Analysis of ***PFAS from Aqueous samples*** Using Automated FREESTYLE XANA PFAS TableTop System and EluCLEAN® PFAS SPE Columns (US EPA 1633 4th draft method)

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Application Note AN0060 | www.LCTech.de
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Content

Key Features

- Excellent recovery rates and low standard deviations for 40 PFAS analytes according to US EPA 1633 (4th draft)
- No detectable PFAS background contamination
- Only one SPE column for clean-up and enrichment needed
- EluCLEAN® PFAS – WAX/GCB SPE column can be used equivalently to the SPE cartridge + dispersive graphitized carbon black used in US EPA 1633 (4th draft)
- EluCLEAN® PFAS – WAX/GCB SPE column contains a weak anion exchanger, mixed-mode polymeric sorbent with an pKa above 8 with optimised parameters for PFAS enrichment, suitable for use in US EPA 1633 (4th draft)
- EluCLEAN® PFAS – Universal SPE column is a special combination phase that improves the recovery rates of PFAS analytes through numerous interactions. It can be used in a wide variety of matrices. It is also available as HP version with an improved matrix reduction e.g. for highly pigmented/colored matrices
- Reliable and robust automation with FREESTYLE XANA-PFAS TableTop
- Fully automated processing of up to 30 samples in one sequence
- Parallel processing of up to 6 samples

LCTech Products

SPE cartridges

Part No.: 20841, 20842, 20843 (Universal) // 20851, 20852, 20853 (Universal HP)

EluCLEAN® PFAS – Universal and Universal HP

Sorbent: special combination phase

Part No.: 20821, 20822, 20823 (150/10 mg/6 mL) // 20831, 20832, 20833 (200/50 mg/6 mL)

EluCLEAN® PFAS – WAX/GCB

Sorbent1: Weak Anion Exchanger, Mixed-Mode Polymeric Sorbent (WAX)

Sorbent 2: Graphitized Carbon Black (GCB)

FREESTYLE XANA PFAS TableTop

Part No.: 20600	FREESTYLE XANA-PFAS TableTop (30 positions)
Part No.: 19372	SPE column adapters for elution into 50 mL Falcon tubes (12 pcs.)
Part No.: 14923-PFAS	Caps for 6 mL SPE PFAS cartridges, reusable (25 pcs.)
Part No.: 13156	Solvent bottle rack, 6 x 1 L
Part No.: 12709	Overflow Sensor for Waste Level Control, with GL 45 Cap

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

AN0058- D-EVA – Automated Evaporation of PFAS samples

AN0059 Analysis of PFAS from Drinking Water Using Automated FREESTYLE XANA-PFAS TableTop System and EluCLEAN® PFAS SPE Columns

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

Figure 2 Equipping the bottle rack of the FREESTYLE XANA PFAS TableTop

Figure 3 Loading the bottles of the FREESTYLE XANA PFAS TableTop

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, usages and disposal. Research has revealed the high toxicity of PFAS compounds and thus the resulting need to regulate the substances. Therefore the analytical interest in these compounds has rapidly increased in the last few years. All methods require solid phase extraction (SPE) prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. All methods apply SPE cartridges containing a weak anion exchanger, mixed-mode polymeric sorbent, whereas the US EPA 1633 (4th draft) and the DoD/QSM 5.1/5.3 additionally use a dispersive clean-up step depending on the type of matrices.

The following application note shows how aqueous samples can be prepared fully automated for LC-MS/MS analysis by applying SPE with the FREESTYLE XANA-PFAS TableTop robotic system according to US EPA 1633 (4th draft). 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 TableTop 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 Tabletop robotic system used a new single SPE cartridge solution with an for PFAS enrichment optimised polymeric sorbent. The EluCLEAN® PFAS – WAX/GCB SPE column contains 150 mg of a weak anion exchanger, mixed-mode polymeric sorbent mixed with 10 mg of graphitized carbon black. Also a version with 200 mg WAX and 50 mg GCB is available for matrices with higher organic matrix content.The SPE cartridge shows excellent recovery rates in combination with low standard deviations and is therefore ideally suited for SPE of PFAS from aqueous, soil and other environmental matrices. It can equivalently replace the dispersive clean-up step + WAX SPE cartridge used in US EPA 1633 (4th draft). The single cartridge solution saves time and costs in PFAS analysis.

In this application note two different types of columns EluCLEAN® PFAS – WAX/GCB SPE column and EluCLEAN® PFAS – Universal SPE, which can be used for a variety of matrices mentioned in US EPA 1633 (4th draft) method, were used. Here, the columns were applied together with FREESTYLE XANA PFAS TableTop to extract PFAS analytes automatically from aqueous samples (Tap water in this case) using the US EPA 1633 (4th draft) method (see also application AN0053 for analysis of PFAS from soil, using the EluCLEAN® PFAS – WAX/GCB SPE column).

2. Experimental

2.1 Sample Preparation

2.1.1 Sample Extraction (Solid Phase Extraction)

250 mL of tap water is collected from tap in 250 mL PE bottles. Acetic acid was added to achieve a pH in range of 4-7. 40 Native PFAS (PFAC MXF-G, Wellington Laboratories) and 24 isotope dilution standard (PFAC HIF ES, Wellington Laboratories) were spiked as mentioned in table below.

Table 1. 40 Native PFAS (PFAC MXF-G, Wellington Laboratories) and 24 isotope dilution standard (PFAC HIF ES, Wellington Laboratories)

Compounds	ng
Labeled compounds	**0.42– 3.33**
11Cl-PF3OUdS, 9Cl-PF3ONS, ADONA, HFPO-DA, NFDHA, PFEESA, PFMB, PFMPA, PFPeA	**2**
PFBA, 4:2FTS, 6:2FTS, 8:2FTS	**4**
N-MeFOSE, N-EtFOSE	**10**
5:3 FTCA, 7:3 FTCA	**20**
FBSA-I, PFECHS, FHXSA-I, P37DMOA, FOUEA, 6:2 diPAP, 8:2 diPAP	**1.67**
PFHxDA, PFODA	**0.835**
L-PFUdS, L-PFTrDS	**3.35**
6:2 PAP, 8:2 PAP, PFDPA	**16.75**
All other PFAS	**1**

The method was designed to comply with the SPE procedure described in US EPA 1633 (4th draft) method.

Table 2. FREESTYLE XANA-PFAS TableTop conditions according to US EPA 1633 (4th draft) method

LCTech FreeStyle - Report on Methods: WAT Type PFAS - EPA

Name: 1633.wat				
Column:	LCTech_6ml.col		Extension cannula:	no
Conditioning 1:	ON			
Volume:	15 ml	Dispensing Speed:	5 ml / min	
Suction Speed:	15 ml / min	Waiting time:	0 min	Port : W2 1% NH4OH MeOH
Conditioning 2:	ON			
Volume:	5 ml	Dispensing Speed:	5 ml / min	
Suction Speed:	15 ml / min	Waiting time:	0 min	Port : W8 0.3M FA MeOH
Conditioning 3:	OFF			
Load 1:	ON	Typ:	empty	
Number of bottles:	1	Transfer Speed	5 ml / min	
1. rinsing cycle				
1x Rinsing volume:	5 ml	Suction Speed:	15 ml / min	
Dispensing Speed:	5 ml / min			Port : 7 Water
2. rinsing cycle				
1x Rinsing volume:	5 ml	Suction Speed:	15 ml / min	
Dispensing Speed:	5 ml / min			Port : 10 0.1 M FA:MeOH 1:1
Washing 1:	OFF			
Washing 2:	OFF			
Drying 1:	ON	stay on actual position		
Time:	45 min			
PFAS RinsElution	ON			
2x Volume:	5 ml	Dispensing Speed:	2 ml / min	
Suction Speed:	15 ml / min	Waiting time:	0 min	Port : 11 1% NH4OH MeOH

20 µL of concentrated acetic acid and 10 µL NIS (MPFAC-HIF-IS) solution were added to each sample eluate and vortexed. The samples were filtered through a syringe filter (25 mm filter, 0.2 µm nylon membrane) into a clean 15 mL polypropylene centrifuge tube.

2.1.2 Evaporation/Concentration

All samples were evaporated to 500 µL - 1 mL using D-EVA Rotational Vacuum Concentrator (temperature: 45°C, vacuum: 20 mbar) and transferred into a 1.5 mL polypropylene vial and kept at 0 – 4 °C for LC-MS/MS analysis.

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 H2O with 2 % MeOH and 0.1 % acetic acid B) 20 mM ammonium acetate MeOH with 2 % H2O and 0.1 % acetic acid	
Gradient Flow rate	0.5 mL/min	
Gradient	Time (min) 0 1 6 13 14 17 20 22 25	% 0 30 45 80 95 95 95 0 0

3. Results

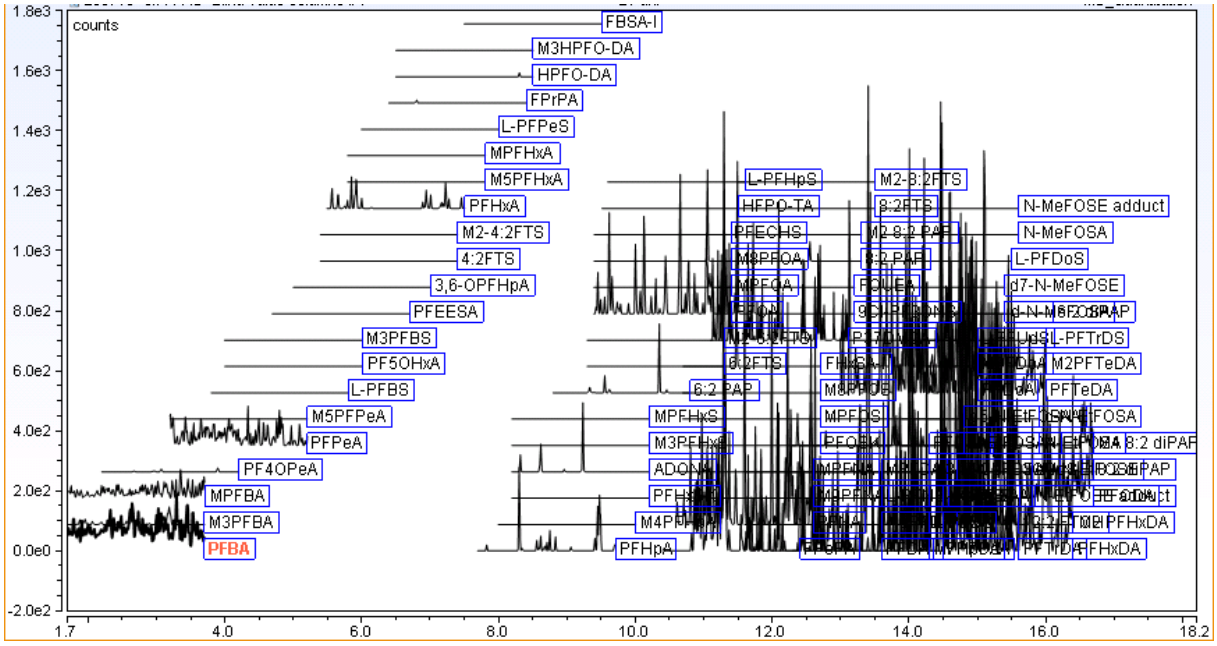


Figure 4. Chromatogram demonstrating EluCLEAN® PFAS –Universal columns are free of blind value when screened for 55 PFAS analytes

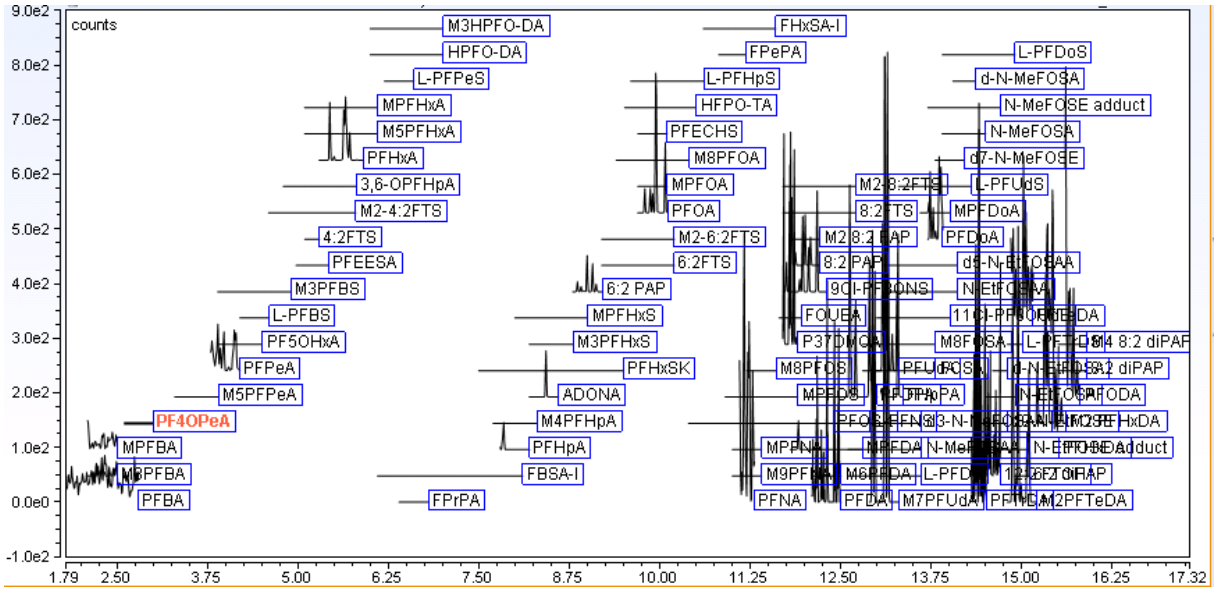


Figure 5. Chromatogram demonstrating EluCLEAN® PFAS – WAX-GCB columns are free of blind value when screened for 55 PFAS analytes

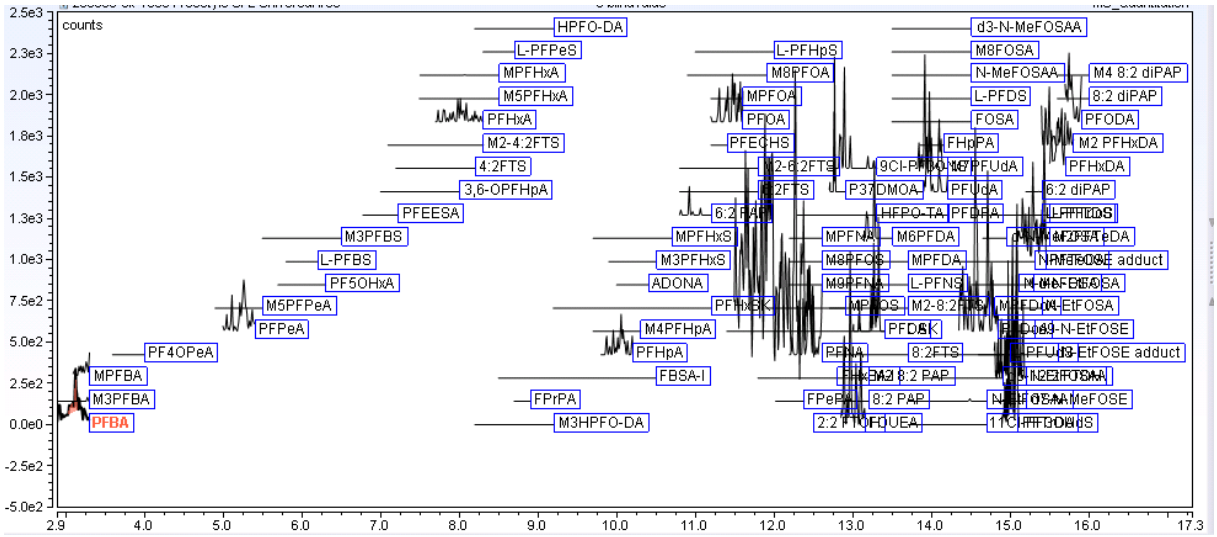


Figure 6. Chromatogram demonstrating FREESTYLE XANA PFAS –TableTop is free of blind value when screened for 55 PFAS analytes

3.1 Analytes, Retention Times and Corresponding Isotope Dilution Standards

Table 5. Overview retention times and corresponding isotope dilution standard

Analyte	Retention time (min)	Isotope Dilution Standard
PFBA	2.86	MPFBA
PFMPA	3.40	M5PFPeA
PFPeA	4.60	M5PFPeA
PFMBA	5.34	M5PFPeA
L-PFBS	5.28	M3PFBS
PFEESA	6.3	M3PFBS
NFDHA	6.8	M3PFBS
4:2FTS	6.8	M2-4:2FTS
PFHxA	7,12	M5PFHxA
L-PFPeS	7.73	M5PFHxA
3:3 FTCA	7.94	M5PFHxA
HPFO-DA	7.92	M3HPFO-DA
PFHpA	9.37	M4PFHpA
PFHxSK	9.66	M3PFHxSK
ADONA	9.64	M4PFHpA
6:2FTS	10.72	M2-6:2FTS
PFOA	10.85	M8PFOA
L-PFHpS	10.97	M8PFOA
5:3 FTCA	11.65	M9PFNA
PFNA	11.93	M9PFNA
PFOSK	12.00	M8PFOS
9Cl-PF3ONS	12.48	M6PFDA / M2-8:2 FTS
8:2FTS	12.78	M2-8:2FTS
PFDA	12.82	M6PFDA
L-PFNS	12.86	M6PFDA
PFUnA	13.57	M7PFUnA
7:3 FTCA	13.60	M7PFUnA
L-PFDS	13.56	d3-N-MeFOSAA
N-MeFOSAA	13.49	d3-N-MeFOSAA
FOSA	13.70	M8FOSA
11Cl-PF3OUdS	13.91	d5-N-EtFOSAA
N-EtFOSAA	13.90	d5-N-EtFOSAA
PFDoA	14.20	MPFDoA
N-MeFOSA	14.57	d-N-MeFOSA
N-MeFOSE	14.50	d7-N-MeFOSE
L-PFDoS	14.69	- / d7-N-MeFOSE
PFTrDA	14.75	M2PFTeDA
N-EtFOSA	14.94	d-N-EtFOSA
N-EtFOSE	14.94	d9-N-EtFOSE
PFTeDA	15.09	MPFTeDA

3.2 Recovery Rates and RSD % of 40 PFAS

Table 6. Recovery rates and RSD

Analytes	Universal		WAX-GCB	
	Recovery rate (%)	RSD (%)	Recovery rate (%)	RSD (%)
PFBA	99	2	102	2
PFMPA	100	4	102	5
PFPeA	103	7	107	4
PFMBA	102	2	107	7
L-PFBS	94	10	98	8
PFEESA	87	12	100	5
NFDHA	100	16	103	9
4:2FTS	94	5	117	6
PFHxA	105	6	113	3
L-PFPeS	101	6	121	9
3:3 FTCA	98	5	112	7
HPFO-DA	97	5	108	3
PFHpA	103	8	99	5
PFHxSK	98	16	108	4
ADONA	103	4	99	3
6:2FTS	95	6	99	5
PFOA	98	5	98	7
L-PFHpS	90	7	87	5
5:3 FTCA	98	5	108	19
PFNA	100	6	100	18
PFOSK	111	8	107	11
9Cl-PF3ONS	94	13	87	6
8:2FTS	86	9	96	10
PFDA	100	16	84	12
L-PFNS	97	24	89	11
PFUnA	101	9	102	13
7:3 FTCA	100	4	100	12
L-PFDS	101	10	95	9
N-MeFOSAA	114	10	101	7
FOSA	99	11	107	14
11Cl-PF3OUdS	85	7	107	10
N-EtFOSAA	103	8	106	18
PFDoA	104	7	103	16
N-MeFOSA	98	14	92	17
N-MeFOSE	85	10	106	20
L-PFDoS	81	6	104	20
PFTrDA	79	2	88	14
N-EtFOSA	102	12	78	8
N-EtFOSE	89	3	87	11
PFTeDA	92	11	96	14

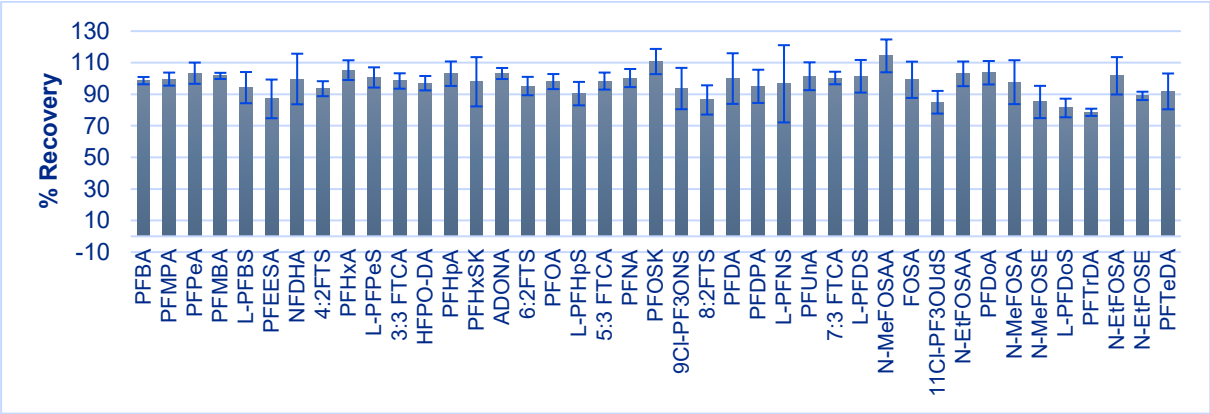


Figure 7. Recovery rates of 40 PFAS (listed in US EPA method 1633 4th draft) from tap water extracted with EluCLEAN® PFAS – Universal SPE column (n=4, spiked concentration=1-20 ng in 250 mL tap water) using FREESTYLE XANA PFAS TableTop automated SPE

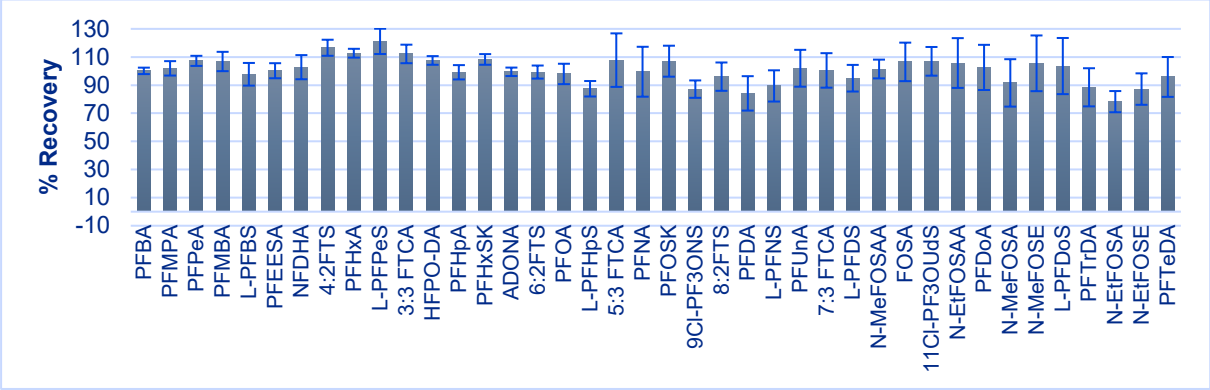


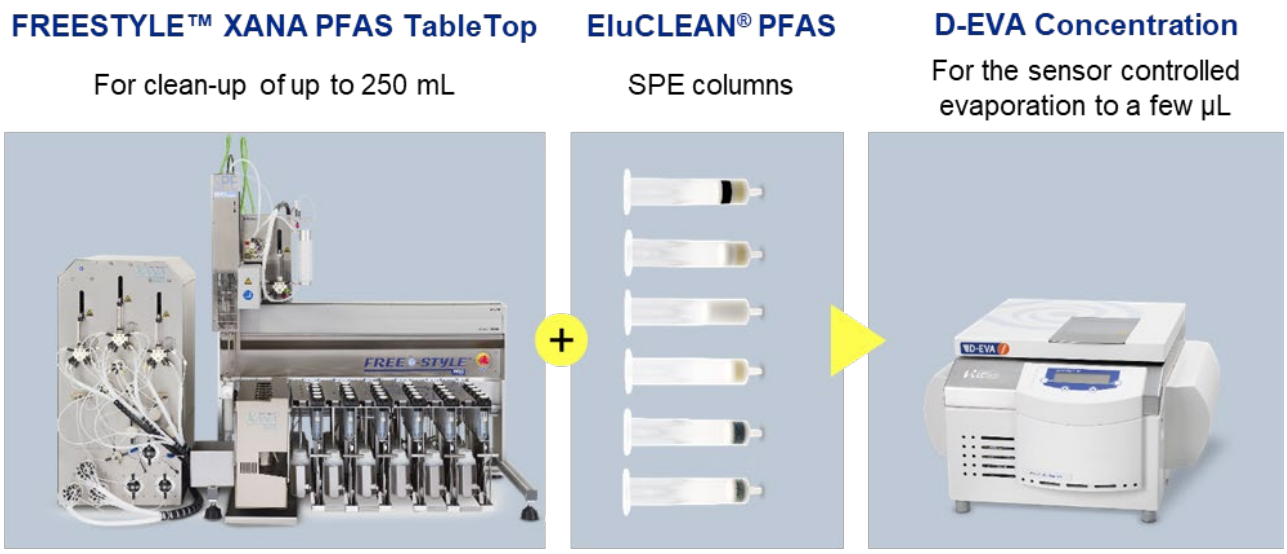
Figure 8. Recovery rates of 40 PFAS (listed in US EPA method 1633 4th draft) from tap water extracted with EluCLEAN® PFAS – WAX/GCB (200/50 mg) SPE column (n=4, spiked concentration=1-20 ng in 250 mL tap water) using FREESTYLE XANA PFAS TableTop automated SPE

4. Conclusion

EluCLEAN® PFAS – Universal, EluCLEAN® PFAS – WAX/GCB cartridges and FREESTYLE XANA PFAS TableTop have no detectable PFAS background contamination. The cartridge EluCLEAN® PFAS – Universal and EluCLEAN® PFAS – WAX/GCB are suitable for US EPA 1633 (4th draft) according to their performance. Recoveries of samples are very well in between the acceptable criteria of 70 - 130 %. Therefore, the desired accuracy is given.

EluCLEAN® PFAS – Universal and EluCLEAN® PFAS – WAX/GCB cartridges combined with FREESTYLE XANA PFAS TableTop are therefore ideally suited to be used for the enrichment and clean-up of PFAS from aqueous matrices. This is the time and cost saving alternative for every laboratory.

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



5. References

[1] 4th Draft Method 1633*: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS; EPA Document No. EPA 821-D-23-001, July 2023.
*Finalized for the Aqueous Matrices: Wastewater, Surface Water and Groundwater

Any Questions?
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