Enhanced sensitivity for PFAS using a hybrid autosampler approach with LC-MSMS

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NEMC 2024
P28

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Introduction

The challenge of low PFAS detection and quantification limits

The analysis of PFAS (per- and polyfluorinated alkyl substances) often requires sample preparation techniques like solid phase extraction (SPE), especially in case of drinking water analysis with low detection limit requirements. The final samples ready for LC/MS/MS analysis are therefore usually dissolved in 80-100% organic solvents.[1] Additionally, recommended drinking water concentration limits are getting even lower, lately[2], especially considering the current Interim Health Advisory Levels for PFOA (0.004 ppt) and PFOS (0.02 ppt) published by the US EPA.[3]

Injecting high sample volumes could improve sensitivity and therefore allow lower detection limits but this is limited by undesirable solvent effects caused by the high elution strength of the sample solvent in case of common reversed phase liquid chromatography.

Overcoming solvent effects in a convenient way

Feed Injection, as an alternative injection principle to the common flow through injection allows much higher injection volumes without negative impact on the peak shape, even when the sample is dissolved in 100% organic solvents. This is achieved by infusing the sample into the mobile phase stream with a special valve resulting in a dilution.[4]

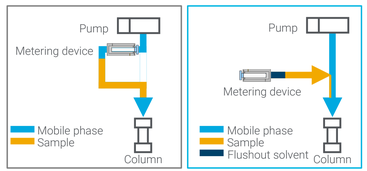

Figure 1. Classic flowthrough injection (left) vs. Feed Injection (right)

Positive impact of Feed Injection on chromatographic performance

In case of PFAS analysis, the benefits of Feed Injection have recently been demonstrated.[5]

To maximize the impact, 100% aqueous mobile phases, columns that can tolerate these conditions and slow feed speeds of 10-20% of the mobile phase flow rate can be applied.

As a result, the chromatographic performance is significantly improved:

- Linear correlation between injection volume and peak height
- No analyte breakthrough (maximum sensitivity)
- Great peak shape (no issues with automatic integration)
- No interference of fronting main peaks with branched isomer peaks

Introduction

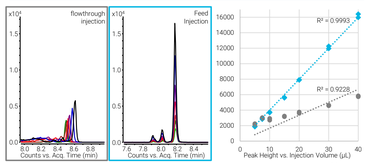

Figure 2. EtFOSAA (comparison of flowthrough injection with Feed Injection)[5]

Experimental

Hardware

LC-MS system:
1290 Infinity II High Speed Pump (G7120A)
1260 Infinity II Hybrid Multisampler (G7167C)
1290 Infinity II Multicolumn Thermostat (G7116B)
G6495D triple quadrupole LC-MS
Converted for low PFAS background with the Agilent PFC free HPLC upgrade kit (5004-0006)

Column:
InfinityLab Poroshell 120 Aq-C18 4.6 x 100 mm, 2.7 µm (695975-742)

Guard Column:
InfinityLab Poroshell 120 Aq-C18 3.0 mm, 2.7 µm (823750-953)

Delay Column:
InfinityLab PFC Delay Column, 4.6 x 30 mm (5062-8100)

SPE Cartridges:
Bond Elut PFAS WAX, 200 mg, 6 mL (5610-2151)

Samples

200mL water spiked with PFAS standards at various concentrations

SPE Method

- Flush cartridge 2x with 5mL 1% NH_4OH in MeOH
- Condition cartridge with 5mL 0.3M formic acid
- Load Sample
- Rinse 2x with 5mL water
- Dry under vacuum
- Elute 2x with 5mL 1% NH_4OH in MeOH
- Evaporate to 1mL
- Transfer to vial

Analytes

51 PFAS analytes (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnDS, PFDoDS, 3:3FTCA, 5:3FTCA, 7:3FTCA, 4:2FTS, 6:2FTS, 8:2FTS, 6:2FTOH, 8:2FTOH, 6:2FTAB, FBSA, FHxSA, FOSA, HFPO-DA, HFPO-TA, ADONA, 6:2Cl-PFESA, 8:2Cl-PFESA PFMPA, PFMBA, C6O4, NFDHA, PFEESA, MeFOSAA, EtFOSAA, MeFOSA, EtFOSA, MeFOSE, EtFOSE, PFecHxS)

Internal Standards

Isotopically labelled PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnA PFDoA, PFTeA, PFBS, PFHxS, PFOS, 4:2 FTS, 6:2 FTS, 8:2 FTS, MeFOSA, EtFOSA, MeFOSE, EtFOSE, MeFOSAA, EtFOSAA

Results and Discussion

LC-MS/MS method

Mobile Phase A (MP A): water with 5mM ammonium acetate
Mobile Phase B (MP B): methanol

Column Temperature: 45 °C

Feed Injection:
Injection Volume: 40µL
Feed Speed: 15 % of flow (adaptive)
Automatic Overfeed Volume
Flushout Solvent: Mobile Phase A
Wash Solvent: Mobile Phase B
Inner / outer wash: 150 µL / 6s
Reconditioning with Mobile Phase A

MS settings: PFAS MRM Database for LC/TQ (G1736AA) was used for all LCMS settings

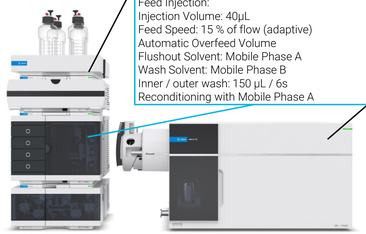

Table 1. Mobile Phase Gradient

Time	%B
2.0	2%
3.0	50%
7.0	72%
10.0	90%
14.4	90%
14.41	2%
17.0	2%

Table 2. Ion source parameters

Parameter	For most PFAS	For FTOHs and C6O4
Gas Temperature	250 °C	80 °C
Gas Flow	12 L/min	20 L/min
Nebulizer Pressure	30 PSI	60 PSI
Sheath Gas Temperature	370 °C	200 °C
Sheath Gas Flow	12 L/min	8 L/min
Capillary Voltage	2350 V	3000 V
Ion Funnel Settings	Variable	Variable

Results and Discussion

Maximizing Sensitivity

Combining the benefits of Feed Injection on the chromatographic end with the use of SPE with a high concentration factor and the most current state-of-the-art triple quadrupole MS instruments enables access to extremely low PFAS detection limits.

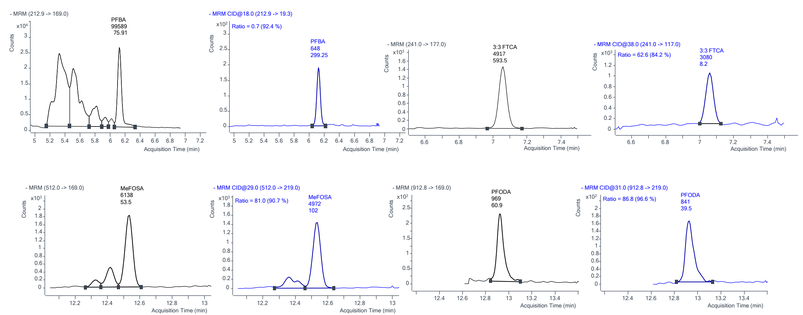

Figure 3. Chromatograms of select PFAS analytes from a drinking water sample spiked at 0.1 ng/L (S/N ratios and peak areas listed below analyte names; qualifier transitions in blue)

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Results and Discussion

Ultra-trace detection limits

In case of some of the most commonly analyzes PFAS, even much lower concentrations can reliably be detected. This demonstrates, that even the challenging EPA Health Advisory Levels can be met or undercut.

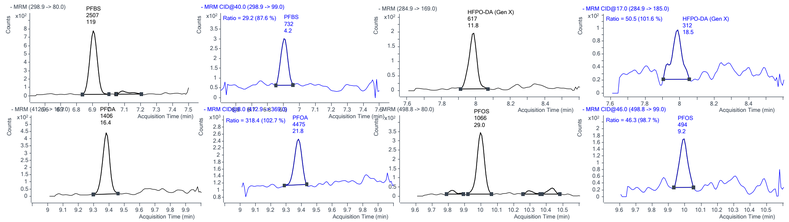

Figure 4. Chromatograms of select PFAS analytes from a drinking water samples spiked at 4 pg/L (S/N ratios and peak areas listed below analyte names; qualifier transitions in blue)

Table 3. Results from a drinking water sample spiked at 4pg/L (N = 9)

Analyte	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFTeDA	PFOcDA	PFBS	PFPeS	PFHxS	PFHpS
Average Peak Area	6921	12546	7392	4470	1480	2376	3117	4211	295	89	2561	1617	1314	1187
% RSD	9.8	11.1	4.2	6.0	4.0	21.3	12.4	22.3	19.7	22.4	3.0	5.0	5.0	7.5
Average S/N	18.5	420	36.2	23.3	54.0	2.4	8.2	6.0	86.1	17.8	104	86.6	55.0	109
Analyte	**PFOS**	**PFNS**	**PFDS**	**PFUnDS**	**PFDoDS**	**3:3FTCA**	**5:3FTCA**	**7:3FTCA**	**4:2FTS**	**6:2FTS**	**8:2FTS**	**FBSA**	**FHxSA**	**FOSA**
Average Peak Area	1034	989	754	759	689	267	2649	2348	1066	1798	844	5695	2443	3606
% RSD	6.7	8.3	10.9	14.6	14.4	11.7	5.2	9.3	9.4	10.0	20.9	3.4	7.5	6.8
Average S/N	41.5	174	124	44.2	7.4	26.4	25.8	17.0	143	44.8	10.0	3264	2958	761
Analyte	**HFPO-DA**	**HFPO-TA**	**ADONA**	**6:2Cl-PFESA**	**8:2Cl-PFESA**	**PFMPA**	**PFMBA**	**NFDHA**	**PFEESA**	**MeFOSAA**	**EtFOSAA**	**MeFOSA**	**MeFOSE**	**PFecHxS**
Average Peak Area	683	1858	9771	2073	1654	5624	5506	4731	4663	1158	1090	901	1199	2172
% RSD	10.8	18.0	1.8	7.8	7.0	2.5	3.1	4.9	2.0	13.3	14.3	13.5	12.9	7.4
Average S/N	34.9	44.8	298	11.9	31.6	68.2	130	26.3	215	20.7	8.0	17.2	10.9	34.7

Out of the 51 PFAS tested, good to very good results were obtained at a spiking concentration of 4 pg/L in case of the 42 PFAS listed in the table above. At a spiking concentration of 100 pg/L, good to very good results were obtained for all other analytes, except EtFOSE and 6:2FTOH, 8:2FTOH and C6O4, with an LOQ of 250 pg/L. In case of the latter three, special ion source parameters had to be used for good results.

Conclusions

Feed Injection can reduce solvent effects significantly in the analysis of PFAS, allowing high injection volumes of samples dissolved in organic solvents. Combining the chromatographic benefits of Feed Injection with SPE sample enrichment and highest sensitivity triple quadrupole LCMS instrumentation allows lowest possible PFAS detection and quantification limits.

- **Sharp and symmetric peaks (including challenging analytes like PFBA) at high injection volumes**
- **Extremely low detection limits (4 pg/L = 4 ppq) achievable for many of the most common PFAS analytes**

References

[1] M. Zarębska, S. Bajkacz, Poly- and perfluoroalkyl substances (PFAS) - recent advances in the aquatic environment analysis, Trends in Analytical Chemistry (2023), doi: https://doi.org/10.1016/j.trac.2023.117062
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[4] Performance Characteristics of the Agilent 1260 Infinity II Hybrid Multisampler. Agilent Technologies Technical Overview, publication number 5994-5952EN, 2023.
[5] More Sensitive Quantification of PFAS by LC/MS with the Agilent 1260 Infinity II Hybrid Multisampler. Agilent Technologies Application Note, publication number 5994-6994EN, 2024.