

SOLUTIONS BY



Determination of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Water with FREESTYLE XANA and LC-MS/MS

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1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are used in numerous industrial and consumer products because of their special chemical properties. The group of substances includes more than 3000 different compounds.

For more than 60 years, PFAS are used to provide everyday objects and materials with non-stick coatings. They make surfaces fat- and water-repellent and are extremely versatile. Not only functional clothing in the textile industry, but also technical devices and even pizza boxes or disposable coffee cups are often coated with PFAS. Other typical applications for PFAS would be fire-fighting foams, polishes, waxes and paint.

PFAS are anthropogenic and considered non-biodegradable. They get into the environment during their manufacturing process and also during their use and disposal. Therefore, PFAS can be found globally (even in polar regions) in the environment, in wildlife, in human tissues and blood.

Sewage plants are mostly not able to purify waste water with PFAS, so it accumulates in the sewage sludge, that is often used in agriculture. The chemicals can reach the groundwater and get absorbed again through plants.

Humans mostly take in PFAS through food or contaminated drinking water. They can remain in the human body for years without being degraded.

Animal studies showed, that PFAS can be carcinogenic, toxic for reproduction and weaken the immune system. It is still not clear, which effects PFAS have on the human body and what concentration must be reached to have an effect.

The topic of PFAS is present more than ever and will be even more relevant in the future as the European Commission has requested the EFSA to reevaluate the risks of PFAS to human health. This could result in much lower detection limits and a wider range of analytes as known before.

The following application note shows how water samples can be prepared fully automated for LC-MS/MS analysis by applying SPE with the FREESTYLE XANA robotic system according to DIN 38407-42. By the application of fully automated parallel sample preparation, multiple samples can be processed at the same time. Thus, a high sample throughput at low demand of personnel resources is obtained.



2. Method Development

2.1 Reagents

- 0.1 % ammonia in methanol
- Acetonitrile (HPLC-grade)
- Methanol (HPLC-grade)
- Water (HPLC-grade)
- 1 % formic acid in acetone – acetonitrile (1:1, v/v)
- 5 mM ammonium acetate in water
- 5 mM ammonium acetate in methanol
- Native PFAS precision and recovery standard solution (conc. 2.0 µg/mL in MeOH), Wellington Laboratories
- Mass-labelled PFAS standard solution/mixture (conc. 2.0 µg/mL in MeOH), Wellington Laboratories

2.2 Sample Preparation Standard/Blanks

Each standard was diluted to 200 ng/mL in methanol, as working solution. After that, 500 mL of water were spiked with 25 µL of each standard's working solution. Accordingly, the sample has a concentration of 10 ng/L for each analyte, which is the lower application limit according to DIN 38407-42.

The blanks were prepared as follows:

All volumes of the solvents used in the application were put into a vial and evaporated to dryness, with a gentle stream of nitrogen. After that, the vial was rinsed with 1 mL acetonitrile. The solution again was concentrated to dryness under a gentle stream of nitrogen and redissolved with a final volume of 1 mL methanol/water (6:4, v/v).

The same procedure was applied with the methanol/water (6:4, v/v) solution.

2.3 Instrumentation

2.3.1 FREESTYLE XANA System

The FREESTYLE XANA system consists of the xyz-robotic platform FREESTYLE BASIC and the SPE module which are modified and thus virtually free of fluorocarbon compounds for PFAS sample preparation.

In the following the required items for a processing of 24 samples are listed together with their corresponding part numbers.

1.	1 x FREESTYLE BASIC, 6 Solvents	P/N	12663-12-PFAS
2.	1 x FREESTYLE SPE Module	P/N	12668-PFAS
3.	1 x FREESTYLE XANA	P/N	14692-PFAS
4.	2 x Special Rack for up to 12 SPE Columns	P/N	14047
5.	3 x Clamping Adapter for 3 mL SPE Cartridges	P/N	14892
6.	1 x CHROMABOND HR-XAW, 3 mL, 60 mg	P/N	17029
7.	2 x Reusable Polyethylene Caps for SPE Cartridges	P/N	16970
8.	2 x Reusable Needles, Stainless Steel	P/N	13382
9.	1 x Frame 100 mm	P/N	11915
10.	1 x Tray, 4 mL, 60 Positions	P/N	11926
11.	1 x 4 mL Screw-Thread Vials	P/N	V0004



2.5 Analytical Set-up

2.5.1 HPLC System and Settings

- Agilent Infinity II 1290 (Modules G7116B, G7167B, G7120A)
- API 5500 Triple Quad, Turbo Spray (ESI)
- Scan type: SMRM
- MRM detection window: 60 sec
- Polarity: negative
- Curtain gas: 30 psig
- Ion spray voltage: -4500 V
- Temperature: 400 °C
- Gas 1 (nebulizer): 50 psig
- Gas 2 (turbo gas): 60 psig
- CAD gas: medium

2.5.2 Chromatographic Conditions

- Column: EC 100/2 NUCLEOSHELL® Bluebird RP18 2.7 µm
- Eluent A: 5 mM ammonium acetate in water
- Eluent B: 5 mM ammonium acetate in methanol
- Gradient: hold 20 % B for 1.0 min, in 8.0 min to 95 % B, hold 95 % B for 3.0 min, in 0.1 min to 20 % B, hold 20 % B for 2.9 min
- Flow rate: 0.33 mL/min
- Temperature: 40 °C
- Injection volume: 10 µL (Concentration: 5 ng/mL in water/methanol (40/60, v/v))

2.5.3 Eluent Exchange

- Sample concentration under a gentle stream of nitrogen to dryness
- Rinsing with 1 mL acetonitrile
- Sample concentration under a gentle stream of nitrogen to dryness
- Fill up to a final volume of 1 mL with methanol/water (6:4, v/v)

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2.5.5 Software Protocol

In the following the FREESTYLE XANA method protocol is shown.



Name: PFAS_XANA.wat				
Column:	LCTech_3ml.col	Extension cannula:	yes	
Conditioning 1:	ON	Dispensing Speed:	1 ml / min	
Volume:	2 ml	Waiting time:	0.25 min	Port : W4 0,1% ammonia/MeOH
Suction Speed:	10 ml / min			
Conditioning 2:	ON	Dispensing Speed:	1 ml / min	
Volume:	2 ml	Waiting time:	0.25 min	Port : W2 MeOH
Suction Speed:	10 ml / min			
Conditioning 3:	ON	Dispensing Speed:	1 ml / min	
Volume:	2 ml	Waiting time:	0.25 min	Port : W8 HPLC-H2O
Suction Speed:	10 ml / min			
Load 1:	ON	Typ:	empty	
Number of bottles:	1	Transfer Speed	5 ml / min	
rinsing cycle included				
Rinsing volume:	2 ml	Suction Speed:	10 ml / min	
Dispensing Speed:	50 ml / min			Port : W8 HPLC-H2O
Washing 1:	ON	stay on actual position		
Volume:	2 ml	Dispensing Speed:	1 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0.25 min	Port : W3 1% Ameisen/Ac/Acn
Washing 2:	ON	stay on actual position		
Volume:	2 ml	Dispensing Speed:	1 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0.25 min	Port : W2 MeOH
Drying 1:	ON	stay on actual position		
Time:	1 min			
Washing 3:	OFF			
Elution 1:	ON	Dispensing Speed:	1 ml / min	
Volume:	1.2 ml	Waiting time:	0.25 min	Port : 9 0,1% ammonia/MeOH
Suction Speed:	15 ml / min	Vial Type:	Type1@4	
Drying 2:	OFF			
Washing 4:	OFF			
Drying 3:	OFF			
Elution 2:	ON	Dispensing Speed:	1 ml / min	
Volume:	1.2 ml	Waiting time:	0.25 min	Port : 9 0,1% ammonia/MeOH
Suction Speed:	15 ml / min	Fill in vial of Elution 1		
Drying 4:	ON	Drying volume: 10 ml	Speed: 50 ml / min	
defined by volume				
EVA :	OFF			
Tube filling volume before elution		1 ml		

Figure 1: FREESTYLE XANA method protocol.

3. Results

3.1 Recovery Rates

Fourteen selected PFAS were processed on the FREESTYLE XANA system in order to see whether all compounds are recovered and do not adhere to the system.

Table 1: Recovery data of 14 selected PFAS compounds according to DIN 38407-42.

No	Component Name	Recovery [%]	STD [%]
1	Polyfluorobutanoic acid (PFBA)	80	3,8
2	Polyfluoropentanoic acid (PFPeA)	106	1,1
3	Polyfluorohexanoic acid (PFHxA)	101	1,3
4	Polyfluoroheptanoic acid (PFHpA)	106	1,5
5	Polyfluorooctanoic acid (PFOA)	107	1,6
6	Polyfluorononanoic acid (PFNA)	102	1,8
7	Polyfluorodecanoic acid (PFDA)	66	2,0
8	Polyfluorobutane sulfonate (PFBS)	102	1,4
9	Polyfluorohexane sulfonate (PFHxS)	107	2,1
10	Polyfluorooctane sulfonate (PFOS)	79	1,7
11	¹³ C ₄ perfluorobutanoic acid (¹³ C ₄ PFBA)	84	2,3
12	¹³ C ₂ perfluorohexanoic acid (¹³ C ₂ PFHxA)	108	1,6
13	¹³ C ₄ perfluorooctanoic acid (¹³ C ₄ PFOA)	113	1,3
14	¹³ C ₄ perfluorooctanesulfonic acid (¹³ C ₄ PFOS)	85	1,8

3.2 Background Concentrations for PFAS

One of the key challenges in analysing PFAS is to keep the background of analytes as low as possible. Unfortunately, PFAS are widely spread all over the globe and will thus be found ubiquitously. Furthermore, any fluorocarbon materials, commonly used in laboratory systems due to their unique chemical properties, are prone to a release of small amounts of PFAS that may significantly increase background levels.

Therefore, any fluorocarbon materials of the FREESTYLE SPE as well as the XANA system were consequently removed and replaced by polyethylene or polypropylene, respectively.

In order to prove the effectiveness of this measure, PFAS background levels of neat solvents and pure water samples were compared.

From the Table 2 shown below, it is evident that any analyte concentration measured is already found in the neat solvents and does not originate from the system!

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For the determination of the background concentration of the solvents and solutions, the processing was as described in 2.2.

For the determination of the background concentration of the FREESTYLE XANA system, the application was processed with 500 mL of unspiked water and the same solvents, that were processed as described in 2.2.

Table 2: Comparison of PFAS background concentrations of neat solvent and water samples processed with the FREESTYLE XANA system

Component Name	Background FREESTYLE [ng/mL]	Background Solvent [ng/mL]
PFBA	0	0
PFPeA	0,09	0,06
PFHxA	0,02	0,04
PFHpA	0,02	0,02
PFOA	0,05	0,05
PFDA	0,01	0,01
PFNA	0,01	0,01
PFBS	0	0
PFHxS	0	0
PFOS	0,01	0,01
PFDS	0,01	0,01

3.1 Chromatograms

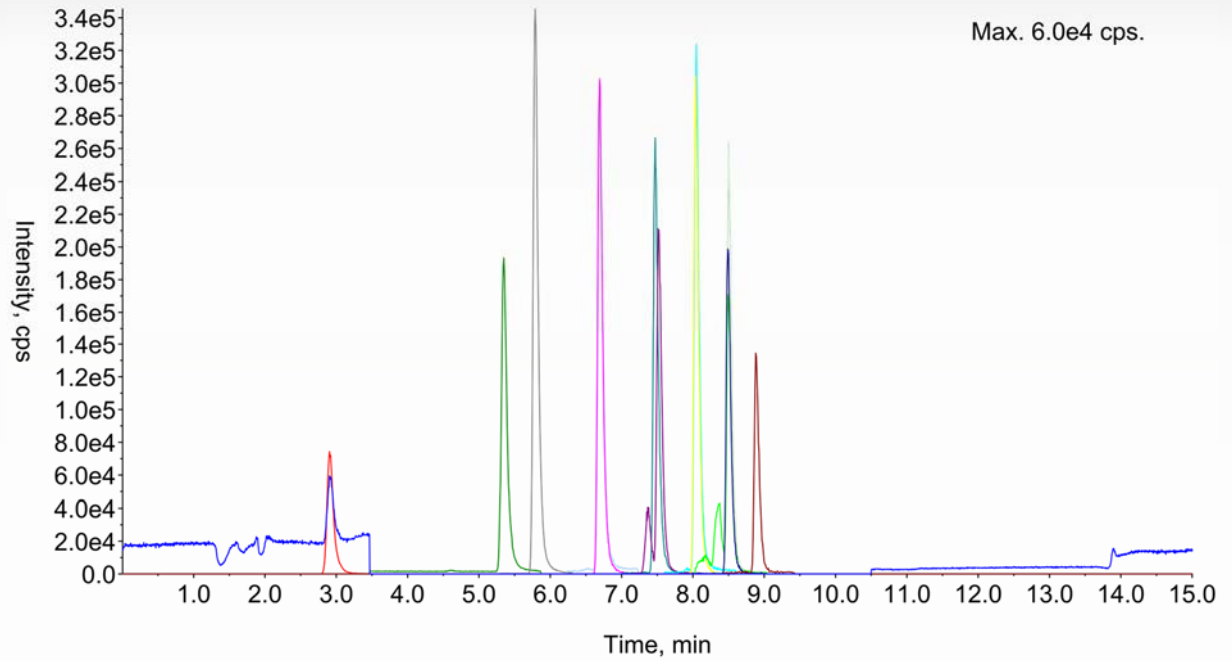


Figure 2: Chromatogram of spiked sample over FREESTYLE XANA

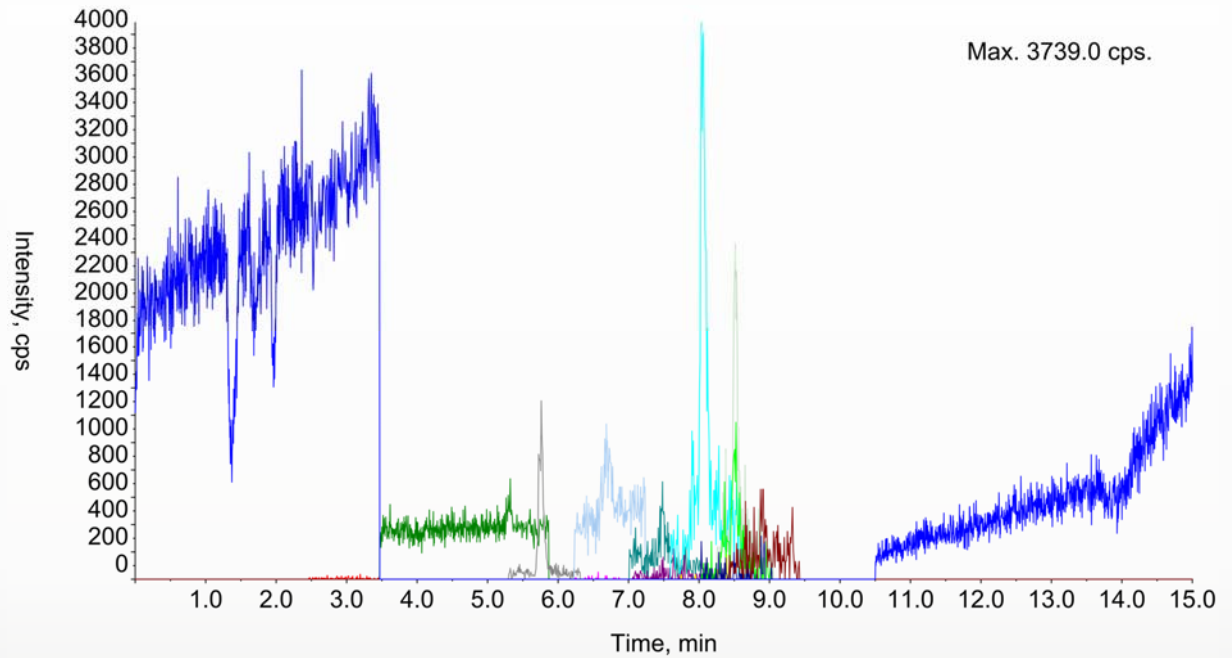


Figure 3: Chromatogram of blank solvent

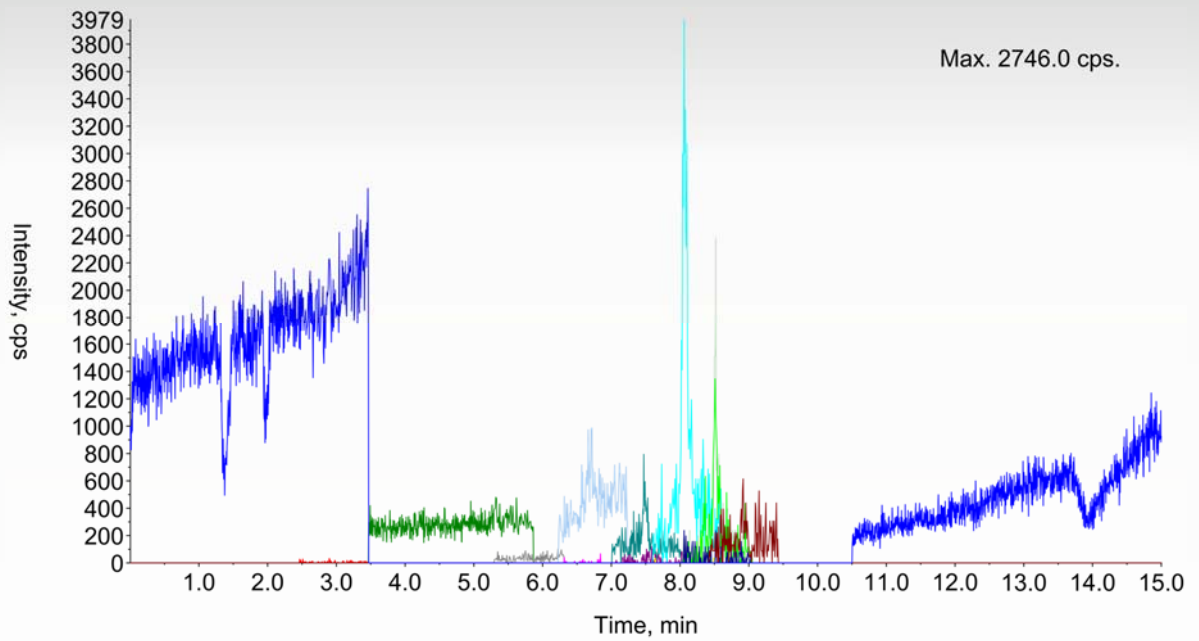


Figure 4: Chromatogram of blank sample over FREESTYLE XANA

Table 3: LC-MS/MS parameters

No	Component Name	Retention Time (Min.)	MRM-Transition		
			Q1	Q2	Q3
1	PFBA	2,57	212,9	168,8	-
2	PFPeA	4,68	262,9	219	-
3	PFHxA	5,88	312,9	268,7	119
4	PFHpA	6,65	362,9	318,6	168,8
5	PFOA	7,23	412,9	368,8	168,8
6	PFNA	7,7	462,9	418,9	218,9
7	PFDA	8,1	512,8	468,9	218,8
14	PFBS	5,02	298,9	79,9	98,9
15	PFHxS	6,69	398,9	79,8	98,8
17	PFOS	7,69	498,9	79,9	98,9
25	M4-PFBA	2,56	216,9	171,9	-
26	M4-PFOA	7,23	416,8	371,8	171,9
27	M2-PFHxA	5,88	314,9	269,9	120
30	M4-PFOS	7,68	502,8	79,9	98,9

4. Conclusions

In the application note, PFAS can be processed fully automated according to DIN 38407-42. Due to the specifically designed PFAS system, which is virtually free of fluorocarbon compounds, any blank values stemming from the system are minimised. This was shown by PFAS background measurements of the instrument that were equal to the values obtained with neat solvent.

Furthermore, the analytical targets of the DIN method were found with good recoveries and very low standard variations because of the reliable and robust automation.

As the FREESTYLE SPE module is an integral part of the FREESTYLE XANA, it is evident that a processing on the SPE system only is possible as well.



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