

LC-MS/MS analysis of PFAS in human serum

Analysis of PFAS in human serum using a YMC-Triart C18 column in combination with an online SPE column-switching method

Poly- and perfluoroalkyl substances (PFAS) are widely used because of their special properties which include heat resistance and water and fat repellency. Commonly known products in which PFAS can be found include non-sticky cookware, water-repelling textiles or glossy magazines. More than 4,000 substances are available on the market, which all are industrially originated.

Due to their molecular structure which contains carbon-fluorine bonds, PFAS benefit from a high stability, both thermal as well as chemical. However, this stability also leads to a persistency in the environment as they are not fully degradable which makes them persistent organic pollutants (POPs). These POPs not only accumulate in the environment but also in the human body since they are absorbed from food, water or air.

Reference:

S.F. Nakayama, T. Isobe, M. Iwai-Shimada, Y. Kobayashi, Y. Nishihama, Y. Taniguchi, M. Sekiyama, T. Michikawa, S. Yamazaki, H. Nitta, M. Oda, H. Mitsubuchi, M. Sanefuji, S. Ohga, N. Mise, A. Ikegami, R. Suga, M. Shimono, "Poly- and perfluoroalkyl substances in maternal serum: Method development and application in Pilot Study of the Japan Environment and Children's Study", J. Chromatogr. A, 2020, 1618:460933.

As a result, they are found predominantly in human blood or organs, which is why they need to be strictly monitored and regulated. As they are recently been assumed to be potentially carcinogenic, the daily uptake limit was lowered especially for two substances. Due to the adverse health effects of PFAS, a highly sensitive method is of particular relevance. This application note shows the analysis of 28 PFAS in human serum, based on the study of Nakayama et al. An automated solid phase extraction (SPE) system was coupled to an online SPE column-switching LC-MS/MS method. Anion-exchanging scrubber columns were placed downstream of the LC pumps A, C and D in order to eliminate possible contamination from the system. A YMC-Triart C18 column used as the analytical column shows excellent results for this sensitive analysis.

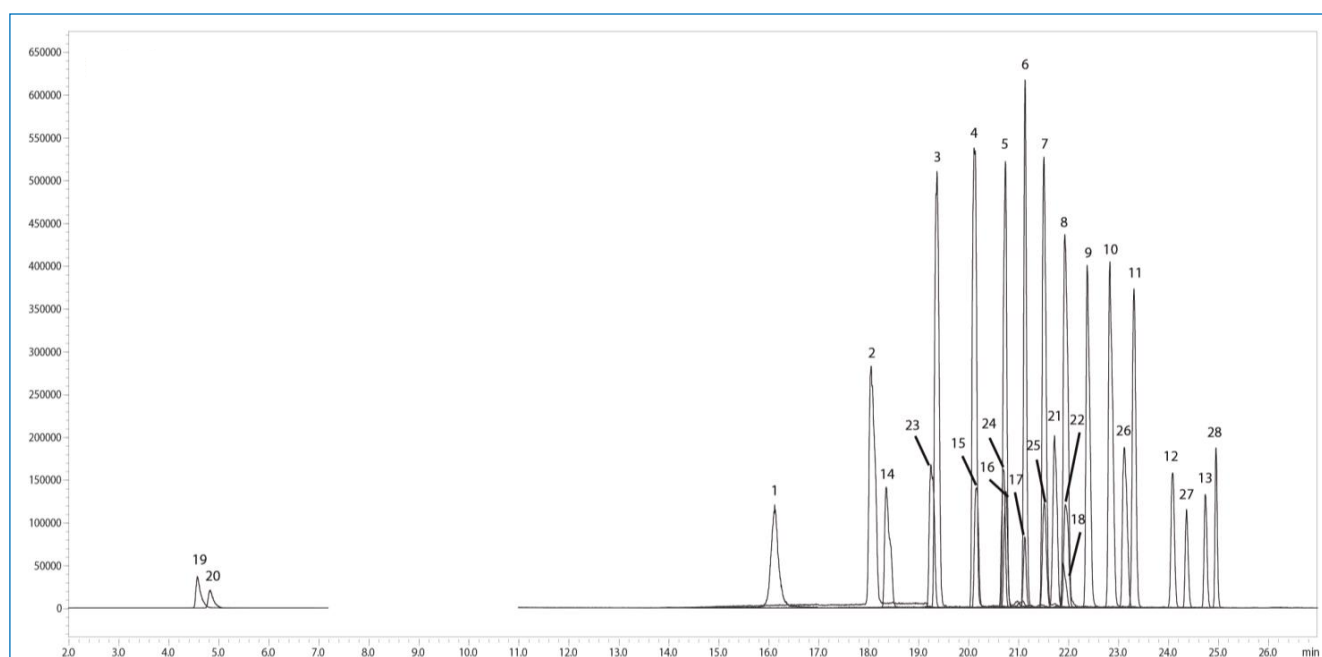


Figure 1: Separation of 28 PFAS using a YMC-Triart C18 column as an analytical column.

List of PFAS separated in Figure 1:

- | | |
|---|---|
| 1. Perfluorobutanoic acid (PFBA) | 15. Perfluorohexane sulphonic acid (PFHxS) |
| 2. Perfluoropentanoic acid (PFPA) | 16. Perfluoroheptane sulphonic acid (PFHpS) |
| 3. Perfluorohexanoic acid (PFHxA) | 17. Perfluorooctane sulphonic acid (PFOS) |
| 4. Perfluoroheptanoic acid (PFHpA) | 18. Perfluorodecane sulphonic acid (PFDS) |
| 5. Perfluorooctanoic acid (PFOA) | 19. <i>N</i> -Methyl perfluorooctane sulphonamide (MeFOSA-M) |
| 6. Perfluorononanoic acid (PFNA) | 20. <i>N</i> -Ethyl perfluorooctane sulphonamide (EtFOSA-M) |
| 7. Perfluorodecanoic acid (PFDA) | 21. <i>N</i> -Methyl perfluorooctane sulphonamido acetic acid (MeFOSA-A) |
| 8. Perfluoroundecanoic acid (PFUnA) | 22. <i>N</i> -Ethyl perfluorooctane sulphonamido acetic acid (EtFOSA-A) |
| 9. Perfluorododecanoic acid (PFDoA) | 23. 4:2 Fluorotelomer sulphonic acid (4:2 FTS) |
| 10. Perfluorotridecanoic acid (PFTrDA) | 24. 6:2 Fluorotelomer sulphonic acid (6:2 FTS) |
| 11. Perfluorotetradecanoic acid (PFTeDA) | 25. 8:2 Fluorotelomer sulphonic acid (8:2 FTS) |
| 12. Perfluorohexadecanoic acid (PFHxDA) | 26. 6:2 Polyfluoroalkyl phosphoric acid diester (6:2 diPAP) |
| 13. Perfluorooctadecanoic acid (PFODA) | 27. 8:2 Polyfluoroalkyl phosphoric acid diester (8:2 diPAP) |
| 14. Perfluorobutane sulphonic acid (PFBS) | 28. Perfluorooctane sulphonamido ethanol-based phosphate ester (diSAmPAP) |

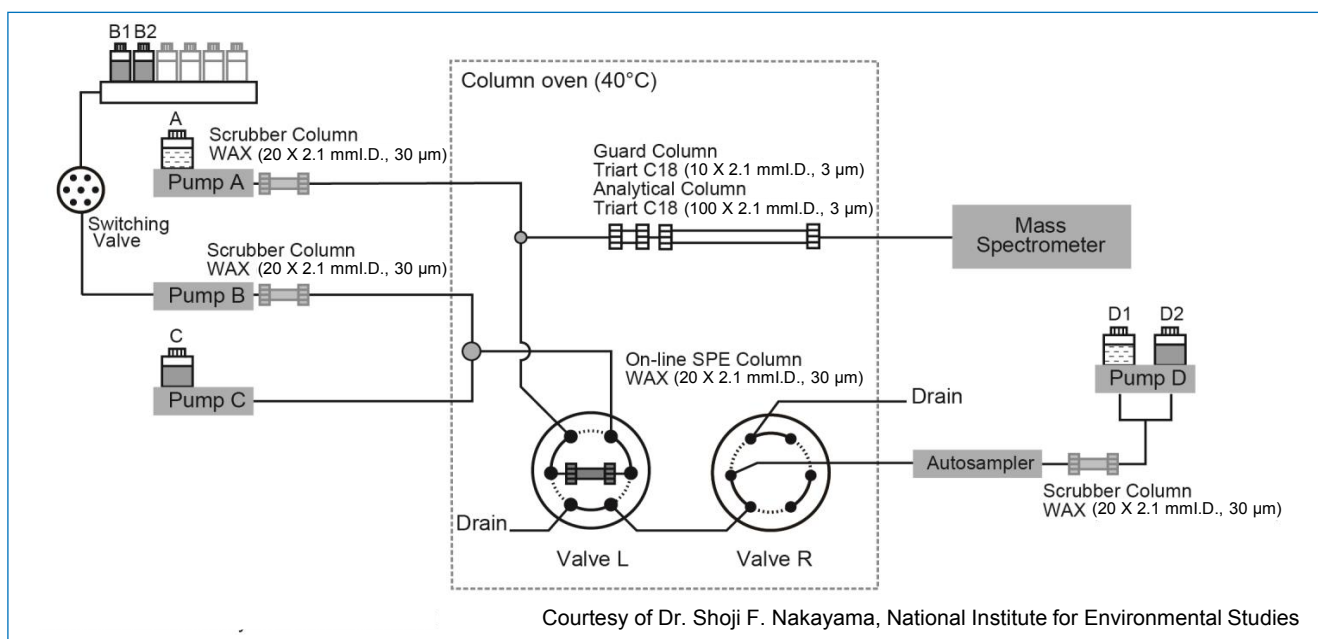


Figure 2: Instrumental configuration.

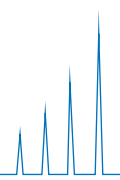


Table 1: Gradient conditions.

Time (min)	Ternary gradient				Flow rate	Binary gradient		Flow rate	Valve position	
	Pump A (%)	Pump B1 (%)	Pump B2 (%)	Pump C (%)	Pump A+B+C (mL/min)	Pump D1 (%)	Pump D2 (%)	Pump D (mL/min)	Valve L	Valve R
0.00	12.5	87.5	0.0	0.0	0.3	100	0.0	1.0	1	0
2.00	12.5	87.5	0.0	0.0	0.3	100	0.0	1.0	0	1
2.01	12.5	87.5	0.0	0.0	0.3	0.0	0.0	0.0	0	1
4.00	12.5	87.5	0.0	0.0	0.3	0.0	0.0	0.0	0	1
5.50	12.5	87.5	0.0	0.0	0.3	0.0	0.0	0.0	1	0
5.51	12.5	0.0	87.5	0.0	0.3	0.0	0.0	0.0	1	0
7.00	12.5	0.0	87.5	0.0	0.3	0.0	0.0	0.0	1	0
7.01	92.5	0.0	0.0	7.5	0.5	0.0	0.0	0.0	1	0
7.50	92.5	0.0	0.0	7.5	0.5	0.0	0.0	0.0	0	1
10.00	92.5	0.0	0.0	7.5	0.5	0.0	0.0	0.0	0	1
10.01	92.5	0.0	0.0	7.5	0.3	0.0	0.0	0.0	0	1
18.00	45	0.0	47.5	7.5	0.3	0.0	0.0	0.0	0	1
20.00	27.5	0.0	65	7.5	0.3	0.0	0.0	0.0	1	0
20.01	*a	0.0	*a	12.5	0.3	0.0	100	1.0	1	0
24.00	*a	0.0	*a	12.5	0.3	0.0	100	1.0	1	0
24.01	*a	0.0	*a	12.5	0.3	100	0.0	1.0	1	0
25.00	0.0	0.0	87.5	12.5	0.3	100	0.0	1.0	1	0
25.01	12.5	87.5	0.0	0.0	0.3	100	0.0	1.0	1	0
27.00	12.5	87.5	0.0	0.0	0.3	100	0.0	1.0	1	0

*a: 20.00–25.00 min linear gradient (Pump A + Pump B2)

Table 2: Chromatographic conditions

Column:	YMC-Triart C18 (3 µm, 12 nm) Analytical column 100 x 2.1 mm ID Guard column 10 x 2.1 mm ID
Part nos.:	TA12S03-10Q1PTH and TA12S03-01Q1GC
Eluent:	LC Pump A: 2.5 mM ammonium acetate LC Pump B1: methanol LC Pump B2: 2.5 mM ammonium acetate in 95% methanol LC Pump C: 0.1% ammonia in methanol
Temperature:	40°C
Detection:	negative ESI
Injection:	400 µL (4 ng/mL standard solution)
Sample Load:	LC pump D1: water
Online SPE	
column wash:	LC pump D2: 0.5% ammonia in 90% methanol
System:	LCMS-8060 (Shimadzu Corporation)

