

# Recommended Procedures for the Preparation of PFAS Compounds For UCMR 5

## Procedure for EPA 537.1

| Step         | Amount | Reagent / Description                                          | Flow Rate (mL/min) | Elute To | Notes                        |
|--------------|--------|----------------------------------------------------------------|--------------------|----------|------------------------------|
| Pre-Treat    | 0.94 g | Tris(hydroxymethyl)aminomethane                                |                    |          | Add to sample container      |
| Pre-Treat    | 660 µL | Concentrated Hydrochloric Acid                                 |                    |          | Add to sample container      |
| Condition    | 15 mL  | Methanol                                                       | 15                 | Waste    | Do not let the column go dry |
| Equilibrate  | 18 mL  | Reagent Water                                                  | 15                 | Waste    | Do not let the column go dry |
| Equilibrate  | 3 mL   | Reagent Water                                                  | 0                  |          |                              |
| Load         | 250 mL | Sample                                                         | 15                 | Waste    |                              |
| Wash         | 7.5 mL | Reagent Water                                                  | 15                 | Waste    | Rinse the sample container   |
| Wash         | 7.5 mL | Reagent Water                                                  | 15                 | Waste    | Rinse the sample container   |
| Dry          |        | Using air under -10 to -15 in. Hg vacuum for 5 min.            |                    |          |                              |
| Elute        | 4 mL   | Methanol                                                       | Dropwise           | Collect  | Rinse the sample container   |
| Elute        | 4 mL   | Methanol                                                       | Dropwise           | Collect  | Rinse the sample container   |
| Evaporate    |        | Bring to dryness. See concentration procedure.                 |                    |          |                              |
| Reconstitute | 990 µL | Methanol/Water (96/4, v/v)                                     |                    |          |                              |
| Analyze      | 10 µL  | Add internal standard solutions, mix, and analyze on LC-MS/MS. |                    |          |                              |

## Procedure for EPA 533

| Step         | Amount  | Reagent / Description                                                          | Flow Rate (mL/min) | Elute To | Notes                        |
|--------------|---------|--------------------------------------------------------------------------------|--------------------|----------|------------------------------|
| Pre-Treat    | Varies  | Glacial Acetic Acid                                                            |                    |          | Adjust pH to 7.0 ± 1         |
| Condition    | 10 mL   | Methanol                                                                       | 10                 | Waste    | Do not let the column go dry |
| Condition    | 10 mL   | 0.1 M Phosphate Buffer                                                         | 10                 | Waste    | Do not let the column go dry |
| Equilibrate  | 3 mL    | 0.1 M Phosphate Buffer                                                         | 10                 |          | Add directly to the columns  |
| Equilibrate  | 3 mL    | Reagent Water                                                                  | 10                 |          | Add directly to the columns  |
| Load         | 250 mL  | Sample                                                                         | 5                  | Waste    |                              |
| Wash         | 10 mL   | 1 g/L NH <sub>4</sub> OAc in Reagent Water                                     | 5                  | Waste    | Rinse the sample container   |
| Wash         | 1 mL    | Methanol                                                                       | 5                  | Waste    | Rinse the sample container   |
| Dry          |         | Using air under -15 to -20 in. Hg vacuum for 5 min. (approximately 25 mL/min). |                    |          |                              |
| Elute        | 5 mL    | NH <sub>4</sub> OH/Methanol (2%, v/v)*                                         | Dropwise           | Collect  | Rinse the sample container   |
| Elute        | 5 mL    | NH <sub>4</sub> OH/Methanol (2%, v/v)*                                         | Dropwise           | Collect  | Rinse the sample container   |
| Evaporate    |         | Bring to dryness. See concentration procedure.                                 |                    |          |                              |
| Reconstitute | 0.99 mL | 80:20 Methanol/Water (v/v)                                                     |                    |          |                              |
| Analyze      |         | Add isotope performance standards and analyze on LC-MS/MS.                     |                    |          |                              |

\*This solution should be made fresh daily.

## Concentration Procedure

| Parameter              | Setting                           |
|------------------------|-----------------------------------|
| Inlet Pressure Setting | 6–9 bar (0.6–0.9 MPa; 87–130 psi) |
| Bath Temperature       | 60 °C                             |
| Evaporation Mode       | Method (Ramp Gradient)            |
| Manifold Setup         | 48 Positions                      |
| Rack Row Position      | 120 mm                            |
| Step 1                 | 2.5 L/min for 15 min              |
| Step 2                 | 3.0 L/min for 15 min              |
| Step 3                 | 3.5 L/min for 45 min              |

For even more information on the procedure above, visit [Biotage.com](https://www.biotage.com) for the full application note.



## Ordering Information

| Part Number                              | Description                                                        | Quantity |
|------------------------------------------|--------------------------------------------------------------------|----------|
| <b>Extraction System and Accessories</b> |                                                                    |          |
| <b>121-2015ML</b>                        | Biotage® VacMaster™ 20 Sample Processing Station (With 15 mL 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>120-1111</b>                          | ISOLUTE® Column Adapters (PFAS) 1, 3, 6 mL Columns                 | 10       |
| <b>121-2195</b>                          | Biotage® VacMaster™ Trap Kit, 10 L                                 | 1        |
| <b>Concentration</b>                     |                                                                    |          |
| <b>415000</b>                            | TurboVap® LV Automated Solvent Evaporation System                  | 1        |
| <b>414964</b>                            | TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes)             | 1        |
| <b>352281SP</b>                          | Pressure Regulator                                                 | 1        |
| <b>PFAS Consumables</b>                  |                                                                    |          |
| <b>19-6615</b>                           | Bulk 15 mL Tubes With Leak Proof Screw Caps                        | 100      |
| <b>101-0050-C</b>                        | ISOLUTE® 101 SPE Columns, 500 mg/6 mL (For EPA 537.1)              | 30       |
| <b>604-0020-C-533</b>                    | EVOLUTE® PFAS 533 200 mg/6 mL (For EPA 533)                        | 30       |
| <b>604-0050-C-533</b>                    | EVOLUTE® PFAS 533 500 mg/6 mL (For EPA 533)                        | 30       |



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