

Analysis of *PFAS from Drinking Water* Using Automated FREESTYLE XANA-PFAS System and EluCLEAN PFAS SPE Columns

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Key Features

- Excellent recovery rates and low standard deviations for 18 PFAS analytes according to US EPA 537.1 and 25 PFAS analytes according to US EPA 533
- EluCLEAN PFAS - SDVB column is an equivalent to the SPE cartridge mentioned in US EPA 537.1
- EluCLEAN PFAS - WAX SPE column contains a weak anion exchanger, mixed-mode polymeric sorbent with an pKa above 8, suitable for use in ISO 21675, ISO 25101, DIN 38407-42, US EPA 533
- Reliable and robust automation with FREESTYLE XANA-PFAS
- Fully automated processing of up to 24 samples in one sequence
- Parallel processing of up to 7 samples
- No detectable PFAS background contamination

LCTech Products

SPE cartridges

Part No.:20801, 20802, 20803

EluCLEAN® PFAS – SDVB

Sorbent: Poly(Styrene-Co-Divinylbenzene) Polymer (SDVB)

500 mg/6 mL

Part No.:20811, 20812, 20813

EluCLEAN® PFAS – WAX

Sorbent: Weak Anion Exchanger, Mixed-Mode Polymeric Sorbent (WAX)

500 mg/6 mL

Part No.:20871, 20872, 20873

EluCLEAN® PFAS – WAX

Sorbent: Weak Anion Exchanger, Mixed-Mode Polymeric Sorbent (WAX)

200 mg/6 mL

FREESTYLE XANA-PFAS configuration

Part No.: 12663-12-PFAS FREESTYLE BASIC, PFAS-version, 12-port valve (for up to 6 solvents)

Part no.: 12668-PFAS FREESTYLE SPE Module, PFAS-version

Part no.: 14692-PFAS FREESTYLE XANA, PFAS-version

Part no.: 14047 Special SPE rack for up to 12 SPE cartridges

Part no.: 14923-PFAS Reusable Caps for 6 mL SPE cartridges

Part no.: 12103 Frame for below mentioned tray

Part no.: 13751 Tray for 30 pcs 50 mL-Saarstedt/Falcon tubes



Part no.: 19372	Special clamping adapter for 6 mL SPE cartridges
Part no.: 14643	Plunger removal tool for 6 mL cartridges
Part no.: 19455	Holder for 250 mL bottles (Thermo/Nalgene)

Other Relevant LCTech Application Notes and Product Information

AN0052 Analysis of PFAS from Drinking Water Using EluCLEAN PFAS SPE Columns

AN0053 Analysis of PFAS from Soil Using EluCLEAN PFAS SPE Columns

AN0045 D-EVA – Automated Evaporation of PFAS compliant to US-EPA 537.1

Figure 1. FREESTYLE XANA PFAS with EluCLEAN PFAS – SPE columns





1. Introduction

Per- and polyfluorinated alkyl substances (PFAS) products have been in use for more than 60 years. They get into the environment during their manufacturing process and also during their use and disposal. The analytical interest in these compounds has rapidly increased in the last few years. Research has revealed the high toxicity of PFAS compounds and thus the resulting need to regulate the substances. The current and upcoming regulations in the EU and US make it necessary to test drinking water for PFAS content. Different methods for PFAS analysis in the EU and US exist. For example ISO 21675, ISO 25101, DIN 38407-42, US EPA 533 [1] and US EPA 537.1 [2]. All methods require solid phase extraction (SPE) prior liquid chromatography-tandem mass spectrometry (LC-MS/MS). The European methods and US EPA 533 using SPE cartridges containing a weak anion exchanger, mixed-mode polymeric sorbent, whereas the US EPA 537.1 calls for a styrene divinylbenzene polymer (SDVB).

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-PFAS robotic system according to US EPA 537.1 and US EPA 533. 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. The FREESTYLE XANA-PFAS robotic system is a solution especially for PFAS applications by avoiding fluorine-containing plastics such as PTFE and is thus solving the challenge: No blank values from the system were measurable.

The FREESTYLE XANA-PFAS robotic system was used in combination with two newly introduced SPE cartridges containing optimised polymeric sorbents for PFAS enrichment. The EluCLEAN PFAS - SDVB SPE column is a 500 mg containing SDVB based sorbent which is used for US EPA 537.1. The ELUCLEAN PFAS - WAX SPE column is a 500 mg containing weak anion exchanger, mixed-mode polymeric sorbent which is used for US EPA 533.

For both methods excellent recovery rates in combination with low standard deviations were achieved.



2. Experimental

2.1 Sample Preparation

2.1.1 Sample Pre-Treatment

a) According to US EPA 537.1

250 mL of drinking water is collected from tap in 250 mL PE bottles. No further treatment was done. 18 Native PFAS (ES 5631, Cambridge Isotope Laboratories) and 4 surrogate standards (ES-5632, Cambridge Isotope Laboratories) were spiked to get the final concentrations of 40 ng/L and 80 ng/L, respectively.

b) According to US EPA 533

250 mL of drinking water is collected from tap in 250 mL PE bottles. Ammonium acetate was added to achieve a 1 g/L concentration. 25 Native PFAS (EPA-533PAR, Wellington Laboratories) and 16 isotope dilution standard (EPA-533ES, Wellington Laboratories) were spiked to get the final concentration of 20 ng/L and 20 - 80 ng/L, respectively.

2.1.2 Solid Phase Extraction

SPE columns were placed on a SPE rack of the FREESTYLE system. Sample bottles were placed in the XANA bottle rack. Solvent bottles were connected with respective ports. The fully automated method (see below) was applied and the eluate was collected for subsequent evaporation.

a) According to US EPA 537.1

The method was designed to comply with the SPE procedure described in US EPA 537.1 method.

Table 1. FREESTYLE XANA-PFAS conditions according to US EPA 537.1

LC Tech FreeStyle - Report on Methods: WAT Type PFAS - EPA



Column:	LC Tech_6ml.col	Extension cannula:	no
Conditioning 1:	ON	Dispensing Speed:	5 ml / min
Volume:	15 ml	Waiting time:	2 min
Suction Speed:	30 ml / min	Port :	W4 MeOH PFAS
Conditioning 2:	ON	Dispensing Speed:	5 ml / min
Volume:	20 ml	Waiting time:	2 min
Suction Speed:	30 ml / min	Port :	W10 Water PFAS
Conditioning 3:	OFF		
Load 1:	ON	Typ:	empty
Number of bottles:	1	Transfer Speed	5 ml / min
1. rinsing cycle included		Suction Speed:	30 ml / min
2x Rinsing volume:	7.5 ml	Port :	W10 Water PFAS
Dispensing Speed:	15 ml / min		
Washing 1:	OFF		
Washing 2:	OFF		
Drying 1:	ON	stay on actual position	
Time:	35 min		
PFAS RinsElution	ON	Dispensing Speed:	15 ml / min
1x Volume:	8.0 ml	Waiting time:	0 min
Suction Speed:	30 ml / min	Port :	W4 MeOH PFAS



b) According to US EPA 533

The method was designed to comply with the SPE procedure described in US EPA 533 method.

Table 2. FREESTYLE XANA-PFAS conditions according to US EPA 533

LC Tech FreeStyle - Report on Methods: WAT Type PFAS - EPA



Column:	LC Tech_6ml.col	Extension cannula:	no
Conditioning 1:	ON		
Volume:	10 ml	Dispensing Speed:	5 ml / min
Suction Speed:	30 ml / min	Waiting time:	2 min
			Port : W4 MeOH PFAS
Conditioning 2:	ON		
Volume:	13 ml	Dispensing Speed:	5 ml / min
Suction Speed:	30 ml / min	Waiting time:	2 min
			Port : W1 0.1 M Phosphate buffer
Conditioning 3:	OFF		
Load 1:	ON	Typ:	empty
Number of bottles:	1	Transfer Speed	5 ml / min
1. rinsing cycle included			
1x Rinsing volume:	10 ml	Suction Speed:	30 ml / min
Dispensing Speed:	15 ml / min		Port : W3 1 g/L AA aq.
2. rinsing cycle included			
1x Rinsing volume:	1 ml	Suction Speed:	30 ml / min
Dispensing Speed:	15 ml / min		Port : W4 MeOH PFAS
Washing 1:	OFF		
Washing 2:	OFF		
Drying 1:	ON	stay on actual position	
Time:	35 min		
PFAS RinsElution	ON		
1x Volume:	10.0 ml	Dispensing Speed:	15 ml / min
Suction Speed:	30 ml / min	Waiting time:	0 min
			Port : W9 2% NH4OH MeOH

2.1.3 Evaporation/Concentration

All samples were evaporated to dryness using D-EVA Rotational Vacuum Concentrator (temperature: 45°C, vacuum: 20 mbar).

2.1.4 Reconstitution

a) According to US EPA 537.1

Appropriate amount of 96:4 % (vol/vol) methanol:water solution was added to bring the volume to 1 mL (2 x 500 uL extraction: 500 uL MeOH + 460 uL MeOH and 40 uL H₂O) and transferred into a 1.5 mL PP vial.

b) According to US EPA 533:

Sample was reconstituted in 1 mL of 20 % reagent water in methanol (v/v) and transferred into a 1.5 mL PP vial.

10 uL of respective isotopic dilution standards were added and vortexed. The vial was closed with a PP cap and stored at 0 - 4 °C for LC-MS/MS.



2.2 Instrumentation

2.2.1 MS Conditions

Table 3. MS Conditions

Parameter	Value
MS	TSQ Quantis (Thermo)
Polarity	Negative
Spray voltage	2500 V
Sheath Gas	50 Arb
Aux Gas	10
CID Gas	2 mTorr
Ion transfer tube temp	325 °C
Vaporizer Temp	300 °C
Q1 resolution	0.7 FWHM
Q3 resolution	1.2 FWHM
Cycle time	0.5 sec
Chromatographic peak width	6 sec

2.2.2 LC Instrument Conditions

Table 4. LC Conditions

Parameter	Value																				
LC	Thermo Scientific Vanquish Flex UHPLC system																				
Analytical column	Accucore RP-MS, 2.1*100 mM, 2.6 µm																				
Delay column	Agilent ZOBRAx Eclipse plus C18, 4.6*50 mm; 3.5 µm																				
Column temperature	45 °C																				
Injection volume	5 µL																				
Mobile Phase	A) 20 mM ammonium acetate H ₂ O with 2 % MeOH and 0.1 % acetic acid B) 20 mM ammonium acetate MeOH with 2 % H ₂ O and 0.1 % acetic acid																				
Gradient Flow rate	0.5 mL/min																				
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>% B</th> </tr> </thead> <tbody> <tr><td>0</td><td>0</td></tr> <tr><td>1</td><td>30</td></tr> <tr><td>6</td><td>45</td></tr> <tr><td>13</td><td>80</td></tr> <tr><td>14</td><td>95</td></tr> <tr><td>17</td><td>95</td></tr> <tr><td>20</td><td>95</td></tr> <tr><td>22</td><td>0</td></tr> <tr><td>25</td><td>0</td></tr> </tbody> </table>	Time (min)	% B	0	0	1	30	6	45	13	80	14	95	17	95	20	95	22	0	25	0
Time (min)	% B																				
0	0																				
1	30																				
6	45																				
13	80																				
14	95																				
17	95																				
20	95																				
22	0																				
25	0																				



Table 5. Recovery rates and RSD of native PFAS for both methods

Drinking water				
Analyte	EPA 533		EPA 537.1	
	Recovery rates in %	RSD in %	Recovery rates in %	RSD in %
PFBA	75	6	-	-
PFMPA	109	5	-	-
PFPeA	111	5	-	-
L-PFBS	102	5	110	4
PFMBA	110	5	-	-
PFEESA	103	3	-	-
NFDHA	102	7	-	-
4:2FTS	116	5	-	-
PFHxA	111	6	108	3
L-PFPeS	111	8	-	-
HPFO-DA	111	6	108	5
PFHpA	102	5	110	4
PFHxSK	103	6	117	5
ADONA	105	5	115	5
6:2FTS	113	6	-	-
PFOA	107	7	111	4
L-PFHpS	110	6	-	-
PFNA	111	6	110	5
PFOSK	104	8	104	6
9CI-PF3ONS	96	5	103	8
8:2FTS	112	4	-	-
PFDA	103	5	106	7
PFUnA	97	4	101	9
11CI-PF3OUdS	84	4	88	9
PFDaA	91	6	95	6
N-MeFOSAA	-	-	96	9
N-EtFOSAA	-	-	92	9
PFTTrDA	-	-	91	10
PFTeDA	-	-	86	10



Table 6. Recovery rates and RSD of labelled PFAS for both methods

Analyte	Drinking water			
	EPA 533		EPA 537.1	
	Recovery rates [%]	RSD [%]	Recovery rates [%]	RSD [%]
MPFBA	75	1	-	-
M5PFPeA	103	6	-	-
M3PFBS	103	7	-	-
M2-4:2FTS	114	8	-	-
M5PFHxA	102	4	-	-
MPFHxA	-	-	97	2
M3HPFO-DA	101	7	106	7
M4PFHpA	101	6	-	-
M3PFHxS	104	5	-	-
M2-6:2FTS	110	4	-	-
M8PFOA	102	4	-	-
M9PFNA	99	8	-	-
M8PFOS	95	3	-	-
M2-8:2FTS	120	5	-	-
M6PFDA	104	4	-	-
MPFDA	-	-	95	6
M7PFUnA	98	5	-	-
MPFDoA	91	6	-	-
d5-N-EtFOSAA	-	-	86	8

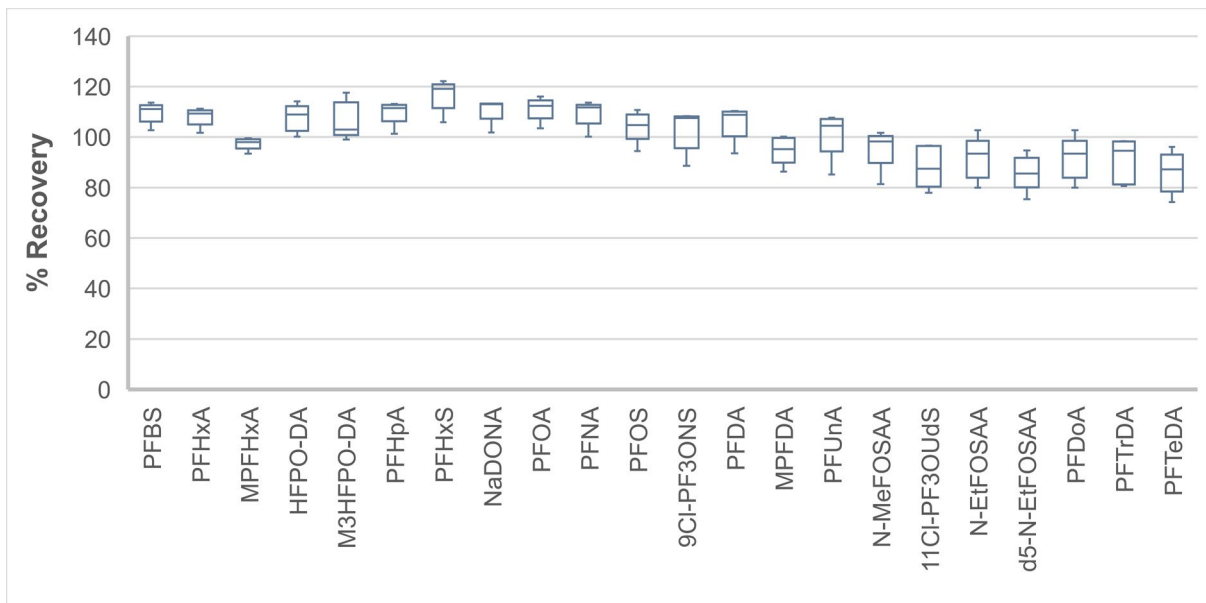


Figure 4. Recovery rates of 18 PFAS (listed in US EPA method 537.1) + 4 surrogates from drinking water with EluCLEAR SDVB columns (n = 6)

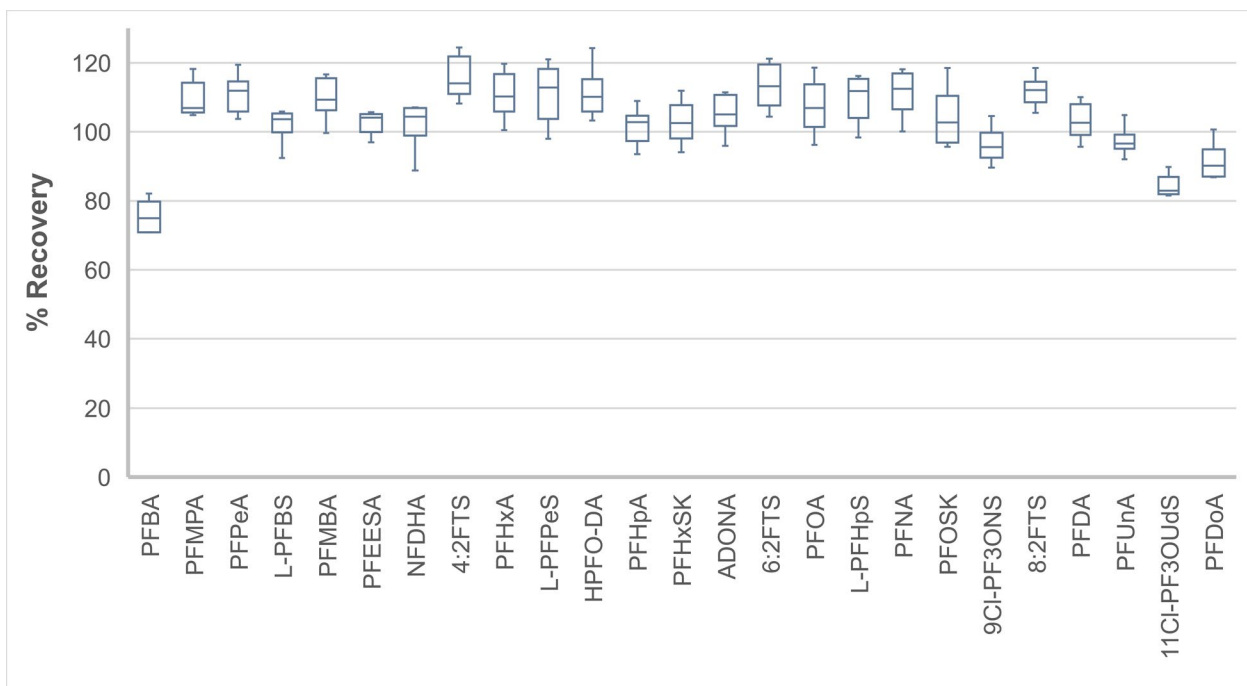


Figure 5. Recovery rates 25 PFAS (listed in US EPA method 533) from drinking water with EluCLEAR-WAX Column (n = 6, c = 20 ng/L) using FREESTYLE XANA automated SPE

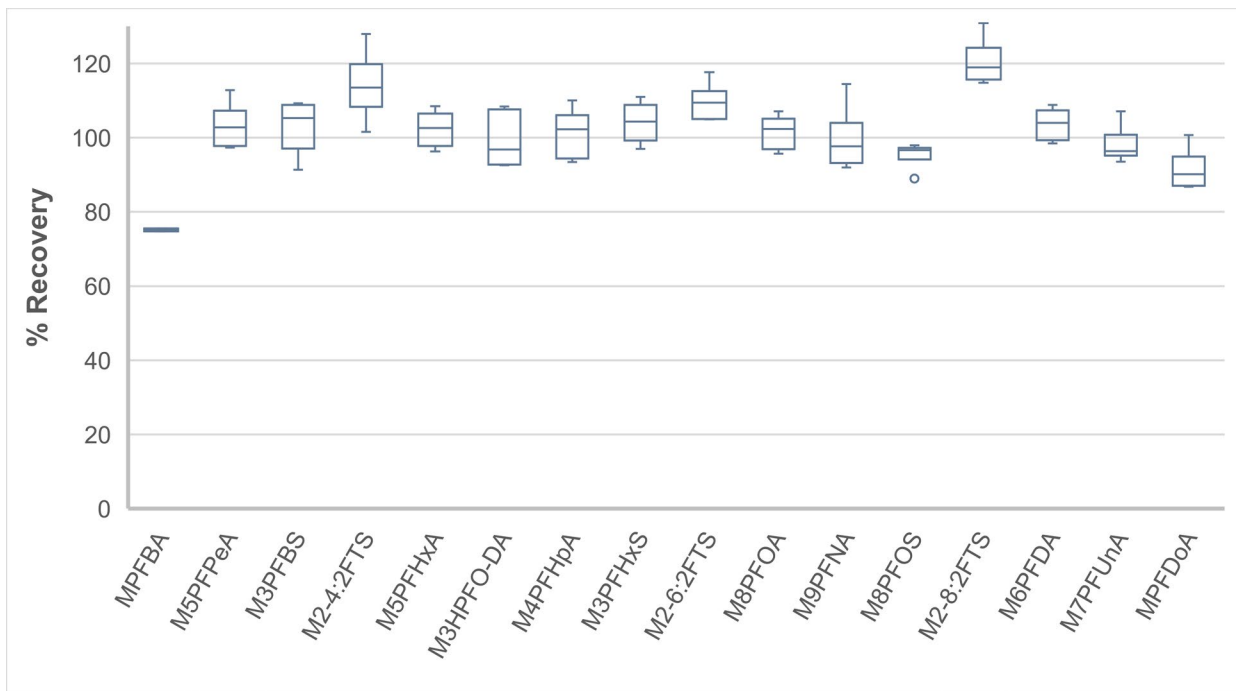


Figure 6. Recovery rates 16 isotope dilution PFAS standards (listed in US EPA method 533) from drinking water with EluCLEAN-WAX Column (n = 6, c = 20-80 ng/L) using FREESTYLE XANA automated SPE



4. Conclusion

Due to the specifically designed PFAS system, which is virtually free of fluorocarbon compounds, any blank values stemming from the system are minimised, which was shown by PFAS background measurements (see Figure 2 and 3). In addition, EluCLEAN PFAS SPE cartridges have no detectable PFAS background contamination.

The analytical targets of the US EPA 537.1 (with EluCLEAN PFAS-SDVB) and US EPA 533 (with EluCLEAN PFAS-WAX) method were successfully fulfilled. Recoveries for samples with a concentration of 40 ng/L for US EPA 537.1 with a range of 86 - 117 % and 20 ng/L samples for US EPA 533 with a range of 75 - 116 % were very well in between the acceptable criteria of 70 - 130 %. Therefore, the desired accuracy is given. Precision, measured by % RSD of replicate extracts, was also well within the range of requirements, with all RSDs at 10 % or below.

The results show that a reliable and robust PFAS analysis can be processed via a fully automated sample preparation on FREESTYLE XANA-PFAS robotic system in combination with EluCLEAN PFAS SPE cartridges.

5. References

[1] METHOD 533: DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY ISOTOPE DILUTION ANION EXCHANGE SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY; EPA Document No. 815-B-19-020, December 2019.

[2] METHOD 537.1 DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS), EPA Document No.: EPA/600/R-20/006, Version 2.0, March 2020

Any Questions?
Do not hesitate to contact us: