Agilent

MassHunter PFAS MRM Database (for analysis of Per/Polyfluoroalkyl substances)

Quick Start Guide

What is the MassHunter PFAS MRM Database?

The MassHunter PFAS MRM Database lets you screen 108 native and labeled per- and polyfluoroalkyl substance analytes with up to 4 transitions each with enhanced sensitivity, all in a single LC/MS analysis. The MassHunter PFAS MRM Database and example methods help to minimize development time for your analysis.

The MassHunter PFAS MRM Database contains target compounds for EPA, ASTM, ISO methods, and EU DWD plus emerging PFAS classes methods and regulations.

Working with MassHunter PFAS MRM Database

The MassHunter PFAS MRM Database helps minimize method development time for your PFAS analysis, when used with the Agilent recommended LC/MS configuration and accessories. It stores up to 4 MRM transitions (a precursor and product ion) per compound of the PFAS analytes included in the database, and their optimized fragmentor and collision energy settings from the Agilent 6400 Series Triple Quadrupole LC/MS and Ultivo LC/TQ instruments. Method development can simply be achieved by importing target compounds from the database to the MassHunter Data Acquisition program.

For some compounds in the MassHunter PFAS MRM Database, several transitions are provided. If you want to convert an MRM method to a dMRM or tMRM method, please refer to the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide*.

MassHunter PFAS MRM Database Product Content

MassHunter PFAS MRM Database Quick Start Guide

The Quick Start Guide provides an introduction to the MassHunter PFAS MRM Database.

MassHunter PFAS MRM Database Media

This media contains:

- PFAS MRM database for 6470B, 6495C, and Ultivo LC/TQ
- Example methods for 6470B, 6495C, and Ultivo LC/TQ
- PFAS example data

MassHunter MRM Database Familiarization Media

Download the MassHunter MRM Database Familiarization Media from SubscribeNet or get it from your Agilent representative.

This media contains:

- Checkout Mix example data, database, methods, and reports.
- *MassHunter MRM/dMRM/tMRM Database Familiarization Guide*

Optional Products

PFC-free HPLC Conversion Kit *(optional)*

Your Agilent Infinity HPLC instrument must be set up to remove the interference of the PFAS compounds leaching from the tubings and solvents. Agilent recommends the use of:

- **PFC-Free HPLC Conversion Kit** (p/n 5004-0006), which includes PTFE-free tubings and a delay column.

Checkout Test Mix and Column (*optional*)

You can purchase the optional Checkout Test Mix and column to collect your own data to use with the Familiarization exercises. If you purchased the Installation and Familiarization option, these items are included.

- **LC TOF/QTOF/QQQ Pesticide Checkout Test Mix** (p/n 5190-0469), which includes 6 ampoules containing a Pesticides LC/MS Checkout test mix of 20 components in total (3 ampoules of Positive and 3 ampoules of Negative). Instructions to prepare the sample is found in the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide*.
- **Agilent ZORBAX RRHD Eclipse Plus C18, 95Å, 2.1 x 100 mm, 1.8 µm, 1200 bar pressure limit** (p/n 959758-902)

Where to find more information

MassHunter MRM/dMRM/tMRM Database Familiarization Guide Use the exercises in this guide to learn how to use your MassHunter MRM, dMRM, or tMRM Database with MassHunter Data Acquisition, Qualitative Analysis, and Quantitative Analysis programs. You use the example Checkout Mix data, method files, and database to learn how to find and identify compounds in a data file. The Checkout Mix data files, methods, and database are based on the Pesticides Checkout Test Mix, which contains a wide variety of compound classes. Find it in the **\MassHunter\Documents** folder of the Familiarization media, or in the **\MassHunter\Documents** folder on your computer after installation of the Familiarization media.

Agilent Data Acquisition Familiarization Guide Use this guide to learn more about tMRM acquisition.

Agilent web site Check **www.agilent.com** for more information. Look for **PFAS MRM Database**.

Installation

Before you begin

- Make sure these MassHunter programs are installed.
  - MassHunter Data Acquisition

    - for Ultivo LC/TQ, 1.1 or later

    - for 6470B, 10.1 or later

    - for 6495C, 10.0 SR1 or later
  - MassHunter Quantitative Analysis 10.0 or later.
  - MassHunter Qualitative Analysis 10.0 or later.
- Make sure you know which drive the **\MassHunter** folder is installed on your computer. By default, this folder is created on the **D:** drive.

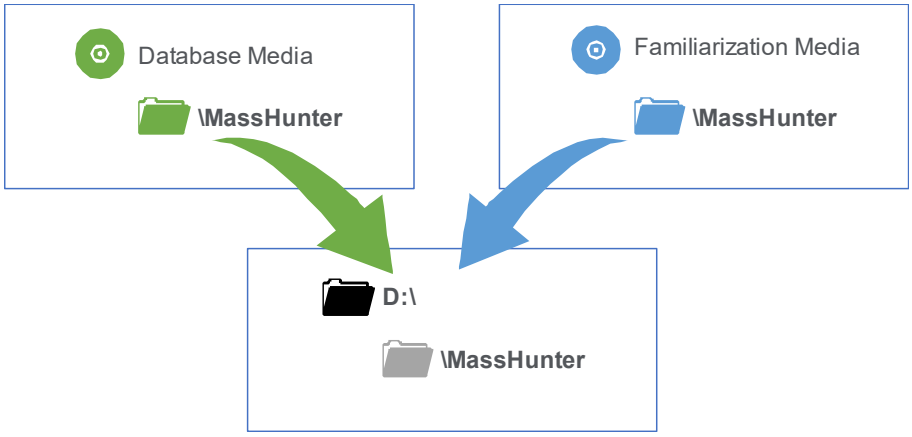
To install the MassHunter PFAS MRM Database files

1 Copy the **\MassHunter** folder into the root folder of your MassHunter drive. By default, the folder is **D:**.

The **\MassHunter** folder already exists on your computer. Make sure to copy the **\MassHunter** folder from the media to the same location where the **\MassHunter** folder already exists.

2 To install the Familiarization files, from the Familiarization media, copy the **\MassHunter** folder into the root folder of your MassHunter drive. By default, the folder is **D:**.

Check the instructions that come with the Familiarization media for the most current installation instructions.



To uninstall MassHunter database files

You need only delete folders and not individual files. The appropriate individual files are removed when the folders are removed.

- To remove the PFAS MRM Database files:
  - **a** In the **\MassHunter** folder on your computer, search for **PFAS MRM Database**.
  - **b** Delete all folders that contains that term.
- To remove Familiarization files:
  - **a** In the **\MassHunter** folder on your computer, search for **Checkout Mix**.
  - **b** Delete all folders that contains that term.

MRM Database Workflow

Figure 1 summarizes the workflow, which includes incremental method development from MRM, over to dynamic MRM (dMRM) to triggered MRM (tMRM) methods, including identification of retention times (RT), trigger parameters, and secondary transitions.

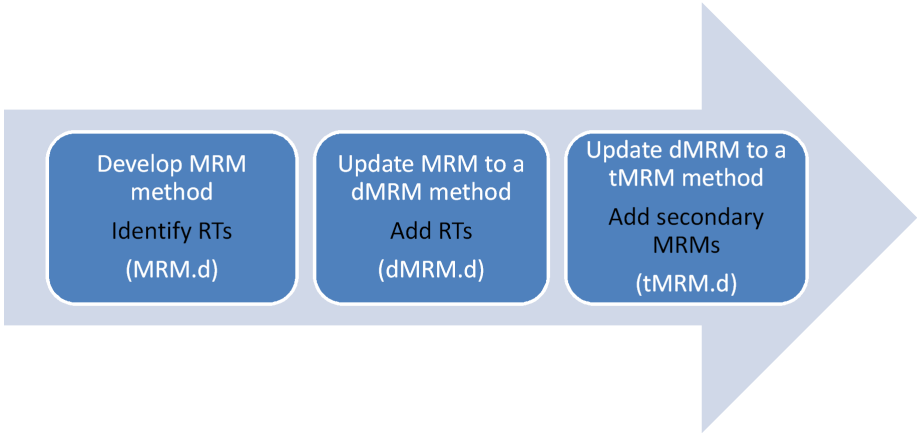


Figure 1. MRM to dMRM to tMRM Method Development Workflow for single standard mix

Refer to the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide* for more details.

Single Standard Mix Workflow

You can use this complete workflow to create an MRM, dMRM, or tMRM method to analyze a single standard mix:

1. Use the database to create the MRM method for the primary transitions.
2. Establish the Retention Times, and then update the MRM method to a dMRM method using the **Update DMRM Method** command. Save as a dMRM method.
3. Check the dMRM editor for any overlaps in retention time. If needed, adjust the cycle time settings and/or the Retention Time windows.
4. Acquire data to make sure that the dMRM method is valid.
5. Update the dMRM method with trigger parameters. Save as a tMRM method.
6. Add the secondary transitions.

After you have set up methods to analyze a single standard mix, you can adapt the same procedures for your unique multi-component analysis.

Multiple Standard Mix Workflow

Some analyses include multiple standard mixes.

To develop a method to analyze multiple compound mixes in one analytical run:

1. Create and optimize each dMRM or tMRM method for each standard mix separately. Use the same LC chromatographic method.
2. Combine these dMRM or tMRM methods. (Copy and paste transition tables of each dMRM or tMRM method into a single acquisition method.)
3. Re-optimize the parameters for overlapping dMRM or tMRM transitions for compounds that co-elute.

For ease of use, optimize no more than 50 compounds at a time in each **MRM -> dMRM -> tMRM** workflow.

Getting Started

This section describes the steps to get started with the MassHunter PFAS MRM Database. In this section, you prepare to use the MassHunter PFAS MRM Database for the first time, set up your method with content from the MassHunter PFAS MRM Database, and then set up a worklist.

The MassHunter PFAS MRM Database product includes two example methods for each model, as described in **"Example Methods"** on page 24. The example methods give you appropriate results only if you use the same HPLC setup that was used to create the methods. Follow the steps in this section to create your own methods with your existing HPLC setup.

Step 1. Prepare to use the PFAS database

1 Before you use the database or run the Familiarization exercises for the first time, do a Preventative Maintenance on your system.

2 Install the **PFC-free HPLC Conversion Kit** to prevent the interference of PFAS compounds possibly leaching from tubings and solvents.

NOTE

If you plan to use the **LC TOF/QTOF/QQQ Pesticide Checkout Test Mix (p/n 5190-0469)** to collect your own checkout data, Agilent recommends that you collect data before you install the **PFC-free HPLC Conversion Kit**. The Familiarization exercises use a different column than the **PFC-free HPLC Conversion Kit**.

Step 2. Set up LC method parameters

This step assumes a **Agilent ZORBAX RRHD Eclipse Plus C18, 95Å, 2.1 x 100 mm, 1.8 µm, 1200 bar pressure limit (p/n 959758-902)**. Adjust your method as needed.

1. Prepare your samples and transfer the sample solutions to 1-mL polypropylene vials for analysis.
2. Set up the mobile phases. The mobile phases used to create the **Example Methods** are:
   - Solvent A: 5 mM ammonium formate in water
   - Solvent B: methanol
3. In the MassHunter Data Acquisition program, open the method of your choice or create a new method.

   You can also use the ***model*_PFAS_BaseMethod.m** found in the **\MassHunter\Methods\PFAS MRM Database 1.0** folder. Note that the Base Methods and the Comprehensive Methods are dMRM methods. These dMRM methods contain Retention Times based on instrument setup as described in **"Example Methods"** on page 24. If your instrument setup is different, expected Retention Times may differ from those in the Base Methods.

   If you start with one of the **Example Methods**, you need to save the method to a different name.
4. Check that the method is set up to make the appropriate injection volume. The default injection volumes used in the **Example Methods** are:
   - Ultivo LC/TQ: 5 µL
   - 6470B LC/TQ: 3 µL
   - 6495C LC/TQ: 2 µL
5. Set up the gradient.

   See **Figure 2** on page 13 for the gradient used in the **Example Methods**. The Stop time is 14.5 minutes with a post time of 2.5 minutes.
6. Set the Column Compartment temperature. The temperature used to create the **Example Methods** is 55°C.

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	85.00	15.00	0.400	1000.00
1.00	85.00	15.00	0.400	---
1.50	45.00	55.00	0.400	---
5.50	30.00	70.00	0.400	---
7.00	20.00	80.00	0.400	---
12.00	0.00	100.00	0.400	---
14.40	0.00	100.00	0.400	---
14.50	85.00	15.00	0.400	---

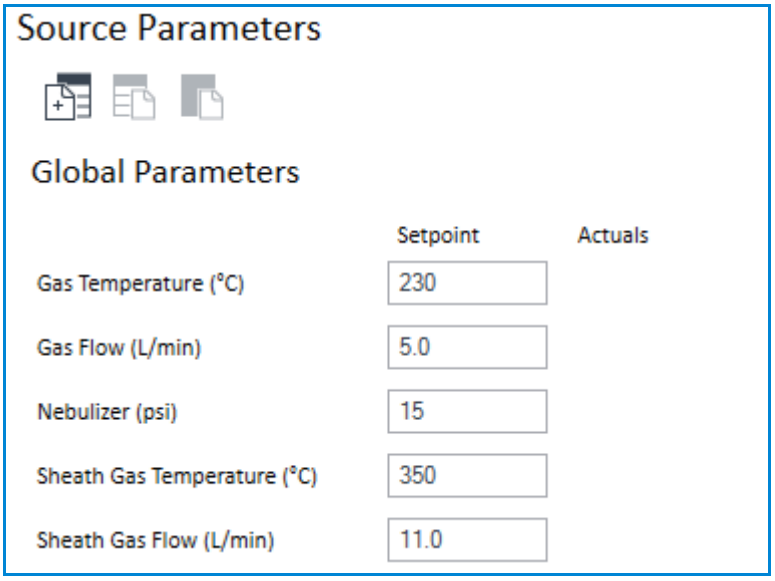
Figure 2. Gradient for 1290 Infinity II LC system with Agilent Eclipse Plus C18, 2.1 mm × 100 mm, 1.8-µm ZORBAX LC column (p/n 959758-902)

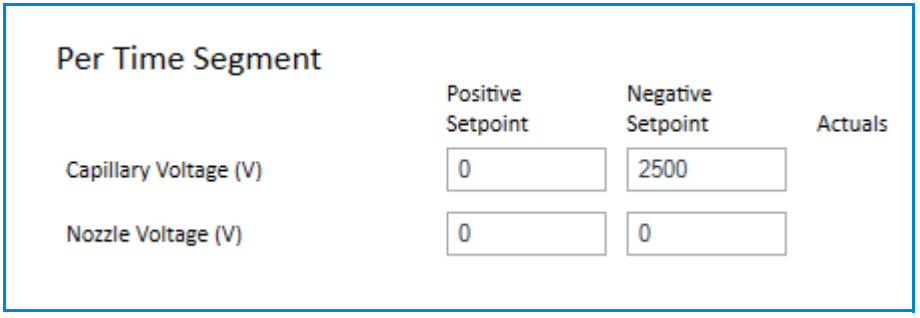
Step 3. Set up LC/MS ion source parameters

- Set up the ion source parameters in the MS part of the method tab.

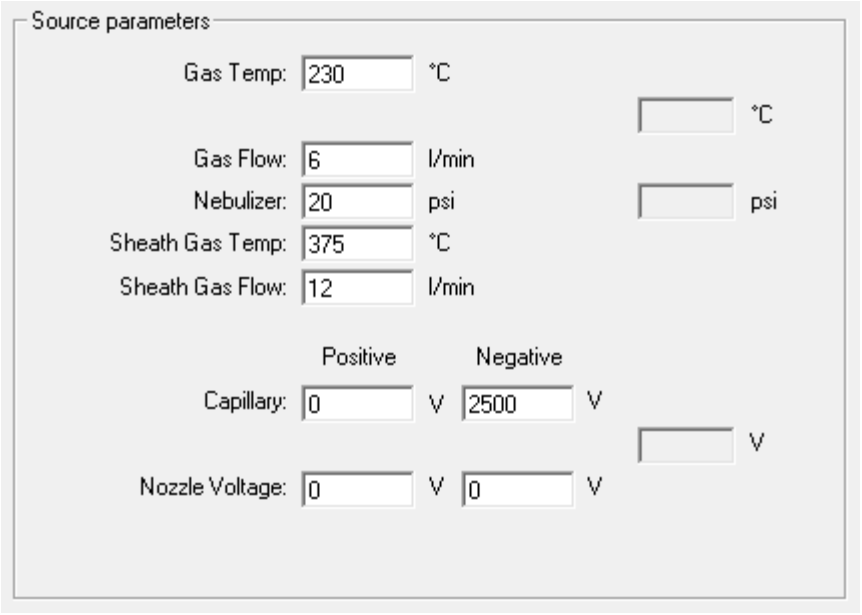
  For a multicomponent method, the ion source parameters shown in the next figures are used to achieve the best overall sensitivity for all compounds in the database for each instrument. You can adjust the method to optimize for individual or selected compounds.

Ion source parameters for Ultivo QQQ with an Agilent Jet Stream source



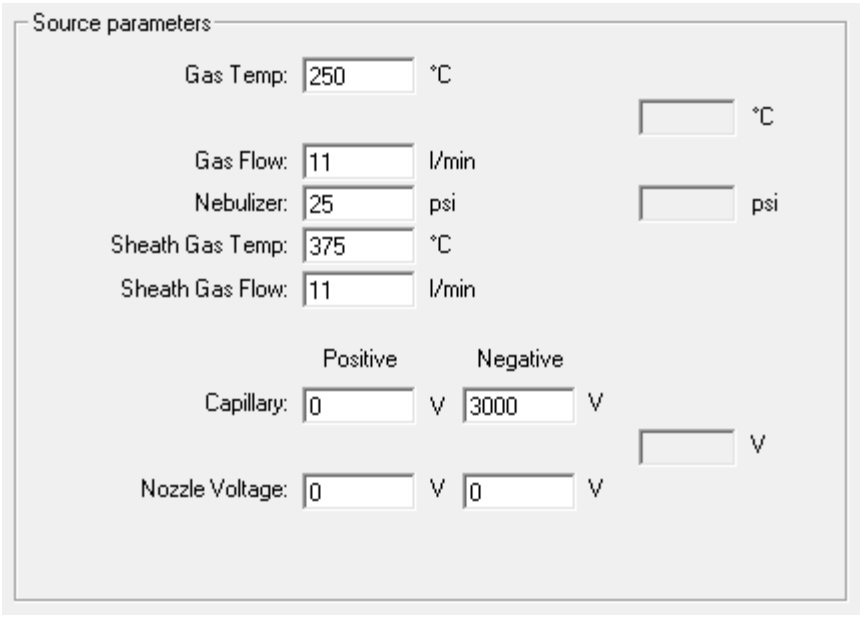


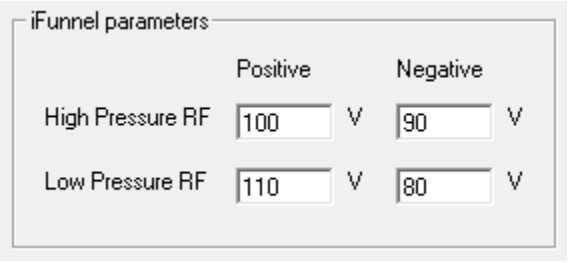
Ion source parameters for 6470B with an Agilent Jet Stream source



Under **Time segments**, make sure that **Delta EMV (-)** is set to **200**.

Ion source parameters for 6495C QQQ with an Agilent Jet Stream source





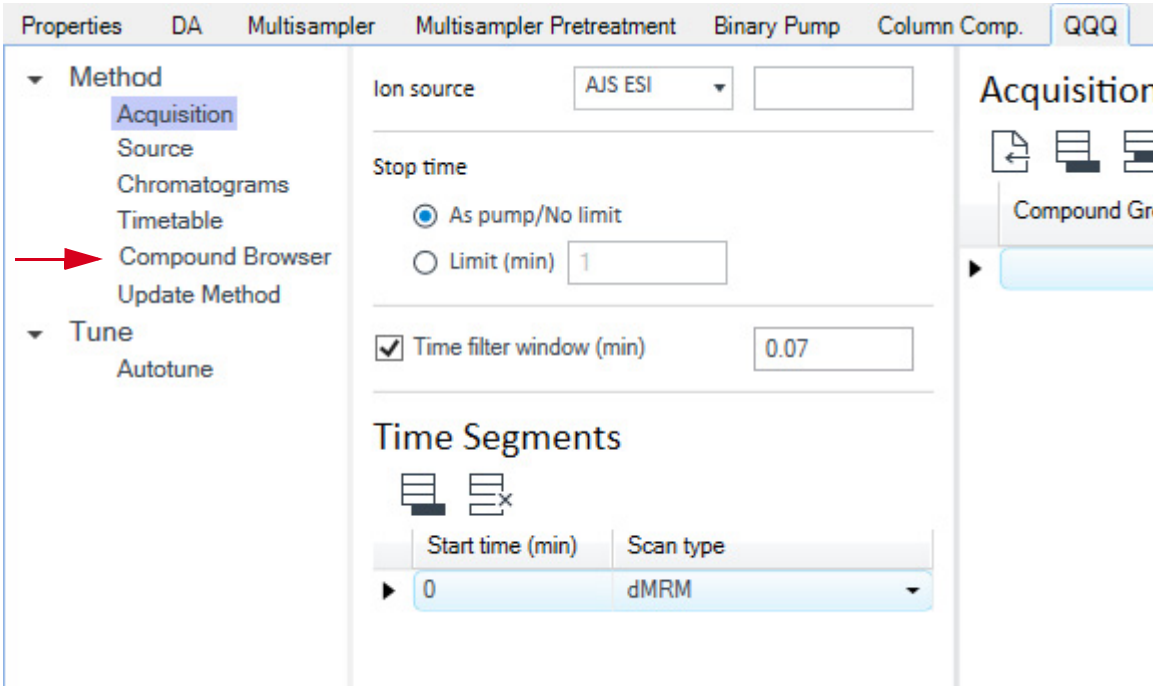
Under **Time segments**, make sure that **Delta EMV (-)** is set to **200**.

For the 6495C, the **Source** tab includes iFunnel parameters. These iFunnel parameters ensure the best overall sensitivity for PFAS compounds. You can use the **Source and iFunnel Optimizer** program to optimize for individual or selected compounds. Refer to the MassHunter Data Acquisition *online Help*.

Step 4. Set up the MRM method

Ultivo LC/TQ

1 In the Method Editor window, click **QQQ > Acquisition**.



2 Select **Compound Browser**.

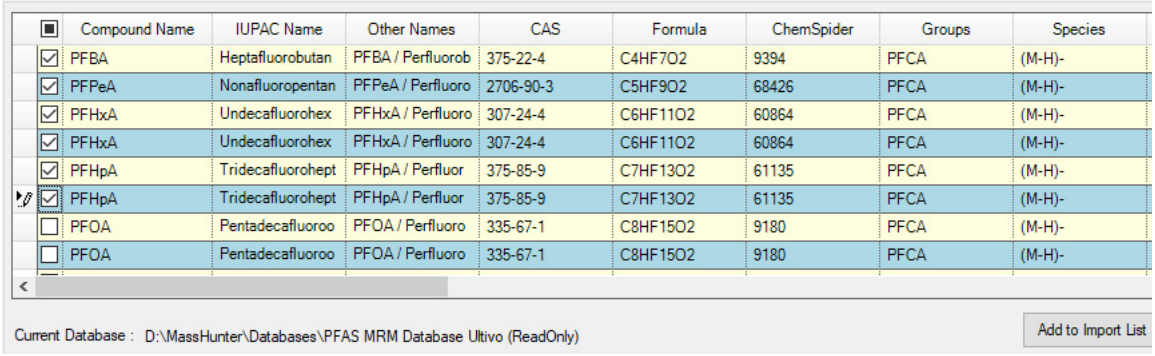
3 In the Compound Browser, click **File > Open Database**.

4 From the folder **\MassHunter\Databases\PFAS MRM Database 1.0**, open **PFAS_MRM_Database_Ultivo_1.1**. Click **OK**.

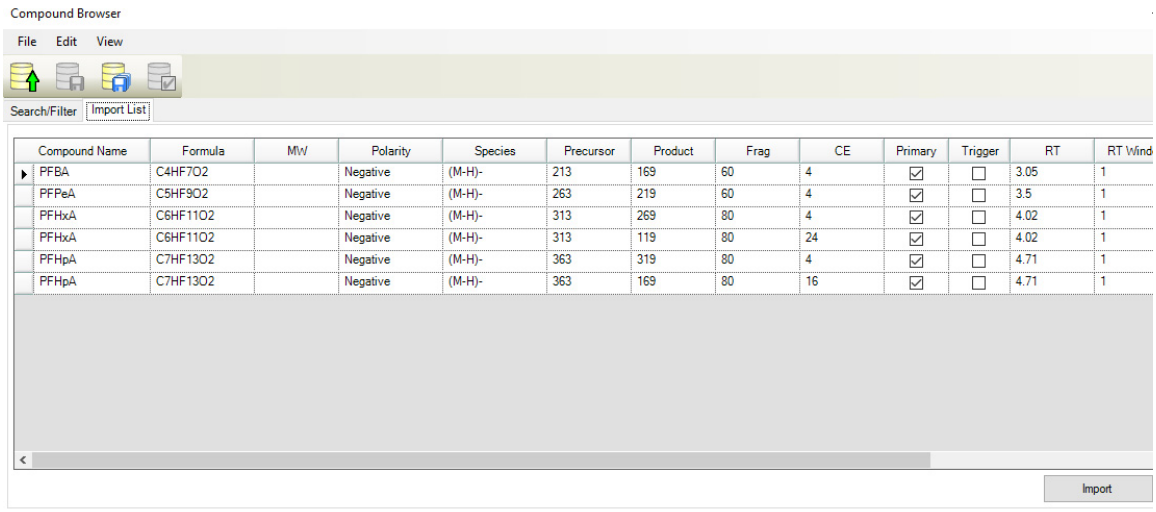
5 Select the list of transitions from the database:

a Check the box next to each **Compound Name** of interest.

b Click **Add to Import List**.



c Click **Import**.



6 Save the method.

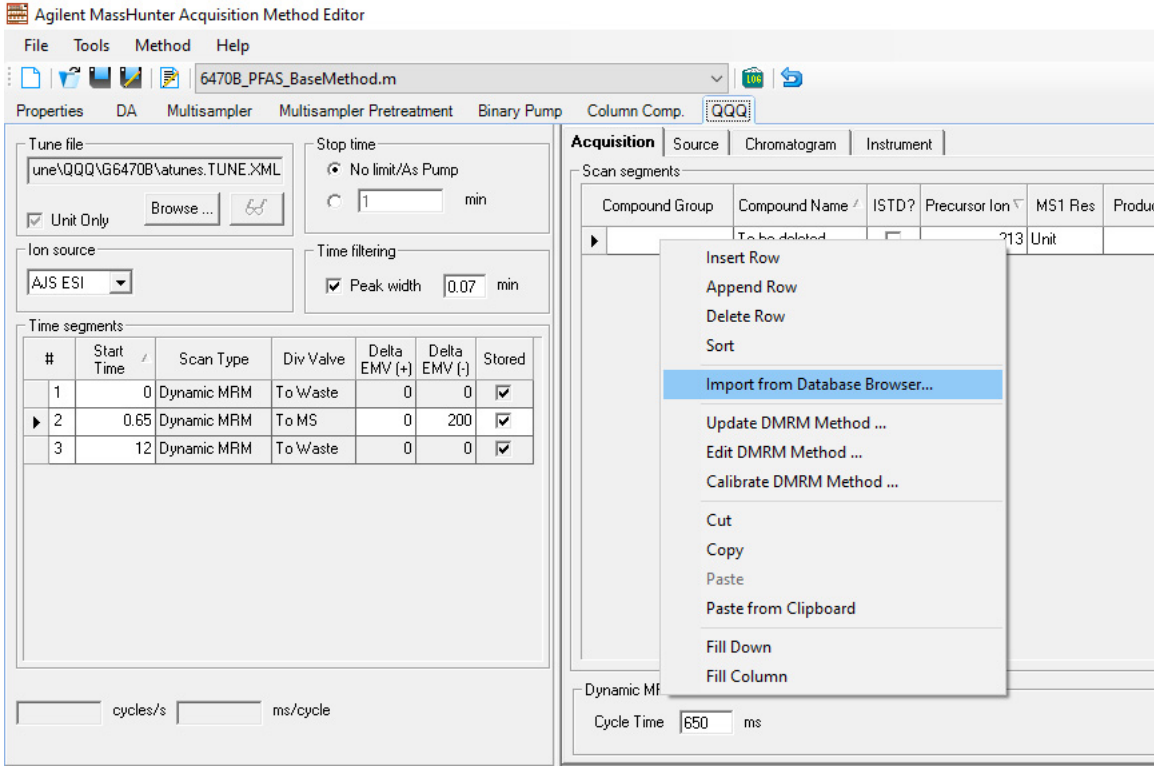
If you started with a PFAS example Base Method, save the method to a different name.

Refer to the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide* for more details.

6470B

1 In the Method Editor window, click **QQQ > Acquisition**.

2 Right-click the scan segment table and click **Import from Database Browser**.



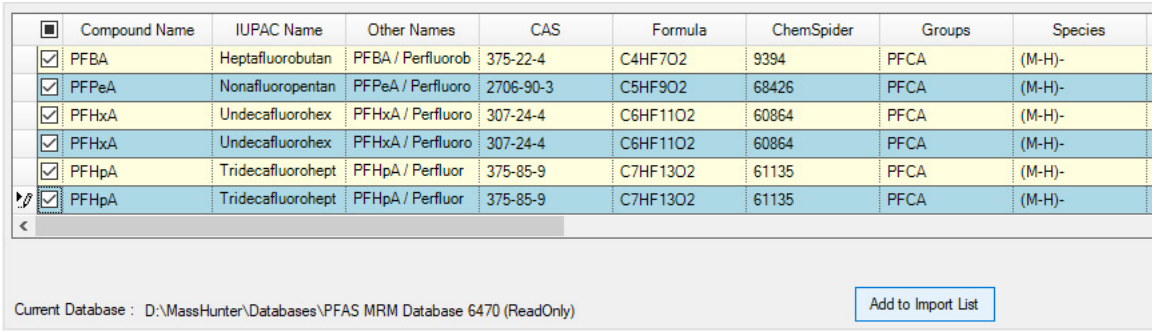
3 In the Database Browser, click **File > Open Database**.

4 From the folder **\MassHunter\Databases\PFAS MRM Database 1.0**, open **PFAS_MRM_Database_6470B_10.1**. Click **OK**.

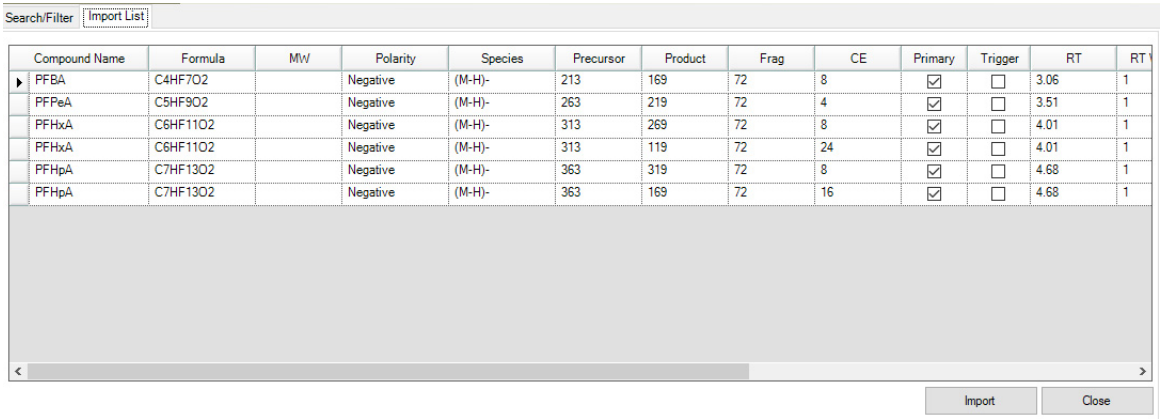
5 Select the list of transitions from the database:

a Check the box next to each **Compound Name** of interest.

b Click **Add to Import List**.



c Click **Import**.



6 Save the method.

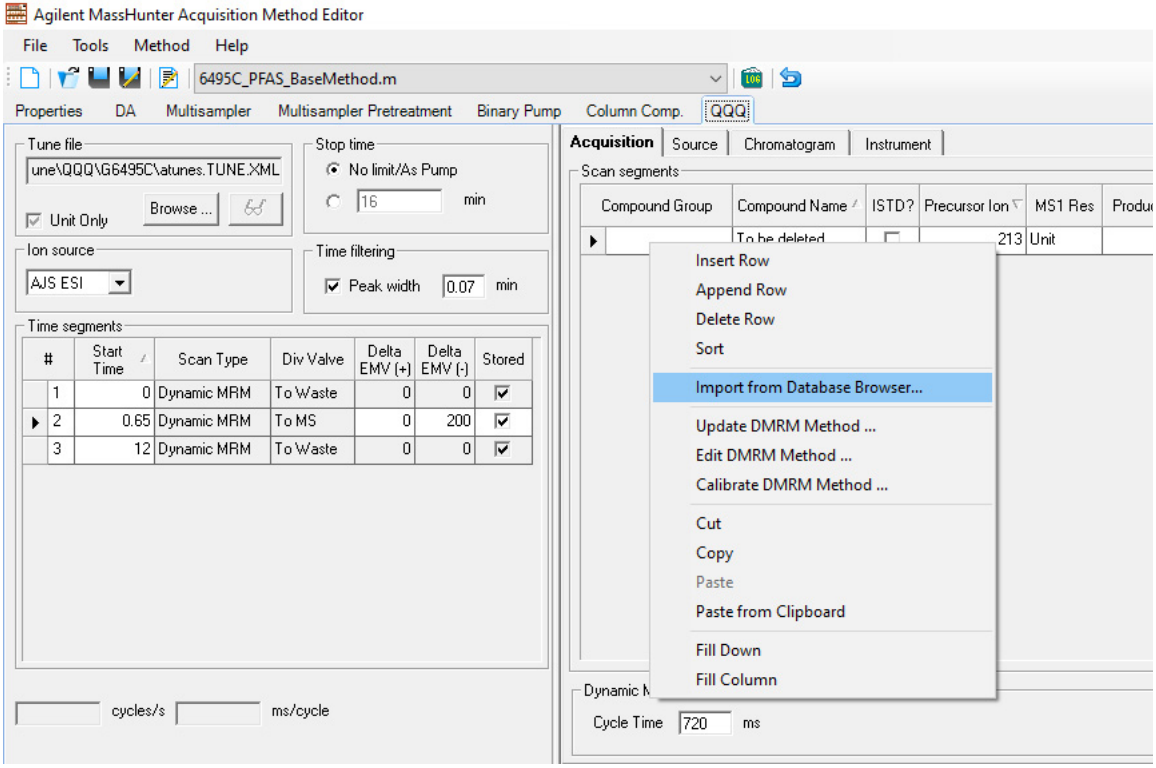
If you started with a PFAS example Base Method, save the method to a different name.

Refer to the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide* for more details.

6495C

1 In the Method Editor window, click **QQQ > Acquisition**.

2 Right-click the scan segment table and click **Import from Database Browser**.



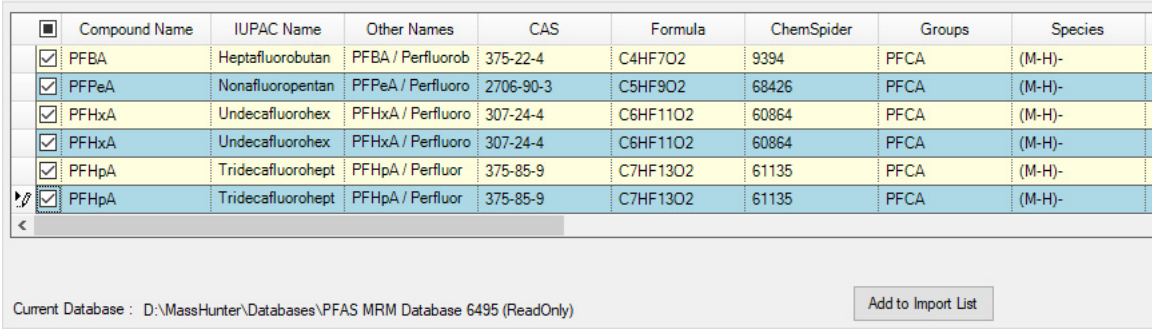
3 In the Database Browser, click **File > Open Database**.

4 From the folder **\MassHunter\Databases\PFAS MRM Database 1.0**, open **PFAS_MRM_Database_6495C_10.0**. Click **OK**.

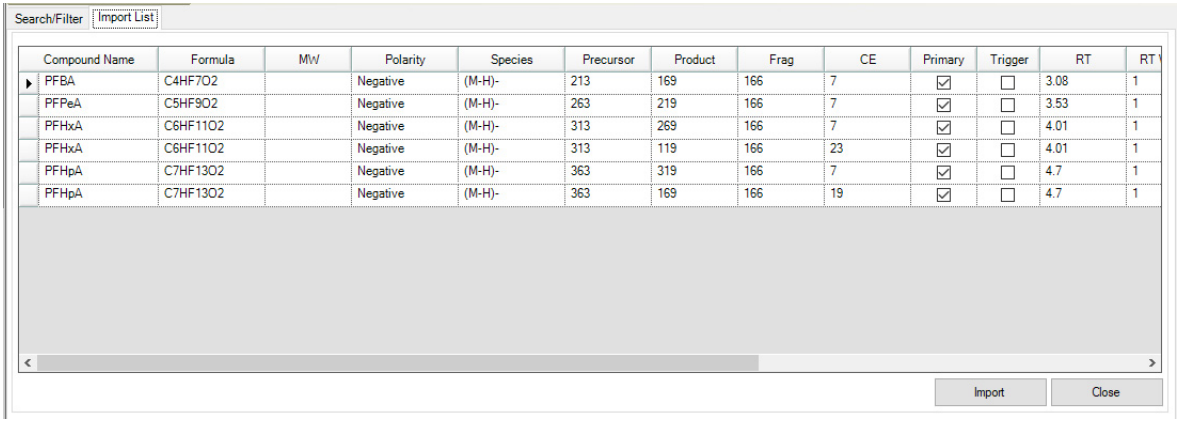
5 Select the list of transitions from the database:

a Check the box next to each **Compound Name** of interest.

b Click **Add to Import List**.



c Click **Import**.



6 Save the method.

If you started with a PFAS example Base Method, save the method to a different name.

Refer to the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide* for more details.

Step 5. Set up a worklist to run the methods

- Set up the worklist as needed for your samples. An example is shown in **Figure 3**.

	☑	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (µl)
1	✔	No_Injection	No Injection	6470B_PFAS_Comprehensive.m	No_Injection_01.d	Blank		As Method
2	✔	Solvent_Blank	P1-A1	6470B_PFAS_Comprehensive.m	Solvent_Blank.d	Blank		As Method
3	✔	CAL 1	P1-A2	6470B_PFAS_Comprehensive.m	CAL 1.d	Calibration	1	As Method
4	✔	CAL 2	P1-A3	6470B_PFAS_Comprehensive.m	CAL 2.d	Calibration	2	As Method
5	✔	CAL 3	P1-A4	6470B_PFAS_Comprehensive.m	CAL 3.d	Calibration	3	As Method
6	✔	CAL 4	P1-A5	6470B_PFAS_Comprehensive.m	CAL 4.d	Calibration	4	As Method
7	✔	CAL 5	P1-A6	6470B_PFAS_Comprehensive.m	CAL 5.d	Calibration	5	As Method

Figure 3. Worklist

The Base Methods are dMRM methods, but if you start with your own MRM method, then you can create dMRM and tMRM methods from the MRM method and optimize the parameters. Follow the steps in the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide*.

Refer to the MassHunter Data Acquisition *Familiarization Guide* or *online Help* for more information.

NOTE

If needed, increase the Delta RT for compounds that have isomers or a shift in retention time.

In a standard method development workflow, the trigger parameters in the tMRM methods such as Threshold, Trigger Entrance, Trigger Delay and Trigger Window are created for analysis of standards in solvent. These trigger parameters must be rechecked for matrix samples as signal abundances and noise characteristics of some MRM transitions can be different in samples with complex matrix compared to solvent standards.

Example Methods

The MassHunter PFAS MRM Database includes two example methods, the PFAS Base Method and the PFAS Comprehensive Method.

The **PFAS Base Method** includes empty scan segment table to allow import of selected analytes from the database.

The **PFAS Comprehensive Method** includes the MRM settings for all the analytes from the database.

You can find these methods in the **\MassHunter\Methods\PFAS MRM Database 1.0** folder on your computer (post-installation) and the MassHunter PFAS MRM Database media.

Use these methods as a basis for your own analysis, or create your own methods using the steps in**"Getting Started"** on page 11.

For more detailed instructions, see the *MassHunter MRM/dMRM/tMRM Database Familiarization Guide*, and the MassHunter Data Acquisition *Familiarization Guide* and *online Help*.

This topic describes the LC parameters that were used to create the example methods. The MS parameters are described in **"Step 3. Set up LC/MS ion source parameters"** on page 14.

System and column

Component	Description
HPLC System	1290 Infinity II LC System
Conversion kit	PFC-free HPLC Conversion Kit (p/n 5004-0006)
LC column	ZORBAX RRHD Eclipse Plus C18, 95Å, 2.1 x 100 mm, 1.8 µm, 1200 bar pressure limit (p/n 959758-902)
Guard column	ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1200 bar pressure limit, UHPLC guard (p/n 821725-901)

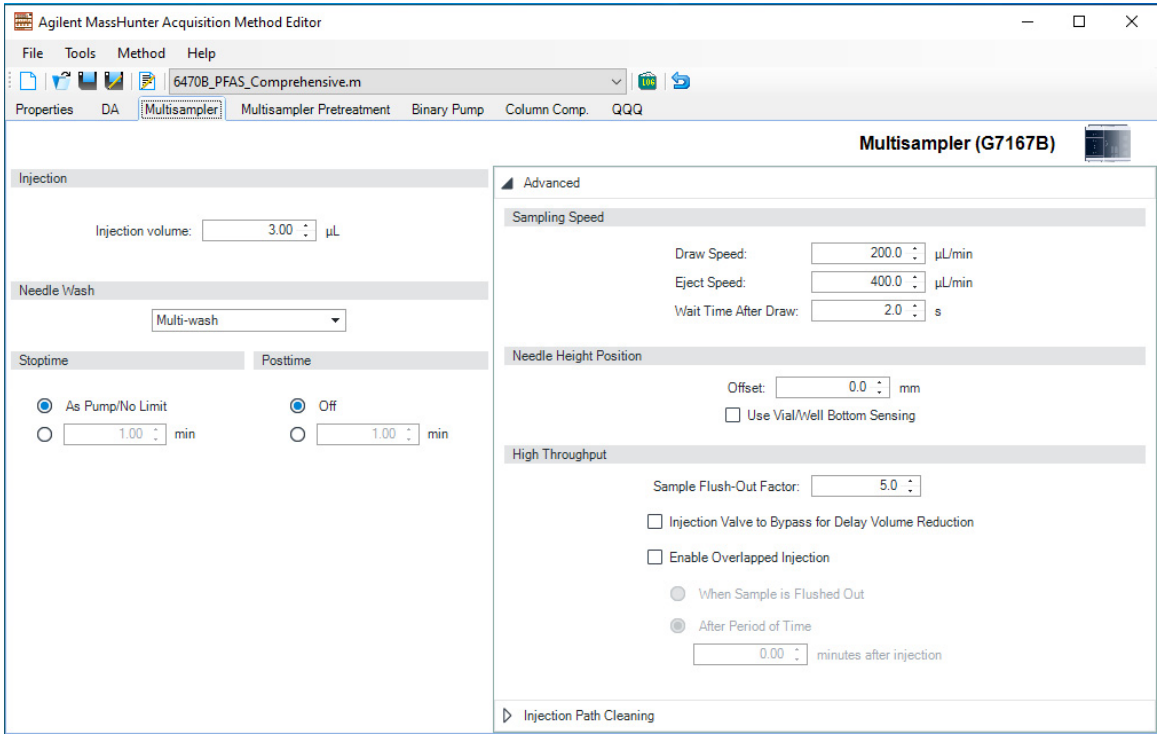
G7167B Multisampler

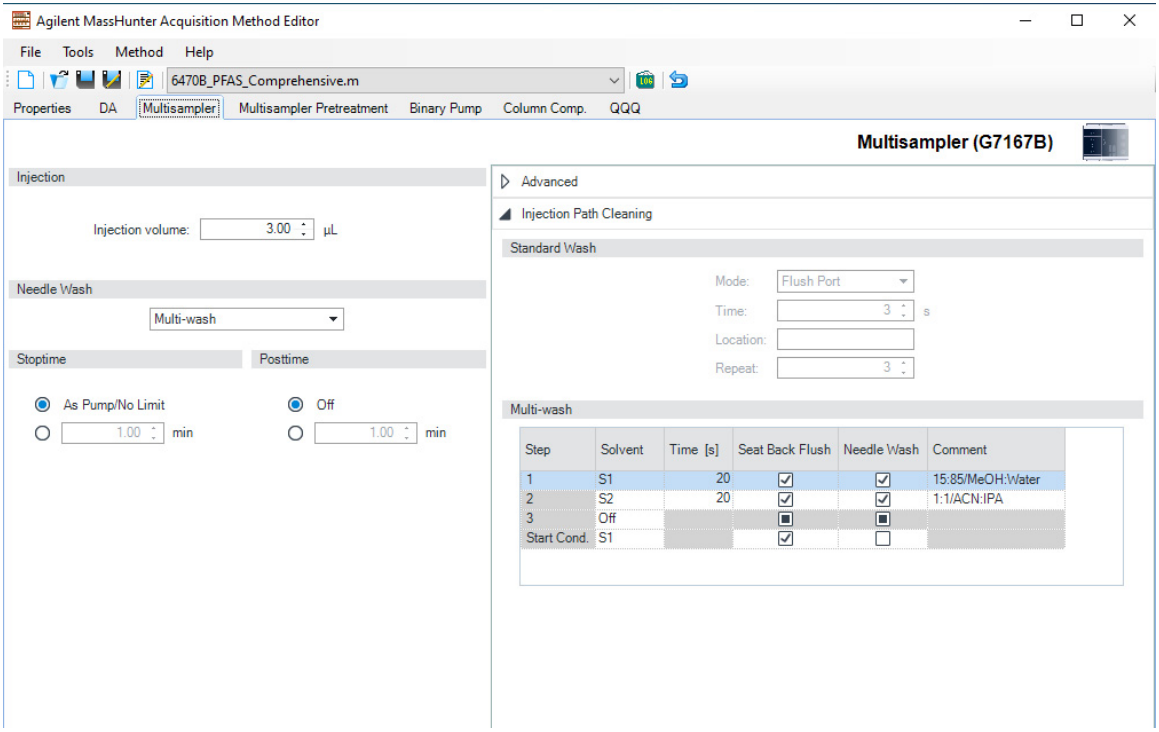
Table 1. G7167B Multisampler parameters

Parameter	Value				
Thermostat temperature	5°C				
Injection volume	Ultivo LC/TQ: 5 µL 6470B LC/TQ: 3 µL 6495C LC/TQ: 2 µL				
Draw speed	200 µL/min				
Eject speed	400 µL/min				
Wait time after draw	2.0 s				
Needle height offset	0.0 mm				
Use vial/well bottom sensing	No				
Needle wash	Multi-wash				
Wash solvent 1 (S1)	15:85 methanol:water				
Wash solvent 2 (S2)	1:1 acetonitrile:2-propanol				
Multi-wash program	**Step**	**Solvent**	**Time (s)**	**Seat Back Flush**	**Needle wash**
	1	S1	20	☑	☑
	2	S2	20	☑	☑
	3	Off	—	▣	▣
	4	S1	—	☑	☐
Stop time	As Pump/No Limit				
Post time	Off				

These parameters are shown in the Method Editor window in the next two images.

Note that the **Injection volume** shown is for the 6470B. The volume varies with the instrument model, as shown in **Table 1**.


Agilent MassHunter Acquisition Method Editor
File
Tools
Method
Help
6470B_PFAS_Comprehensive.m
Properties
DA
Multisampler
Multisampler Pretreatment
Binary Pump
Column Comp.
QQQ
Multisampler (G7167B)
Injection
Injection volume:
3.00
µL
Needle Wash
Multi-wash
Stoptime
Posttime
As Pump/No Limit
1.00
min
Off
1.00
min
Advanced
Sampling Speed
Draw Speed:
200.0
µL/min
Eject Speed:
400.0
µL/min
Wait Time After Draw:
2.0
s
Needle Height Position
Offset:
0.0
mm
Use Vial/Well Bottom Sensing
High Throughput
Sample Flush-Out Factor:
5.0
Injection Valve to Bypass for Delay Volume Reduction
Enable Overlapped Injection
When Sample is Flushed Out
After Period of Time
0.00
minutes after injection
Injection Path Cleaning

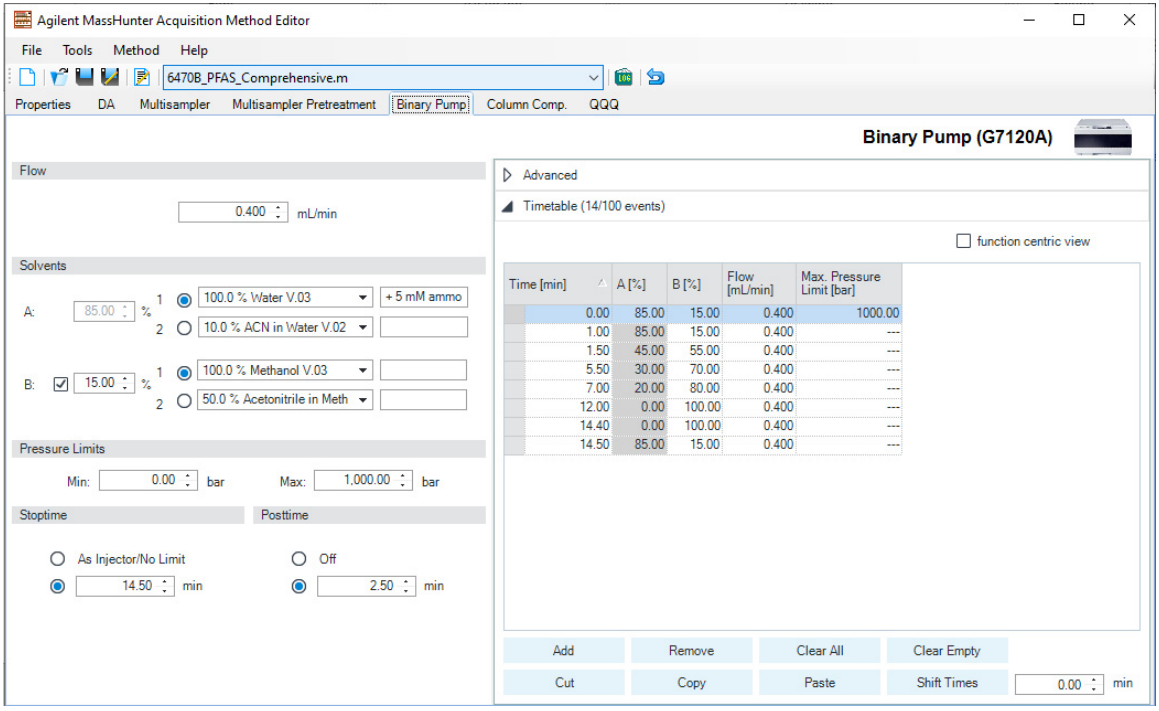

Agilent MassHunter Acquisition Method Editor
File
Tools
Method
Help
6470B_PFAS_Comprehensive.m
Properties
DA
Multisampler
Multisampler Pretreatment
Binary Pump
Column Comp.
QQQ
Multisampler (G7167B)
Injection
Injection volume:
3.00
µL
Needle Wash
Multi-wash
Stoptime
As Pump/No Limit
1.00
min
Posttime
Off
1.00
min
Advanced
Injection Path Cleaning
Standard Wash
Mode:
Flush Port
Time:
3
s
Location:
Repeat:
3
Multi-wash
Step
Solvent
Time [s]
Seat Back Flush
Needle Wash
Comment
1
S1
20
15:85/MeOH:Water
2
S2
20
1:1/ACN:IPA
3
Off
Start Cond.
S1

G7120A Binary Pump

Table 2. G7120A Binary Pump parameters

Parameter	Value		
Flow rate	0.4 mL/min		
Solvent A	5 mM ammonium acetate in water		
Solvent B	methanol		
Minimum pressure limit	0 bar		
Maximum pressure limit	1000 bar		
Stop time	14.5 min		
Post time	2.5 min		
Timetable	**Time (min)**	**A (%)**	**B (%)**
	0.00	85	15
	1.00	85	15
	1.50	45	55
	5.50	30	70
	7.00	20	80
	12.00	0	100
	14.40	0	100
	14.50	85	15

These parameters are shown in the Method Editor window in the next image.

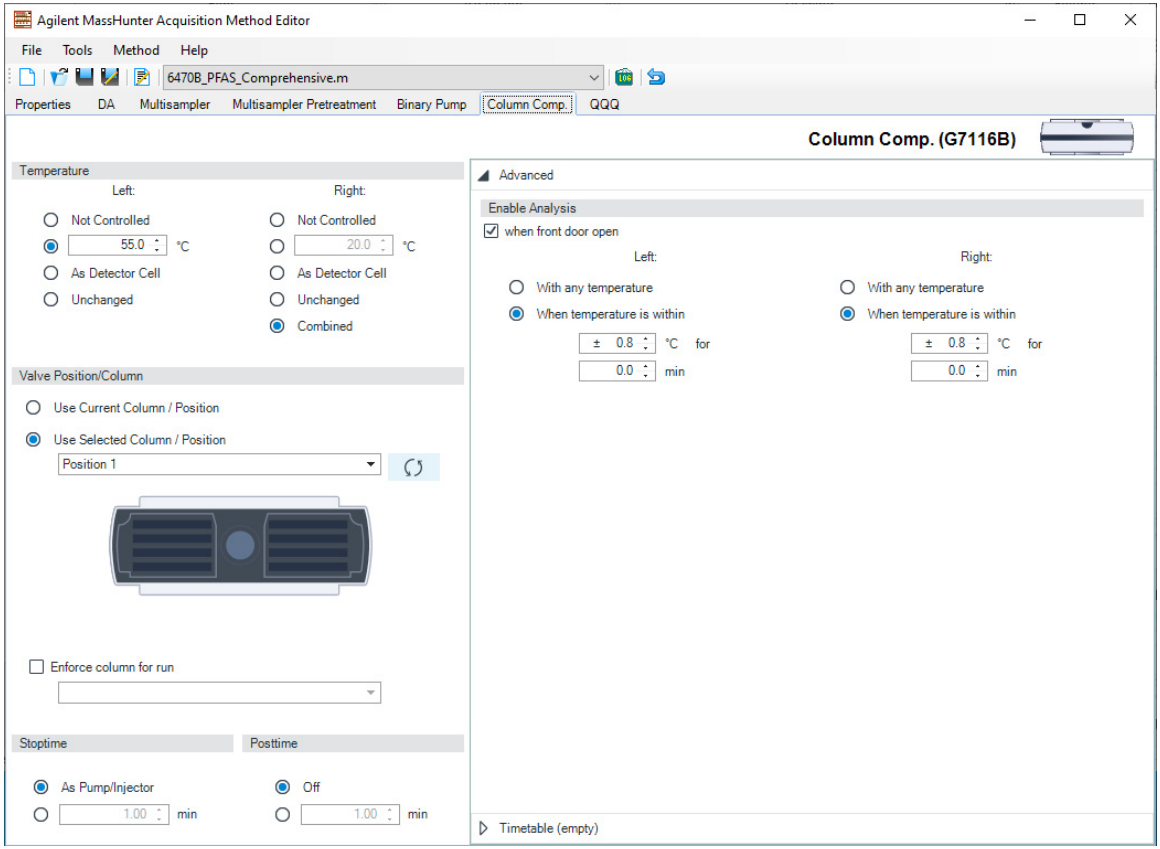

Agilent MassHunter Acquisition Method Editor
File Tools Method Help
6470B_PFAS_Comprehensive.m
Properties DA Multisampler Multisampler Pretreatment Binary Pump Column Comp. QQQ
Binary Pump (G7120A)
Flow
0.400 mL/min
Solvents
A: 85.00 % 1 100.0 % Water V.03 + 5 mM ammo
2 10.0 % ACN in Water V.02
B: 15.00 % 1 100.0 % Methanol V.03
2 50.0 % Acetonitrile in Meth
Pressure Limits
Min: 0.00 bar Max: 1,000.00 bar
Stoptime
As Injector/No Limit
14.50 min
Posttime
Off
2.50 min
Advanced
Timetable (14/100 events)
function centric view
Time [min] A [%] B [%] Flow [mL/min] Max. Pressure Limit [bar]
0.00 85.00 15.00 0.400 1000.00
1.00 85.00 15.00 0.400 ---
1.50 45.00 55.00 0.400 ---
5.50 30.00 70.00 0.400 ---
7.00 20.00 80.00 0.400 ---
12.00 0.00 100.00 0.400 ---
14.40 0.00 100.00 0.400 ---
14.50 85.00 15.00 0.400 ---
Add Remove Clear All Clear Empty
Cut Copy Paste Shift Times 0.00 min

G7116B Column Compartment

Table 3. G7116B Column Compartment parameters

Parameter	Value
Temperature	Left: 55°C Right: Combined
Temperature allowance	Left and Right: ± 0.8°C

These parameters are shown in the Method Editor window in the next image.



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In This Guide

This Quick Start Guide provides an introduction to the MassHunter PFAS MRM Database. This guide applies to PFAS MRM Database 1.0, and includes these database versions:

- PFAS_MRM_Database_6470B_10.1
- PFAS_MRM_Database_6495C_10.0
- PFAS_MRM_Database_Ultivo_1.1

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