Waters™

PFAS Analysis Kit for ACQUITY UPLC Systems

User Guide

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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice (GLP) and consult your organization's standard operating procedures as well as your local requirements for safety.

Safety hazard symbol notice

The  symbol indicates a potential hazard. Consult the documentation for important information about the hazard and the appropriate measures to prevent and control the hazard.

High voltage hazard

Warning: To avoid electric shock, do not remove protective panels from the device. The components within are not user-serviceable.

Power cord replacement hazard

Warning: To avoid electric shock, observe these precautions:

- The power cord functions as the safety disconnect device. Position the equipment so that you can reach the power cord easily.
- Use SVT-type power cords in the United States and HAR-type power cords, or better, in Europe. For requirements elsewhere, contact your local Waters distributor.
- Do not replace power cords with inadequately rated power cords.
- Inspect the power cords for damage and replace them if necessary.
- Power-off and unplug a system module or stand-alone device before performing any maintenance operation on it.

CO_2 hazards to humans

Carbon dioxide exhibits three primary hazards for humans:

- Toxicity, at levels above 10,000 ppm (PEL 5000 ppm)
- Frostbite, from uncontrolled release of pressurized CO_2 to atmosphere, or contact with accumulated dry ice at a leak site
- Asphyxiation, caused by displacement of oxygen

For locations in which CO_2 is used and stored, the United States Occupational Safety and Health Administration (OSHA) has established the permissible exposure limit (PEL). Installing an ambient-air sensor and alarm capable of detecting CO_2 levels to 10,000 ppm and triggering an alarm at 5000 ppm (instead of, or in addition to, installing an oxygen monitor) complies with the PEL. When another asphyxiant, such as N_2, is used or stored in the same room as CO_2, Waters recommends installing an oxygen monitor with both audible and visual alarms in addition to the ambient-air sensor.

Before proceeding with any monitoring safety configuration, consult your environmental health and safety manager regarding applicable local, federal, and international safety regulations and requirements.

Using this kit

When using this kit, follow standard quality control (QC) procedures and the guidelines presented in this section.

Applicable symbols

The following symbols can be present on the device, system, or packaging.

Symbol	Definition
	Manufacturer
	Date of manufacture
	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	UK Conformity Assessed marking confirms that a manufactured product is in conformity with the applicable requirements for products sold within Great Britain
	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Environmentally friendly use period (China RoHS): indicates the number of years from the date of manufacture until the product, or components within the product, are likely to be discarded or degrade into the environment
	Consult instructions for use
	Alternating current

Symbol	Definition
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste. For compliance with Waste Electrical and Electronic Equipment legislation, contact Waters Corporation for the correct disposal and recycling instructions
	For indoor use only
	No pushing
	Do not connect to an LC system
	Indicates the maximum load you can place on that item (for example, 10kg)
	Serial number
	Part number, catalog number
	For in vitro diagnostic use

Audience and purpose

This document is for laboratory personnel. It includes procedures and information for installing, operating, and maintaining the PFAS Analysis Kit for Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC) Systems.

Intended use

Waters designed the PFAS Analysis Kit for ACQUITY UPLC systems to analyze trace amounts of per- and polyfluoroalkyl substances (PFAS) in a variety of environmental, food, and other matrices.

Calibrating

To calibrate liquid chromatography (LC) systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must include the entire range of QC samples, typical specimens, and atypical specimens.

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not an operator's guide, accompanies the instrument, consult the instrument's online Help system for calibration instructions.

Quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report this data until you are certain that the instrument performs satisfactorily.

ISM classification: ISM group 1 class B

This classification was assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instrument requirements.

Group 1 products apply to intentionally generated and/or used conductively coupled radiofrequency energy that is necessary for the internal functioning of the equipment.

Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low-voltage, power-supply network.

This equipment complies with the emission and immunity requirements described in the relevant parts of IEC/EN 61326: Electrical equipment for measurement, control, and laboratory use — EMC requirements.

Table of contents

1 Overview

Use the PFAS Analysis Kit for ACQUITY UPLC systems to quantify trace levels of PFAS in drinking, ground, surface, waste water as well as other environmental, food, and various matrices. The kit minimizes contamination from system components and separates background contaminants from the analytes of interest.

1.1 Purpose

The PFAS Analysis Kit for ACQUITY UPLC systems enables the quantitative analysis of PFAS in a variety of matrices including but not limited to water, soil, food, and other environmental matrices. It is designed to minimize interference from PFAS found in many laboratory instrument components, common HPLC solvents, and laboratory water. The kit contains tubing, fittings, filters, and other components to replace those that can contribute to contamination.

The kit also contains a per-and polyfluoroalkyl substances (PFAS) isolator column that helps isolate the background contaminants from the analytes in a sample. The isolator column is in the flow path between the mixer of the binary solvent manager (BSM) or quaternary solvent manager (QSM) and the injector of the sample manager.

During analysis, the isolator column retains the system PFAS contaminants, separating them from the analytes of interest. The increased retention time of the PFAS system contaminants makes them easily distinguishable by mass analysis.

For examples of TICs and multiple-reaction monitoring (MRM) chromatograms from the analysis of standard PFAS compounds using the PFAS Analysis Kit and method refer to the application note, *ACQUITY UPLC System Solution for Quantifying Trace Levels of Perfluorinated Compounds with an ACQUITY PFC Analysis Kit* (720002813), on www.waters.com.

1.2 Kit contents

Notice: To help minimize PFAS contamination and bacterial growth in the system's solvent supply, follow the recommendations in this guide for appropriate glassware and bottle closures.

The PFAS Analysis Kit includes the items described in the following table. Some elements are described in subsequent sections of this guide. Because there are multiple kits, the contents of the kit vary.

Table 1–1: Contents of the PFAS Analysis Kit

Part	Description
Caps, solvent	High-density polyethylene:

Table 1–1: Contents of the PFAS Analysis Kit (continued)

Part	Description
	• Closed-thread (7 colors) • Open-thread (7 colors) Accessories: • Filters, 10-µm (7) • Liners, inner (7) • Plugs, liner (14)
Cartridges, extraction	One of the following, depending on specific kit ordered, except Base Hardware or Direct Inject Kit: • Oasis **W**eak **A**nion-e**X**change (WAX); 6 cc,150 mg, 30-µm particle size, 30/pk • Oasis WAX; 6 cc, 500 mg, 60-µm particle size, 30/pk • Oasis WAX; 6cc, 500 mg 30-µm particle size, 30/pk • Sep-Pak PS2; 6 cc, 500 mg, 80-µm particle size, 50/pk
Column, analytical	ACQUITY UPLC, Bridged Ethylene Hybrid (BEH), C18, 2.1 × 100 mm OR ACQUITY UPLC CSH Phenyl-Hexyl 130Å 1.7 µm, 2.1 x 100 mm Alternative columns are available on www.waters.com.
Kit, PFAS Analysis Retrofit	**See also:** Section PFAS Analysis Retrofit Kit (Page 12).
Reservoirs, solvent	Borosilicate glass: • Clear, 1000 mL (4)
Standards, PFAS	**See also:** Section PFAS standards (Page 12). Not included in all kits!
Vials (with polyethylene, septumless caps)	Polypropylene, screw-neck, 700 µL, 12 × 32 mm (pack of 100). Not included in all kits!

1.3 PFAS Analysis Retrofit Kit

Use the PFAS Analysis Base Hardware Kit for UPLC systems to install a complete flow path suitable for the trace analysis of PFAS.

Table 1–2: Key contents of the PFAS Analysis Base Hardware Kit

Part	Description
Isolator Column, Atlantis Premier BEH C18 AX	2.1 × 50 mm, 5 µm
Assembly, Extension Loop	50 µL
Tool, nut extender	Flangeless, short
Tubing assembly, column-to-detector	Polyetheretherketone (PEEK)
Tubing assembly, solvent inlet	PEEK (set of 7)
Tubing assembly, valve-to-isolator column	Coiled, stainless steel

1.4 PFAS standards

These are analytical standards you can use for calibration and to manually prepare your quality control.

Refer to Table A, "PFAC30PAR; Components and Concentrations", on page 3 of the *Wellington Laborites Certificate of Analysis Documentation for PFAC30PAR* document included with the PFAS Analysis Kit [1].

1.5 Compatible ACQUITY UPLC System configurations

The PFAS Analysis Kit is compatible with a variety of ACQUITY UPLC Systems (H-Class, I-Class, Plus, or Premier versions) and comprises the following components:

Note: This kit does not support the ACQUITY UPLC column manager or detectors with Teflon flow cells, such as the ACQUITY UPLC photodiode array (PDA) or TUV detectors.

- BSM, with or without *i*[2] *Valves*
- Column heater or high-temperature column heater
- Sample manager
- Sample organizer (optional)

[1] P/Ns of the PFAS Analysis Kit that include PFASC30PAR: 176004548, 176004549, 176004550, 176004554, 176001767

- Tandem Quadrupole (TQ) mass spectrometer (MS) (TQ mass spectrometer including or like the Xevo TQ-S micro, Xevo TQ-XS, or Xevo TQ Absolute)

1.6 Software control

The PFAS Analysis Kit was developed on ACQUITY UPLC Systems using MassLynx software, but its use is not dependent on MassLynx or any other software.

2 Setting up the system hardware

Before installing the PFAS Analysis Kit on an existing system, you must first remove some components. Installing the kit establishes a complete flow path for analyzing PFAS-containing samples while minimizing interference from background contamination.

Notice: To avoid damaging the kit hardware, use the PFAS Analysis Kit only for applications involving the analysis of PFAS.

Requirement: Verify that the standard ACQUITY UPLC installation procedure is complete before installing the kit on a new system.

See also: The *ACQUITY UPLC System Operator's Guide* (71500082502) for basic ACQUITY hardware installation procedures.

Table 2–1: Where to find required materials

Location	Material
Kit, ACQUITY UPLC Startup	• 1/4-inch open-end wrench • 5/8-inch open-end wrench
Kit, PFAS Analysis	• Column, isolator • Tool, nut extender (flangeless) • Tubing assemblies (stainless steel, labeled “Mixer to PFC Isolator” and “Valve to Isolator Column”

2.1 Preparing an existing system

If you are adding the PFAS Analysis Kit to an existing ACQUITY UPLC system, perform the following procedure before installing the kit components. The following instructions are for Flow Through Needle (FTN) sample managers. Specific port numbers may change depending on the sample manager used.

To prepare an existing system:

1. Using the open-end wrenches, remove the stainless-steel tubing that extends from the mixer outlet of the Solvent Manager (Figure 2–1: Pump outlet connections (Page 15)) to port 5 of the sample manager injector valve (Figure 2–2: Injector valve connections (Page 15)). Remove the fitting in port 6 of the injector valve of the tube connecting to the active preheater.

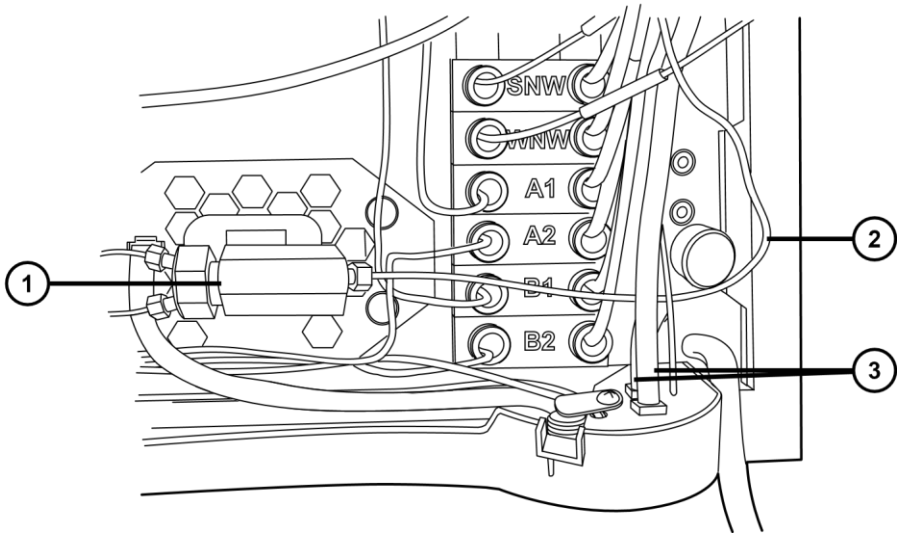
Tips:

- Store the tubing for future use.
- If you need more room to maneuver inside the binary solvent manager, you can remove the ends of the preinstalled Tygon tubing (running from the process waste port) and the PharMed

tubing (running from the needle-clean system waste port) from the pass-through of the binary solvent manager drip tray.

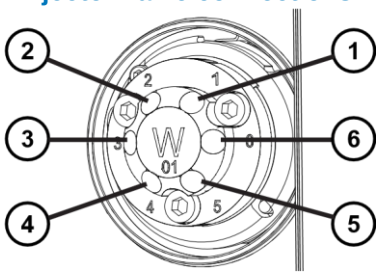
Figure 2–1: Pump outlet connections

Note: Image shown illustrates I-Class system connections. Connections for H-Class systems are labeled identically, with the exception of there being a "Purge/WNW" connection in place of one for "SNW".



1. Mixer
2. Tubing to port 5 of the sample manager injector valve
3. Waste tubing (Tygon and PharMed)

Figure 2–2: Injector valve connections



1. Injection port
2. Waste
3. Sample syringe

4. Needle
5. Valve to isolator column tubing
6. Active preheater connection

2. Using the flangeless-nut extender tool, remove the Teflon solvent tubing from the BSM's A1, B1, A2, B2, strong needle wash (SNW), weak needle wash (WNW), and seal wash (SW) ports.
3. Remove the solvent tubing and solvent bottles from the system and store them for future use.
4. Using the 1/4-inch wrench, remove the tubing that connects the analytical column to the existing detector.
5. Remove from the flow path any detectors employing Teflon flow cells (TUV or PDA, for example). To ensure adequate tubing lengths and distances for the PFAS Analysis Kit components, physically remove these detectors from the ACQUITY stack.

Requirements:

- Do not install any detectors that have flow cells made of Teflon.
- Do not place additional detectors in the ACQUITY stack.

2.2 Installing the solvent reservoirs

Notice: To prevent contamination of solvent reservoirs, heed these measures.

- Always wear particle-free, powder-free, non-latex gloves when handling system components
- Select, prepare, and handle solvents and samples correctly
- For best results, use polypropylene bottles and caps
- Avoid any bottle with a Teflon-lined cap
- Do not wash reservoirs in detergent, alongside other glassware, or in washing facilities that can retain detergent residue; store glassware separately from common-use glassware
- Use clean fittings, tubing, and columns
- Keep laboratory air clean

To install solvent reservoirs, caps, and tubing:

1. From the PFAS Analysis Kit, unwrap the appropriate PEEK solvent tubing assemblies for the BSM’s A1, B1, A2, B2, SNW, WNW, and SW ports.

   Tip: ACQUITY I-Class tubing is pre-labeled, additional labels are included for H-Class tubing.

   Figure 2–3: PEEK solvent tubing assembly, example labels

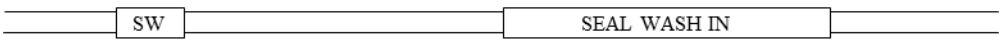


Table 2–2: Tubing assembly labels and colors

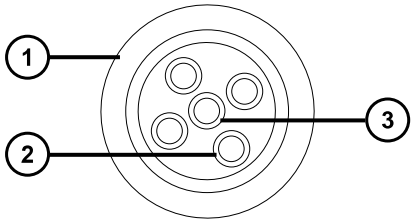
Solvent type	Label [a]	Color
Strong needle wash	SNW	White
Weak needle wash	WNW	Orange
Solvent A1	A1	Yellow
Solvent A2	A2	Blue
Solvent B1	B1	Red
Solvent B2	B2	Green
Seal wash	SW	Brown

a. For kit installations on H-Class systems, use additional labels supplied for that instrument.

Recommendation: Match the cap color to the color on the tubing labels and the degasser port.

2. Install the bottle cap and solvent filter on one end of the PEEK tubing as follows:
    a. Unwind one end of the PEEK tubing, and then slide the outer cap onto it with the threads facing toward the end of the tubing.
    b. Slide the end of the tubing through an open hole in the inner liner.

Figure 2–4: Inner bottle cap liner



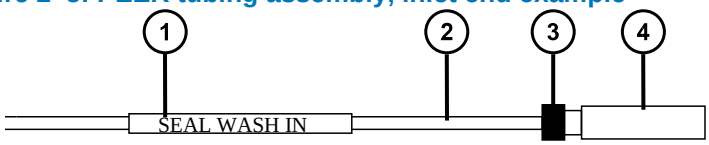
1 Inner liner

2 Open hole in inner liner

3 Center port with 10-µm filter attached

    c. Insert the solvent filter fitting and then the solvent filter onto the end of the tubing.

Figure 2–5: PEEK tubing assembly, inlet end example



1. Label
2. PEEK tubing
3. Inlet filter fitting
4. Inlet (solvent) filter

d. Push the tubing all the way into the solvent filter, and then finger-tighten the fitting.

e. If other solvent lines require the same solvent, repeat steps a through d.

f. Insert plugs into the remaining open ports on the inner liner.

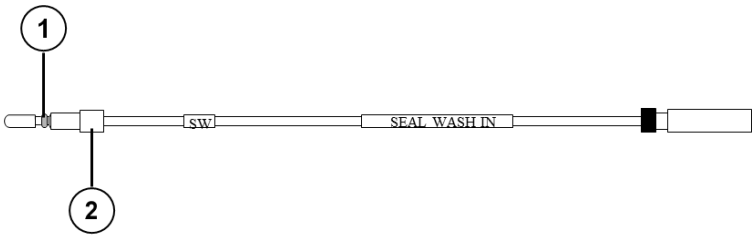
3. Install the tubing in the appropriate degasser port as follows:

   a. Install the compression screw on the degasser end of the tubing assembly.

   b. Slide the lock ring and ferrule onto the tubing, with the metal end nearest the compression screw.

   Tip: Finger-tighten the compression screw, and then use the flangeless nut extender tool to tighten it an additional 1/4-turn.

Figure 2–6: PEEK tubing assembly, degasser end example



1. Locking ring and ferrule
2. PEEK compression screw

4. If the reservoir has a cap and plastic ring around its neck, remove them.
5. Rinse the reservoir with organic solvent, MS-grade water, and, finally, with the intended solvent.

   Tip: If more aggressive cleaning is required, see section Thorough cleaning of solvent reservoirs (Page 38).

6. Fill the reservoir with solvent.

   See: Section Preparing eluents and wash solvents (Page 25).

7. Insert the solvent filter and tubing in the reservoir.

   Requirement: Ensure that the entire filter is submerged in the solvent.

8. Fit the inner liner in the outer cap and tighten the reservoir's cap.
9. Place the reservoir in the solvent tray.

2.3 Installing the columns and tubing

To install the columns and tubing:

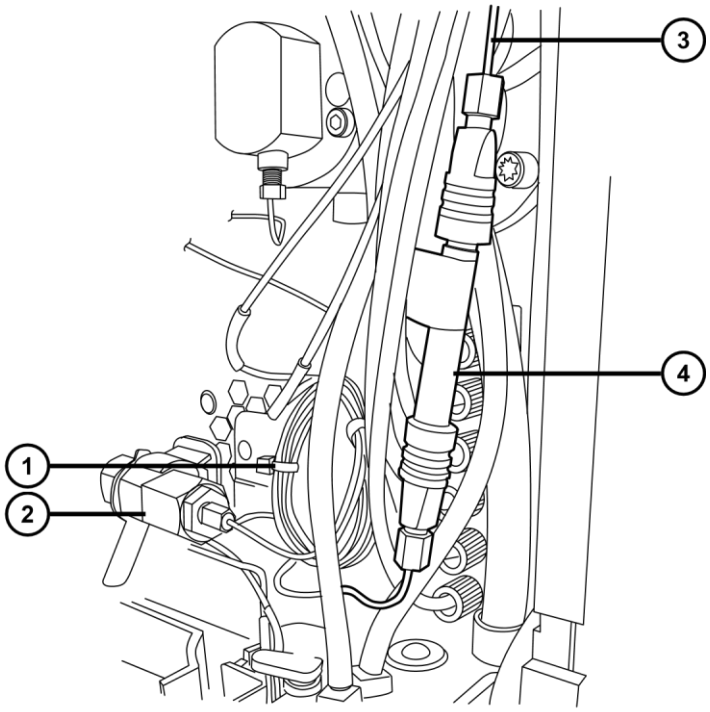
1. In the PFAS Analysis Kit, locate the 50-µL extension loop and two stainless steel tubing assemblies labeled *Mixer to PFC Isolator* and *Valve to Isolator Column*.

Requirement: Remove the black, protective O-rings from the stainless steel tubings before installing them.

2. Using the 5/8-inch and 1/4-inch wrenches, attach one end of the coiled, stainless steel tubing assembly labeled *Mixer to PFC Isolator* to the inlet of the PFAS isolator column provided with the PFAS Analysis Kit.
3. Attach the remaining stainless steel tubing assembly, labeled *Valve to Isolator Column*, to the PFAS isolator column outlet.

   Note: The following figure shows an example of I-Class system connections (the order of connections for H-Class systems is similar).

Figure 2–7: BSM connections

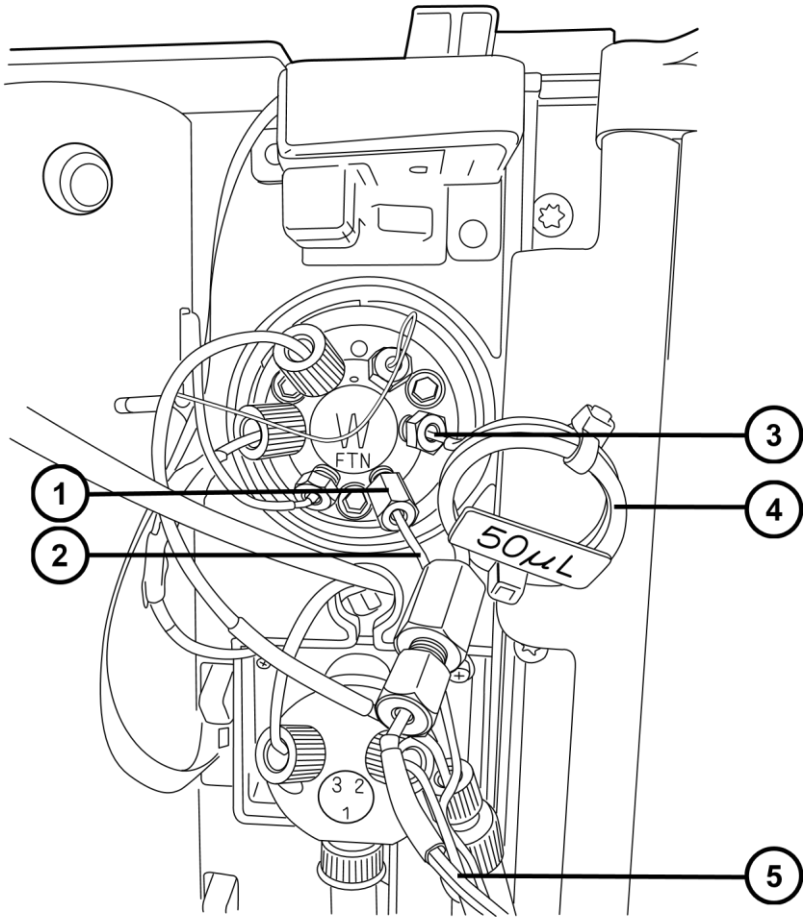


1. Coiled tubing, Mixer to PFAS Isolator
2. Mixer
3. Tubing, Valve to Isolator Column
4. PFAS isolator column

4. Attach the free end of the coiled tubing assembly to the mixer outlet port and attach the free end of the *Valve to Isolator Column* tubing assembly to port 5 of the injector valve. Orient the tubing as shown in the Figure 2–8: Injector valve connections (Page 21).

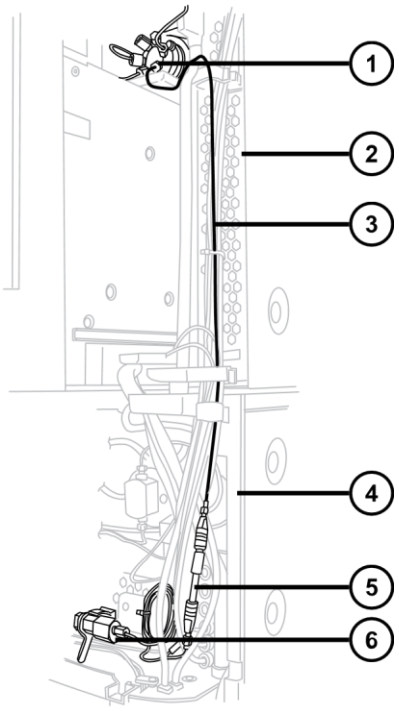
Requirement: Place the ends of the Tygon tubing (running from the process waste port) and the PharMed tubing (running from the needle clean system waste port) in the passthrough of the binary solvent manager drip tray, if you removed them to install the PFAS Analysis Kit.

Figure 2–8: Injector valve connections



1. Port 5
2. Tubing, Valve to Isolator Column
3. Port 6
4. 50-µL extension loop
5. Tube to active preheater

Figure 2–9: Flow path through PFAS isolator



1. Injector valve port 5
2. Sample Manager
3. Solvent flow path
4. BSM
5. PFAS isolator column
6. Mixer

5. Attach the 50-µL extension loop to port 6 of the sample manager injection valve. Attach the active preheater tubing to the fitting on the 50-µL extension loop.
6. Install the analytical column in the column compartment.
7. Using the 1/4-inch wrench, connect the PEEK column-to-detector tubing assembly to the outlet of the analytical column, routing it to a waste container until the flushing procedure is completed.

See also: Section Flushing the system (Page 24).

2.4 Removing the PFAS analysis components

You must remove the PFAS analysis components to resume analyzing compounds other than PFAS.

To remove the PFAS analysis components:

1. Disconnect the end of the "Valve to Isolator column" tubing from injector valve port 5 of the sample manager.
2. Disconnect the end of the coiled "Mixer to PFC isolator" tubing from the solvent manager mixer.
3. Remove the PFAS isolator with the stainless-steel tubing from the system.

   Tip: The PEEK tubing can remain.
4. Reinstall the original stainless-steel tubing connecting the mixer and the injector.
5. Check for leaks.
6. Verify system performance.

   See also: The *ACQUITY UPLC System Operator's Guide* (71500082502) for detailed instructions on verifying system performance.
7. Store the PFAS analysis components for future use.

3 Preparing the system for analysis

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Notice: To avoid damaging the kit hardware, use the PFAS Analysis Kit only for applications involving the analysis of PFAS.

To minimize background PFAS contamination, clean the system thoroughly before performing an analysis. Carefully prepare eluents and wash solvents in the glassware provided with the kit.

3.1 Flushing the system

Notice: To remove background contamination, flush both existing and newly installed systems thoroughly before performing a PFAS analysis.

Notice: To avoid contamination, use MS-grade (or better) solvents and 18-MΩ-cm resistivity water for PFAS analysis. Purify the solvents by solid-phase extraction (SPE), if necessary.

Before performing a PFAS analysis, flush the ACQUITY UPLC system thoroughly to remove background contamination that can interfere with the analysis. Flushing is especially important when the system has been used for other types of analyses and it is also required for newly installed systems.

To flush the system:

1. After installing the PFAS Analysis Kit components, prepare the solvents as follows:
    a. Place all solvent lines, except the seal wash, in a reservoir containing four liters of100% MS-grade methanol.
    b. Prepare a solution of 10% methanol by mixing 100 mL of methanol with 900 mL of deionized water in a solvent reservoir, and then place the seal wash line in the 10% methanol reservoir.
    c. Verify that the column outlet tubing is routed to a waste container.
2. Open the MassLynx application and log on.

    See also: MassLynx user documentation for instructions on how to complete basic software tasks.
3. On the MassLynx toolbar, click **Instrument** > **MS Console**.
4. In the ACQUITY UPLC System tree, click **Binary Solvent Manager** > **Control** > **Prime seal wash** > **Yes**.

    Result: Seal wash priming begins and continues for 15.0 min.

5. In the ACQUITY UPLC System instrument tree, click **Sample Manager**, and then set the column temperature to **50 °C**.
6. In the ACQUITY UPLC System instrument tree, click **Binary Solvent Manager**.
7. In the Binary Solvent Manager window, prime the solvents A1 and B1 as follows:
    a. Click **Control** > **Prime A/B solvents**.
    b. Select solvent lines **A1** and **B1** for **3 minutes**, and then click **OK**.
    c. Click **Control** > **Set flow**.
    d. Specify a flow rate of 0.4 mL/min for 50% A1 and 50% B1, and then click the green check mark.
8. After four hours, repeat step 6 and step 7 for the A2 and B2 solvent lines.
9. While the system is flushing, during step 6 through step 8, prime the syringes as follows:
    a. Click **Sample Manager** > **Control** > **Prime syringes**.
    b. Select **Sample syringe and wash syringes**, type 99 for the number of cycles, and then click **OK**.
    c. Allow approximately three hours.
10. After the syringe priming cycles complete, wash the needle as follows:
    a. Click **Control** > **Wash needle**.
    b. Type 500 for both SW and WW, and then click **OK**.
    c. Repeat nine times.
11. When the flushing procedure is complete, connect the Column to Detector tubing (attached to the analytical column outlet) to the MS.
12. Run five blank injections before running an analytical sample.

3.2 Preparing eluents and wash solvents

Notice: To avoid contamination, use MS-grade (or better) solvents and 18-MΩ-cm resistivity water for PFAS analysis. Purify the solvents by solid-phase extraction (SPE), if necessary.

Success with the PFAS Analysis Kit requires specific eluents and wash solvents. Use appropriate bottles and closures to minimize contamination and bacterial growth. Carefully prepare the wash solvents and eluents, as described in the following tables, in the appropriate glassware.

Table 3–1: Wash solvents for PFAS analyses on the ACQUITY UPLC system

Solvent line	Solvent
SNW	1 L of 90:10 methanol/water

WNW	1 L of 50:50 water/methanol
SW	1 L of 50:50 water/methanol (use 5-minute washes; can be placed in WNW reservoir)

Table 3–2: Eluents for PFAS analyses on the ACQUITY UPLC system

Solvent line	Eluent
A1	1 L of 2-mM ammonium acetate in water
B1	1 L of 2-mM ammonium acetate in methanol
A2	Same as A1 (can place solvent line in A1 reservoir)
B2	Same as B1 (can place solvent line in B1 reservoir)

3.2.1 Preparing the solvent for line A1

Required materials

- 1-L class A graduated measuring cylinder with cap (including plugs for mixing)
- 1-L reservoir bottle
- 18-MΩ-cm resistivity water
- Ammonium acetate
- Calibrated analytical balance
- LC-MS-grade methanol

To prepare the solvent for line A1:

1. Weigh 0.1542 g of ammonium acetate on an analytical balance, and then transfer it to a 1L solvent reservoir.
2. Using a graduated cylinder, add 1000 mL of water to the reservoir.
3. Cap the bottle and plug any holes to prevent leaks.
4. Shake the solvent bottle to mix the contents.

3.2.2 Preparing the solvent for line B1

Required materials

- 1-L class A graduated measuring cylinder with cap (including plugs for mixing)
- 1-L reservoir bottle
- Ammonium acetate
- Calibrated analytical balance

- LC-MS-grade methanol

To prepare the solvent for line B1:

1. Weigh 0.1542 g of ammonium acetate on an analytical balance, and then transfer to a 1-L solvent reservoir.
2. Using a graduated cylinder, add 1000 mL of methanol to the reservoir.
3. Cap the bottle and plug the holes to prevent leaks.
4. Shake the solvent bottle to mix the contents.

4 Verifying system performance

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

By running a standard mixture of PFAS on a system equipped with the PFAS Analysis Kit, you verify that the system is performing correctly. This chapter assumes you are using a Waters TQ detector MS.

See also: The MS user documentation for specific information on using it with the PFAS Analysis Kit.

4.1 Preparing the standards

Required materials

- 18-MΩ-cm resistivity water
- LC-MS-grade methanol
- PFAS calibration standards from the analysis kit

See also: Section PFAS standards (Page 12).

- Pipettor, with polypropylene tips, capacity 5 to 1000 µL
- Vials, polypropylene, screw-neck, 12 × 32-mm, 700-uL, with polyethylene, septumless caps

To prepare the verification standard and blanks:

1. Prepare a 50:50 water:methanol solution by mixing 2 mL of water and 2 mL of methanol. Label this solution Diluent.
2. Using a pipettor, transfer 50 µL of PFAS calibration standard to a 700-µL polypropylene vial.
3. Add 450 µL of methanol to the vial so that the concentration of the resulting mixture is 100 ng/mL. Label this vial PFAS stock 1.
4. In a new 700 µL polypropylene vial, pipette 395 µL of Diluent, and then add 5 µL of PFAS stock 1. Label this sample Test solution #1.
5. Pipette 500 µL of Diluent into a new polypropylene vial and label as Blank.

4.2 Performing the verification test

Requirement: Before you run a test to verify whether the system is operating properly for PFAS analysis, ensure that the system is properly flushed by following the instructions in section Flushing the system (Page 24).

When flushing of the system is complete, run a verification test with the standards prepared as described in section PFAS standards (Page 12).

4.2.1 Preparing the chromatography method and running samples

Recommendations:

- Set up a refined MS method, with multiple MRM time windows, allowing more time to scan target MRM transitions at the target PFAS peak, which yields better signal-to-noise ratios and peak detection.
- To achieve optimal sensitivity for each PFAS analyte, use IntelliStart's Develop Method functions, which fine-tune the MRM transitions, cone voltage, and collision energy for each perfluorinated compound.

  See also: The MassLynx or waters_connect for Quantitation online Help and any of the Xevo mass spectrometer Quick Reference Cards (for example, Xevo TQ Absolute Quick Reference Card 715007385) or Overview Guides (for example, Xevo TQ Absolute Guide 715007384).
- At system startup, and before each run, monitor the level of background PFAS contamination, and verify the performance of the isolator column by running five blank injections and a PFAS standard.

To prepare the chromatography method and run samples (blank and PFAS standard):

1. Place the blank sample vial in the sample tray position 2:1 and the "Test solution #1" (1.25 ng/mL PFAS standard) sample vial in tray position 2:2.
2. Create an inlet method as described in the following table:

   Table 4–1: Channel settings for MRM of 28 mass pairs page

Component	Precursor	Fragment	Dwell	Cone (V)	CE (V)
PFBA	212.9	169	Auto	10	10
PFPeA	262.9	219	Auto	10	5
PFHxA	312.9	269	Auto	5	10
PFHpA	362.9	319	Auto	15	10
PFOA	412.9	369	Auto	10	10
PFNA	462.9	419	Auto	10	10
PFDA	512.9	469	Auto	15	10
PFUdA	562.9	519	Auto	25	10

Table 4–1: Channel settings for MRM of 28 mass pairs page (continued)

Component	Precursor	Fragment	Dwell	Cone (V)	CE (V)
PFDoA	612.9	569	Auto	30	10
PFTrDA	662.9	619	Auto	5	10
PFTeDA	712.9	669	Auto	10	15
PFBS	298.9	80.1	Auto	15	30
PFPeS	348.9	80.1	Auto	10	30
PFHxS	398.9	80.1	Auto	10	35
PFHpS	448.9	80.1	Auto	15	35
PFOS	498.9	80.1	Auto	15	40
PFNS	548.9	80.1	Auto	20	40
PFDS	598.9	80.1	Auto	25	40
FOSA	497.9	78.2	Auto	40	30
N-MeFOSAA	569.9	418.9	Auto	35	20
N-EtFOSAA	584	418.8	Auto	15	20
4_2 FTS	326.9	307.0	Auto	15	15
6_2 FTS	426.9	407.0	Auto	15	20
8_2 FTS	526.9	506.8	Auto	15	25
NaDONA	376.9	251.0	Auto	10	10
9Cl-PF3ONS	530.9	350.9	Auto	15	25
11Cl-PF3OUdS	630.9	450.8	Auto	30	30
HFPO-DA	285.0	119.0	Auto	5	35

3. In the MassLynx project inlet file (*PFC_inlet_9min*), verify that the BSM and sample manager instrument methods match the values in the following table:

Table 4–2: UPLC conditions

Parameter	Value
Purge	50:50 water/methanol
Needle wash	90:10 methanol/water (6 sec pre- and postinjection)
SW	50:50 water/methanol (5.0 min)
Column temperature	35.0 °C
Sample temperature	10.0 °C
Injection volume	10 µL

Table 4–2: UPLC conditions (continued)

Parameter	Value
Column	ACQUITY UPLC BEH C18 2.1 x 100 mm
Mobile phase A1	2 mM ammonium acetate in water
Mobile phase B1	2 mM ammonium acetate in methanol
Run time	22.00 min

4. Confirm that you added five blank samples and your verification standard samples to the sample queue.

   Tip: To add a new test sample to the sample queue, click **Samples** > **Add**, and then specify the appropriate sample information. Use optimized MS information.

   Recommendation: At system startup, always run five blank samples before your analysis, to equilibrate the system and monitor the level of background PFAS contamination.

5. Confirm the following selections:

   - MS tune file is *PFC_analysis*
   - Injection volume in the sample list is 10 µL
   - Mobile phases match the solvents in the following table:

Table 4–3: Gradient method

Time (min)	Flow rate (mL/ min)	%A	%B	Curve
0	0.3	95	5	6
1	0.3	75	25	6
6	0.3	50	50	6
13	0.3	15	85	6
14	0.3	5	95	6
17	0.3	5	95	6
18	0.3	95	5	6
22	0.3	95	5	6

6. Prime the A and B pumps for 3.0 min.
7. Start the flow at 0.3 mL/min with 95% solvent A for 3.0 min and inspect all connections and columns for leaks.
8. In the MassLynx window, click **Run**.

4.3 Processing and interpreting the data

The system is performing correctly when the following statements about your processed data are true:

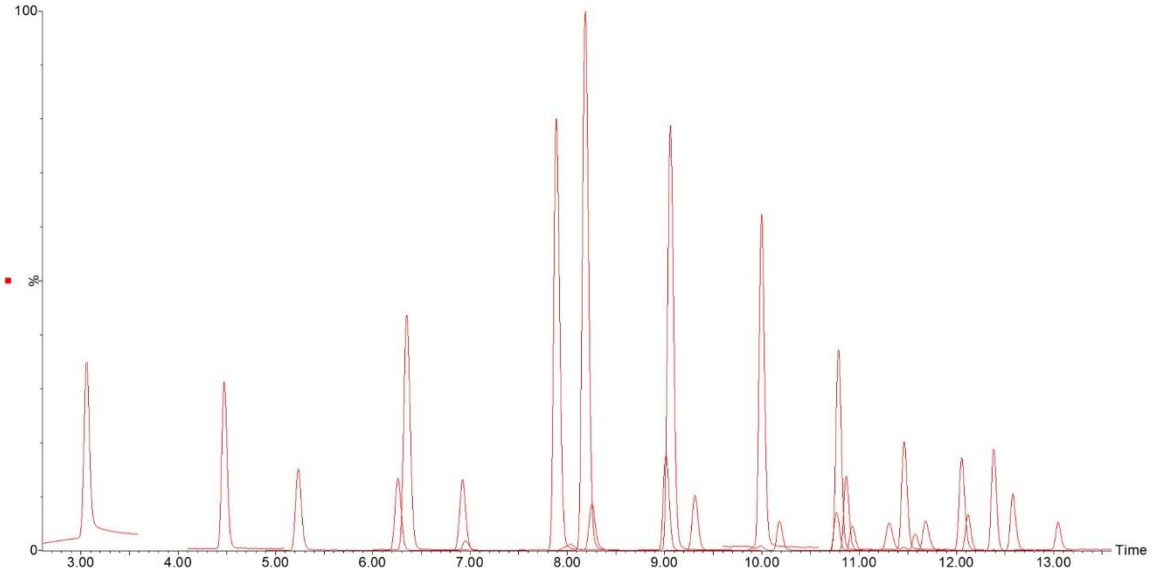
- The retention time difference between the perfluorooctanoic acid (PFOA) analyte peak and the leading PFOA contaminant peak is at least 0.4 min.
- The PFOA and perfluorononanoic acid (PFNA) peaks are baseline-resolved.

To process data from the verification standard:

1. In the MassLynx sample list, click the line number of the standard for which you are processing data.
2. From the sample list menu, click **Chromatogram**.

   Result: The Chromatogram window opens and the TIC for the standard appears.

Figure 4–1: Chromatogram window, full PFAS mix (example)



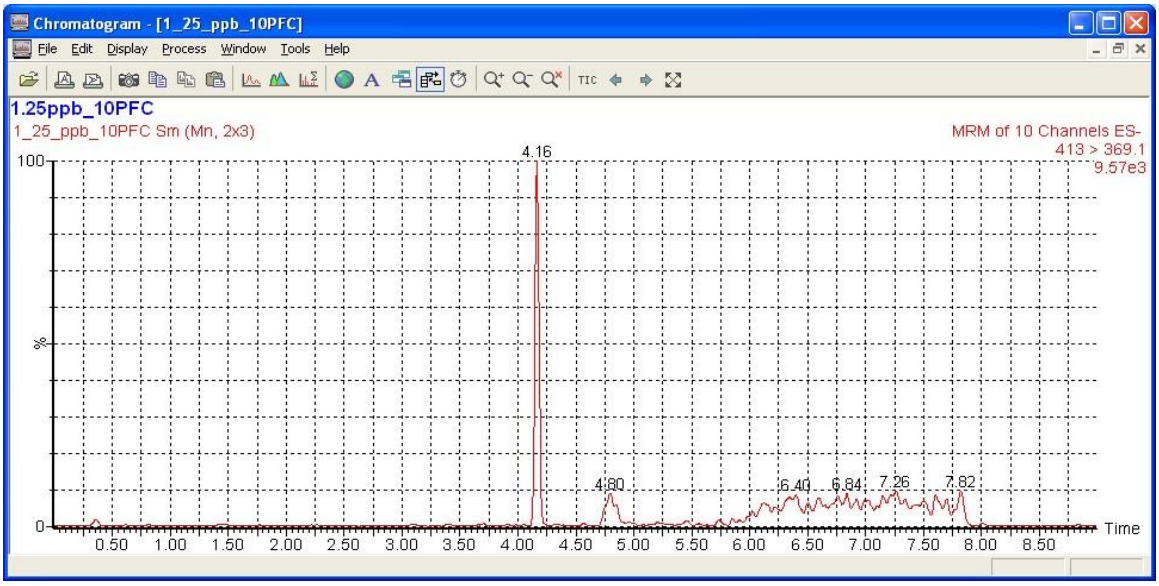
3. Click **Display** > **Mass**, and then, from the Function list, double-click **Mass transition for PFOA (412.0 > 369)**.
4. Click **Add Trace (or Replace trace)** > **OK**.
5. Click **Process** > **Smooth**, and then specify 3 for the window size and 2 for the number of smoothing operations.
6. Click **Mean** > **OK**.

   Result: The chromatogram for PFOA appears.

   Tip: To delete the TIC and the unsmoothed chromatogram, click within the plot area (a colored square appears to the left of the plot), press the Delete key, and then click **OK**.
7. Click **Display** > **View**.

   Result: The Chromatogram window opens.
8. From the Grid menu, select **Dot**, and then click **OK**.

Figure 4–2: Chromatogram window, smoothed PFOA (example)



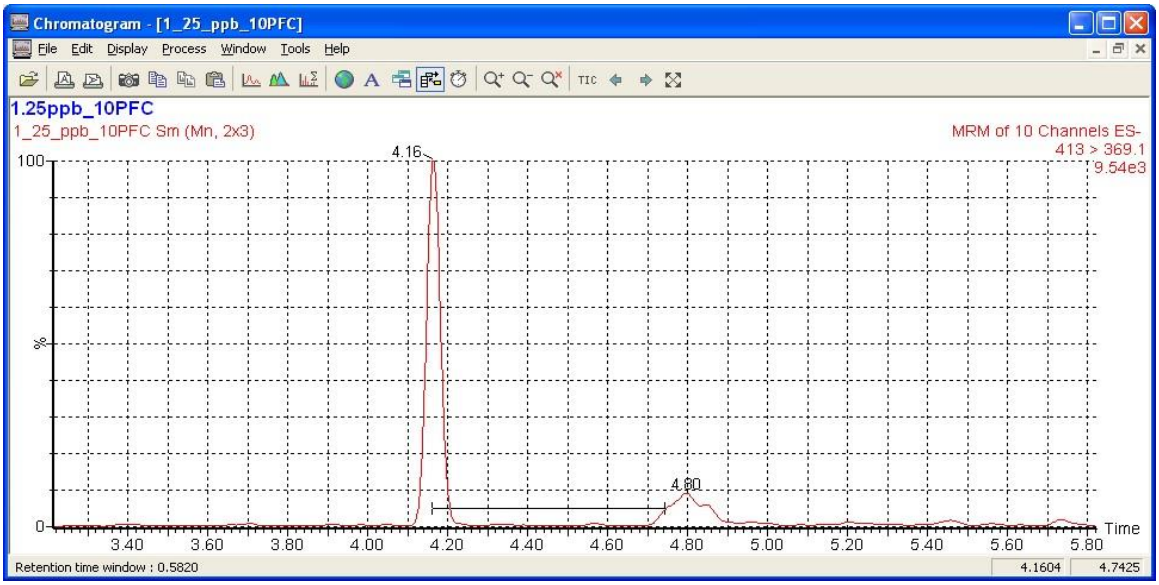
9. Expand the view of the analyte peak and the leading contaminant peaks.

   Note: To draw a box around the peaks of interest, click the top-left corner of the region of interest, and then drag diagonally downward to the axis, without extending the box below the axis.

10. Measure the distance between the apex of the PFOA peak and the half-height of the first contaminant peak.

    Tip: Click and drag a horizontal line between the two points. The retention time difference between the two points appears in the bottom-left corner of the Chromatogram window.

Figure 4–3: Chromatogram window, measuring the difference in retention time (example)



11. Verify that the analytical peak and contamination front are baseline-resolved.

    Tip: Peaks that are not baseline-resolved could indicate a problem.

    See also: Section Troubleshooting PFAS analysis (Page 36).

12. In the Chromatogram window, click **Display > Mass**, and then double-click the **mass transition for PFNA** in the Function list (463 > 419).
13. Click **Add Trace** > **OK**.
14. Click **Process** > **Smooth**, and then specify 3 as the window size and 2 as the number of smooths.
15. Click **Mean** > **OK**.

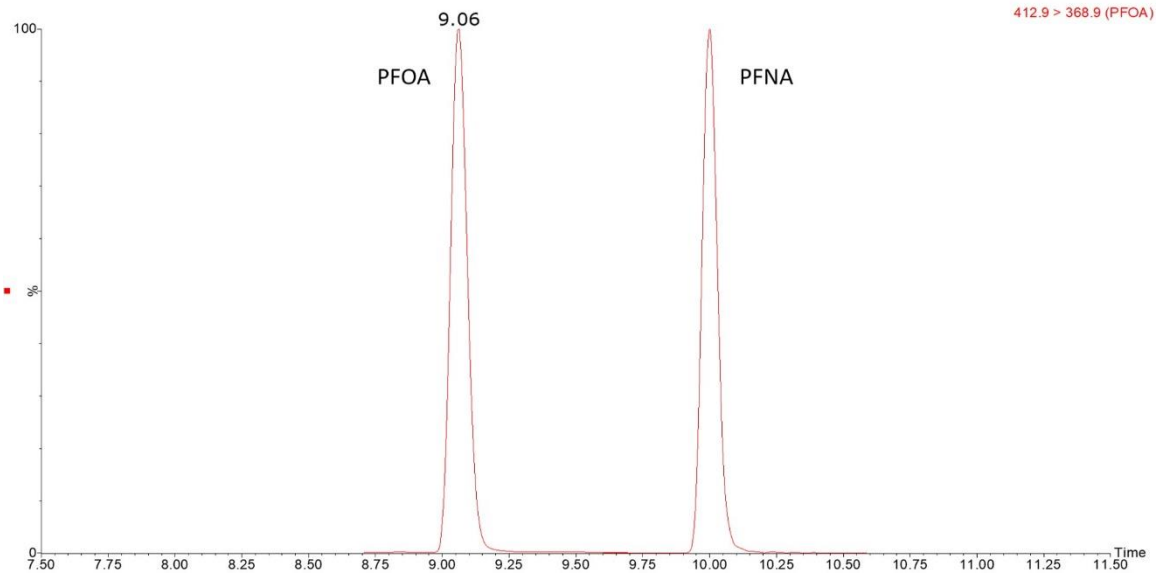
    Result: The smoothed chromatogram for PFNA appears.

    Tip: To delete the unsmoothed PFNA chromatogram, click within the plot area (a colored square appears to the left-hand side of the plot), press the Delete key, and then click **OK**.

16. Click **Display** > **View**.
17. In the Chromatogram window, select **Overlay Graphs**, and then click **OK**.

    Result: The Chromatogram window opens.

    Figure 4–4: Chromatogram window, PFOA and PFNA peaks, 5-minute method (example)



18. Examine the PFOA and PFNA peaks in the plots that you overlaid and verify that they are baseline-resolved.

    Tip: Peaks that are not baseline-resolved can indicate a problem.

    See also: Section Troubleshooting PFAS analysis (Page 36).

5 Troubleshooting

This section provides information about how to solve potential analysis problems.

See also:

- *Controlling Contamination in LC/MS Systems* (715001307)
- For troubleshooting information on your specific system, visit support.waters.com.

5.1 Troubleshooting PFAS analysis

Consult the table below for help solving problems with your analysis.

Table 5–1: Potential analysis problems and probable solutions

Symptom	Possible cause	Solution
Contaminant peaks are larger than expected and are comparable to analyte peaks	System was not sufficiently flushed	Repeat the methanol flushing procedure until the background contamination is at a reasonable level. **See also:** Section Flushing the system (Page 24).
Retention time difference between PFOA (analyte) peak and leading PFOA contaminant peak is less than 0.4 min; analyte peak shapes are not baseline-resolved	Incorrect flow rate	Verify the method. Adjust the flow rate to the correct value.
	Incorrect solvent composition	Verify the method and the solvent supply. Adjust the solvent composition as needed.
	PFAS isolator column is not performing correctly	Replace the PFAS isolator column.
Retention time difference between PFOA (analyte) peak and leading PFOA contaminant peak is less than 0.4 minutes; analyte peak shapes are not baseline-resolved; noticeable broadening of peaks	Analytical column is not performing correctly	Compare current resolution of PFOA and PFNA peaks to the resolution at installation. Verify column efficiency. Replace column if needed.

Table 5–1: Potential analysis problems and probable solutions (continued)

Symptom	Possible cause	Solution
PFOA and PFNA peaks are not baseline-resolved	Incorrect flow rate	Verify the method. Adjust the flow rate to the correct value.
	Incorrect solvent composition	Verify the method and the solvent supply. Adjust the solvent composition as needed.
	Analytical column is not performing correctly	Compare current resolution of PFOA and PFNA peaks to the resolution at installation. Verify column efficiency. Replace column if needed.
PFAS peaks appear in blank runs along with delayed background contaminant peaks	PFAS contamination in injector	**Notice:** To remove background contamination, flush both existing and newly installed systems thoroughly before performing a PFAS analysis. Repeat the flushing procedures specific to the sample manager. Prime the syringes and wash the needle until the peaks no longer appear. **See also:** Section Flushing the system (Page 24).
	PFAS contamination in sample vial	Obtain clean sample vials. For the best results, use polypropylene vials and caps. Avoid any vial with a Teflon-lined cap.
	PFAS contamination in solvents	**Notice:** To avoid contamination, use MS-grade (or better) solvents and 18-MΩcm resistivity water for PFAS analysis. Purify the solvents by solid-phase extraction (SPE), if necessary.

Table 5–1: Potential analysis problems and probable solutions (continued)

Symptom	Possible cause	Solution
PFAS peaks appear in blank runs along with delayed background contaminant peaks		Use higher purity solvents and Waters Oasis MAX or HLB SPE cartridges (if necessary) for your analysis.

5.2 Thorough cleaning of solvent reservoirs

When a container's history is unknown, you must clean it thoroughly.

Recommendations:

- Do not wash reservoirs in detergent, alongside other glassware, or in washing facilities that can retain detergent residue.
- Store glassware separately from common-use glassware.

5.2.1 Cleaning solvent reservoirs

Required materials

LC-MS-grade water

Methanol

MS-grade formic acid (10%)

Nitric acid (highest quality available)

To thoroughly clean solvent reservoirs:

Important: Ensure that organic and nitric acid do not come into contact.

1. Sonicate with MS-quality 10% formic or nitric acid.
2. Sonicate with water.
3. Sonicate with methanol.
4. Sonicate with water.
5. Repeat steps 1 through 4 twice.
6. Rinse with organic solvent and MS-grade water, and then with the solvent that the reservoir will contain.

5.2.2 Cleaning solvent reservoirs contaminated with microbial growth

Required materials

Methanol

To clean solvent reservoirs contaminated with microbial growth:

1. Treat the reservoirs in an autoclave.
2. Remove and replace all filters and tubing between the solvent reservoir and the instrument.
3. Purge the system with methanol and let it stand overnight.

A. Safety advisories

Waters products display safety symbols that identify hazards associated with the product's operation and maintenance. The symbols also appear in product manuals with statements that describe the hazards and advise how to avoid them. This appendix presents all safety symbols and statements that apply to Waters' product offerings. The symbols and statements can apply to a specific product or apply to other products within the same system.

A.1 Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with the misuse of an instrument or device. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appears in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.

Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

Warning: (Risk of burn injury from contacting hot surfaces.)

Warning: (Risk of electric shock.)

Warning: (Risk of fire.)

Warning: (Risk of sharp-point puncture injury.)

Warning: (Risk of hand crush injury.)

Warning: (Risk of injury caused by moving machinery.)

Warning: (Risk of exposure to ultraviolet radiation.)

Warning: (Risk of contacting corrosive substances.)

Warning: (Risk of exposure to a toxic substance.)

Warning: (Risk of personal exposure to laser radiation.)

Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Warning: (Risk of tipping.)

Warning: (Risk of explosion.)

Warning: (Risk of high-pressure gas release.)

A.1.1 Specific warnings

A.1.1.1 Burst warning

This warning applies to Waters instruments and devices fitted with nonmetallic tubing.

Warning: To avoid injury from bursting, nonmetallic tubing, heed these precautions when working in the vicinity of such tubing when it is pressurized:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to compounds with which it is chemically incompatible: tetrahydrofuran, nitric acid, and sulfuric acid, for example.
- Be aware that certain compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.

A.1.1.2 Biohazard warning

The following warning applies to Waters instruments and devices that can process biologically hazardous materials. Biologically hazardous materials are substances that contain biological agents capable of producing harmful effects in humans.

Warning: To avoid infection from blood-borne pathogens, inactivated microorganisms, and other biological materials, assume that all biological fluids that you handle are infectious.

Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Warning: To avoid injury when working with hazardous materials, consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

A.1.1.3 Biohazard and chemical hazard warning

This warning applies to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.

Warning: To avoid personal contamination with biologically hazardous, toxic, or corrosive materials, you must understand the hazards associated with their handling.
Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards*.

To avoid injury when working with hazardous materials, consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials and follow good laboratory practices.

A.2 Notices

Notice advisories appear where an instrument, device, or component can be subject to use or misuse that can damage it or compromise a non-clinical sample's integrity (warning symbols accompany risks to clinical sample integrity). The exclamation point symbol and its associated statement alert you to such risk.

Notice: To avoid damaging the case of the instrument or device, do not clean it with abrasives or solvents.

A.3 Bottles Prohibited symbol

The Bottles Prohibited symbol alerts you to the risk of equipment damage caused by solvent spills.

Prohibited: To avoid equipment damage caused by spilled solvent, do not place reservoir bottles directly atop an instrument or device or on its front ledge. Instead, place the bottles in the bottle tray, which serves as secondary containment in the event of spills.

A.4 Required protection

The Use Eye Protection and Wear Protective Gloves symbols alert you to the requirement for personal protective equipment. Select appropriate protective equipment according to your organization’s standard operating procedures.

Requirement: Use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

A.5 Warnings that apply to all Waters instruments and devices

When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.

Warning: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

Avertissement: Toute modification sur cette unité n’ayant pas été expressément approuvée par l’autorité responsable de la conformité à la réglementation peut annuler le droit de l’utilisateur à exploiter l’équipement.

Warnung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Advertencia: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

警告： 未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

警告： 未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

경고**:** 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

警告**:** 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which reduces the rupture pressure of the tubing.

Avertissement: Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l’instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Warnung: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.

- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

Avvertenza: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Apagar cualquier llama que pudiera haber encendida en las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告： 当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

警告： 當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。

- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

경고**:** 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告**:** 圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

This warning applies to Waters instruments fitted with nonmetallic tubing or operated with flammable solvents.

Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Avertissement: L’utilisateur doit être informé que si le matériel est utilisé d’une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d’être défectueuses.

Warnung: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Avvertenza: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告： 使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。

警告： 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

경고**:** 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告**:** ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

A.6 Warnings that address the replacement of fuses

The following warnings pertain to instruments and devices equipped with user-replaceable fuses. Information describing fuse types and ratings sometimes, but not always, appears on the instrument or device.

Finding fuse types and ratings when that information appears on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.

Avertissement: pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boite à fusible de l'instrument.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.

Avvertenza: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.

Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.

警告： 为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。

警告： 為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。

경고**:** 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告**:** 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Finding fuse types and ratings when that information does not appear on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating indicated in the “Replacing fuses” section of the Maintenance Procedures chapter.

Avertissement: pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués dans la rubrique "Remplacement des fusibles" du chapitre traitant des procédures de maintenance.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.

Avvertenza: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".

Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características indicados en la sección "Sustituir fusibles".

警告： 为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。

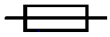
警告： 為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。

경고**:** 화재의 위험을 막으려면 유지관리 절차 단원의 “퓨즈 교체” 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告**:** 火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

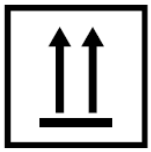
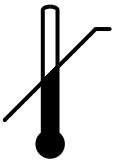
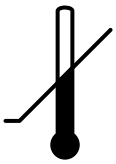
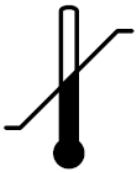
A.7 Electrical symbols

The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument's front or rear panels.

Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Alternating current (three phase)
	Safety ground
	Frame or chassis terminal connection
	Fuse
	Functional ground
	Input
	Output
	Indicates that the device or assembly is susceptible to damage from electrostatic discharge (ESD)

A.8 Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

Symbol	Description
	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation