



Rapid Perfluorinated Alkyl Acid Analysis by LC-MS/MS Increases Sample Throughput

By Dr. Shun-Hsin Liang, Senior LC Applications Scientist

- Raptor C18 SPP 5 μm core-shell silica particle columns offer excellent resolution for fluorochemicals with short total cycle times.
- Meets the requirements for EPA Method 537 and ISO 25101.
- Unique, robust Raptor C18 column design increases instrument uptime.

Perfluorinated alkyl acids are man-made fluorochemicals used as surface-active agents in the manufacture of a variety of products, such as firefighting foams, coating additives, textiles, and cleaning products. They have been detected in the environment globally and are used in very large quantities around the world. These fluorochemicals are extremely persistent and resistant to typical environmental degradation processes. As a result, they are widely distributed across the higher trophic levels and are found in soil, air, groundwater, municipal refuse, and landfill leachates. The toxicity, mobility, and bioaccumulation potential of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), in particular, pose potential adverse effects for the environment and human health.

Due to exposure risk and the likelihood of these detrimental impacts, PFOS and PFOA have both come under regulatory scrutiny, and many countries have restricted their use and are establishing threshold levels in drinking water and other matrices. In Directive 2013/39/EU (an amendment to the Water Framework Directive), PFOS is named as a “Priority Substance” with very challenging environmental quality standards. EU Directive 2006/122/ECOF now prohibits the manufacture and use of PFOS in the European Union, and this directive also considers PFOA to present a risk profile that is comparable to PFOS. Perfluorinated alkyl substances (PFAS) in general are not currently regulated in the United States, but the U.S. EPA has issued a Health Advisory for PFOS and PFOA, and most U.S. states and cities monitor for the presence of PFAS in drinking water.

Perfluorinated alkyl acid analysis can be challenging because these compounds are chemically different from most other environmental contaminants. They are difficult to quantify because some are more volatile than others, and they also tend to be more hydrophilic and somewhat reactive. In addition, fluorochemicals are present in polytetrafluoroethylene (PTFE) materials, so excluding the use of any PTFE labware throughout the sampling and analytical processes (including HPLC solvent inlet tubing) is essential for accurate analysis. Typically, perfluorinated alkyl acids are analyzed by LC-MS/MS methods, such as EPA Method 537, ISO 25101, DIN 38407-42, ASTM D7979, or ASTM D7968, but long analysis times can significantly limit sample throughput. These methods all provide example chromatographic conditions, with analysis times ranging anywhere from 15-27 minutes in length, depending on the standard. However, each method also permits flexibility when defining analytical parameters and choosing a column, which allows laboratories to optimize their conditions to meet method requirements as efficiently as possible.

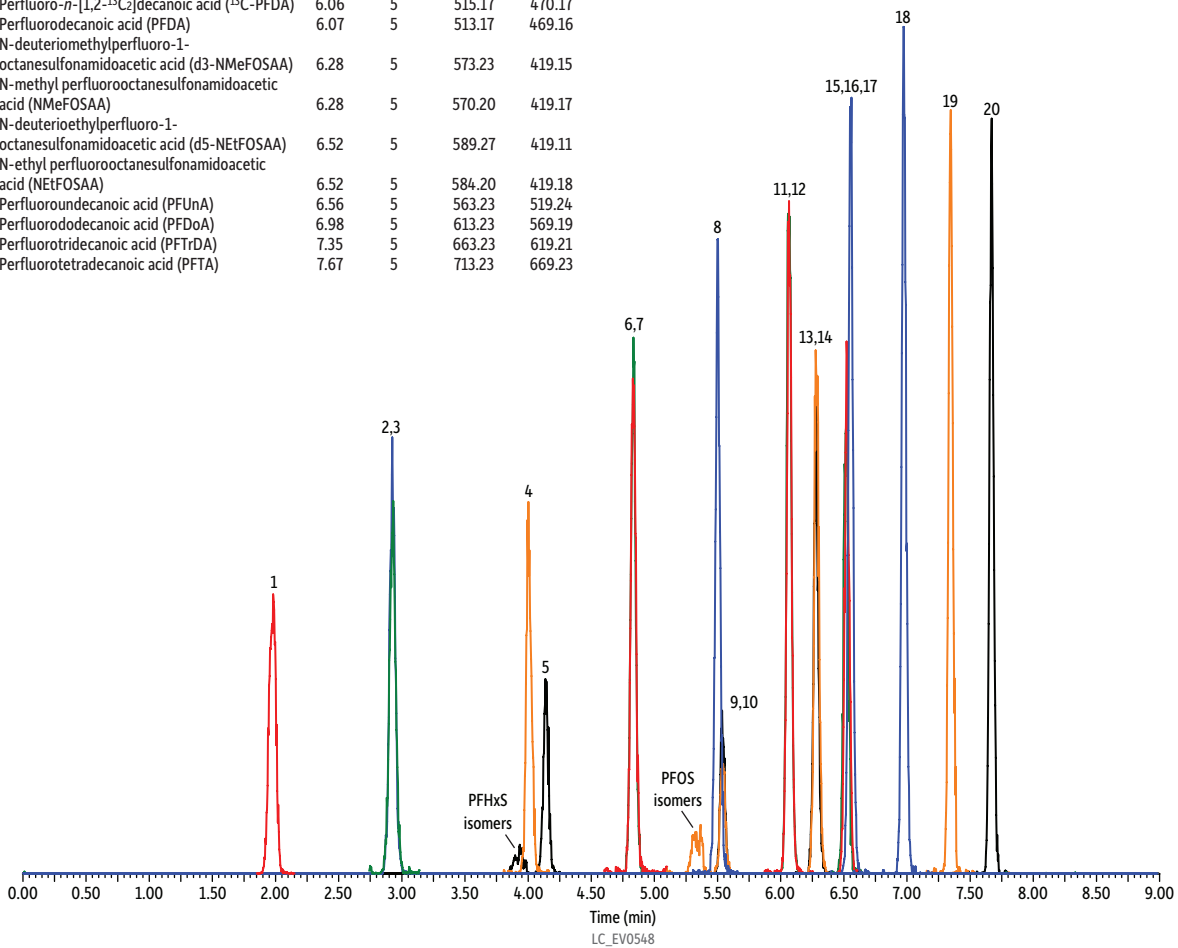
Taking advantage of the faster, more efficient separations that are possible with superficially porous particle (SPP) columns, the method presented here offers a fast analysis with sufficient resolution to permit adequate MS dwell time for all compounds within a specific retention time window. As shown in Figure 1, all target perfluorinated alkyl acids were analyzed on a Raptor C18 column with a total cycle time of 9 minutes without sacrificing peak resolution or selectivity. All analytes elute as highly symmetrical peaks that can be accurately identified, integrated, and quantified by MS/MS. This method also easily resolves PFHxS and PFOS branched isomers for standards requiring their summation with the unbranched compounds as a part of reporting (e.g., EPA Method 537).

If PFOA and PFOS are the only target fluorochemicals, the chromatographic conditions can be further optimized. The chromatogram in Figure 2 demonstrates that a fast, <2-minute separation with a total cycle time of just 4.5 minutes can be achieved using a 2.1 x 100 mm Raptor C18 column. Alternatively, a shorter column (2.1 x 50 mm) or a column with smaller particles (e.g., 2.7 μm particles) can be used for an even faster analysis.

Whether labs conducting perfluorinated alkyl acid analysis by LC use longer target analyte lists or focus just on PFOA and PFOS, the excellent peak shapes and separations achieved here result in consistent, accurate quantification with much shorter analysis times. By switching to a Raptor C18 column, labs can process more samples per hour while still meeting fluorochemical method requirements.

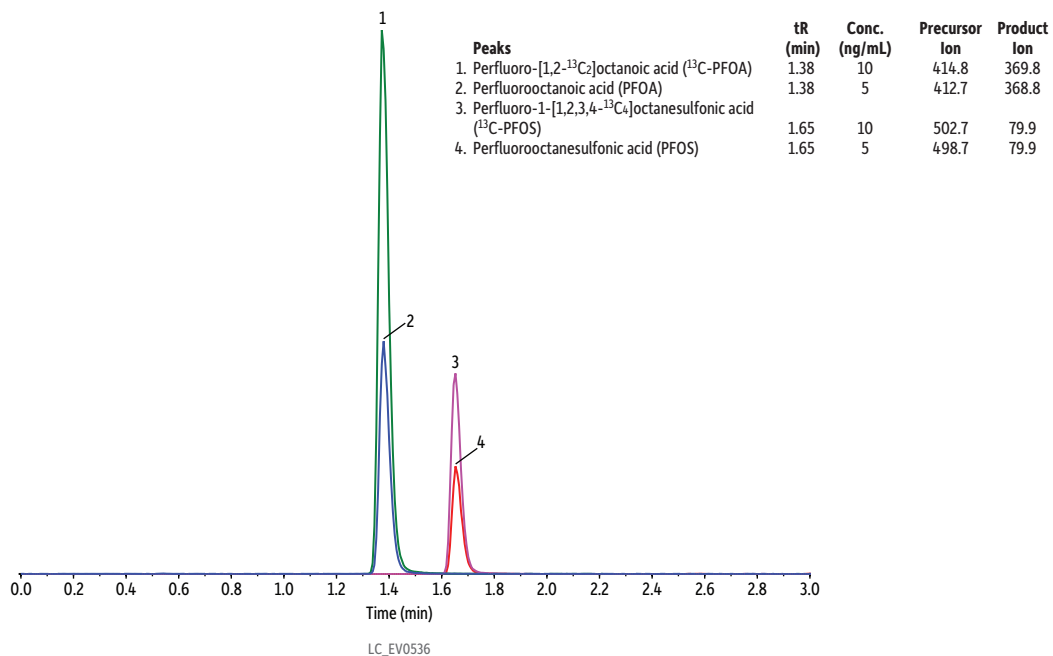
Figure 1: Decrease analysis time for comprehensive perfluorinated alkyl acid analysis using a 5 μm Raptor C18 column and these instrument conditions.

Peaks	tr (min)	Conc. (ng/mL)	Precursor Ion	Product Ion
1. Perfluorobutanesulfonic acid (PFBS)	1.98	10	299.10	79.99
2. Perfluoro- <i>n</i> -[1,2- ¹³ C ₂]hexanoic acid (¹³ C-PFHxA)	2.92	10	315.13	270.13
3. Perfluorohexanoic acid (PFHxA)	2.93	10	313.10	269.12
4. Perfluoroheptanoic acid (PFHpA)	4.00	5	363.16	319.09
5. Perfluorohexanesulfonic acid (PFHxS)	4.14	10	399.13	79.98
6. Perfluoro-[1,2- ¹³ C ₂]octanoic acid (¹³ C-PFOA)	4.83	5	415.13	370.10
7. Perfluorooctanoic acid (PFOA)	4.83	5	413.16	369.10
8. Perfluorononanoic acid (PFNA)	5.50	5	463.16	419.19
9. Perfluoro-1-[1,2,3,4- ¹³ C ₄]octanesulfonic acid (¹³ C-PFOS)	5.54	5	503.13	79.98
10. Perfluorooctanesulfonic acid (PFOS)	5.54	10	499.17	79.98
11. Perfluoro- <i>n</i> -[1,2- ¹³ C ₂]decanoic acid (¹³ C-PFDA)	6.06	5	515.17	470.17
12. Perfluorodecanoic acid (PFDA)	6.07	5	513.17	469.16
13. N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid (d3-NMeFOSAA)	6.28	5	573.23	419.15
14. N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)	6.28	5	570.20	419.17
15. N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid (d5-NEtFOSAA)	6.52	5	589.27	419.11
16. N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)	6.52	5	584.20	419.18
17. Perfluoroundecanoic acid (PFUnA)	6.56	5	563.23	519.24
18. Perfluorododecanoic acid (PFDoA)	6.98	5	613.23	569.19
19. Perfluorotridecanoic acid (PFTrDA)	7.35	5	663.23	619.21
20. Perfluorotetradecanoic acid (PFTA)	7.67	5	713.23	669.23



Column: Raptor C18 (cat.# 9304552); Dimensions: 50 mm x 2.1 mm ID; Particle Size: 5 μm; Pore Size: 90 Å; Temp.: 40 °C; **Sample:** Diluent: 96:4 Methanol:water; Conc.: 5–10 ng/mL; Inj. Vol.: 2 μL; **Mobile Phase:** A: 5 mM ammonium acetate in water; B: Methanol; **Gradient (%B):** 0.00 min (25%), 8.00 min (85%), 8.01 min (25%), 9.00 min (25%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Source: Electrospray; Ion Mode:ESI-; Mode: MRM; **Instrument:** Waters Acquity

Figure 2: Separate PFOS and PFOA to baseline in a fast, <2 min analysis (4.5 min total cycle time).



LC_EV0536

Column Raptor C18 (cat.# 9304512)
 Dimensions: 100 mm x 2.1 mm ID
 Particle Size: 5 µm
 Pore Size: 90 Å
 Temp.: 40 °C

Sample
 Diluent: Water:methanol (50:50)
 Conc.: 5-10 ng/mL
 Inj. Vol.: 5 µL

Mobile Phase
 A: 5 mM ammonium acetate in water
 B: Methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.4	40	60
2.50	0.4	5	95
2.51	0.4	40	60
4.50	0.4	40	60

Detector MS/MS
 Ion Mode: ESI-
 Mode: MRM
Instrument UHPLC



Raptor C18 LC Columns (USP L1)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 μm Columns			
30 mm	9304232	—	—
50 mm	9304252	930425E	—
100 mm	9304212	930421E	—
150 mm	9304262	—	—
2.7 μm Columns			
30 mm	9304A32	9304A3E	9304A35
50 mm	9304A52	9304A5E	9304A55
100 mm	9304A12	9304A1E	9304A15
150 mm	9304A62	9304A6E	9304A65
5 μm Columns			
30 mm	—	930453E	—
50 mm	9304552	930455E	9304555
100 mm	9304512	930451E	9304515
150 mm	9304562	930456E	9304565
250 mm	—	—	9304575