Agilent

Agilent Triple Quadrupole LC/MS System

Introduction Workbook

Notices

Manual Part Number

G1960-90143

October 2024

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Agilent Technologies, Inc

5301 Stevens Creek Blvd.

Santa Clara, CA 95051

www.agilent.com

Software Revision

This guide is valid for MassHunter 12.1 and greater, until superseded.

Instrument Manufacturing

Manufactured by Agilent Technologies Singapore Pte. Ltd. No. 1 Yishun Avenue 7, Singapore 768923

Operating Temperature

Operating Temperature: 15 to 35 °C

Storage Temperature: -40 to 70 °C

Warranty

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

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1 Introduction

About this Workbook

This workbook provides instructions for the Ultivo, 6475A, and 6495D LC/TQ systems running MassHunter Data Acquisition 12.1 or higher.

For more information on the software and detailed instructions on the workflow not covered in this workbook, see the Online Help.

This workbook is your introductory guide for the set-up and execution of basic procedures with the LC/TQ and MRM method development workflow. This workbook is divided into chapters, each building upon the last, so we recommend that each chapter is completed in succession. During each chapter, lessons are guided by an Agilent-certified service professional.

By completing this learning event, you will have an introductory level of experience in the use of an Agilent Triple Quadrupole LC/MS System.

This introduction covers:

- Reviewing hardware components and software procedures
- Performing a checktune
- Acquiring and analyzing a sample
- Performing routine maintenance

How to use this Workbook

This learning experience introduces basic concepts in a learning-by-doing, guided manner. Each chapter uses step-by-step instructions.

Exercises to be completed are marked like this:

Exercise Name

Exercise Instructions

Task steps look like this:

1 Tasks or items needed to complete tasks look like this.

If you are expected to enter any information or if something is important, it is set in italicized type like this:

Type *Blank One* in the field.

If you are expected to press a key on the keyboard or button on the software screen, the key is displayed in bold like this:

Press **Enter**.

Cross-references appear in blue:

(For example, Link)

Before You Begin

This introduction workbook is recommended for all participating end users.

- Download the *Agilent Triple Quadrupole LC/MS System User Guide* by scanning the code or navigating to **https://aglt.co/LCMSUserDocs.**
- Use the *Agilent Triple Quadrupole LC/MS System Introduction Workbook* and Introduction Checklist with your Agilent-certified service professional and keep for future reference.

Additional Resources

User Documentation

Data analysis and library management documentation can be found by scanning the code or navigating to **https://aglt.com/DALibMgmtDocs**.

Instrument documentation, step-by-step videos, and more can be found by scanning the code or navigating to **https://aglt.co/LCMSUserDocs.**

Agilent Triple Quadrupole LC/MS Supplies

Use this quick reference list to keep your shelves stocked by navigating to **https://aglt.co/LCTQSupplies**.

Where to find more information

Agilent Community

To get answers to your questions, join over 10,000 users in the Agilent Community. Review curated support materials organized by platform technology. Ask questions to industry colleagues and collaborators. Get notifications on latest videos, documents, tools, and webinars relevant to your work.

https://community.agilent.com/

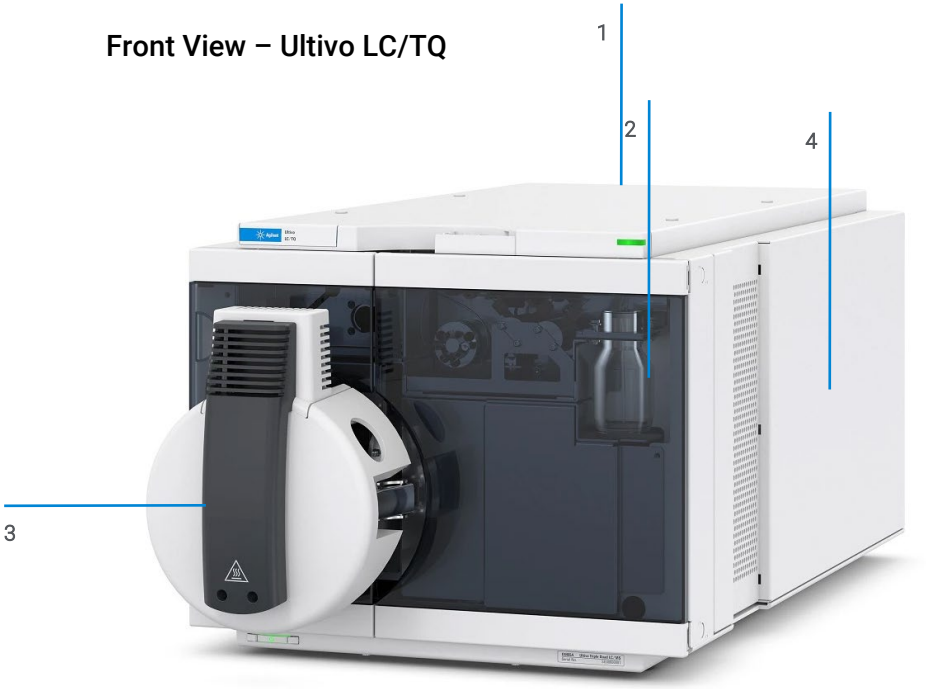
2 Hardware

Overview

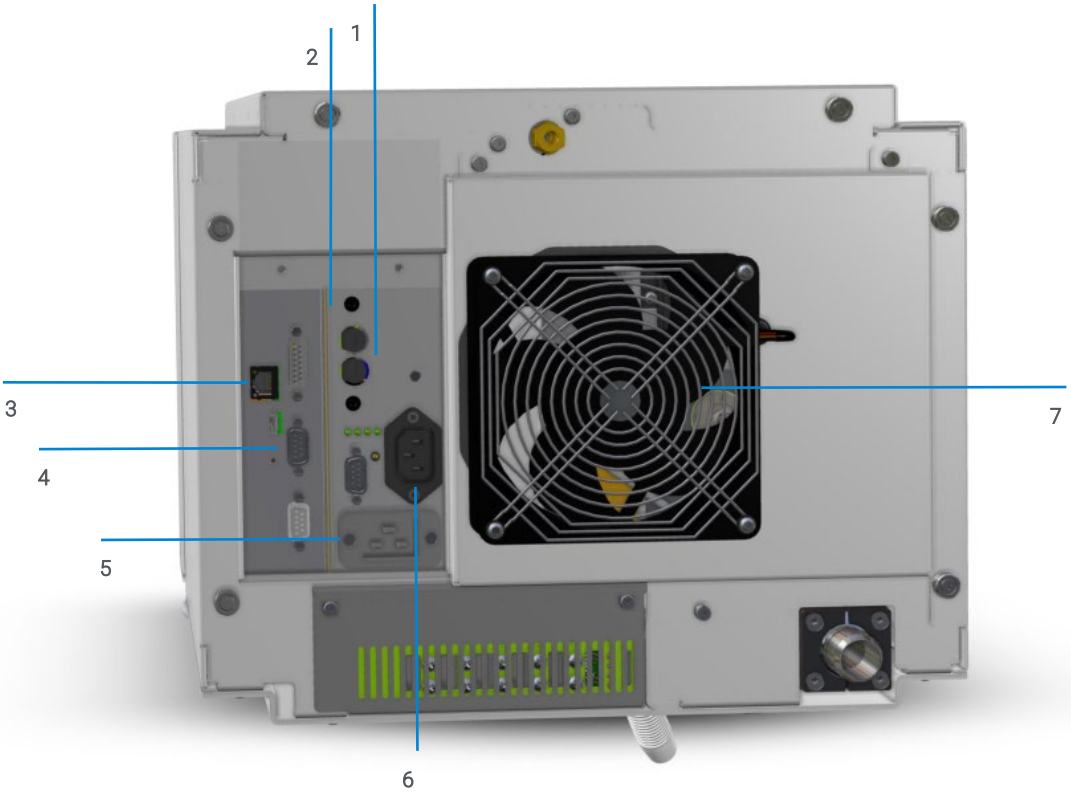
In this section, you will identify basic hardware components and their locations for the 6400 Series triple quadrupole LC/MS system.

Fill in the Blank:
Work with your Agilent Service Engineer and/or use the *Agilent Triple Quadrupole LC/MS System User Guide* to label the flagged components below for your installed instrument(s).

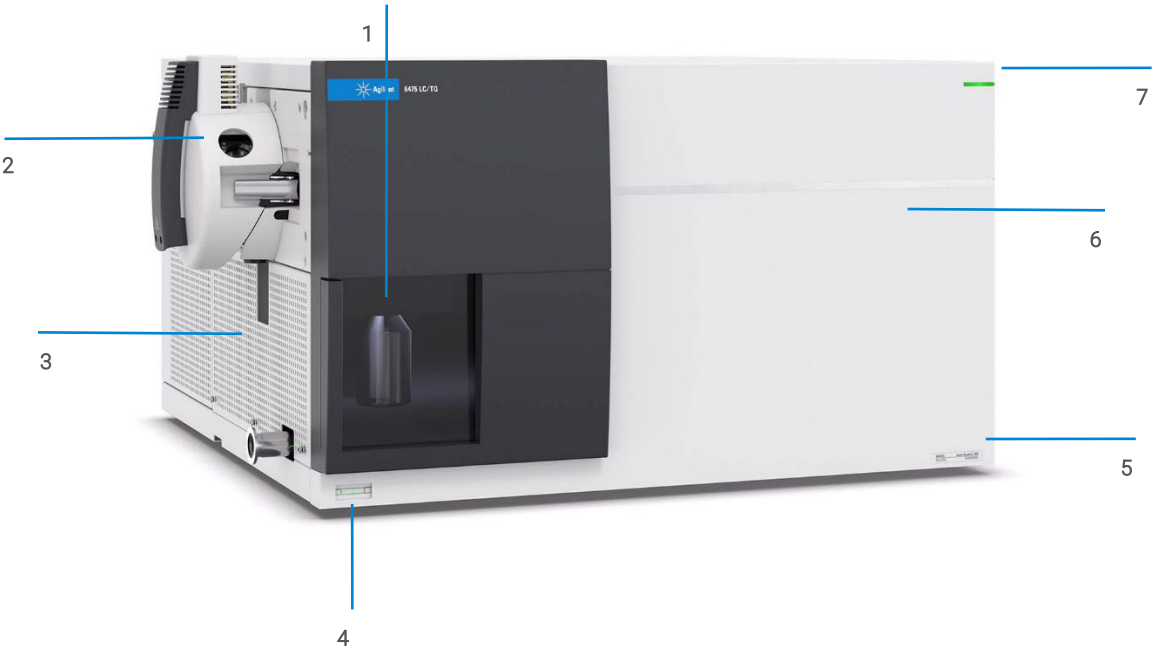
Front View – Ultivo LC/TQ



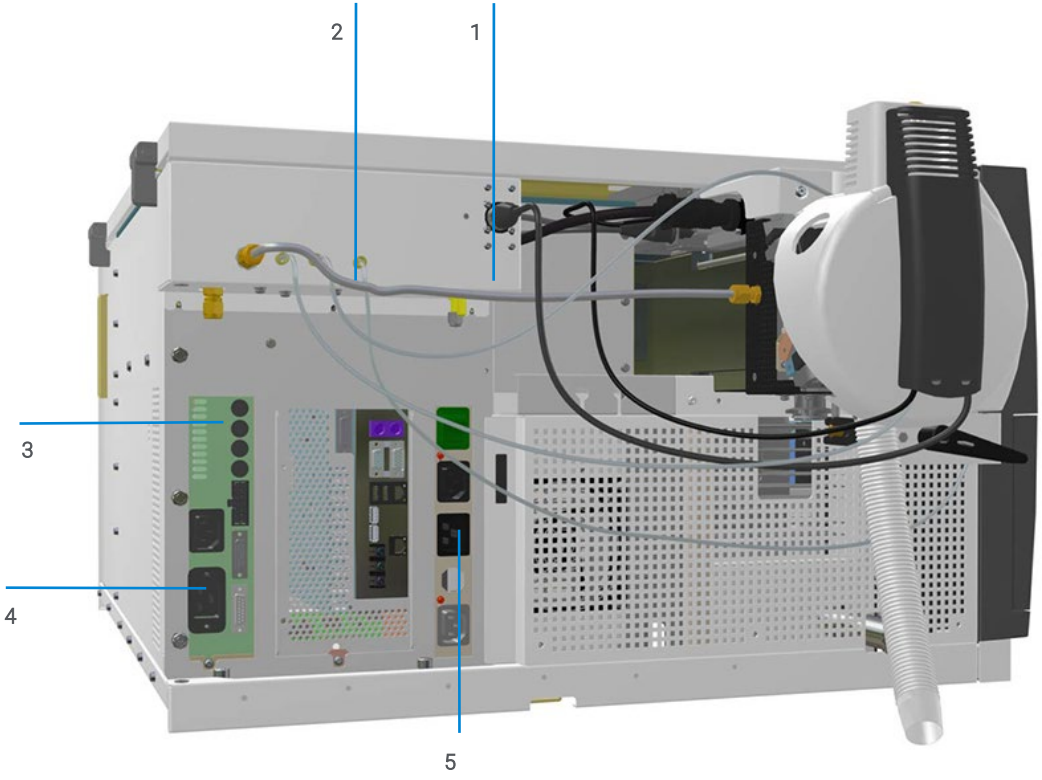
Rear View - Ultivo LC/TQ



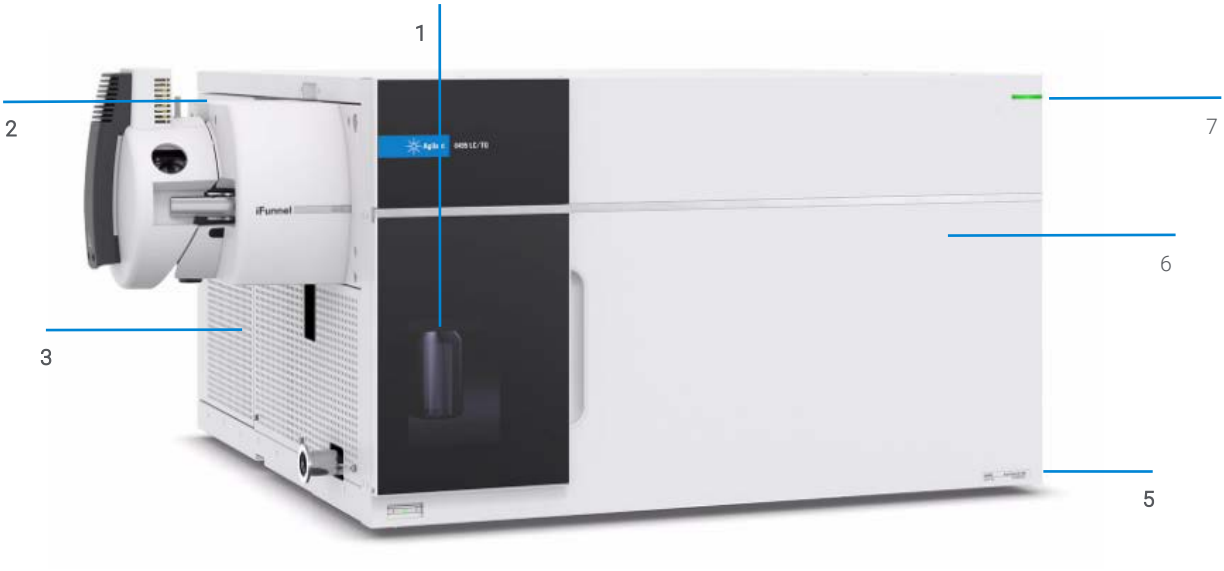
Front View - 6475A



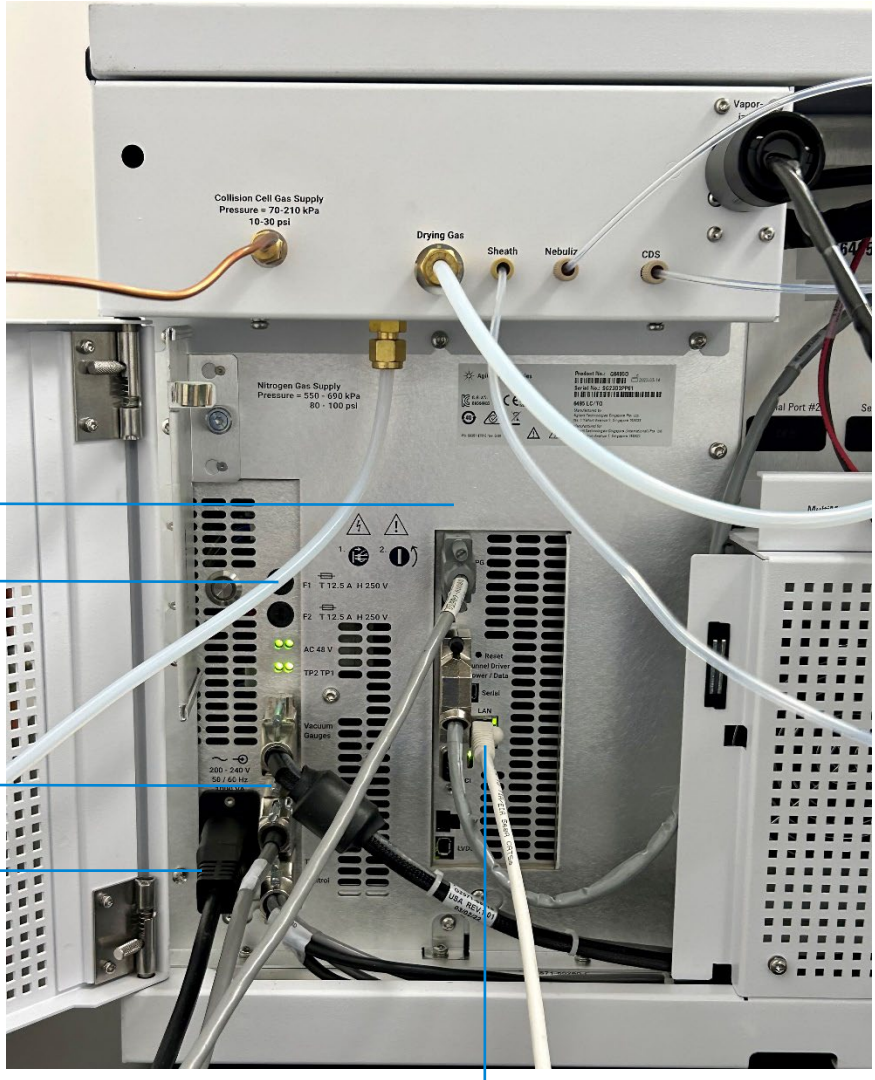
Side View – 6475A



Front View – 6495D



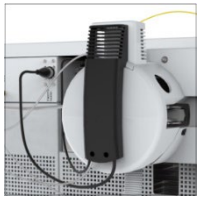
Side View – 6495D



Basic Components

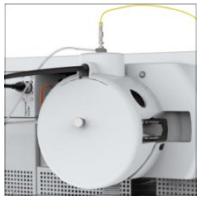
Ionization Source

Agilent liquid chromatography/mass spectrometry (LC/MS) ion sources enable analysis of a wide range of samples quickly and accurately.

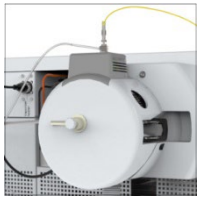


- Agilent Jet Stream (AJS ESI) source

- Electrospray Ionization (ESI) source



- Atmospheric Pressure Chemical Ionization (APCI) source



- Multimode source (MMI)

- Agilent nanospray ESI source

Hardware Introduction

1 List the type of Ionization Source in use:

---

2 It is reviewed on _____ page of the user guide and includes the following parts:

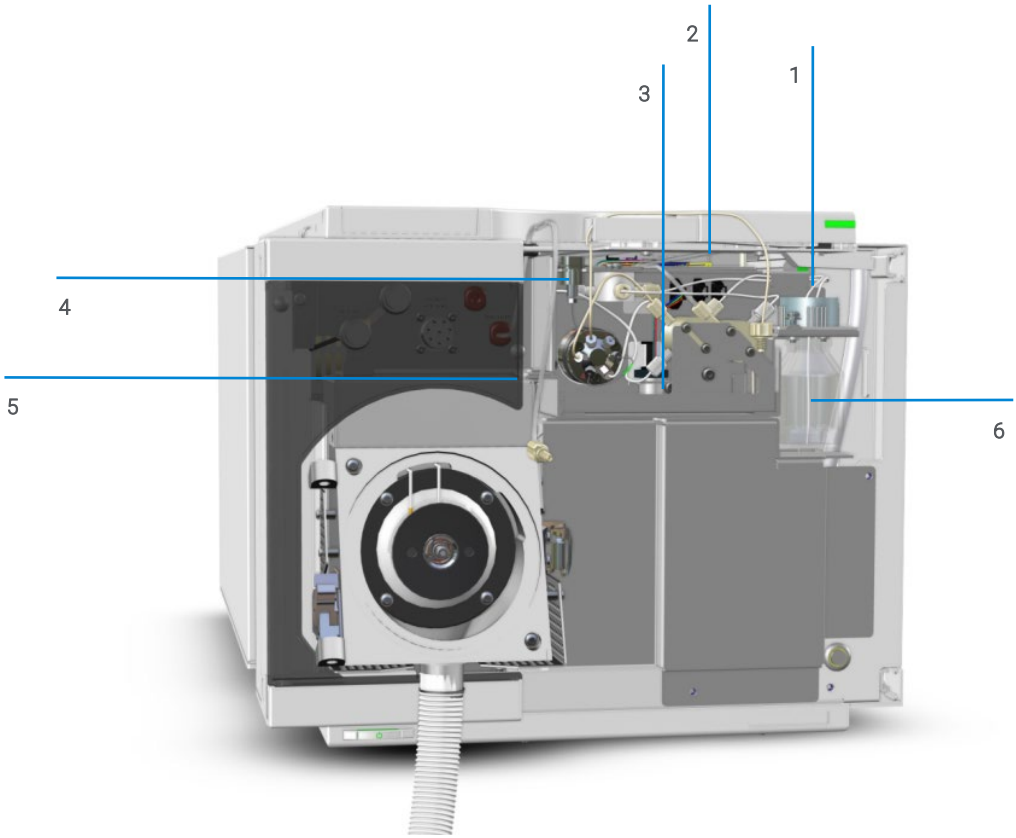
---

3 List the name and part number of the proper tune solution for this system:

Calibrant Delivery System (CDS)/Bottle

The calibrant delivery system (CDS) introduces calibration solution for automated mass calibration of the mass spectrometer, to ensure that the mass accuracy of the system is maintained throughout batch acquisition.

Ultivo



6475A

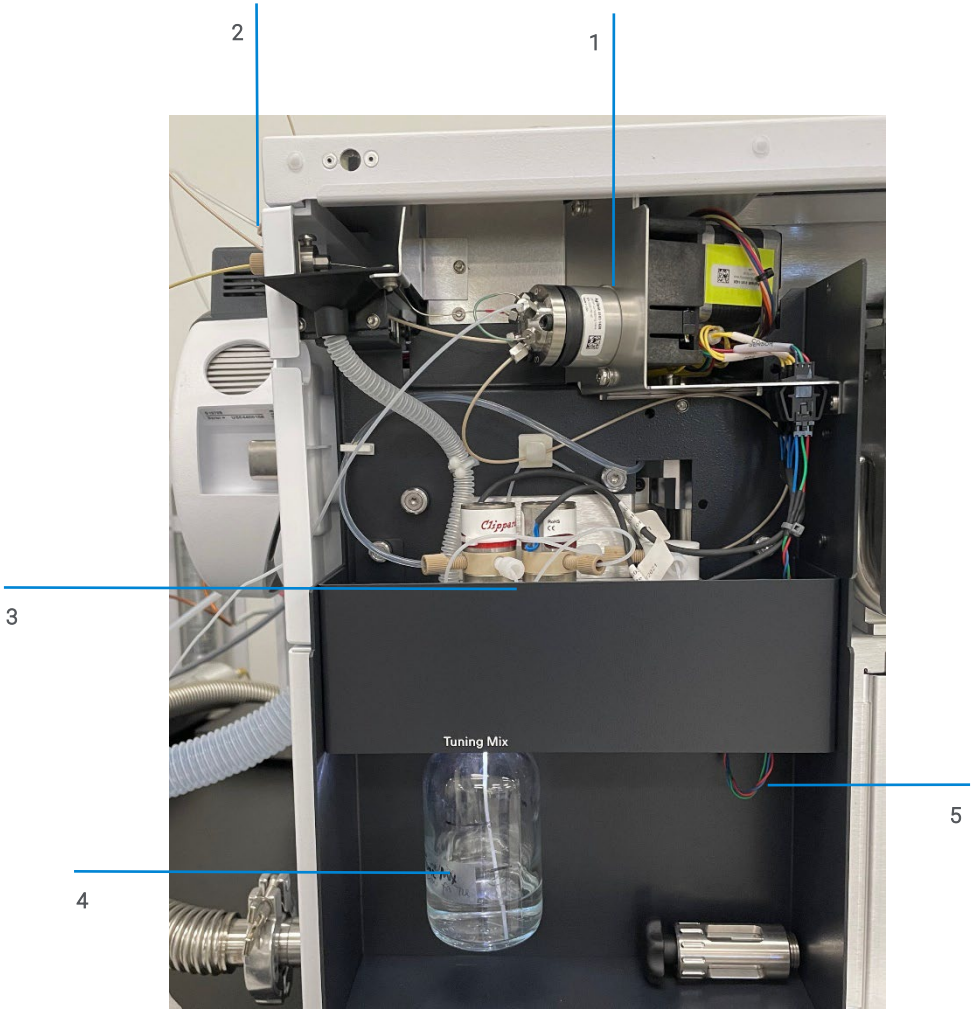
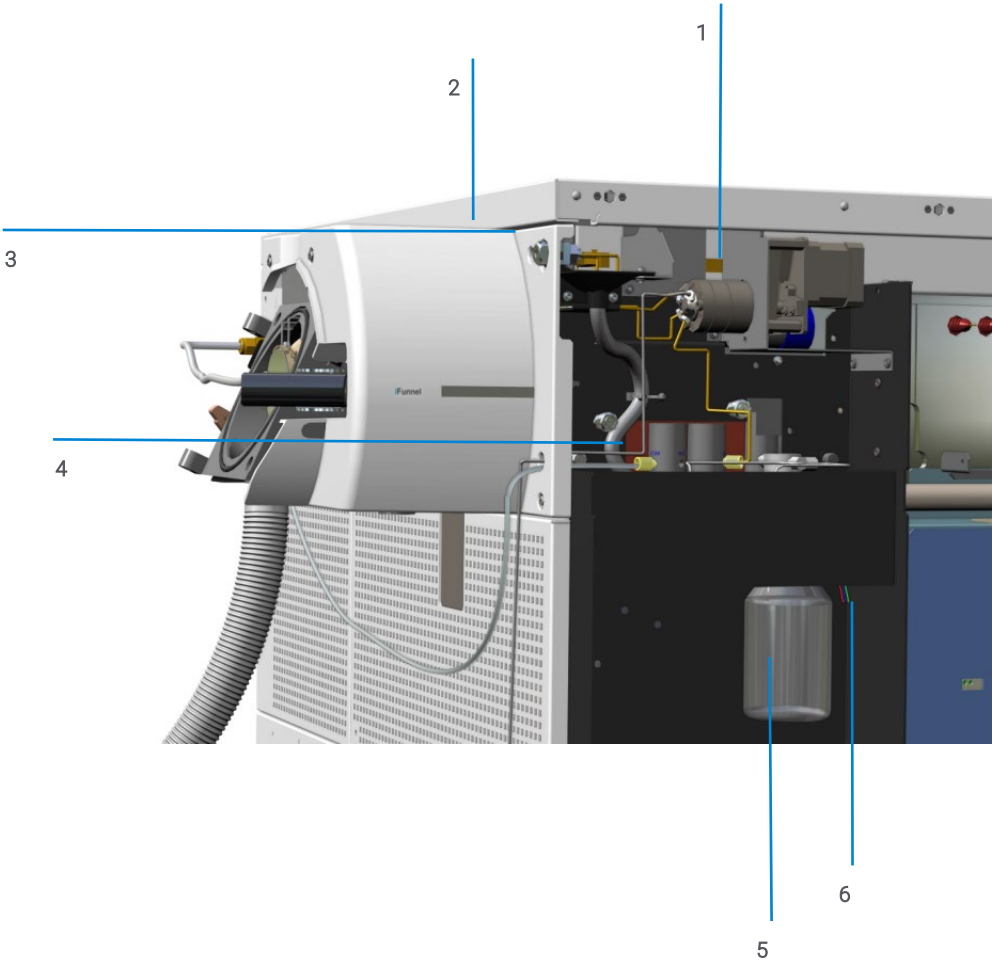


Figure 1. Front cover removed.

6495D



Hardware Introduction

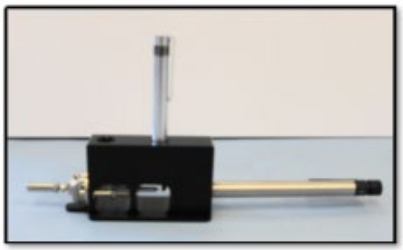
1 Practice removing and attaching the calibrant bottle.

2 How often is the calibrant bottle checked and refilled?

Nebulizer

A nebulizer is a device for producing a fine mist of charged droplets that converts a liquid sample into an aerosol for introduction into the vacuum system.

APCI & APPI Nebulizer



Nebulizer adjustment kit
Use to check the condition and concentricity of the needle, and to adjust the needle position

ESI, MM, & AJS Nebulizer

View The Needle

1 Find your nebulizer type per the user guide or the document that comes with the kit. List the part number below:

Rough Pump

MS40+ (Ultivo and 6475A)

MS120+ (6495D)

Locate The Oil Sight

Using the user guide, fill out the following information:

1 The oil level should be _____ the marks for Max and Min.

2 Check that the pump oil is _____ and the color is _____ than amber.

3 If the pump oil is _____ or full of _____ replace it.

This page is intentionally left blank.

3 Software Basics

Overview

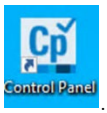
The OpenLab Control Panel is the administrative and management center for MassHunter Data Acquisition software:

- Full instrument status information of your entire laboratory.
- Central configuration and administration of users, instruments, and security settings.
- Full system documentation and built-in reports.

You will review:

- Starting the software
- Navigation overview
- Closing the connections
- Creating projects
- Creating and configuring instruments
- Launching instruments
- Offline method editor
- Creating shortcuts

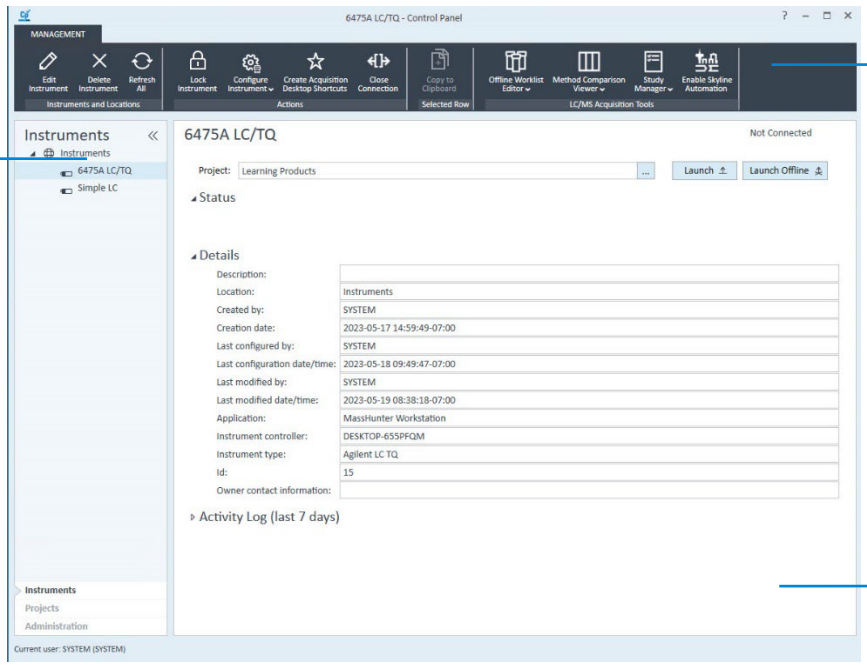
Software Start-Up

1 From the desktop, double-click the **OpenLab Control Panel** icon 

.

2 The navigation pane opens by default and can be minimized or expanded based on your preference.

User Interface and General Navigation



Ribbon

Panes

Instruments – Controls specific instruments.

Projects – Create paths to save project data.

Administration – Add and remove configuration

Main Window

- To minimize the pane, click **<<**. When minimized, the tab currently selected is displayed vertically.
- To expand the pane, click **>>**.
- You can drag and drop items in the Instruments and Projects pane. The existing privileges of the instrument or project are not retained when moving. The user must have the proper privileges to perform this function.

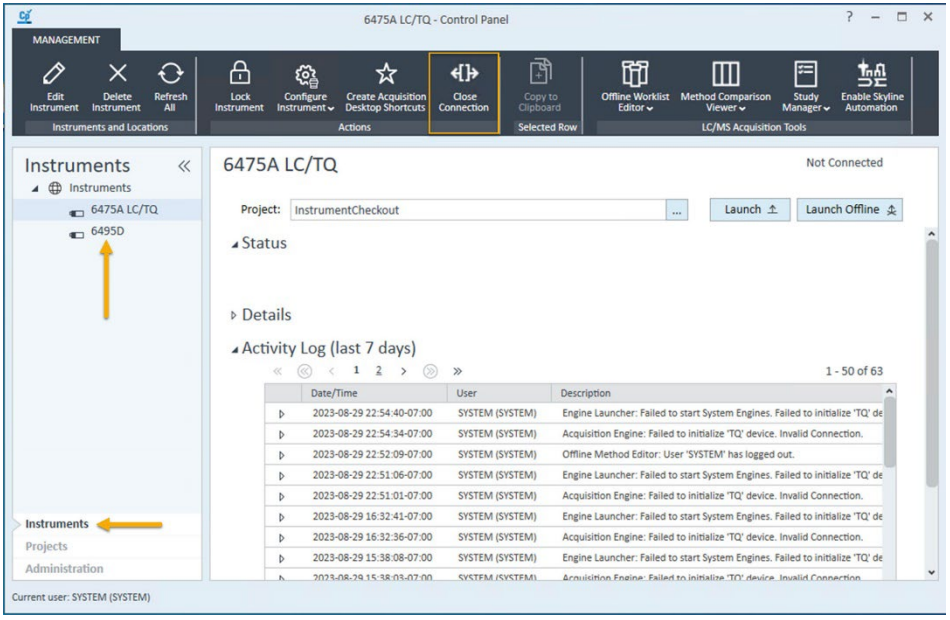
Close Connection

Use the Close Connection function to sever the connection between the instrument and the configured Instrument Controller (AIC or Workstation).

1 Click **Instruments**.

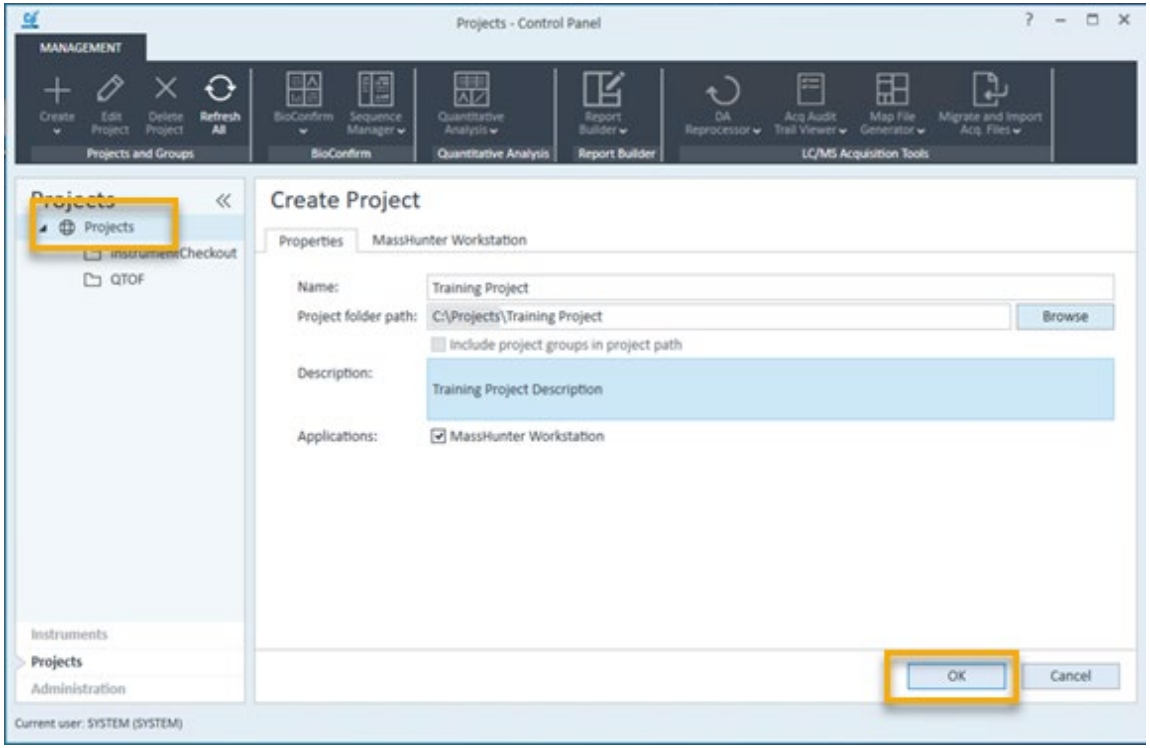
2 Select the instrument to close.

3 Click **Close Connection**.



Creating and Configuring Projects

1. Click **Projects** and select Projects
2. In the Name text box, type *Training Project*.
3. In the Project folder path text box, leave the default folder path.
4. In the Description text box, type a description of the project, for this example *Training Project Description*.
5. Click the **MassHunter Workstation** tab and review the available options. Do not change the defaults.
6. Click **OK**



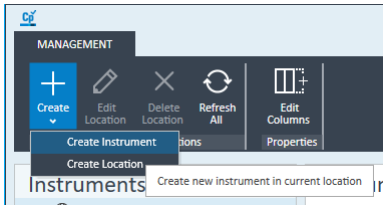
Instruments

Use the Control Panel to connect and control the instruments you want to use with the software.

Create an Instrument

1 Click Instruments and select any location.

2 Click Create > Create Instrument.



3 Enter the required data in the Create Instrument pane.

   a Name: *6495D LC/TQ (or proper model)*

   b Instrument Type: Agilent LC TQ

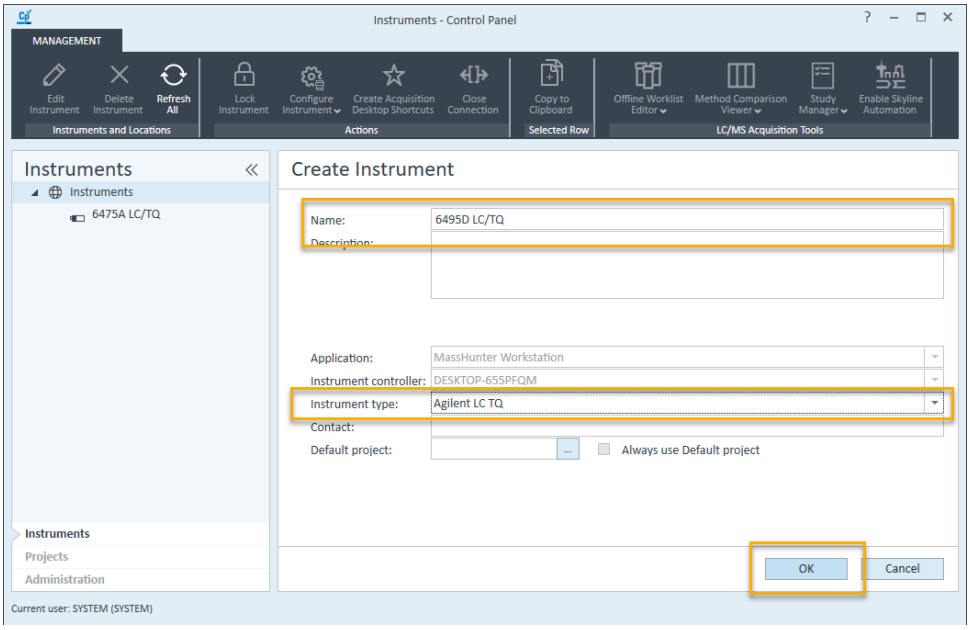
NOTE

Do not select a default project, you will be prompted to select a project when you launch the instrument.

4 Click OK.

5 Click … to select the TrainingProject project from the Select Project dialog box.

6 Click OK. The instrument is displayed in the navigation pane.

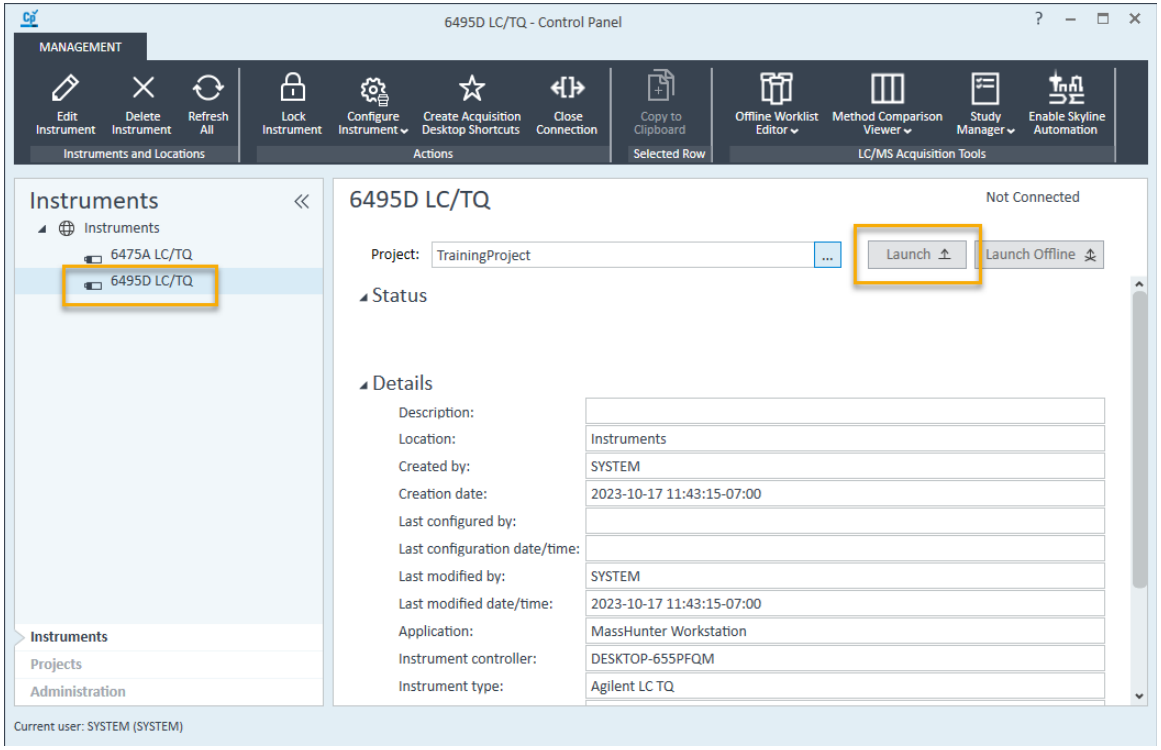

Instruments - Control Panel
MANAGEMENT
Edit Instrument
Delete Instrument
Refresh All
Instruments and Locations
Lock Instrument
Configure Instrument
Create Acquisition Desktop Shortcuts
Close Connection
Actions
Copy to Clipboard
Selected Row
Offline Worklist Editor
Method Comparison Viewer
Study Manager
Enable Skyline Automation
LC/MS Acquisition Tools
Instruments
Instruments
6475A LC/TQ
Instruments
Projects
Administration
Current user: SYSTEM (SYSTEM)
Create Instrument
Name:
6495D LC/TQ
Description:
Application:
MassHunter Workstation
Instrument controller:
DESKTOP-655PFQM
Instrument type:
Agilent LC TQ
Contact:
Default project:
Always use Default project
OK
Cancel

Launching an Instrument

Once you have added an instrument, launch the instrument to begin acquisition from the instrument table or the instrument details page, or launch an instrument directly from your desktop shortcut.

1 Click **Instruments** and select an instrument from the left panel.

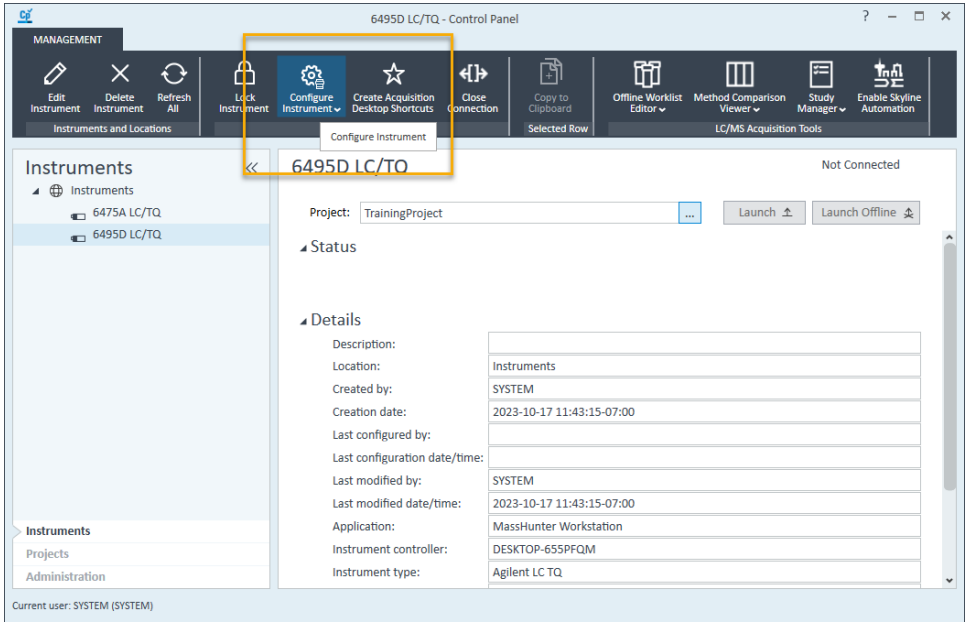
2 In the instrument windows, click the **Launch** button.



Create an instrument shortcut:

1 In the Control Panel, click Instruments and select the **6495D LC/TQ instrument or proper instrument name.** Verify that the correct Project is selected.

2 Click **Create Acquisition Desktop Shortcuts** in the Actions group on the ribbon. Two icons are added to the desktop with the name of the instrument and whether it is online or offline.



4 Tuning

Overview

When the LC/MS triple quadrupole is used as a detector for the LC, a mass spectrum is associated with each data point in the LC chromatogram. To obtain high quality, accurate mass spectra, the LC/MS triple quadrupole must be optimized to:

- Maximize sensitivity.
- Maintain acceptable resolution.
- Ensure accurate mass assignment.

What is tuning in LC/MS?

Tuning is the process of adjusting LC/MS triple quadrupole parameters to achieve the optimized goals listed above.

Tuning acts as a diagnostic tool to indicate the service or cleaning requirements of the spectrometer; it provides a chronicle of system performance, and the matching of fragments from a known calibration compound to adjust the mass axis so it agrees with the expected mass assignments.

What is the difference between autotune and checktune?

A checktune is run each day an analysis is performed. A checktune can be used to determine if the tuning mix ion masses are properly assigned and if the response or sensitivity of these ions is within expectations. In other words, A checktune performs a single profile scan of the tune masses and compares the peak widths and mass axes with target values to make sure they are correct before you start your acquisition. Checktune can be performed in either positive or negative ionization mode, or both.

Autotune only needs to run after preventative maintenance or if you find a problem with checktune. Periodically run an autotune to ensure that the mass spectrometer is working correctly. Autotune can be performed in negative and/or positive ionization.

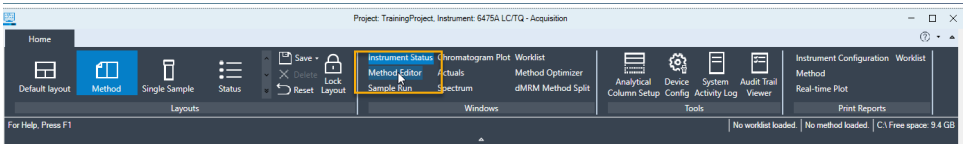
Frequent tuning, automated or manual, is not required. Once tuned, the LC/MS triple quadrupole is stable. Tuning should be needed no more often than monthly, weekly at most.

Wait ~12 hours after pumpdown before tuning or operating your LC/MS triple quadrupole system. The analyzer takes about 12 hours to reach thermal equilibrium. Tune files that are created, or data that is acquired, before the LC/MS triple quadrupole system is at thermal equilibrium may have incorrect mass assignments and other inaccuracies.

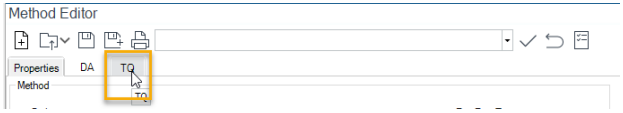
Calibrating the LC/TQ (checktune)

A checktune can be run with the following ion sources: ESI, AJS ESI, MMI, and APCI.

1 In MassHunter Data Acquisition window, click **Method Editor**.



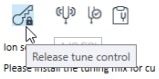
2 Click the **TQ tab**.



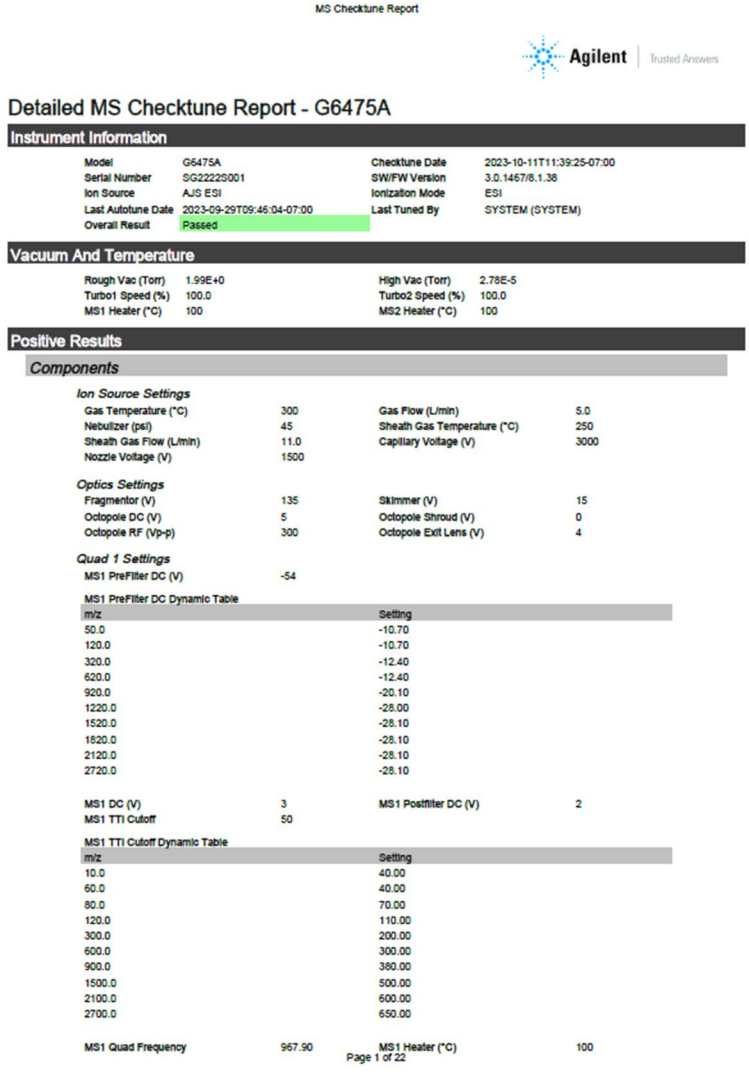
NOTE The process to complete a tune differs for the models covered in this introduction. Consult the user guide for detailed instructions to complete the checktune for the specific model installed

3 When the tune completes, review the report.

4 Click **Release tune control** in the toolbar to release control of the TQ instrument.



Example detailed checktune report



MS Checktune Report

Agilent | Trusted Answers

Detailed MS Checktune Report - G6475A

Instrument Information

Model	G6475A	Checktune Date	2023-10-11T11:39:25-07:00
Serial Number	SG2222S001	SW/FW Version	3.0.1467/8.1.38
Ion Source	AJS ESI	Ionization Mode	ESI
Last Autotune Date	2023-09-29T09:45:04-07:00	Last Tuned By	SYSTEM (SYSTEM)
Overall Result	Passed		

Vacuum And Temperature

Rough Vac (Torr)	1.99E+0	High Vac (Torr)	2.78E-5
Turbo1 Speed (%)	100.0	Turbo2 Speed (%)	100.0
MS1 Heater (°C)	100	MS2 Heater (°C)	100

Positive Results

Components

Ion Source Settings

Gas Temperature (°C)	300	Gas Flow (L/min)	5.0
Nebulizer (psi)	45	Sheath Gas Temperature (°C)	250
Sheath Gas Flow (L/min)	11.0	Capillary Voltage (V)	3000
Nozzle Voltage (V)	1500		

Optics Settings

Fragmentor (V)	135	Skimmer (V)	15
Octopole DC (V)	5	Octopole Shroud (V)	0
Octopole RF (Vp-p)	300	Octopole Exit Lens (V)	4

Quad 1 Settings

MS1 PreFilter DC (V) -54

MS1 PreFilter DC Dynamic Table

m/z	Setting
50.0	-10.70
120.0	-10.70
320.0	-12.40
620.0	-12.40
920.0	-20.10
1220.0	-28.00
1520.0	-28.10
1820.0	-28.10
2120.0	-28.10
2720.0	-28.10

MS1 DC (V)	3	MS1 Postfilter DC (V)	2
MS1 TTI Cutoff	50		

MS1 TTI Cutoff Dynamic Table

m/z	Setting
10.0	40.00
60.0	40.00
80.0	70.00
120.0	110.00
300.0	200.00
600.0	300.00
900.0	380.00
1500.0	500.00
2100.0	600.00
2700.0	650.00

MS1 Quad Frequency	967.90	MS1 Heater (°C)	100

Page 1 of 22

Review a Tune Report

Generate a detailed tune report and save

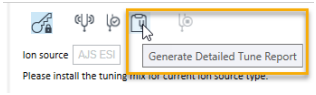
Generate a detailed tune report after you have run autotune or checktune.

1 In the Method Editor window, select the **TQ** tab.

2 Click the **Tune > Autotune** section in the left pane.

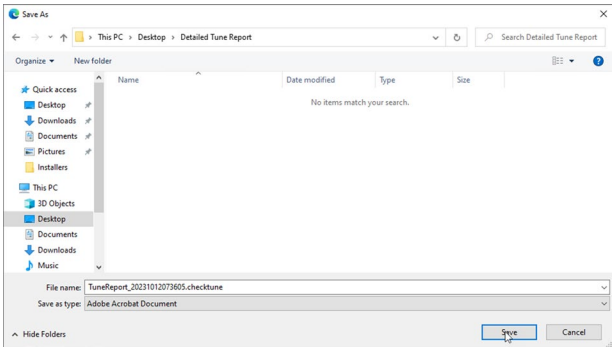
3 Click **Request tune Control** in the toolbar in the Autotune section.

4 Click **Generate Detailed Tune Report** in the toolbar.



5 The Detailed Tune report opens. Review the report in the browser window and click **Save As** to save the report in the desired directory.

6 Using the Save As dialog box, enter a file name and click **Save**.



7 Click **Release tune control** in the toolbar to release control of the TQ instrument.

NOTE

Only the polarities that were last autotuned or checktuned appear in the tune reports saved with the data files. For 6495D, prior detailed tune reports can be accessed through the Autotune section or Checktune section. For all other instruments, save tune reports manually before tuning in a single polarity.

Example detailed autotune report

Detailed MS Autotune Report - G6495D

Instrument Information

Model	G6495D	**Autotune Date**	2023-09-25T17:50:04-07:00
Serial Number	SG2305D301	**SW/FW Version**	3.1.551/9.4.56
Ion Source	AJS ESI	**Ionization Mode**	ESI
Tune Mode	Standard Quadrupole	**Overall Result**	Passed

Vacuum And Temperature

Rough Vac (Torr)	3.13E+0	**High Vac (Torr)**	2.33E-5
Turbo1 Speed (%)	100.0	**Turbo2 Speed (%)**	100.0
MS1 Heater (°C)	100	**MS2 Heater (°C)**	100

Positive Results

Components

Ion Source Settings

Gas Temperature (°C)	220	**Gas Flow (L/min)**	14.0
Nebulizer (psi)	20.0	**Sheath Gas Temperature (°C)**	150
Sheath Gas Flow (L/min)	11.0	**Capillary Voltage (V)**	3000
Nozzle Voltage (V)	1500		

iFunnel

Fragmentor (V)	166		
High Pressure iFunnel DC Drop (V)	10	**High Pressure iFunnel RF (Vp-p)**	150
Low Pressure iFunnel DC Drop (V)	100	**Low Pressure iFunnel RF (Vp-p)**	60
iFunnel Exit DC (V)	15		

High Pressure iFunnel Optics Modes

Mode	Setting
Fragile	50.00
Standard	100.00
Large Molecule	210.00

Low Pressure iFunnel Optics Modes

Mode	Setting
Fragile	50.00
Standard	100.00
Large Molecule	210.00

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Electron Multiplier Voltage (EMV)

To change the electron multiplier voltage (EMV), it is best practice to find these two values in the most recent detailed report. Using the detailed checktune report, answer the following questions:

1 What is the electron multiplier voltage standard (list both polarities if applicable.)?

---

2 In the dynamic gain table, what is the maximum gain and the corresponding voltage?

---

3 A best practice for troubleshooting and maintenance is to monitor the vacuum levels over time using data from the detailed tune reports. Locate the levels for the rough and high vacuum for your system.

a Rough vacuum:

---

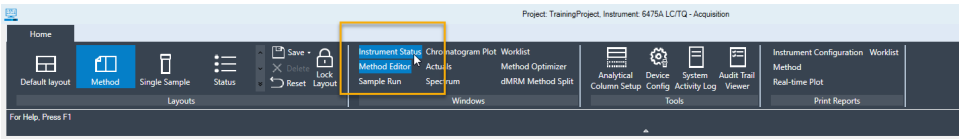
b High vacuum:

---

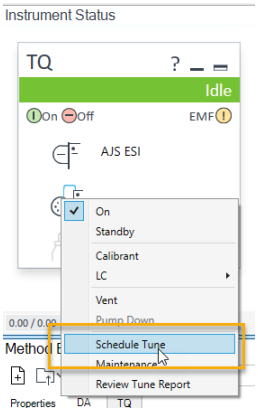
4 Locate the abundance in MS1 Peak Width Unit, Scan Speed Normal and list low middle and high mass numbers:

Scheduling a checktune

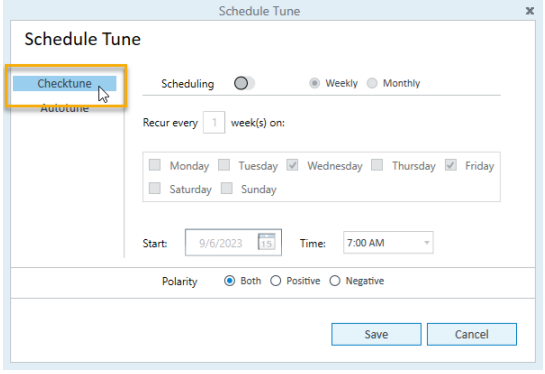
1 On the tool ribbon, click Instrument Status in the Windows section to display the Instrument Status window.



2 Right-click the TQ device in the Instrument Status window. Click **Schedule Tune**. The Schedule Tune Dialog Box opens.



3 Select Checktune in the left pane. The right pane shows the information for scheduling a checktune.



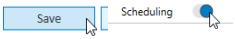
NOTE

The polarity is chosen for this instrument using the functionality **“Configure Tune - 6495D only” on page 43** of the user guide**.**

4 Click the Scheduling slider to switch on Scheduling. Select Weekly for this exercise.

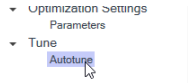
5 Select a day of the week and a Start date and time to indicate how often to schedule the checktune.

6 Click **Save.**

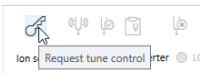


Stop checktune

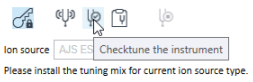
1 Click the Tune > Autotune section in the left pane.



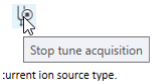
2 Click **Request tune Control** in the toolbar in the Autotune section. This button locks control of the TQ instrument. You cannot start a single sample run or a worklist when Tune has control of the TQ instrument.



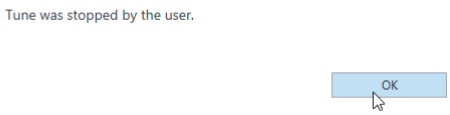
3 Click **Checktune the instrument** in the toolbar in the TQ Autotune section.



4 Before the tune completes, click **Stop Tune acquisition**.



5 When the checktune stops, the Tune Status window displays a date and time with the text “Tune was stopped by the user.” and a dialog box with the same message. Click **OK**.



6 Click **Release tune control** in the toolbar to release control of the TQ instrument.

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5 Using Methods

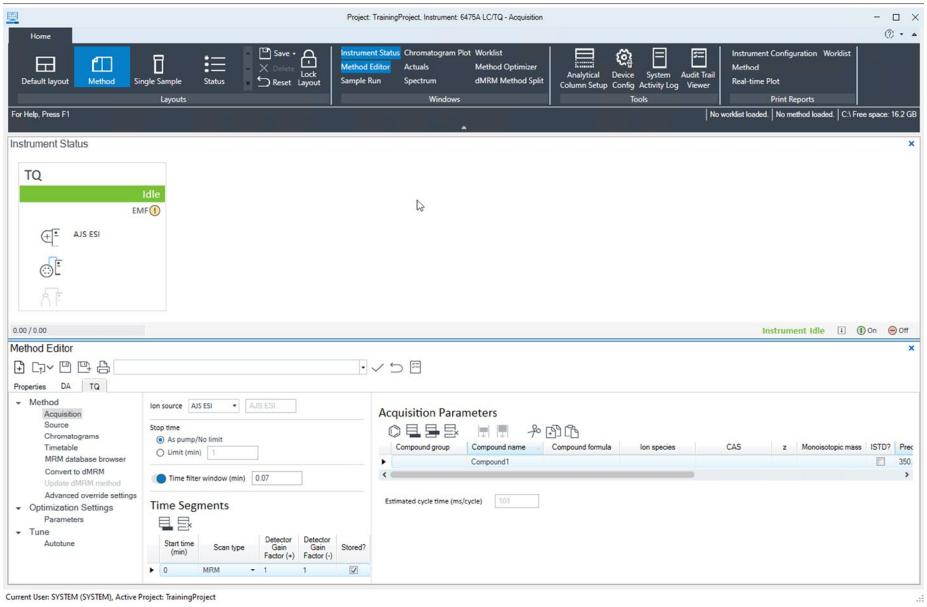
Overview

MassHunter Data Acquisition methods include the parameters for each component associated with your instrument.

The Method Editor Window

1. Launch the acquisition software: select OpenLab Control Panel > Instruments (bottom-left corner) > your instrument > click Launch. Alternatively, if available double-click the desktop shortcut.
2. In the Windows section, select Method Editor.

   The Method Editor window opens in the Main Window.



Set Up and Run an Acquisition Method

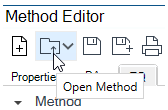
Working with the Default Method

Once an analysis has been created or opened, the default.m method is available to start from or apply a previously created method. The default method represents a good starting point for method development.

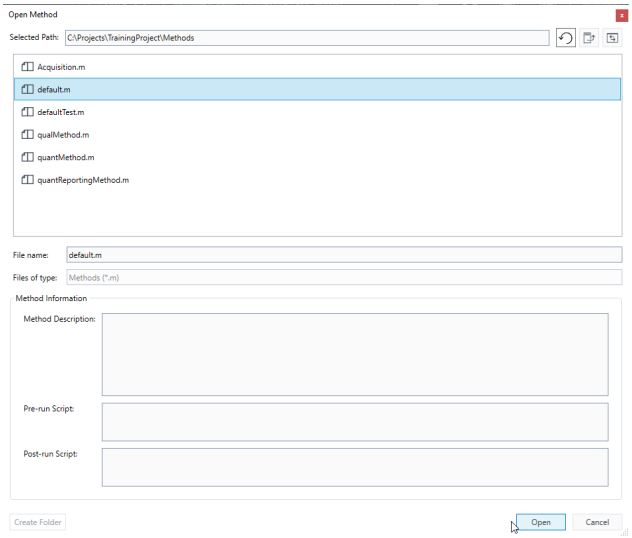
Load the default method

1 In the Method Editor window, click the **TQ** tab.

2 Click **Open Method** to review the methods available.



3 Select **default.m** and click **Open**.



4 Under Method, review all default Method subsections.

- **a** Acquisition - Set TQ acquisition parameters.
- **b** Source - Set source parameters for the TQ.
- **c** Chromatograms - Specify plots to display in the Chromatogram Plot window during the run.

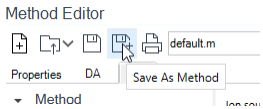
d Timetable - Specify when the diverter valve is set To MS and when it is set To waste.

e MRM database browser - Starts the MRM Database Browser program.

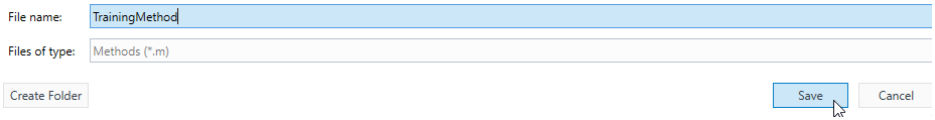
f Convert to dMRM - . This option is only available if the Scan type is dMRM or tMRM.

g Instrument Mode - Select an instrument mode to be saved with the method.

5 Click **Save As Method**.



6 Enter a **File name,** for this example *Training Method,* then click **Save**.



NOTE

After modifying or viewing a method using the drop-down list, you must apply the method to send the parameters to the instruments.

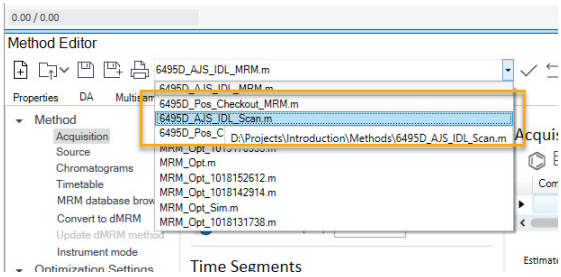
7 Click **Apply**.

Set up a Scan Method

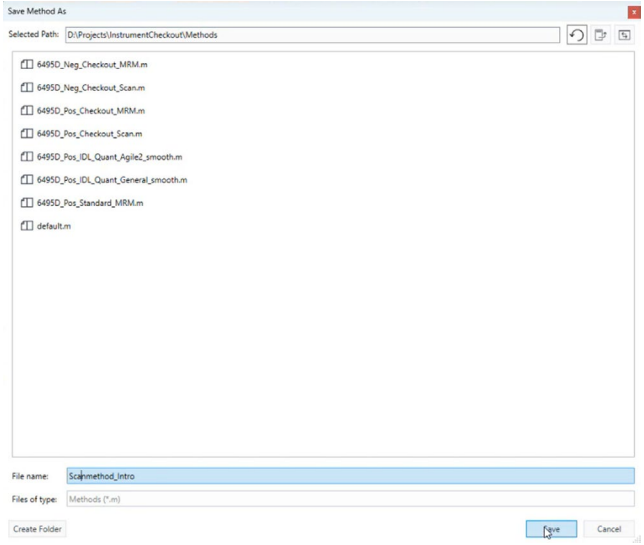
Load an existing method and Save As new method

In this exercise, you will use an existing method (the scan checkout method used during installation) to see if there is a signal for Reserpine at m/z 609 within the spectrum.

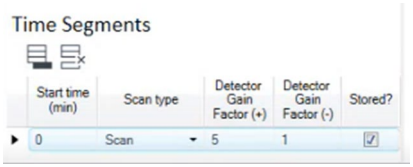
1 Under Method Editor, click **Recently opened methods** and select the checkout method used in installation, for example 6495D_AJS_IDL.Scan.m. The method loads into the Method Editor.



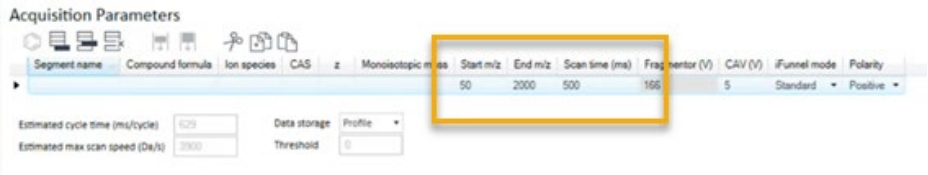
2 Click **Save as Method**, enter *Scanmethod_Intro* for the file name. Click **Save**.



3 Select TQ > Acquisition, then under Time Segments, confirm the settings as follows:



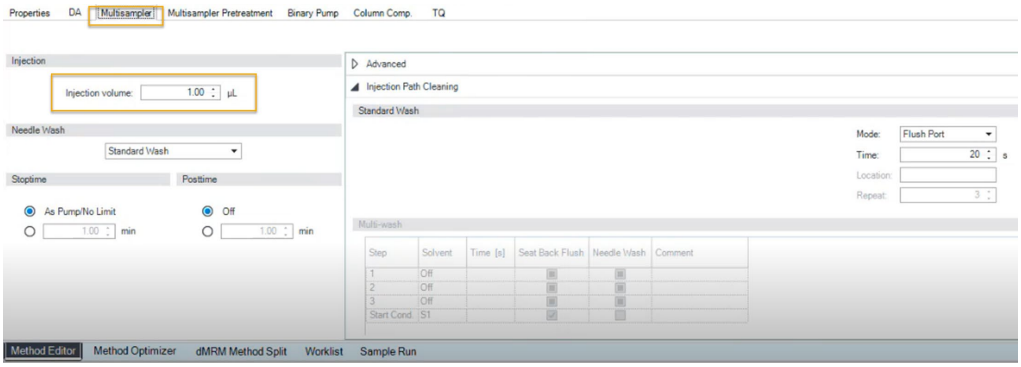
4 In Acquisition Parameters, review the acquisition settings, noting Start m/z and End m/z.



NOTE

Values will vary based on your instrument model.

5 Click Pump/Sampler/Column Comp settings. to review the settings programmed for the LC pump, noting the injection volume.



6 To send the current parameters shown in the Method Editor window to the LC and MS instruments, click Apply.

Running Methods

Run a Scan Method

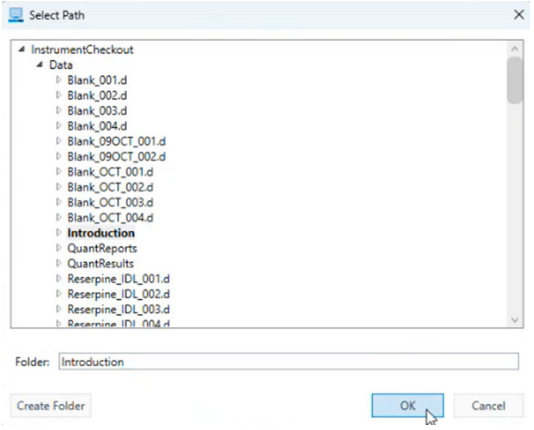
In this exercise, you will acquire data using MassHunter Data Acquisition software and then use MassHunter Qualitative Analysis software to identify a precursor ion for Reserpine.

1. Place the checkout sample (first-level dilution), prepared during the system checkout, into a vial location of the sampler and note the location.
2. In the main window, click the Sample Run tab to display the Sample Run window.
3. In the Sample Run window, specify the following information:
   - **a** Sample Name: Dilution 1
     Sample Position: Vial 4 (or applicable position)
   - **b** Sample Injection Volume: Select As Method to use the volume specified in the method applied in the last step.
   - **c** Data File Name: Introduction_Scan_001.d

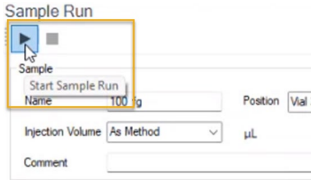
NOTE

(optional) Select Auto Increment to automatically increment the file name if that file exists

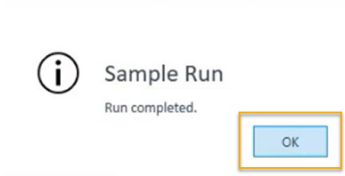
   - **d** Data File Path: Set to D:\Projects\InstrumentCheckout\Data\Introduction. Create a folder if necessary.



4 Click **Start Sample Run**.



5 Click **OK** when the run completes.



Monitor the Status Windows

As data is being acquired, use the instrument status monitors and online signal displays available in the Instrument Status and Real-time Plot Panes to observe changes in modules.

1 View the Chromatogram Plot and note the retention time for Reserpine.

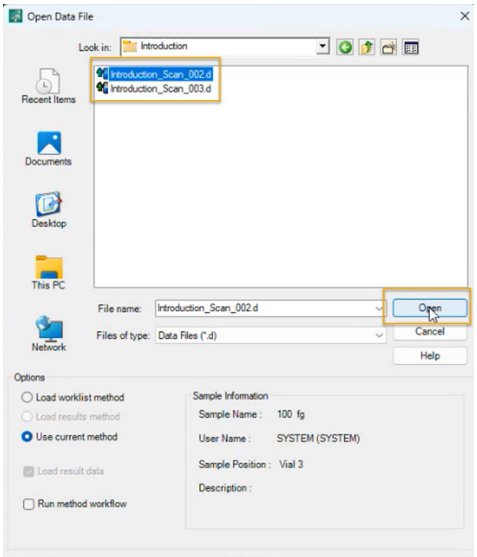
2 Observe the Spectrum window while the samples runs. Discuss with your Agilent-certified service professional the changes observed over time.

Review the data using Qualitative Analysis

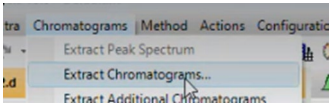
1 From the desktop, double-click the Qualitative Analysis icon 

.

2 In the Open Data File window, browse to the data file directory created earlier (Introduction), select the data file to review, and click **Open**.

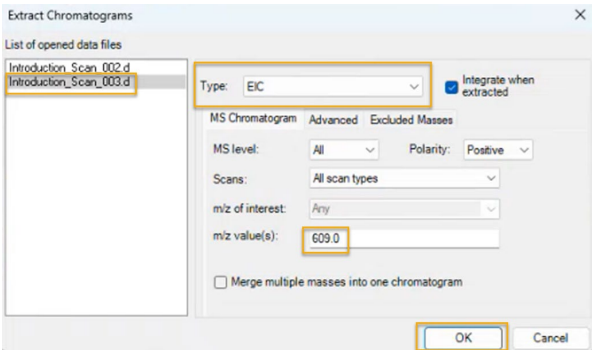


3 In the main window, click **Chromatograms > Extract Chromatograms**.

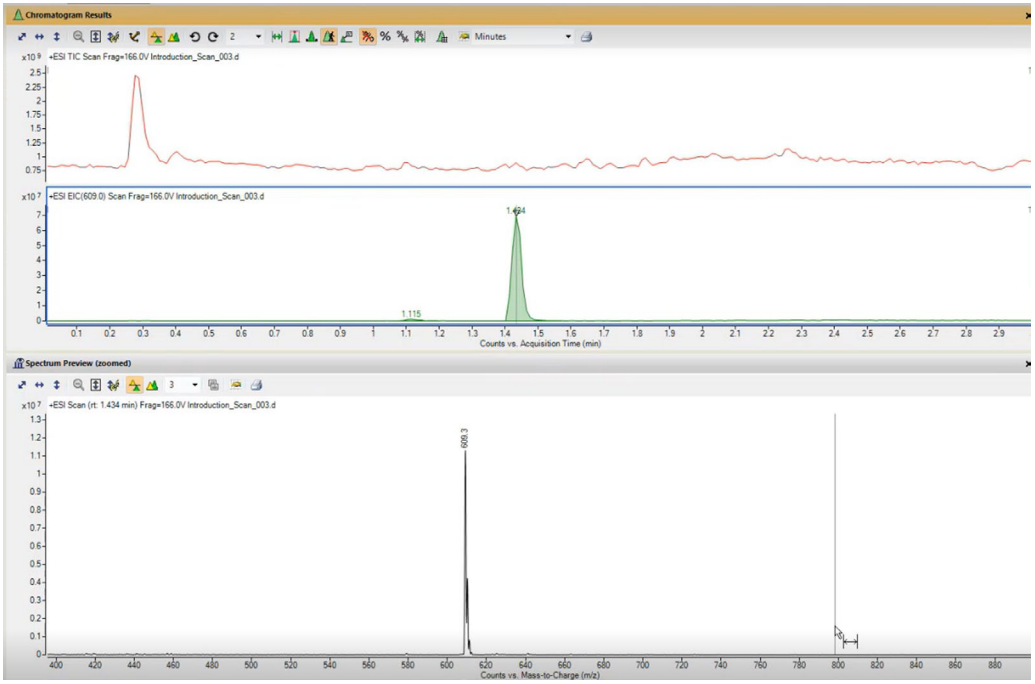


4 In the Extract Chromatograms dialog box, click **Type**: and select **EIC**.

5 Enter the m/z value: *609*, then click **OK**.



6 Review the results.



Review the Results.

1 What is the retention time for Reserpine?

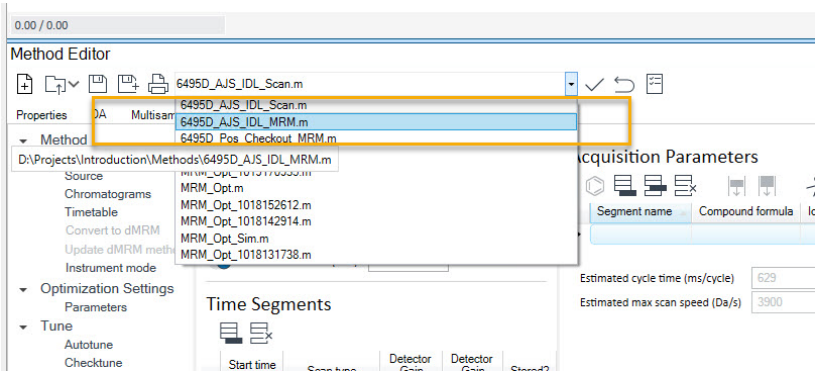
2 What is the m/z observed for Reserpine in the mass spectrum?

Set up an MRM Method

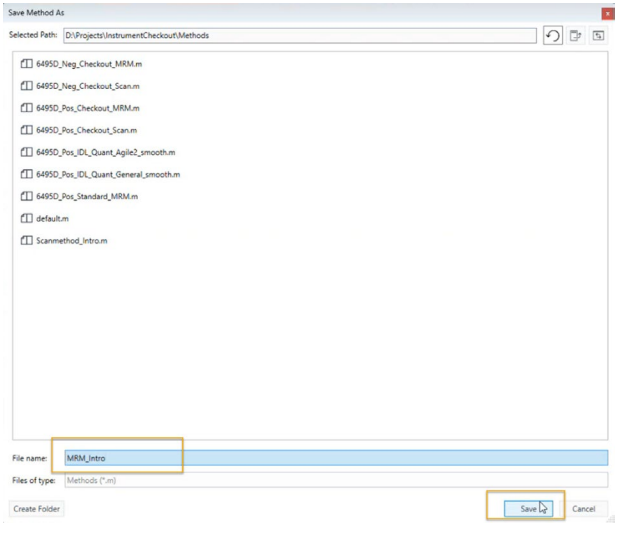
Load an existing method and Save As new method

Now that the precursor ion is identified, start with a known MRM (multiple reaction monitoring) method using product ion m/z195.

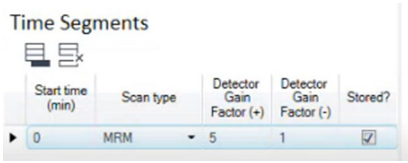
1 In MassHunter Data Acquisition, under Method Editor, click **Recently opened methods** and select the checkout MRM method used during installation, for example, 6495_ADS_IDL.MRM.m. The method loads into the Method Editor.



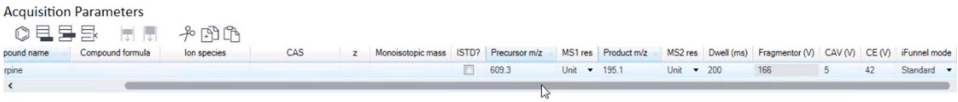
2 Click **Save as Method**, enter *MRM_Intro* for the file name. Click **Save**.



3 Select TQ > Acquisition, then under Time Segments, confirm the settings as follows.

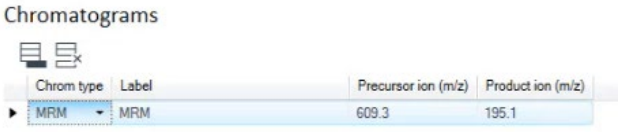


4 In Acquisition Parameters, review the acquisition settings, noting Precursor m/z, Product m/z, Fragmentor, and Collision Energy.



NOTE Values will vary based on your instrument model.

5 Click Chromatograms and confirm the settings below.



6 Save the method, then click Apply to send the current parameters shown in the Method Editor window to the LC and MS instruments.

7 Place the checkout sample (third level dilution) prepared during the system checkout into a vial location of the sampler and note the location.

8 In the main window, click the Sample Run tab to display the Sample Run window.

9 In the Sample Run window, specify the following information:

a Sample Name: *Dilution 3*
Sample Position: Vial 2 (or applicable position)

b Sample Injection Volume: Select As Method to use the volume specified in the method applied in the last step.

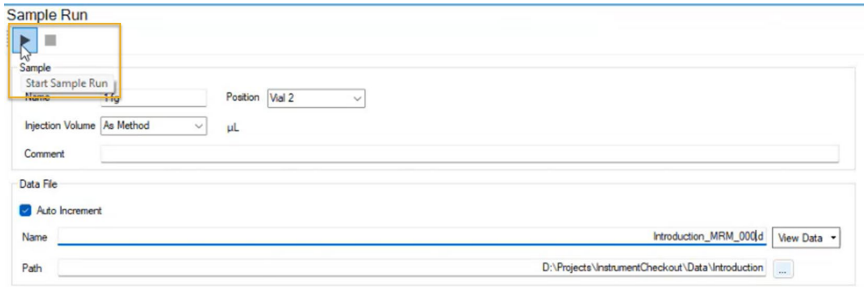
c Data File Name: *Introduction_MRM_000.d*

NOTE

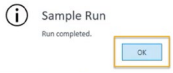
(optional) Select Auto Increment to automatically increment the file name if that file exists.

d Data File Path: Set to **D:\Projects\InstrumentCheckout\Data\Introduction**.

10 Click **Start Sample Run.**

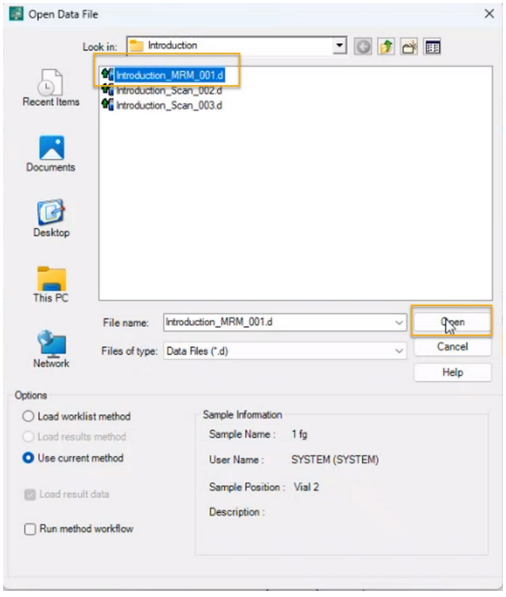


11 Click **OK** when the run completes.

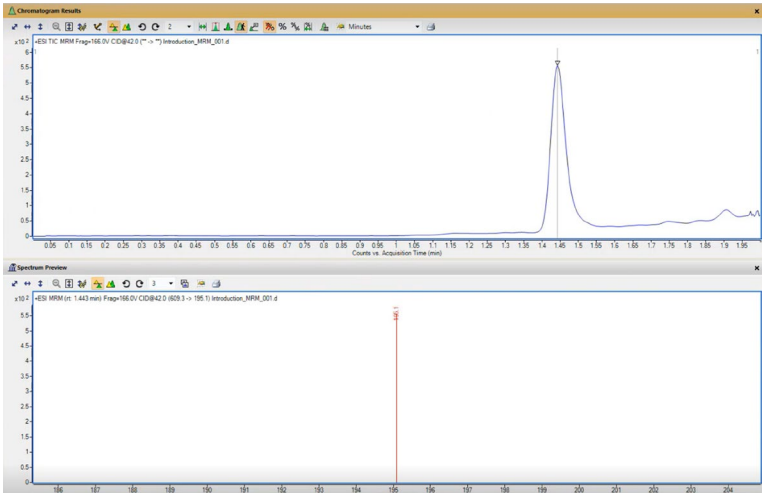


Reviewing the MRM data using Qualitative Analysis

1 In Qualitative Analysis, click **Open Data File** and browse to the data file directory, select the data file to review, and click **Open**.



2 Walk through the Chromatogram results to review the mass spectrometry data.



Review the Results.

1 What is the retention time for Reserpine?

---

2 Which ion is present in the mass spectrum?

Offline Method Editor program

It allows you to edit a method while the system is running a worklist or a single sample. The method shows the devices currently connected to the system. You cannot edit the parameters of any devices which are not currently connected. The system engines must be running.

To get here, do one of the following:

- In Agilent Control Panel, select an LC/MS instrument and click Launch Offline.
- In Agilent Control Panel, create an Acquisition shortcut, and then open that shortcut from your Desktop.

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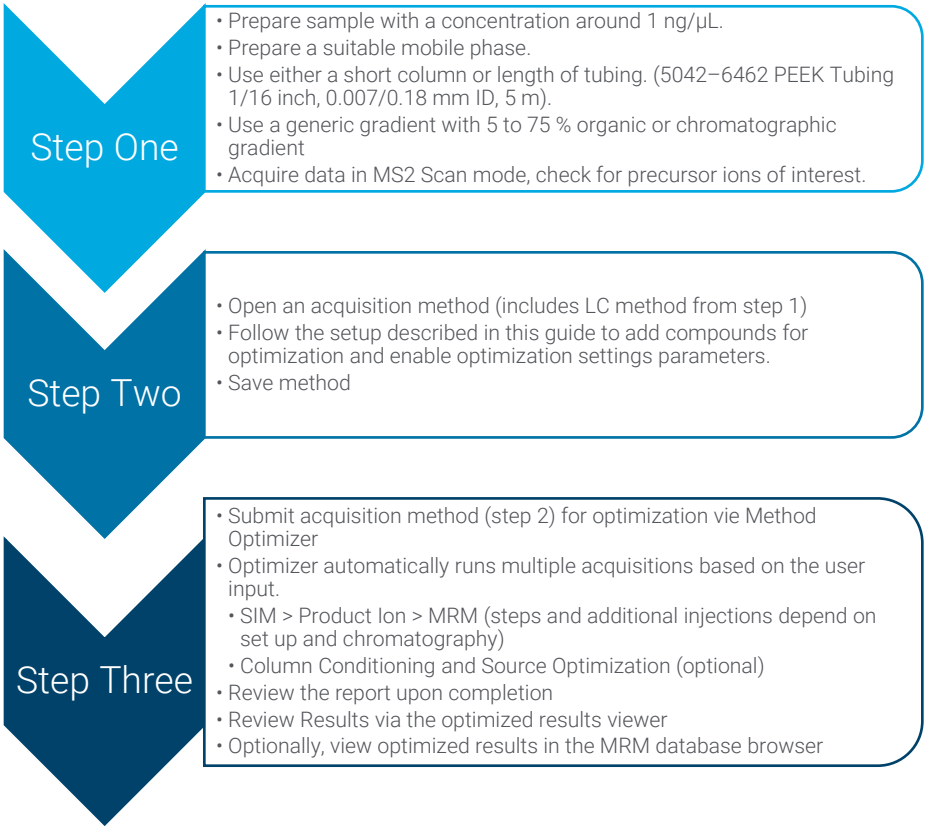
6 Optimizing Methods

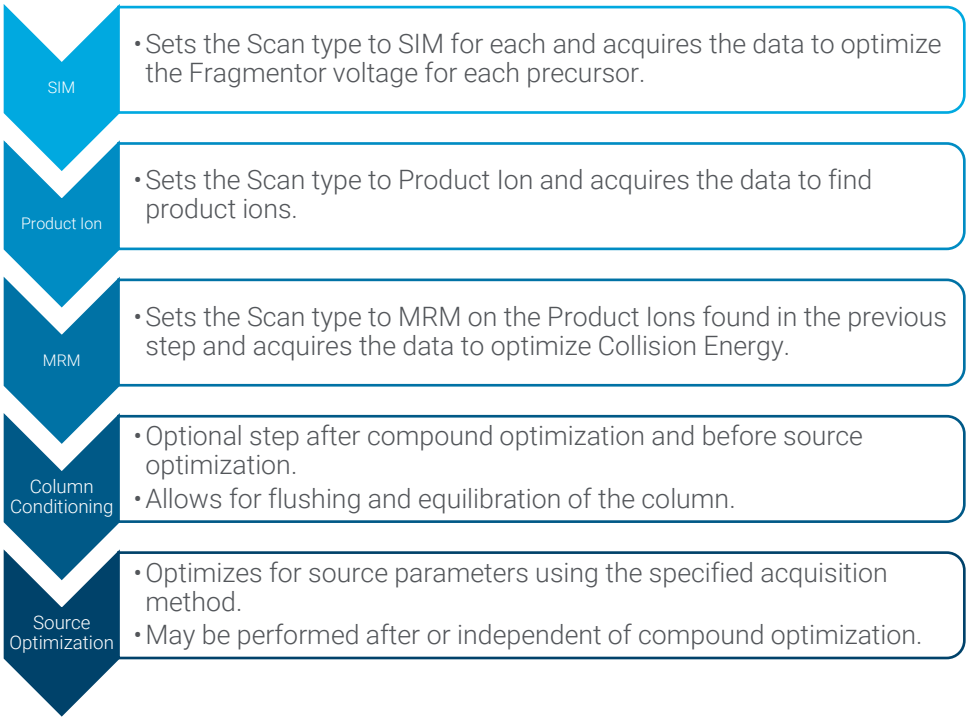
MassHunter Optimizer lets you automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on a triple quadrupole mass spectrometer instrument for each individual compound analyzed. Specifically, it automates the selection of the best precursor ions, the optimization of the fragmentor voltage for each precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify. You can also optimize source parameters.

The Optimizer workflow



Optimizing Methods

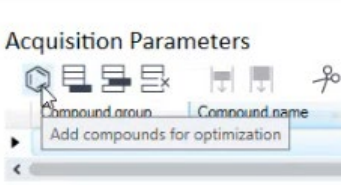
When Optimizer starts, it automatically proceeds through the following scan types, in the order shown. Steps may be skipped depending on model and type of optimization performed. If needed, additional injections will be added automatically.



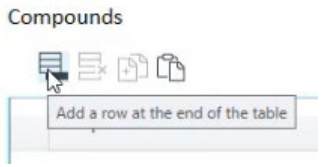
Optimize a method for a known sample:
Optimizer allows the quick development of methods using optimal instrument parameters. Starting with known compounds in a mixture, use the automated function to produce a report and an optimized MRM method. Fragmentor (V) (if applicable), product ion, and collision energy are optimized via compound optimization and capillary voltage via source optimization in the following exercise.

1 In MassHunter Acquisition, save the MRM method created in the prior exercise as *MRM_Opt.m*

2 Under Method Editor > Acquisition Parameters, click **Add compounds for optimization**.



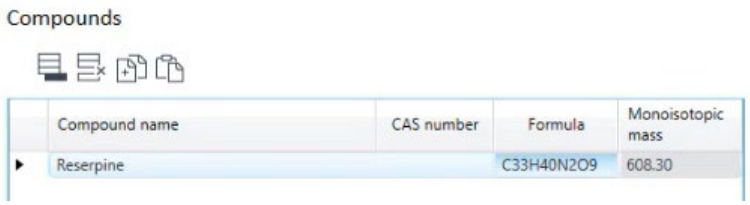
3 The Add compounds for optimization window opens. Click **Add a row at the end of the table**.



4 In the Compounds section, click in the fields and enter the following information:

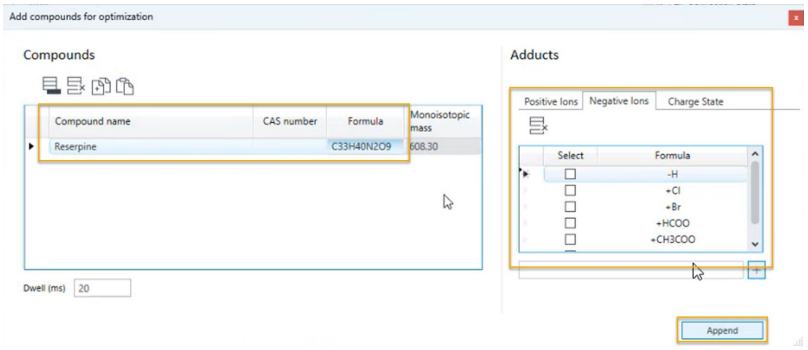
a **Compound name**: *Reserpine*

b **Formula:** *C33H40N2O9*

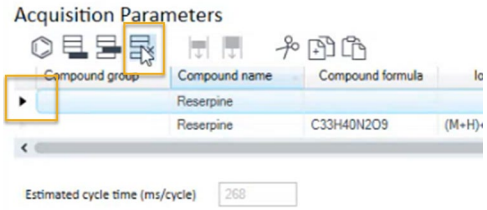


5 In the Adducts section, click the Positive Ions tab and select **+H**, then the Negative Ions tab and deselect **-H**.

6 Click **Append** to add the information to the acquisition parameters pane.

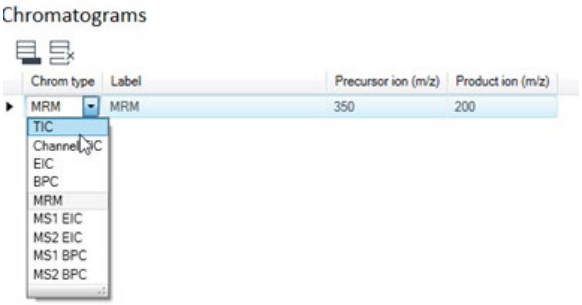


7 Remove the original parameter line by selecting the line, then click **Remove**.

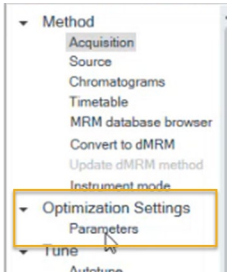


8 Review the imported data. Note the default values and added information.

9 Click **Chromatograms**, click the **Chrom type** drop-down arrow and select **TIC**.



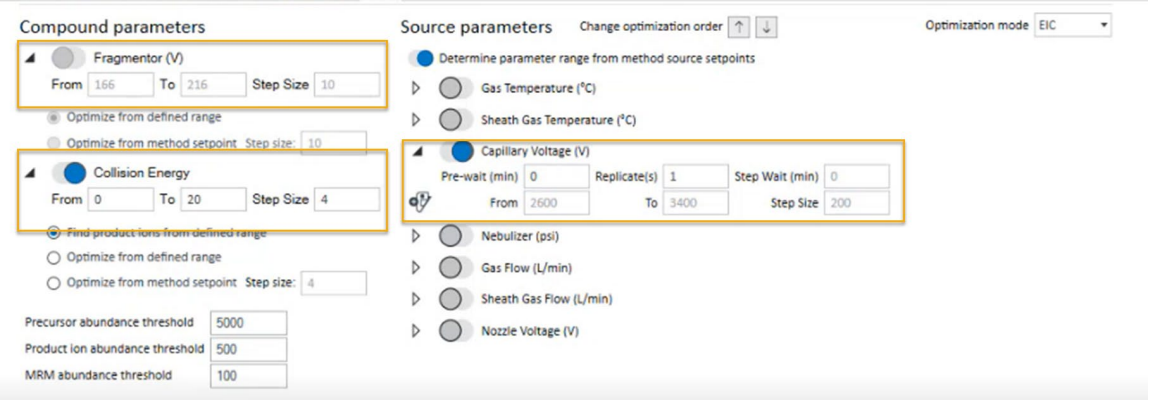
10 On the left pane, navigate to **Optimization Settings > Parameters**.



11 In Compound Parameters, enable and set the following:

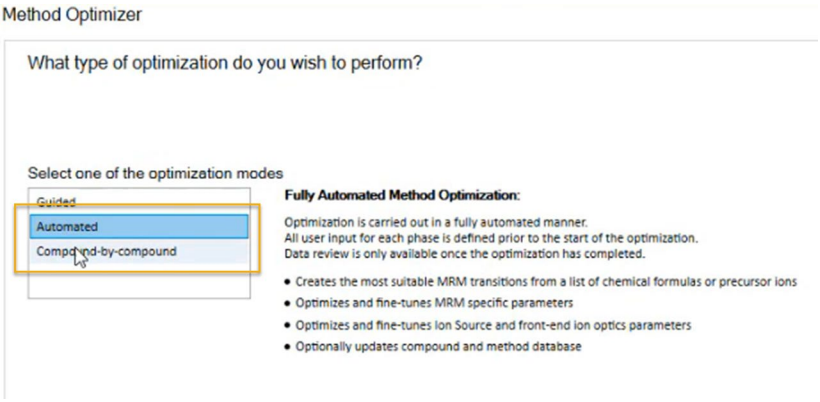
- **a** Fragmentor V (if available): From 120, to 220, step size: 5
- **b** Collision Energy Range: From 0, to 50, step size: 5
- **c** Capillary Voltage: Leave defaults

Settings	Ultivo	6475A	6495D
Fragmentor (V)	130 to 190	150 to 250	166 (fixed)
Collision Energy	30 to 50	30 to 50	30 to 50
Capillary Voltage	3600 to 4400	3500 - 4300	3100 to 3900



12 Click **Save** to save the method.

13 Click the **Method Optimizer** tab and select *Automated*.

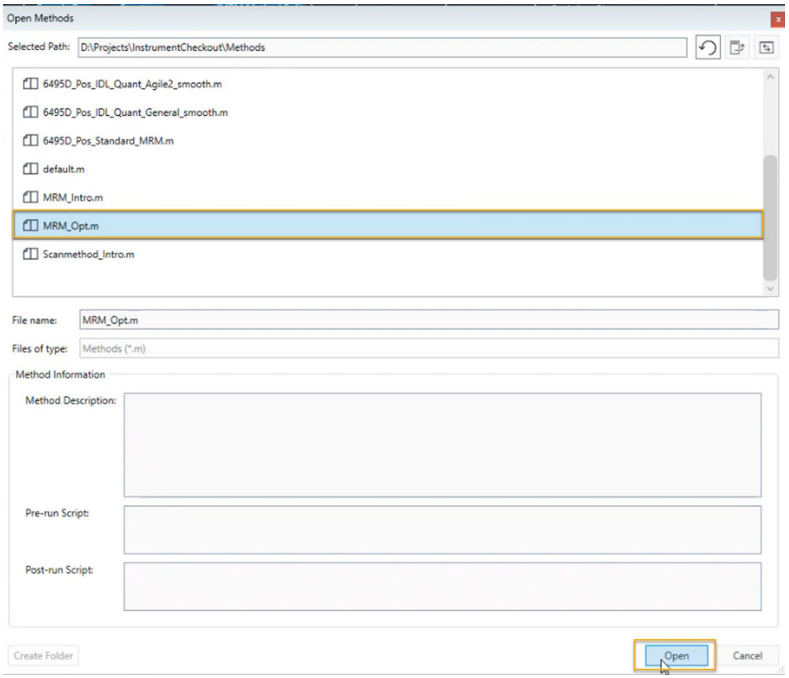


14 Click **Next**.

15 Select **MRM Database or enter a new database** field and enter *Introduction*.

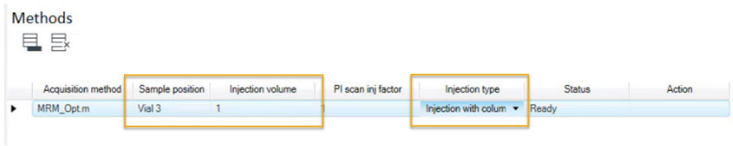
16 Under Methods, click **Add a row at the end of the table**.

17 In the Open Methods dialog box, select **MRM_Opt.m** and click **Open**.



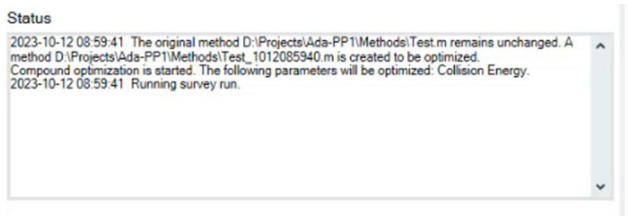
18 In the Methods pane, verify the following settings:

- **a** Sample position: *Dilution 1*
- **b** Injection volume: *1*
- **c** PI scan inj factor: *1*
- **d** Injection type: **Injection with column**

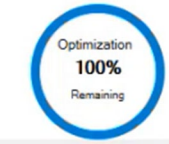


19 Click **Next**. Method optimization begins.

20 As the Method Optimization runs, review the Status.



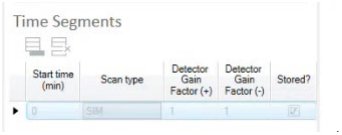
21 Observe over time the Optimization Remaining.



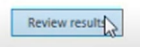
22 Navigate to **Method Editor > Acquisition** to observe the mass spectrum processing.

a Review the TIC plots.

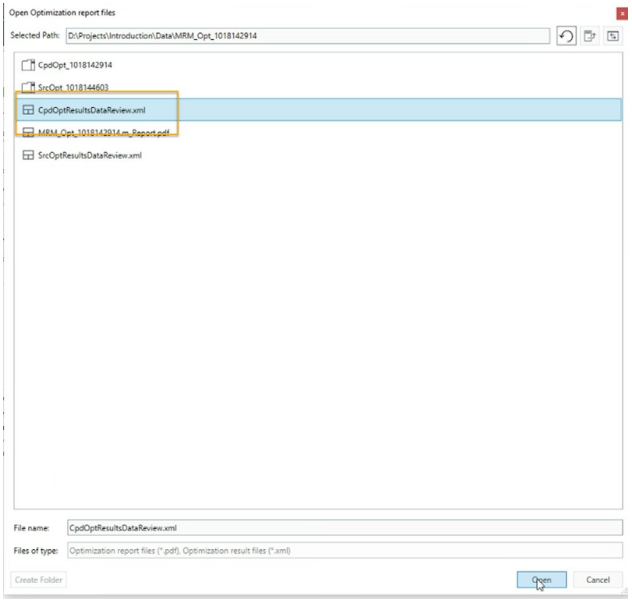
b Review the Scan Type being used in the Time Segment panel



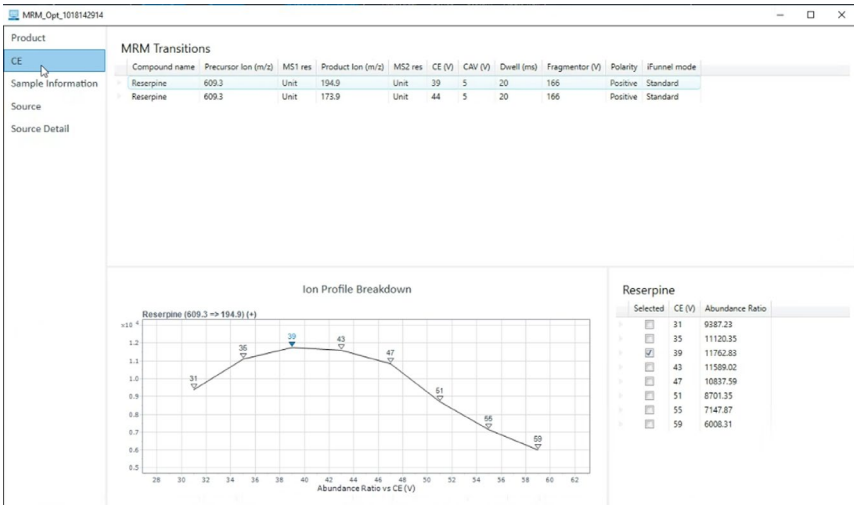
23 Once complete, click **Review results** to review the generated optimized MRM report.



24 Select a report file from the Open optimization report files window and click **Open**.

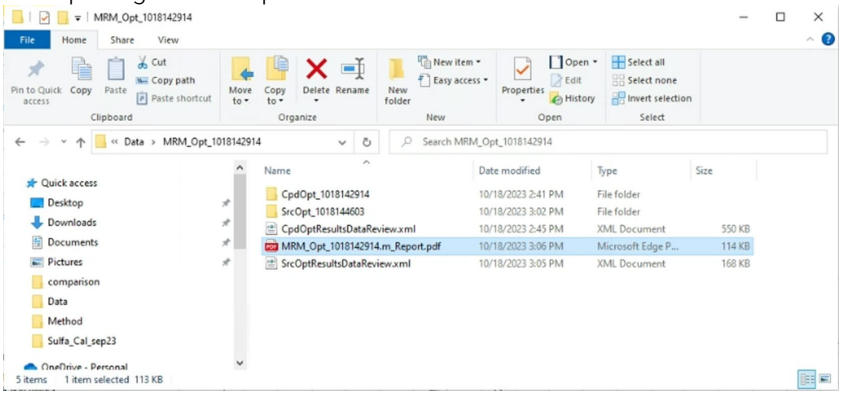


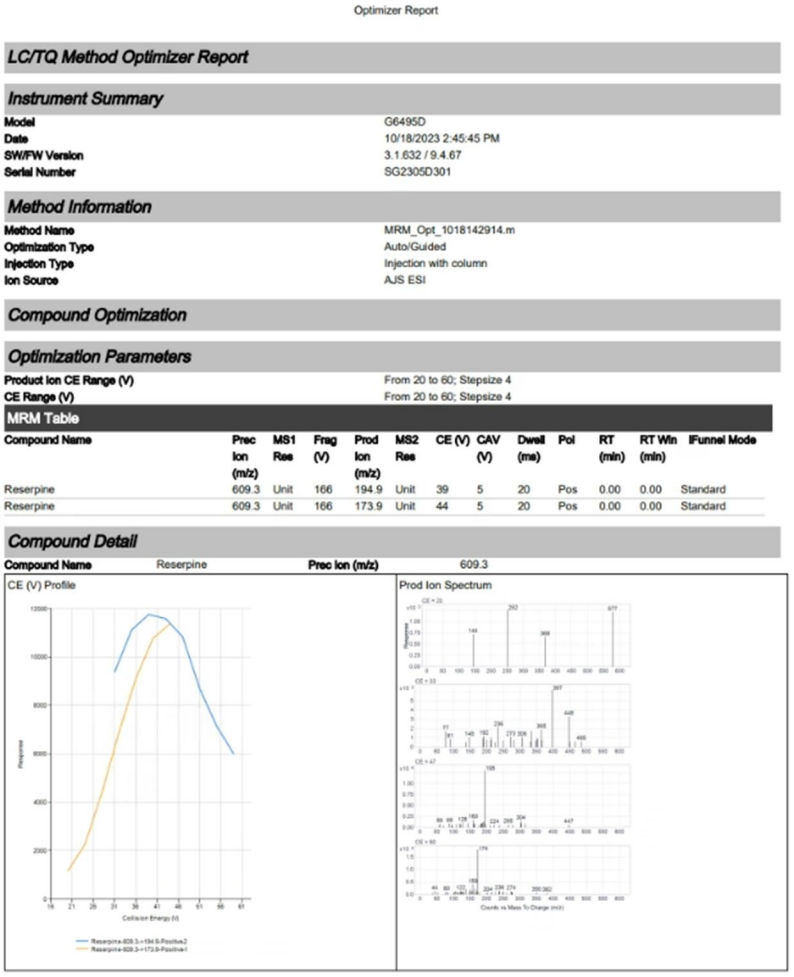
25 Review the results.



NOTE

A full report can be viewed by browsing to the directory for the project and opening the full report with a PDF viewer.





Optimizer Report

LC/TQ Method Optimizer Report

Instrument Summary

Model	G6495D
Date	10/18/2023 2:45:45 PM
SW/FW Version	3.1.632 / 9.4.67
Serial Number	SG2305D301

Method Information

Method Name	MRM_Opt_1018142914.m
Optimization Type	Auto/Guided
Injection Type	Injection with column
Ion Source	AJS ESI

Compound Optimization

Optimization Parameters

Product Ion CE Range (V)	From 20 to 60; Stepsize 4
CE Range (V)	From 20 to 60; Stepsize 4

MRM Table

Compound Name	Prec Ion (m/z)	MS1 Res	Frag (V)	Prod Ion (m/z)	MS2 Res	CE (V)	CAV (V)	Dwell (ms)	Pol	RT (min)	RT Win (min)	IFunnel Mode
Reserpine	609.3	Unit	166	194.9	Unit	39	5	20	Pos	0.00	0.00	Standard
Reserpine	609.3	Unit	166	173.9	Unit	44	5	20	Pos	0.00	0.00	Standard

Compound Detail

Compound Name Reserpine **Prec Ion (m/z)** 609.3

CE (V) Profile

Prod Ion Spectrum

Method Editor Optimization

1. What Scan types are used during optimization?
2. Identify the optimized values for the sample in results viewer and the optimized method:
    - Fragmentor (V)
    - Collison Energy
    - Capillary Voltage

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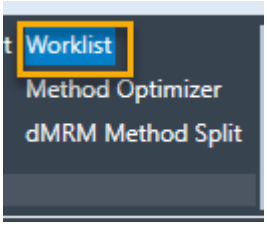
7 Run a Worklist

Overview

Use the Worklist window to create a list of samples to run. A worklist can be created using either the Study Manager Program or the Worklist window. See Study Submitter Dialog Box for more information on creating a worklist through the Study Manager. Use this procedure to inject multiple samples by creating a new worklist.

Create and edit a worklist

1 In MassHunter Acquisition, click **Worklist** to show the Worklist window.

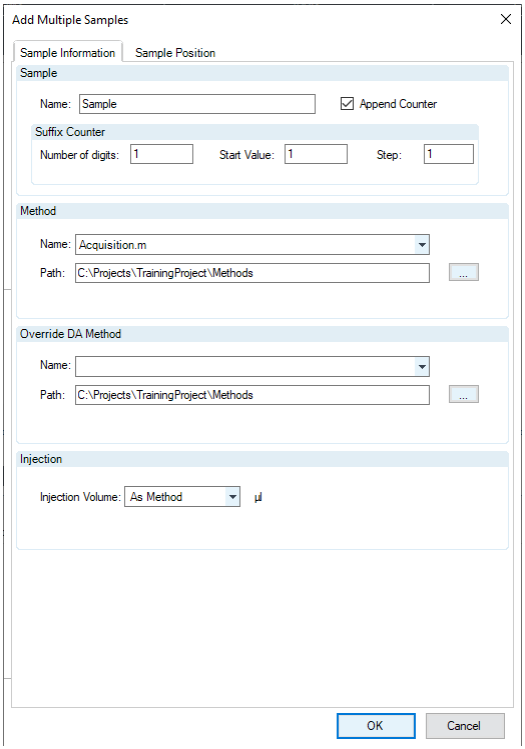


2 In the worklist window, click **(Add Multiple Samples)**. The Add Multiple Samples dialog box opens.

NOTE

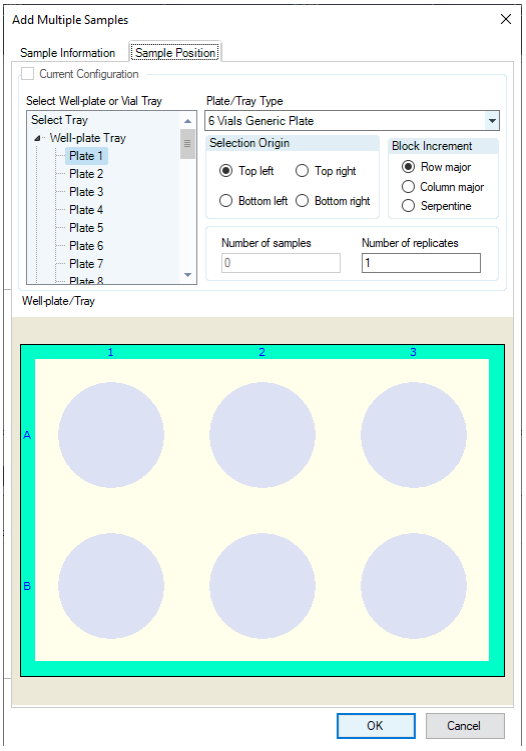
Samples can also be added one-by-one (user only needs to run a few samples, or several replicates of the same sample).

3 Enter all the information on the Sample Information tab.



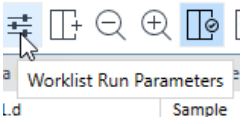
Add Multiple Samples

1 On the Sample Position tab, specify the sample vial locations (make sure the specific sample tray type has been configured by right clicking the autosampler device image).

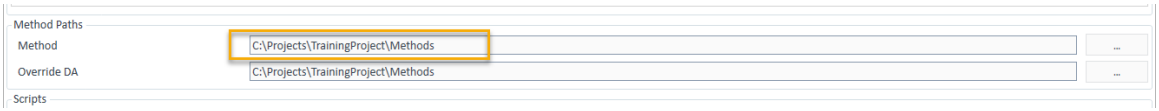


2 Specify the locations and click **OK**.

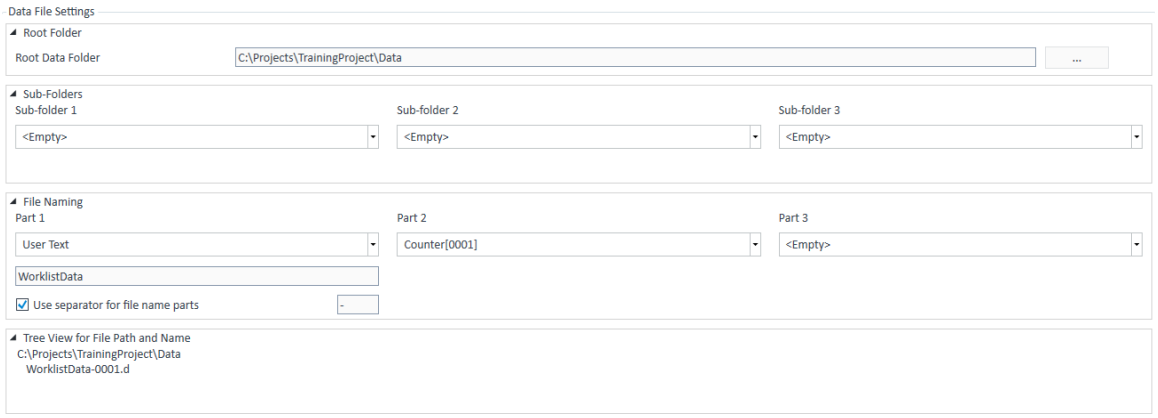
3 To set up the worklist run, click **Worklist Run Parameters**.



4 On the Run Parameters tab, type the paths for the method.

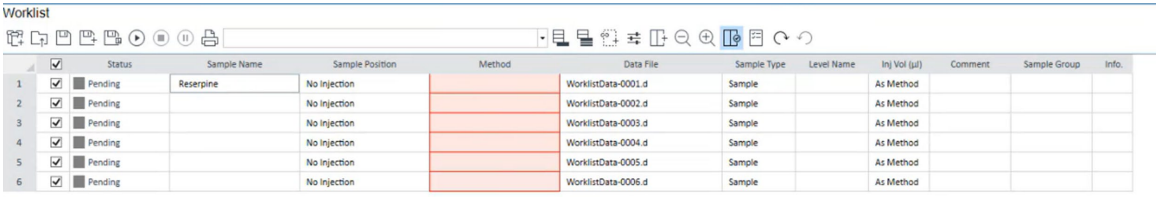


5 On the Data File Settings section, expand all options and enter or select the folders for the data files. Select the File Naming options. Click **OK**.



NOTE For information on the Intelligent Reflex tab, see the online Help

a Optional. On the Additional Parameters tab, enter a comment, and click **OK**.



6 To start the worklist, click **Run Worklist**.

NOTE

To use an acquisition method that has a different data analysis (DA) method than the method entered in the worklist, show that the column called Override DA Method in the worklist using the Show/Hide/Order Columns dialog box. In this column, browse for and select the method containing the DA parameters you want to use for the sample. The DA part of this method is used instead of the DA part of the current method. Or select this method in the Add Multiple Samples dialog box.

Study Manager

The Study Manager application lets you create a queue of studies to execute sequentially. A study is a collection of samples and operations that are grouped and contains the following information:

- System folder that contains platform files
- Data files
- Optimizer output files and methods
- Quant results
- Quant method File

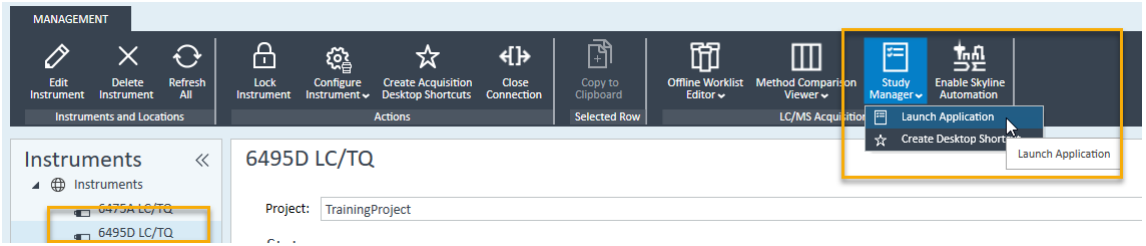
The study types create and run a worklist that to analyze the samples. Specify more information to apply to the study, including a location to place all the files generated by that study or create several types of studies. When the Study Manager application starts, the data acquisition engines are automatically started.

NOTE

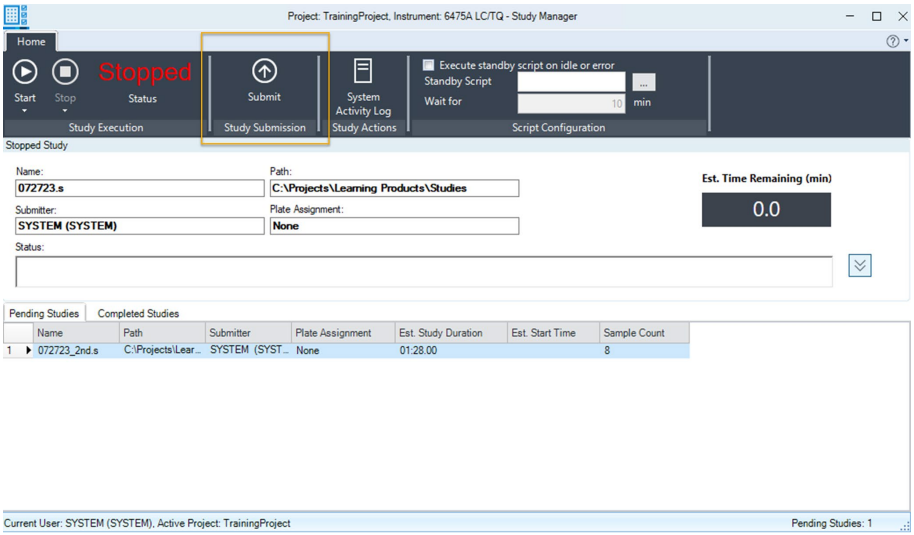
The Intelligent Reflex workflows are not supported in the Study Manager program

Create a Worklist Only Study

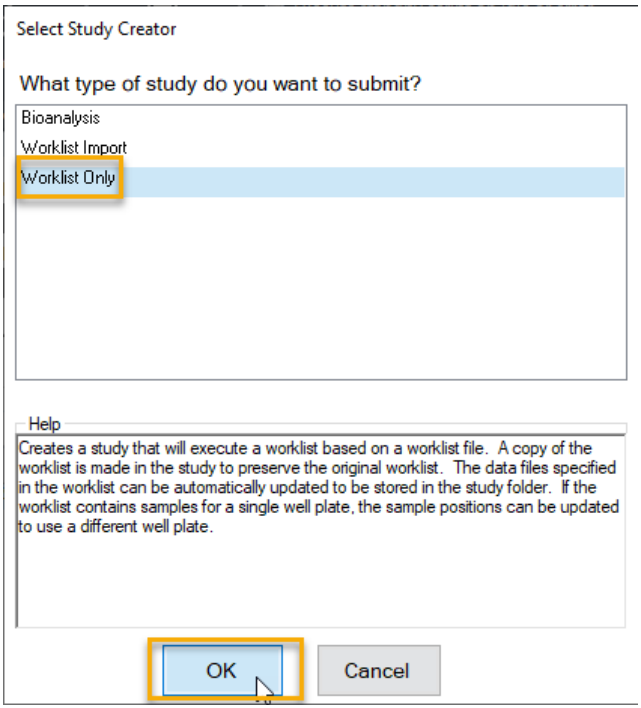
1 In OpenLab Control Panel, select the instrument then click **Study Manager > Launch Application**.



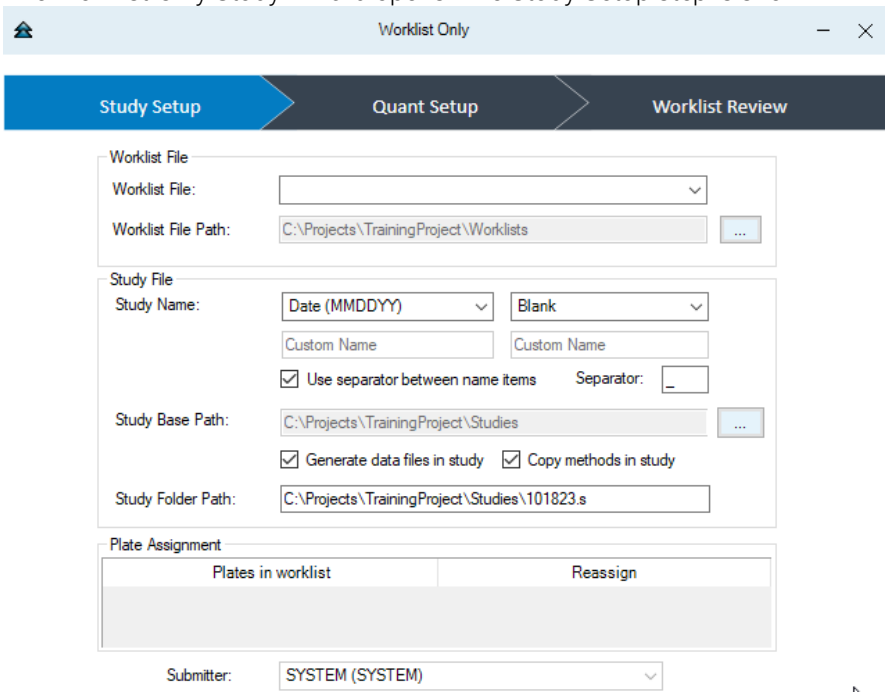
2 To select a study, click **Submit**.



3 In Study Creator, select a **Worklist Only**, then click **OK**.



4 The Worklist-Only Study Wizard opens. The Study Setup step is shown.



Demonstration of Study Manager

Working with your Agilent-certified service professional, create a study to execute a work list based on a worklist file.

1. Can sample positions be updated to used alternate microplates?
2. What benefits are there to specifying the name of the study or the name of the person submitting the study?

8 Use MassHunter Quantitative Analysis to Generate Calibration Curves

Overview

In this exercise, set up a quantitation method for a batch of acquired data files. Conduct the exercise with the DrugsOfAbuse data files and learn how to perform the following tasks:

- Set up a Batch Table containing unknown sample and calibration data files for drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up target compounds.
  - View the MRM transitions and chromatographic parameters for the compounds in the data file.
  - Set up an internal standard for each of the compounds.
  - Set up quantitation for the method.
  - Create levels from calibration samples.
  - Set up qualifier ions and the calibration curve.
- Quantitate the batch and save the results.

Before You Begin These Exercises

Be sure the data files that you will be using as you complete the exercises in this document are on your PC.

- If the default MassHunter Quantitative Analysis Software Supplemental installation was completed, the data files needed for these exercises should be present in MassHunter/Data/QuantExamples.
- If the default MassHunter Quantitative Analysis Software Supplemental installation was not completed, you can copy the data from the installation media (Supplemental/MassHunter/Data/QuantExamples) to the Data folder within the Training Project created in the prior exercise.

Set up a New Batch

Set up a Batch Table containing data files for three unknown samples and several calibration samples of drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.

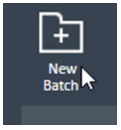
Create a batch to hold samples

1 To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ) icon** on your desktop.

2 Select a Project and click **OK**.

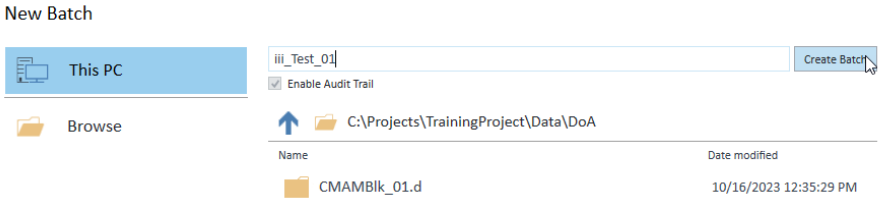
Quantitative Analysis for QQQ opens.

3 Click **New Batch.** The system opens the New Batch dialog box.



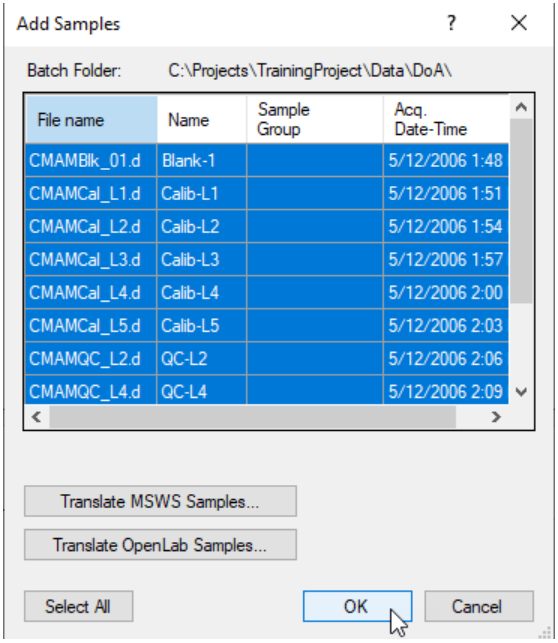
4 Navigate to the folder \Your Directory\DrugsOfAbuse\DoA.

5 Enter a batch file name, for this example *iii_Test_01* and click **Create Batch**.

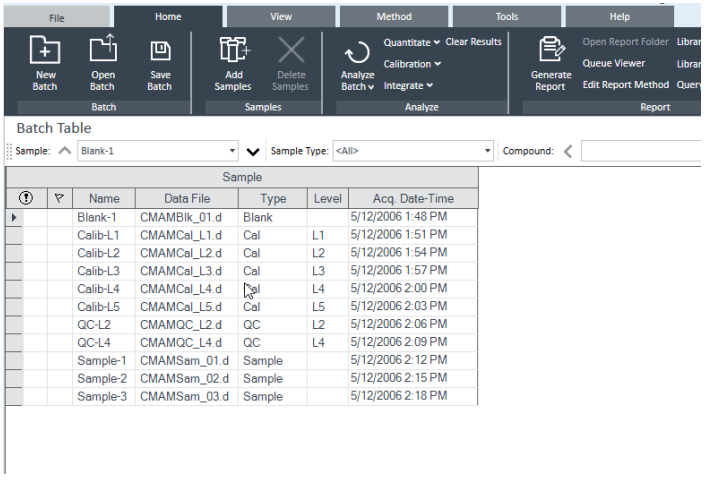


Add all the samples in the DrugsOfAbuse folder to the batch

1 All Samples are selected. Click **OK** to add them to the batch.



2 The Batch Table now contains the blank, calibration, QC, and unknown samples.

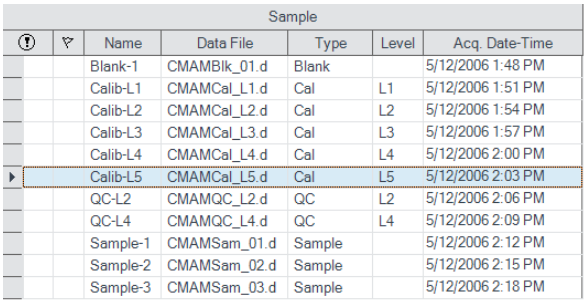


Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on the calibration data file with the highest concentration of sample.

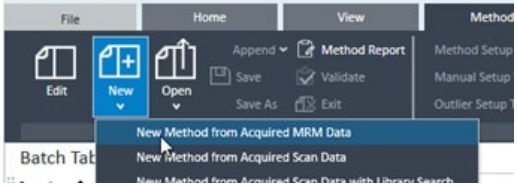
Create a method from acquired MRM data.

1 Use the cursor to highlight the calibration standard that has the highest concentration level.



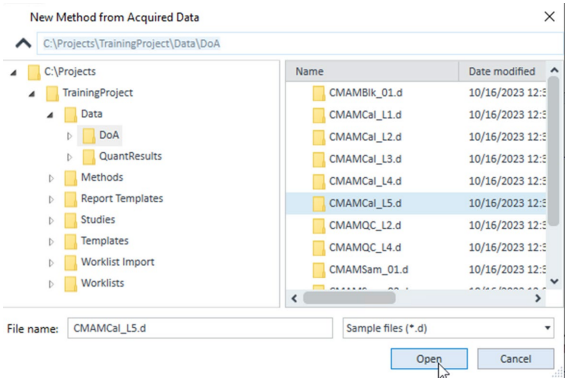
Sample					
		Name	Data File	Type	Level	Acq. Date-Time
		Blank-1	CMAMBlk_01.d	Blank		5/12/2006 1:48 PM
		Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM
		Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM
		Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM
		Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM
▸		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM
		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM
		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM
		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM

2 Click the Method tab, then New -> New Method from Acquired MRM Data.



The system displays the new method From Acquired Data dialog box.

3 Navigate to the C:\Projects\TrainingProject\Data\DoA directory, select CMAMCal_L5.d and click Open to import acquisition method information.

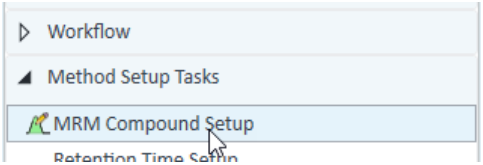


Set up Target Compounds

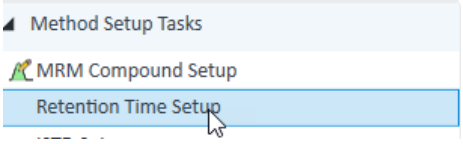
With this task, learn to inspect the MRM transitions and the RT data for the new quantitation method, which you can change for individual target compounds and set up an ISTD compound for each target compound.

Check the new quantitation method created from the imported acquisition method for MRM transitions

1 Under Method Tasks in the sidebar to the left of the Method Table window, click Method Setup Tasks > MRM Compound Setup.

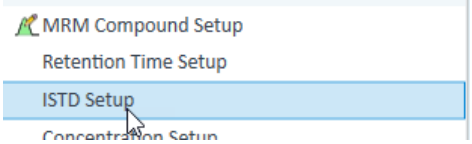


2 To inspect the imported retention time data, click Method Setup Tasks > Retention Time Setup.

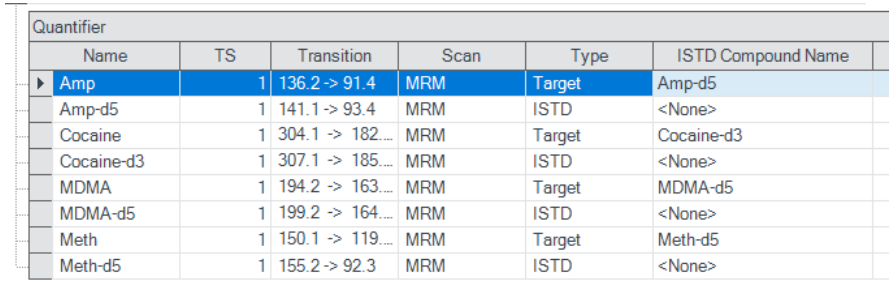


Set up ISTD compounds. Assign the corresponding deuterated compound as the internal standard (ISTD) for each target compound.

1 Click Method Setup Tasks > ISTD Setup.



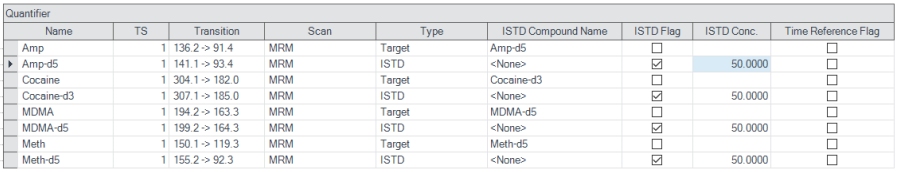
2 For each target compound row, click the down arrow in the **ISTD Compound Name cell** and select the ISTD name associated with the target compound.



Quantifier

Name	TS	Transition	Scan	Type	ISTD Compound Name
Amp	1	136.2 -> 91.4	MRM	Target	Amp-d5
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<None>
Cocaine	1	304.1 -> 182....	MRM	Target	Cocaine-d3
Cocaine-d3	1	307.1 -> 185....	MRM	ISTD	<None>
MDMA	1	194.2 -> 163....	MRM	Target	MDMA-d5
MDMA-d5	1	199.2 -> 164....	MRM	ISTD	<None>
Meth	1	150.1 -> 119....	MRM	Target	Meth-d5
Meth-d5	1	155.2 -> 92.3	MRM	ISTD	<None>

3 Type the ISTD concentration (**ISTD Conc.**) for each ISTD compound (*50.0000* in this example).



Quantifier

Name	TS	Transition	Scan	Type	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Reference Flag
Amp	1	136.2 -> 91.4	MRM	Target	Amp-d5	☐		☐
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<None>	☑	50.0000	☐
Cocaine	1	304.1 -> 182.0	MRM	Target	Cocaine-d3	☐		☐
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	<None>	☑	50.0000	☐
MDMA	1	194.2 -> 163.3	MRM	Target	MDMA-d5	☐		☐
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	<None>	☑	50.0000	☐
Meth	1	150.1 -> 119.3	MRM	Target	Meth-d5	☐		☐
Meth-d5	1	155.2 -> 92.3	MRM	ISTD	<None>	☑	50.0000	☐

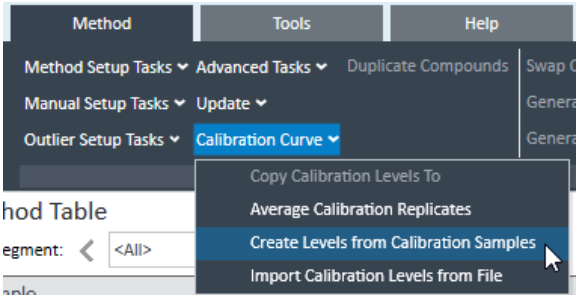
Set up Quantitation

This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels.
- Qualifier ions.
- Calibration curve fit.

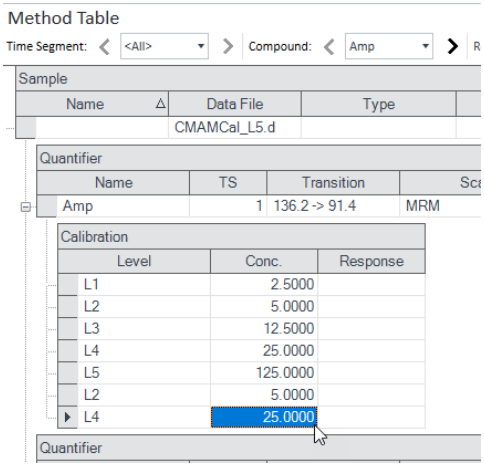
Create five calibration levels for the first compound

1 From the main menu, select Calibration Curve > Create Levels from Calibration Samples.



The Calibration table opens under each Quantifier in the Method Table.

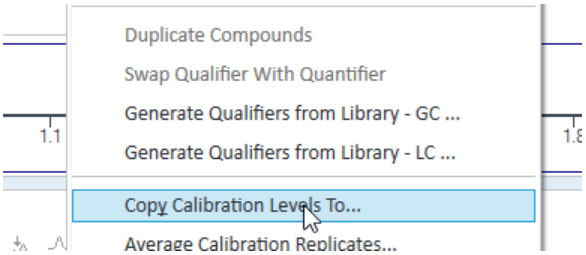
2 For one of the Quantifiers, change the default concentrations to the actual concentration for each level.



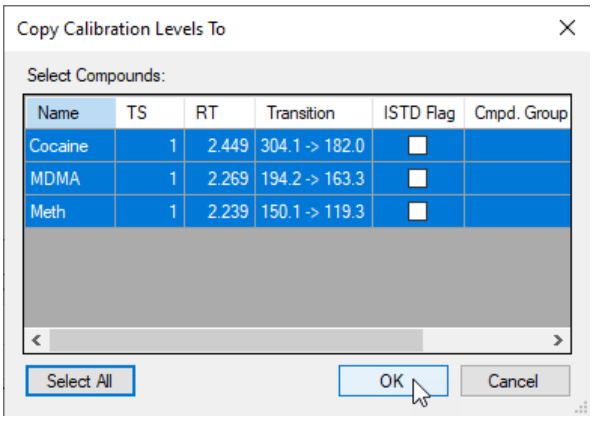
- L1–2.5000
- L2–5.0000
- L3–12.5000
- L4–25.0000
- L5–125.0000

Copy the calibration levels and concentrations to the other compounds

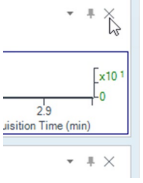
1 Right-click in the Quantifier table and select **Copy Calibration Levels To...**The system displays the Copy Calibration Levels To dialog box.



2 Click **Select All**, and then click **OK**.



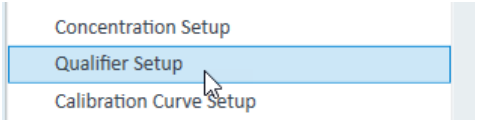
3 Close the **Compound Information** window and the **Sample Information** window in the lower half of the Quantitative Analysis main view.

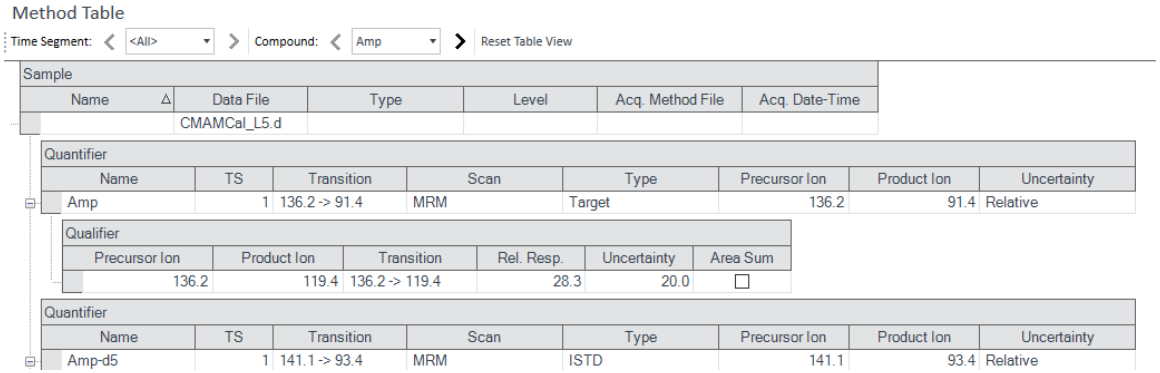


4 Browse the **Method Table** to compare the calibration concentration setup among the four target compounds, Amp, Cocaine, Meth, and MDMA.

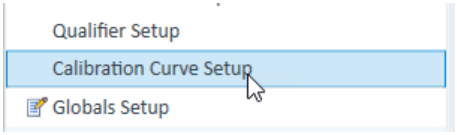
Set up qualifier ions and a calibration curve

1 Under **Method Setup Tasks,** click **Qualifier Setup**, and inspect the Qualifier setup parameters.

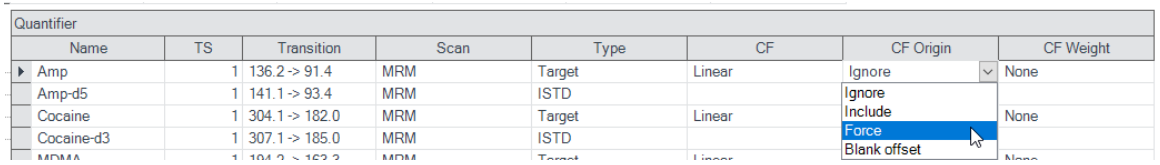




2 Under **Method Setup Tasks**, click **Calibration Curve Setup**.



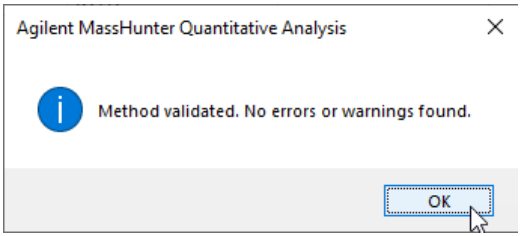
3 For each target compound, change the **CF Origin** to **Force**.



Validate the method

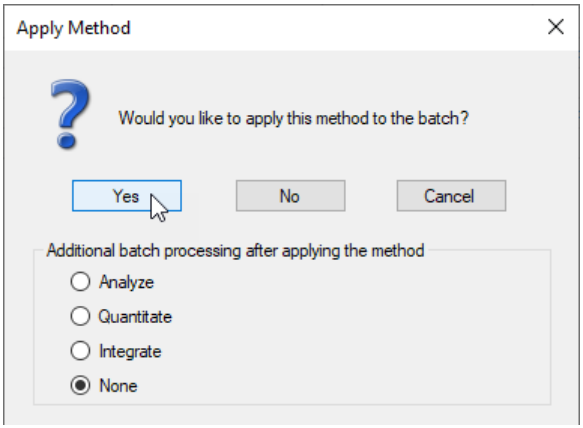
1 Under **Save/Exit**, click **Validate** to validate the method setup.

2 After the validation message appears, click **OK**.



3 Click **Save/Exit** > **Exit**.

4 Select **None** under **Additional batch processing after applying the method** and click **Yes** to the **Would you like to apply this method to the batch**? prompt.

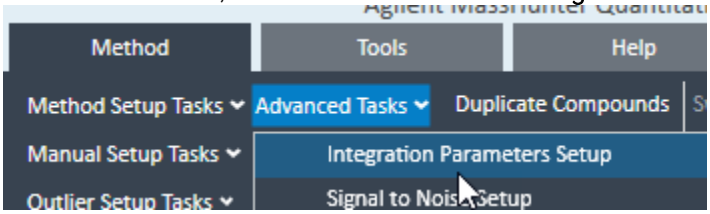


Set the Integrator

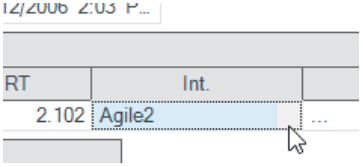
Change the method's integrator to MS/MS

1 On the **Method** tab, click **Edit**.

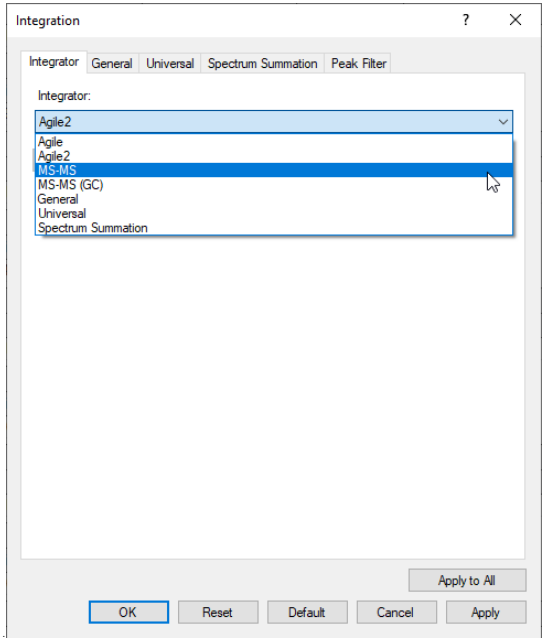
2 On the **Method** tab, select **Advanced Tasks** > **Integration Parameters Setup**.



3 In the **Method Table**, click the box located on the right side of the **Int.** value.



4 Select **MS/MS** from the drop-down menu.



5 Click **Apply to All**.

6 Click OK.

7 Click Exit.

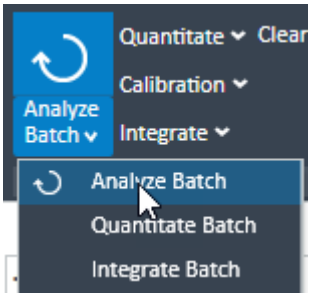
8 Select None under Additional batch processing after applying the method and click Yes to the Would you like to apply this method to the batch? prompt.

Analyze and Save the Batch

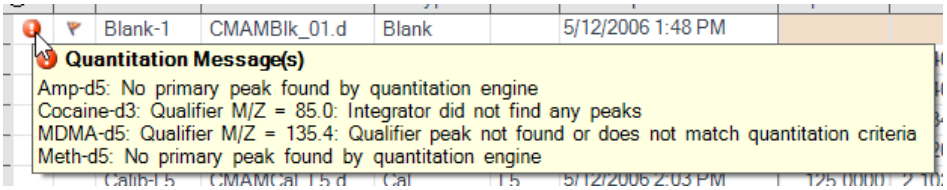
In this exercise, you quantitate the batch and then save the results.

Analyze the batch and inspect the results for each compound.

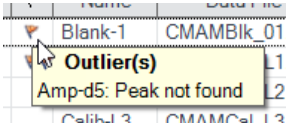
1 On the Home tab, click Analyze Batch.



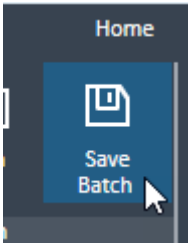
2 Pass the cursor over the quantitation message for Sample 1.



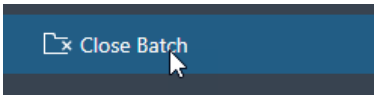
3 Pass the cursor over the flags for the first two calibration standards.



4 On the Home tab, click Save Batch.



5 Click **File > Close Batch** to close the batch.



Review Quantitation Results

The tasks in this exercise show you how to inspect the sample and compound data in a batch file, customize result layouts, export your data to Microsoft Excel, and preview and print the data.

Use the DrugsOfAbuse batch in this exercise.

Navigate the Batch Table Results

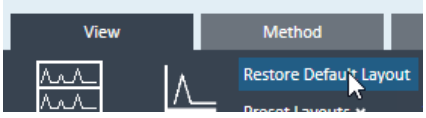
This task shows you how to scroll through your samples and compounds, while observing changes in the Batch Table and compound information data. It also shows you how to display various sample types.

Open the batch file iii_Test_01.batch.bin, created in Exercise 1

1 On the Home tab, click Open Batch.

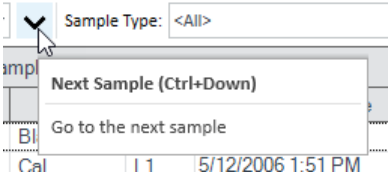
2 Navigate to \Your Directory\DrugsOfAbuse and click iii_Test_01.batch.bin

3 On the View tab, click Restore Default Layout.

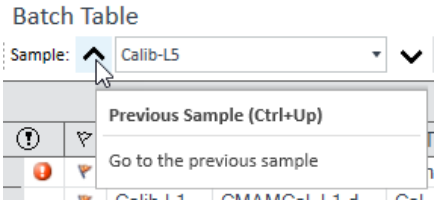


Scroll from sample to sample until you reach the end of the Batch Table, and then return to Cal-L5

1 Click the Next Sample arrow in the Batch Table Standard toolbar until the system displays the desired sample. Inspect the changes in the Compound Information window.



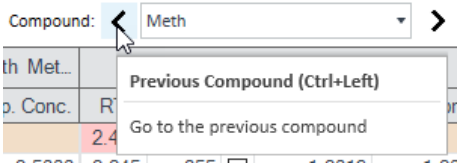
2 Return to Cal-L5, clicking the **Previous Sample icon** in the Batch Table Standard toolbar if needed.



3 Select any cell in the row for sample **Calib_L4** in the Batch Table window to view the changes.

Scroll from compound to compound through all four compounds

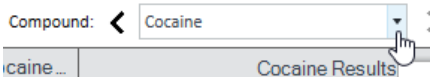
1 Click the **Next Compound** or **Previous Compound** arrow in the toolbar until the system displays the desired compound.



2 Inspect the changes in the **Batch Table**, **Compound Information**, and **Calibration Curve** windows.

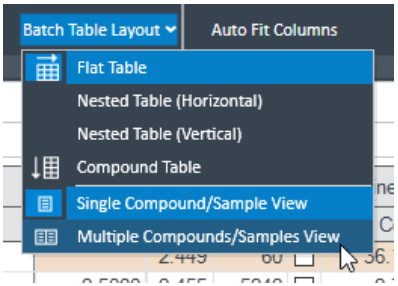
3 Click the down arrow next to the **Compound** list.

4 Click **Cocaine**.



Examine results for multiple compounds

1 On the View tab, select Batch Table Layout > Multiple Compounds/Sample View.



2 Click the Cal-L4 cell and note the difference in RT in the Compound Information window for each compound.

		Sample					Amp Results			Meth Results			MDMA Results			Cocaine Results		
		Name	Data File	Type	Level	Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
		Blank-1	CMAMBlk_01.d	Blank		5/12/2006 1:48 PM							2.284	1.9296		2.433	11.8235	
		Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.141	3.3187	132.7	2.247	2.5936	103.7	2.276	2.2824	91.3	2.453	2.3087	92.3
		Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	2.140	5.7493	115.0	2.248	5.1011	102.0	2.277	4.6561	93.1	2.454	4.2682	85.4
		Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	2.134	13.6808	109.4	2.247	15.1623	121.3	2.277	11.2728	90.2	2.459	11.5607	92.5
		Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	2.022	26.7561	107.0	2.228	27.2574	109.0	2.264	24.8702	99.5	2.449	25.2511	101.0
		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	2.101	124.4844	99.6	2.237	124.2764	99.4	2.271	125.1668	100.1	2.448	125.0768	100.1
		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	2.142	5.2293	104.6	2.248	5.2414	104.8	2.276	4.8567	97.1	2.453	4.2831	85.7
		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	2.135	27.8039	111.2	2.246	27.7713	111.1	2.276	23.0331	92.1	2.455	24.5377	98.2
		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM	2.080			2.286	3.2639		2.315	5.6138		2.408		
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM	2.143	4.8977		2.250	5.8102		2.280	5.1778		2.460	4.3735	
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM	2.105	14.2183		2.236	14.1876		2.267	10.7772		2.446	10.9299	

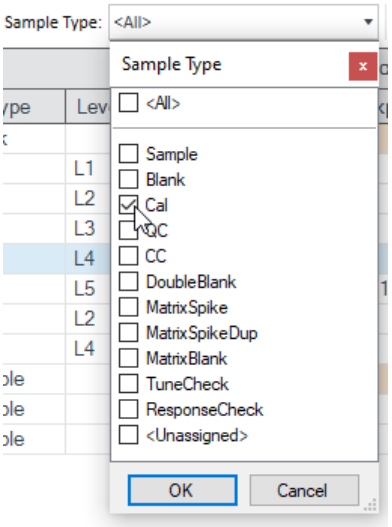
View selected sample types

1 On the View tab, select Batch Table Layout > Single Compound/Sample View.

2 If necessary, click the down arrow next to the Compound list, and click Cocaine.

3 Click the down arrow in the Sample Type drop-down list. The Sample Type dialog box is displayed.

4 Clear the **<All>** check box and mark the **Cal** check box.



5 Click **OK**. The Batch Table should contain only the Cal standards for cocaine.

6 Click the down arrow in the **Sample Type** drop-down list.

7 Click **<All>**, and then click **OK**. The system marks all the check boxes and displays all sample types.

Change Result Window Layouts

This task shows you how to customize your layout and how to recreate the default layout.

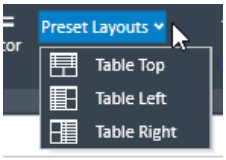
Use layout icons on the toolbar

1 Use layout options on the View Tab to position the Batch Table, Compound Information, and Calibration Curve windows.

   a On the View tab, select Preset Layouts > Table Left.

   b On the View tab, select Preset Layouts > Table Right.

   c On the View tab, select Preset Layouts > Table Top.

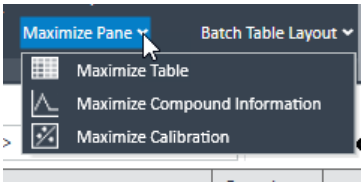


2 Use layout icons on the View Tab to maximize each individual window:

   a On the View tab, select Maximize Pane > Maximize Table.

   b On the View tab, select Maximize Pane > Maximize Compound Information.

   c On the View tab, select Maximize Pane > Maximize Calibration.

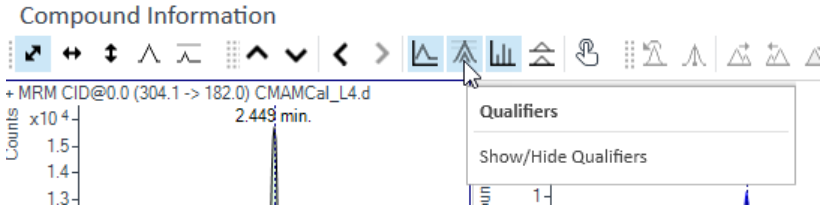


3 On the View tab, click Restore Default Layout.

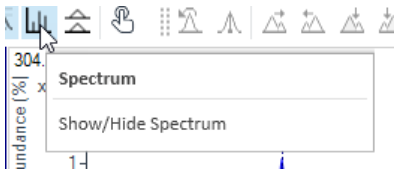
Change the panes in the Compound Information window for Cal-L4

1 In the Batch Table, select the Cal-L4 row.

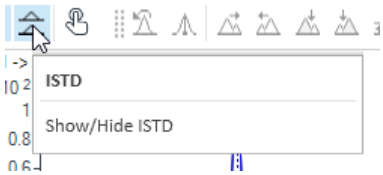
2 In the Compound Information toolbar, click the **Show/Hide Qualifiers** icon.



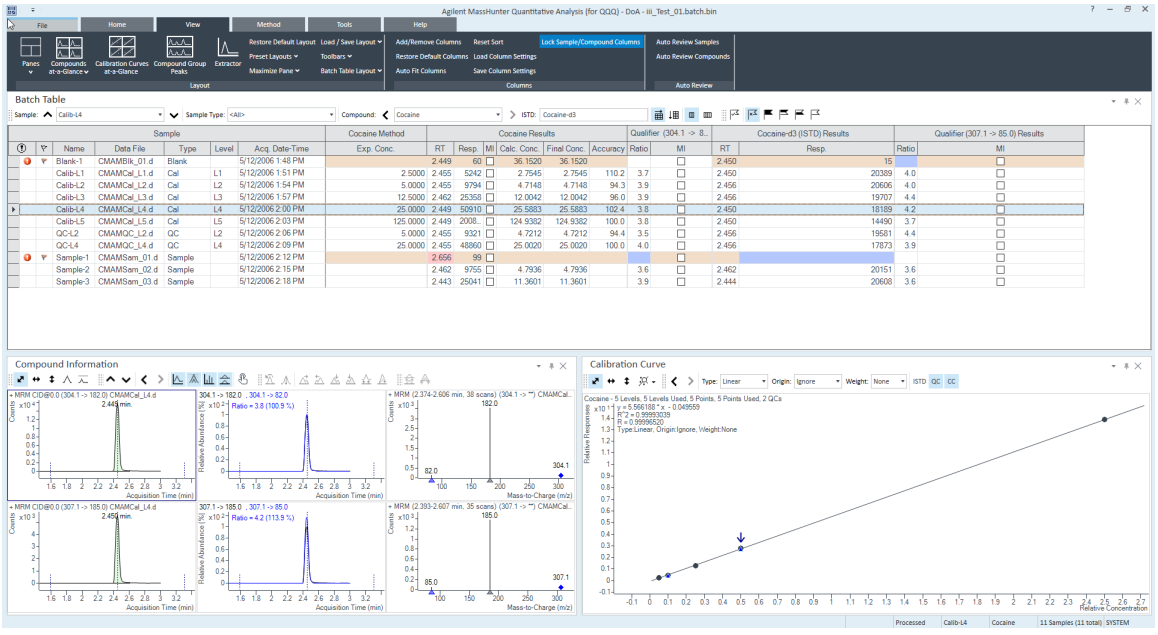
3 Click the **Show/Hide Spectrum** icon.



4 Click the **Show/Hide ISTD** icon.



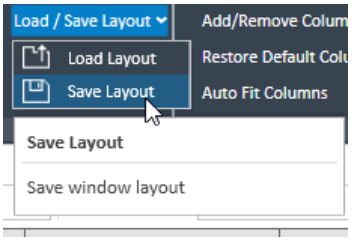
5 The layout and results look like those in the following figure.



Save the default layout without the calibration curve

1 Close the Calibration Curve window.

2 On the View tab, select Load/Save Layout > Save Layout. The system displays the Save Layout File dialog box.



3 Name the layout file Batch Table plus Compound Information and click Save.

Load the newly created layout

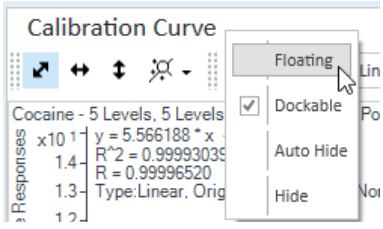
1 On the View tab, click Restore Default Layout.

2 On the View tab, select Load/Save Layout > Load Layout. The system displays the Load Layout dialog box.

3 Click Batch Table plus Compound Information and click Open.

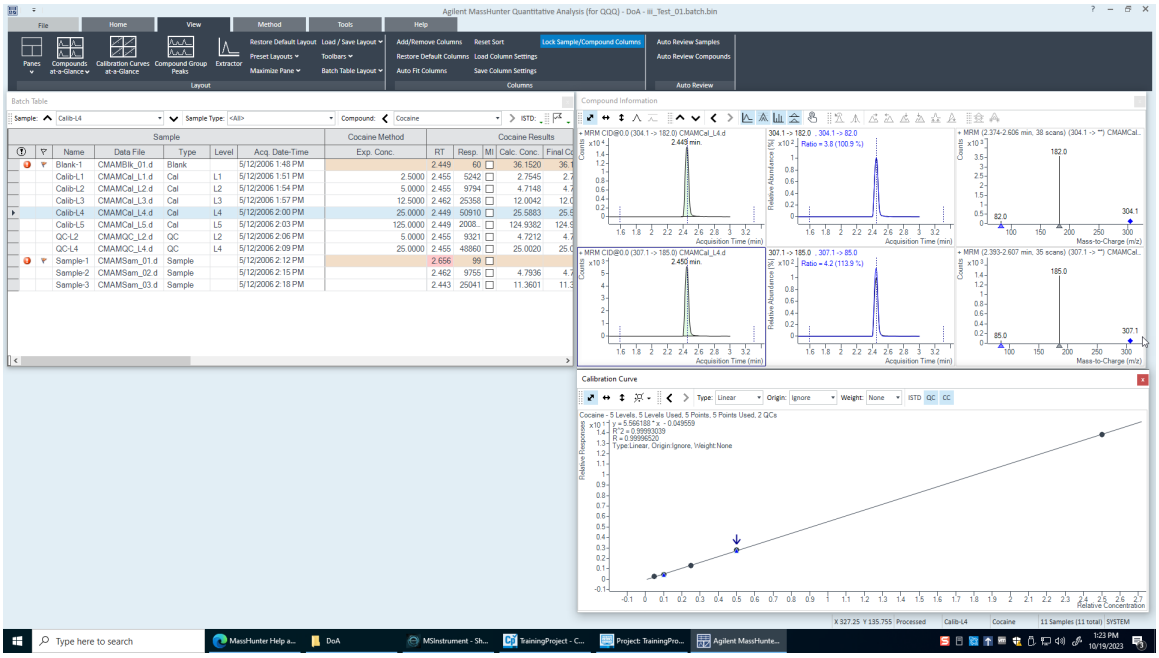
4 On the View tab, click Restore Default Layout.

5 Right-click inside the title bar of the Calibration Curve window, and then mark the Floating check box.



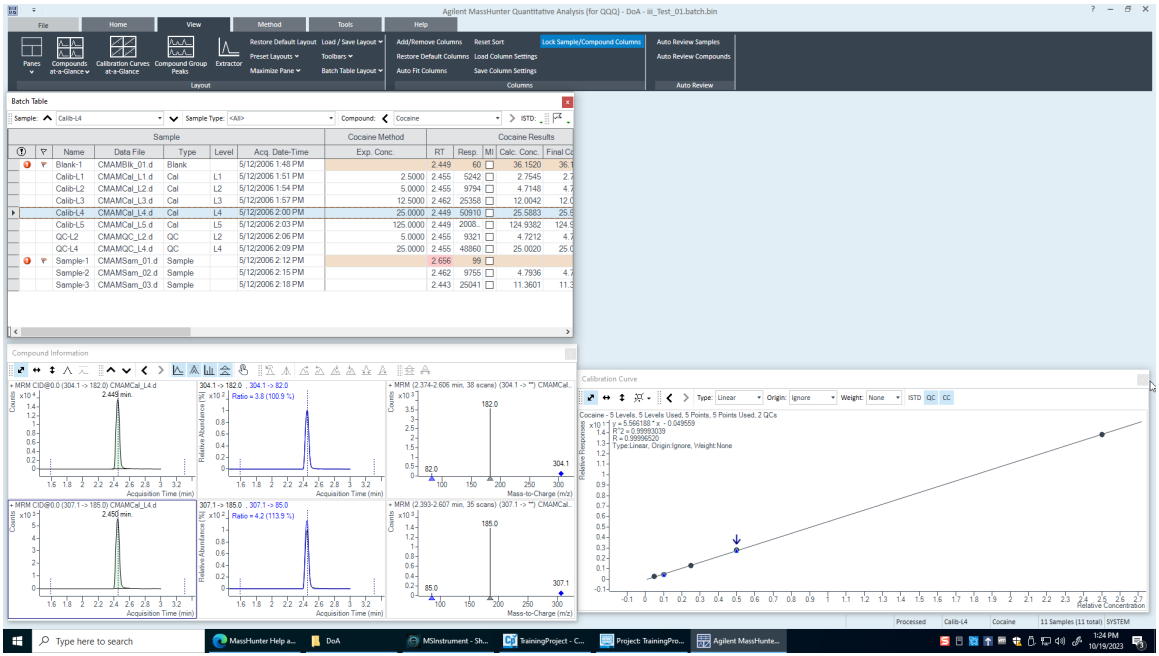
6 Right-click the title bar of the Compound Information window, and then mark the Floating check box.

7 Resize the windows to match the layout below.



8 Right-click inside the title bar of the Compound Information window, and then clear the **Floating** check box.

9 Resize the windows to match the layout in below.



10 Right-click inside the title bar of the **Calibration Curve** window and clear the **Floating** check box.

11 Move the **Compound Information** window so that the layout corresponds to the one pictured at the start of the task.

Recreate (do not restore) the default layout

1 Maximize the program main view.

- Anchor the **Calibration Curve** window first, and then the Compound Information window, to recreate the default layout.
- If after anchoring the two windows, the calibration curve is on the left side, right-click the title bar of the **Calibration Curve** window and drag it to the right. A gray rectangle shows where this window will be placed within the main view.
- Drag the calibration curve to the bottom-right corner of the main view.

2 On the View tab, click **Restore Default Layout**.

Use Three Tools to Evaluate Results

In this exercise, you will use three tools to help you evaluate and obtain more accurate quantitation results:

- Curvefit Assistant, which calculates all combinations of curves and presents results with an equation and confidence band.
- Parameterless integrator, so you do not have to figure out the parameters to change to improve the integration.
- Outlier messages to help you easily detect result values that are out of the specified range.

The DrugsOfAbuse batch is used in this exercise.

Adjust the Calibration Curve Fit

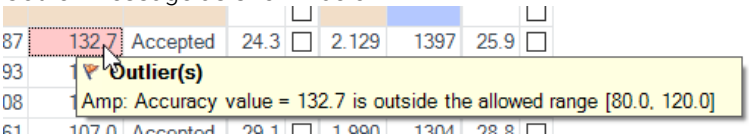
This task shows you how to find the accuracy outlier for a compound, adjust its curve fit, and reanalyze the batch.

1 If necessary, open the batch file. iii_Test_01.batch.bin. On the Home tab, click **Open Batch**.

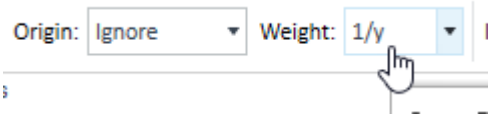
2 Navigate to **\Your Directory\ DrugsOfAbuse** and click **iii_Test_01.batch.bin**.

3 Make sure the **Batch Table** is set to single compound display mode, and the displayed target compound is **Amp**.

4 Point to the cell in the **Calib-L1** row and the **Accuracy** column to display the Outlier message as shown below.



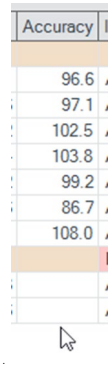
5 In the **Calibration Curve**, set **Origin** to **Ignore**, and **Weight** to **1/y**. The program displays a new window curve fit formula and R2 value.



Analyze the batch and inspect the results in the Batch Table

1 On the Home tab, click Analyze Batch.

2 Inspect the results in the Batch Table after batch analysis.



3 Click Next Compound in the Batch Table toolbar to view individual compounds, such as Cocaine, MDMA, and Meth.

4 Examine the quantitation results, especially the values in the Accuracy column.

Change the curve fit for methamphetamine and analyze the batch

1 In the Calibration Curve Fit window, set Origin to Ignore, and Weight to 1/y.

The Quantitative Analysis program displays a revised curve fit formula and R2 value.

2 On the Home tab, click Analyze Batch. The Batch Table displays the new results after batch analysis.

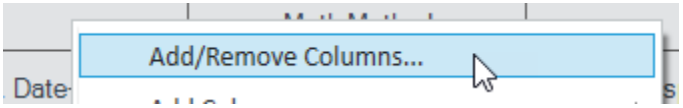
Integrate Without Parameter

This task shows you how to inspect data for proper integration. You learn how to perform the following tasks:

- Add integration columns to the Batch Table
- View default integration values
- Closely examine the chromatogram, looking for such details as:
- Outlier messages
- Baseline parameters
- Peak labels

Add integration columns to the Batch Table

3 Right-click anywhere in the **Batch Table** and click **Add/Remove Columns**.

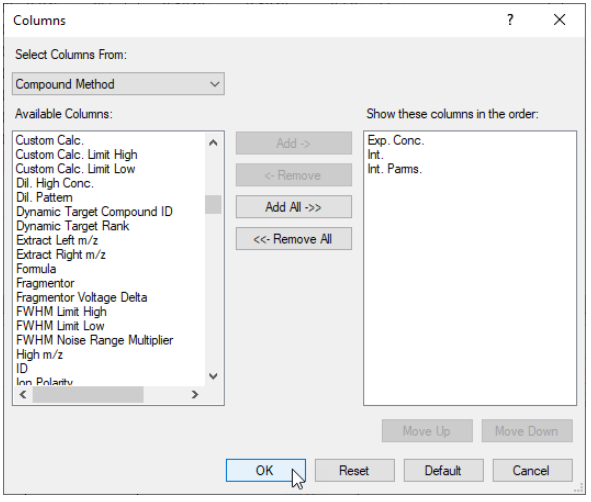


The system displays the **Columns** dialog box.

4 From the **Select Columns From** drop-down list, select **Compound Method**.

5 From the **Available Columns** list, select **Int.** (Integrator Type) and **Int. Parms.** (Integrator Parameters) and click **Add**.

6 The Quantitative Analysis program moves the selected columns to the **Show these columns in the order** list.

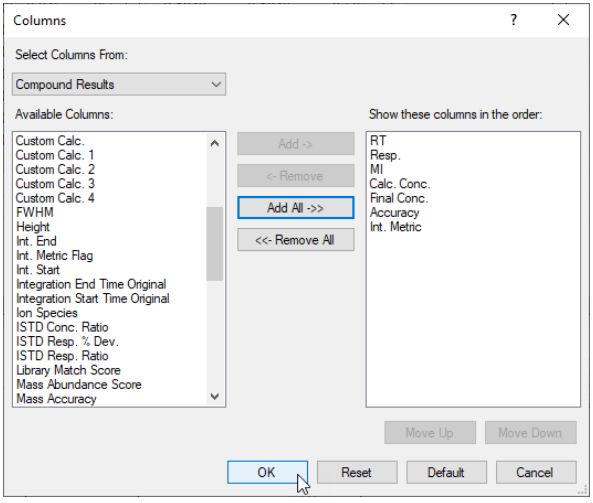


7 From the **Select Columns From** drop-down list, select **Compound Results**.

8 From the **Available Columns** list, select **Int. Metric** (Integrator Metric) and click **Add**.

9 The system moves the selected column to the **Show these columns in the order** list.

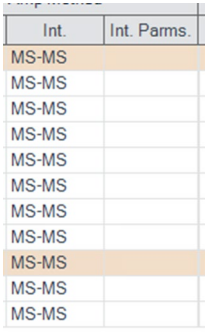
10 Click **OK**.



View the default integration values for amphetamine

1 Click **Previous Compound** in the Batch Table toolbar to view amphetamine (Amp).

2 Examine the default values in the **Int**. and **Int. Parms** columns in the **Batch Table.**



3 Examine the default values in the **Int. Metric** column in the **Batch Table.**

Amp Method			Amp Results						
Exp. Conc.	Int.	Int. Parms.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric
	MS-MS				☐				
2.5000	MS-MS		2.141	658	☐	2.4161	2.4161	96.6	Accepted
5.0000	MS-MS		2.140	1059	☐	4.8556	4.8556	97.1	Accepted
12.5000	MS-MS		2.134	2673	☐	12.8162	12.8162	102.5	Accepted
25.0000	MS-MS		2.022	4952	☐	25.9394	25.9394	103.8	Accepted
125.0000	MS-MS		2.101	18605	☐	124.0262	124.0262	99.2	Accepted
5.0000	MS-MS		2.142	1006	☐	4.3336	4.3336	86.7	Accepted
25.0000	MS-MS		2.135	4716	☐	26.9911	26.9911	108.0	Accepted
	MS-MS		2.080	6	☐				Rejected
	MS-MS		2.143	1004	☐	4.0008	4.0008		Accepted
	MS-MS		2.105	2590	☐	13.3556	13.3556		Accepted

View integration problems for cocaine and MDMA

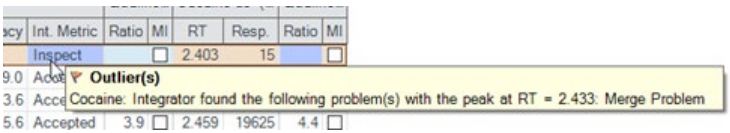
1 Close the **Calibration Curve** window.

2 Enlarge the chromatogram portion of Compound Information toolbar so that only the quantifier and qualifier chromatograms appear. Click the **Show/Hide Spectrum** icon.

3 Also click the **Show/Hide ISTD** icon.

4 Click the **Next Compound** icon in the Batch Table toolbar until the system displays the compound b.

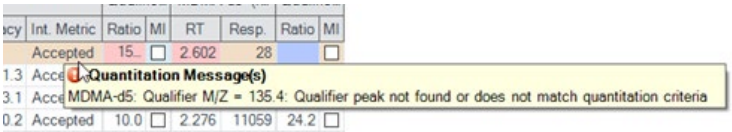
5 Select the **Blank-1 row**, and mouse over the word **Inspect** in the **Int. Metric** column for that row.



The system displays any outlier message for that data, as well as the integrated chromatogram for cocaine.

6 Click the **Next Compound** icon in the Batch Table Standard toolbar or the Previous Compound icon in the Batch Table Standard toolbar until the system displays the compound MDMA.

7 Select the Blank-1 row and point to the **Int. Metric** column.



The system displays any outlier message for that data, as well as the integrated chromatogram for MDMA.

Change the noise algorithm

1 Right-click anywhere in the **Batch Table** and click **Add/Remove Columns**.

The system displays the Columns dialog box.

2 From the **Select Columns From** drop-down list, select **Compound Method**

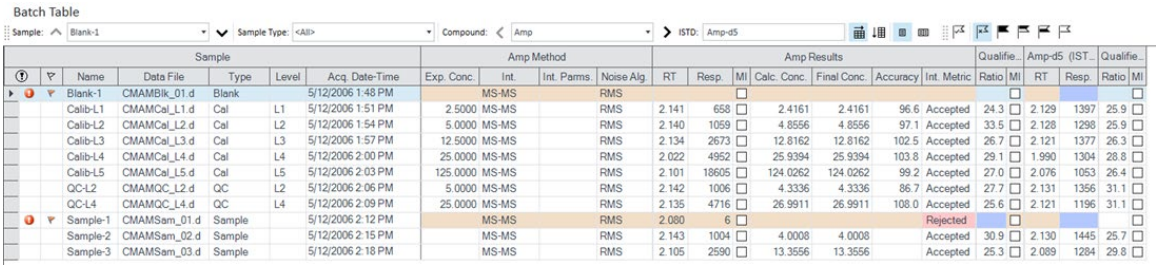
3 From the **Available Columns** list, select **Noise Alg**. (Noise Algorithm Type) and click **Add**.

The system moves the selected column to the Show these columns in the order list.

4 Click **OK**.

5 Click the **Previous Compound** icon in the **Batch Table** toolbar until the system displays the compound Amp.

6 Examine the values in the **Noise Alg**. and S/N (**signal-to-noise ratio)** columns.


Batch Table

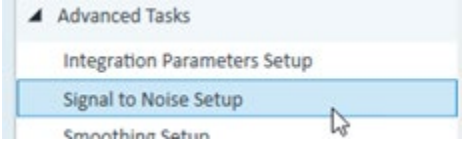
Sample: Blank-1 Sample Type: <All> Compound: Amp ISTD: Amp-d5

Sample						Amp Method				Amp Results							Qualifie...		Amp-d5 (IST...		Qualifie...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	Int.	Int. Parms.	Noise Alg.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI
▸		Blank-1	CMAMBlk_01.d	Blank		5/12/2006 1:48 PM		MS-MS		RMS			☐						☐				☐
		Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000	MS-MS		RMS	2.141	658	☐	2.4161	2.4161	96.6	Accepted	24.3	☐	2.129	1397	25.9	☐
		Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	5.0000	MS-MS		RMS	2.140	1059	☐	4.8556	4.8556	97.1	Accepted	33.5	☐	2.128	1298	25.9	☐
		Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	12.5000	MS-MS		RMS	2.134	2673	☐	12.8162	12.8162	102.5	Accepted	26.7	☐	2.121	1377	26.3	☐
		Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	25.0000	MS-MS		RMS	2.022	4952	☐	25.9394	25.9394	103.8	Accepted	29.1	☐	1.990	1304	28.8	☐
		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	125.0000	MS-MS		RMS	2.101	18605	☐	124.0262	124.0262	99.2	Accepted	27.0	☐	2.076	1053	26.4	☐
		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	5.0000	MS-MS		RMS	2.142	1006	☐	4.3336	4.3336	86.7	Accepted	27.7	☐	2.131	1356	31.1	☐
		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000	MS-MS		RMS	2.135	4716	☐	26.9911	26.9911	108.0	Accepted	25.6	☐	2.121	1196	31.1	☐
		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM		MS-MS		RMS	2.080	6	☐				Rejected		☐				☐
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM		MS-MS		RMS	2.143	1004	☐	4.0008	4.0008		Accepted	30.9	☐	2.130	1445	25.7	☐
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM		MS-MS		RMS	2.105	2590	☐	13.3556	13.3556		Accepted	25.3	☐	2.089	1284	29.8	☐

Practice changing the noise algorithm from RSM to ASTM for amphetamine in the method

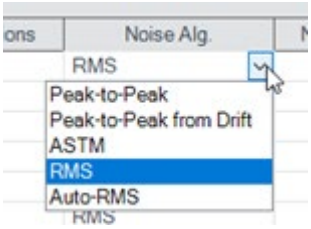
1 On the **Method** tab, click **Edit**.

2 In the **Method Tasks** column, click **Advanced Tasks** > **Signal to Noise Setup**.



The system displays the integrator parameters in the Method Table.

3 In the **Method Table**, click the drop-down arrow in the **Noise Alg.** column for Amp.



A list of available noise algorithms appears.

4 Click **ASTM**.

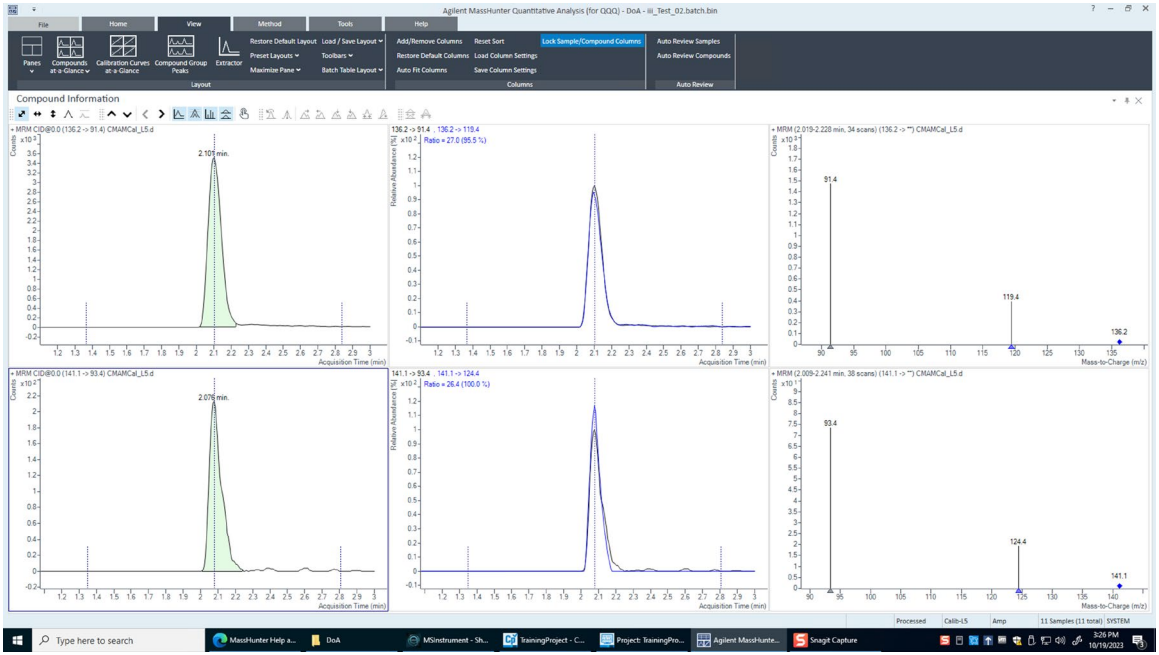
5 Under **Method Tasks/Save/Exit**, click **Exit**.

6 At the **Would you like to apply this method to the batch**? prompt, click **No**. The system displays Batch Analysis mode.

Turn off the baseline (highest concentration standard) and then back on for amphetamine

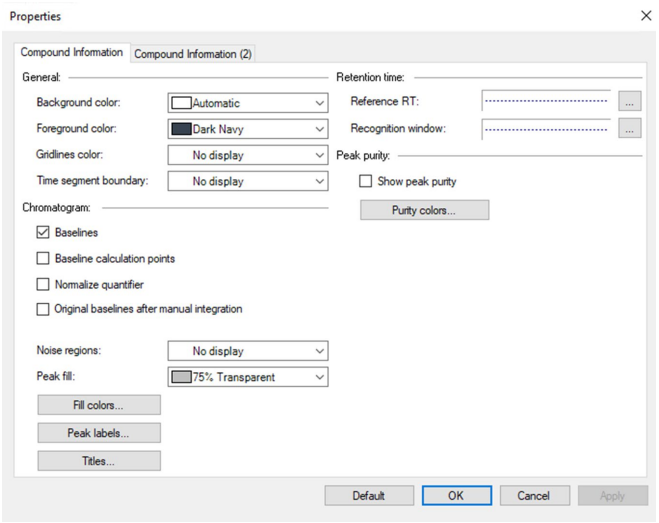
1 Select sample **Calib-L5** (if it is not already selected), and on the **View** tab, select **Maximize Pane > Maximize Compound Information**.

Make sure that only the Compound Information pane is visible in the window.



2 Right-click either of the chromatograms to open the shortcut menu.

3 Click Properties at the bottom of the shortcut menu to open the Properties dialog box.



4 Clear the **Baselines** check box in the Properties dialog box.

5 Click the **Apply** button and observe the peak without the baseline.

Inspect the calculation points for the baseline for amphetamine

1 Mark the **Baselines** check box in the Properties dialog box.

2 Click the **Apply** button and observe the peak with the baseline drawn.

3 Mark the **Baseline Calculation Points** check box in the Properties dialog box.

4 Click **Apply** and observe where the baseline starts and stops.

5 Clear the **Baseline Calculation Points** check box in the Properties dialog box.

6 Click **Apply** and observe the chromatograms.

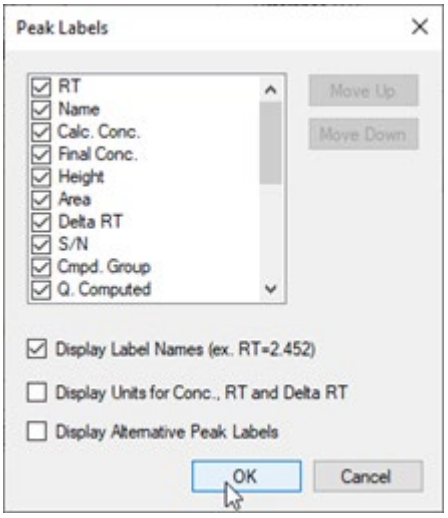
7 Compare the chromatograms with and without Baseline Calculation Points.

Display the peak labels for amphetamine

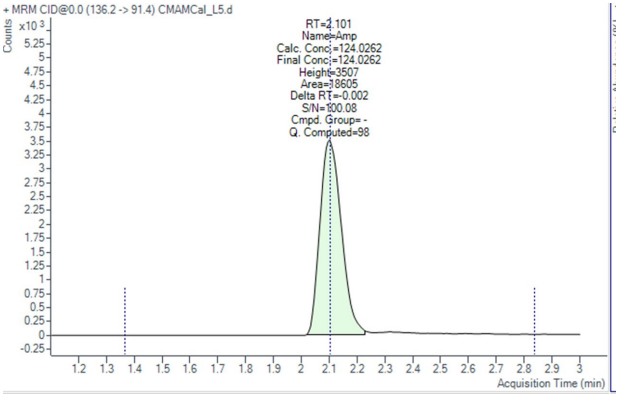
1 From the Properties dialog box, click **Peak Labels**. The system displays the Peak Label dialog box.

2 Mark all the **Peak Labels** check boxes, and the **Display Label Names** check box.

3 Click **OK**.



4 Click the **Apply** button in the Properties dialog box. The peak labels should now match those shown in the example below.



5 Click **Peak Labels** in the Properties dialog box. The system displays the Peak Labels dialog box.

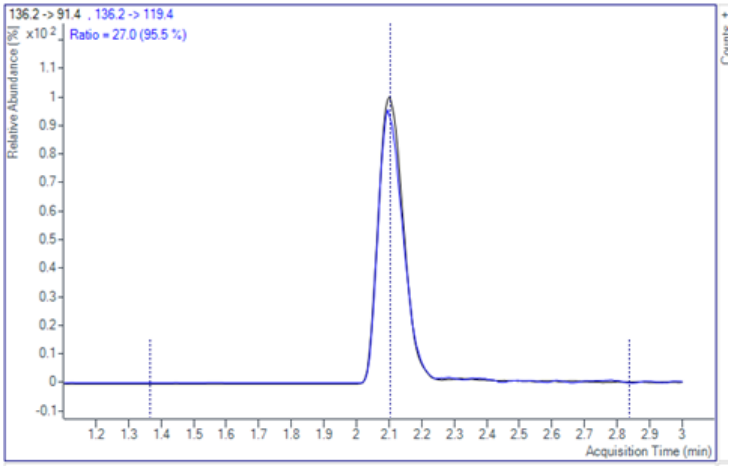
6 Clear all the **Peak Labels** check boxes except RT (retention time). Clear the **Display Label Names** check box and click **OK**.

7 Click **Apply** in the Properties dialog box and observe the change in Peak Labels.

Display the qualifier chromatogram on the right-side before and after normalization

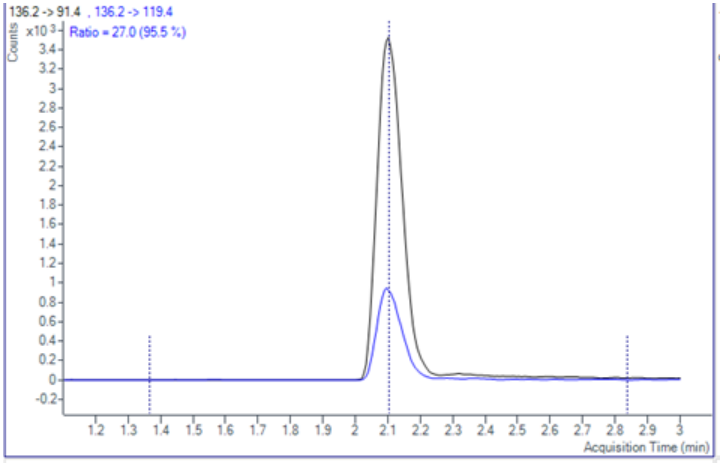
1 Click the Compound Information (2) tab. In the Qualifiers area, mark the Normalize check box.

2 Click Apply and observe that the two peaks now converge and appear as one peak.



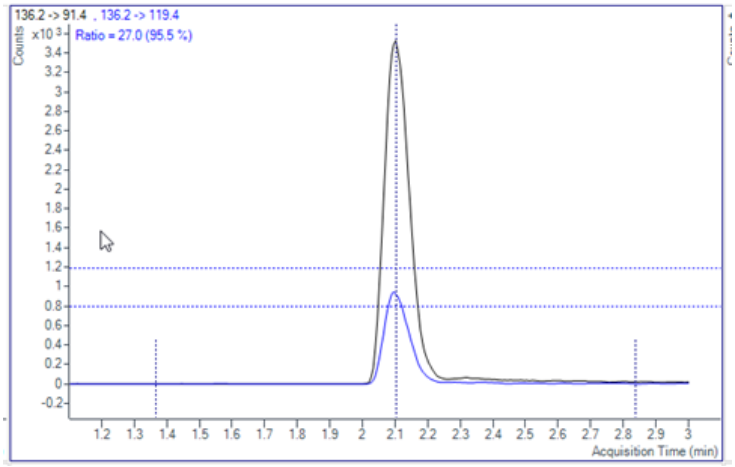
3 Clear the Normalize Qualifiers check box of the Properties dialog box.

4 Click Apply to display the qualifier second quantifier peaks again.



View the uncertainty band

1 Select the type of uncertainty band that you would like to display from the drop-down menu in the **Uncertainty Band** field of the Properties dialog box. Click Apply and the uncertainty band appears in the qualifier chromatogram.



2 Select **No** display from the **Uncertainty Band** drop-down menu of the Properties dialog box. Click **Apply** to remove the uncertainty band from the qualifier chromatogram.

3 Click **OK** to close the Properties dialog box.

4 Compare the qualifier chromatogram with and without the **Uncertainty band**.

Remove the Int. and Int. Parms columns from the Batch Table

1 On the **View** tab, click Restore Default Layout.

2 Right-click the **Compound Method** section of the Batch Table and click **Add/Remove Columns**.

3 From the right list, select **Int. and Int. Parms. (Compound Methods).**

4 Click **Remove**, and then **OK.**

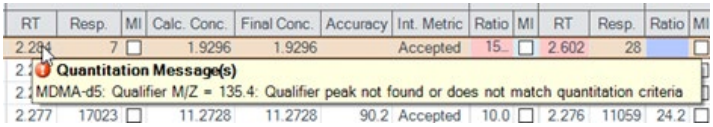
Detect Outliers

This task shows you how to fine-tune the accuracy range for a compound and hide and show results with outlier flags.

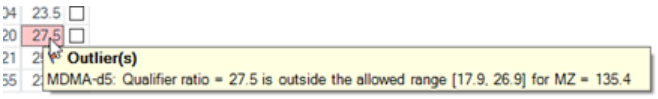
View outlier information for MDMA

1 Click **Next Compound** in the Batch Table toolbar until the system displays the compound MDMA.

2 Select the **Blank-1** row and point the cursor to the RT column.



3 Examine the outlier information in the Qualifier ... Results > Ratio column for Sample 1, as shown in the example below.



Change the accuracy range for amphetamine in the method, and reanalyze the batch

1 Click the **Previous Compound** icon in the toolbar until the system displays the compound Amp.

2 Select the **Calib-L5** row in the table.

3 On the **Method** tab, click **Edit**.

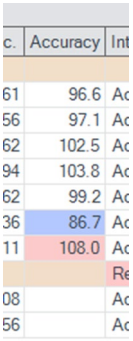
4 In the **Method Tasks** column, click **Outlier Setup Tasks > Accuracy**.

5 Set the **Accuracy Max % Dev** value to *5%* for Amp.

6 In the **Method Tasks** column, click **Save/Exit** > **Exit**, then select None under Additional batch processing after applying the method, and click **Yes** to the Would you like to apply this method to the batch? prompt.

7 Press **F5** to analyze the batch.

8 Red (high) and blue (low) outlier values now appear in the Accuracy column for Amp.



c.	Accuracy	Int
61	96.6	Ac
56	97.1	Ac
62	102.5	Ac
94	103.8	Ac
62	99.2	Ac
36	86.7	Ac
11	108.0	Ac
		Re
08		Ac
56		Ac

Using the following set of outlier flag icons

1 Click the **Display rows that have High outliers** icon on the toolbar to display only samples with high outliers.

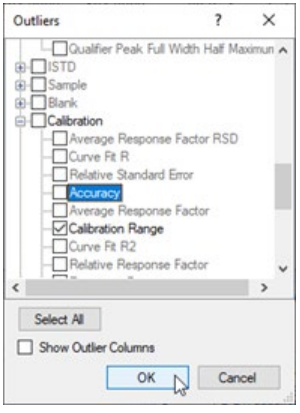
2 Click the **Turn off outlier filter** icon to display all samples.

3 Click the **Display rows that have High/Low outliers** icon on the toolbar to display only samples with low outliers.

4 Click the **Display rows that have High/Low outliers** icon again to display all the samples.

5 Click the **Select Outliers** icon to bring up the Outliers dialog box.

6 Clear the **Accuracy** and **Retention Time** check boxes and click **OK**.



7 Click the Select Outliers icon to bring up the Outliers dialog box.

8 Mark the Accuracy and Retention Time check boxes, and click OK

Generate Quantitation Reports

This exercise helps you learn how to do these tasks:

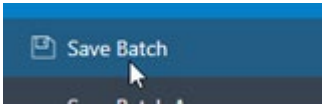
- Generate report methods using one or more report templates
- Generate a report

The DrugsOfAbuse batch is used in this exercise.

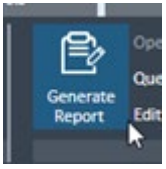
Quantitate the samples for this batch and save your results

1 On the Home tab, click Analyze Batch.

2 Click File > Save to save the batch.



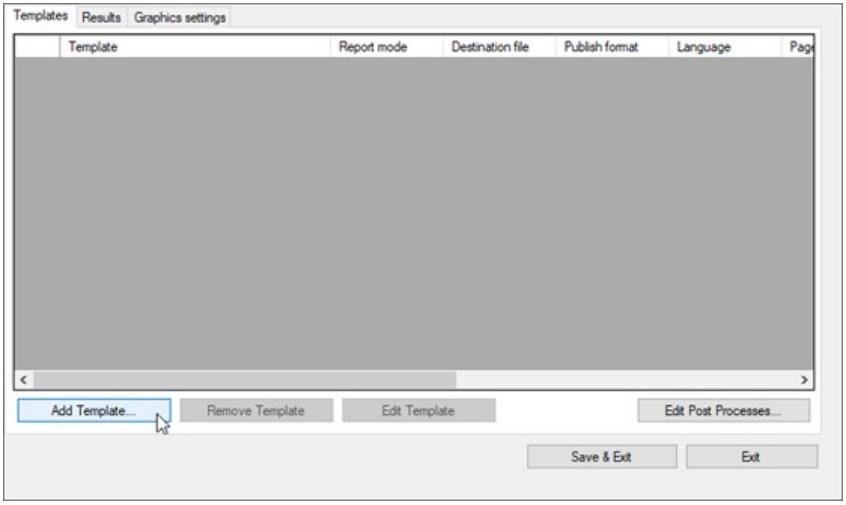
3 On the Home tab, click Generate Report. The system displays the Generate Report dialog box.



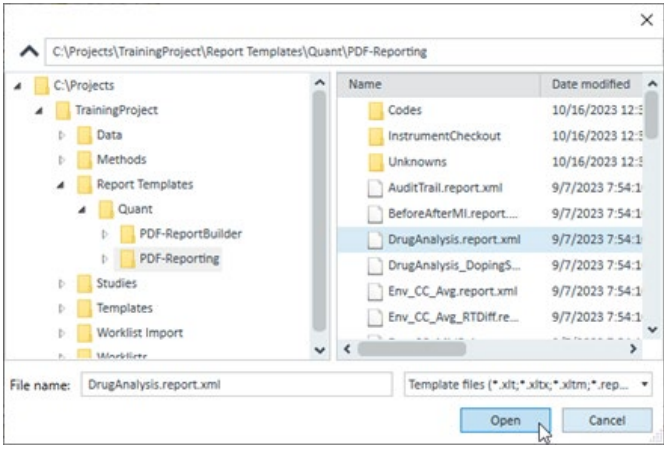
Note the Report Folder directory, which is where the report is saved.

4 Under the Report Method field, click the New button to create a report method.

5 Click the **Add Template button** in the Report Method Edit dialog box to open the browser.



6 Navigate to the **MassHunter/Report Templates/Quant/PDF-Reporting** directory, select **DrugAnalysis.report.xml** and click **Open**.

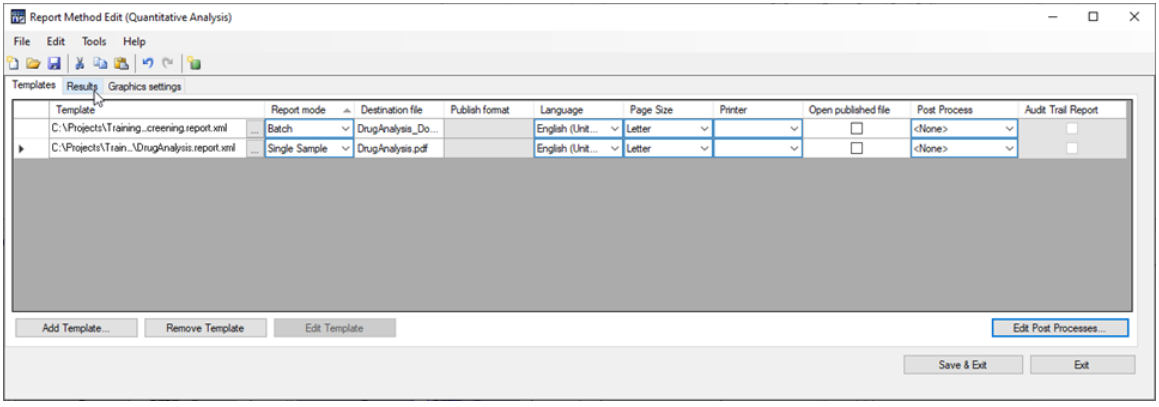


The program adds the template to the Template field in the Report Method Edit pane.

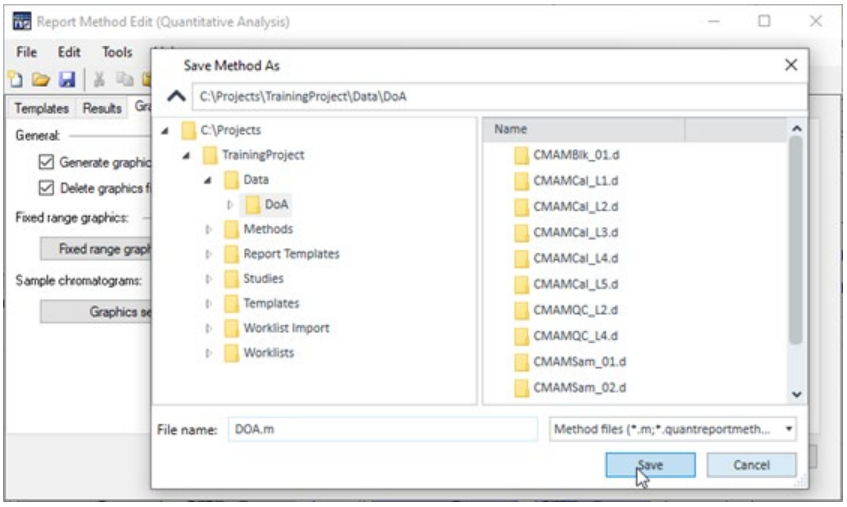
7 Repeat steps d and e to add **DrugAnalysis_DopingScreening.report.xml.**

Edit the report method to create single sample and batch PDF reports

1. In the **Report Method Edit** dialog box, on the **DrugAnalysis.report** template line, **Report Mode** field, select **Single Sample** from the drop-down menu.
2. On the **DrugAnalysis _Doping Screening.report** template line, select **Batch** from the drop-down menu in the **Report Mode** field.
3. Select your language from the drop-down menu in the **Language** field.
4. Select a paper size from the drop-down menu in the **Paper Size** field.
5. Select the **Results** tab of the **Report Method Edit** window.

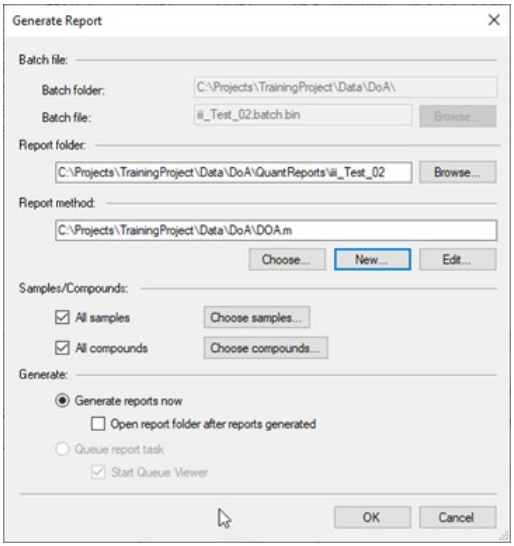


6. Leave the default settings for the rest of the graphic setting fields.
7. Click the save icon in the **Report Method Edit** window.
8. Name the report method **DOA.m**.
9. Click **Save & Exit** to close the Report Method Edit dialog box to return to the Generate Report window.



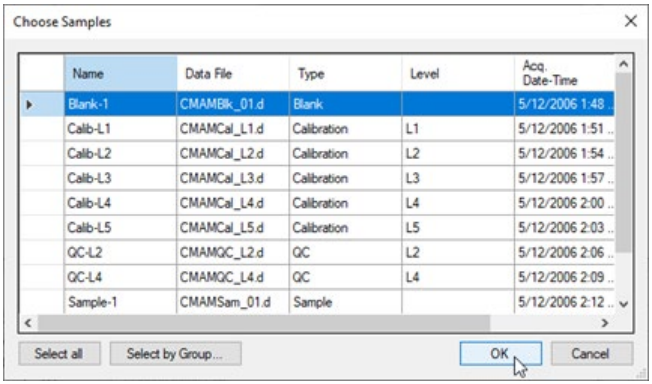
Generate a report from the method

1 Verify that the method you just created is in the **Report Method** field.

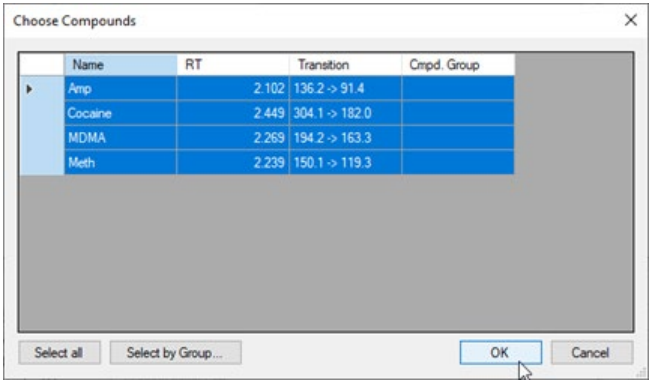


2 In the **Samples/Compounds** field, uncheck **All Samples**, to open the batch table.

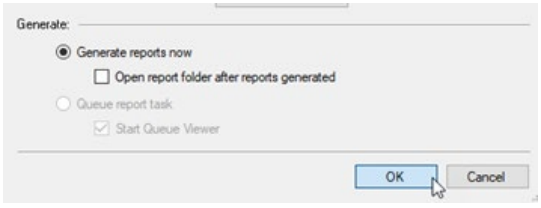
3 Highlight one of the samples in the batch table window and click **OK**.



4 Click **All Compounds** to show all the compounds in the sample you have selected.



5 Select **Generate reports now** and click **OK** to generate the report. Double-click a file to open and display the report.



6 Double-click a file to open and display the report.

9 Maintenance

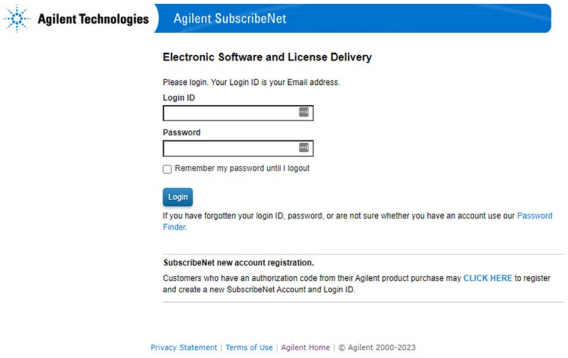
Instrument Maintenance

Register on Agilent SubscribeNet (New Account Registration)

NOTE

If you already have a SubscribeNet account set up for previously purchased Agilent products, it is not necessary to set up additional accounts.

1 Using a web browser, navigate to https://agilent.subscribenet.com/ The site loads.



2 Click the CLICK HERE link to register.

3 Enter the following required information to create the account, along with the authorization code received from the product purchase.

- **a** Authorization Code
- **b** Email
- **c** Company
- **d** Department
- **e** First Name
- **f** Last name
- **g** Phone
- **h** Address
- **i** City State/Province
- **j** Country

4 Click Submit.

You will receive an email to activate your account.

Perform Back Up and Platform Best Practices

- ☐ Safely store the software media provided for the system.
- ☐ Set up Data/Computer image backup regularly.
  - Microsoft Back up and Restore Options (https://t.ly/r2995)
- ☐ Disable power management options and automatic utilities.
  - Set power options to Put the computer to sleep = Never
  - Set Windows Update to "Check for updates but let me choose whether to download and install them."
- ☐ Turn on Windows Firewall.
  - Select the "Notify me when Windows Firewall block a new program." check box.

Locate the tune solution bottle and properly store tune solution

Locate the tune solution bottle and confirm that it is safely stored in the appropriate temperature conditions.

Perform daily cleaning of Ionization Source and Spray Chamber

Perform this maintenance daily or at the end of each shift or anytime you suspect carryover contamination from one sample or analysis to another.

After determining the Source type, find the instructions for cleaning in the user guide for the source in use.

Review routine procedures in the user guide

Perform maintenance daily or at the end of each shift or anytime you suspect carryover contamination from one sample or analysis to another.

Remove, clean, and replace the capillary

Review the steps in the user guide.

Add a new user defined EMF counter

Using the online help, set up a user defined EMF counter by entering a new threshold value for a selected EMF item.

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