

Agilent Triple Quadrupole LC/MS System

Introduction Workbook



Notices

Manual Part Number

G1960-90141

October 2023

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Software Revision

This guide is valid for MassHunter 12.1, until superseded.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

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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

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Instrument Maintenance

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Introduction

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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

Introduction

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>.

Introduction

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

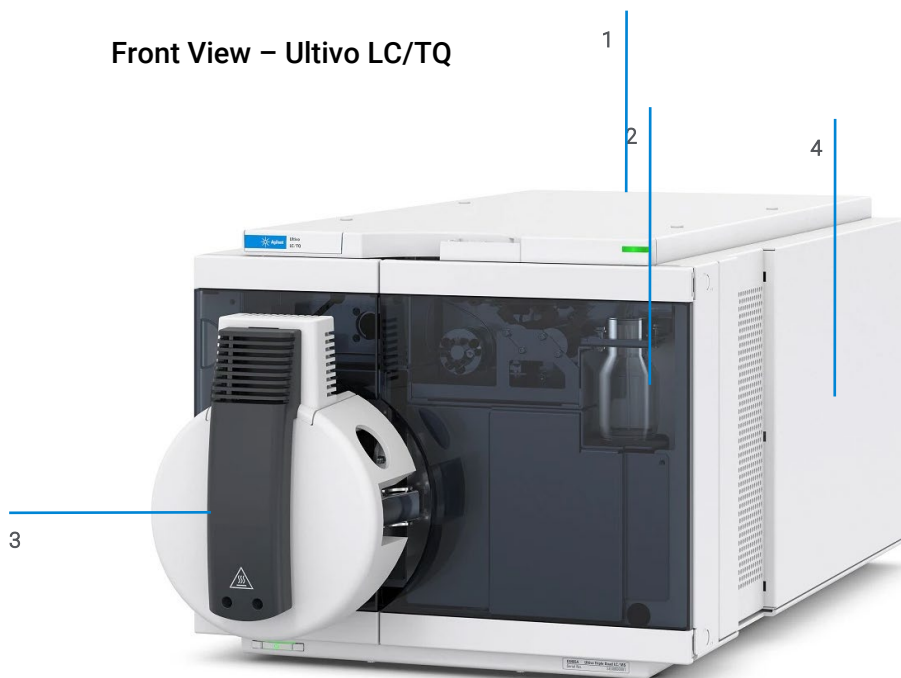
In this section, you will identify basic hardware components and their locations for the 6400 Series triple quadrupole LC/MS system.



Fill in the Blank:

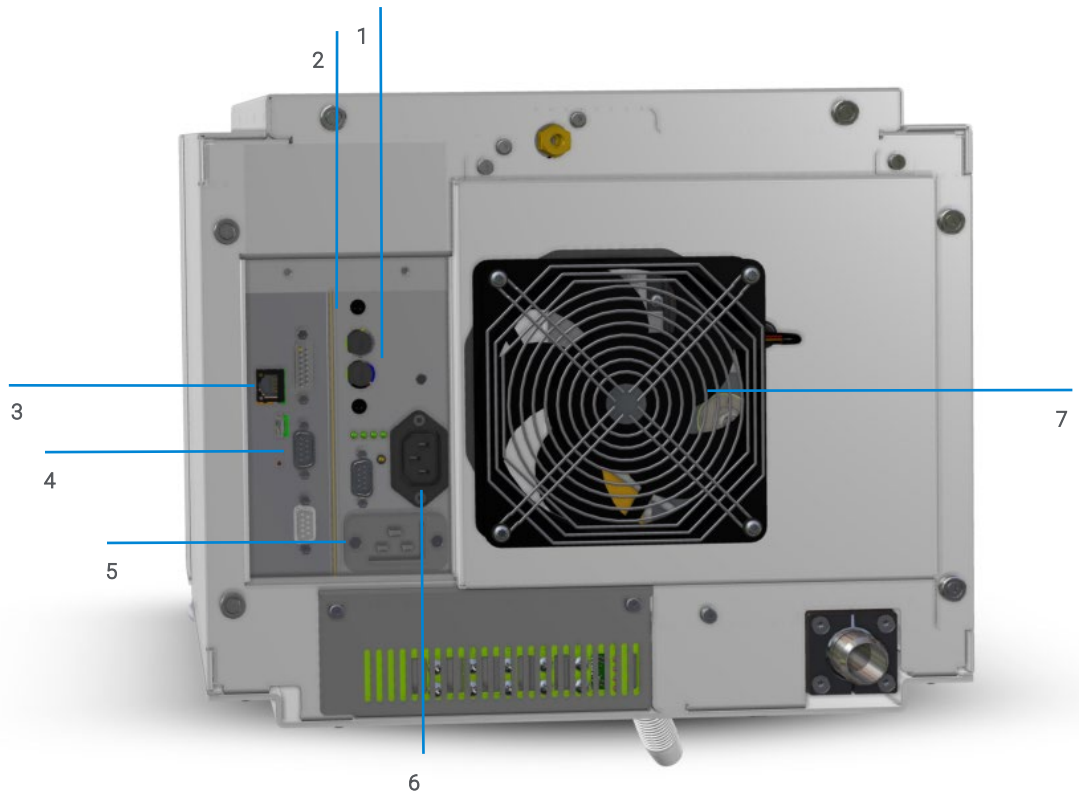
Work with your Agilent Service Engineer and/or use the *Agilent Triple Quadrupole LC/MS System User Guide* to label the flagged components below for your installed instrument(s).

Front View – Ultivo LC/TQ



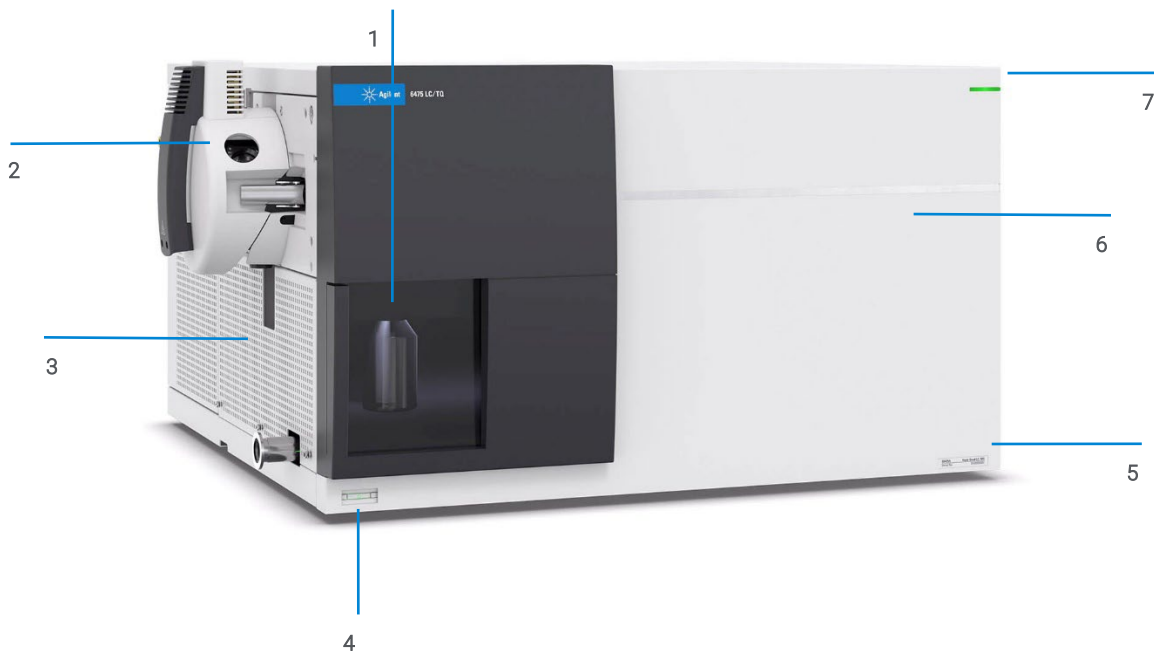
Hardware

Rear View - Ultivo LC/TQ



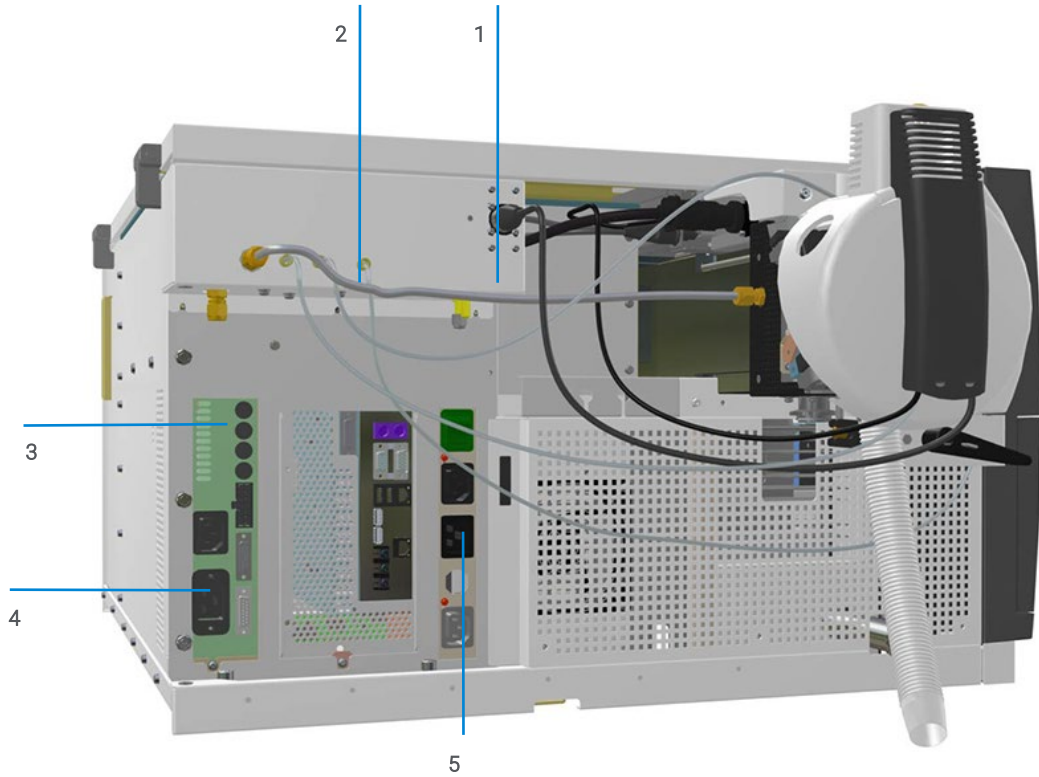
Hardware

Front View - 6475A



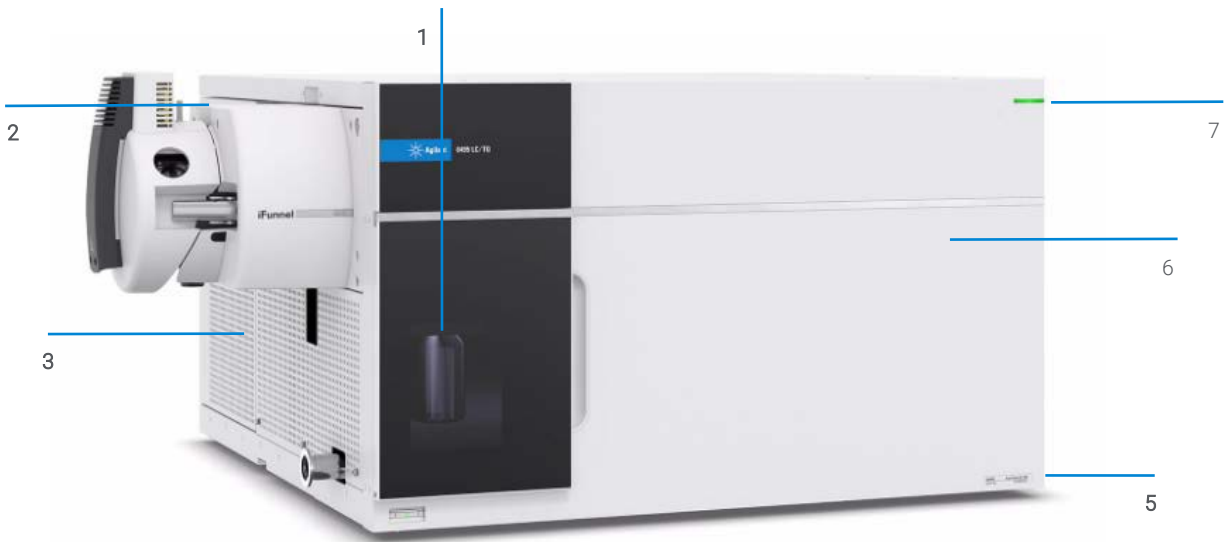
Hardware

Side View – 6475A



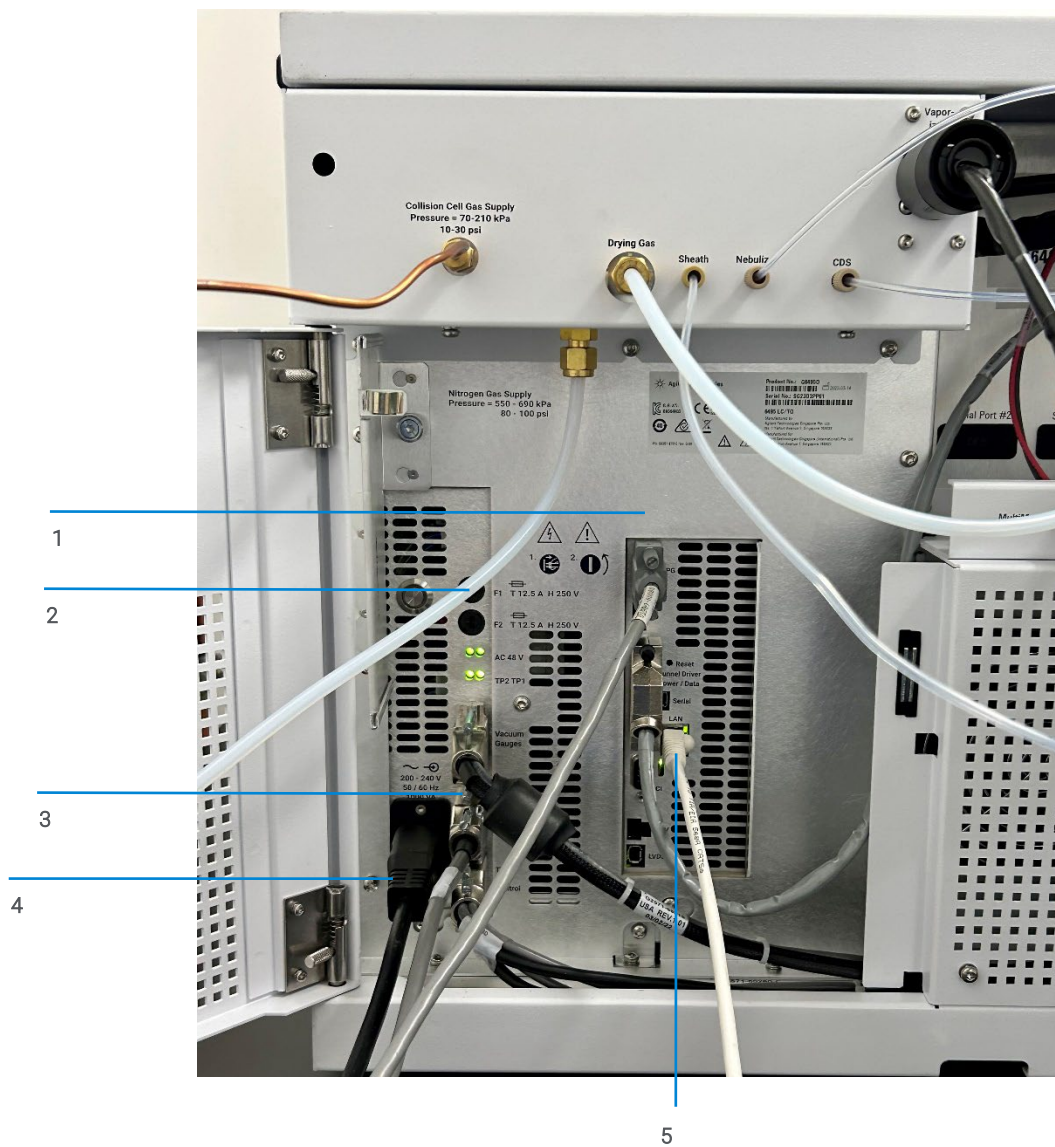
Hardware

Front View – 6495D



Hardware

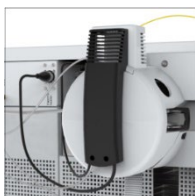
Side View – 6495D



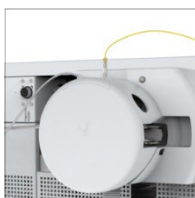
Basic Components

Ionization Source

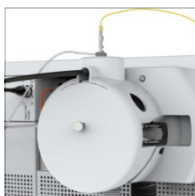
Agilent liquid chromatography/mass spectrometry (LC/MS) ion sources enable analysis of a wide range of samples quickly and accurately.



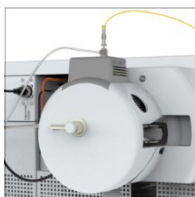
- Agilent Jet Stream (AJS ESI) source



- Electro spray Ionization (ESI) source



- Atmospheric Pressure Chemical Ionization (APCI) source



- Multimode source (MMI)



- Agilent nanospray ESI source

Hardware



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:

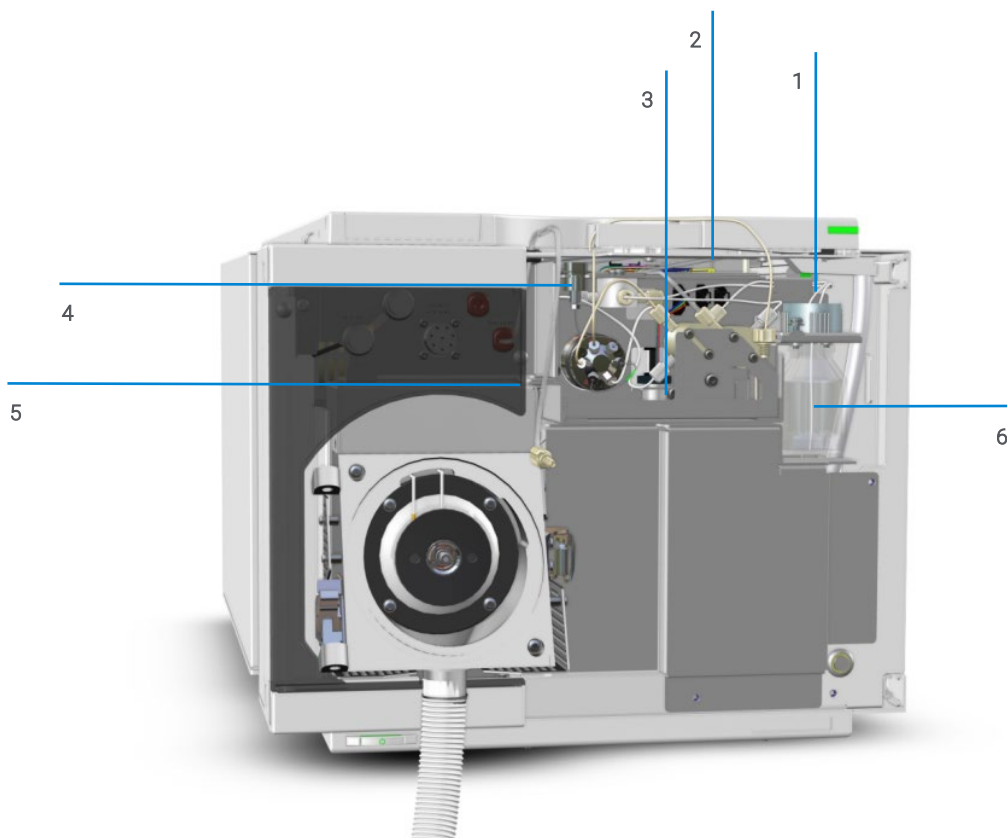
3 List the name and part number of the proper tune solution for this system:

Hardware

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



Hardware

6475A

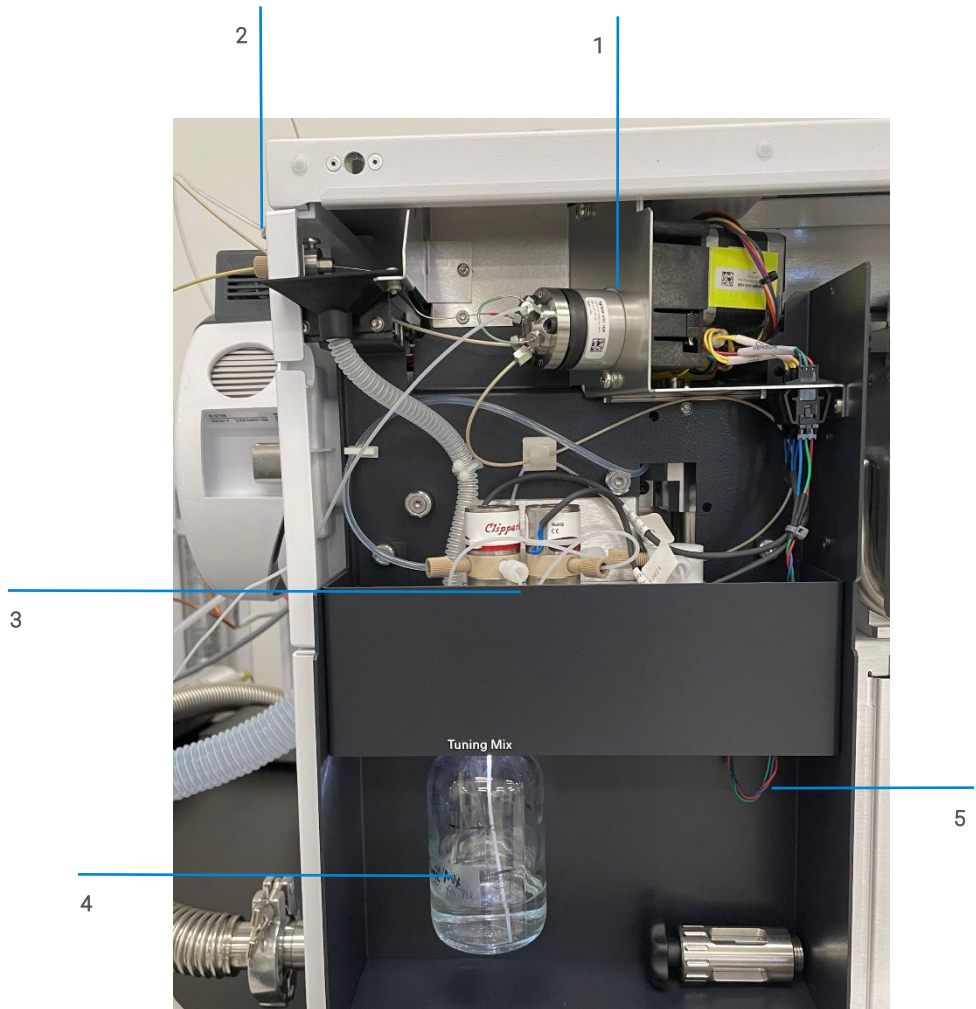
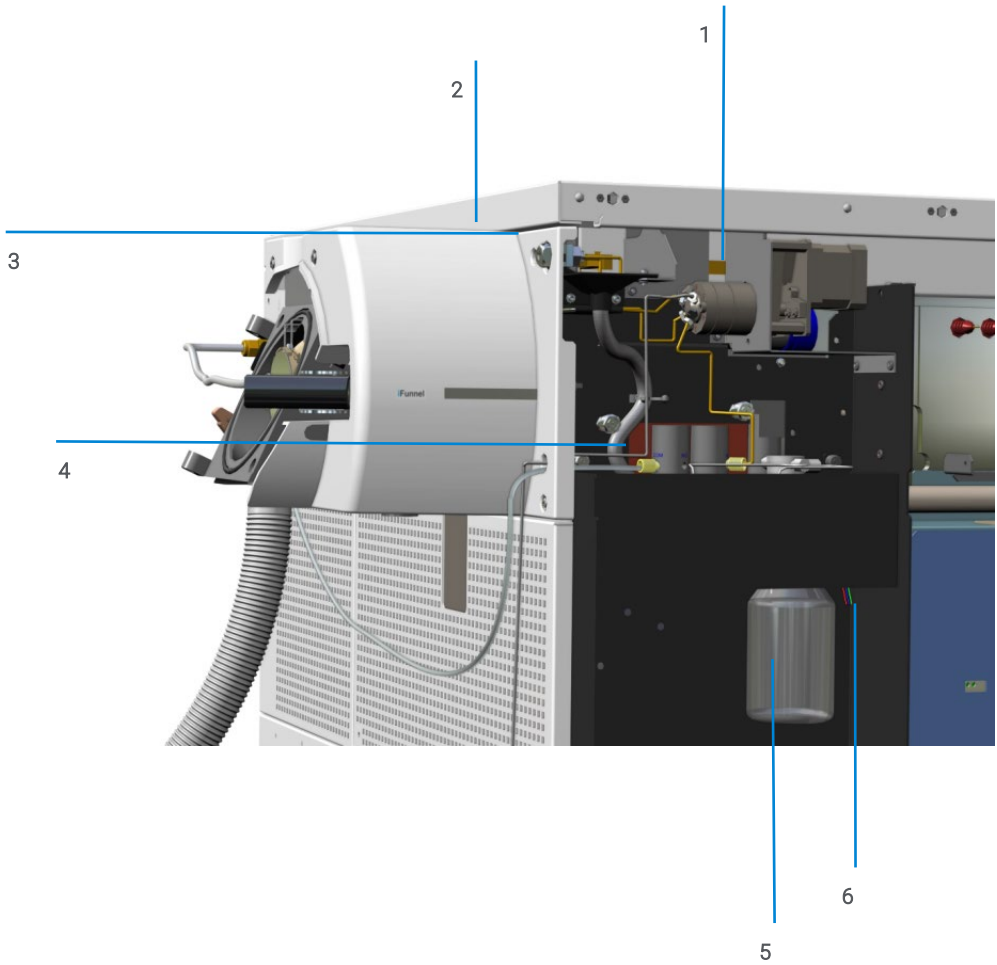


Figure 1. Front cover removed.

Hardware

6495D



Hardware



Hardware Introduction

- 1 Practice removing and attaching the calibrant bottle.
 - 2 How often is the calibrant bottle checked and refilled?
-

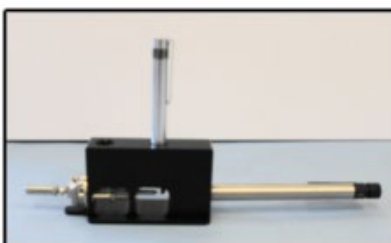
Hardware

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:

Hardware

Rough Pump

MS40+ (Ultivo and 6475A)



MS120+ (6495D)



Hardware



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 Basics



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

Panes

Instruments – Controls specific instruments.

Projects – Create paths to save project data.

Administration – Add and remove configuration

6475A LC/TQ	
Description:	Instruments
Location:	SYSTEM
Created by:	SYSTEM
Creation date:	2023-05-17 14:59:49-07:00
Last configured by:	SYSTEM
Last configuration date/time:	2023-05-18 09:49:47-07:00
Last modified by:	SYSTEM
Last modified date/time:	2023-05-19 08:38:18-07:00
Application:	MassHunter Workstation
Instrument controller:	DESKTOP-655FPQJM
Instrument type:	Agilent LC TQ
Id:	15
Owner contact information:	

Ribbon

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.

Software Basics

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**.

6475A LC/TQ - Control Panel

MANAGEMENT

Edit Instrument Delete Instrument Refresh All Lock Instrument Configure Instrument Create Acquisition Desktop Shortcuts Close Connection Copy to Clipboard Offline Worklist Editor Method Comparison Viewer Study Manager Enable Skyline Automation

Instruments and Locations Actions Selected Row LC/MS Acquisition Tools

Instruments 6475A LC/TQ Not Connected

Project: InstrumentCheckout Launch Launch Offline

Status

Details


Activity Log (last 7 days) 1 - 50 of 63

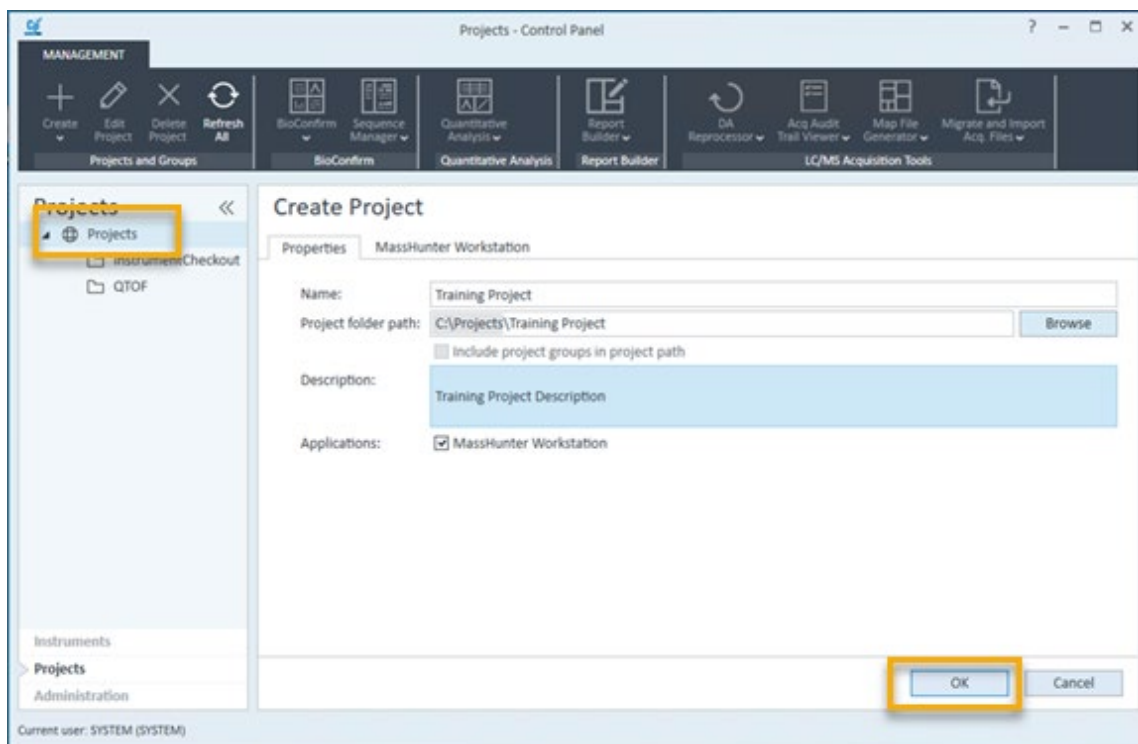
Date/Time	User	Description
2023-08-29 22:54:40-07:00	SYSTEM (SYSTEM)	Engine Launcher: Failed to start System Engines. Failed to initialize 'TQ' de
2023-08-29 22:54:34-07:00	SYSTEM (SYSTEM)	Acquisition Engine: Failed to initialize 'TQ' device. Invalid Connection.
2023-08-29 22:52:09-07:00	SYSTEM (SYSTEM)	Offline Method Editor: User 'SYSTEM' has logged out.
2023-08-29 22:51:06-07:00	SYSTEM (SYSTEM)	Engine Launcher: Failed to start System Engines. Failed to initialize 'TQ' de
2023-08-29 22:51:01-07:00	SYSTEM (SYSTEM)	Acquisition Engine: Failed to initialize 'TQ' device. Invalid Connection.
2023-08-29 16:32:41-07:00	SYSTEM (SYSTEM)	Engine Launcher: Failed to start System Engines. Failed to initialize 'TQ' de
2023-08-29 16:32:36-07:00	SYSTEM (SYSTEM)	Acquisition Engine: Failed to initialize 'TQ' device. Invalid Connection.
2023-08-29 15:38:08-07:00	SYSTEM (SYSTEM)	Engine Launcher: Failed to start System Engines. Failed to initialize 'TQ' de
2023-08-29 15:38:03-07:00	SYSTEM (SYSTEM)	Acquisition Engine: Failed to initialize 'TQ' device. Invalid Connection.

Current user: SYSTEM (SYSTEM)



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**



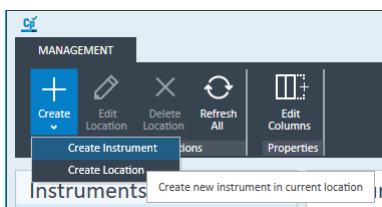
Instruments

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.

Software Basics

The screenshot displays the 'Instruments - Control Panel' window. The top toolbar includes 'MANAGEMENT' (Edit, Delete, Refresh, Lock, Configure, Create Acquisition Desktop Shortcuts, Close Connection) and 'LC/MS Acquisition Tools' (Copy to Clipboard, Offline Worklist Editor, Method Comparison Viewer, Study Manager, Enable Skyline Automation). The left sidebar shows a tree view with 'Instruments' expanded, containing '6475A LC/TQ'. The main area is titled 'Create Instrument' and contains the following fields:

- Name: 6495D LC/TQ
- Description: (empty)
- Application: MassHunter Workstation
- Instrument controller: DESKTOP-655PFQM
- Instrument type: Agilent LC TQ
- Contact: (empty)
- Default project: (empty) Always use Default project

At the bottom right, the 'OK' button is highlighted with a yellow box, and the 'Cancel' button is also visible.

Current user: SYSTEM (SYSTEM)

Software Basics

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.

6495D LC/TQ - Control Panel

MANAGEMENT

Edit Instrument Delete Instrument Refresh All Lock Instrument Configure Instrument Create Acquisition Desktop Shortcuts Close Connection Copy to Clipboard Offline Worklist Editor Method Comparison Viewer Study Manager Enable Skyline Automation

Instruments and Locations Actions Selected Row LC/MS Acquisition Tools

Instruments Instruments 6475A LC/TQ 6495D LC/TQ

6495D LC/TQ Not Connected

Project: TrainingProject Launch Launch Offline

Status

Details

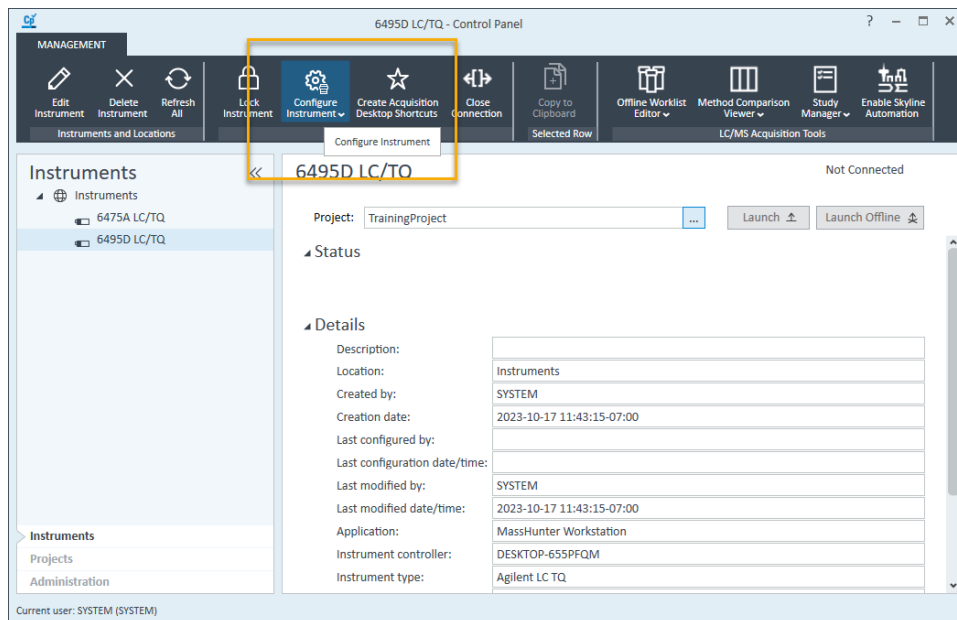
Description:	
Location:	Instruments
Created by:	SYSTEM
Creation date:	2023-10-17 11:43:15-07:00
Last configured by:	
Last configuration date/time:	
Last modified by:	SYSTEM
Last modified date/time:	2023-10-17 11:43:15-07:00
Application:	MassHunter Workstation
Instrument controller:	DESKTOP-655PFQM
Instrument type:	Agilent LC TQ

Current user: SYSTEM (SYSTEM)

Software Basics

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.

Tuning

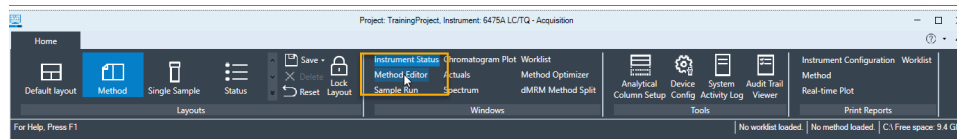
Frequent tuning, automated or manual, is not required. Once tuned, the LC/MS triple quadrupole is stable. Tuning should be needed no more often than monthly, weekly at most.

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.

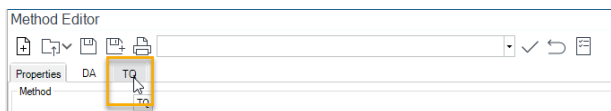
Calibrating the LC/TQ (checktune)

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**.



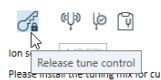
- 2 Click the **TQ** tab.



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



Detailed MS Checktune Report - G6475A

Instrument Information

Model	G6475A	Checktune Date	2023-10-11T11:39:25-07:00
Serial Number	GG2222S001	SWFW Version	3.0.1467/8.1.38
Ion Source	AJS ESI	Ionization Mode	ESI
Last Autotune Date	2023-09-29T09:46:04-07:00	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

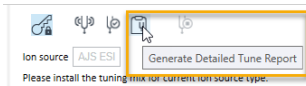
MS1 Quad Frequency	967.90	MS1 Heater (°C)	100
--------------------	--------	-----------------	-----

Review a Tune Report

Generate a detailed tune report and save

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

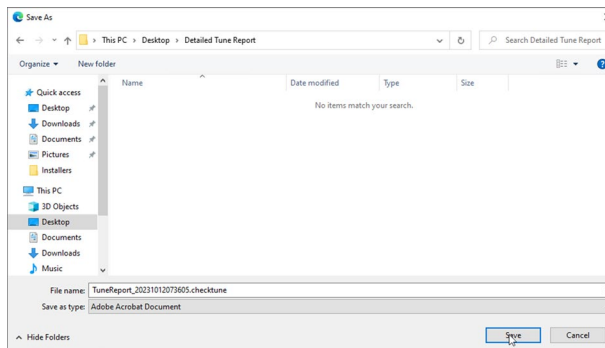
- 1 In the Method Editor window, select the **TQ** tab.
- 2 Click the **Tune > Autotune** section in the left pane.
- 3 Click **Request tune Control** in the toolbar in the Autotune section.
- 4 Click **Generate Detailed Tune Report** in the toolbar.



- 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.

Tuning

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/PW Version	3.1.551/0.4.56
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

Tuning



Electron Multiplier Voltage (EMV)

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

- 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 prevention 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:

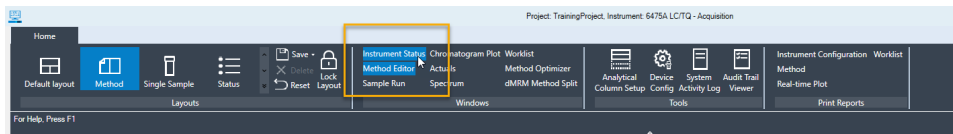
- b High vacuum:

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

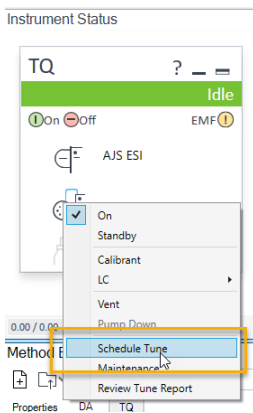
Tuning

Scheduling a checktune

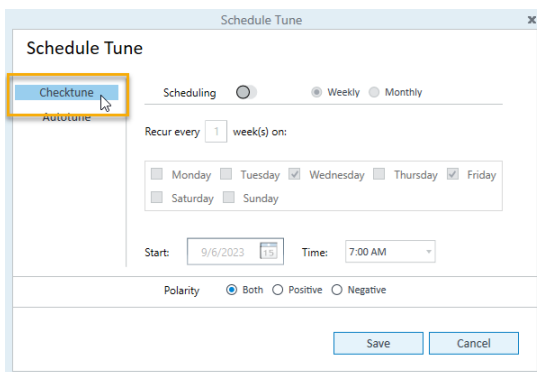
- 1 On the tool ribbon, click Instrument Status in Windows to display the Instrument Status window.



- 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.



Tuning

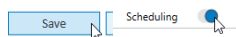
NOTE

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.

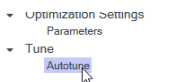


- 5 Select a day of the week and a Start date and time to indicate how often to schedule the checktune.
- 6 Click **Save**.

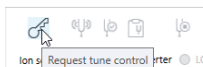


Stop checktune

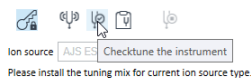
- 1 Click the Tune > Autotune section in the left pane.



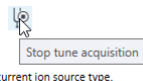
- 2 Click **Request tune Control** in the toolbar in the Autotune section. This button locks control of the TQ instrument. You cannot start a single sample run or a worklist when Tune has control of the TQ instrument.



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



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



Tuning

- 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**.

Tune was stopped by the user.



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

Tuning

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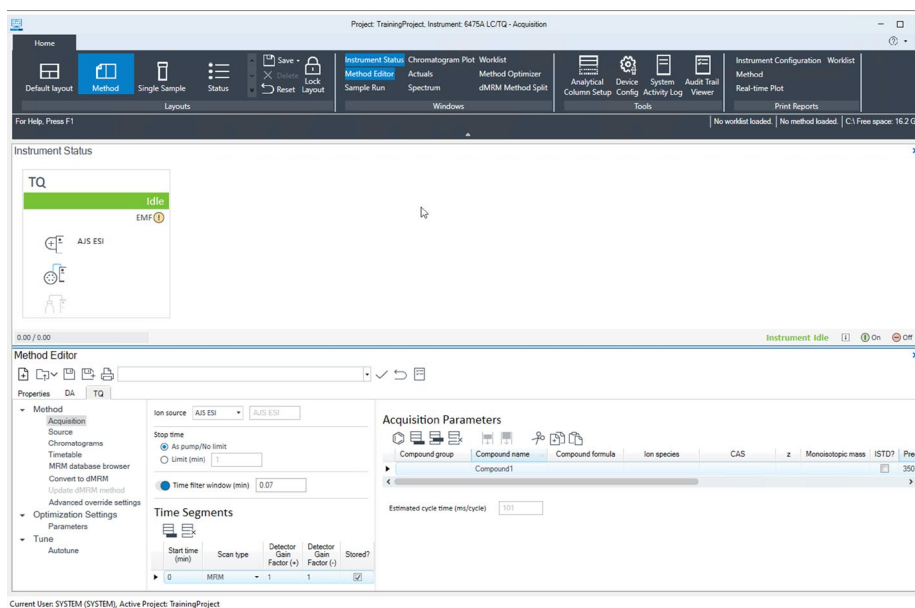
5 Using Methods

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

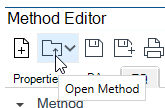


Working with the Default Method

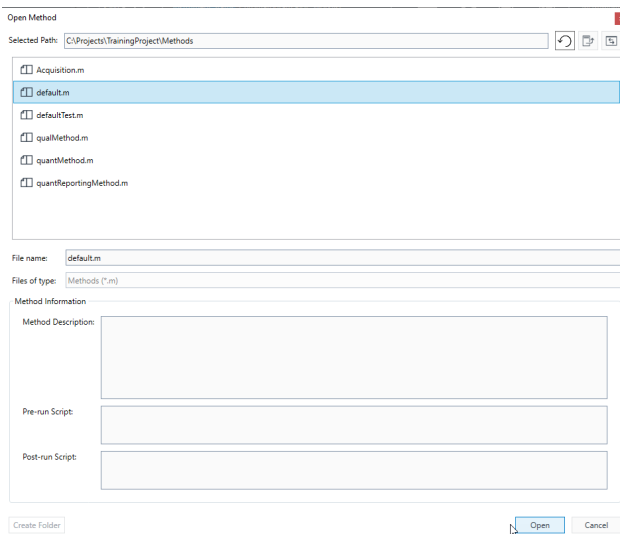
Once an analysis has been created or opened, the default.m method is available to start from or apply a previously created method. The default method represents a good starting point for method development.

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**.

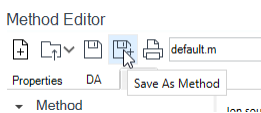


- 4 Under Method, review all default Method subsections.
 - a Acquisition - Set TQ acquisition parameters.
 - b Source - Set source parameters for the TQ.
 - c Chromatograms - Specify plots to display in the Chromatogram Plot window during the run.

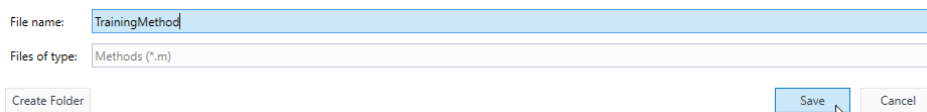
Using Methods

- 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**.



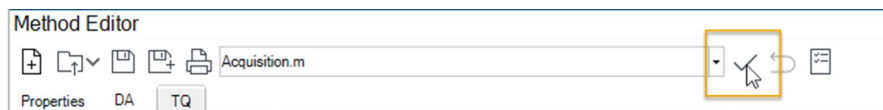
6 Enter a **File name**, for this example *Training Method*, then click **Save**.



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**.



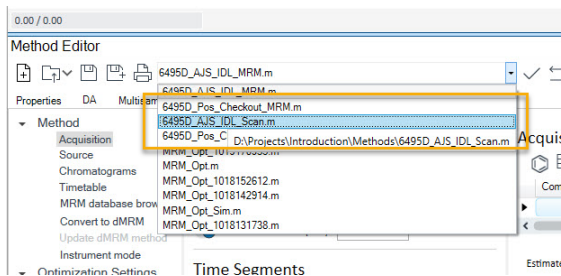
Set up a Scan Method



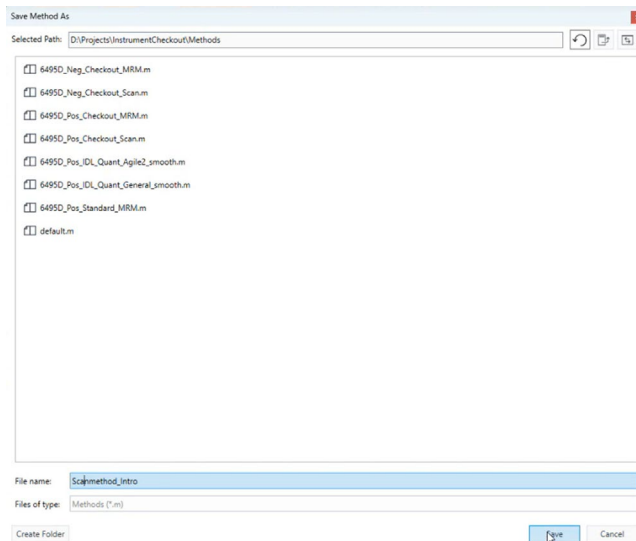
Load an existing method and Save As new method

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.

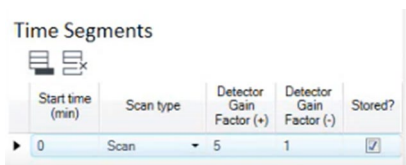


- 2 Click **Save as Method**, enter *Scanmethod_Intro* for the file name. Click **Save**.



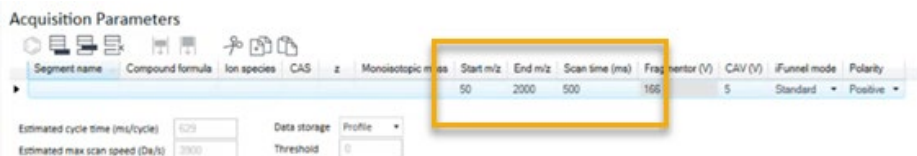
Using Methods

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



Start time (min)	Scan type	Detector Gain Factor (+)	Detector Gain Factor (-)	Stored?
0	Scan	5	1	<input checked="" type="checkbox"/>

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



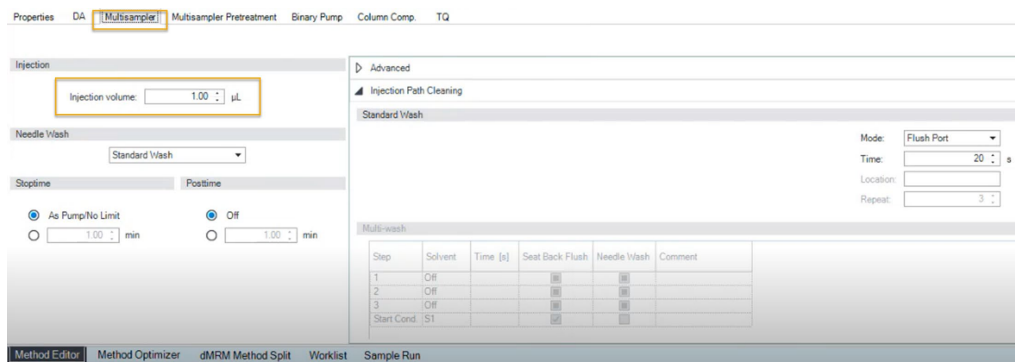
Segment name	Compound formula	Ion species	CAS	z	Monoisotopic mass	Start m/z	End m/z	Scan time (ms)	Fragmentor (V)	CAV (V)	Funnel mode	Polarity
						50	2000	500	166	5	Standard	Positive

Estimated cycle time (ms/cycle): 629
Estimated max scan speed (Da/s): 2000
Data storage: Profile
Threshold: 0

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.



Properties DA **Multisampler** Multisampler Pretreatment Binary Pump Column Comp. TQ

Injection
Injection volume: 1.00 µL

Needle Wash
Standard Wash

Stop time
 As Pump/No Limit
 1.00 min

Posttime
 Off
 1.00 min

Advanced
Injection Path Clearing
Standard Wash
Mode: Flush Port
Time: 20 s
Location:
Repeat: 3

Multi-wash

Step	Solvent	Time (s)	Seat Back Flush	Needle Wash	Comment
1	Off		<input type="checkbox"/>	<input type="checkbox"/>	
2	Off		<input type="checkbox"/>	<input type="checkbox"/>	
3	Off		<input type="checkbox"/>	<input type="checkbox"/>	
Start Cond.	S1		<input type="checkbox"/>	<input type="checkbox"/>	

Method Editor | Method Optimizer | dMRM Method Split | Worklist | Sample Run

- 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

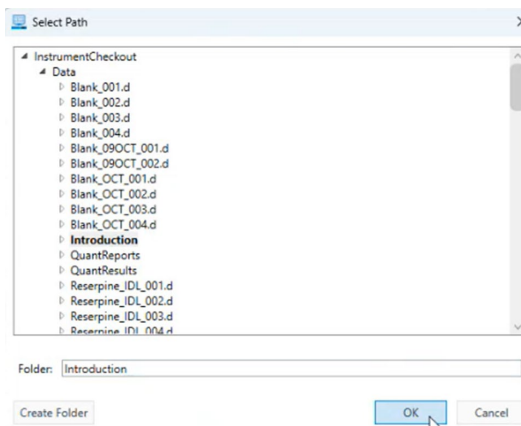
In this exercise, you will acquire data using MassHunter Data Acquisition software and then use MassHunter Qualitative Analysis software to identify a precursor ion for Reserpine.

- 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

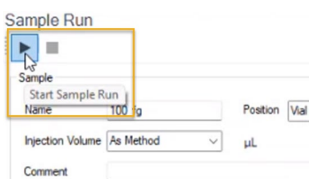
(optional) Select Auto Increment to automatically increment the file name if that file exists

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

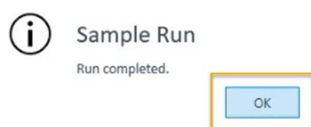


Using Methods

- 4 Click **Start Sample Run**.



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



Monitor the Status Windows

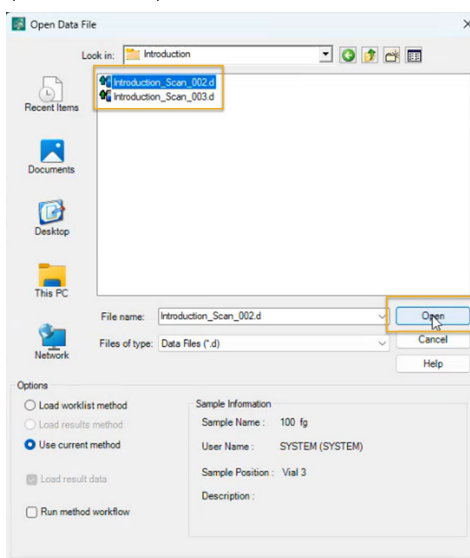
As data is being acquired, use the instrument status monitors and online signal displays available in the Instrument Status and Real-time Plot Panes to observe changes in modules.

- 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.

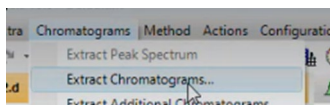
Review the data using Qualitative Analysis



- 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**.

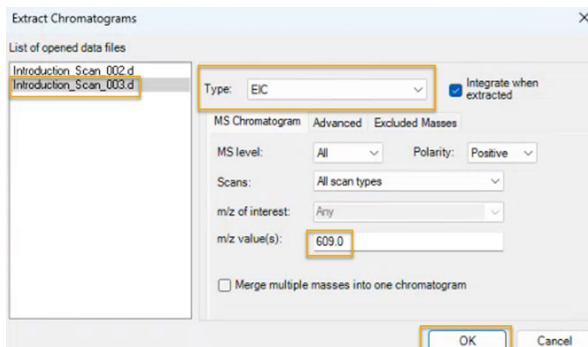


- 3 In the main window, click **Chromatograms > Extract Chromatograms**.

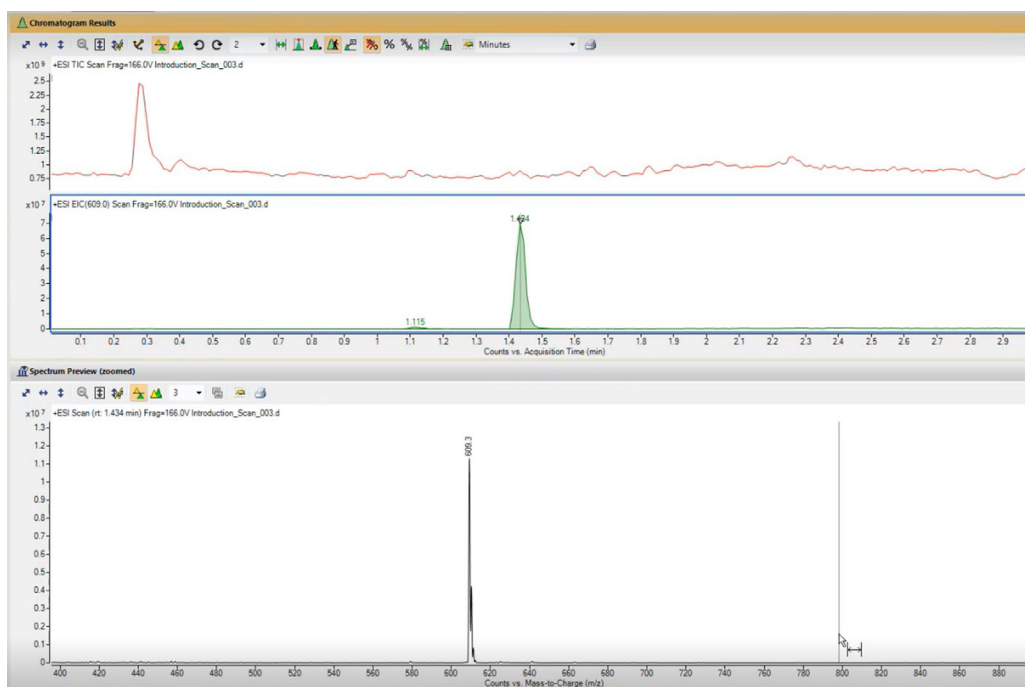


- 4 In the Extract Chromatograms dialog box, click **Type:** and select **EIC**.
- 5 Enter the m/z value: 609, then click **OK**.

Using Methods



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?

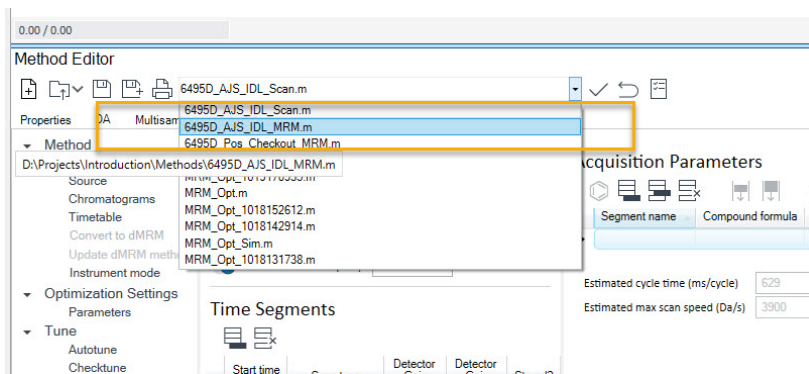
Set up an MRM Method



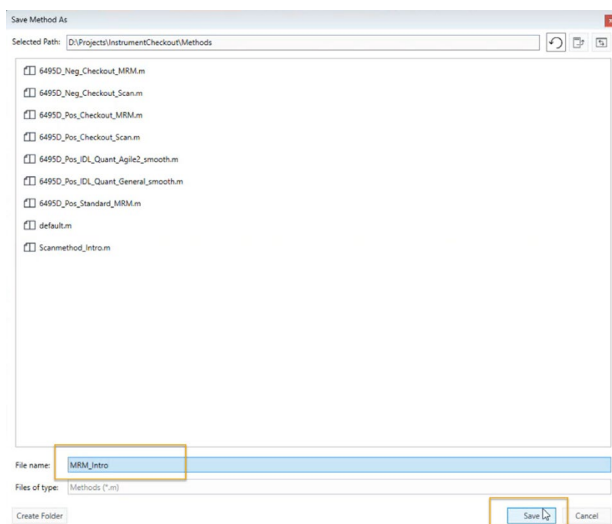
Load an existing method and Save As new method

Now that the precursor ion is identified, start with a known MRM (multiple reaction monitoring) method using product ion m/z 195.

- 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**.



Using Methods

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

Start time (min)	Scan type	Detector Gain Factor (+)	Detector Gain Factor (-)	Stored?
0	MRM	5	1	<input checked="" type="checkbox"/>

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

Acquisition Parameters	Compound formula	Ion species	CAS	z	Monoisotopic mass	ISTD?	Precursor m/z	MS1 res	Product m/z	MS2 res	Dwell (ms)	Fragmentor (V)	CAV (V)	CE (V)	IFunnel mode
rpine						<input type="checkbox"/>	609.3	Unit	195.1	Unit	200	166	5	42	Standard

NOTE

Values will vary based on your instrument model.

- 5 Click **Chromatograms** and confirm the settings below.

Chrom type	Label	Precursor ion (m/z)	Product ion (m/z)
MRM	MRM	609.3	195.1

- 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.

Using Methods

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**.

Sample Run

Start Sample Run

Name: Position: Val 2

Injection Volume: As Method μ L

Comment:

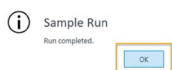
Data File

Auto Increment

Name: Introduction_MRM_000.d View Data

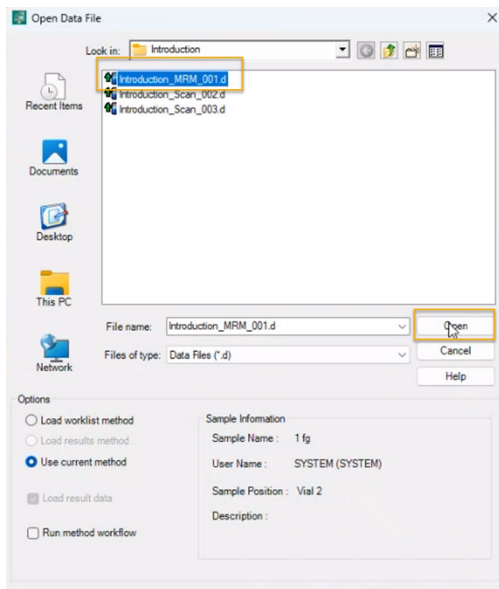
Path: D:\Projects\InstrumentCheckout\Data\Introduction

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



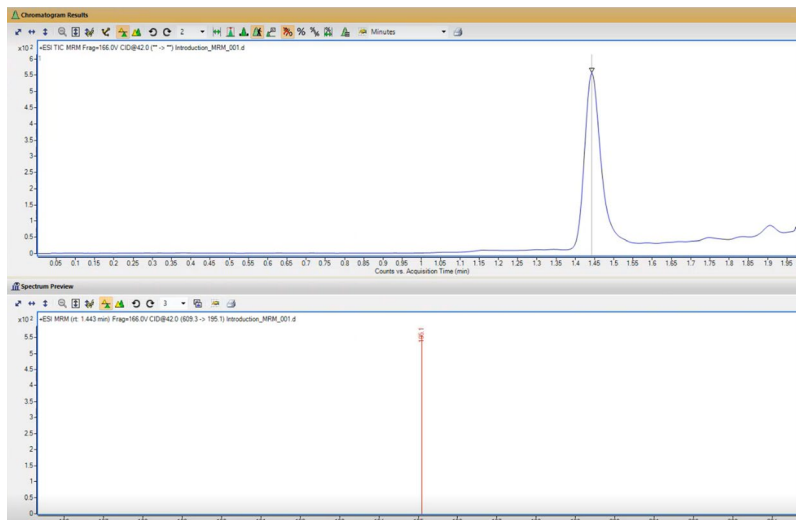
Reviewing the MRM data using Qualitative Analysis

- 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.

Using Methods



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.

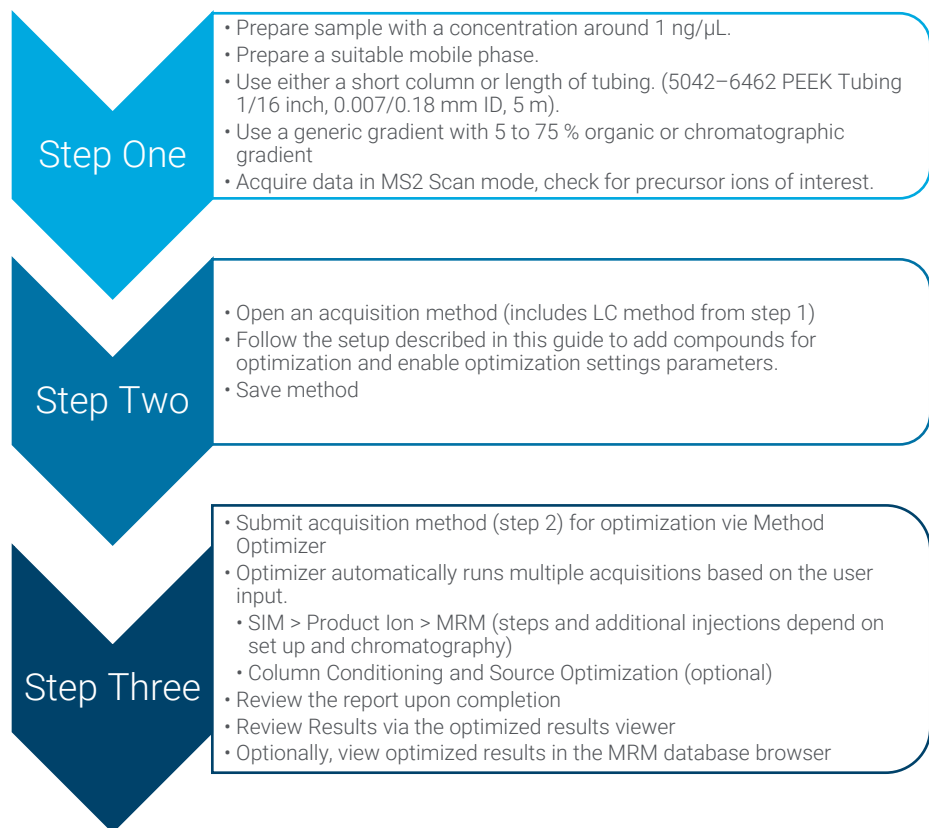
Using Methods

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6 Optimizing Methods

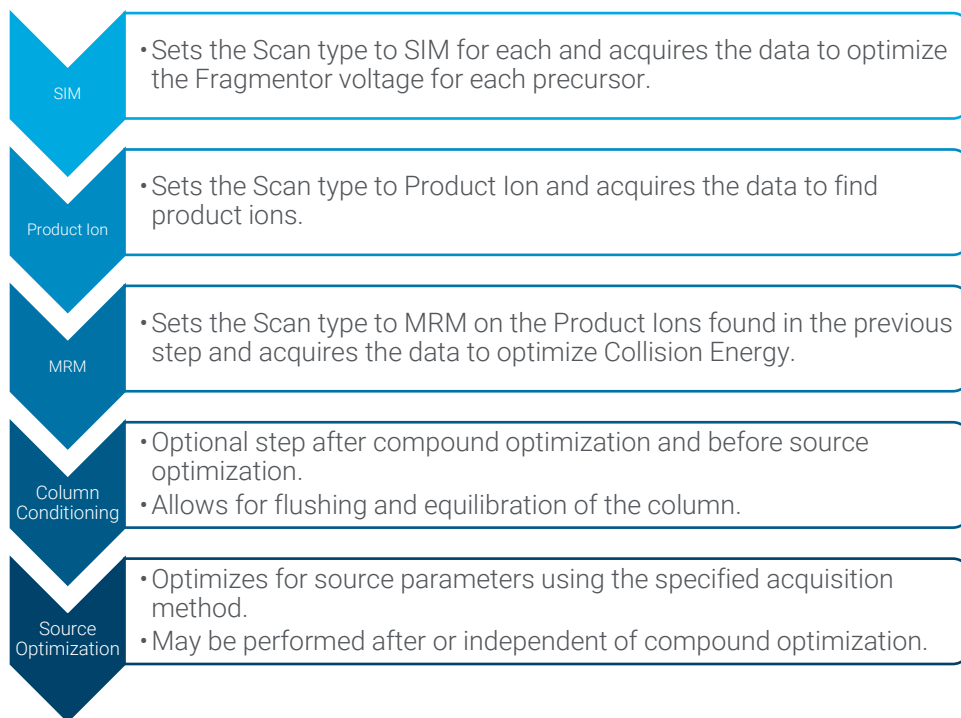
MassHunter Optimizer lets you automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on a triple quadrupole mass spectrometer instrument for each individual compound analyzed. Specifically, it automates the selection of the best precursor ions, the optimization 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.



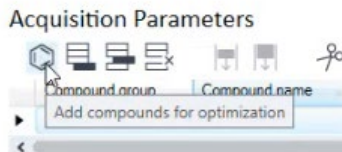
Optimize a method for a known sample:

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*

Optimizing Methods

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



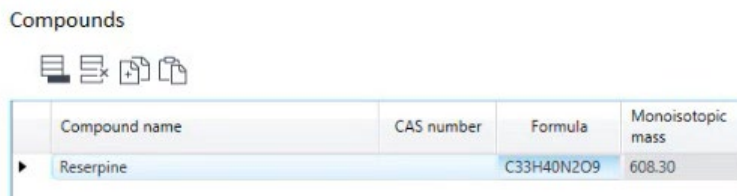
- The Add compounds for optimization window opens. Click **Add a row at the end of the table**.



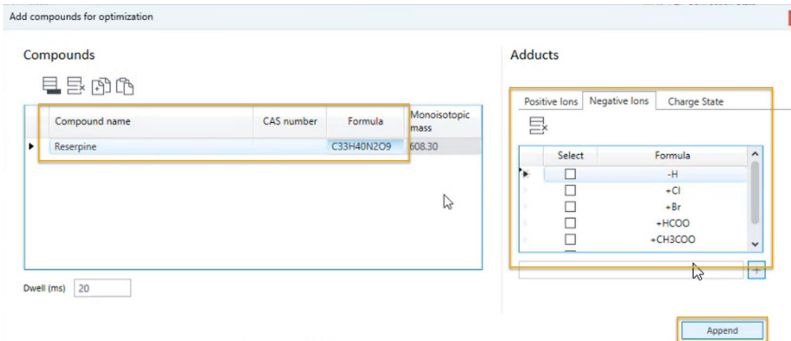
- In the Compounds section, click in the fields and enter the following information:

a Compound name: *Reserpine*

b Formula: *C33H40N2O9*

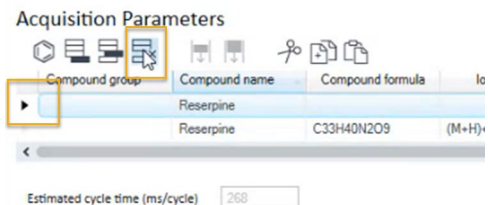


- In the Adducts section, click the Positive Ions tab and select **+H**, then the Negative Ions tab and deselect **-H**.
- Click **Append** to add the information to the acquisition parameters pane.

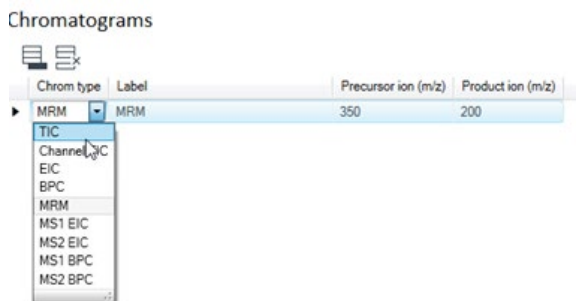


Optimizing Methods

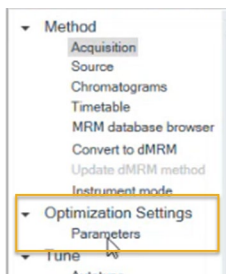
- 7 Remove the original parameter line by selecting the line, then click **Remove**.



- 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

Optimizing Methods

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

The screenshot shows the 'Compound parameters' and 'Source parameters' sections of the optimization software. In the 'Compound parameters' section, the 'Fragmentor (V)' and 'Collision Energy' settings are highlighted with orange boxes. The 'Fragmentor (V)' is set from 166 to 216 with a step size of 10. The 'Collision Energy' is set from 0 to 20 with a step size of 4. In the 'Source parameters' section, the 'Capillary Voltage (V)' setting is highlighted with an orange box, showing a range from 2600 to 3400 with a step size of 200. Other parameters like Gas Temperature, Sheath Gas Temperature, Nebulizer, Gas Flow, Sheath Gas Flow, and Nozzle Voltage are listed but not selected.

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

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

Method Optimizer

The screenshot shows the 'Method Optimizer' dialog box with the question 'What type of optimization do you wish to perform?'. Under 'Select one of the optimization modes', three options are listed: 'Guided', 'Automated', and 'Compound-by-compound'. The 'Automated' option is selected and highlighted with an orange box. To the right, under 'Fully Automated Method Optimization:', there is a description and a list of features:

- Optimization is carried out in a fully automated manner. All user input for each phase is defined prior to the start of the optimization. Data review is only available once the optimization has completed.
- Creates the most suitable MRM transitions from a list of chemical formulas or precursor ions
- Optimizes and fine-tunes MRM specific parameters
- Optimizes and fine-tunes Ion Source and front-end ion optics parameters
- Optionally updates compound and method database

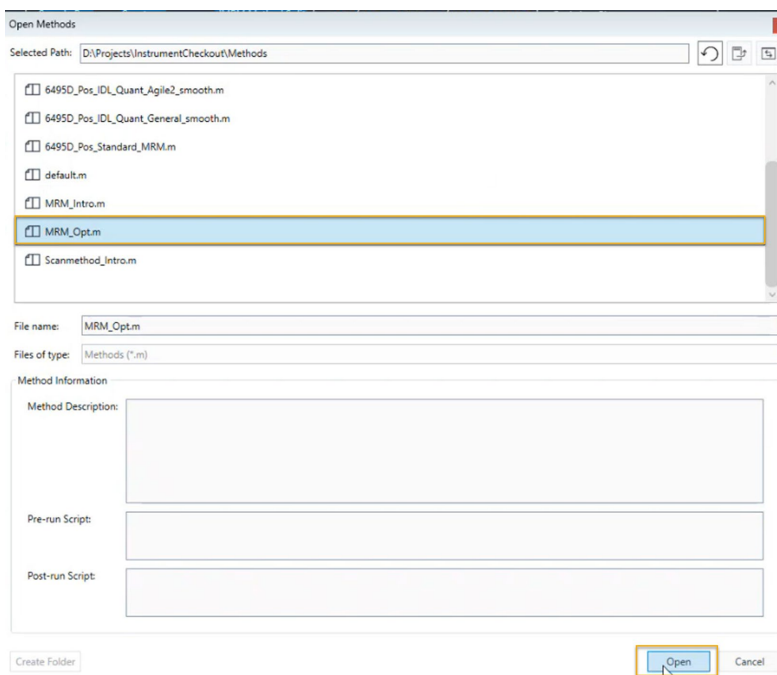
14 Click **Next**.

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.

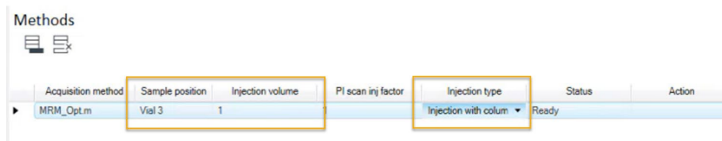
Optimizing Methods

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



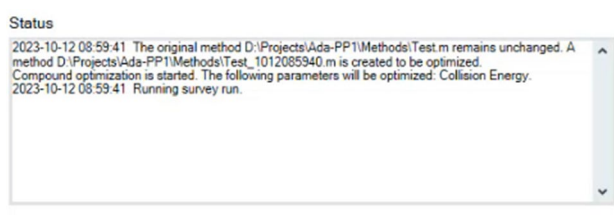
18 In the Methods pane, verify the following settings:

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



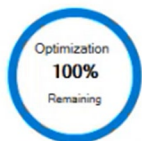
19 Click **Next**. Method optimization begins.

20 As the Method Optimization runs, review the Status.



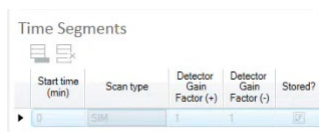
Optimizing Methods

21 Observe over time the Optimization Remaining.



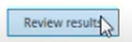
22 Navigate to **Method Editor > Acquisition** to observe the mass spectrum processing.

- a Review the TIC plots.
- b Review the Scan Type being used in the Time Segment panel

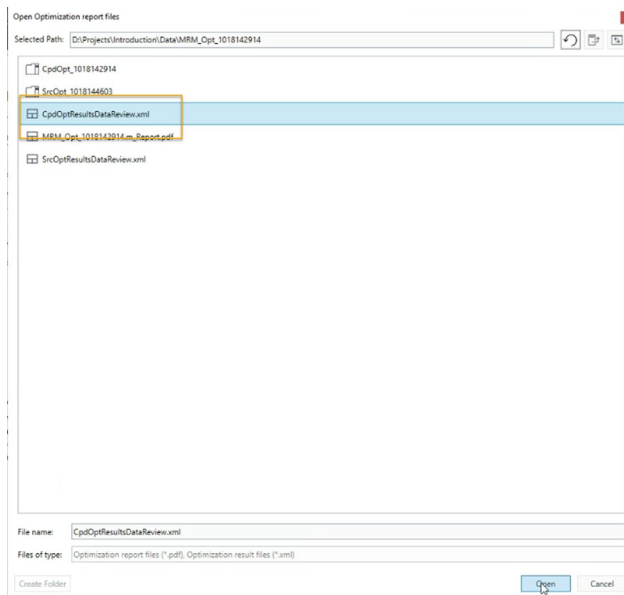


Start time (min)	Scan type	Detector Gain Factor (+)	Detector Gain Factor (-)	Stored?
0	SIM	1	1	[X]

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

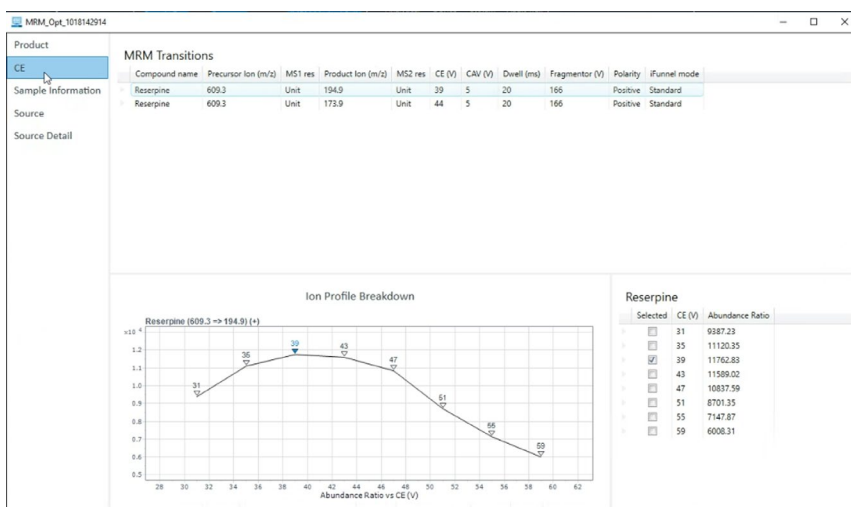


24 Select a report file from the Open optimization report files window and click **Open**.



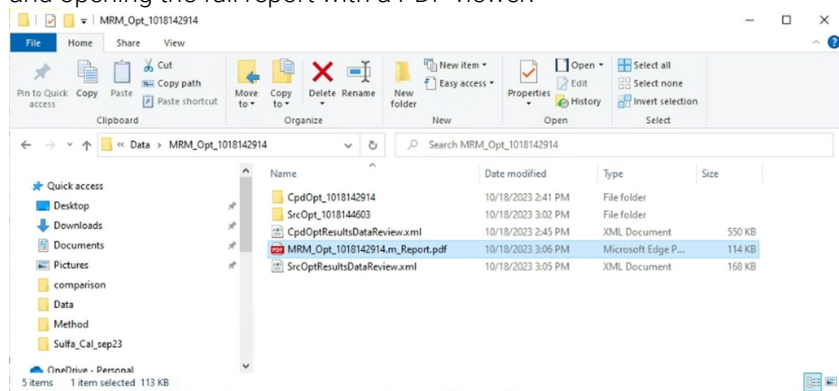
Optimizing Methods

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.



Optimizing Methods

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

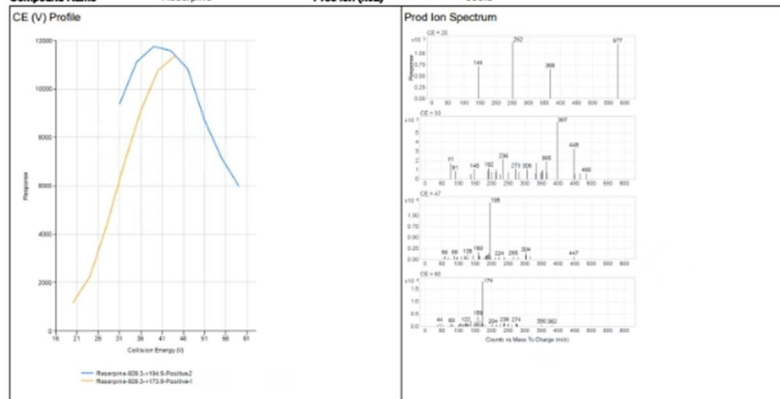
Product Ion CE Range (V) From 20 to 60; Stepsize 4
 CE Range (V) From 20 to 60; Stepsize 4

MRM Table

Compound Name	Prec Ion (m/z)	MS1 Res	Frag (V)	Prod Ion (m/z)	MS2 Res	CE (V)	CAV (V)	Dwell (ms)	Poi	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



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)
 - Collision Energy
 - Capillary Voltage

Optimizing Methods

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7

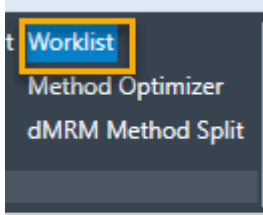
Run a Worklist


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).

Run a Worklist

- 3 Enter all the information on the Sample Information tab.

Add Multiple Samples

Sample Information Sample Position

Sample

Name: Append Counter

Suffix Counter

Number of digits: Start Value: Step:

Method

Name:

Path:

Override DA Method

Name:

Path:

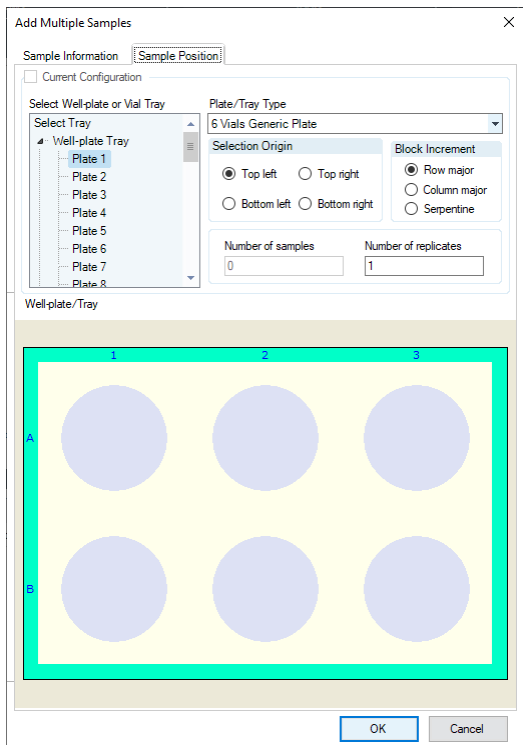
Injection

Injection Volume: µl

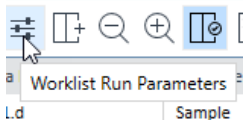
Run a Worklist

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**.



Run a Worklist

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



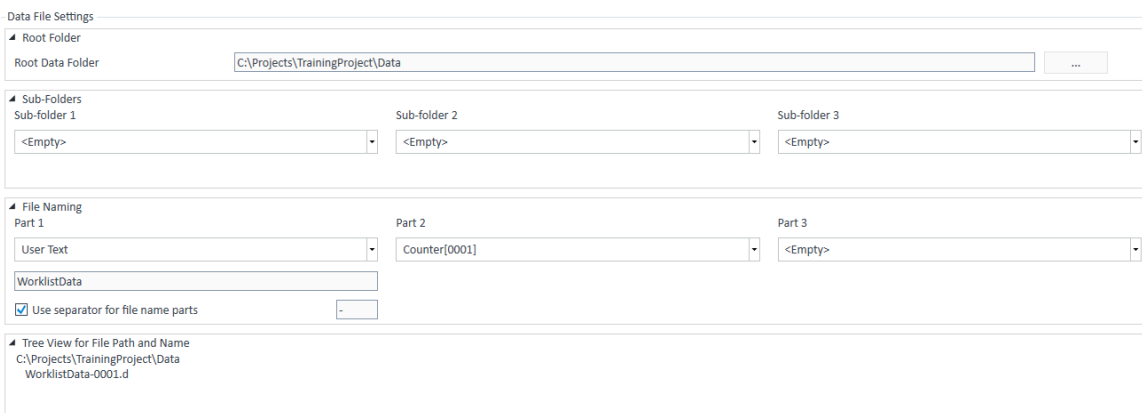
Method Paths

Method

Override DA

Scripts

- 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**.



Data File Settings

Root Folder
Root Data Folder

Sub-Folders
Sub-folder 1 Sub-folder 2 Sub-folder 3

File Naming
Part 1 Part 2 Part 3

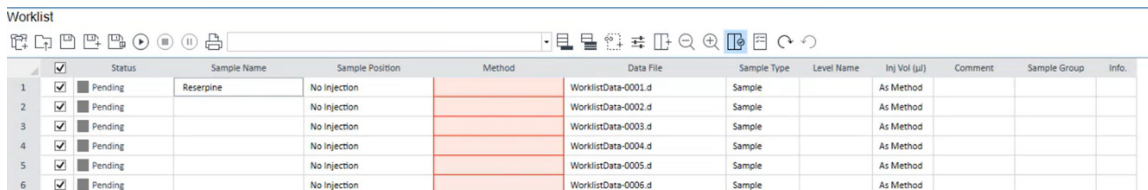
 Use separator for file name parts

Tree View for File Path and Name
C:\Projects\TrainingProject\Data
WorklistData-0001.d

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**.



Worklist

	Status	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	Inj Vol (µl)	Comment	Sample Group	Info.
1	<input checked="" type="checkbox"/> Pending	Reserpine	No Injection		WorklistData-0001.d	Sample		As Method			
2	<input checked="" type="checkbox"/> Pending		No Injection		WorklistData-0002.d	Sample		As Method			
3	<input checked="" type="checkbox"/> Pending		No Injection		WorklistData-0003.d	Sample		As Method			
4	<input checked="" type="checkbox"/> Pending		No Injection		WorklistData-0004.d	Sample		As Method			
5	<input checked="" type="checkbox"/> Pending		No Injection		WorklistData-0005.d	Sample		As Method			
6	<input checked="" type="checkbox"/> Pending		No Injection		WorklistData-0006.d	Sample		As Method			

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



Run a 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.

Run a Worklist

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

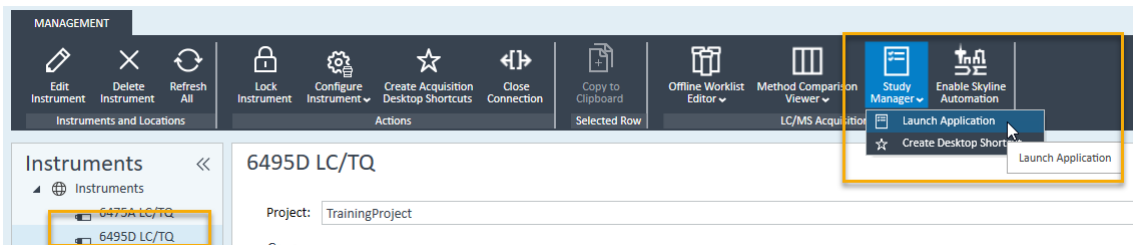
The study types create and run a worklist that to analyze the samples. Specify more information to apply to the study, including a location to place all the files generated by that study or create several types of studies. When the Study Manager application starts, the data acquisition engines are automatically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

NOTE

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

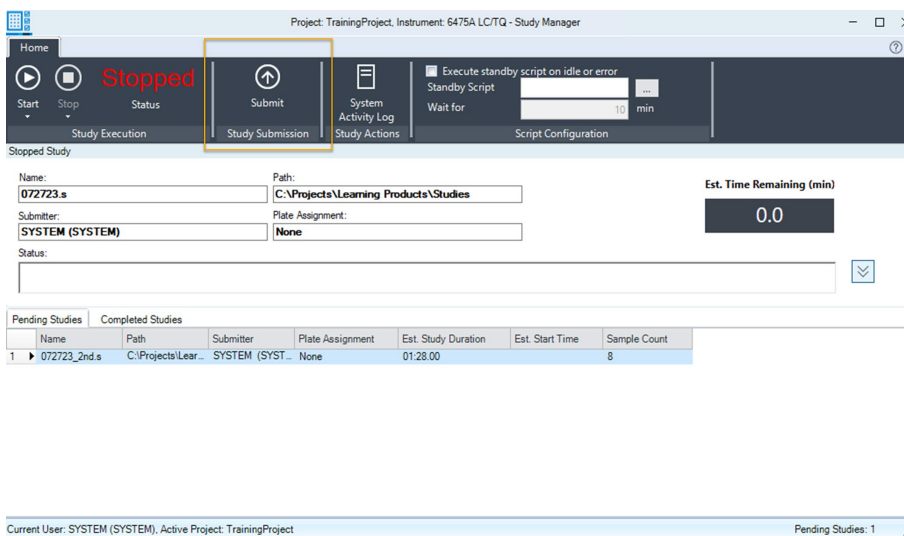
Create a Worklist Only Study

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

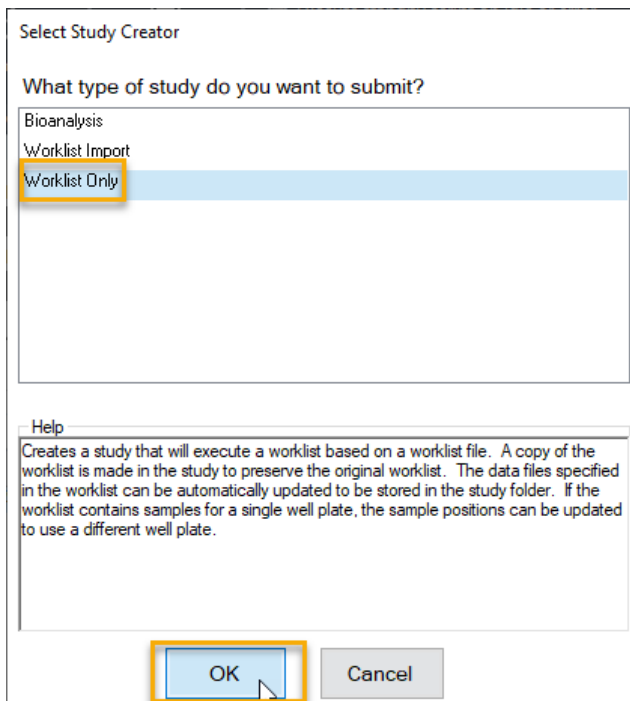


Run a Worklist

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

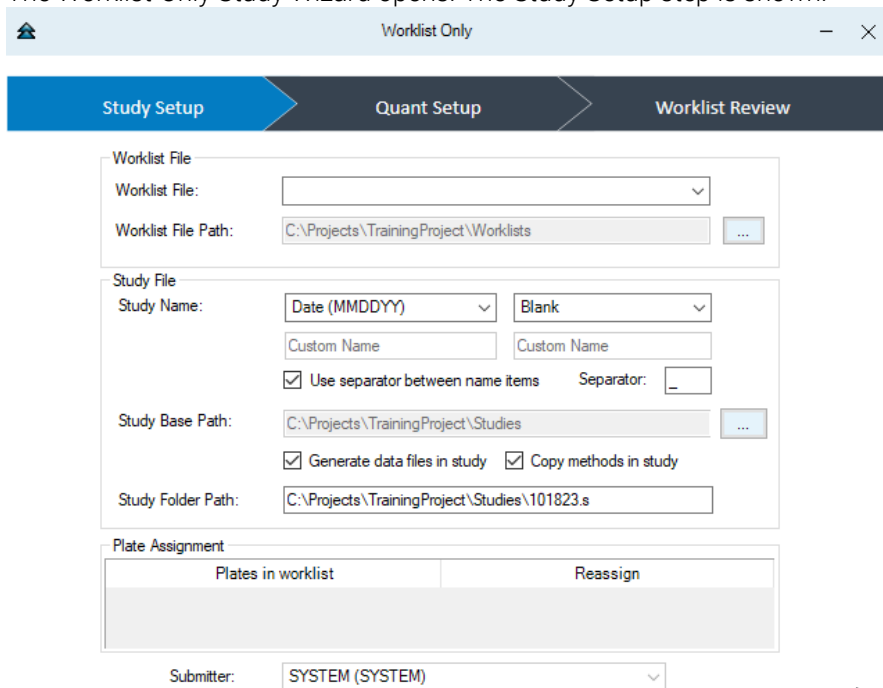


- 3 In Study Creator, select a **Worklist Only**, then click **OK**.



Run a Worklist

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



The screenshot shows the 'Worklist Only' window with three steps: 'Study Setup' (highlighted in blue), 'Quant Setup', and 'Worklist Review'. The 'Study Setup' section contains the following fields and options:

- Worklist File:** A dropdown menu.
- Worklist File Path:** A text box containing 'C:\Projects\TrainingProject\Worklists' and a browse button (...).
- Study File:**
 - Study Name:** Two dropdown menus: 'Date (MMDDYY)' and 'Blank'.
 - Two 'Custom Name' text boxes.
 - Use separator between name items. Separator: .
- Study Base Path:** A text box containing 'C:\Projects\TrainingProject\Studies' and a browse button (...).
- Generate data files in study
- Copy methods in study
- Study Folder Path:** A text box containing 'C:\Projects\TrainingProject\Studies\101823.s'.

Plate Assignment

Plates in worklist	Reassign

Submitter: A dropdown menu showing 'SYSTEM (SYSTEM)'.



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 Qualitative 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.

Use Qualitative Analysis to Generate Calibration Curves

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.

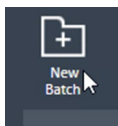
Create a batch to hold samples

- 1 To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ) icon** on your desktop.
- 2 Select a Project and click **OK**.



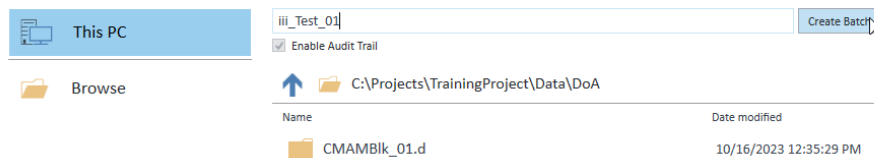
Quantitative Analysis for QQQ opens.

- 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**.

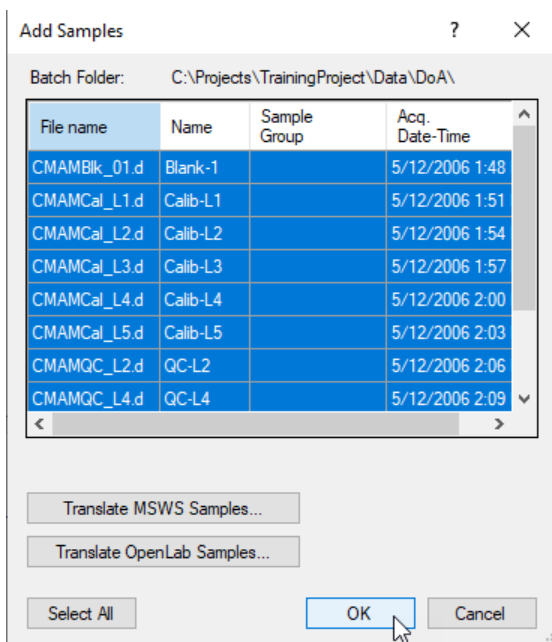
New Batch



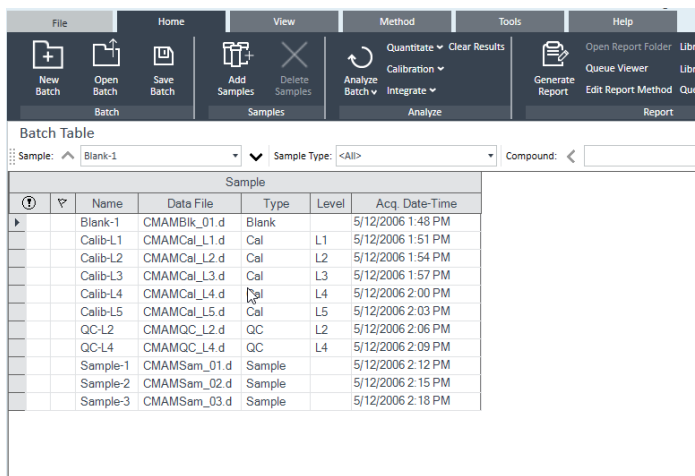
Use Qualitative Analysis to Generate Calibration Curves

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

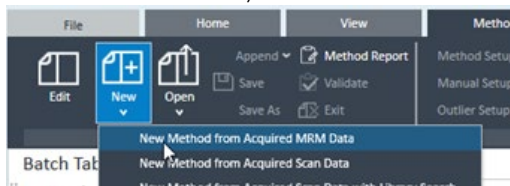
This task shows you how to set up a new quantitation method based on the calibration data file with the highest concentration of sample.

Create a method from acquired MRM data.

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

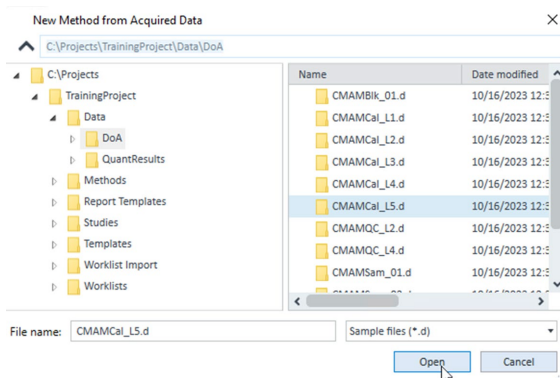
Sample						
	Name	Data File	Type	Level	Acq. Date-Time	
	Blank-1	CMAMBlk_01.d	Blank		5/12/2006 1:48 PM	
	Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	
	Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	
	Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	
	Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	
▶	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 **CMAMCalL5.d** and click **Open** to import acquisition method information.

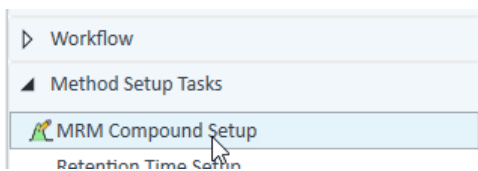


Set up Target Compounds

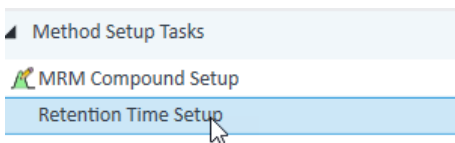
With this task, you learn to inspect the MRM transitions and the RT data for the new quantitation method, which you can change for individual target compounds. You also learn to set up an ISTD compound for each target compound.

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**.

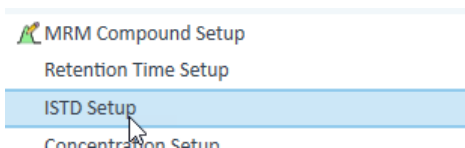


- 2 To inspect the imported retention time data, click **Method Setup Tasks > Retention Time Setup**.



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**.



Use Qualitative Analysis to Generate Calibration Curves

- 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>	

- 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	<input type="checkbox"/>		<input type="checkbox"/>	
▶ Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<None>	<input checked="" type="checkbox"/>	50.0000	<input type="checkbox"/>	
Cocaine	1	304.1 -> 182.0	MRM	Target	Cocaine-d3	<input type="checkbox"/>		<input type="checkbox"/>	
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	<None>	<input checked="" type="checkbox"/>	50.0000	<input type="checkbox"/>	
MDMA	1	194.2 -> 163.3	MRM	Target	MDMA-d5	<input type="checkbox"/>		<input type="checkbox"/>	
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	<None>	<input checked="" type="checkbox"/>	50.0000	<input type="checkbox"/>	
Meth	1	150.1 -> 119.3	MRM	Target	Meth-d5	<input type="checkbox"/>		<input type="checkbox"/>	
Meth-d5	1	155.2 -> 92.3	MRM	ISTD	<None>	<input checked="" type="checkbox"/>	50.0000	<input type="checkbox"/>	

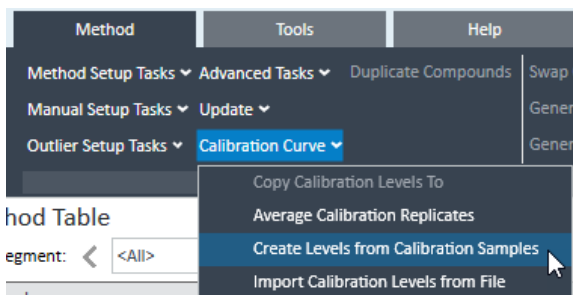
Set up Quantitation

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

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

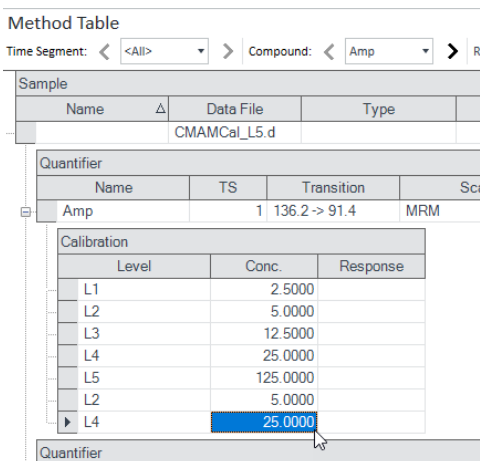
Create five calibration levels for the first compound

- 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.

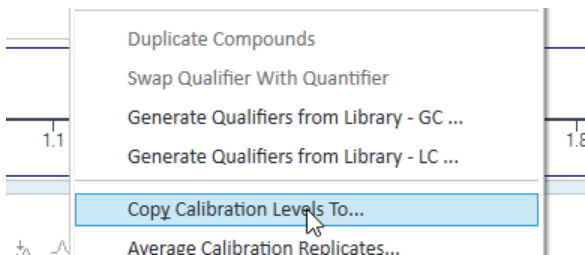


- L1–2.5000
- L2–5.0000
- L3–12.5000
- L4–25.0000
- L5–125.0000

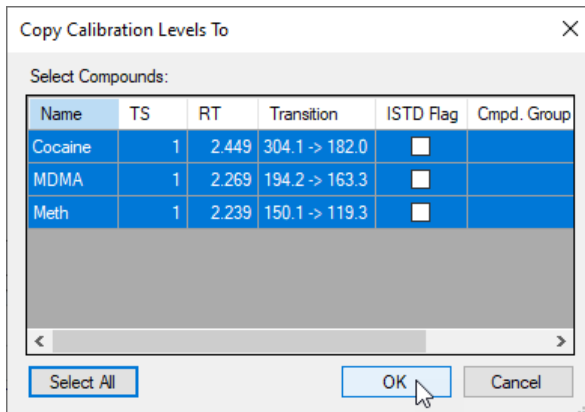
Use Qualitative Analysis to Generate Calibration Curves

Copy the calibration levels and concentrations to the other compounds

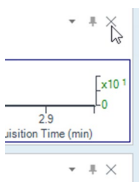
- 1 Right-click in the Quantifier table and select **Copy Calibration Levels To...**. The system displays the Copy Calibration Levels To dialog box.



- 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.

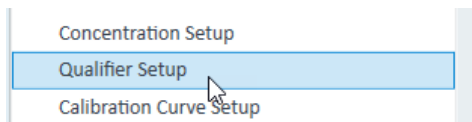


- 4 Browse the **Method Table** to compare the calibration concentration setup among the four target compounds, Amp, Cocaine, Meth, and MDMA.

Use Qualitative Analysis to Generate Calibration Curves

Set up qualifier ions and a calibration curve

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

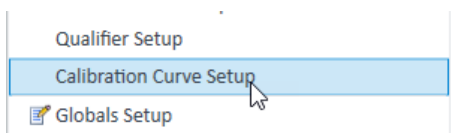


Method Table

Time Segment: < <All> > Compound: < Amp > Reset Table View

Sample								
Name	Δ	Data File	Type	Level	Acq. Method File	Acq. Date-Time		
		CMAMCal_L5.d						
Qualifier								
Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	Uncertainty	
Amp	1	136.2 -> 91.4	MRM	Target	136.2	91.4	Relative	
Qualifier								
Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Area Sum			
136.2	119.4	136.2 -> 119.4	28.3	20.0	<input type="checkbox"/>			
Qualifier								
Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	Uncertainty	
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	141.1	93.4	Relative	

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



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

Qualifier								
Name	TS	Transition	Scan	Type	CF	CF Origin	CF Weight	
▶ Amp	1	136.2 -> 91.4	MRM	Target	Linear	Ignore	None	
Amp-d5	1	141.1 -> 93.4	MRM	ISTD		Ignore	None	
Cocaine	1	304.1 -> 182.0	MRM	Target	Linear	Include	None	
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD		Force	None	
MORPH	1	184.2 -> 163.2	MRM	Target	Linear	Blank offset	None	

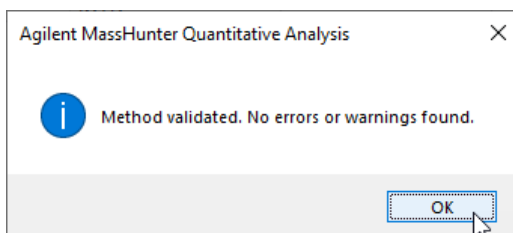
Validate the method

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

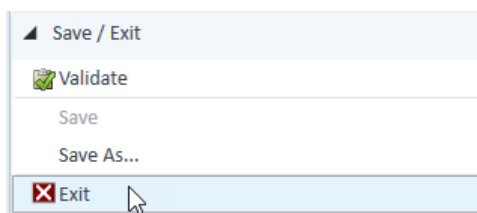


Use Qualitative Analysis to Generate Calibration Curves

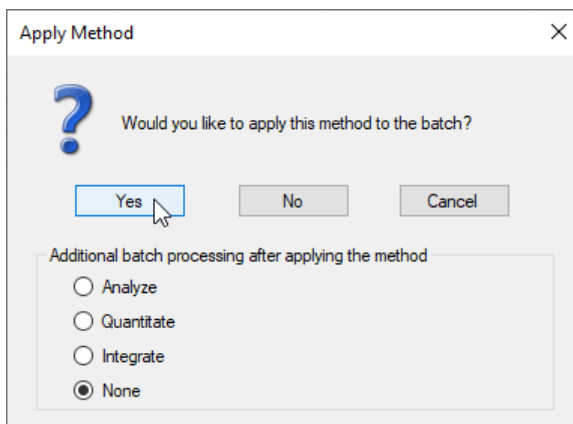
- 2 After the validation message appears, click **OK**.



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



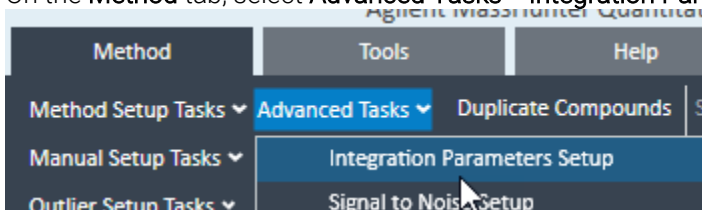
- 4 Select **None** under **Additional batch processing after applying the method** and click **Yes** to the **Would you like to apply this method to the batch?** prompt.



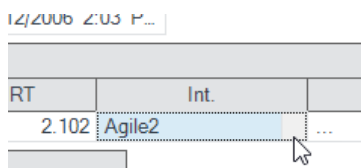
Set the Integrator

Change the method's integrator to MS/MS

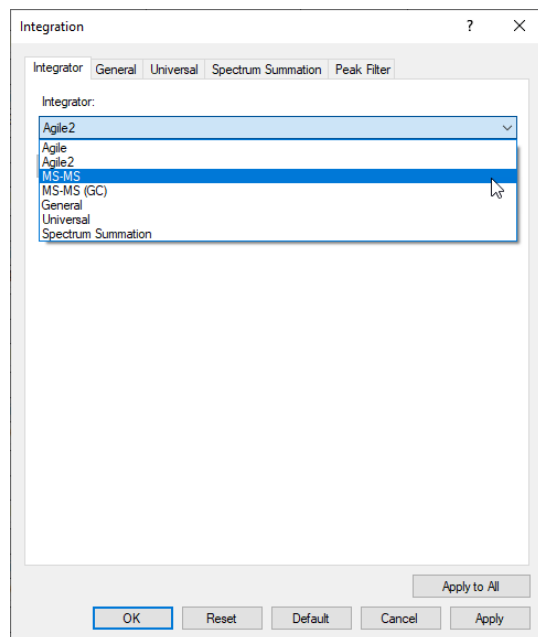
- 1 On the **Method** tab, click **Edit**.
- 2 On the **Method** tab, select **Advanced Tasks > Integration Parameters Setup**.



- 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**.

Use Qualitative Analysis to Generate Calibration Curves

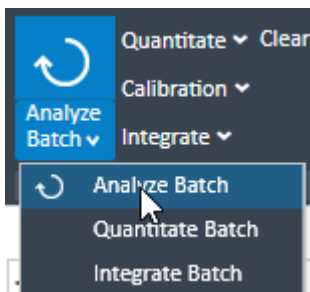
- 6 Click **OK**.
- 7 Click **Exit**.
- 8 Select **None** under **Additional batch processing after applying the method** and click **Yes** to the **Would you like to apply this method to the batch?** prompt.

Analyze and Save the Batch

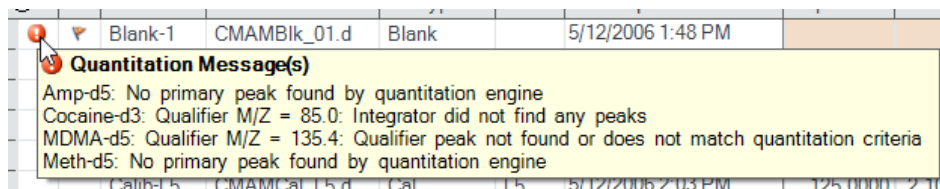
In this exercise, you quantitate the batch and then save the results.

Analyze the batch and inspect the results for each compound.

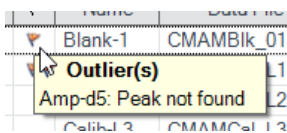
- 1 On the Home tab, click **Analyze Batch**.



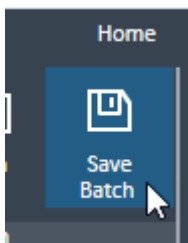
- 2 Pass the cursor over the quantitation message for Sample 1.



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

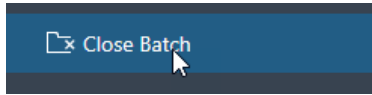


- 4 On the Home tab, click **Save Batch**.



Use Qualitative Analysis to Generate Calibration Curves

- 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.

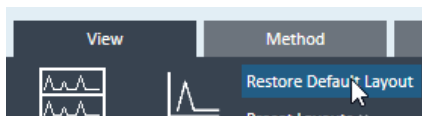
Use the **DrugsOfAbuse** batch in this exercise.

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.

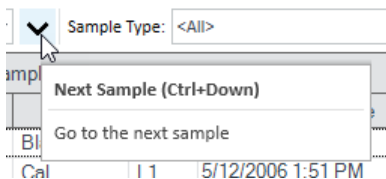
Open the batch file **iii_Test_01.batch.bin**, created in Exercise 1

- 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**.



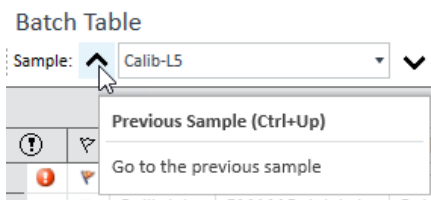
Scroll from sample to sample until you reach the end of the Batch Table, and then return to Cal-L5

- 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.



Use Qualitative Analysis to Generate Calibration Curves

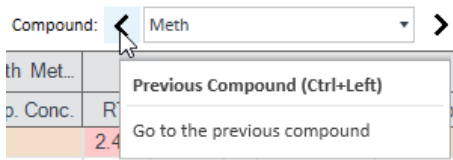
- 2 Return to Cal-L5, clicking the **Previous Sample** icon in the Batch Table Standard toolbar if needed.



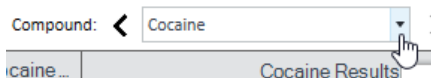
- 3 Select any cell in the row for sample **Calib_L4** in the Batch Table window to view the changes.

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.



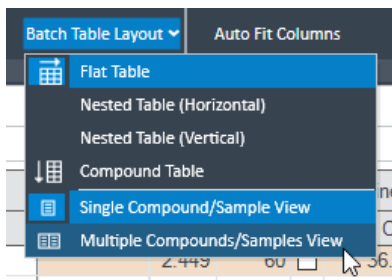
- 2 Inspect the changes in the **Batch Table**, **Compound Information**, and **Calibration Curve** windows.
- 3 Click the down arrow next to the **Compound** list.
- 4 Click **Cocaine**.



Use Qualitative Analysis to Generate Calibration Curves

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.

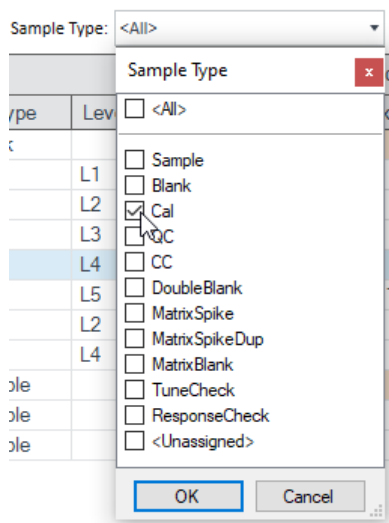
Sample						Amp Results			Meth Results			MDMA Results			Cocaine Results			
Info	▼	Name	Data File	Type	Level	Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
!	▼	Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM							2.284	1.9296		2.433	11.8235	
	▼	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.

Use Qualitative Analysis to Generate Calibration Curves

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



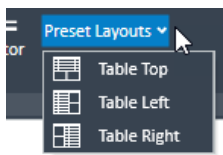
- 5 Click **OK**. The Batch Table should contain only the Cal standards for cocaine.
- 6 Click the down arrow in the **Sample Type** drop-down list.
- 7 Click **<All>**, and then click **OK**. The system marks all the check boxes and displays all sample types.

Change Result Window Layouts

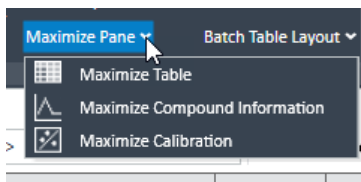
This task shows you how to customize your layout and how to recreate the default layout.

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**.



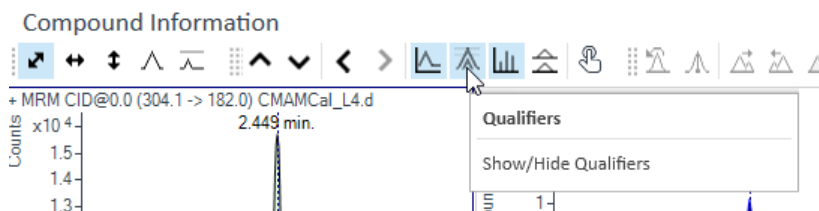
- 3 On the **View** tab, click **Restore Default Layout**.

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

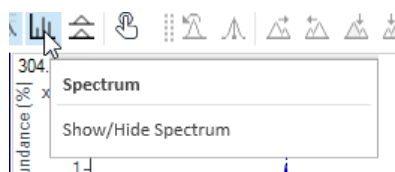
- 1 In the **Batch Table**, select the **Cal-L4** row.

Use Qualitative Analysis to Generate Calibration Curves

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



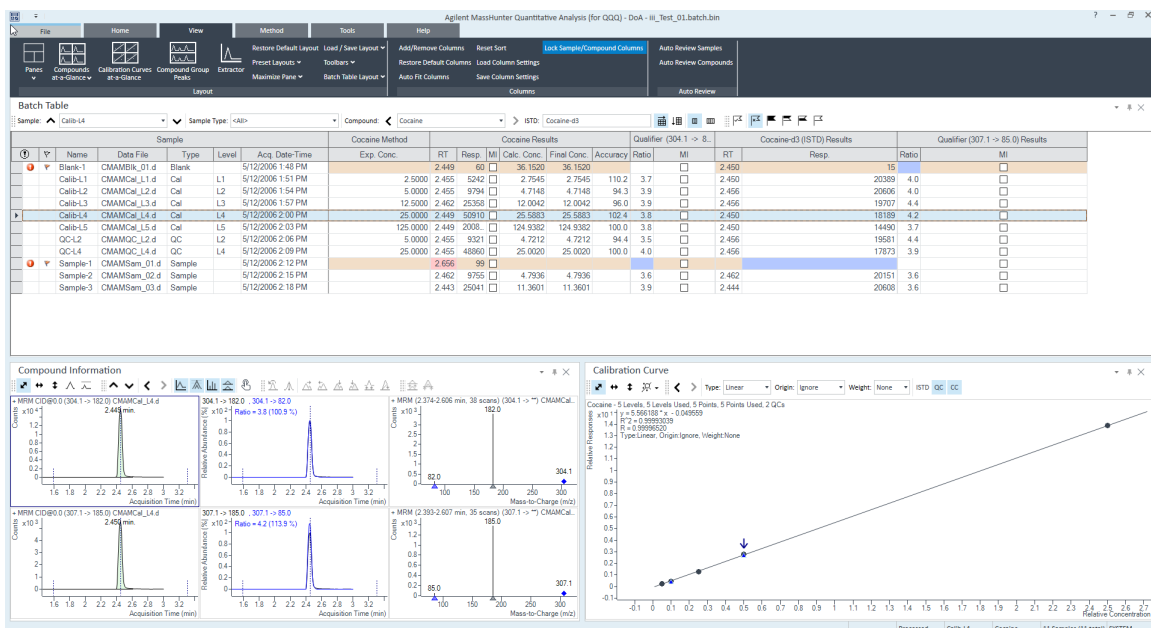
- 3 Click the **Show/Hide Spectrum** icon.



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



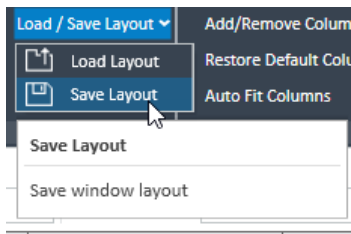
- 5 The layout and results look like those in the following figure.



Use Qualitative Analysis to Generate Calibration Curves

Save the default layout without the calibration curve

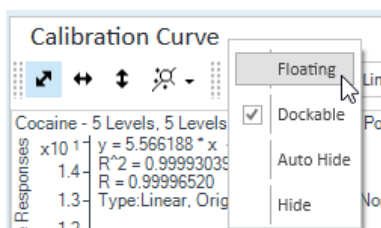
- 1 Close the **Calibration Curve** window.
- 2 On the **View** tab, select **Load/Save Layout > Save Layout**. The system displays the **Save Layout File** dialog box.



- 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.

Use Qualitative Analysis to Generate Calibration Curves

7 Resize the windows to match the layout below.

The screenshot displays the Agilent MassHunter Quantitative Analysis interface. The main window shows a data table with columns for Sample Name, Data File, Type, Level, Acq. Date-Time, Cocaine Method, Exp. Conc., RT, Resp. MI, Calc. Conc., and Final Co. The table lists various calibration standards (Calb-L1 to Calb-L5, Calb-L2, Calb-L3, Calb-L4, Calb-L5) and samples (Sample-1 to Sample-3). The Cocaine Results section shows RT, Resp. MI, Calc. Conc., and Final Co. for each entry.

On the right, the Compound Information window displays three mass spectra plots. The top plot shows a peak at 2.449 min with a ratio of 3.8 (100.9%). The middle plot shows a peak at 2.450 min with a ratio of 4.2 (119.9%). The bottom plot shows a peak at 2.450 min with a ratio of 4.2 (119.9%).

At the bottom, the Calibration Curve window shows a linear plot of Relative Response versus Relative Concentration. The plot includes a linear regression line and the equation $y = 5.58653x + 0.046959$ with $R^2 = 0.9999039$. The plot also shows the Type: Linear, Origin: Ignore, and Weight: None.

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

Use Qualitative Analysis to Generate Calibration Curves

9 Resize the windows to match the layout in below.

Sample	Name	Data File	Type	Level	Acq. Date-Time	Cocaine Method	Exp. Conc.	RT	Resp. MI	Calc. Conc.	Final Conc.
Blank-1	CMAMBlk_01.d	Blank			5/12/2006 1:48 PM			2.449	60	36.1520	36.1
Calib-L1	CMAMCal_L1.d	Cal	L1		5/12/2006 1:51 PM		2.5000	2.455	5242	2.7545	2.7
Calib-L2	CMAMCal_L2.d	Cal	L2		5/12/2006 1:54 PM		5.0000	2.455	9794	4.7140	4.7
Calib-L3	CMAMCal_L3.d	Cal	L3		5/12/2006 1:57 PM		12.5000	2.462	25358	12.0042	12.0
Calib-L4	CMAMCal_L4.d	Cal	L4		5/12/2006 2:00 PM		25.0000	2.449	50910	25.5883	25.5
Calib-L5	CMAMCal_L5.d	Cal	L5		5/12/2006 2:03 PM		125.0000	2.449	2008	124.9382	124.9
QC-L2	CMAMQC_L2.d	QC	L2		5/12/2006 2:06 PM		5.0000	2.455	9321	4.7212	4.7
QC-L4	CMAMQC_L4.d	QC	L4		5/12/2006 2:09 PM		25.0000	2.455	48860	25.0020	25.0
Sample-1	CMAMSam_01.d	Sample			5/12/2006 2:12 PM			2.656	99		
Sample-2	CMAMSam_02.d	Sample			5/12/2006 2:15 PM			2.462	9755	4.7936	4.7
Sample-3	CMAMSam_03.d	Sample			5/12/2006 2:18 PM			2.443	25041	11.3601	11.5

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**.

Compound: < Amp > ISTD: Amp-d5

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

87	132.7	Accepted	24.3	2.129	1397	25.9
93	Outlier(s)					
08	Amp: Accuracy value = 132.7 is outside the allowed range [80.0, 120.0]					
61	107.0	Accepted	20.1	1.000	1204	28.8

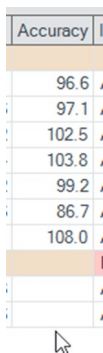
- 5 In the **Calibration Curve**, set **Origin** to **Ignore**, and **Weight** to **1/y**. The program displays a new window curve fit formula and R2 value.

Origin: Ignore Weight: 1/y

Use Qualitative Analysis to Generate Calibration Curves

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.



The screenshot shows a vertical list of numerical values under the heading 'Accuracy'. The values are: 96.6, 97.1, 102.5, 103.8, 99.2, 86.7, and 108.0. The list is presented in a scrollable format with a mouse cursor at the bottom.

Accuracy
96.6
97.1
102.5
103.8
99.2
86.7
108.0

- 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.

Use Qualitative Analysis to Generate Calibration Curves

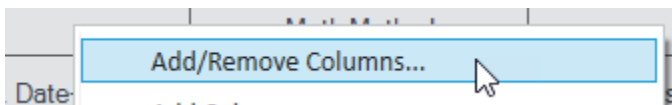
Integrate Without Parameter

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

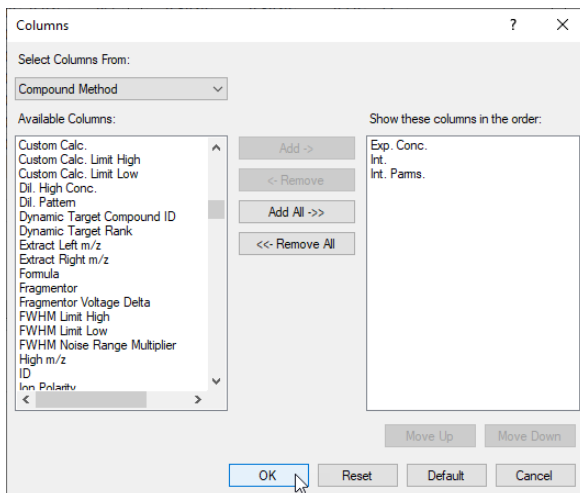
Add integration columns to the Batch Table

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



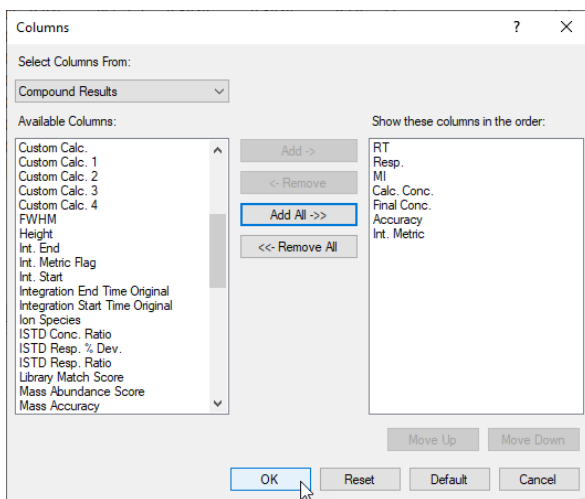
The system displays the **Columns** dialog box.

- 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.



Use Qualitative Analysis to Generate Calibration Curves

- From the **Select Columns From** drop-down list, select **Compound Results**.
- From the **Available Columns** list, select **Int. Metric** (Integrator Metric) and click **Add**.
- The system moves the selected column to the **Show these columns in the order** list.
- Click **OK**.



View the default integration values for amphetamine

- Click **Previous Compound** in the Batch Table toolbar to view amphetamine (Amp).
- Examine the default values in the **Int.** and **Int. Parms** columns in the **Batch Table**.

Int.	Int. Parms.
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	

Use Qualitative Analysis to Generate Calibration Curves

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

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

View integration problems for cocaine and MDMA

- Close the **Calibration Curve** window.
- Enlarge the chromatogram portion of Compound Information toolbar so that only the quantifier and qualifier chromatograms appear. Click the **Show/Hide Spectrum** icon.
- Also click the **Show/Hide ISTD** icon.
- Click the **Next Compound** icon in the Batch Table toolbar until the system displays the compound b.
- Select the **Blank-1** row, and mouse over the word **Inspect** in the **Int. Metric** column for that row.

Qty	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI
	Inspect	<input type="checkbox"/>		2.403	15		<input type="checkbox"/>
9.0	Ado						
3.6 Accept Outlier(s)							
3.6 Accept Cocaine: Integrator found the following problem(s) with the peak at RT = 2.433: Merge Problem							
5.6	Accepted	3.9	<input type="checkbox"/>	2.459	19625	4.4	<input type="checkbox"/>

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

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

Qty	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI
	Accepted	15	<input type="checkbox"/>	2.602	28		<input type="checkbox"/>
1.3	Accept						
3.1 Accept Quantitation Message(s)							
3.1 Accept MDMA-d5: Qualifier M/Z = 135.4: Qualifier peak not found or does not match quantitation criteria							
0.2	Accepted	10.0	<input type="checkbox"/>	2.276	11059	24.2	<input type="checkbox"/>

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

Use Qualitative Analysis to Generate Calibration Curves

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.

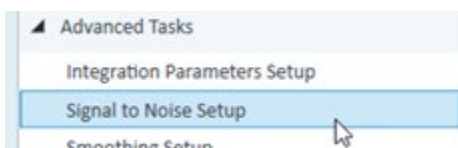
Batch Table

Sample: Blank-1 Sample Type: <A> 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. Params.	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

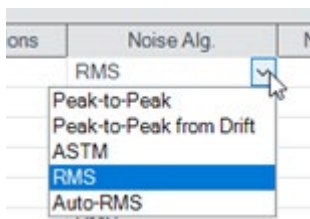
- 1 On the **Method** tab, click **Edit**.
- 2 In the **Method Tasks** column, click **Advanced Tasks > Signal to Noise Setup**.



The system displays the integrator parameters in the Method Table.

Use Qualitative Analysis to Generate Calibration Curves

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



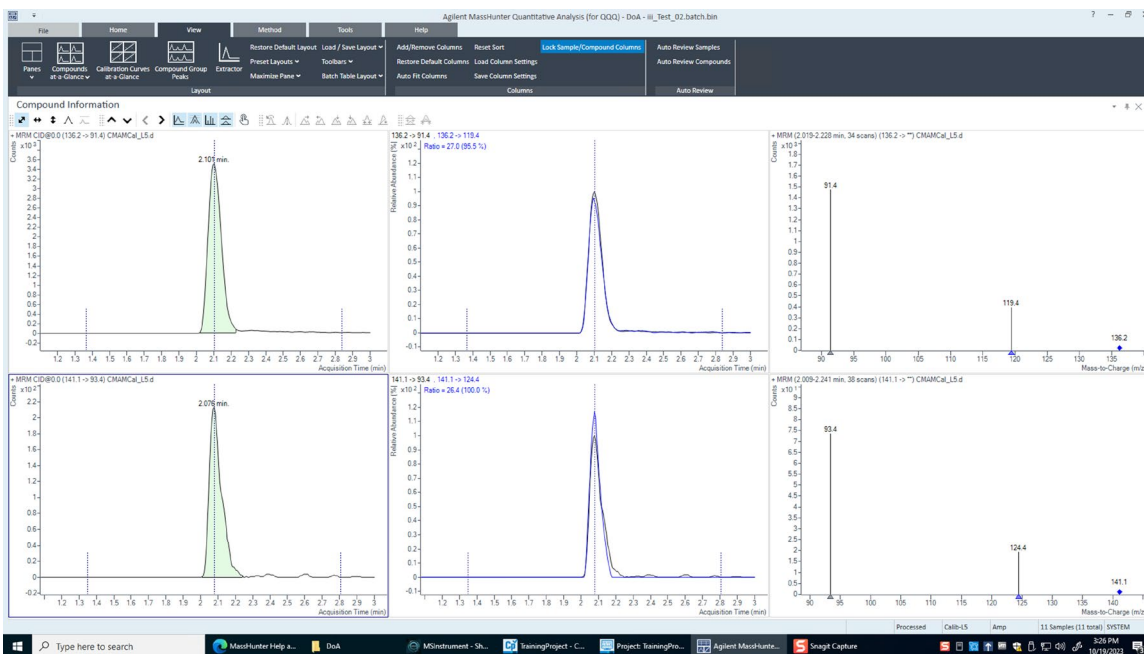
A list of available noise algorithms appears.

- 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

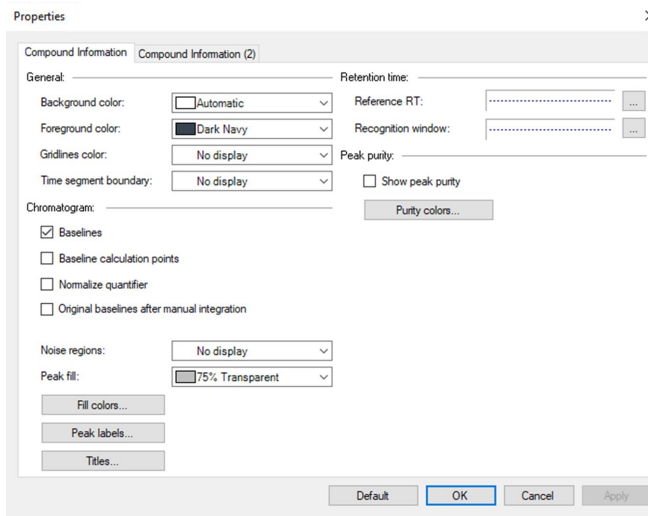
- 1 Select sample **Calib-L5** (if it is not already selected), and on the **View** tab, select **Maximize Pane > Maximize Compound Information**.

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



Use Qualitative Analysis to Generate Calibration Curves

- 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

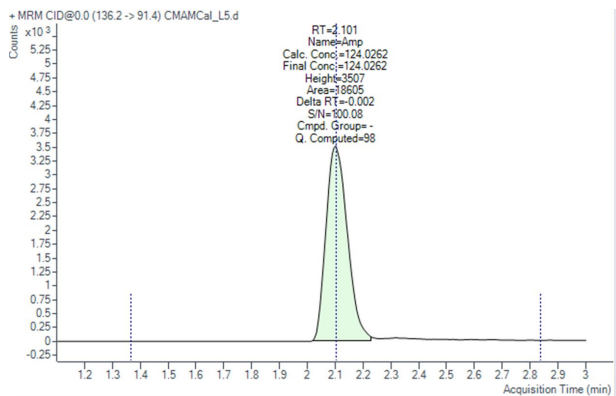
- 1 From the Properties dialog box, click **Peak Labels**. The system displays the Peak Label dialog box.
- 2 Mark all the **Peak Labels** check boxes, and the **Display Label Names** check box.

Use Qualitative Analysis to Generate Calibration Curves

- Click OK.



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

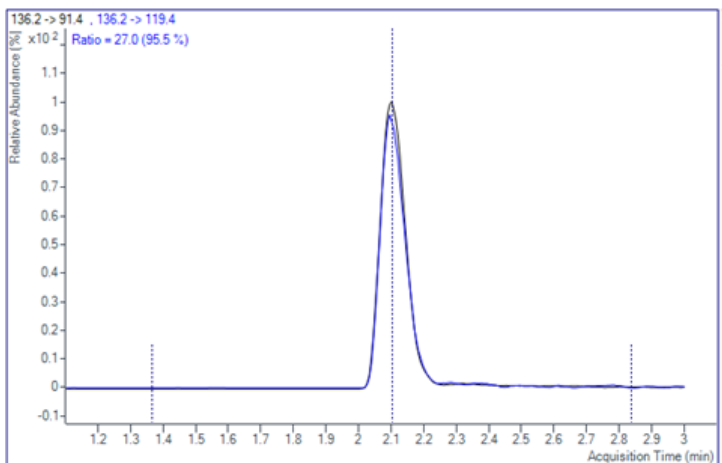


- Click **Peak Labels** in the Properties dialog box. The system displays the Peak Labels dialog box.
- Clear all the **Peak Labels** check boxes except RT (retention time). Clear the **Display Label Names** check box and click **OK**.
- Click **Apply** in the Properties dialog box and observe the change in Peak Labels.

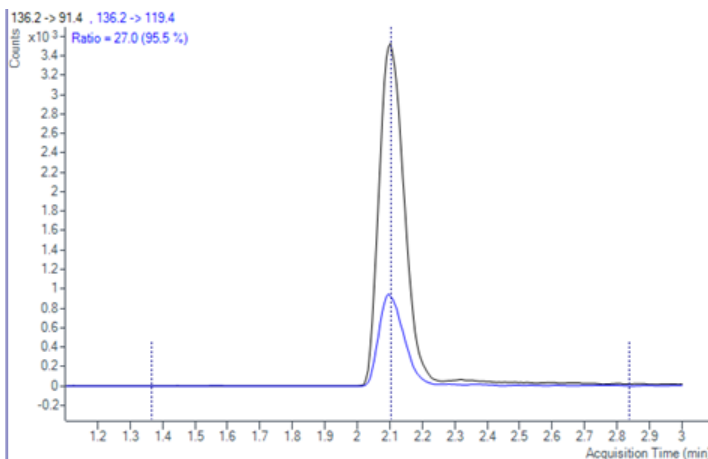
Use Qualitative Analysis to Generate Calibration Curves

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.



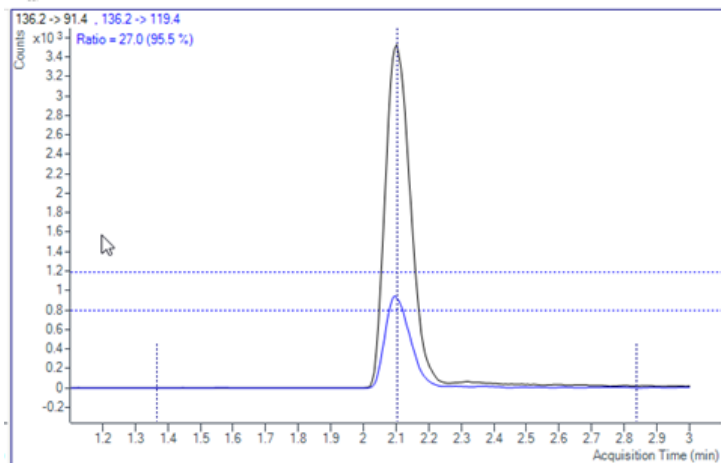
- 3 Clear the **Normalize Qualifiers** check box of the **Properties** dialog box.
- 4 Click **Apply** to display the qualifier second quantifier peaks again.



Use Qualitative Analysis to Generate Calibration Curves

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**.

Use Qualitative Analysis to Generate Calibration Curves

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.

RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI
2.284	7	<input type="checkbox"/>	1.9296	1.9296	Accepted	15.	<input type="checkbox"/>	2.602	28	<input type="checkbox"/>		
Quantitation Message(s)												
2 MDMA-d5: Qualifier M/Z = 135.4: Qualifier peak not found or does not match quantitation criteria												
2.277	17023	<input type="checkbox"/>	11.2728	11.2728	90.2	Accepted	10.0	<input type="checkbox"/>	2.276	11059	24.2	<input type="checkbox"/>

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

34	23.5	<input type="checkbox"/>
20	27.5	<input type="checkbox"/>
21	Outlier(s)	
55	2 MDMA-d5: Qualifier ratio = 27.5 is outside the allowed range [17.9, 26.9] for MZ = 135.4	

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.

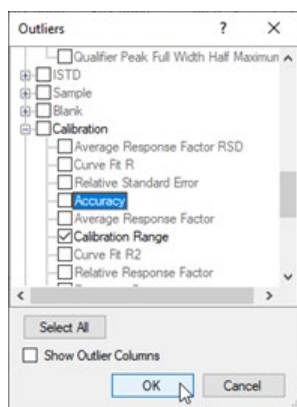
Use Qualitative Analysis to Generate Calibration Curves

- 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
		Rt
08		Ac
56		Ac

Using the following set of outlier flag icons

- Click the **Display rows that have High outliers**  icon on the toolbar to display only samples with high outliers.
- Click the **Turn off outlier filter**  icon to display all samples.
- Click the **Display rows that have High/Low outliers**  icon on the toolbar to display only samples with low outliers.
- Click the **Display rows that have High/Low outliers**  icon again to display all the samples.
- Click the **Select Outliers**  icon to bring up the Outliers dialog box.
- Clear the **Accuracy** and **Retention Time** check boxes and click **OK**.



Use Qualitative Analysis to Generate Calibration Curves

- 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

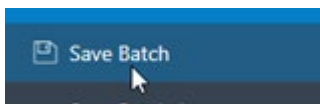
This exercise helps you learn how to do these tasks:

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

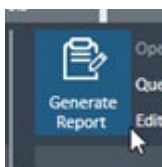
The DrugsOfAbuse batch is used in this exercise.

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.

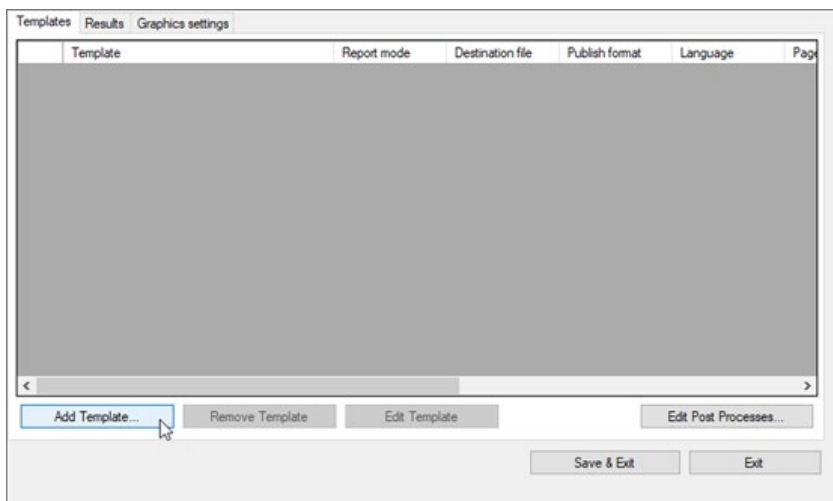


Note the Report Folder directory, which is where the report is saved.

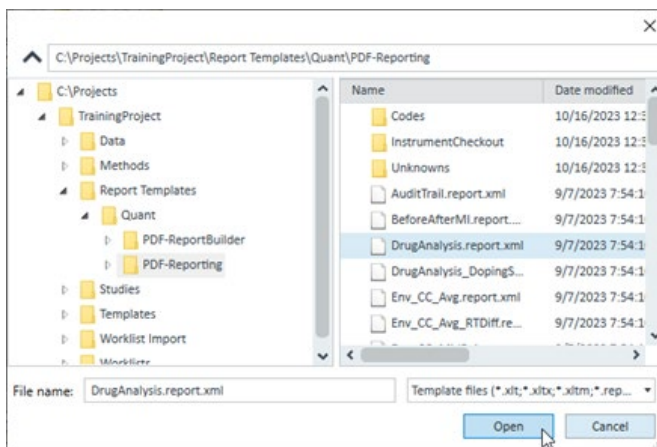
- 4 Under the Report Method field, click the **New** button to create a report method.

Use Qualitative Analysis to Generate Calibration Curves

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



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



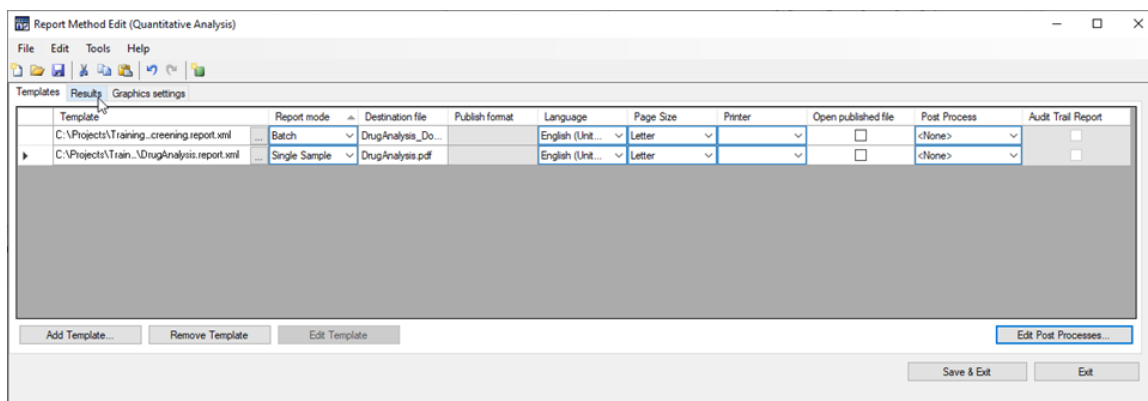
The program adds the template to the Template field in the Report Method Edit pane.

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

Use Qualitative Analysis to Generate Calibration Curves

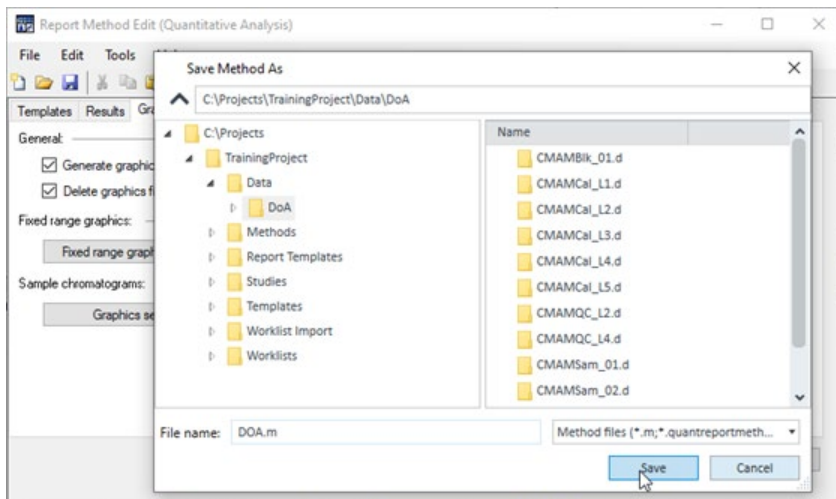
Edit the report method to create single sample and batch PDF reports

- 1 In the **Report Method Edit** dialog box, on the **DrugAnalysis.report** template line, 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.



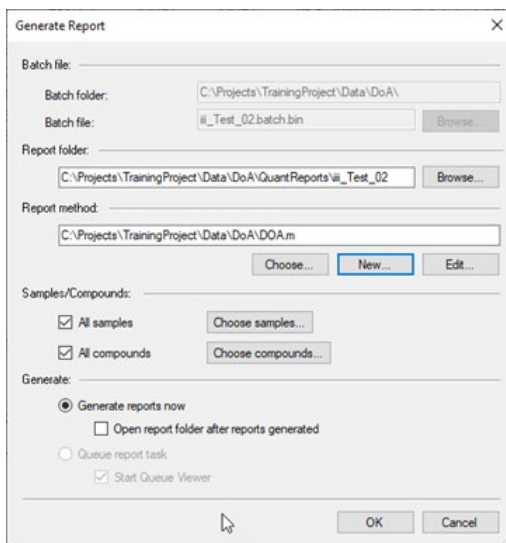
- 6 Leave the default settings for the rest of the graphic setting fields.
- 7 Click the save icon in the **Report Method Edit** window.
- 8 Name the report method **DOA.m**.
- 9 Click **Save & Exit** to close the Report Method Edit dialog box to return to the Generate Report window.

Use Qualitative Analysis to Generate Calibration Curves



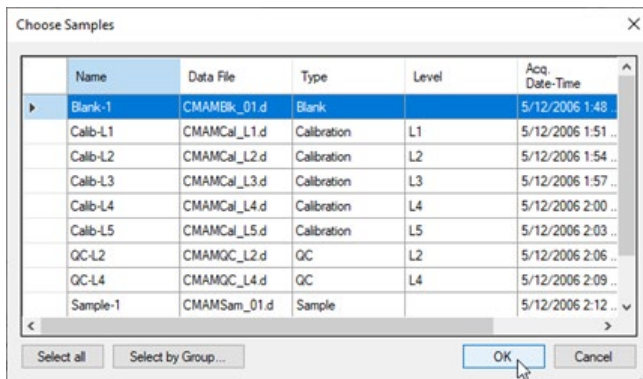
Generate a report from the method

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

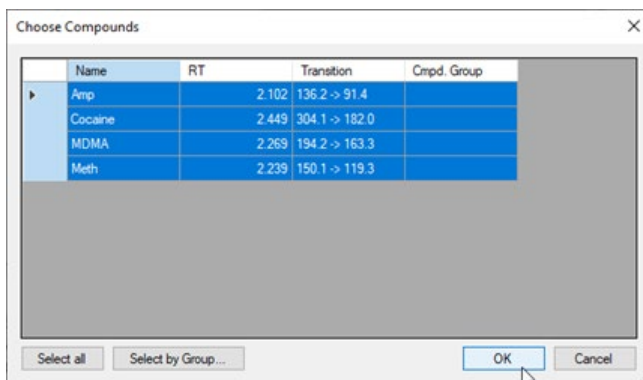


- 2 In the **Samples/Compounds** field, uncheck **All Samples**, to open the batch table.
- 3 Highlight one of the samples in the batch table window and click **OK**.

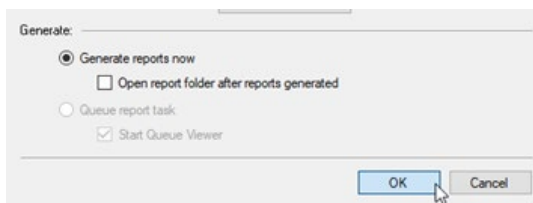
Use Qualitative Analysis to Generate Calibration Curves



- 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.

Agilent Technologies Agilent SubscribeNet

Electronic Software and License Delivery

Please login. Your Login ID is your Email address.

Login ID

Password

Remember my password until I logout

Login

If you have forgotten your login ID, password, or are not sure whether you have an account use our [Password Finder](#).

SubscribeNet new account registration.

Customers who have an authorization code from their Agilent product purchase may [CLICK HERE](#) to register and create a new SubscribeNet Account and Login ID.

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- 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.

Maintenance

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) (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.

Maintenance



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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