D-EVA – Automated Evaporation of PFAS Samples

(Compliant to US EPA 1633, 537.1, 533, DoD QSM 5.4, DIN 3840742 and 38414-14, EU 2020/2184, ISO 21675-2019)

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Content

Highlights

- 55 PFAS analytes were evaporated using D-EVA
- D-EVA evaporation is contamination free and without loss of analytes
- D-EVA programs can be optimized to get any desired final volume
- 52 samples in 15 mL Falcon™ Tubes and 23 sample in 50 mL Falcon™ Tubes can be processed at a time
- 10 mL and 40 mL methanol can be evaporated to less than 1 mL within 1.5 and 3 hours, respectively
- The whole process runs without any supervision

LCTech Products

D-EVA Vacuum Concentrator

Part No.: 16900	Rotational Vacuum Concentrator D-EVAporation
	Rotor with 53 positions
Part No.: 16742	Fixed angle rotor, 53 positions
Part No.: 20776	Sensor for 53 position angle rotor
	Rotor with 24 positions
Part No.: 16802	Fixed angle rotor, 24 positions
Part No.: 20775	Sensor for 53 position angle rotor

Other Relevant LCTech Application Notes and Product Information:

AN0053 Analysis of PFAS from Soil Using EluCLEAN PFAS SPE Columns

AN0054 Analysis of PFAS from Drinking Water Using Automated FREESTYLE XANA-PFAS System and EluCLEAN PFAS SPE Columns

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

AN0059-Analysis-PFAS-Drinking-Water-Automated-FREESTYLE-XANA-PFAS-TableTop-EluCLEAN-PFAS-SPE-Columns

AN0060-Analysis-PFAS-1633- Drinking water-EluCLEAN-PFAS-SPE-Column

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are exclusively man-made chemicals which, due to their longevity, are continuously accumulating in the environment. There are more than 10,000 different compounds which are already known in this class of chemicals. The best known PFAS are the perfluorinated alkyl sulfonates, including the perfluorooctane sulfonic acid (PFOS) and the perfluorinated carboxylic acids including the perfluorooctanoic acid (PFOA). PFAS are anthropogenic and considered non-biodegradable. They were popular for decades due to their physico-chemical properties (thermally and chemically stable, water- and grease-repellent). They get into the environment during their manufacturing process and also during their use and disposal, e.g. in fire extinguishing foams, in the production/impregnation of outdoor clothing, in ski waxes, in the coating of food packaging, in make-up and lipsticks etc. - to name just a few areas of application. 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. As a result, PFAS can be found everywhere today: in water, in soils, in dusts, in food and ultimately also in the blood of humans. Sufficient research has shown that they can be deteriorating to human health too.

The sample preparation for PFAS analytes is complex and delicate for various matrices due to its wide range of chemical properties. To include a wide range of PFAS in a single method is a challenging process. They can be polar to non-polar depending on the carbon length which effect its water solubility. Long chain PFAS are very sticky on any surface. Furthermore, volatile PFAS are sensitive during evaporation process.
LCTech has perfected the sample preparation from extraction to sample concentration, with technical solutions for all parts of the whole workflow. The extraction efficiency for long-chain PFAS from solids has been significantly increased with the X-TRACTION, the EluCLEAN® PFAS SPE columns have been optimised compared to the standard columns, the risk of contamination from the laboratory environment has been drastically reduced in the evaporation step with the D-EVA and, finally, the devices have been designed for throughput with a high degree of automation.

The following application note describes a method for the D-EVA to evaporate PFAS samples in methanol. The D-EVA is a vacuum centrifuge from Christ enhanced by LCTech GmbH coming with a vacuum pump from Vacuubrand and a high efficient Cryotrap that makes the system indipendent from a fume hood. There are two different rotors for the vacuum centrifuge. One can be used with 52 x 15 mL plastic tubes, the other with up to 23 x 50 mL plastic tubes. Common Falcon™ Tubes or tubes from Greiner fit perfectly into these rotors. Finally, there is a sensor for a desired final volume. This means the samples can be evaporated unsupervised to a desired final volume. The schematic representation is shown in Fig 1.

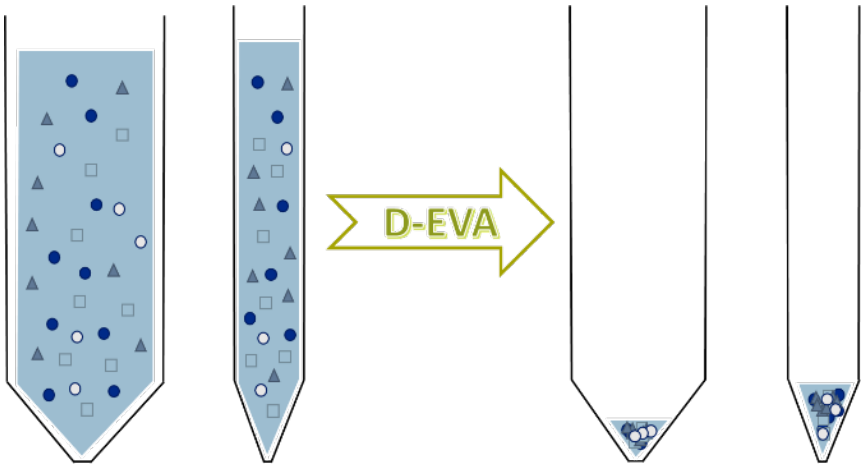


Figure 1. Schematic representation of evaporation in D-EVA

For instance, 23 samples with volumes up to 40 mL methanol in Falcon™ Tubes can be evaporated simultanously down to 400-900 µL (with a sensor) within less than 3 hours without any loss of analyte of interest.

1.1 Principle of the D-EVA

By lowering the atmospheric pressure with vacuum, the boiling point of the solvents (to be evaporated) can be reduced. So whenever solvent is put under a vaccum, the evaporation process is more rapid compared to normal atmospheric conditions. However, during evaporation, the transition from the liquid to the gas phase will lower the temperature of the solvent (enthalpy of evaporation). When the temperature is too low the evaporation process will be slow or stopped. Hence, to accelerate the evaporation process D-EVA has infrared lamps in the vaccum chamber which will switch on to maintain a certain temperature. This centrifugal evaporation process under vaccum at low temperature will evapore the sample without any loss of analyte and further without any cross-contamination.

At the end of the evaporation process, after the solvent has evaporated the temperature in the sensor vial rises rapidly. D-EVA regulates the evaporation on the basis of this temperature curve. For this purpose, LCTech GmbH has designed a sensor that precisely monitors the course of evaporation in a reference Falcon™ Tube as shown in Fig 2. As soon as the sensor detects a rapid rise in temperature, the vacuum chamber of the centrifuge is ventilated to raise the boiling point and the IR-lamps are switched off. This ends the active evaporation.

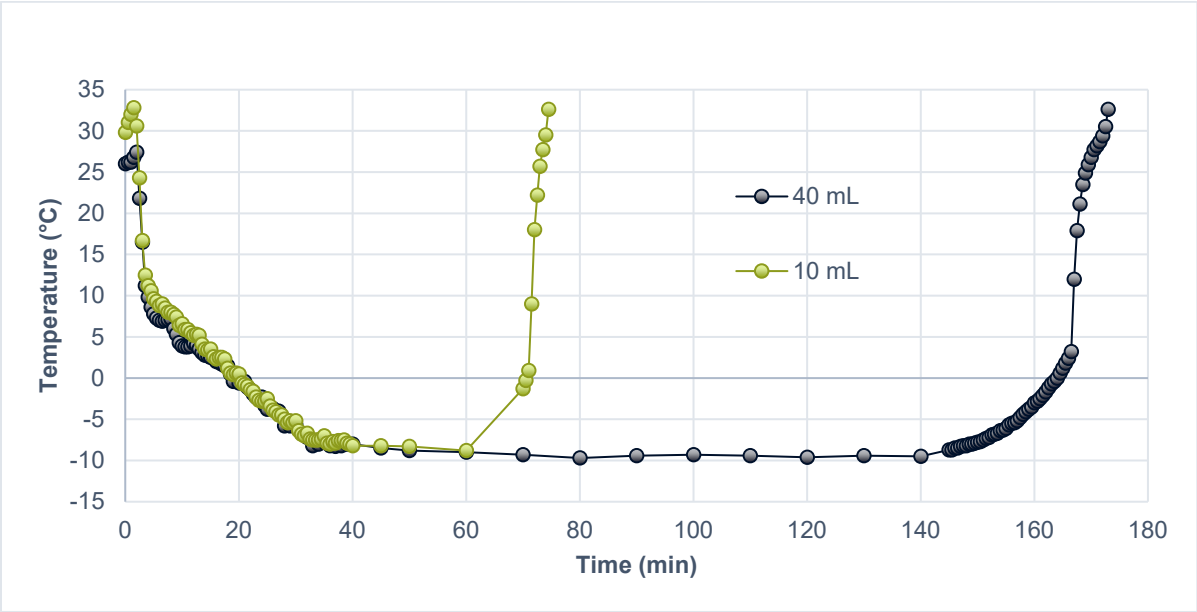


Figure 2. Temperature of sample during evaporation of 10 and 40 mL MeOH in 15 mL and 50 mL Falcon™ Tubes in D-EVA

Figure 3. Rotor for DEVA for 15 mL and 50 mL Falcon™ tubes

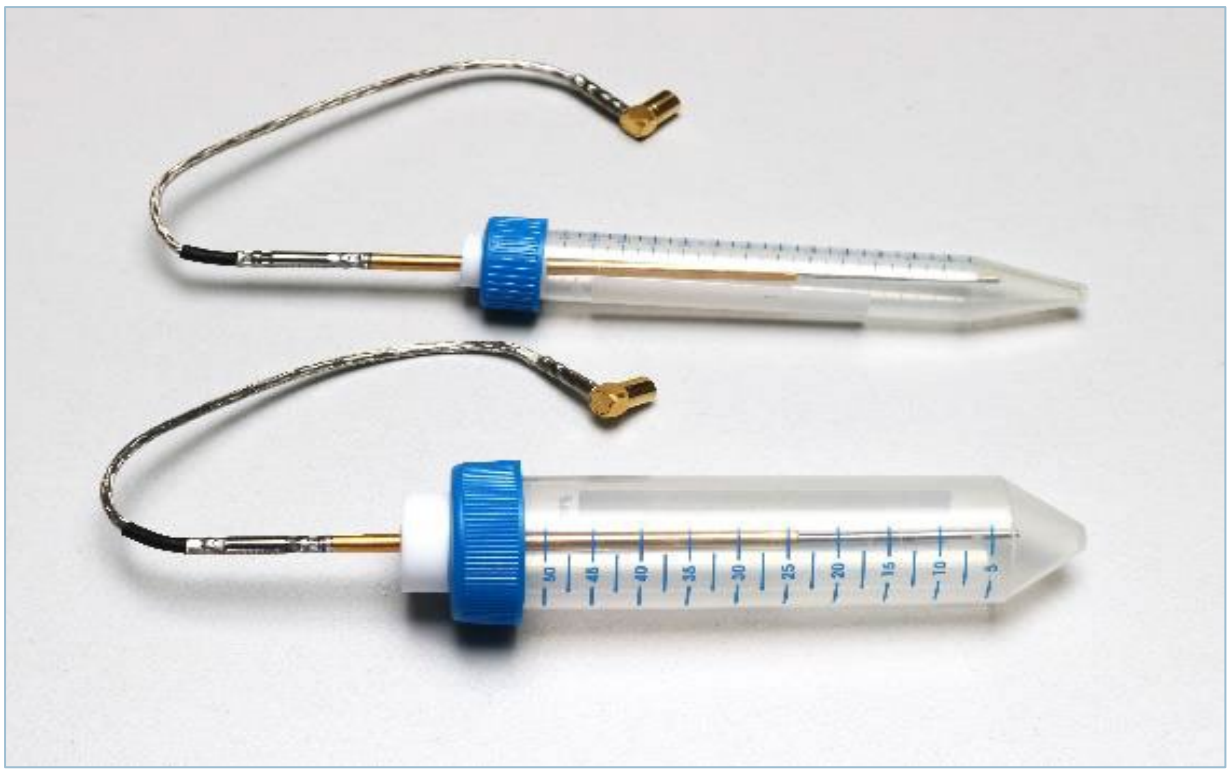


Figure 4. Sensors for DEVA for 15 mL and 50 mL Falcon™ tubes

1.2 Benefits of the D-EVA

1. **Robustness – compensation of residual water**
   - high tolerance against residual water in the sample

2. **Speed – high sample throughput**
   - 52 samples in 15 mL Falcon™ Tubes or 23 sample in 50 mL Falcon™ Tubes can be processed at a time

3. **Recoveries – better than with nitrogen blow-down**
   - no generation of aerosols
   - no additional rinsing steps
   - no loss of light sensitiv analytes

4. **No supervision – no losses due to overheating**
   - automatic stop

5. **No aerosols – no cross-contamination**
   - generation of aerosols is inhibited by the centrifugal force

6. **No safety cabinet needed**
   - closed system with solvent trap

7. **Higher signal intensity – lower LOQ**
   - can reach to lower final volume in µL

2. Method Development

2.1 Reagents and Materials

Standard solutions (e.g. from Wellington Laboratories)

- Mixture of 55 native PFAS analytes standard solution
- Mixture of 6 labelled IS solution

Solvents

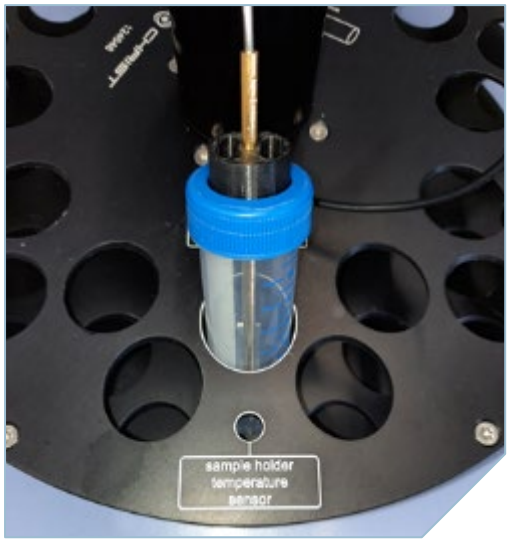
- Methanol HPLC grade (from Biosolve)

Materials

- D-EVA, Martin Christ enhanced by LCTech GmbH P/N 16900
- Angle Rotor, 24 positions P/N 16802
- Angle Rotor, 53 position P/N 16742
- Sensor for 24 position fixed angle rotor P/N 20775
- Sensor for 53 position fixed angle rotor P/N 20776
- 50 mL centrifuge tubes (PP) e.g. Falcon™ Tubes
- 15 mL centrifuge tubes (PP) e.g. Falcon™ Tubes
- UHPLC-MS/MS, Thermo Fisher Scientific GmbH

2.2 D-EVA Instrumentation

The centrifuge is running its rotors at 800 rpm. To prevent an imbalanced running of the motor, a balanced positioning of the vials in the rotor is recommended. However, an exact balancing of the sample vials with a scale is not necessary due to low rotation speed of the motor and further it was designed to handle a certain percentage of imbalance.
All positions of the rotors i.e. the inner ring and the outer ring can be used. The distance between the lamps and the sample tubes is not significant hence the efficiency of the infrared lamps is not influenced.



The white ring on the rotor marks the position for the tube with the sensor. A small spy hole and a connector for the sensor is to be found on this position as well. The sensor comes with a modified Falcon™ Tube and cap. The tube has to be filled with the same solvent and same volume according to that of the samples. The filled sensor tube is inserted into the marked position and connected with the plug of the rotor. The position of the sensor tube is correct, when the tip of the sensor is visible in the spyhole and the cable of the sensor is directed counterclockwise.

Figure 5. Spyhole in rotor near sensor

2.3 Sample Preparation

Native PFAS standards are spiked in 50 mL Falcon™ Tubes filled with 40 mL methanol. 4 samples each on 3 different days are evaporated in the D-EVA with the methanol program (which will be described in next section D-EVA Programs) down to a maximum of 1000 µL. The efficiency of an evaporation/up-concentration method can be evaluated by quantifying the recovery of analytes of interest (SUR) spiked to the sample right before the evaporation. The remaining end volume samples were transferred to HPLC vial without vortexing them. Internal standards were added and vortexed in HPLC vials. Then the samples were measured in LC-MSMS.

2.4 D-EVA Programs

D-EVA can be set up with many programs. Below are the examples of programs, which were used to evaporate PFAS samples in methanol.

Table 1 Methanol (Main)

Section	Unit	Start	1 step	2 step	3 step	
Time	h:m		00:03	00:30	03:00	
Temperature	°C	40	45	45	45	
Pressure	mbar		55	20	20	
Safety pressure	mbar		130	130	130	
Speed	min^{-1}		800	800	800	Stop at 30°C

Duration: 2h 20 mins

Table 2 Methanol (Short)

Section	Unit	Start	1step	2 step	
Time	h:m		00:04	00:05	
Temperature	°C	40	45	45	
Pressure	mbar		10	10	
Safety.pressure	mbar		500	90	
Speed	min^{-1}		800	800	Stop at 30°C

Duration: 10 mins

These programs can be used for up to 40 mL sample in the 50 mL - Falcon™ Tubes (23 samples for 50 mL Falcon™ Tube rotor) and up to 12 mL sample in the 15 mL Falcon™ Tubes (53 samples for 15 mL Falcon™ Tubes rotor). The final volume can be optimized to the needs of the lab by changing the volume of the solvent in the reference tube and rising or lowering the stopping temperature. Further, it can als be optimized via the temperature sensor position in the Falcon™ Tube.

The pressure in these programs is optimized for pure methanol as solvent. If other solvents are mixed with the methanol an overboiling is possible. Then rising the pressure for evaporation will help.

2.5 Cross-Contamination

D-EVA evaporation works on vaccum centrifuge and principally it should be free of contamination. Experiments were carried out in order to check if the process within D-EVA is in reality free of contamination. 40 mL MeOH (without spiking) was processed along with the samples in parallel. The end volume was transferred to HPLC vial and measured in LC-MS/MS.

3. Results

3.1 Residual volume

Table 3 shows the residual volumes of the samples after the evaporation with the methanol program. The first four samples have been further evaporated with a short methanol program to much lower final volume.

Table 3. Residual volume after evaporation of spiked sample in 40 mL methanol

Sample	Final volume [µL]	Final volume after an additional short run [µL]
1	450	12
2	600	200
3	520	250
4	610	280
5	380	
6	400	
7	400	
8	500	
9	610	
10	800	
11	880	
12	900	
Mean	588	
Deviation	178	

3.2 Cross-Contamination

55 PFAS were scrutinized for cross-contamination. None could be detected after evaporating MeOH in D-EVA as shown by the blank chromatogram in Fig 2. Evaporation in D-EVA is free of any cross-contamination during the evaporation process for PFAS application.

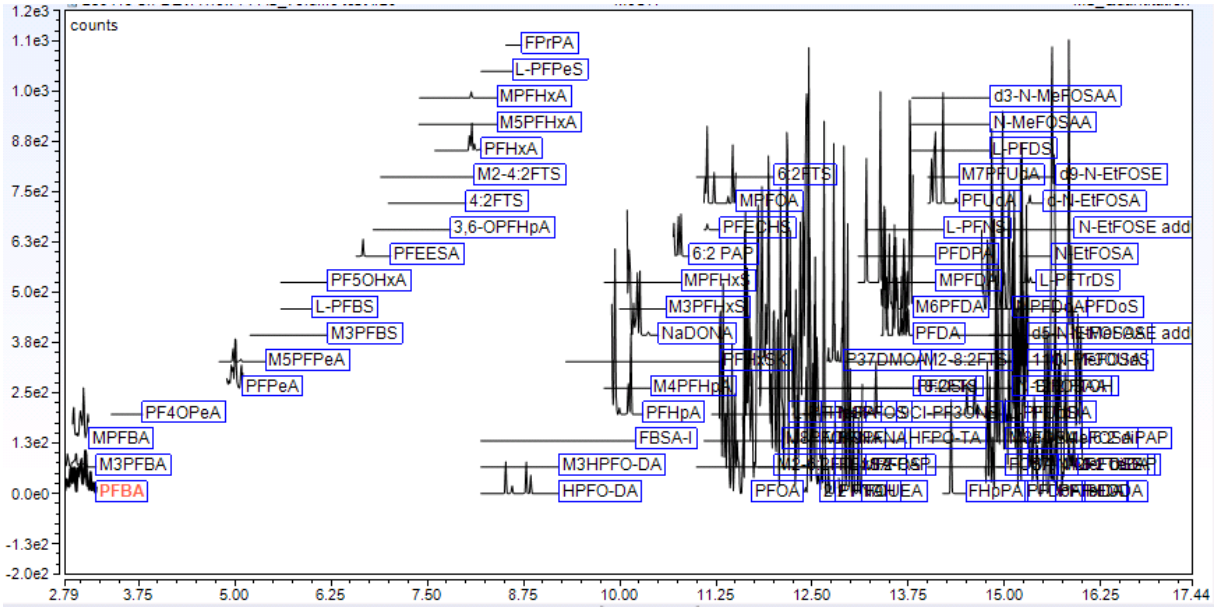


Figure 6. Chromatogram showing D-EVA is free of PFAS blind value when screened for 55 PFAS during evaporation of 40 mL MeOH to 0.1 mL

3.3 Recoveries

The recoveries of the 40 PFAS analytes mentioned in US EPA 1633 draft 4 method are shown in Fig 7. The recoveries were calculated for different final volume groups of samples, which were transferred into HPLC vials without vortexing. For 200-280 µL final volume, the recoveries were in the range of 60 %. Further, for 380-500 µL the recoveries improved, but were still below 80 %. Finally, for 610-910 µL the recoveries are excellent (above 80% for all analytes). Note: These recoveries were measured just with transferring the residual volume without vortexing or rinsing at the end.

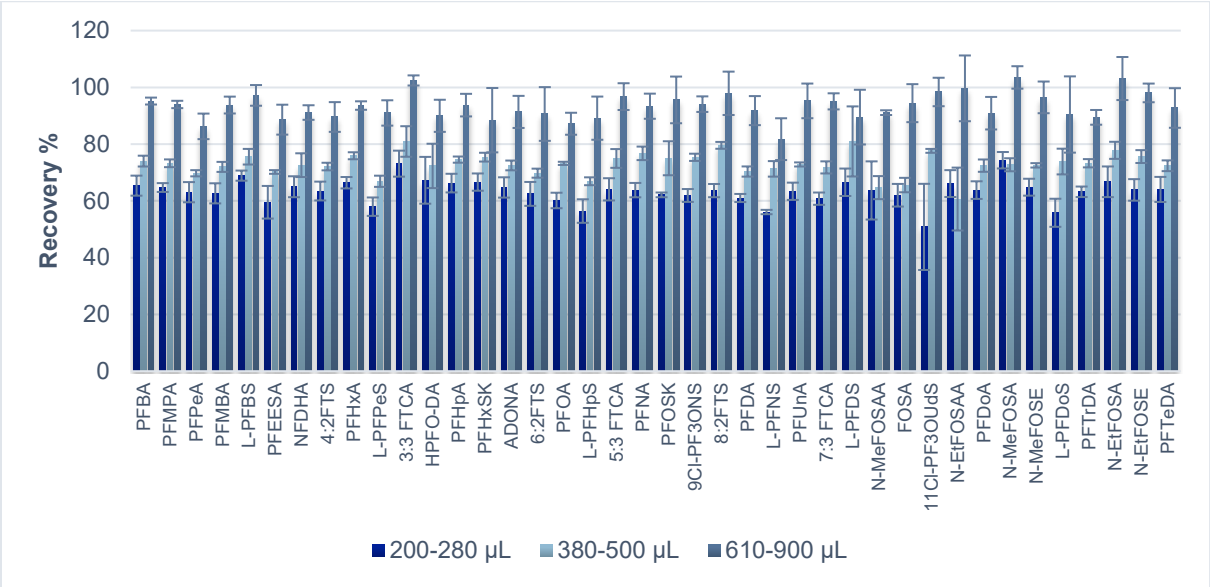


Figure 7 Recoveries of 40 PFAS (mentioned in USEPA 1633 method) with a final volume of 400 – 900 µL (without vortexing) (n = 3)

Figure 8 shows the recoveries of some volatile PFAS of the sample correlating to the final volume. The recoveries are below 80% for up to 280 µL final volume. However, when the final volume is maintained above 380 µL the recoveries will be above 80 %.

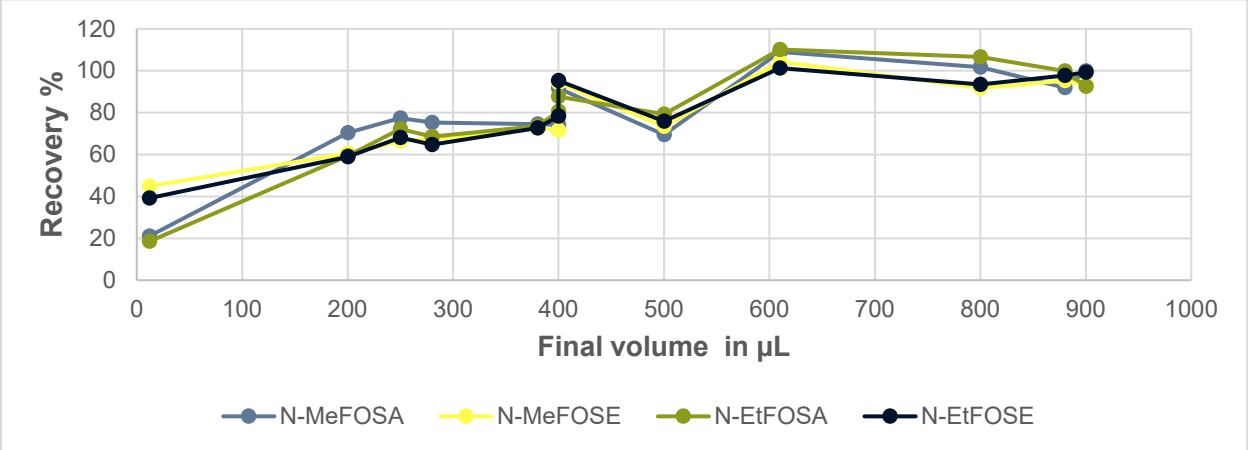


Figure 8. Recoveries of the volatile PFAS related to the final volume without vortexing

Fig. 9 shows the recoveries correlation to final volumes similar to Fig. 2 but with 15 other PFAS analytes. However, in this experiment a second rinsing with 500 µL MeOH was done to recover any leftovers from the Falcon™ Tubes. Higher leftovers were observed for samples having a low final volume as presented in Fig. 8.

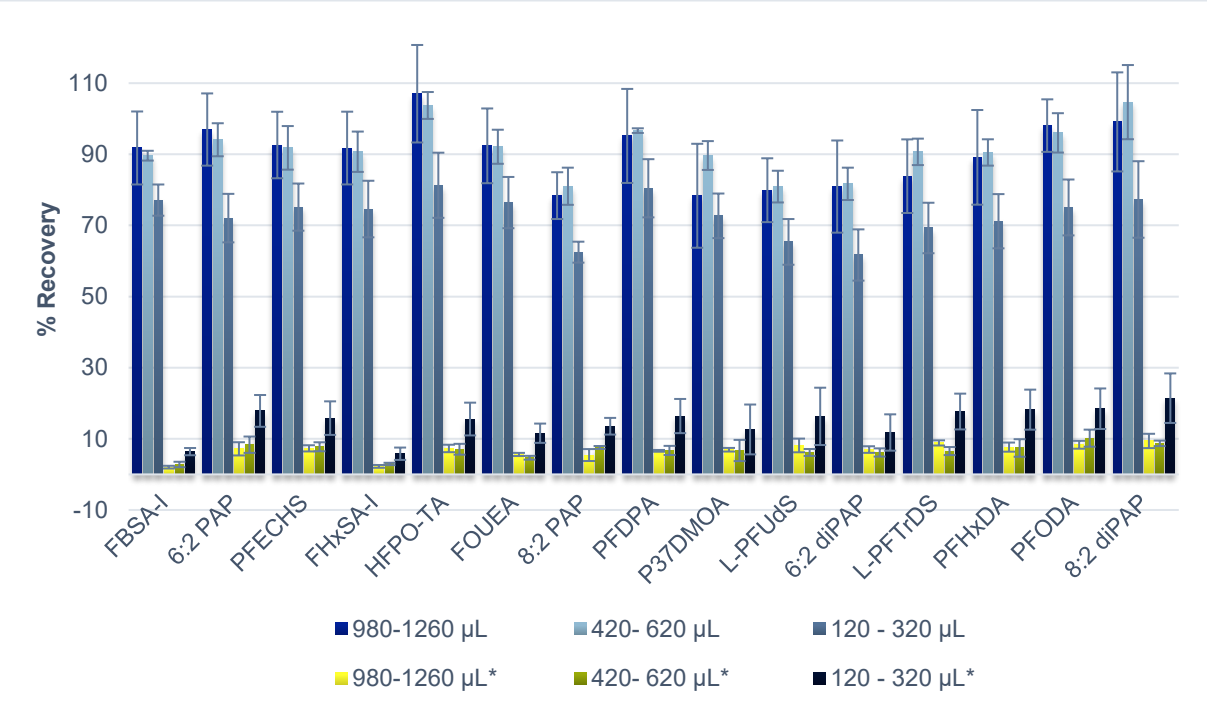


Figure 9. Recoveries of additional 15 PFAS, transferred from D-EVA to vial without rinsing and *with additional 500 µL MeOH rinsing to recover the all leftovers

Note: When an evaporation to total dryness is required like in method US EPA 537.1 or 533 (Method was used in AN0054 Analysis of PFAS from Drinking Water Using Automated FREESTYLE XANA-PFAS System and EluCLEAN PFAS SPE Columns), it can be carried out easily under 3 hours (with a little longer program).

4. Conclusion

According to this study, the following can be concluded:

- 55 PFAS analytes were evaporate using D-EVA
- D-EVA evaporation is contamination free and without loss of analytes
- D-EVA programs can be optimized to get any desired final volume
- 52 samples in 15 mL Falcon™ Tubes or 23 sample in 50 mL Falcon™ Tubes can be processed at a time
- 10 mL and 40 mL methanol can be evaporated to less than 1 mL within 1.5 and 3 hours
- The whole process runs without any supervision

During this study, the transfer of final volumes was done without vortexing the Falcon™ Tubes. However, in real practice it is recommended to vortex before transfer to ensure efficient transfer. Especially, for real world samples with a water content, it is very important to vortex the sample before transfer and rinse the evaporation tube one more time. This is of particular importance for long chain PFAS which are getting more adherent to the wall of Falcon™ Tubes when the water content is getting higher.

Any Questions?
Do not hesitate to contact us:

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