

SOLUTIONS BY



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

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1. Introduction

The following application note can be used as an extension to the AN0033_LCTech_Applicationnote_FREESTYLE_XANA_PFAS that shows how water samples can be prepared fully automated for LC-MS/MS analysis by applying SPE with the FREESTYLE XANA system. 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 could be achieved.

Beside the fact, that a safety cabinet is needed, one of the most time consuming steps is the evaporation of 8 mL methanol to dryness by means of a heated water bath under a gentle N₂ flow to concentrate and for analysis to redissolve the sample in methanol/water 96:4 (v/v), according to US-EPA 537.1. Due to the high sample throughput of the FREESTYLE XANA, the main goal of this application is to evaporate faster than the recommended method US-EPA 537.1, without any losses of analytes, better signal intensity and without any supervision.

Application goals:

Robustness – compensation of residual water

- high tolerance against residual water in the sample

Speed – high sample throughput

- 75 min are needed for up to 22 samples in parallel
- 120 min are needed with N₂ evaporation

Recoveries – better than with N₂

- no generation of aerosols
- no additional rinsing steps
- no loss of light sensitiv analytes

No supervision – no losses due to overheating

- automatic stop

No aerosols – no cross-contamination

- generation of aerosols is inhibited by the centrifugal forces

No safety cabinet needed

- closed system, only an exhaust gas loop behind the cryotrap, no organic
- solvent vapors are released into atmosphere

Higher signal intensity – lower LOQ

- no generation of aerosols
- lower final volume, 250 µL instead of 1 mL

2. Method Development

To verify the efficiency of an evaporation method, possible losses have to be quantified. Therefore 8 mL methanol as test sample has been spiked with deuterated standard solution (surrogate standard) right at the beginning of the the evaporation to simulate a SPE cleanup with 100 % recovery. According to US-EPA 537.1, the methanol has to be evaporated to dryness and to be redissolved in methanol/water 96:4 (v/v). Spiked with a recovery standard solution (internal standard) as 100 % indication for calculating any losses, there should be a final volume of only 500 μL (1 mL according to US-EPA 537.1) The redissolved sample has to be transferred into a GC vial with a micropipette equipped with a plastictip.

2.1. Reagents and Materials

- Standard solutions (e.g. from Wellington Laboratories)
 - Internal standard (IS) in methanol:
 - $^{13}\text{C}_2$ -PFOA (1 ng/ μL)
 - $^{13}\text{C}_4$ -PFOS (3 ng/ μL)
 - d_3 -NMeFOSAA (4 ng/ μL)
 - Surrogate standard (SUR) in methanol:
 - $^{13}\text{C}_2$ -PFHxA (1 ng/ μL)
 - $^{13}\text{C}_2$ -PFDA (1 ng/ μL)
 - d_5 -NEtFOSAA (4 ng/ μL)
 - $^{13}\text{C}_3$ -HPFO-DA (1 ng/ μL)
 - Laboratory Fortified Blank (LFB) in methanol
 - Lower range (SUR 0.4 – 1.6 ng/500 μL)
 - Medium range (SUR 4 – 16 ng/500 μL)
 - Upper range (SUR 16-64 ng/500 μL)
 - Analyte Primary Dilution Standard (PDS)
 - The analyte PDS contains all the method analytes of interest at various concentration (0.5 – 2.5 ng/ μL)
- Solvents
 - Methanol HPLC grade (e.g. from J.T.Baker)
 - Water HPLC grade (e.g. from J.T.Baker)

- D-EVA, Martin Christ enhanced by LCTech GmbH
 - Angle Rotor, 24 positions P/N 16900
 - Sensor for below mentioned vial P/N 16802
 - Centrifuge tube, GPI 24-400 P/N 16738
 - Centrifuge tube, GPI 24-400 P/N 16452
 - 50 mL centrifuge tubes (PP) e.g. Falcontubes™
- UHPLC-MS/MS, Thermo Fisher Scientific

2.2. Sample Preparation

- Blanks: Falcontubes™ filled with 8 mL methanol HPLC grade
- Samples: Falcontubes™ filled with 8 mL methanol HPLC grade and spiked with Surrogate Standard in lower range, medium range or upper range.

2.3. Instrumentation

2.3.1. Evaporation

The sensor centrifuge vial and samples are filled with the same solvent mixture. Due to the thermal capacity of the temperature sensor in the reference vial, which is made of glass, the volume in that vial has to be approximately 200 % of the volume of the sample.

Due to the varying vapour pressure of the solvents, the cryotrap has to be emptied before changing evaporation methods. Otherwise, the evaporation is slower and insufficient recoveries could be achieved.

The centrifuge is running at 800 rpm only. Therefore, an exact balancing of the sample vials with a scale is not necessary. However, to prevent an imbalanced running of the motor, a balanced positioning of the vials in the rotor is strongly recommended.

The efficiency of the infrared lamps is not influenced by the Falcontubes™, so the distance between the lamps and the sample tubes is not significant. Therefore, all positions of the rotor can be used!

2.3.1.1 D-EVA Programs:

Methanol:

Sensor tube: 16 mL methanol
 Sample tubes: 8 mL methanol

Methanol	Start	1	2	3
t [min]	45	00:03	00:30	02:00
T [°C]		45	45	45
p [mbar]		55	20	20
ps [mbar]		130	130	130
Rotor [rpm]		800	800	800

Program stopping temperature: 40 °C

Final Volume: 1 - 5 µL

Run Time: ~77 min

Program "short":

Sensor tube: twice amount of residual volume of the sample tubes
 Sample tubes: residual volume

Short	Start	1	2
t [min]	45	00:03	00:07
T [°C]		45	45
p [mbar]		20	20
ps [mbar]		140	90
Rotor [rpm]		800	800

Program stopping temperature: 30 °C

Final Volume: 0 µL

Run Time: ~11 min

2.3.1.2 Sample transfer into GC vials:

The concentrated extract has to be redissolved in methanol/water 96:4 % (v/v) spiked with internal standard to bring the volume to the indicated volume and vortex. Transfer the solution with a plastic pipette into the autosampler Vial

2.3.1.3 Analysis with LC-MS/MS heated ESI according to the m/z values and gradient LC conditions for PFAS analysis.

2.4. Experiment procedure

2.4.1. Efficiency and Robustness

2.4.1.1. On the first day, 2 samples spiked with SUR at medium range are evaporated with a heated water bath (65 °C) and N₂ as reference test method. 2 solvent blanks, 8 samples spiked with SUR at medium range and 2 samples spiked with SUR at upper range are evaporated with the D-EVA. All evaporated samples are redissolved in methanol/water 96:4 % (v/v) spiked with internal standard.

2.4.1.2. On the second day, the experiment of the first day has been repeated, plus 2 additional samples spiked with SUR at lower range are tested as well. All evaporated samples are redissolved in methanol/water 96:4 % (v/v) spiked with internal standard.

2.4.1.3. On the third day, 6 samples spiked with SUR at medium range and 2 solvent blanks are evaporated with the D-EVA and are resolved in methanol/water 96:4 % (v/v) spiked with internal standard.

2.4.1.2. On the fourth day 6 samples spiked with SUR and PDS at medium range are evaporated with the D-EVA and are resolved in methanol/water 96:4 % (v/v) spiked with internal standard.

All re-dissolved samples of all 4 days have been analysed with the UHPLC-MS/MS. As acceptance criteria for the experiments, the calculated recoveries have to be comparable or better than the reference test.

2.4.2. Cross-contamination

An upper range spiked sample is positioned in the inner row of the rotor. In counter rotation direction 1 blank positioned in the inner and 1 blank in the outer row of the rotor. All samples are redissolved in methanol/water 96:4 % (v/v) with internal standard. The calculated recoveries are corresponding to the level of cross-contamination and should not exceed 1 %.

2.4.2.1. Lower final volume for lower limits of quantification (optional)

The concentrated extract has to be redissolved in 1 mL methanol/water 96:4 % (v/v) according to US-EPA 537.1. Because evaporation with the D-EVA avoids the generation of aerosols and keeps the analytes in the solvent there is no need of rinsing the centrifuge tube. So all performance tests have been redissolved in 500 µL methanol/water 96:4 % (v/v) and as a linearity test samples have been re-eluted in 500 µL, 250 µL, 100 µL and 50 µL methanol/water 96:4 % (v/v) spiked with internal standard solution. The calculated recoveries are corresponding to the level of efficiency.

2.4.3. Residual water in sample

Due to the fact that a water containing sample will carry some water through the whole process it is important to know how much water the D-EVA can handle without any losses.

8 samples with different amounts of water, spiked with SUR at medium range are filled up to a final volume of 8 mL as follows:

- 0 mL water + SUR and methanol up to 8 mL
- 0.1 mL water + SUR and methanol up to 8 mL
- 0.25 mL water + SUR and methanol up to 8 mL
- 0.5 mL water + SUR and methanol up to 8 mL
- 1 mL water + SUR and methanol up to 8 mL
- 2 mL water + SUR and methanol up to 8 mL
- 3 mL water + SUR and methanol up to 8 mL
- 4 mL water + SUR and methanol up to 8 mL

All samples are evaporated with the D-EVA, program "methanol". Any sample near dryness is ready. All other samples are evaporated with the D-EVA, program "short". Again all samples near dryness are ready, all other samples are evaporated with program "short" again. When all samples are near dryness, they are resolved in methanol/water 96:4 % (v/v) spiked with internal standard and analysed with the UHPLC-MS/MS.

The signal intensity of the measurement has to be checked and the recoveries of the SUR have to be within the acceptance criteria 70 % to 130 % recommended by US-EPA 537.1.

3. Results

3.1. Efficiency and Robustness

To evaporate medium range samples with N₂ with a recovery > 80 % needs at least 130 minutes, as too high temperature or too high N₂ flow leads to aerosols and loss of analytes! The evaporation via D-EVA stops with a final volume of 1-5 µL within 77 minutes. An evaporation to total dryness is not necessary, but if needed due to formal reasons, it can be carried out easily with a short program within additional 11 min.

The following figures (Figure 1 to 3) are presenting a summary of all results of the first three days. Figure 4 is showing the recoveries of the analytes recommended by US-EPA 537.1.

Detailed information see Appendix 5.1

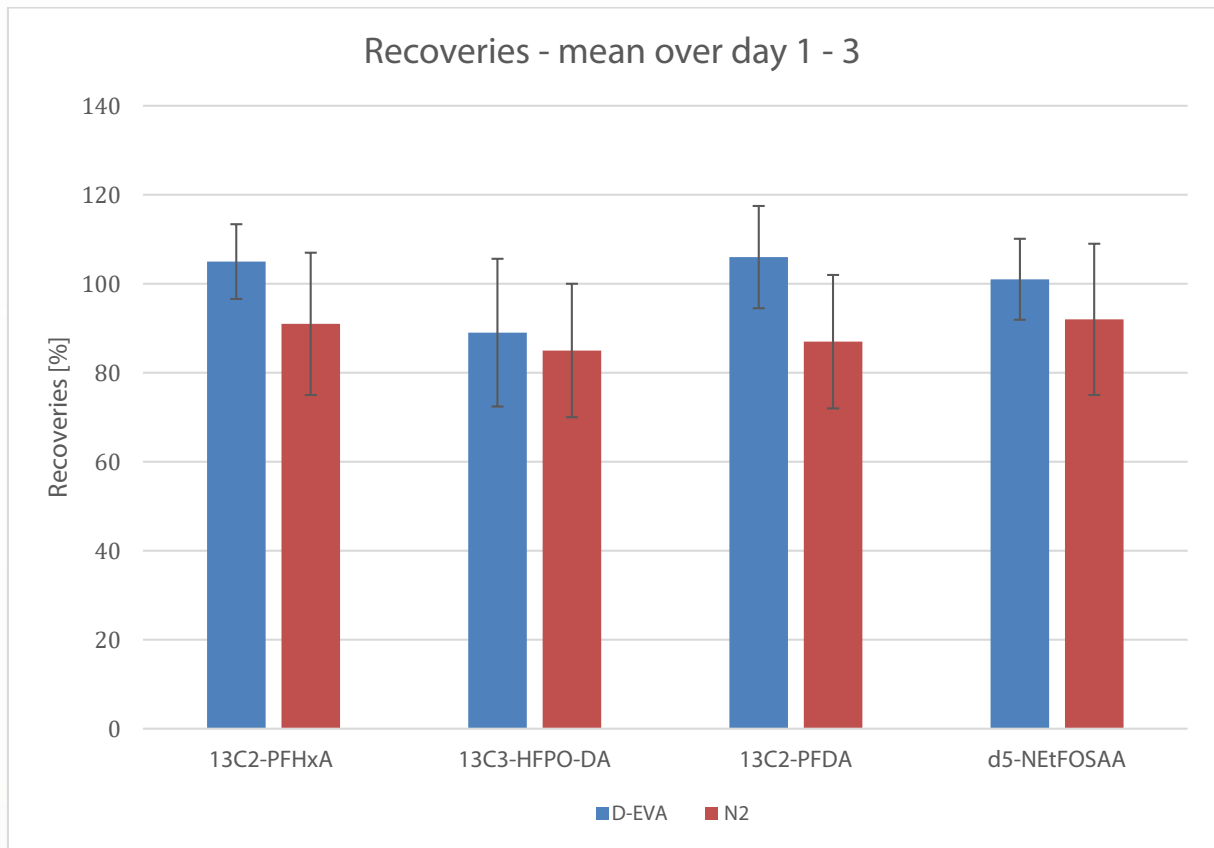


Figure 1) Mean of the recoveries of day 1 to day 3 with D-EVA & N₂

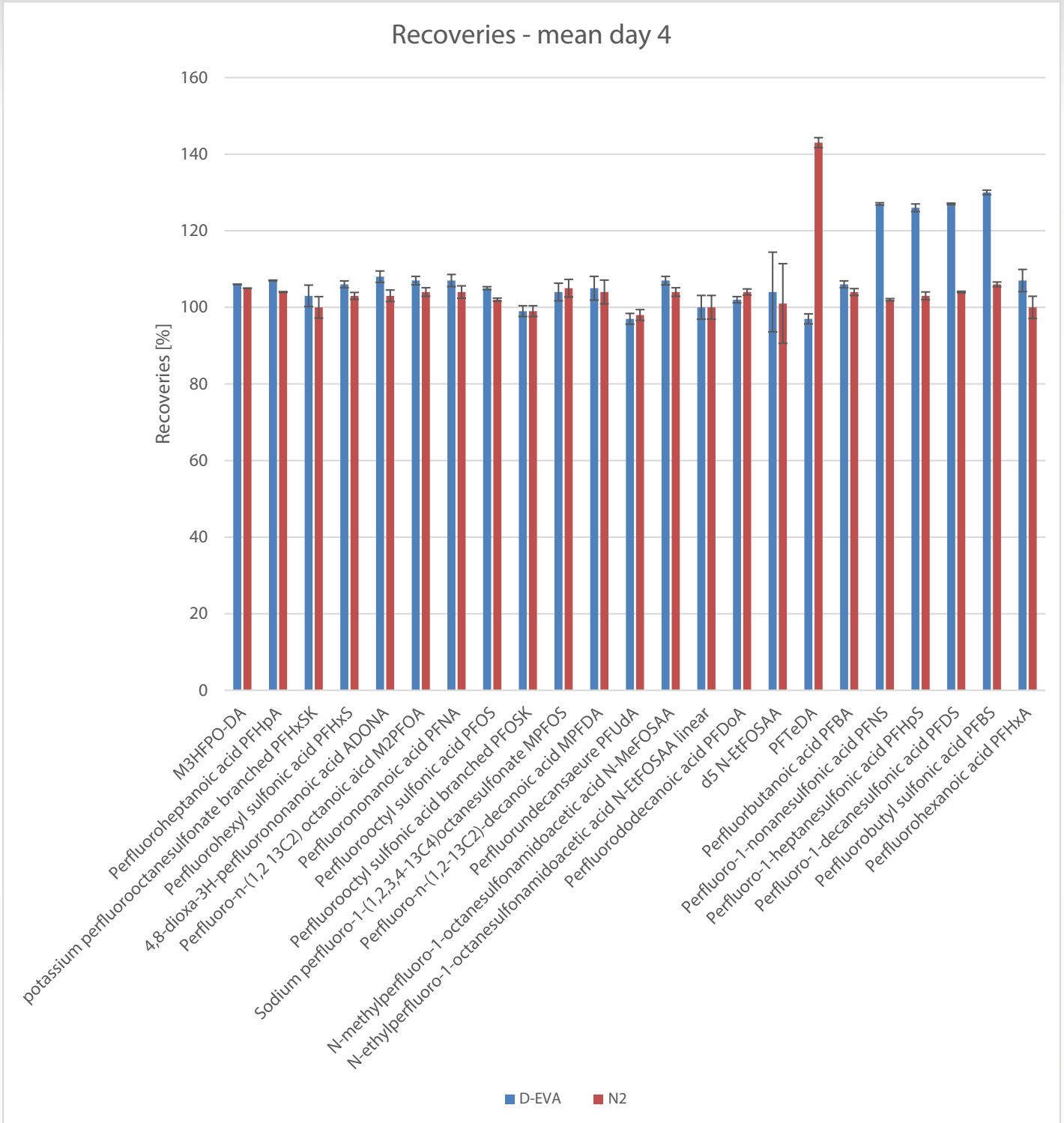


Figure 2) Summary of all recoveries day 4

The recoveries of n=12 samples are very reproducible. The results are all within the acceptance criteria 70 % to 130 % of the US-EPA 537.1.

3.2 Cross-contamination

Cross-contamination

[%]	¹³ C ₂ -PFHxA	¹³ C ₃ -HFPO-DA	¹³ C ₂ -PFDA	d ₅ -NEtFOSAA
upper range - 1	101	96	102	98
blank methanol	0.000	0.000	0.000	0.000
blank cross-1<1	0.012	0.000	0.013	0.232
blank cross-1<2	0.002	0.000	0.022	0.064
upper range - 2	87	73	87	86
blank cross-2<1	0.000	0.000	0.049	0.350
blank cross-2<2	0.000	0.000	0.000	0.125
upper range - 3	102	121	104	108
blank methanol	0.000	0.000	0.000	0.000
blank cross-3<1	0.000	0.000	0	0.164
blank cross-3<2	0.000	0.000	0.051	0.000
upper range - 4	103	108	104	102
blank methanol	0.000	0.000	0.000	0.000
blank cross-4<1	0.000	0.000	0.000	0.065
blank cross-4<2	0.000	0.000	0.000	0.098
upper range - 1	101	96	102	98
blank methanol	0.000	0.000	0.000	0.000
blank cross-1<1	0.012	0.000	0.013	0.232
blank cross-1<2	0.002	0.000	0.022	0.064
upper range - 2	87	73	87	86
blank cross-2<1	0.000	0.000	0.049	0.350
blank cross-2<2	0.000	0.000	0.000	0.125

The calculated recoveries in the Blank Cross samples do not exceed 1 % absolut. There is no cross-contamination detected!

3.3 Lower final volumes for lower LOQ (optional)

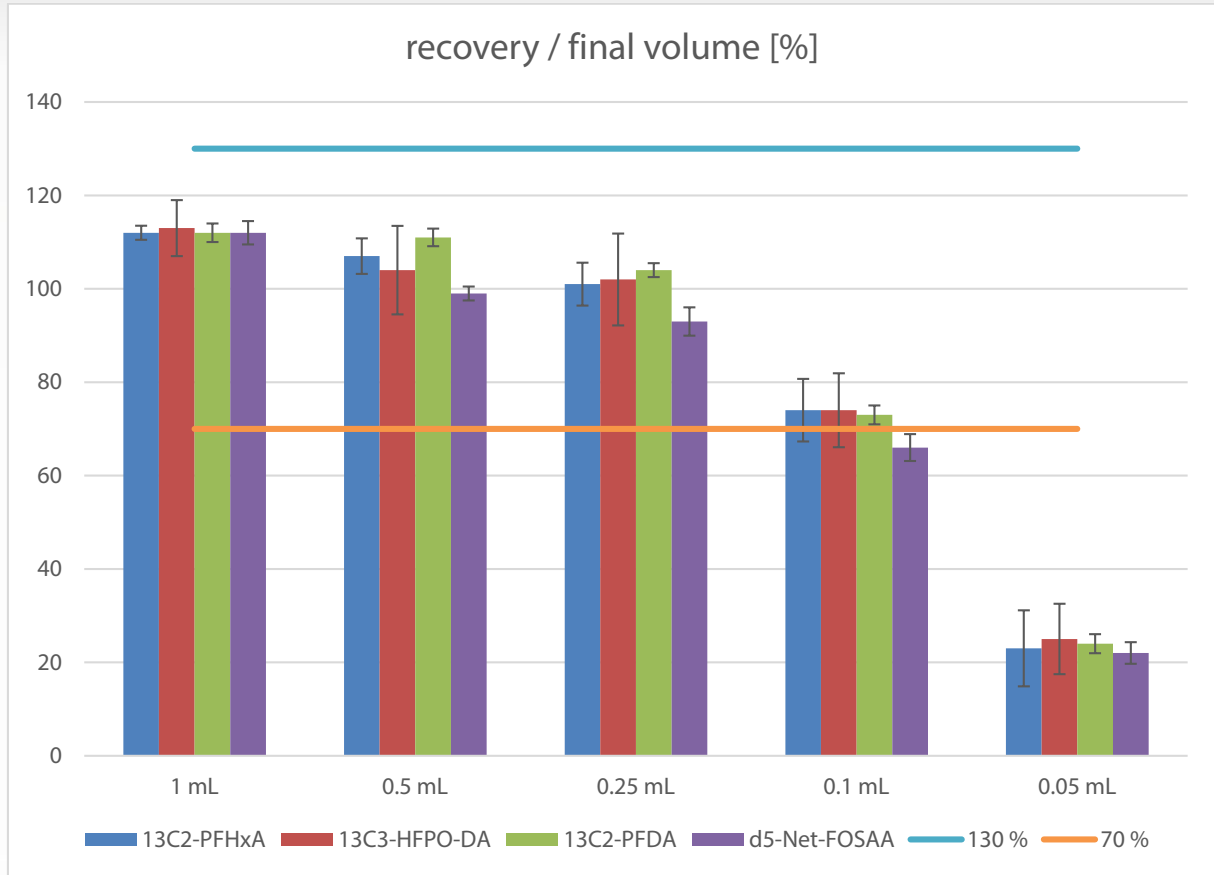


Figure 3) Linearity test – final volume (n=6 per level)

The recoveries shown in figure 3 are within the acceptance criteria 70 % to 130 % according to US-EPA 537.1 for the first three levels. Down to 250 μ L final volume is easy possible with high precision and high recoveries of the analytes.

3.4 Residual water in sample

	1 programm „short“	to near dryness
0 mL water	100	104
0 mL water (2)	101	106
0.1 mL water	99	105
0.1 mL water (2)	96	107
0.25 mL water	87	100
0.25 mL water (2)	88	99
0.5 mL water	80	100
0.5 mL water (2)	78	97
1 mL water	53	101
1 mL water (2)	55	99
2 mL water	38	78
2 mL water (2)	39	78
3 mL water	29	58
3 mL water (2)	34	57
4 mL water	25	44
4 mL water (2)	24	44

Figure 4) Effect of higher volumes of residual water on the signal intensity

Note: The residual volume of 2 and 4 mL water in the sample with only one additional program “short” via D-EVA has been higher than 1 mL. Running several times program “short” still left a residual volume of ca. 1 mL for the 4 mL test.

Evaporating the samples containing different amounts of water is showing a significant effect on the measurement uncertainty. Figure 4 shows, that the standard program of the D-EVA can easily handle up to 0.5 mL of water after the clean-up. Higher amounts of water in the sample needs additional runs of program “short” of the D-EVA, or a longer program “methanol”, adding the expected amount of water into the reference tube.

Figure 4 shows, that with the additional runs the D-EVA can handle up to 2 mL of water. The next diagrams are showing the corresponding recoveries to the different amounts of water.

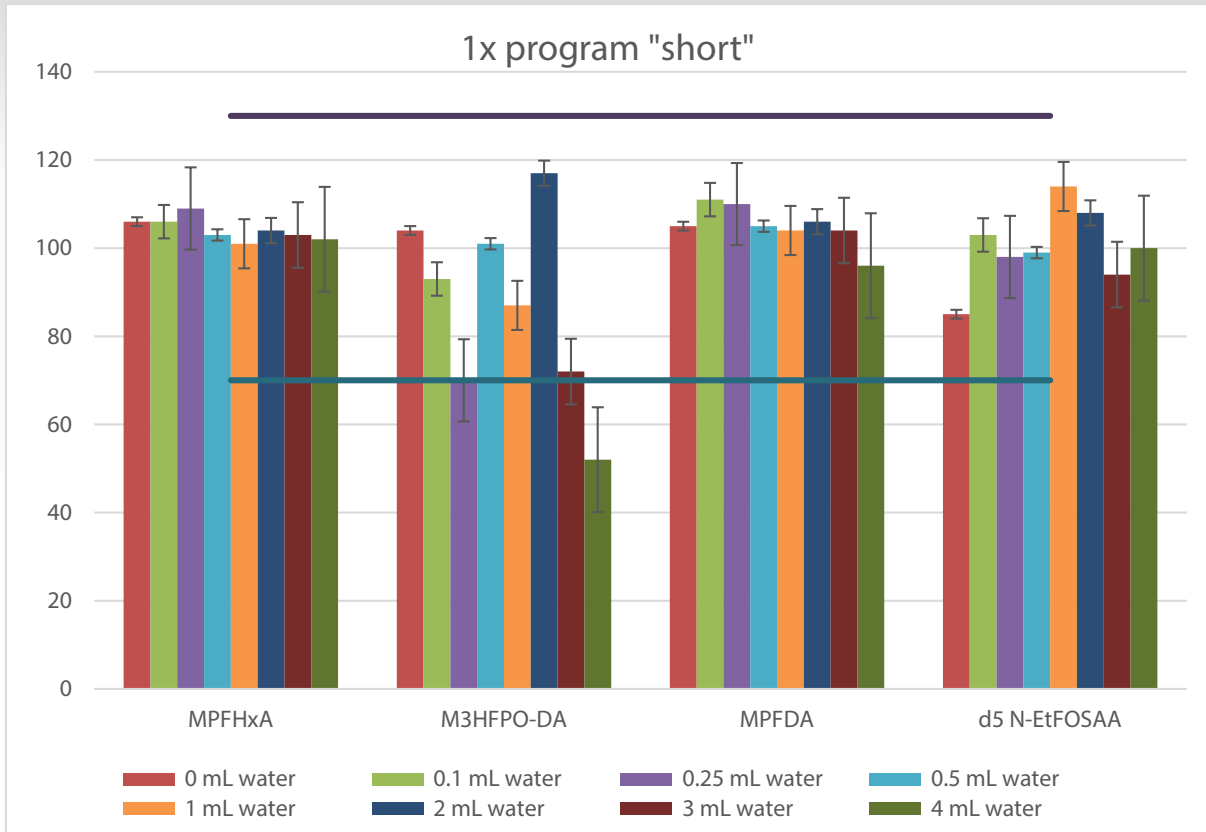


Figure 5) Robustness against residual water in sample, with only 1 program "short"

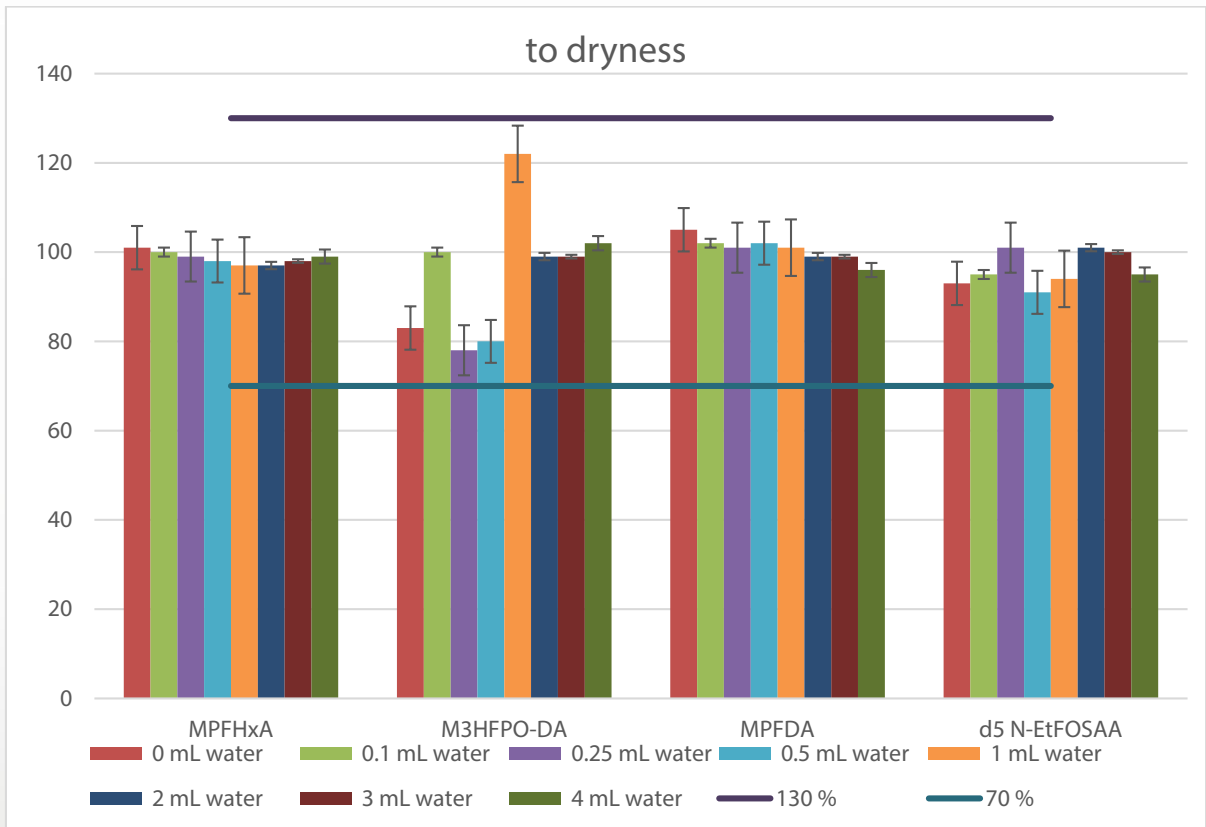


Figure 6) Robustness against residual water in sample, with program "short" to dryness (n=2)

Running the samples with different amounts of water with the standard program of the D-EVA, 1x "methanol" + 1x "short" is showing a greater uncertainty for the recoveries than evaporating to nearly dryness with additional program "short". Comparing the most difficult analyte M₃HFPO-DA because of its low intensity on the UHPLC-MS/MS, shows that the recoveries are spreaded over a range of 50 – 120 %.

The standard method of the D-EVA can easily handle an amount of 0.25 mL of water after the Clean-Up. If there is water expected in the sample, this amount of water can be added to the reference tube for a longer "methanol"-method, to reduce the amount of programs "short" and to save time.

Evaporating to near dryness via additional programs "short" improves the recoveries to 75 – 120 % within the acceptance criteria of the EPA537.1! The D-EVA can handle 25 % water in 8 mL methanol.

4. Conclusions

	N2	D-EVA
Speed + optional 11 min	130 min	77 min
Recoveries	82-116 %	89-105 %
Cross-contamination	Not tested	Not detected
Tolerance against residual water	Not tested	25% water in the sample
No rinsing necessary to transfer in GC vial	Not possible	No rinsing needed
Throughput	6 – 18 parallel	23 parallel
Supervision	Yes	No
Lower LOQ	Not tested	Factor 4 lower LOQ

LCTech D-EVA is a brilliant solution for fast, parallel and reproducible evaporation of PFAS samples without any cross-contamination. The system concentrates samples with vacuum and energy supply via infrared light to a low volume and prevents evaporation to dryness due to a special LCTech sensor. There are no aerosols due to the centrifugal force, so no rinsing is necessary and the volume of the final sample is only 500 µL instead of 1 mL, resulting in a better signal/noise at the HPLC-system.

The sensor allows the system to stop automatically at the defined volume, as described in this application note. The subsequent transfer into the insert of a GC vial is easy to handle.

If needed the D-EVA shows a great robustness against unwanted residual water in the sample. The repetition of program "short" after the initial evaporation program, has no negative impact on analyte recovery but improves analytical sensitivity by a smaller volume error and corresponding correction by internal standards.

5. Appendix

5.1. Results efficiency and robustness (SUR, IS)

Results efficiency and robustness (SUR, IS)

Day 1:	¹³ C ₂ - PFHxA	¹³ C ₃ - HFPO- DA*	IS ¹³ C ₂ - PFOA	IS ¹³ C ₄ - PFOS	¹³ C ₂ - PFDA	IS d ₃ - NMe- FOSAA	d ₅ -Net- FOSAA
Midrange IS4+SS4/500	100	100	100	100	100	100	100
Midrange in resolving+IS 4SS+495	108	112	99	98	100	103	105
Upper range 40SS+4IS	100	100	100	100	100	100	100
Midrange N2-1	109	82	104	97	104	99	116
Midrange N2-2	103	92	101	97	98	101	101
Midrange Methanol-8 mL-1	100	72	103	96	101	101	102
Midrange Methanol-8 mL-2	96	98	106	97	96	97	96
Midrange Methanol 2-1	101	76	105	96	101	99	103
Midrange Methanol 2-2	95	75	100	102	95	98	100
Midrange Methanol 2-3	93	74	106	99	102	96	96
Midrange Methanol 2-4	100	83	105	98	101	97	103
Midrange Methanol 2-5	101	66	101	97	102	102	104
Midrange Methanol 2-6	102	88	98	98	99	105	94
Midrange Methanol 2-7	104	61	101	101	112	98	102
Midrange Methanol 2-8	94	83	103	99	94	98	96
Upper range Methanol 2-1	101	96	97	99	102	104	98
Upper range Methanol 2-2	87	73	99	101	87	100	86

*) small areas for this analyte increases the uncertainty

**) outlier (Grubbs' Test)

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Results efficiency and robustness (SUR, IS) (no further results for the N2 -evaporation and solvent):

Day 2:	¹³ C ₂ -PFHxA	¹³ C ₃ -HFPO-DA*	IS ¹³ C ₂ -PFOA	IS ¹³ C ₄ -PFOS	¹³ C ₂ -PFDA	IS d ₃ -NMe-FOSAA	d ₅ -Net-FOSAA
Blank methanol	0	0	0	0	0	0	0
Blank 1	0	0	98	106	0	96	1
Blank 2	0	0	104	97	0	99	1
Low range 1	126	94	103	95	127	102	123
Low range 2	119	168	103	95	119	102	141
Midrange 1	116	143 **	96	109	123	94	134 **
Midrange 2	119	129 **	101	102	125	97	117
Midrange 3	120	112	97	98	125	105	116
Midrange 4	120	106	103	103	124	94	120
Midrange 5	112	116	97	98	119	106	112
Upper range 1	102	121	101	100	104	100	108
Upper range 2	103	108	101	100	104	100	102

*) small areas for this analyte increases the uncertainty

***) outlier (Grubbs' Test)

Results efficiency and robustness (SUR, IS) (no further results for the N2-Evaporation and solvent):

Day 3:	¹³ C ₂ -PFHxA	¹³ C ₃ -HFPO-DA	IS ¹³ C ₂ -PFOA	IS ¹³ C ₂ -PFOA	¹³ C ₂ -PFDA	IS d ₃ -NMeFOSAA	d ₅ -NEtFOSAA
Blank 1	0	0	98	105	0	97	0.3
Blank 2	0	0	101	100	0	98	0
Midrange 1	102	103	104	103	99	94	95
Midrange 2	102	77	103	100	101	100	94
Midrange 3	101	90	100	97	91	103	92
Midrange 4	105	108	105	105	98	108	91
Midrange 5	104	96	103	100	98	107	95
Midrange 6	104	101	109	108	102	107	95

5.2 Results efficiency and robustness (PDS)

Analytes	N2		D-EVA											
	1	2	1	2	3	4	5	6	7	8	9	10	11	12
M3HFPO-DA	105	106	105	103	105	109	106	108	110	109	110	103	108	108
Perfluoroheptanoic acid PFHpA	104	105	110	106	107	114	102	105	110	109	112	106	105	105
potassium perfluorooctane-sulfonate branched PFHxSK	100	104	102	103	103	106	103	103	104	109	106	103	104	104
Perfluorohexyl sulfonic acid PFHxS	103	108	106	104	105	110	105	106	107	109	109	104	106	69
4,8-dioxa-3H-perfluorononanoic acid ADONA	103	104	108	106	107	111	105	107	107	111	113	104	106	107
Perfluorooctanoic acid PFOA	118	107	96	96	99	101	94	95	102	104	100	92	104	100
Perfluoro-n-(1,2-13C2) octanoic acid M2PFOA	104	107	106	106	107	112	104	108	107	110	109	107	106	104
Perfluorononanoic acid PFNA	104	108	105	104	109	110	107	109	109	113	105	103	103	106
Perfluorooctyl sulfonic acid PFOS	102	106	103	103	106	108	103	107	107	108	106	99	101	104
Perfluorooctyl sulfonic acid branched PFOSK	99	102	102	94	93	102	101	100	101	98	103	95	97	95
potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate 9Cl-PF3	114	102	96	93	101	99	94	98	103	101	96	93	95	107
Sodium perfluoro-1-(1,2,3,4-13C4)octanesulfonate MPFOS	105	106	104	100	106	108	104	105	103	108	108	102	102	104
Perfluoro-n-(1,2-13C2)-decanoic acid MPFDA	104	105	105	108	105	109	103	103	102	109	106	101	104	106
Perfluoroundecansaeure PFUDA	98	106	94	90	96	100	100	99	102	105	100	100	92	95
N-methylperfluoro-1-octanesulfonamidoacetic acid N-MeFOSAA	104	107	106	105	107	112	103	107	107	110	109	106	106	70
N-ethylperfluoro-1-octanesulfonamidoacetic acid N-EtFOSAA linear	100	109	103	94	102	104	96	99	103	110	107	99	84	102
Perfluorododecanoic acid PFDoA	104	111	100	103	102	108	97	101	102	103	101	95	93	90
d5 N-EtFOSAA	101	113	102	106	104	109	100	103	103	84	102	82	95	99
PFTeDA	143	204	73	108	125	112	109	56	65	67	168	83	61	64
Perfluorbutanoic acid PFBA	104	108	104	104	105	110	107	104	106	108	113	104	106	107
Perfluoro-1-nonanesulfonic acid PFNS	102	115	129	126	125	127	127	127	124	125	129	128	116	121
Perfluoro-1-heptanesulfonic acid PFHpS	103	117	125	124	127	125	126	130	126	124	126	122	112	122
Perfluoro-1-decanesulfonic acid PFDS	104	119	127	125	121	128	133	128	124	124	134	128	117	124
Perfluorobutyl sulfonic acid PFBS	106	119	128	128	131	128	130	133	125	123	131	129	121	125
Perfluorohexanoic acid PFHxA	100	110	100	99	120	113	94	114	110	104	111	103	109	111
Perfluoro-n-(1,2-13C2) hexanoic acid MPFHxA	118	111	88	97	88	93	86	93	91	95	94	93	97	97

5.3 Lower final volumes

Final volume	¹³ C ₂ -PFHxA	¹³ C ₃ -HFPO-DA	IS ¹³ C ₂ -PFOA	IS ¹³ C ₂ -PFOA	¹³ C ₂ -PFDA	IS d ₃ -NMe-FOSAA	d ₅ -Net-FOSAA
1 mL 1	110	107	107	96	110	97	118
1 mL 2	113	119	103	101	113	96	106
0.5 mL 1	102	92	101	99	110	100	102
0.5 mL 2	109	97	104	99	111	97	102
0.5 mL 3	114	113	99	103	116	98	101
0.5 mL 4	105	95	100	101	115	100	92
0.5 mL 5	105	108	104	102	106	94	99
0.5 mL 6	106	117	102	100	110	98	99
0.25 mL 1	99	90	102	101	102	97	94
0.25 mL 2	105	104	102	105	108	93	98
0.25 mL 3	98	98	101	98	98	101	88
0.25 mL 4	109	116	104	99	109	96	93
0.25 mL 5	96	113	99	103	102	98	90
0.25 mL 6	98	92	102	96	103	102	92
0.1 mL 1	85	79	99	98	84	102	74
0.1 mL 2	70	63	104	96	73	100	64
0.1 mL 3	66	67	103	95	64	102	60
0.1 mL 4	71	77	99	95	72	106	63
0.1 mL 5	80	87	103	102	80	95	74
0.1 mL 6	69	72	103	93	65	104	60
0.05 mL 1	21	23	106	98	21	96	20
0.05 mL 2	19	21	105	92	21	103	18
0.05 mL 3	22	22	102	92	23	106	20
0.05 mL 4	41	41	104	96	40	100	36
0.05 mL 5	19	22	100	95	20	105	18
0.05 mL 6	17	18	105	97	18	98	17

5.4 Residual water in sample

"methanol" + 1 program "short"	¹³ C ₂ - PFHx A	¹³ C ₃ - HFPO DA	IS ¹³ C ₂ - PFOA	IS ¹³ C ₂ - PFOA	¹³ C ₂ - PFDA	IS d ₃ -NMe- FOSAA	d ₅ -Net- FOSAA
0 mL water	105	107	104	102	106	94	83
0 mL water (2)	106	101	101	97	105	102	87
0.1 mL water	102	90	100	99	112	101	93
0.1 mL water (2)	109	96	105	96	110	100	113
0.25 mL water	108	59	102	93	111	105	98
0.25 mL water (2)	109	82	103	98	109	99	97
0.5 mL water	101	92	102	101	104	97	93
0.5 mL water (2)	105	109	101	99	106	100	105
1 mL water	100	90	100	99	100	101	111
1 mL water (2)	103	85	96	94	108	110	118
2 mL water	103	157	102	97	106	101	116
2 mL water (2)	104	78	93	96	105	111	100
3 mL water	110	111	107	93	114	100	98
3 mL water (2)	97	32	94	98	95	107	89
4 mL water	99	79	96	94	98	110	118
4 mL water (2)	105	26	98	98	95	103	82

"methanol" to nearly dryness	¹³ C ₂ - PFHx A	¹³ C ₃ - HFPO DA	IS ¹³ C ₂ - PFOA	IS ¹³ C ₂ - PFOA	¹³ C ₂ - PFDA	IS d ₃ -NMe- FOSAA	d ₅ -Net- FOSAA
0 mL water	102	75	102	101	107	97	96
0 mL water (2)	99	92	99	99	102	102	91
0.1 mL water	103	100	102	93	105	105	96
0.1 mL water (2)	97	100	99	98	100	102	95
0.25 mL water	98	93	99	97	103	104	105
0.25 mL water (2)	100	63	100	95	99	106	98
0.5 mL water	98	57	97	92	101	111	95
0.5 mL water (2)	98	102	102	95	103	104	88
1 mL water	92	118	94	93	98	113	91
1 mL water (2)	102	125	96	98	104	106	97
2 mL water	96	113	96	101	98	104	104
2 mL water (2)	97	85	98	105	101	97	98
3 mL water	96	62	102	92	95	106	96
3 mL water (2)	100	136	106	90	104	104	104
4 mL water	103	93	92	98	97	110	114
4 mL water (2)	95	111	93	102	96	105	77

6. General Accessories & Spare Parts

6.1. D-EVA Vacuum Concentrator

Rotational-Vacuum-Concentrator D-EVAporation P/N 16900

6.1.1. Rotor with 48 Positions

- | | | |
|------------------------------------|-----|----------|
| 1. Fixed angle rotor, 48 positions | P/N | 16742 |
| 2. Sensor for below mentioned vial | P/N | 16741 |
| 3. Centrifuge tube, GL14 | P/N | 15781 |
| 4. Screw cap GL 14 | P/N | V0014-SL |
| 5. Seal for centrifuge tube GL 14 | P/N | V0014-D |

6.1.2. Rotor with 24 Positions

- | | | |
|------------------------------------|-----|----------|
| 1. Fixed angle Rotor, 24 positions | P/N | 16802 |
| 2. Sensor for below mentioned vial | P/N | 16738 |
| 3. Centrifuge tube, GPI 24-400 | P/N | 16452 |
| 4. Screw cap GPI 24-400 | P/N | V0024-SL |
| 5. Seal for centrifuge tube GPI 24 | P/N | V0025-D |

6.1.3. Rotor with 12 Positions

- | | | |
|------------------------------------|-----|-------|
| 1. Fixed angle Rotor, 12 positions | P/N | 16929 |
| 2. Sensor for below mentioned vial | P/N | 16755 |
| 3. Centrifuge tube, GL 32 | P/N | 16725 |
| 4. Screw Cap, GL 32 | P/N | 16754 |

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