

# Manual Extraction of PFAS in Drinking Water Following DIN 38407-42

## Authors

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## Equipment and Materials Used

### Biotage

- » Biotage® VacMaster™ 20 Sample Processing Station (with 15 mL rack), p/n 121-2015ML, fitted with polypropylene (PFAS free) stopcocks (p/n 121-0009-PP)
- » Biotage® VacMaster™ LVE Kit (PFAS) for 1, 3, 6 mL SPE Columns (p/n 121-2190)
- » EVOLUTE® PFAS 500 mg/6 mL SPE Columns, p/n 614-0050-CP
- » EVOLUTE® PFAS 150 mg/6 mL SPE Columns, p/n 614-0015-CP
- » TurboVap® LV Automated Solvent Evaporation System, p/n 415000
- » TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes), p/n 414964

### Wellington Laboratories

- » ISO 21675:2019 Labelled Stock Solution, 1.2 mL, p/n ISO 21675-LSS
- » ISO 21675:2019 Native Stock Solution, 1.2 mL, p/n ISO 21675-NSS

## Ordering Information

Part Number	Description	Qty
<b>121-2015ML</b>	Biotage® VacMaster™ 20 Sample Processing Station With 15 mm Rack	1
<b>121-2190</b>	Biotage® VacMaster™ LVE Kit (PFAS) for 1, 3, 6 mL SPE Columns	1
<b>121-0009-PP</b>	Polypropylene (PFAS) Stopcocks	10
<b>614-0050-CP</b>	EVOLUTE® PFAS 500 mg/6 mL columns	30
<b>614-0015-CP</b>	EVOLUTE® PFAS 150 mg/6 mL columns	30
<b>614-0006-BP</b>	EVOLUTE® PFAS 60 mg/3 mL columns	50
<b>415000</b>	TurboVap® LV Automated Solvent Evaporation System	1
<b>414964</b>	TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes)	1

## Analytes

**Table 1.** Listing of Target Analytes and Internal Standards.

Target Analyte	Acronym	CAS
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluoroundecanoic acid <sup>1</sup>	PFUnA	2058-94-8
Perfluorododecanoic acid <sup>1</sup>	PFDoA	307-55-1
Perfluoroheptanesulfonic acid <sup>1</sup>	PFHpS	375-92-8
Perfluorodecanesulfonic acid <sup>1</sup>	PFDS	335-77-3
1H,1H,2H,2H-perfluorooctanesulfonic acid <sup>1</sup>	H4PFOS	27619-97-2
Internal Standard		
Perfluoro-n-[1,2,3,4- <sup>13</sup> C <sub>4</sub> ]butanoic acid	<sup>13</sup> C <sub>4</sub> -PFBA	
Perfluoro-n-[1,2,3,4,5- <sup>13</sup> C <sub>5</sub> ]pentanoic acid	<sup>13</sup> C <sub>5</sub> -PFPeA	
Perfluoro-n-[1,2- <sup>13</sup> C <sub>2</sub> ]hexanoic acid	<sup>13</sup> C <sub>2</sub> -PFHxA	
Sodium perfluoro-1-[1,2,3- <sup>13</sup> C <sub>3</sub> ]hexanesulfonate	<sup>13</sup> C <sub>3</sub> -PFHxS	
Sodium perfluoro-1-[1,2,3- <sup>18</sup> O <sub>2</sub> ]hexanesulfonate	<sup>18</sup> O <sub>2</sub> -PFHxS	
Perfluoro-n-[1,2,3,4- <sup>13</sup> C <sub>4</sub> ]heptanoic acid	<sup>13</sup> C <sub>4</sub> -PFHpA	
Perfluoro-[1,2- <sup>13</sup> C <sub>2</sub> ]octanoic acid	<sup>13</sup> C <sub>2</sub> -PFOA	
Sodium perfluoro-1-[1,2,3,4- <sup>13</sup> C <sub>4</sub> ]octanesulfonate	<sup>13</sup> C <sub>4</sub> -PFOS	
Perfluoro-n-[ <sup>13</sup> C <sub>5</sub> ]nonanoic acid	<sup>13</sup> C <sub>5</sub> -PFNA	
Perfluoro-n-[1,2- <sup>13</sup> C <sub>2</sub> ]decanoic acid	<sup>13</sup> C <sub>2</sub> -PFDA	

<sup>1</sup>Target compounds are from the expanded list given in DIN 38407-42 Section 2.1.

## Solution Preparation

### Ammonia/Methanol Solution

1. Add 400  $\mu\text{L}$  of  $\text{NH}_4\text{OH}$  for every 100 mL of methanol to a clean beaker.
2. Agitate to homogenize.
3. Prepare new solution daily.

### Acetate Buffer

1. Measure out 499.5 mL of reagent water in a clean beaker.
2. Add 0.193 g of  $\text{NH}_4\text{Ac}$ .
3. Sonicate the solution for 5 minutes until the salt is fully dissolved.
4. Add 570  $\mu\text{L}$  of glacial acetic acid.
5. Agitate to homogenize the solution.

### Working Spiking Solution

1. Dilute 100  $\mu\text{L}$  of the native stock solution with 900  $\mu\text{L}$  of methanol to achieve a 10 ppt solution.



## Summary of SPE Method

### SPE Column Format

EVOLUTE® PFAS 500 mg/6 mL or EVOLUTE® PFAS 150 mg/6 mL

### Sample Pre-Treatment

Adjust the pH of each sample to 3 using glacial acetic acid. Add targets and internal standards.

### Conditioning

Condition each column with 0.1 %  $\text{NH}_4\text{OH}$  in methanol (10 mL) followed by methanol (10 mL).

### Equilibration

Equilibrate each column with reagent water (10 mL).

### Sample Loading

Load sample at a flow rate of 5 mL/min.

### Wash

Rinse the sample container with acetate buffer solution (10 mL) and load onto the column. Repeat using reagent water (10 mL).

### Dry

Dry the column for 5 minutes at a flow rate of 5 mL/min.

### Elution

Rinse the sample container with methanol (5 mL) and use to elute the analytes from the column at a flow rate of 2 mL/min. Repeat using 0.1 %  $\text{NH}_4\text{OH}$  in methanol (5 mL).

### Post Extraction

Concentrate the extract to a volume of 1 mL. Add IS and mix prior to analysis.

**Note:** EVOLUTE® PFAS 60 mg/3 mL columns may be used however sample and solvent volumes should be adjusted. Refer to DIN 38407-42 for the appropriate amounts.

## Sample Preparation Procedure

- Clean all parts of the Biotage® VacMaster™ system per the procedure given in Appendix A.
- Set up and fill new sample containers with water; 250–500 mL are typical for this method.
- Add glacial acetic acid to each of the sample containers to reduce the pH to 3 (approximately 100 µL for 250 mL sample volumes and 200 µL for 500 mL sample volumes).
- Verify the pH of the sample is 3 using pH paper. To reduce the possibility of contamination, a duplicate volume was collected and adjusted to the appropriate pH and the same volume of acid was added to the sample container.
- Prepare for the determination of the initial sample volume by either marking the level of the sample on the container or by weighing the sample container.
- Add 20 µL of the undiluted Labeled Stock Solution to each of the sample containers. If desired, fortify a sample using target analytes: the addition of 125 µL or 37.5 µL of the native stock solution will yield either 50 ppt or 15 ppt concentrations respectively, while the addition of 50 µL of the working spiking solution will yield a 2 ppt concentration. If the mixes used were different than the ones outlined in this note, adjust the concentration or spiking amounts as needed.
- Load the desired EVOLUTE® PFAS columns onto the Biotage® VacMaster™. Seal any unused positions using VacMaster Port Sealing Plugs (p/n 121-0005)
- Rinse each column with 10 mL of 0.1 % NH<sub>4</sub>OH in methanol and apply vacuum at 10 mL/min to pull it to waste. Do not allow the sorbent to go dry.
- Rinse each column with 10 mL of methanol and apply vacuum at 10 mL/min to pull it to waste. Do not allow the sorbent to go dry.
- Rinse each column with 10 mL of reagent water and apply vacuum at 10 mL/min to send it to waste. Do not allow the water level to drop below the top of the packing.
- Using the Biotage® VacMaster™ LVE Kit, place one end of the cleaned tubing into the bottom of each of the sample containers, and secure in position using the clips provided.
- Load the samples onto the columns using a flow rate of 5 mL/min.
- Once the sample has been fully loaded, rinse the sample containers using 10 mL of acetate buffer solution, swirl to ensure the full rinsing of the container, and load the aliquot onto the column at a rate of 5 mL/min.
- Rinse the sample containers using 10 mL of reagent water, swirl to ensure the full rinsing of the container, and load the aliquot onto the column at a rate of 5 mL/min.
- Dry the column for 5 minutes at a rate of 5 mL/min.
- Load 15 mL centrifuge tubes into the rack corresponding to each of the column positions and load into the VacMaster.
- Rinse each sample container using 5 mL of methanol and swirl to ensure the full rinsing of the container. Load the aliquot through the appropriate column and collect at a dropwise rate.
- Rinse each sample container using 5 mL of 0.1% NH<sub>4</sub>OH in methanol and swirl to ensure the full rinsing of the container. Load the aliquot through the appropriate column and collect at a dropwise rate.
- Determine the initial sample volume by either using a graduated cylinder and filling the sample container to the original mark or by taking an additional weight of the container.
- Transfer the centrifuge tubes to the TurboVap® LV system and concentrate the samples to just under 1 mL using nitrogen according to the parameters in Table 2.
- Bring the final extract to 1 mL and transfer to an autosampler vial.
- Load the extract onto a calibrated LC-MS/MS system and process using the conditions given in the below sections.

**Table 2.** TurboVap® LV Concentration Protocol.

<b>Bath Temp:</b>	60 °C
<b>Evaporation Mode</b>	Method (Ramp Gradient)
<b>Manifold Setup</b>	48 positions
<b>Rack Row Height</b>	120 mm*
<b>Step 1:</b>	1.5 L/min for 20 min
<b>Step 2:</b>	3.0 L/min for 15 min
<b>Step 3:</b>	3.5 L/min for 45 min

\*The nozzle position was adjusted such that it was as far to the right as possible to give the user a clear view of the vortex within the tube.

