

Preparing the Biotage® Extrahera™ HV-5000 for PFAS analysis: Cleaning procedure

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large group of man-made chemicals that have been widely used for many years in industrial processes and everyday products. Their resistance to heat, water, and oil makes them useful in applications such as firefighting foams, non-stick cookware, stain-resistant textiles, and food packaging.

However, these same properties also make PFAS very persistent in the environment. They can accumulate over time and may pose risks to human health and ecosystems, which has led to increasing regulatory and scientific attention.

As a result, accurate detection and measurement of PFAS in environmental, biological, and food samples is now a key focus for analytical laboratories. One of the main challenges is contamination from background sources. PFAS can be introduced from laboratory materials such as tubing, valves, and solvents, as well as from the surrounding environment.

Even very small amounts of contamination can affect results, leading to false positives, unstable baselines, or high blank values, and ultimately reducing confidence in the data.

Given that PFAS are often present at very low levels, typically in the low parts-per-trillion (ppt) or even parts-per-quadrillion (ppq) range, careful sample preparation is critical to ensuring the reliability and accuracy of analytical results.

Therefore, necessary precautions should be taken, and adequate cleaning procedures for consumables and instrumentation are required. Automated solid-phase extraction (SPE) platforms, such as the Biotage® Extrahera™ HV-5000, are widely adopted to automate and standardise the preparation of complex sample matrices prior to analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The Biotage® Extrahera™ HV-5000 PFAS cleaning procedure

To ensure reliable PFAS quantitation, the Biotage® Extrahera™ HV-5000 system must undergo a thorough decontamination procedure prior to use. The following protocol provides a comprehensive cleaning process, including solvent bottles, reservoirs, tubing, and pumps, as well as pipette tips. Each part is designed to mitigate the risk of background contamination while maintaining system performance. The steps outlined below are provided as general recommendations and have not been formally validated as a standardised method. Users should assess and adapt the procedure based on their specific application and laboratory requirements.

Solvent bottles

The selection of solvent containers is a critical first step in minimising PFAS contamination; only PFAS-free solvent bottles should be employed. Prior to use, all solvent bottles should be rinsed thoroughly. This is done by adding 50 – 100 mL of high-purity methanol, capping the container, shaking vigorously for 5 – 10 seconds, and discarding the rinse. The procedure is then repeated in triplicate to ensure effective decontamination.

Solvent reservoirs

Solvent reservoirs represent another potential source of contamination and should, where possible, be dedicated or newly procured for PFAS applications. If reuse is unavoidable, reservoirs should be cleaned by filling to approximately 50 % of capacity with methanol and agitated to maximise contact with the surface, ensuring full coverage. The rinse is then discarded and the procedure repeated three times.



Tubing & pumps

Given the high surface area and direct solvent contact, the Biotage® Extrahera™ HV-5000 tubing and pump system also needs particular attention. For effective cleaning, five PFAS-free solvent bottles, each containing at least 150 mL of methanol, are prepared and connected to the corresponding pumps. Clean reservoirs are placed in position 5 of the system. Using 'Maintenance' → 'Flush Solvent Inlets', each pump is flushed with 35 mL of methanol. The solvent is then discarded and the process is repeated three times. This ensures that residual PFAS, as well as potential carryover from previous use, is effectively purged.

5000 µL tips on Biotage® Extrahera™ HV-5000

The PFAS background of Biotage® Extrahera™ 5000 µL tips can vary between manufacturing batches, potentially introducing low-level contamination into the analytical workflow. To mitigate this, it is recommended as best practice to perform a dedicated cleaning cycle prior to first use.

The exact method settings required to perform the following tip-cleaning procedure are provided in the section "Extrahera™ HV-5000 Settings" below, in order to facilitate the implementation of the PFAS Tip Cleaning Method.

Method overview

1. Tip loading

Fill positions 1 and 2 of the deck completely with 5000 µL tips.

2. Solvent configuration

Fill 3 PFAS-free solvent bottles with at least 300 mL methanol and connect to solvent pumps S1, S2 and S3. Navigate to Maintenance → Flush Solvent Inlets, then:

- Assign S1 = PFAS Cleaning Solvent 1
- Assign S2 = PFAS Cleaning Solvent 2
- Assign S3 = PFAS Cleaning Solvent 3

3. Running the 'PFAS Tip Cleaning' method

Only solvent tips 1–3 are required for the method. A flow-through 24-well plate may be placed in position 3 to act as the extraction medium. The number of rows selected corresponds to the number of wash cycles per tip; it is recommended to perform a minimum of two and a maximum of four washes per tip.



4. Automated cleaning sequence

During execution, the system will:

- Collect tips from column 1, position 1.
- Aspirate 5 mL of PFAS Cleaning Solvent 1.
- Dispense the solvent into the designated extraction medium.
- Repeat the process for the specified number of wash cycles.
- Return the cleaned tips to their rack, then repeat for columns 2 and 3.

Note: Tip conditioning is intentionally omitted to prevent the first wash from reintroducing contaminants into clean solvent. Minor solvent dripping may occur during the first aliquot; solvent handling performance remains unaffected. It's important to ensure that no samples or extraction media are present to prevent cross-contamination.

5. Rack rotation and continuation

- After completion of columns 1–3, the system will pause.
- Rotate the tip rack in position 1 so that clean tips occupy positions 4–6.
- Resume the method to initiate cleaning of the next set of tips.
- Upon the next pause, exchange the tip rack in position 1 with that in position 2 and resume the procedure.

Repeat the cleaning and rotation steps until all tip racks have been processed.

6. Post-cleaning procedure

Upon completion of the tip-cleaning method:

Remove solvent bottles from the pump connections and flush each pump with approximately 35 mL of air to clear residual solvent. Dispose of remaining methanol in solvent reservoirs following the appropriate laboratory waste-handling protocols.



Extrahera™ HV-5000 Settings

Method for PFAS cleaning protocol of Biotage® Extrahera™ HV-5000.

General sample tab

Method name
PFAS Tip Cleaning

Sample plate/rack
16 x 100 mm Test Tube...

Extraction media
3 mL Column Rack, 24 ...

Pretreatment
☐ Off

Conditioning
☒ On

Equilibration
☒ On

Load
☐ Off

Wash
☒ On

Elution
☒ On

General

Pretreatment

Conditioning (3)

Equilibration (3)

Load

Wash (3)

Elution (3)

Sample type
Aqueous sample

Two solvent tip types?
☐ No

Sample pipette tip type, pos 2
5000 µL Biotage Tip

Solvent pipette tip type, pos 1
5000 µL Biotage Tip

Starting sample volume in plate/rack (µL)
0

Solvent pipette tip type, pos 3

Reuse tips for pretreatment mix and sample load?
☐ No

Method comment

Settings

Sample type	Aqueous sample
Starting sample vol. (µL)	0
Method comment	-

Pretreatment

No. of steps	0
Pause after last step	No

Conditioning tab

Method name
PFAS Tip Cleaning

Sample plate/rack
16 x 100 mm Test Tube...

Extraction media
3 mL Column Rack, 24 ...

Pretreatment
☐ Off

Conditioning
☒ On

Equilibration
☒ On

Load
☐ Off

Wash
☒ On

Elution
☒ On

General

Pretreatment

Conditioning (3)

Equilibration (3)

Load

Wash (3)

Elution (3)

Number of steps
3

Pressure (bar)
0.5

Solvent tips
Pos 1

Dispose solvent tips after each step?
☐ No

1 Solvent
PFAS Clean 1 - Methanol
Volume (µL)
5000
Collect in position
D (W1)
Positive pressure time (s)
2
Advanced pressure settings
Edit...
Repeat (number of times)
1
Pause after this step?
☐ No

2 Solvent
PFAS Clean 2 - Methanol
Volume (µL)
5000
Collect in position
D (W1)
Positive pressure time (s)
0
Advanced pressure settings
Edit...
Repeat (number of times)
1
Pause after this step?
☐ No

3 Solvent
PFAS Clean 3 - Methanol
Volume (µL)
5000
Collect in position
D (W1)
Positive pressure time (s)
0
Advanced pressure settings
Edit...
Repeat (number of times)
1
Pause after this step?
☒ Yes

Conditioning 1, 2 & 3

Positive pressure (bar)	0.5
Positive pressure time (s)	2
Volume	5000
Collect in position	D
Advanced pressure	Yes
Pressure (bar)	0
Positive pressure time (s)	2
Plate dry after 2nd wash (s)	No

Advanced pressure settings tab

(Used for all steps)

< Back

Edit Advanced Pressure Settings

Use advanced pressure settings?
☒ Yes

Number of steps
1

Pressure (bar)
0.0

Positive pressure time (s)
2

Plate dry?
☐ No

Plate dry time (s)
0

Solvent

1	PFAS cleaning solvent 1
2	PFAS cleaning solvent 2
3	PFAS cleaning solvent 3

Spin 'Solvent tips – position 1'



Equilibration tab

Method name: PFAS Tip Cleaning

Sample plate/rack: 16 x 100 mm Test Tube...

Extraction media: 3 mL Column Rack, 24 ...

General | Pretreatment | Conditioning (3) | Equilibration (3) | Load | Wash (3) | Elution (3)

Pretreatment: Off

Conditioning: On

Equilibration: On

Load: Off

Wash: On

Elution: On

1	2	3
Solvent: PFAS Clean 1 - Methanol	Solvent: PFAS Clean 2 - Methanol	Solvent: PFAS Clean 3 - Methanol
Volume (µL): 5000	Volume (µL): 5000	Volume (µL): 5000
Collect in position: D (W1)	Collect in position: D (W1)	Collect in position: D (W1)
Positive pressure time (s): 0	Positive pressure time (s): 0	Positive pressure time (s): 0
Advanced pressure settings: Edit...	Advanced pressure settings: Edit...	Advanced pressure settings: Edit...
Repeat (number of times): 1	Repeat (number of times): 1	Repeat (number of times): 1
Pause after this step?: No	Pause after this step?: No	Pause after this step?: Yes

Swap tip rack in position 1

Equilibration 1, 2 & 3	
Positive pressure (bar)	0.5
Positive pressure time (s)	2
Volume	5000
Collect in position	D
Advanced pressure	Yes
Pressure (bar)	0
Positive pressure time (s)	2
Plate dry after 2nd wash (s)	No

Solvent	
1	PFAS cleaning Solvent 1
2	PFAS cleaning Solvent 2
3	PFAS cleaning Solvent 3

Wash tab

Method name: PFAS Tip Cleaning

Sample plate/rack: 16 x 100 mm Test Tube...

Extraction media: 3 mL Column Rack, 24 ...

General | Pretreatment | Conditioning (3) | Equilibration (3) | Load | Wash (3) | Elution (3)

Pretreatment: Off

Conditioning: On

Equilibration: On

Load: Off

Wash: On

Elution: On

1	2	3
Solvent: PFAS Clean 1 - Methanol	Solvent: PFAS Clean 2 - Methanol	Solvent: PFAS Clean 3 - Methanol
Volume (µL): 5000	Volume (µL): 5000	Volume (µL): 5000
Collect in position: D (W1)	Collect in position: D (W1)	Collect in position: D (W1)
Positive pressure time (s): 0	Positive pressure time (s): 0	Positive pressure time (s): 0
Advanced pressure settings: Edit...	Advanced pressure settings: Edit...	Advanced pressure settings: Edit...
Repeat (number of times): 1	Repeat (number of times): 1	Repeat (number of times): 1
Pause after this step?: No	Pause after this step?: No	Pause after this step?: Yes

Spin 'Solvent tip – position 1'

Wash 1, 2 & 3	
Positive pressure (bar)	0.5
Positive pressure time (s)	2
Volume	5000
Collect in position	D
Advanced pressure	Yes
Pressure (bar)	0
Positive pressure time (s)	2
Plate dry after 2nd wash (s)	No

Solvent	
1	PFAS cleaning solvent 1
2	PFAS cleaning solvent 2
3	PFAS cleaning solvent 3



Elution tab

Method name: PFAS Tip Cleaning

Sample plate/rack: 16 x 100 mm Test Tube...

Extraction media: 3 mL Column Rack, 24 ...

Off

On

Conditioning

On

On

Off

Equilibration

On

On

Off

Load

On

On

Off

Wash

On

On

Off

Elution

On

General

Pretreatment

Conditioning (3)

Equilibration (3)

Load

Wash (3)

Elution (3)

Number of steps: 3

Pressure (bar): 0.5

Plate dry after last elution?: No

Plate dry time (s): 0

Solvent tips: Pos 1

Dispose solvent tips after each step?: No

1

Solvent: PFAS Clean 1 - Methanol

Volume (µL): 5000

Collect in position: D (W1)

Positive pressure time (s): 0

Advanced settings: Edit...

Repeat (number of times): 1

Pause after this step?: No

2

Solvent: PFAS Clean 2 - Methanol

Volume (µL): 5000

Collect in position: A

Positive pressure time (s): 0

Advanced settings: Edit...

Repeat (number of times): 1

Pause after this step?: No

3

Solvent: PFAS Clean 3 - Methanol

Volume (µL): 5000

Collect in position: A

Positive pressure time (s): 0

Advanced settings: Edit...

Repeat (number of times): 1

Pause after this step?: No

Elution 1, 2 & 3

Positive pressure (bar)	0.5
Positive pressure time (s)	2
Volume	5000
Collect in position	D
Advanced pressure	Yes
Pressure (bar)	0
Positive pressure time (s)	2
Plate dry after 2nd wash (s)	No

Solvent	
1	PFAS cleaning solvent 1
2	PFAS cleaning solvent 2
3	PFAS cleaning solvent 3

Flush solvent inlets tab

< Back

Maintenance - Flush Solvent Inlets

	Solvent		Volume (mL)		
S1	PFAS Clean 1 - Methanol	Clear	15	Flush	Stop
S2	PFAS Clean 2 - Methanol	Clear	15	Flush	Stop
S3	PFAS Clean 3 - Methanol	Clear	15	Flush	Stop
S4		Clear	15	Flush	Stop
S5		Clear	15	Flush	Stop

PFAS solvent pipetting characteristics

< Cancel

Edit Solvent - PFAS Clean 1 - Methanol

Save >

General

5000 µL Bi...

Solvent settings used with tip: 5000 µL Biotage Tip

Flow Rates

Aspiration flow rate (mL/min): 50.00

Dispense flow rate (mL/min): 70.00

Aspirate Post Dispense

Aspirate post dispense? Yes

Aspirate post dispense flow rate (mL/min): 7.50

Aspirate post dispense volume (µL): 50

Air Gap

Lower air gap flow rate (mL/min): 1.00

Lower air gap volume (µL): 15

Upper air gap flow rate (mL/min): 10.00

Upper air gap volume (µL): 250

Upper air gap dispense pause (ms): 1000



Solvent	PFAS cleaning solvent 1	PFAS cleaning solvent 2	PFAS cleaning solvent 3
Reservoir type	Refillable	Refillable	Refillable
Capacity			
Aspiration flow rate (mL min ⁻¹)	50	50	50
Dispense flow rate (mL min ⁻¹)	70	70	70
Lower air gap flow rate (mL min ⁻¹)	1	1	1
Lower air gap volume (µL)	15	15	15
Upper air gap flow rate (mL min ⁻¹)	10	10	10
Upper air gap volume (µL)	250	250	250
Upper air gap dispense pause (ms)	1000	1000	1000
Aspirate post dispense	Yes	Yes	Yes
Aspirate post dispense flow rate (mL/min)	7.5	7.5	7.5
Aspirate post dispense volume (µL)	50	50	50
Conditioning times	0	0	0
Conditioning flow rate (mL min ⁻¹)	-	-	-
Chlorinated	No	No	No
Serial dispense	No	No	No



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