table 4). Interday precision, assessed through six replicate injections of low and high concentration standards, yielded average relative standard deviations (%RSDs) of 3% and 2%, respectively, demonstrating excellent repeatability across the calibration range.

Table 4. Linear range, instrument detection limits, and relative standard deviations for a low- and high-level spikes within linear ranges.

PFAS Class	PFAS Analyte	Linear Range (ng/mL)	IDL (fg Injected)	IDL (pg/mL on column)	Low Conc. RSD (%)	High Conc. RSD (%)
Perfluoroalkane Sulfonamide (FASA)	FBSA	0.01–10	4.7	0.9	1	1
	FBSE	0.05–20	31.4	6.3	2	1
	FHxSA	0.01–10	7.1	1.4	3	2
	N-AP-FHxSA	0.5–200	331.0	66.2	4	4
	FOSA	0.01–10	17.5	3.5	2	1
	N-MeFOSA	0.05–20	35.2	7.0	3	2
	N-EtFOSA	0.05–20	42.6	8.5	4	3
Perfluoroalkane Sulfonamido Acetic Acids (FASAA)	N-MeFOSAA	0.01–10	11.5	2.3	4	1
	N-EtFOSAA	0.01–10	19.3	3.9	3	2
Fluorotelomer Carboxylic Acid (FTCA)	3:3 FTCA	0.2–200	146.0	29.2	9	3
	5:3 FTCA	0.2–200	137.0	27.4	8	3
	6:2 FTCA	0.2–200	156.0	31.2	1	5
	7:3 FTCA	0.2–200	67.5	13.5	7	2
	8:2 FTCA	0.2–200	250.0	50.0	3	6
	10:2 FTCA	0.2–200	496.0	99.2	6	8
Fluorotelomer Alcohol (FTOH)	HFAG	0.01–10	14.0	2.8	3	1
Fluorotelomer Sulfonic Acid (FTSA)	4:2 FTSA	0.01–10	14.3	2.9	4	1
	6:2 FTSA	0.01–10	48.8	9.8	4	2
	8:2 FTSA	0.01–10	11.7	2.3	2	1
	10:2 FTSA	0.01–10	28.7	5.7	8	2
Fluorotelomer Unsaturated Carboxylic Acid (FTUCA)	FHUEA	0.05–20	142.0	28.4	2	3
	FOUEA	0.01–10	21.3	4.3	3	1
	FDUEA	0.01–10	9.2	1.8	2	1
Other	Capstone A	0.5–200	311.0	62.2	2	2
	Capstone B	0.5–200	989.0	197.8	6	3
Fluorotelomer Phosphate Ester (PAP)	8:2 PAP	0.5–200	723.0	144.6	4	6
	6:2 diPAP	0.05–20	41.8	8.4	2	2
	8:2 diPAP	0.05–20	102.0	20.4	3	1
	diSAmPAP	0.5–200	547.0	109.4	7	5

PFAS Class	PFAS Analyte	Linear Range (ng/mL)	IDL (fg Injected)	IDL (pg/mL on column)	Low Conc. RSD (%)	High Conc. RSD (%)
Per- and polyfluoroalkyl carboxylic acid (PFCA) and H-substitued PFCAs	PFBA	0.01–10	30.0	5.0	5	4
	5H PFPeA	0.5–200	291.0	58.2	17	6
	PFPeA	0.01–10	7.4	1.5	1	1
	PFHxA	0.01–10	5.0	1.0	2	1
	PFHpA	0.01–10	5.9	1.2	2	1
	PFOA	0.01–10	4.4	0.9	1	1
	PFNA	0.01–10	7.9	1.6	2	1
	PFDA	0.01–10	5.2	1.0	2	1
	PFUdA	0.01–10	11.1	2.2	1	1
	PFDoA	0.01–10	8.0	1.6	1	1
	PFTrA	0.01–10	9.1	1.8	1	1
	PFTeA	0.01–10	9.8	2.0	2	1
	PFPeDA	0.01–10	13.1	2.6	2	1
	PFHxDA	0.01–10	13.7	2.7	1	1
	PFODA	0.01–10	13.1	2.6	1	1
Other	NFPA	0.5–200	588.0	117.6	7	8
Per- and polyfluoroalkyl ether carboxylic acids (PFECA)	PFMPA	0.01–10	5.6	1.1	3	1
	PFMBA	0.01–10	3.7	0.7	1	1
	HFPO-DA	0.01–10	22.9	4.6	3	2
	NFDHA	0.05–20	124.0	24.8	3	4
	NaDONA	0.01–10	4.0	0.8	5	1
	PFTODA	0.5–200	638.0	127.6	8	5
Per- and polyfluoroalkyl ether alcohols (PFEOH)	PFDOD	0.01–10	13.5	2.7	4	2
Per- and polyfluoroalkyl ether sulfonic acids (PFESA)	PFEESA	0.01–10	5.6	1.1	1	1
	Nafion Byproduct	0.01–10	23.7	4.7	1	0
	9Cl PF3OUdS	0.01–10	5.1	1.0	1	2
	11Cl_PF3OUdS	0.01–10	7.6	1.5	3	1
Perfluoroalkyl phosphonic acid (PFPA)	PFHxPA	0.5–200	536.0	107.2	3	2
	PFOPA	0.5–200	563.0	112.6	3	2
	PFDPA	0.5–200	937.0	187.4	3	3
Perfluoroalkyl phosphinic acid (PFPiA)	6:6 PFPiA	0.01–10	16.3	3.3	4	1
	6:8 PFPiA	0.01–10	25.4	5.1	2	2
	8:8 PFPiA	0.01–10	26.0	5.2	3	2
Per- and polyfluoroalkyl sulfonic acid (PFSA)	PFEtS	0.5–200	224.0	44.8	1	4
	PFPrS	0.05–20	83.6	16.7	5	3
	PFBS	0.01–10	4.5	0.9	1	1
	PFPeS	0.01–10	6.4	1.3	2	1
	PFHxS	0.01–10	6.3	1.3	3	1
	PFHpS	0.01–10	10.0	2.0	2	1
	PFOS	0.01–10	9.4	1.9	4	2
	PFNS	0.01–10	10.7	2.1	2	1
	PFDS	0.01–10	8.8	1.8	3	2
	PFUnDS	0.01–10	16.4	3.3	4	2
	PFDoS	0.01–10	10.2	2.0	3	1
	PFTrDS	0.01–10	6.4	1.3	4	2

Note: IDLs were calculated by multiplying the %RSD of 6 replicated injections of analytes at lowest concentration of linear range by fg injected and the value of a one-sdied students t-test at 99% confidence level for n = 6.
Note: Low concentration RSDs were calculated at the bottom of the linear range while high concentration RSDs were calculated at the top for each analyte with n = 6.

Challenges in mixed standard preparation

During extended method evaluation, intraday relative standard deviations (RSDs) increased significantly for several compounds over a two-week period, including legacy PFAS such as PFOA and PFHxA. Upon comparison of individual stock solutions with the master mix, several compounds were identified as either degrading over time or containing unintended PFAS contaminants originating from the original stock vials (Table 5).

Table 5. Percentage of other compounds detected in MRM injections of stock concentrations of standards (100 ppb).

Standard (100 ppb)	PFEts	6:2 FTSA	6:6 PFPi	8:8 PFPi
PFHxPA	–	–	5.35%	–
PFOPA	–	–	–	4.12%
6:8 PFPi	–	–	5.12%	4.23%
Nafion Byproduct	31.02%	–	–	–
Capstone A	30.06%	27.60%	–	–

Note: Percentages are calculated by comparing peak areas of analytes detected to corresponding injections of the same compound (e.g. 6:6 PFPiA peak found in PFHxPA compared to 100 ppb injection of 6:6 PFPiA area)

Previous studies have reported that x:2 FTCAs and FTUCAs are prone to degradation in methanolic solutions. To mitigate this, these compounds are now commonly dissolved in isopropyl alcohol (IPA). In alignment with this approach, a separate stock solution of FTCAs and FTUCAs was prepared in IPA, then diluted in methanol and combined with other analytes immediately prior to spiking into samples and calibration standards. Additionally, fresh calibration curves were generated for all compounds during each extraction batch to minimize the impact of degradation.

Contamination was also observed in several commercial stock solutions. Notably, Nafion Byproduct and Capstone A were found to contain significant levels of PFPrS (~30 ppb in 100 ppb stock), while Capstone A also contained 6:2 FTSA (~25 ppb). Given their nature as byproducts, the presence of additional PFAS is not unexpected. However, due to these interferences, these compounds were excluded from the master mix and validated separately.

Similarly, PFHxPA, PFOPA, and 6:8 PFPiA were found to contain measurable levels of 6:6 and 6:8 PFPiA (~5 ppb in 100 ppb stock) and were also removed from the master mix. Validation and calibration for these five analytes, along with Capstone B and PFDPA, were conducted independently from the main group of 67 analytes, which showed no significant degradation or contamination.

Despite being excluded from the master mix, transitions for these compounds were retained in the dynamic MRM (dMRM) method to enable detection in incurred samples. Moving forward, calibration curves for these analytes were only generated during routine analysis if they were detected in samples, to prevent cross-contamination of other calibration sets. We recommend performing full MRM scans of individual PFAS standards when working with large, targeted panels to identify and mitigate potential cross-contamination.

Injection program

During method development, the addition of acid (specifically formic acid, though acetic acid was also evaluated) significantly improved the peak shape of early-eluting compounds such as PFBA and PFPrS. In the absence of acid, these analytes exhibited pronounced tailing and inconsistent retention times due to matrix effects. However, incorporating acid into the sample extraction protocol[12] led to improved peak symmetry and enhanced ionization efficiency. While smaller compounds such as 3:3 FTCA and PFBA showed reduced ionization under these conditions, larger PFAS compounds benefitted from increased signal intensity with the addition of 1% formic acid to the extraction solvent.

This enhancement, however, came with a trade-off: increased matrix co-extraction, resulting in elevated background signals. To mitigate this, an injection program was implemented in MassHunter Acquisition. This program introduced acid directly into the sample stream to improve chromatographic performance without exacerbating matrix effects. As detailed in Table 2, the program sequentially draws 10 µL of 1% formic acid in water, followed by 5 µL of sample and 1 µL of air prior to injection.

Bile acids separation

All 74 compounds were separated successfully using a 12-minute chromatographic gradient (Figure 2), with particular attention given to the baseline resolution of PFOS from isobaric bile acids—taurodeoxycholic acid (TDCA), tauroursodeoxycholic acid (TUDCA), and taurochenodeoxycholic acid (TCDCA)—which are commonly found in food matrices such as eggs (Figure 2). To ensure consistent and accurate peak identification, spectral summation was applied during sample analysis using MassHunter Qualitative Analysis software (version 12.2). Compound-specific integration windows (e.g., ± 0.1 minute) were established based on injections of pure standards at 100 ng/mL, with baselines defined at the lowest point within the total ion chromatogram (TIC) window. This approach was particularly critical for PFOS, helping to prevent automated peak-picking algorithms from misidentifying the more intense bile acid peaks eluting nearby.

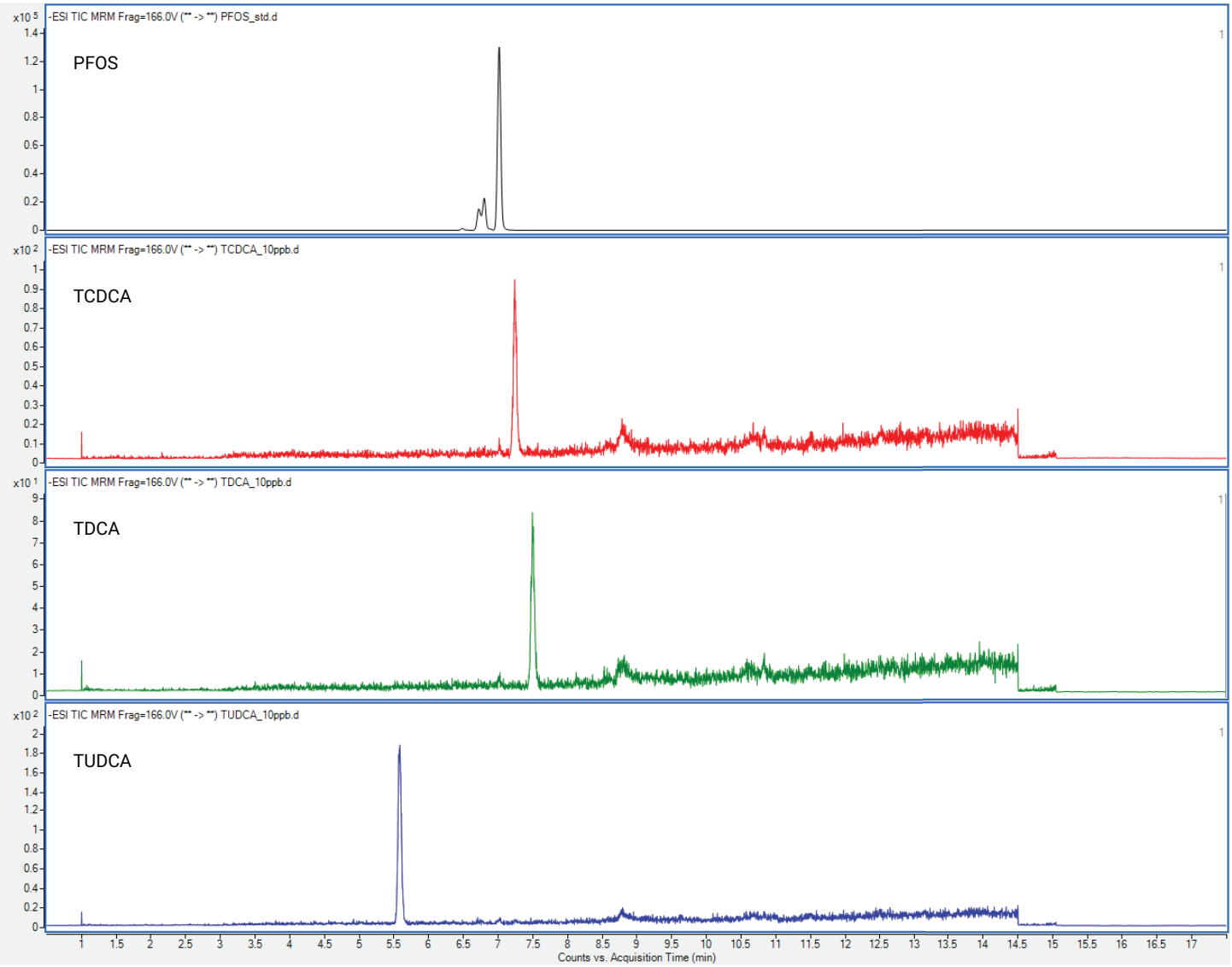

Figure 2. TIC of PFOS and bile acids showing baseline separation for all acids from PFOS.

Conclusion

This study presents a robust and comprehensive LC/MS/MS method for the detection of 74 PFAS compounds across diverse chemical classes. Through careful optimization of mixed standard preparation, chromatographic separation—particularly for bile acids—and injection programming, the method achieves high sensitivity and reproducibility, with most compounds exhibiting instrument detection limits below 10 pg/mL. Although developed for animal-derived matrices, the method demonstrates broad applicability to other sample types. These findings underscore the importance of addressing compound-specific challenges in PFAS analysis and provide a reliable framework for future monitoring efforts in food safety and environmental research.

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