

Agilent Triple Quadrupole LC/MS System

Introduction Workbook



Notices

Manual Part Number

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Agilent Technologies, Inc 5301 Stevens Creek Blvd. Santa Clara, CA 95051 www.agilent.com

Software Revision

This guide is valid for MassHunter 12.1 and greater, until superseded.

Instrument Manufacturing

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

Operating Temperature

Operating Temperature: 15 to 35 °C Storage Temperature: -40 to 70 °C

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Safety Notices

CAUTION

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

WARNING

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

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About this Workbook

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

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

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

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

This introduction covers:

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

How to use this Workbook

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

Exercises to be completed are marked like this:



Exercise Name

Exercise Instructions

Task steps look like this:

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

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

Type Blank One in the field.

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

Press Enter.

Cross-references appear in blue:

(For example, Link)

Before You Begin

This introduction workbook is recommended for all participating end users.



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

Additional Resources

User Documentation



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



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

Agilent Triple Quadrupole LC/MS Supplies



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

Where to find more information



Agilent Community

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

https://community.agilent.com/

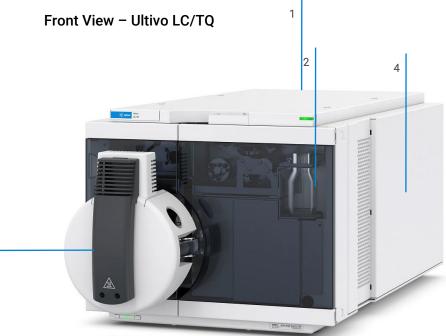
Overview

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:

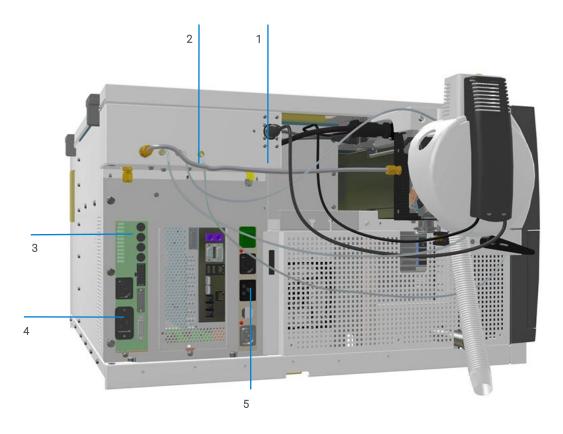
Work with your Agilent Service Engineer and/or use the *Agilent Triple Quadrupole LC/MS System User Guide* to label the flagged components below for your installed instrument(s).





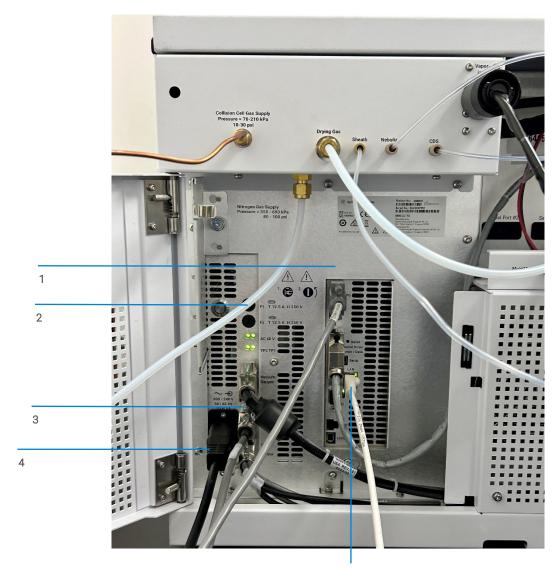


Side View - 6475A





Side View - 6495D



Basic Components

Ionization Source

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







- Agilent Jet Stream (AJS ESI) source
- Electrospray Ionization (ESI)
 source
- Atmospheric Pressure Chemical Ionization (APCI) source
- Multimode source (MMI)

• Agilent nanospray ESI source



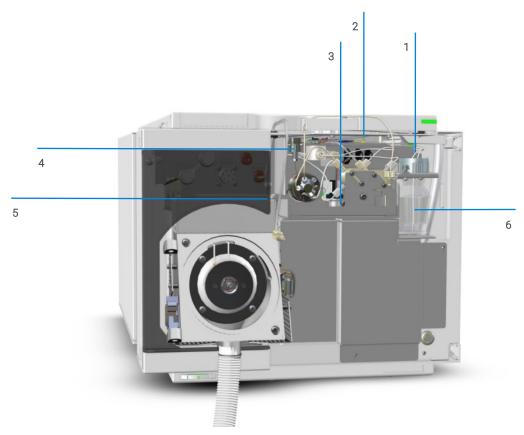
Hardware Introduction

- 1 List the type of Ionization Source in use:
- 2 It is reviewed on _____ page of the user guide and includes the following parts:
- **3** List the name and part number of the proper tune solution for this system:

Calibrant Delivery System (CDS)/Bottle

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

Ultivo



6475A

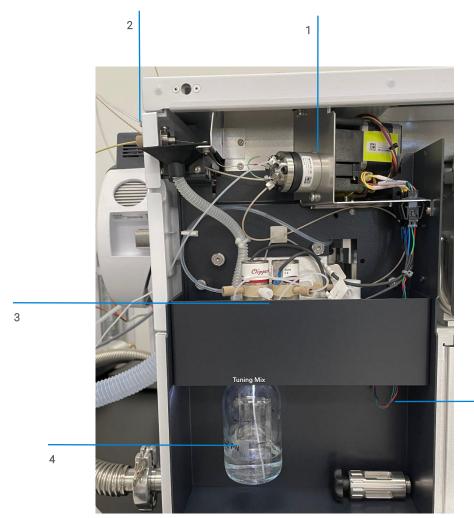
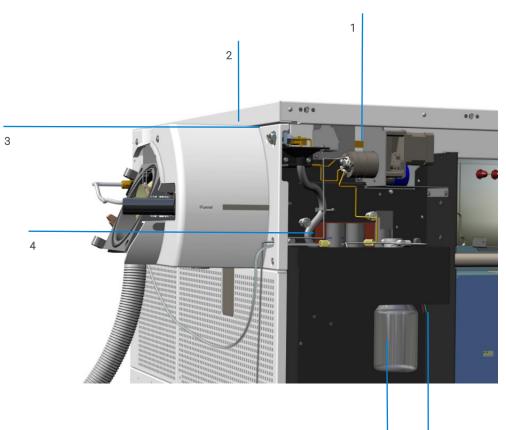


Figure 1. Front cover removed.





6

5



Hardware Introduction

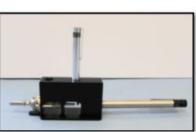
- **1** Practice removing and attaching the calibrant bottle.
- 2 How often is the calibrant bottle checked and refilled?

Nebulizer

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



APCI & APPI Nebulizer



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



ESI, MM, & AJS Nebulizer



View The Needle

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

Rough Pump

MS40+ (Ultivo and 6475A)



MS120+ (6495D)





Locate The Oil Sight

Using the user guide, fill out the following information:

- 1 The oil level should be ______ the marks for Max and Min.
- 2 Check that the pump oil is _____ and the color is _____ than amber.
- **3** If the pump oil is _____ or full of _____ replace it.

This page is intentionally left blank.

Overview

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

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

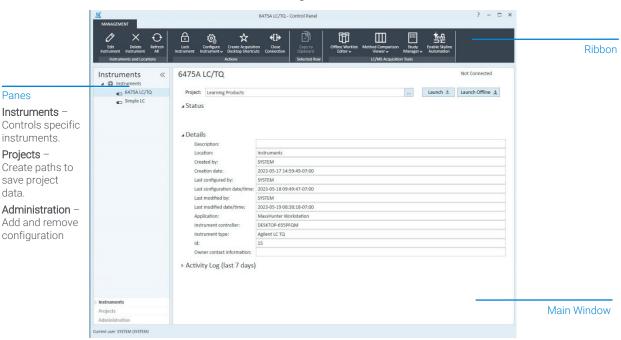
You will review:

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



Software Start-Up

- 1 From the desktop, double-click the OpenLab Control Panel icon
- 2 The navigation pane opens by default and can be minimized or expanded based on your preference.



User Interface and General Navigation

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

Close Connection

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

- 1 Click Instruments.
- 2 Select the instrument to close.
- 3 Click Close Connection.

MANAGEMENT	647	5A LC/TQ - Control Panel		? –	
Edit Delete Refresh Instrument Instrument All Instruments and Locations	Lock Configure Create A	cquisition Shortcuts Connection Selecte	to Offline Worklist Method C Editor - View	Comparison Study Enable Sky wer Manager Automati MS Acquisition Tools	vline
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C 6475A LC/TQ	Project: InstrumentCheck	out		Launch 1 Launch Offline	*
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Creating and Configuring Projects

- 1 Click Projects and select ^(D) 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.



BioConfirm BioConfirm BioConfirm BioConfirm BioConfirm BioConfirm	Cuantitative Analysis • Quantitative Analysis Quantitative Analysis Quantitative Analysis	
increasing and in the		
operation Matthew		
percies maximum	nter Workstation	
Name:	Training Project	
Project folder path:	C:\Projects\Training Project Brow	wse
	Include project groups in project path	
Description:	Training Project Description	
Applications:	MassHunter Workstation	_
		_
	ОК Са	ncel
	Name: Project folder path: Description: Applications:	Project folder path: CAProjects\Training Project Brow Include project groups in project path Description: Training Project Description Applications: Image: Comparison of the second seco

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.

<u>Sé</u> MANAGEMENT		Instrument	ts - Control Panel ? - 🗆 🗙
Edit Delete Refresh Instrument Instrument All Instruments and Locations	Lock Instrument In	Configure Create Acquisition nstrument Desktop Shortcut Actions	Image: Copy to s Connection Copy to Clipboard Offline Worklist Method Comparison Viewer - Study Manager - Enable Shiftine Automation Selected Row LQ/MS Acquisition Tools Copy to Selected Row Copy to Clipboard Copy to Clipboa
Instruments	~	Create Instrume	ent
Instruments			
6475A LC/TQ		Name:	6495D LC/TQ
		Description:	
		Application:	MassHunter Workstation 👻
		Instrument controller:	DESKTOP-655PFQM
		Instrument type:	Agilent LC TQ.
		Contact:	
		Default project:	Always use Default project
Instruments			
Projects			
Administration			OK Cancel
Current user: SYSTEM (SYSTEM)			

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.

Cế MANAGEMENT		64	195D LC/TQ - C	ontrol Pane	I				? –	×
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6495D LC/TQ		⊿ Status	iningProject				 Launch		inch on ine 5	^
		▲ Details Description	on:							. [
		Location:		Instr	uments					
		Created b	y:	SYST	ΈM					
		Creation	date:	2023	3-10-17 11:43:	15-07:00				
		Last confi	gured by:							
		Last confi	guration date/	time:						
		Last mod	ified by:	SYST	EM					
		Last mod	ified date/time	2023	3-10-17 11:43:	15-07:00				
Instruments		Applicatio	on:	Mas	sHunter Work	station				
Projects		Instrume	nt controller:	DES	KTOP-655PFQ	N				
Administration		Instrume	nt type:	Agile	ent LC TQ					•
Current user: SYSTEM (SYSTEM)										

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.

	6495D LC/TQ - Control Panel ? - D	×
Edit Delete Refresh Instrument Instrument All Instruments and Locations	Configure Instrument Configure	
Instruments	KX 6495D LC/TO Not Connected	
6475A LC/TQ	Project: TrainingProject Launch ♪ Launch Offline &	
💼 6495D LC/TQ	4 Status	^
	▲ Details Description: Location: 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	
Instruments	Application: MassHunter Workstation	
Projects	Instrument controller: DESKTOP-655PFQM	
Administration	Instrument type: Agilent LC TQ	~
Current user: SYSTEM (SYSTEM)		

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**.

23	Project TrainingProject, Instrument: 6475A LC/TQ - Acquisition - 🛛 🗙											- 🗆 ×	
Home													() · •
Default layout	1 Method	Single Sample	Status	 ▲ Save * ▲ ▲ Delete Lock ★ Deset Layout 	Instrument Statu Method Editor Sample Run	Chromatogram Actuals Spectrum	Plot Worklist Method Optimizer dMRM Method Split	Analytical Column Setup			SE Audit Trail Viewer	Instrument Configuration Method Real-time Plot	Worklist
	Layouts Windows Tools Print Reports												
For Help, Press F1										No	o worklist load	led. No method loaded. C:\	Free space: 9.4 GB

2 Click the TQ tab.



NOTE

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

- **3** When the tune completes, review the report.
- 4 Click Release tune control in the toolbar to release control of the TQ instrument.



Example detailed checktune report

MS Checktune Report Agilent Trusted Answers Detailed MS Checktune Report - G6475A Instrument Information 2023-10-11T11:39:25-07:00 Model G6475A Checktune Date Serial Number SG2222S001 SW/FW Version 3.0.1467/8.1.38 Ion Source AJS ESI Ionization Mode ESI Last Autotune Date 2023-09-29T09:46:04-07:00 Overall Result Passed SYSTEM (SYSTEM) Last Tuned By Vacuum And Temperature Rough Vac (Torr) 1.99E+0 High Vac (Torr) 2.78E-5 Turbo1 Speed (%) 100.0 MS1 Heater (*C) 100 Turbo2 Speed (%) MS2 Heater (*C) 100.0 100 Positive Results Components Ion Source Settings Gas Temperature (*C) 300 Gas Flow (L/min) 5.0 Nebulizer (psl) 45 Sheath Gas Temperature ("C) 250 Sheath Gas Flow (L/min) 11.0 Capillary Voltage (V) 3000 Nozzie Voitage (V) 1500 **Optics Settings** Fragmentor (V) 135 15 Skimmer (V) 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 -20.10 920.0 1220.0 -28.00 1520.0 -28.10 1820.0 -28.10 -28.10 2120.0 2720.0 -28.10 MS1 DC (V) 3 50 MS1 Postfliter DC (V) 2 MS1 TTI Cutoff 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 2100.0 600.00 650.00 2700.0 MS1 Heater (*C) Page 1 of 22 MS1 Quad Frequency 967.90 100

Review a Tune Report

Generate a detailed tune report and save

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

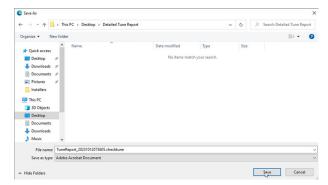
- 1 In the Method Editor window, select the TQ tab.
- 2 Click the Tune > Autotune section in the left pane.
- 3 Click Request tune Control in the toolbar in the Autotune section.
- 4 Click Generate Detailed Tune Report in the toolbar.



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.

Example detailed autotune report



Detailed MS Autotune Report - G6495D

nstrument Info	rmation				
Model	G6495D		Autotune Date	2023-09-25T17	:50:04-07:00
Serial Number	SG2305D301		SW/FW Version	3.1.551/9.4.56	
Ion Source	AJS ESI		Ionization Mode	ESI	
Tune Mode	Standard Quadrup	ole	Overall Result	Passed	
Vacuum And T	emperature				
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 Result	S				
Components					
Ion Sc	ource Settings				
Gas Temperature (°C)		220	Gas Flow (L/min)		14.0
Nebulizer (psi)		20.0	Sheath Gas Temperate	ure (°C)	150
Sheath Gas Flow (L/m	in)	11.0	Capillary Voltage (V)		3000
lozzle Voltage (V)		1500			
iFunne	2				
Fragmentor (V)		166			
High Pressure iFunnel		10	High Pressure iFunnel		150
	DC Drop (V)	100 15	Low Pressure iFunnel	RF (Vp-p)	60
	-				
Funnel Exit DC (V)	Optics Modes			-	
Funnel Exit DC (V) High Pressure iFunnel Mode	Optics Modes		Settin		
Funnel Exit DC (V) High Pressure iFunnel Mode Fragile	Optics Modes		50.00		
Funnel Exit DC (V) High Pressure iFunnel Mode Fragile Standard	Optics Modes		50.00 100.0	0	
Funnel Exit DC (V) High Pressure iFunnel Mode Fragile Standard	Optics Modes		50.00	0	
Funnel Exit DC (V) High Pressure IFunnel Mode Fragile Standard .arge Molecule Low Pressure iFunnel			50.00 100.0 210.0	0	
Funnel Exit DC (V) High Pressure iFunnel Mode Fragile Standard .arge Molecule Lew Pressure iFunnel Mode			50.00 100.0 210.0 Settir	0	
Hum Flessor Failles High Pressure (Funnel Mode Fragile Standard Large Molecule Low Pressure (Funnel Mode Fragile Standard			50.00 100.0 210.0	0 0 19	

Page 1 of 28



Electron Multiplier Voltage (EMV)

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

- **1** What is the electron multiplier voltage standard (list both polarities if applicable.)?
- 2 In the dynamic gain table, what is the maximum gain and the corresponding voltage?
- **3** A best practice for troubleshooting and maintenance is to monitor the vacuum levels over time using data from the detailed tune reports. Locate the levels for the rough and high vacuum for your system.
 - a Rough vacuum:
 - **b** High vacuum:
- **4** Locate the abundance in MS1 Peak Width Unit, Scan Speed Normal and list low middle and high mass numbers:

Scheduling a checktune

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



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

Instrument St	atus
TQ	? _ =
	Idle
	ff EMF()
Ē	AJS ESI
	On Standby
	Calibrant LC +
0.00 / 0.02	Vent Pump Down
Method E	Schedule Tune
₽ Cp1	Review Tune Report
Properties D/	

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

	Schedule Tune	х
Schedule Tur	le	
Checktune S	Scheduling Weekly Monthly Recur every 1 week(s) on: Monday Tuesday Wednesday Thursday Saturday Sunday	
	Polarity Both Positive Negative Save Cancel	

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.

Scheduling

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

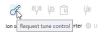


Stop checktune

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.

Ion source AJS ES Checktune the instrument Please install the tuning mix for current ion source type

4 Before the tune completes, click Stop Tune acquisition.



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

Tune was stopped by the user.



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

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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.

- 🗆 × oProject. Instrument: 6475A LC/TQ - Ac @ · . 2 Ξ 2 Status ੰ⊡ Real-time Plot Help, Pre Instrument Status TQ 6 AJS ESI đ 0.00 / 0.00 nt Idle 🗉 🛈 On 😑 Off Method Editor · < 5 E Properties DA TQ Ion source AJS ESI · AJS ESI Acquisition Parameters Compound group Compound name Compound Stop time As pump/No limit O Limit (min) Compound1 350 Convert to dMRM Time filter window (min)
 0.07 Estimated cycle time (ms/cycle) 101 Time Segments
 Start time (min)
 Scan type
 Detector Gain Factor (+)
 Detector Gain Factor (+)
 Stored?

 •
 0
 MRM • 1
 1
 I
 I
 Current User: SYSTEM (SYSTEM), Active Project: TrainingProject

The Method Editor window opens in the Main Window.

→ Tune

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.

Met	hod Ec	ditor		
+	C7~		-	ß
Prop	ertie Or	en M	ethod	٦١
-	Metno			

3 Select default.m and click Open.

Open Method			x
Selected Path:	C:\Projects\TrainingProject\Methods		n 🗗
Acquisit	ion.m		
default.	m		
default	est.m		
🗂 qualMe	thod.m		
quantM	ethod.m		
🖽 quantRe	portingMethod.m		
File name:	default.m		
Files of type:	Methods (*.m)		
Method Inform	nation		
Method De	cription:		
Pre-run Scri	pt:		
Post-run Sc	ipt:		
Create Folder			Open Cancel

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

- **d** Timetable Specify when the diverter valve is set To MS and when it is set To waste.
- e MRM database browser Starts the MRM Database Browser program.
- **f** Convert to dMRM . This option is only available if the Scan type is dMRM or tMRM.
- **g** Instrument Mode Select an instrument mode to be saved with the method.
- 5 Click Save As Method.

Method Editor	
🕂 다~ 🖱 🛱	default.m
Properties DA	Save As Method
 Method 	lon so

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

File name:	TrainingMethod	
Files of type:	Methods (*.m)	
Create Folder	Save Cance	ł

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.

Method Editor	
	⊡√ç⊅ ⊟
Properties DA TQ	

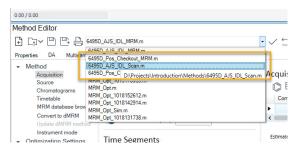
Set up a Scan Method



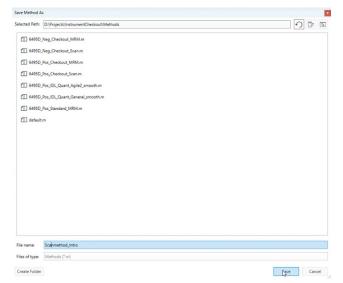
Load an existing method and Save As new method

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

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



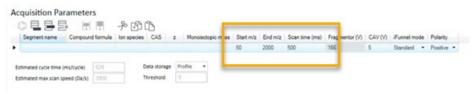
2 Click Save as Method, enter Scanmethod_Intro for the file name. Click Save.



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



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



NOTE

Values will vary based on your instrument model.

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

njection		Advanced								
Injection volume: 1.00 🛟 µL		Injection	Path Cleaning							
		Standard W	ash							
leedle Wash								Mode:	Flush Port	
Standard Was	h 👻							Time:		20 ;
Roptime	Posttime							Location:		
								Repeat		3 ;
 As Pump/No Limit 	Off	Multi-wash								
O 1.00 ; min	O 1.00 (min	Muru-wash								
		Step	Solvent	Time [s]	Seat Back Flush	Needle Wash	Comment			
		1	Off			10				
			Off							
			Off							

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.

Select Path	>
InstrumentCheckout	^
4 Data	
Blank_001.d	
Blank_002.d	
Blank_003.d	
Blank_004.d	
Blank_09OCT_001.d	
Blank_09OCT_002.d	
Blank_OCT_001.d	
Blank_OCT_002.d	
Blank_OCT_003.d	
Blank_OCT_004.d	
Introduction	
QuantReports	
QuantResults	
Reservine_IDL_001.d	
Reservine_IDL_002.d	
Reservine_IDL_003.d	
D Recemine IDI 004 d	~
Folder: Introduction	
rolder. Introduction	
Create Folder	OK , Cancel

4 Click Start Sample Run.

Sample		
Start Sam		
Name	100 g	Position Via
Injection Vo	lume As Method	γµL

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

(j)	Sample Run		
	Run completed.	OK	1
			-



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.

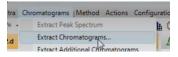
Review the data using Qualitative Analysis



- 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**.

Recent Items	1 Introductio	oduction n_Scan_002.d n_Scan_003.d	- 0	1	E.
Documents					
Desktop					
_					
This PC				_	
This PC	File name:	Introduction_Scan_002.d		-	Open
2		Introduction_Scan_002.d			Oren Cancel
This PC				- (- (45
2				~ (Cancel
Network	Files of type:				Cancel
Network ptions	Files of type:	Data Files (".d)	100 fg		Cancel
Network ptions O Load work	Files of type: dist method	Data Files (*.d) Sample Information	100 fg SYSTEM (SYSTEM)		Cancel
Network ptions O Load work O Load resul	Files of type: dist method lts method nt method	Data Files (".d) Sample Information Sample Name :	SYSTEM (SYSTEM)		Cancel

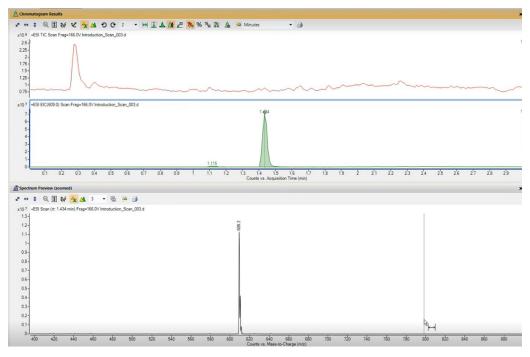
3 In the main window, click Chromatograms > Extract Chromatograms.



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

duction_Scan_002.d duction_Scan_003.d	Type: EIC V Integrate when
	MS Chromatogram Advanced Excluded Masses
	MS level: All
	Scans: All scan types
	m/z of interest: Any
	miz value(s): 609.0
	Merge multiple masses into one chromatogram

6 Review the results.





Review the Results.

- 1 What is the retention time for Reserpine?
- 2 What is the m/z observed for Reserpine in the mass spectrum?

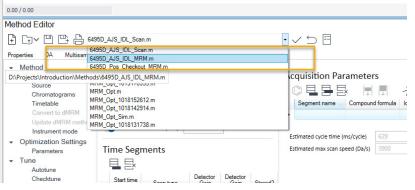
Set up an MRM Method



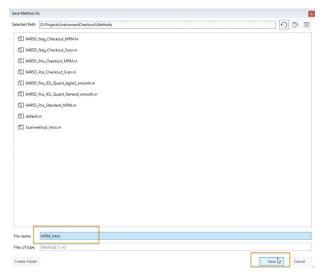
Load an existing method and Save As new method

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

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



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



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



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

Acquisition	Parameters														
OBB		~ D C													
pound name	Compound formula	lon 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							609.3	Unit 💌	195.1	Unit 💌	200	166	5	42	Standard •
(_		_	b.	_		-	_		_	-	_

Values will vary based on your instrument model.

5 Click Chromatograms and confirm the settings below.

Chromatograms

1			
	Chrom type Label	Precursor ion (m/z)	Product ion (m/z)
۲	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.

NOTE

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 Sample Start Sample Run Name Hjection Volume As Method ~	Position Vial 2 ~			
Data File				
Name			Introduction_MRM_000(d	View Data ·
Path		D:\Projects\Instr		

11 Click OK when the run completes.

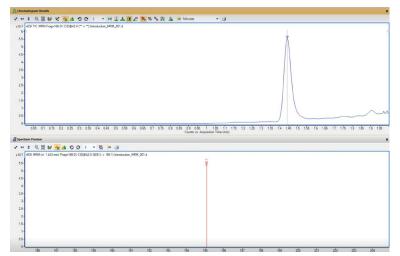
i	Sample Run Run completed.	
	Non compreses.	ОК

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**.

Recent Items	1 Introductio	roduction	0 1	
Documents Desktop				
	File name:	Introduction_MRM_001.d	~	(Sen
Network	Files of type:	Data Files (*.d)	~	Cancel
	Files of type:	Data Files (".d)	~	
ptions		Data Files (".d) Sample Information	~	Cancel
ptions O Load work	list method		~	Cancel
ptions O Load work O Load resul	list method	Sample Information	STEM)	Cancel
otions C Load work Load resul Use curren	list method Its method nt method	Sample Information Sample Name : 1 fg	STEM)	Cancel
ptions Dead work Load resul Use curren Load resul	list method Its method nt method	Sample Information Sample Name : 1 fg User Name : SYSTEM (SY	STEM)	Cancel

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



Introduction Workbook



Review the Results.

- 1 What is the retention time for Reserpine?
- 2 Which ion is present in the mass spectrum?

Offline Method Editor program

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

To get here, do one of the following:

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

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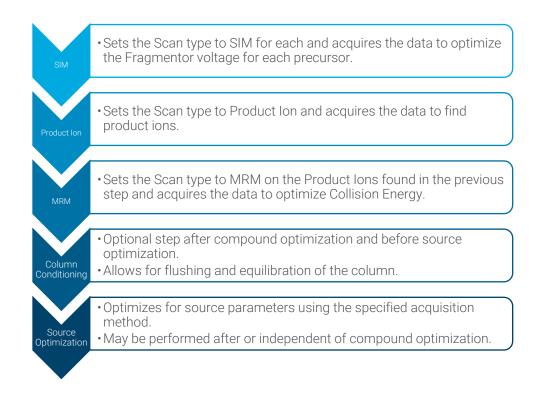
MassHunter Optimizer lets you automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on a triple quadrupole mass spectrometer instrument for each individual compound analyzed. Specifically, it automates the selection of the best precursor ions, the optimization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



Introduction Workbook

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





Optimize a method for a known sample:

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

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

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



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



- **4** In the Compounds section, click in the fields and enter the following information:
 - a Compound name: Reserpine
 - b Formula: C33H40N2O9

Compounds

	Compound name	CAS number	Formula	Monoisotopic mass
•	Reserpine		C33H40N2O9	608.30

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

ſ	Compound name	CAS number	Formula	Monoisotopic mass	Positi		ve lons Charge St	ate
ł	Reserpine		C33H40N2O9	608.30		Select	Formula	^
					1e		-Н	
				La .			+CI	
				H2			+Br +HCOO	
							+CH3COO	~

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.

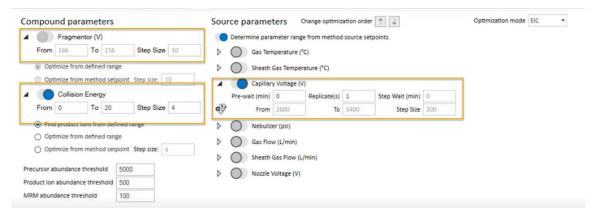
Chrom type Label	Precursor ion (m/z)	Product ion (m/z)
MRM MRM	350	200
TIC Channel 3C EIC		
BPC MRM		

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



- **11** In Compound Parameters, enable and set the following:
 - a Fragmentor V (if available): From 120, to 220, step size: 5
 - **b** Collision Energy Range: From 0, to 50, step size: 5
 - c Capillary Voltage: Leave defaults

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



12 Click Save to save the method.

13 Click the Method Optimizer tab and select Automated.

Method Optimizer

What type of optimization	do you wish to perform?
Select one of the optimization	Fully Automated Method Optimization:
Automated	Optimization is carried out in a fully automated manner.
Comparing the second	All user input for each phase is defined prior to the start of the optimization.
Compayed-by-compound	Data review is only available once the optimization has completed.
	Creates the most suitable MRM transitions from a list of chemical formulas or precursor ior
	 Optimizes and fine-tunes MRM specific parameters
	 Optimizer and fine types in Source and front and in particular
	 Optimizes and fine-tunes Ion Source and front-end ion optics parameters

- 14 Click Next.
- 15 Select MRM Database or enter a new database field and enter Introduction.
- 16 Under Methods, click Add a row at the end of the table.

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

pen Methods		
elected Path:	D:\Projects\InstrumentCheckout\Methods	
6495D	_Pos_IDL_Quant_Agile2_smooth.m	
6495D	Pos_IDL_Quant_General_smooth.m	
f 6495D	_Pos_Standard_MRM.m	
default	Lm	
	Intro.m	
	Optm	
Scanm	ethod_Intro.m	
ile name:	MRM_Optm	
iles of type:	Methods (*.m)	
Aethod Infor	mation	
Method De	escription:	
Pre-run Sci	ript:	
Post-run Si	cript	
Create Folder		Open Cance

- **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

	ethods						
	Acquisition method	Sample position	Injection volume	PI scan inj factor	Injection type	Status	Action
۲	MRM_Opt.m	Vial 3	1		Injection with colum 👻	Ready	

- 19 Click Next. Method optimization begins.
- **20** As the Method Optimization runs, review the Status.

Status	
2023-10-12 08 59:41 The original method D:\Projects\Ada-PP1\Methods\Test.m remains unchan; method D.\Projects\Ada-PP1\Methods\Test_1012085940 m is created to be optimized. Compound optimization is attated. The following parameters will be optimized: Collision Energy. 2023-10-12 08:59:41 Running survey run.	jed. A
	~

21 Observe over time the Optimization Remaining.



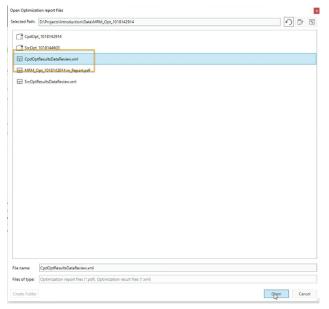
- 22 Navigate to **Method Editor > Acquisition** to observe the mass spectrum processing.
 - a Review the TIC plots.
 - **b** Review the Scan Type being used in the Time Segment panel

Start time (min)	Scan type	Detector Gain Factor (+)	Detector Gain Factor (-)	Stored

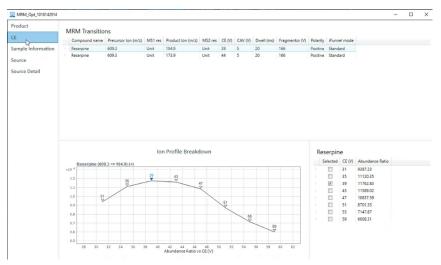
23 Once complete, click **Review results** to review the generated optimized MRM report.

Review result

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



25 Review the results.



NOTE

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

in to Quick Copy Paste Copy path	Move to •	Copy to	New item •	Properties History	Select all		
Clipboard		Organize	New	Open	Select		
← → × ↑ 📙 « Data → MRM_O	pt_10181429	4 v õ	Search MRM_O	pt_1018142914			
	^	Name	Da	te modified	Type	Size	
🖈 Quick access		CpdOpt_1018142914	10		File folder		
Desktop	1	SrcOpt_1018144603			File folder		
👆 Downloads	1	CpdOptResultsDataRe			KML Document	550 KB	
Documents	1	MRM_Opt_1018142914		/18/2023 3:06 PM	Microsoft Edge P	114 KB	
E Pictures	1	SrcOptResultsDataRev	iew.xml 10	/18/2023 3:05 PM	(ML Document	168 KB	
comparison							
Data							
- Method							
Sulfa_Cal_sep23							

LC/TQ Method C	Optimizer Rep	ort											
Instrument Sumr	nary												
Model Date SW/FW Version Serial Number						G6495D 10/18/20 3.1.632 SG2305	23 2:45:4	15 PM					
Method Informat	ion												
Method Name Optimization Type njection Type on Source					í	Auto/Gu	with colu		n				
Compound Optin	nization												
Optimization Par Product ion CE Range (V							to 60; Str						
CE Range (V))						to 60; St						
MRM Table Compound Name		Prec	MS1	Ener	Prod	MS2	CE AA	CAV	Dwell	Pol	RT	DTM	Funnel Mode
		Priec Ion (m/z)	Res	Frag (V)	lon (m/z)	Res	CE (V)	Ś	(ms)	Ро	(min)	(min)	IPUNNEI MOGE
Reserpine Reserpine		609.3 609.3	Unit Unit	166 166	194.9 173.9	Unit Unit	39 44	5 5	20 20	Pos Pos	0.00	0.00	Standard Standard
Compound Deta	11												
Compound Name	Reserpine			Prec lor	n (m/z)		60	9.3					
CE (V) Profile						rtt 1	Ion Spect	trum					



Method Editor Optimization

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

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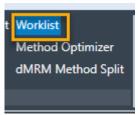
7 Run a Worklist

Overview

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

Create and edit a worklist

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



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

NOTE

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

Run a Worklist

3 Enter all the information on the Sample Information tab.

Sample Information Sample Position	
Sample	
Name: Sample Append Counter	
Suffix Counter	
Number of digits: 1 Start Value: 1 Step: 1	
Method	
Name: Acquisition.m	
Path: C:\Projects\TrainingProject\Methods	
Dverride DA Method	
Name:	
Path: C:\Projects\TrainingProject\Methods	
njection	
Injection Volume: As Method 💌 μl	

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).

	Position		
Current Configuration			
Select Well-plate or Vial Tray	Plate/Tray Type		
Select Tray	6 Vials Generic Plate		
✓ Well-plate Tray	Selection Origin		Block Increment
Plate 1	0.		Row major
Plate 2	Top left O Top	right	Column major
- Plate 3	O Bottom left O Bot	tom right	 Column major Serpentine
Plate 4			O Serpentine
Plate 5 Plate 6	Number of samples	Num	ber of replicates
Plate 7	0	1	
Plate 8	-		
Well-plate/Tray			
1	2		3
A			

- 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.

C:\Projects\TrainingProject\Methods	
C:\Projects\TrainingProject\Methods	

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	C:\Projects\TrainingProject\Da	ta			
▲ Sub-Folders Sub-folder 1		Sub-folder 2		Sub-folder 3	
<empty></empty>	•	<empty></empty>	·	<empty></empty>	•
 File Naming Part 1 User Text 	-	Part 2 Counter[0001]		Part 3 <empty></empty>	
WorklistData		contertoort		compty>	
 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**.

Norkli	ist											
Č7 [h 🖻				- Ę	· 팊 틸 磐 幸 IB Q € <mark>IB</mark> 图 ┍ · · ·						
	\checkmark	Status	Sample Name	Sample Position	Method	Data File	Sample Type	Level Name	lnj Vol (μl)	Comment	Sample Group	Info.
1	\checkmark	Pending	Reserpine	No Injection		WorklistData-0001.d	Sample		As Method			
2	1	Pending		No Injection		WorklistData-0002.d	Sample		As Method			
3	\checkmark	Pending		No Injection		WorklistData-0003.d	Sample		As Method			
4	-	Pending		No Injection		WorklistData-0004.d	Sample		As Method			
5	-	Pending		No Injection		WorklistData-0005.d	Sample		As Method			
6	-	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.

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

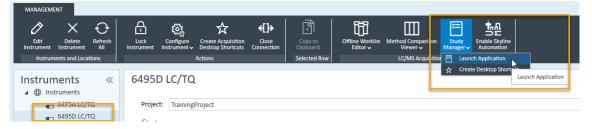
The study types create and run a worklist that to analyze the samples. Specify more information to apply to the study, including a location to place all the files generated by that study or create several types of studies. When the Study Manager application starts, the data acquisition engines are automatically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

				Project:	TrainingProject	Instrument: 6475A LC/T	Q - Study Manager			-	
Home	e										0
Start	Stop • Study	Stopped Status Execution	Subr	mit	System Activity Log Study Action	Standby Script Wait for	dby script on idle or	10 min			
topped					-						
Name:				Path:					Est. Time Remaining (min)		
0727	23.s					roducts\Studies					
Submit				Plate Assignm	nent:				0.0		
SYST	TEM (SYST	EM)		None							
Pending	Studies	Completed Studies								⇒]
choing		Completed Stadies									
N	lame	Path	Submitter	Plate A	Assignment	Est. Study Duration	Est. Start Time	Sample Count			
	lame)72723_2nd.s				Assignment	Est. Study Duration 01:28.00	Est. Start Time	Sample Count 8			
					Assignment		Est. Start Time				

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

Select Study Creator
What type of study do you want to submit?
Bioanalysis
Worklist Import
Worklist Only
Help
Creates a study that will execute a worklist based on a worklist file. A copy of the worklist is made in the study to preserve the original worklist. The data files specified in the worklist can be automatically updated to be stored in the study folder. If the worklist contains samples for a single well plate, the sample positions can be updated to use a different well plate.
OK

Run a Worklist

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

:		Worklist	Only			-	×
Study Setup		Quant S	etup	\geq	Worklist Review		
Worklist File Worklist File: Worklist File P	ath:	C:\Projects\TrainingPro	ject\Worklis	ts	× 		
Study File Study Name:		Date (MMDDYY) Custom Name Use separator betwe		Blank Custom Name ms Separate	>		
Study Base P	ath:	C:\Projects\TrainingPro	oject\Studies		n study		
Study Folder	Path:	C:\Projects\TrainingPro					
Plate Assignme	ent						
	Plates in	worklist		Reassig	n		
Subm	itter:	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?

Overview

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

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

Before You Begin These Exercises

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

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

Set up a New Batch

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

Create a batch to hold samples

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

🗅 Project						
Select project:						
TrainingProject	•					
	ОК					

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

This PC	iii_Test_01	Create Batch					
Browse	📄 Browse 🟫 🖆 C:\Projects\TrainingProject\Data\DoA						
	Name	Date modified					
	CMAMBIk_01.d	10/16/2023 12:35:29 PM					

Add all the samples in the DrugsOfAbuse folder to the batch

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

Add Samples				?	\times
Batch Folder:	C:\Projects	TrainingProject\E)ata \Do A\	λ	
File name	Name	Sample Group	Acq. Date-Tir	me	^
CMAMBlk_01.d	Blank-1		5/12/20	06 1:48	
CMAMCal_L1.d	Calib-L1		5/12/20	06 1:51	
CMAMCal_L2.d	Calib-L2		5/12/20	06 1:54	
CMAMCal_L3.d	Calib-L3		5/12/20	06 1:57	
CMAMCal_L4.d	Calib-L4		5/12/20	06 2:00	
CMAMCal_L5.d	Calib-L5		5/12/20	06 2:03	
CMAMQC_L2.d	QC-L2		5/12/20	06 2:06	
CMAMQC_L4.d	QC-L4		5/12/20	06 2:09	¥
<				>	
Translate MS	WS Samples				
Translate Ope	nLab Sample	s			
Select All		ОК	5	Cancel	

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

B	File	Open Batch Batch	Home	d Delet	e /	Method Quantitate ~ Clear F Calibration ~ Integrate ~ Analyze	Tools Results	Generate Report	Help Open Report Folder L Queue Viewer L Edit Report Method C Report
	ch Ta		•		e Type: </th <th>All></th> <th>▼ Corr</th> <th>npound: 🔇</th> <th></th>	All>	▼ Corr	npound: 🔇	
()	8	Name	Data File	mple Type	Level	Acq. Date-Time	-		
•		Blank-1	CMAMBIk 01.d	Blank	20101	5/12/2006 1:48 PM	1		
		Calib-L1	CMAMCal L1.d	Cal	L1	5/12/2006 1:51 PM	1		
_		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	Ì		
_		Calib-L4	CMAMCal_L4.d	N al	L4	5/12/2006 2:00 PM	İ.		
_		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	1		
_		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	1		
		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	1		
		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			

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

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



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

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

C:\Projects	Name	Date modified
TrainingProject	CMAMBIk_01.d	10/16/2023 12:3
A Data	CMAMCal_L1.d	10/16/2023 12:3
DoA	CMAMCal_L2.d	10/16/2023 12:3
QuantResults	CMAMCal_L3.d	10/16/2023 12:3
Methods	CMAMCal_L4.d	10/16/2023 12:
Report Templates	CMAMCal_L5.d	10/16/2023 12:3
Studies	CMAMQC_L2.d	10/16/2023 12:
Templates	CMAMQC_L4.d	10/16/2023 12:3
Worklist Import	CMAMSam_01.d	10/16/2023 12:
Worklists	<	******
ile name: CMAMCal_L5.d	Sample files (*.d)

Set up Target Compounds

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

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

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

Þ	Workflow
4	Method Setup Tasks
ſ	CMRM Compound Setup
	Retention Time Settin

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

Method Setup Tasks	
KMRM Compound Setu	p
Retention Time Setup	
hs	

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

1 Click Method Setup Tasks > ISTD Setup.



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

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

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

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

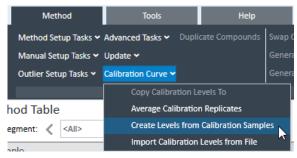
Set up Quantitation

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

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

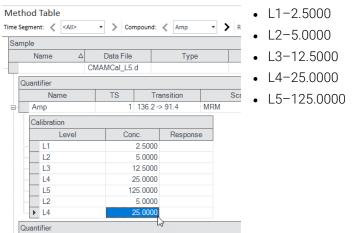
Create five calibration levels for the first compound

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



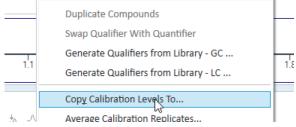
The Calibration table opens under each Quantifier in the Method Table.

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



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.

Name	TS	RT	Transition	ISTD Flag	Cmpd. Group
Cocaine		2.449	304.1 -> 182.0		
MDMA		2.269	194.2 -> 163.3		
Meth		2.239	150.1 -> 119.3		
Vleth	1	2.239	150.1 -> 119.3		

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



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

Set up qualifier ions and a calibration curve

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

Concentration Setup
Qualifier Setup
Calibration Curve Setup

ample													
Name	Δ	Data File		Туре		Level		Acq. Method	File	Acq.	Date-Tim	е	
	CMA	MCal_L5.	d										
Quantifier													
Name		TS	Trans	ition		Scan		Туре	P	recurs	orlon	Product Ion	Uncertainty
Amp	p 1 136.2 -> 91.4		1.4	MRM		Targ	Target		136.2		91.4	Relative	
Qualifier													
Precursor	lon	Prod	uct Ion	Trar	sition	Rel. Resp.		Uncertainty	Area S	Sum			
	136.2		119.4	136.2 ->	119.4	28	8.3	20.0					
Quantifier													
Name		TS	Trans	ition		Scan		Туре	P	recurs	or lon	Product Ion	Uncertainty
Amp-d5		1	141.1 -> 9	3.4	MRM		IST	D			141.1	93.4	Relative

·
Qualifier Setup
Calibration Curve Setup
말 Globals Setup

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

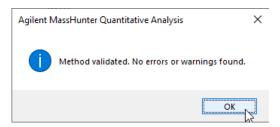
Q	uantifier							
	Name	TS	Transition	Scan	Туре	CF	CF Origin	CF Weight
•	Amp	1	136.2 -> 91.4	MRM	Target	Linear	Ignore 🗸	None
	Amp-d5	1	141.1 -> 93.4	MRM	ISTD		Ignore	
	Cocaine	1	304.1 -> 182.0	MRM	Target	Linear	Include	None
	Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD		Force	
	MDMA	1	104.0 \$ 160.0	MOM	Tornot	Lincor	Blank offset	None

Validate the method

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

Save / Exit		
💞 Validate	N	
Savo	h3-	

2 After the validation message appears, click OK.



3 Click Save/Exit > Exit.

▲ Save / Ex	t	
👸 Validate		
Save		
Save As.		
🔀 Exit	de la companya de la	

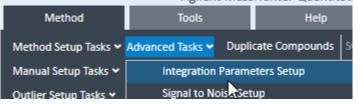
4 Select None under Additional batch processing after applying the method and click Yes to the Would you like to apply this method to the batch? prompt.

Apply Method	×
Would you like to apply this method to the batch?	
Yes No Cancel	
Additional batch processing after applying the method	
○ Analyze	
O Quantitate	
O Integrate	
None	

Set the Integrator

Change the method's integrator to MS/MS

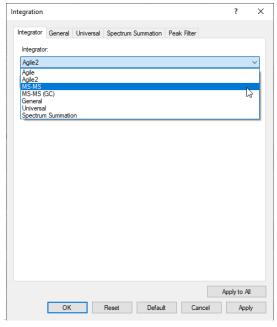
- 1 On the Method tab, click Edit.
- 2 On the Method tab, select Advanced Tasks > Integration Parameters Setup.



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

RT		Int.		
	ļ			
2.102	Agile2			
			(1)	
			10	5

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



5 Click Apply to All.

Introduction Workbook

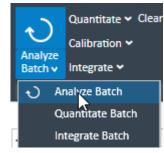
- 6 Click OK.
- 7 Click Exit.
- 8 Select None under Additional batch processing after applying the method and click Yes to the Would you like to apply this method to the batch? prompt.

Analyze and Save the Batch

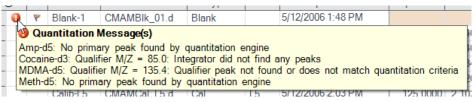
In this exercise, you quantitate the batch and then save the results.

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.



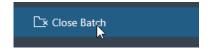
3 Pass the cursor over the flags for the first two calibration standards.

,	Humo	Data Filo
٣	Blank-1	CMAMBlk_01
	Outlier(s)	
A	mp-d5: Peak	not found 2
	Calib.L3	CMAMCel 13

4 On the Home tab, click Save Batch.



5 Click File > Close Batch to close the batch.



Review Quantitation Results

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

Use the DrugsOfAbuse batch in this exercise.

Navigate the Batch Table Results

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

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

- 1 On the Home tab, click **Open Batch**.
- 2 Navigate to \Your Directory\DrugsOfAbuse and click iii_Test_01.batch.bin
- 3 On the View tab, click Restore Default Layout.



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

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

	Sample	Type:	<ali></ali>	
ampl	Next Sam	ple (C	trl+Down)	
BI	Go to the	next s	sample .	
Cal		11	5/12/2006 1:51 PM	

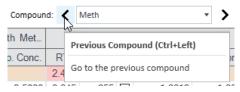
2 Return to Cal-L5, clicking the **Previous Sample icon** in the Batch Table Standard toolbar if needed.

Batcl	h Ta	able		
Sample	: ^	Calib-L5	•	\mathbf{v}
	87	रु Previous Sample (Ctrl+Up)		
	۲ ۲	Go to the previous sample		h
	10r	CELET CHANGE FEED		0-1

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.

Compound:	<	Cocaine	
caine			Cocaine Results

Examine results for multiple compounds

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

Batch	Table Layout 🛩	Auto Fit Columns	
	Flat Table		
	Nested Table (Horizontal)	
	Nested Table (Vertical)	
↓⊞	Compound Tat	le	
_ 🔳	Single Compou	ind/Sample View	ne
==		ounds/Samples View	C
	2.4		, .

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

	Sample						Amp Result	ts		Meth Resul	s		MDMA Resu	ilts	Cocaine Results			
•	Ÿ	Name	Data File	Туре	Level	Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
(9	Blank-1	CMAMBlk_01.d	Blank		5/12/2006 1:48 PM							2.284	1.9296		2.433	11.8235	
	٣	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
R		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
13		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
	9	Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM	2.080			2.286	3.2639		2.315	5.6138		2.408		
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM	2.143	4.8977		2.250	5.8102		2.280	5.1778		2.460	4.3735	
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM	2.105	14.2183		2.236	14.1876		2.267	10.7772		2.446	10.9299	

View selected sample types

- 1 On the View tab, select Batch Table Layout > Single Compound/Sample View.
- 2 If necessary, click the down arrow next to the Compound list, and click **Cocaine**.
- 3 Click the down arrow in the **Sample Type** drop-down list. **The Sample Type** dialog box is displayed.

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

Sample	Type:	<all></all>
		Sample Type
/pe	Lev	<al><al></al></al>
c Die Die Die	L1 L2 L3 L4 L5 L2 L4	Sample Blank Cal VQC CC DoubleBlank MatrixSpike MatrixSpikeDup MatrixBlank TuneCheck ResponseCheck <unassigned></unassigned>
		OK Cancel

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

Change Result Window Layouts

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

Use layout icons on the toolbar

- 1 Use layout options on the View Tab to position the **Batch Table**, **Compound Information**, and **Calibration Curve windows**.
 - a On the View tab, select Preset Layouts > Table Left.
 - **b** On the View tab, select **Preset Layouts > Table Right**.
 - **c** On the View tab, select **Preset Layouts > Table Top**.



- **2** Use layout icons on the View Tab to maximize each individual window:
 - a On the View tab, select Maximize Pane > Maximize Table.
 - **b** On the View tab, select Maximize Pane > Maximize Compound Information.
 - c On the View tab, select Maximize Pane > Maximize Calibration.

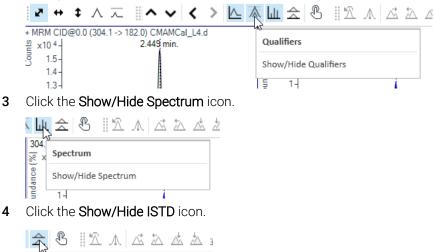


3 On the View tab, click Restore Default Layout.

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

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

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





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

÷ File		Home	View		Method	Tools	Help							est_01.batch.bi					
	~~~		A.A.	- L.		t Load / Save Layout 🛩			ns Reset So	n Ie	ock Sample/Comp	oound Colur	A Jan	iuto Review Sampl	les				
$\square$	A_A_	22	A.A.	Λ_	Preset Lawauts *	Toolbars ~			imns Load Col					iuto Review Comp					
Panes	Compounds at-a-Glance v	Calibration Curves Ci at-a-Glance	ompound Grou Peaks	p Extract	or Maximize Pane 🛩	Batch Table Layout 🛩	Auto Fit Col	lumns	Save Col	umn Settings									
			Layo							Columns			- 1	Auto Review	r				
Batch Ta	able																		- + ×
iample: ٨	Calib-L4		• 🗸 Samp	le Type: <	u>	<ul> <li>Compound:</li> </ul>	< Cocaine			> ISTD: C	locaine-d3		Ē	i 18 🛛 🚥	찌				
		S	ample			Cocaine N	fethod			Cocaine Resu	lts		Qualifier	(304.1 > 8		Cocaine-d3 (ISTD) Results		Qualifier (307.1 -> 85.0) Results	
9	Name	Data File	Туре	Level	Acq. Date-Time	Exp. Cr	onc.	RT	Resp. MI	Calc. Conc.	Final Conc. A	Accuracy	Ratio	MI	RT	Resp.	Ratio	MI	
۳	Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM			2.449	60 🗌	36.1520	36.1520		_		2.450	15			
	Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM		2.5000	2.455	5242	2.7545	2.7545	110.2	3.7		2.450	20389	4.0		
	Calib-L2	CMAMCal_L2.d		L2	5/12/2006 1:54 PM		5.0000	2.455	9794	4.7148	4.7148	94.3			2.456	20606	4.0		
	Calib-L3	CMAMCal_L3.d		L3	5/12/2006 1:57 PM				25358	12.0042	12.0042	96.0			2.456	19707			
	Calib-L4			L4	5/12/2006 2:00 PM				50910	25.5883	25.5883	102.4	3.8		2.450	18189			
	Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM				2008 🔲	124.9382	124.9382		3.8		2.450	14490			
	QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM		5.0000			4.7212	4.7212	94.4	3.5		2.456	19581			
	QC-L4	CMAMQC_L4.d		L4	5/12/2006 2:09 PM				48860	25.0020	25.0020	100.0	4.0		2.456	17873	3.9		
		CMAMSam_01.d			5/12/2006 2:12 PM			2.656											
۳ 0		CMAMSam 02.d			5/12/2006 2:15 PM				9755	4.7936	4.7936		3.6 3.9		2.462	20151 20608			
0 7	Sample-2 Sample-3	CMAMSem_03.d	Sample		5/12/2006 2:18 PM			2.443	25041	11.3601	11.3601		5.5		2.111				
	Sample-3		Sample		5/12/2006 2:18 PM			2.443	25041						2.111	£3333			
Compou ₽ ↔	Sample-3 und Inform \$ ∧ ⊼	nation	> L &		8   A   A   A		1    <u>û</u> A				* × C	alibratio	n Curv			Origin: Ignore      Weight: None	• ISTD		- 4
Compou ▲ ↔ : ARM CIDe ×10 4 12- 1- 0.8- 0.6- 0.4- 0.2- 0	Sample-3	nation	Database (c) ta	04.1 -> 18 ×10 ² f 0.8 0.6 0.4 0.2 0	① ※ ※ 本 本 本 本 本 本 本 本 本 本 本 本 本 本 本 本 本	≥ ≤ 2 3 32	+ MRM (237- # x103 2.5- 1.5- 0.5- 8- 0.5- 8- 0.5- 8- 0.5- 8- 0.5- 1.1- 0.5- 8- 0.5- 1.1- 0.5- 8- 0.5- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1- 1.1				# × C which Call Coc 304.1 300	aine - 5 Lev x10 1 − y = 5 1.4 − R ² 2 R = 1	n Curv	re els Used, 5 Point x - 0.04959	e: Linear s, 5 Points	Origin: Ignore      Weight: None	• ISTD		• 8

#### Introduction Workbook

#### Save the default layout without the calibration curve

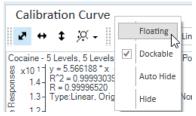
- 1 Close the Calibration Curve window.
- 2 On the View tab, select Load/Save Layout > Save Layout. The system displays the Save Layout File dialog box.

Load / Save Layout 🛩	Add/Remove Colum
Load Layout	Restore Default Colu
Save Layout	Auto Fit Columns
Save Layout	
Save window layou	t

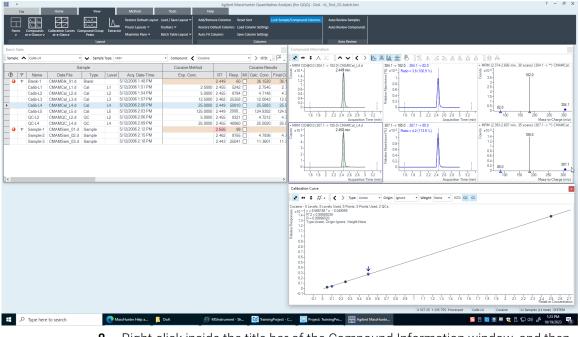
3 Name the layout file Batch Table plus Compound Information and click Save.

#### Load the newly created layout

- 1 On the View tab, click Restore Default Layout.
- 2 On the View tab, select Load/Save Layout > Load Layout. The system displays the Load Layout dialog box.
- 3 Click Batch Table plus Compound Information and click Open.
- 4 On the View tab, click Restore Default Layout.
- 5 Right-click inside the title bar of the **Calibration Curve** window, and then mark the **Floating** check box.

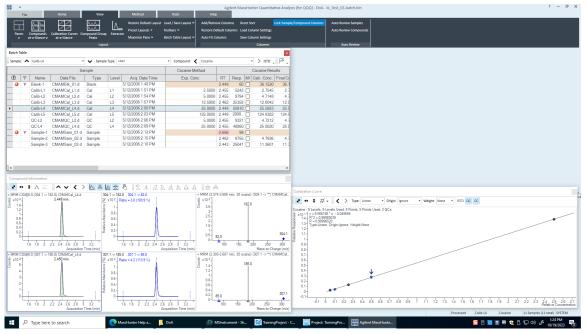


6 Right-click the title bar of the **Compound Information** window, and then mark the **Floating** check box.



7 Resize the windows to match the layout below.

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



9 Resize the windows to match the layout in below.

- 10 Right-click inside the title bar of the **Calibration Curve** window and clear the **Floating** check box.
- **11** Move the **Compound Information** window so that the layout corresponds to the one pictured at the start of the task.

#### Recreate (do not restore) the default layout

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

### **Use Three Tools to Evaluate Results**

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

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

The DrugsOfAbuse batch is used in this exercise.

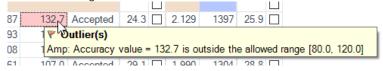
#### Adjust the Calibration Curve Fit

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

- 1 If necessary, open the batch file. iii_Test_01.batch.bin. On the Home tab, click **Open Batch**.
- 2 Navigate to \Your Directory\ DrugsOfAbuse and click iii_Test_01.batch.bin.
- **3** Make sure the **Batch Table** is set to single compound display mode, and the displayed target compound is **Amp**.



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



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



#### Analyze the batch and inspect the results in the Batch Table

- 1 On the Home tab, click Analyze Batch.
- 2 Inspect the results in the Batch Table after batch analysis.

	96.6	5
	97.1	
	102.5	
	103.8	;
:	99.2	2
	86.7	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.

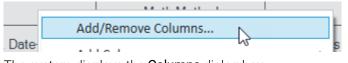
#### **Integrate Without Parameter**

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.

Columns					?	×
Select Columns From:						
Compound Method		$\sim$				
Available Columns:				Show these columns	in the order:	
Custom Calc. Custom Calc. Limit High Custom Calc. Limit Low DII. High Conc. DII. Pattem Dynamic Target Compound ID Dynamic Target Rank Extract Left m/z Extract Right m/z Formula Fragmentor Fragmentor Voltage Delta FWHM Limit Low FWHM Limit L	>	*	Add -> <- Remove Add All ->> < Remove All	Exp. Conc. Int. Int. Parms.		
				Move Up	Move Do	wn
			OK Res	set Default	Canc	el

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

Columns				?	×
Select Columns From:					
Compound Results	$\sim$				
Available Columns:			Show these column	s in the order:	
Custom Calc. Custom Calc. 1 Custom Calc. 2 Custom Calc. 3 Custom Calc. 4 FWHM Height Int. Betor Fag Int. Start Integration End Time Original Integration Start Time Original Integration Start Time Original Integration Start Time Original Integration Start Time Original ISTD Cresp. Ratio ISTD Resp. 7. Dev. ISTD Resp. Ratio Library Match Score Mass Abundance Score Mass Accuracy	< >	Add -> < Remove Add All -> <<- Remove All	RT Resp. MI Calc. Conc. Final Conc. Accuracy Int. Metric		
			Move Up	Move Do	own
		OK Res	et Default	Canc	el

#### View the default integration values for amphetamine

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

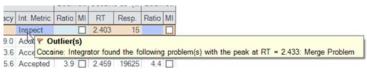
Int.	Int. Parms.
MS-MS	

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

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

#### View integration problems for cocaine and MDMA

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



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

- 6 Click the **Next Compound** icon in the Batch Table Standard toolbar or the Previous Compound icon in the Batch Table Standard toolbar until the system displays the compound MDMA.
- 7 Select the Blank-1 row and point to the Int. Metric column.

		-	100		"			
icy	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI	
	Accepted	15_		2.602	28			
1.3	Acce	uantit	atio	n Mess	age(s)			
3.1	Acce MDM	A-d5:	Qua	lifier M/	Z = 135.	4: Qua	alifier	peak not found or does not match quantitation criteria
0.2	Accepted	10.0		2.276	11059	24.2		

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

#### Change the noise algorithm

- 1 Right-click anywhere in the **Batch Table** and click **Add/Remove Columns**. The system displays the Columns dialog box.
- 2 From the Select Columns From drop-down list, select Compound Method
- **3** From the **Available Columns** list, select **Noise Alg**. (Noise Algorithm Type) and click **Add**.

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

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

Sample	e: ^	Blank-1		✓ Sample	e Type: <a< th=""><th>JI&gt;</th><th>* Compound</th><th>d: &lt; Amj</th><th>þ</th><th></th><th>&gt; 151</th><th>D: Amp-d5</th><th></th><th>i</th><th></th><th>m    [74</th><th>F F</th><th></th><th>3</th><th></th></a<>	JI>	* Compound	d: < Amj	þ		> 151	D: Amp-d5		i		m    [74	F F		3	
			Sa	mple				Amp	Method				Amp	Results		~	Qualifie	Amp-d	5 (IST_	Qualifie.
1	8	Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.	Int.	Int. Parms.	Noise Alg.	RT	Resp. M	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio MI	RT	Resp.	Ratio M
0	*	Blank-1	CMAMBIk_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
1		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
1		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
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
0	٣	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	1	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	1	MS-MS		RMS	2.105	2590	13.3556	13.3556		Accepted	25.3	2.089	1284	29.8

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

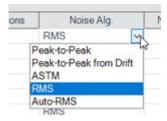
# Practice changing the noise algorithm from RSM to ASTM for amphetamine in the method

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

4	Advanced Tasks
	Integration Parameters Setup
	Signal to Noise Setup
	Smoothing Setun

The system displays the integrator parameters in the Method Table.

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



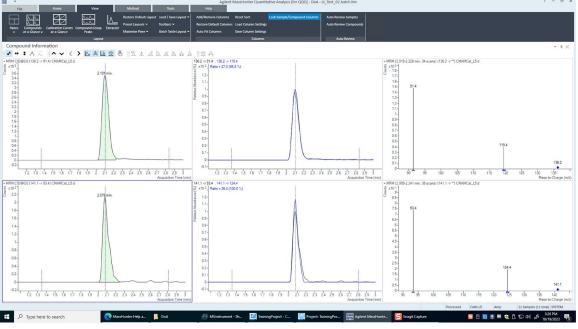
A list of available noise algorithms appears.

- 4 Click ASTM.
- 5 Under Method Tasks/Save/Exit, click Exit.
- 6 At the Would you like to apply this method to the batch? prompt, click No. The system displays Batch Analysis mode.

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

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

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



**Introduction Workbook** 

- 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.

operties					
Compound Information Comp	ound Information (2)				
General:			Retention time:		
Background color:	Automatic	~	Reference RT:		
Foreground color:	Dark Navy	~	Recognition window:		
Gridlines color:	No display	~	Peak purity:		
Time segment boundary:	No display	~	Show peak purity		
Chromatogram:			Purity colors		
Baselines					
Baseline calculation po	ints				
Normalize quantifier					
Original baselines after	manual integration				
Noise regions:	No display	~			
Peak fill:	75% Transparent	~			
Fill colors	]				
Peak labels	]				
Titles	]				
		1	Default OK	Cancel Acol	

- 4 Clear the Baselines check box in the Properties dialog box.
- 5 Click the Apply button and observe the peak without the baseline.

#### Inspect the calculation points for the baseline for amphetamine

- 1 Mark the **Baselines** check box in the Properties dialog box.
- 2 Click the **Apply** button and observe the peak with the baseline drawn.
- 3 Mark the **Baseline Calculation Points** check box in the Properties dialog box.
- 4 Click Apply and observe where the baseline starts and stops.
- 5 Clear the Baseline Calculation Points check box in the Properties dialog box.
- 6 Click Apply and observe the chromatograms.
- 7 Compare the chromatograms with and without Baseline Calculation Points.

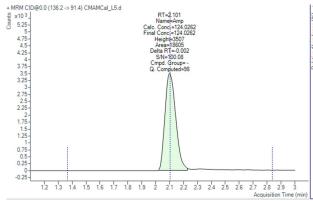
#### Display the peak labels for amphetamine

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

3 Click OK.

RT Name	^	Move Up
Calc. Conc. Final Conc.		Move Down
Height Area		
Delta RT		
S/N Cmpd. Group		
Q. Computed	*	
Display Label Names	(ex. RT=2.45	2)
Display Units for Cond	., RT and De	ata RT
Display Alternative Pe	ale I abala	

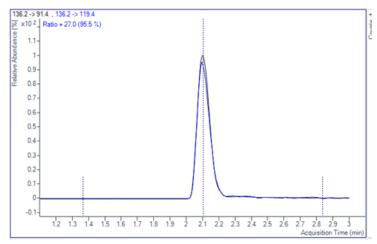
4 Click the **Apply** button in the Properties dialog box. The peak labels should now match those shown in the example below.



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

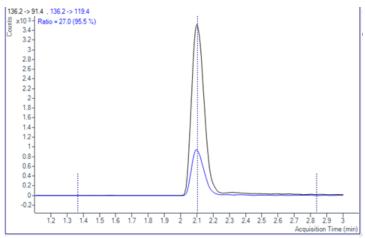
## Display the qualifier chromatogram on the right-side before and after normalization

- 1 Click the **Compound Information (2)** tab. In the Qualifiers area, mark the Normalize check box.
- 2 Click **Apply** and observe that the two peaks now converge and appear as one peak.



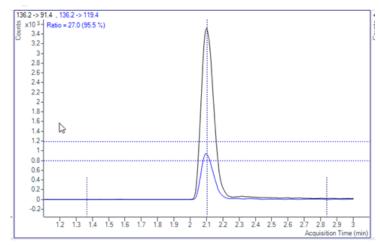
3 Clear the Normalize Qualifiers check box of the Properties dialog box.





#### View the uncertainty band

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



- 2 Select **No** display from the **Uncertainty Band** drop-down menu of the Properties dialog box. Click **Apply** to remove the uncertainty band from the qualifier chromatogram.
- 3 Click OK to close the Properties dialog box.
- 4 Compare the qualifier chromatogram with and without the Uncertainty band.

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

- 1 On the View tab, click Restore Default Layout.
- 2 Right-click the **Compound Method** section of the Batch Table and click **Add/Remove Columns**.
- 3 From the right list, select Int. and Int. Parms. (Compound Methods).
- 4 Click Remove, and then OK.

#### **Detect Outliers**

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

#### View outlier information for MDMA

- 1 Click **Next Compound** in the Batch Table toolbar until the system displays the compound MDMA.
- 2 Select the Blank-1 row and point the cursor to the RT column.



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

04	23.5	
20	27,5	
21	2 W 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

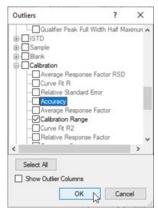
- 1 Click the **Previous Compound** icon in the toolbar until the system displays the compound Amp.
- 2 Select the Calib-L5 row in the table.
- 3 On the Method tab, click Edit.
- 4 In the Method Tasks column, click Outlier Setup Tasks > Accuracy.
- 5 Set the Accuracy Max % Dev value to 5% for Amp.
- 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.

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

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

#### Using the following set of outlier flag icons

- 1 Click the **Display rows that have High outliers** icon on the toolbar to display only samples with high outliers.
- 2 Click the Turn off outlier filter ¹/₁ icon to display all samples.
- 3 Click the **Display rows that have High/Low outliers** icon on the toolbar to display only samples with low outliers.
- 4 Click the **Display rows that have High/Low outliers** icon again to display all the samples.
- 5 Click the Select Outliers icon to bring up the Outliers dialog box.
- 6 Clear the Accuracy and Retention Time check boxes and click OK.



#### Introduction Workbook

- 7 Click the Select Outliers  $\stackrel{\text{\tiny P}}{=}$  icon to bring up the Outliers dialog box.
- 8 Mark the Accuracy and Retention Time check boxes, and click OK

### **Generate Quantitation Reports**

This exercise helps you learn how to do these tasks:

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

The DrugsOfAbuse batch is used in this exercise.

#### Quantitate the samples for this batch and save your results

- 1 On the Home tab, click Analyze Batch.
- 2 Click File > Save to save the batch.



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



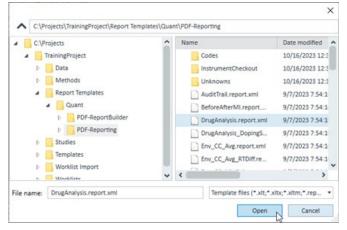
Note the Report Folder directory, which is where the report is saved.

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

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

Template		Report mode	Destination file	Publish format	Language	Pag
						2
Add Template	Remove Template	Edit Temp	slate		Edit Post Processe	

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



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

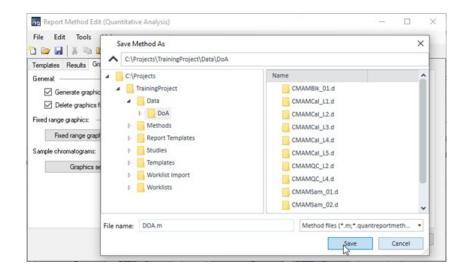
7 Repeat steps d and e to add DrugAnalysis_DopingScreening.report.xml.

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

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

Report Method Edit (Quantitative Analysis)					- 🗆 ×
File Edit Tools Help					
🖆 🗁 🖼 👗 🛍 🌋 🖬 📽					
Templates Results Graphics settings					
Template	Report mode 🔺 Destination file	Publish format Language	Page Size Printer	Open published file Post Process	Audt Trail Report
C:\Projects\Trainingcreening.report.xml	Batch V DrugAnalysis_Do	English (Unit 🗸	Letter V V	None>	×
<ul> <li>C:\Projects\Train\DrugAnalysis.report.xml</li> </ul>	Single Sample 🗸 DrugAnalysis.pdf	English (Unit 🗸	Letter V V	None>	×
Add Template Remove Template	Edit Template				Edit Post Processes
				Save & Ex	t Ext

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



#### Generate a report from the method

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

Generate Report					
Batch file:					
Batch folder:	C:\Projects\TrainingProject\Data\DoA\				
Batch file:	ii_Test_02.batch.bin	Browse			
Report folder:					
C:\Projects\TrainingPr	oject\Data\DoA\QuantReports\ii_Test_02	Browse			
Report method:					
C:\Projects\TrainingPr	oject\Data\DoA\DOA.m				
	Choose New	Edit			
Samples/Compounds:					
All samples	Choose samples				
All compounds	Choose compounds				
Generate:					
Generate reports no	DW				
Open report	folder after reports generated				
O Queue report task					
Start Queue	Viewer				
	ОК	Cancel			

- 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.

	Name	Data File	Туре	Level	Acq. Date-Time	í
۶.	Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48	
	Calib-L1	CMAMCal_L1.d	Calibration	L1	5/12/2006 1:51	
	Calb-L2	CMAMCal_L2.d	Calbration	L2	5/12/2006 1:54	
	Calb-L3	CMAMCal_L3.d	Calibration	L3	5/12/2006 1:57	
	Calb-L4	CMAMCal_L4.d	Calibration	L4	5/12/2006 2:00	
	Calib-L5	CMAMCal_L5.d	Calibration	L5	5/12/2006 2:03	
	QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06	
	QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09	
	Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12	.,
<					>	

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

	Name	RT	Transition	Cmpd. Group	
•	Amp	2.102	136.2 -> 91.4		
	Cocaine	2.449	304.1 -> 182.0		
	MDMA	2.269	194.2 -> 163.3		
	Meth	2.239	150.1 -> 119.3		

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

۲	Generate reports now
	Open report folder after reports generated
	Queue report task
	Start Queue Viewer

**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.

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 Passwore 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.

- 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)
- 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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