



6230 LC/TOF System

Introduction Workbook

with OpenLab CDS Software



Notices

Document Identification

G1960-90155

May 2026

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Software Revision

This workbook is valid for OpenLab CDS Acquisition software 3.0, until superseded.

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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.

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Introduction

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About this Training Workbook

This workbook provides instructions for the Agilent 6230 LC/TOF system running OpenLab Acquisition 3.0 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/TOF and 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 6230 TOF 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**.

Before You Begin

This introduction workbook is recommended for all participating end users.



- Download the *Agilent 6230 LC/TOF System User Guide* by scanning the code or navigating to <https://aglt.co/LCMSUserDocs>.
- Use the *Agilent 6230 LC/TOF 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 Q-TOF LC/MS supplies



Use this quick reference list to keep your shelves stocked by navigating to <https://aglt.co/LCQTOFSupplies>.

Agilent Community

Introduction

Before You Begin



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/>

Where to find more information

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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.

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Overview

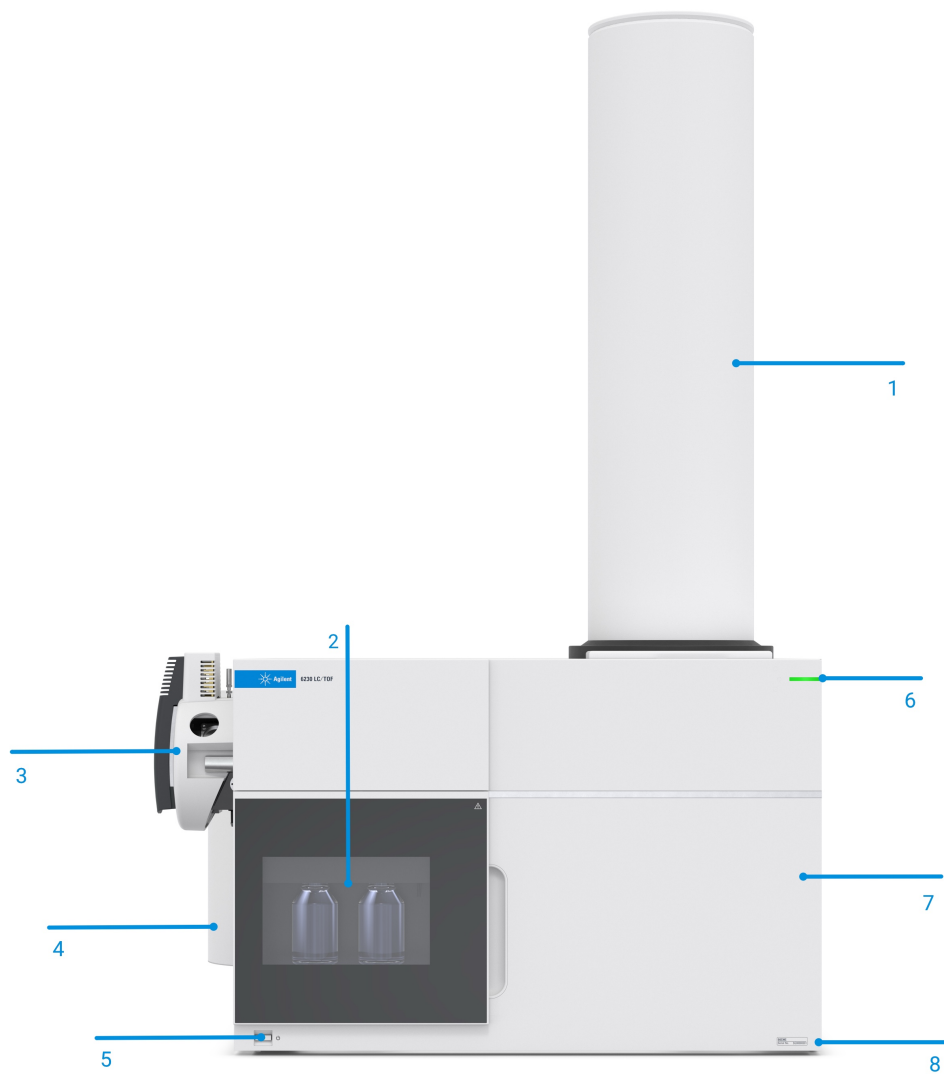
In this section, you will identify basic hardware components and their locations for the 6230 LC/TOF system.



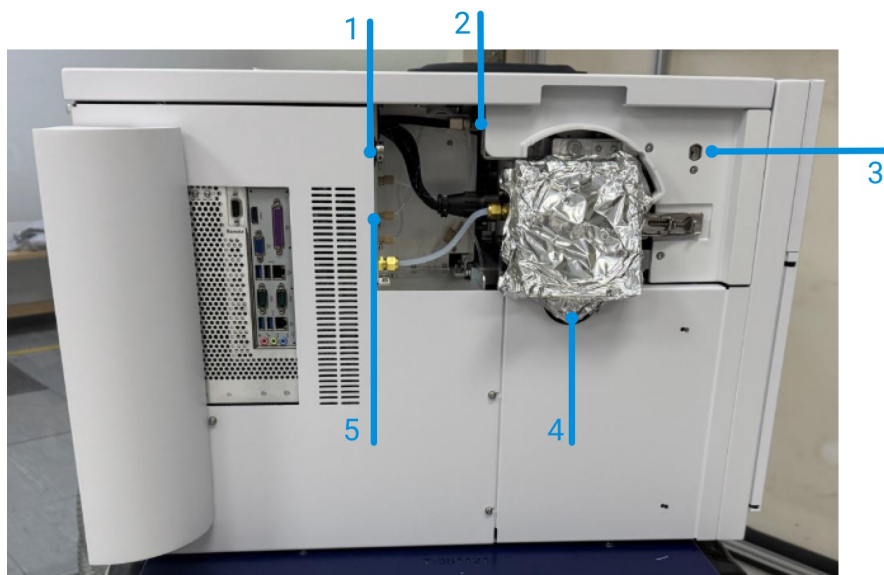
Fill in the Blank

Fill in the Blank: Work with your Agilent Service Engineer and/or use the *Agilent 6230 LC/TOF System User Guide* to label the flagged components below for your installed instrument(s).

Front view



Side view



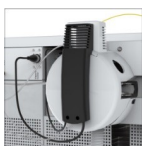
Back view



Basic Components

Ionization source

Agilent liquid chromatography/mass spectrometry (LC/MS) ion sources enable analysis of a wide range of samples quickly and accurately. These easily interchangeable ion sources for Agilent LC/MS systems include the:



- Agilent Jet Stream (Dual AJS ESI)



- Electrospray Ionization (ESI)



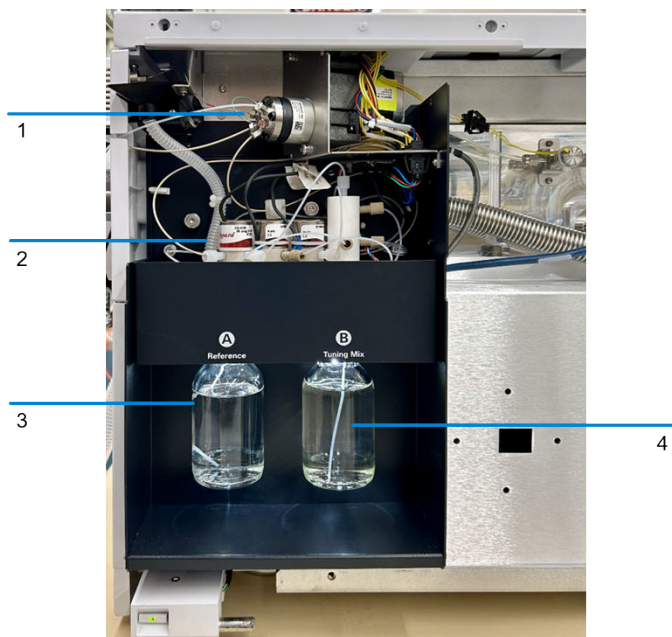
Hardware Introduction

1. List the ionization source in use:

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

Calibrant delivery system

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.



Hardware Introduction

1. Practice removing and attaching the calibrant bottle and the internal reference mass bottle.

2. Are the calibrant bottle and the internal reference mass bottle interchangeable? Why?

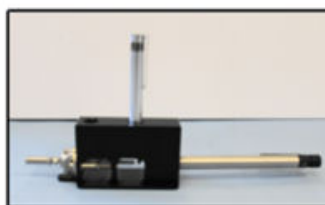
3. How often is the calibrant bottle checked and refilled?

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

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

Rough pump

MS45



IDP-45



For more information on your rough pump, visit <https://www.agilent.com/en/product/vacuum-technologies>.

Waste bottle from LC pump or mass spectrometer



Waste Bottle

1. At what level should you empty the waste bottle?

2. Does the waste bottle have a waste line connected? Why is this important to keep in place?



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The OpenLab Control Panel is the administrative and management center for the OpenLab Acquisition software. Control Panel provides:

- 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

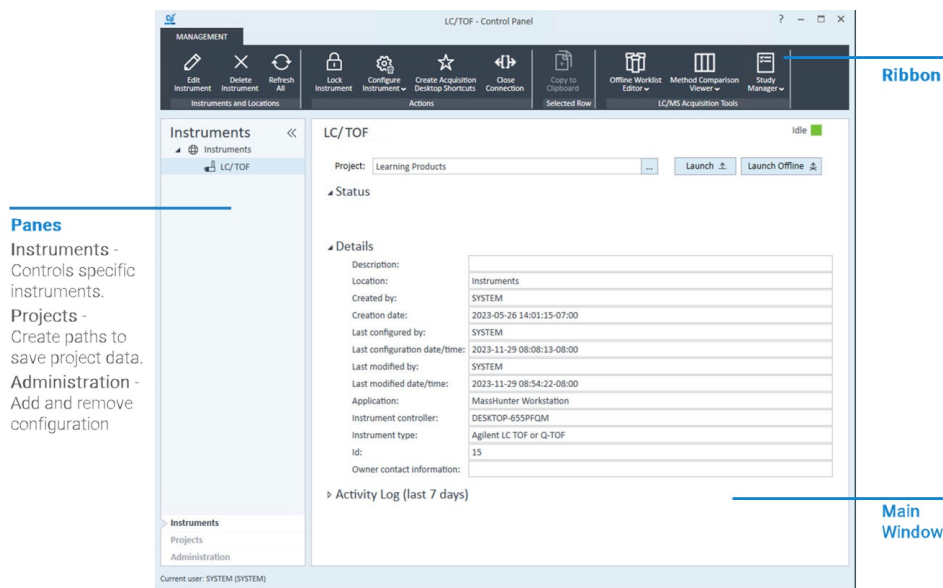


- From the desktop, double-click the **Control Panel** icon.



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

User Interface and General Navigation



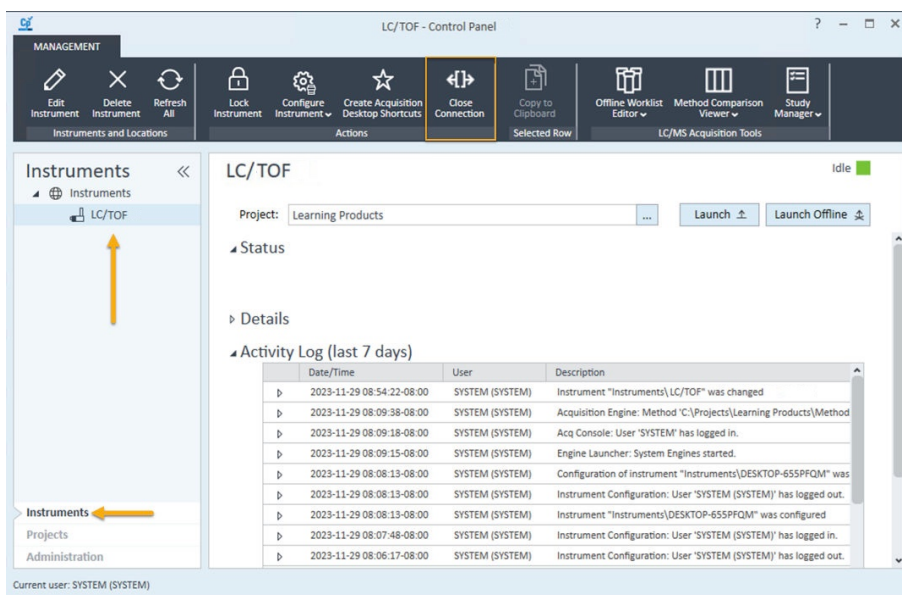
Adjusting the Instruments pane:

- To minimize the pane, click <<. When minimized, the tab currently selected is displayed vertically.
- To expand the pane, click >>.
- Items can be dragged and dropped 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.

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

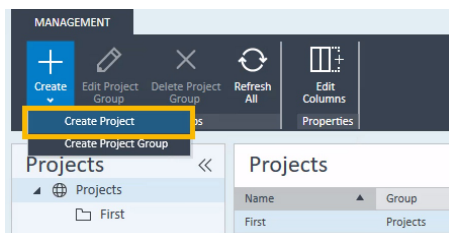


Creating and Configuring Projects



Creating and Configuring Projects

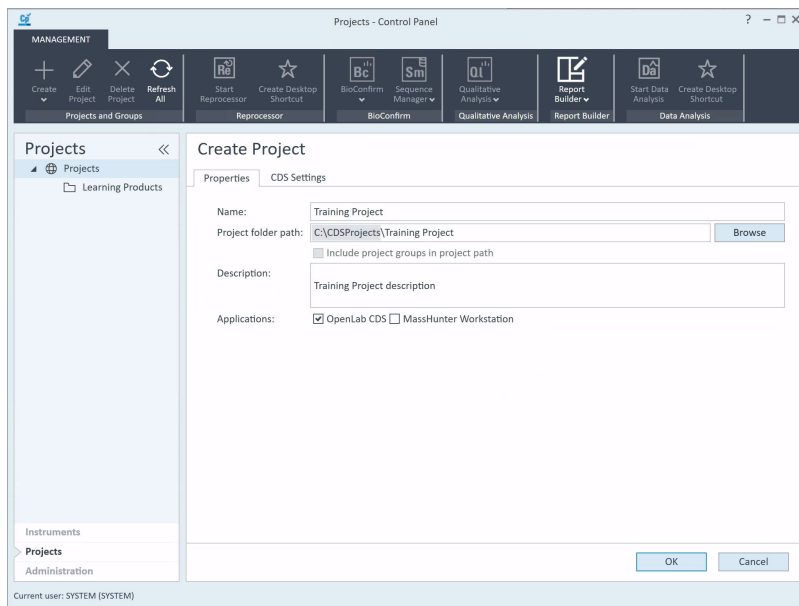
1. Click  **Projects** and select **Projects**.
2. On the ribbon, click **Create Project**.



3. In the **Name** text box, type *Training Project*.
4. In the Project folder path text box, leave the default folder path.
5. (optional) In the Description text box, type a description of the project, for this example *Training Project Description*.
6. If necessary, select the **OpenLab CDS** checkbox.
7. Click the **CDS Settings** tab and review the available options. Do not change the defaults.
8. Click **OK**.

Software Basics

Creating and Configuring Projects



Instruments

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

Assign a project to an instrument

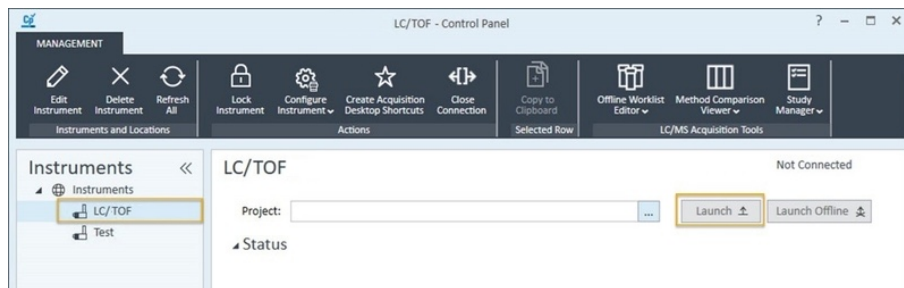


1. Click **Instruments** and select an instrument.
2. Click **Browse** to select the **TrainingProject** project from the Select Project dialog.
3. Click **OK**.

Launch an instrument

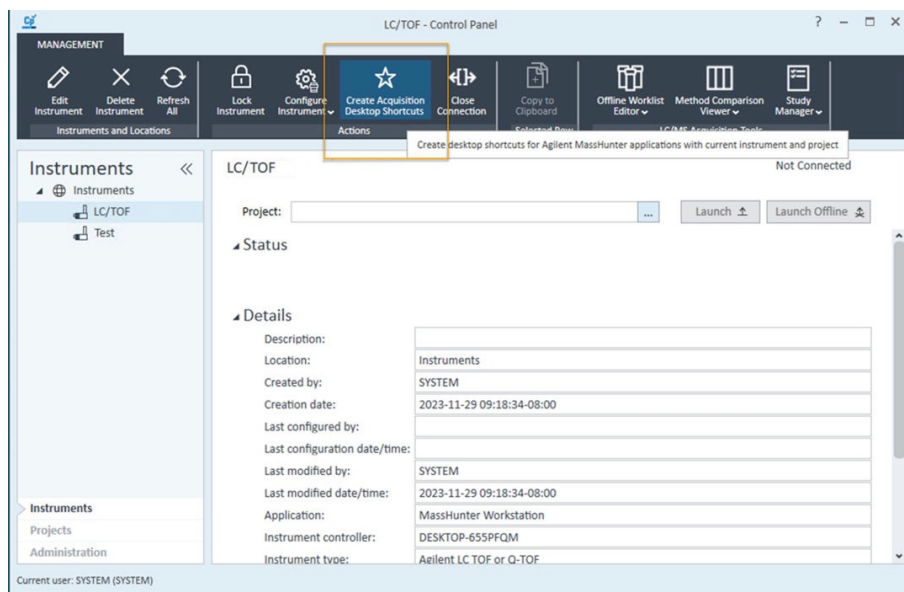
To begin acquisition, launch the instrument from the instrument table or the instrument details page, or launch an instrument directly from your desktop shortcut.

1. Click **Instruments** and select an instrument from the left panel.
2. In the Instruments window, click **Launch**.



Create an instrument shortcut

1. In the Control Panel, click **Instruments** and select an instrument from the left panel. 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.





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Tuning

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In this chapter, you will run a mass calibration and a checktune, and generate a tune report.

Mass Calibration

Mass Calibration performs a TOF mass calibration on the active instrument mode by infusing a solution with known masses into the source and measuring the actual flight times for ions of the masses.

For optimum performance, run a mass calibration before analysis each day, or after changing high-voltage manual tune parameters or adjusting the mass range. During normal operation, mass calibration takes approximately 5 minutes. To ensure the mass accuracy of your data, a mass calibration is required when selecting a different active instrument mode.

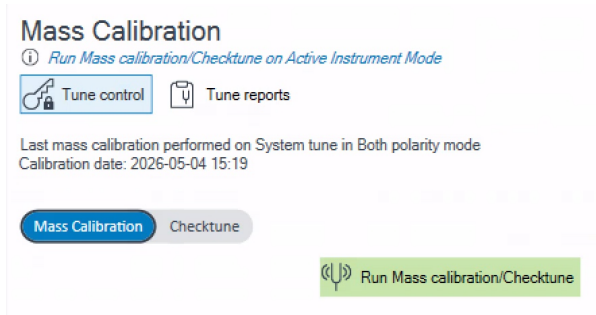
NOTE

Only the active instrument mode can be calibrated.

A mass calibration can only be run on instrument modes that have first been configured and enabled in the Configure Tune panel. System Tune is always enabled by default.

Follow these steps to perform a mass calibration:

1. In the Method Editor window, click the **MS TOF/Q-TOF** tab.
2. Click **Tune > Mass Calibration** in the left pane.
3. Click  **Tune control**.
4. Select **Mass Calibration**.



Tuning

Mass Calibration

5. Click  **Mass calibration/Checktune**. When the tune completes, review the report.
6. Click  **Tune control** to release control of the LC/TOF instrument.

Checktune

Checktune is used to determine whether the instrument is correctly tuned by applying the current tune parameters to the source and detector to confirm that the signal intensity and resolution is within the expected range. A checktune performs a TOF mass calibration and checks the detector for the currently selected active instrument mode. A checktune is automatically performed when running an autotune.

During normal operation, a checktune will take approximately 20 minutes.

A checktune can be run with the following ion sources:

- ESI
- Dual AJS ESI

NOTE

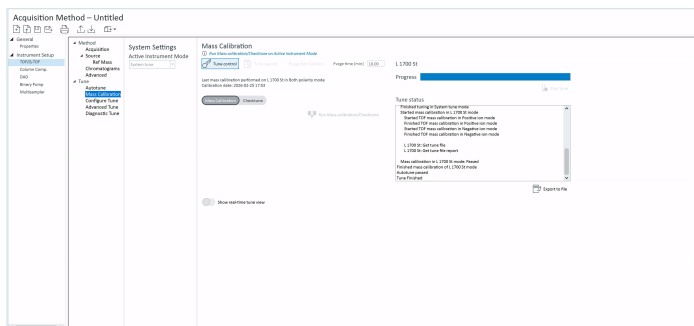
A checktune is only run on the currently selected instrument mode in the Active Instrument Mode drop-down.

A Checktune can only be run on instrument modes that have first been configured and enabled in the Configure Tune panel. System Tune is always enabled by default.

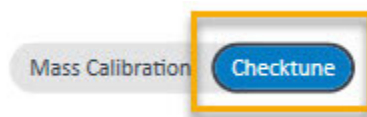
Follow these steps to perform a checktune:

1. In the Method Editor window, click the **MS TOF/Q-TOF** tab.
2. Click **Tune > Mass Calibration** in the left pane.

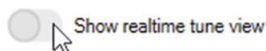
3. Click  **Tune control** **Tune control**.



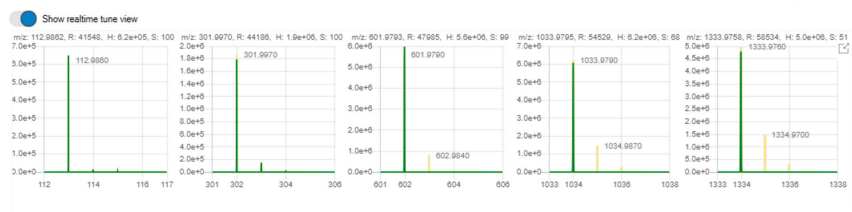
4. Select **Checktune**.



5. Activate **Show realtime tune view** to view the real time data acquisition.



6. Click  **Run Mass calibration/Checktune**. View the progress of the tune in the Real Time tune results.

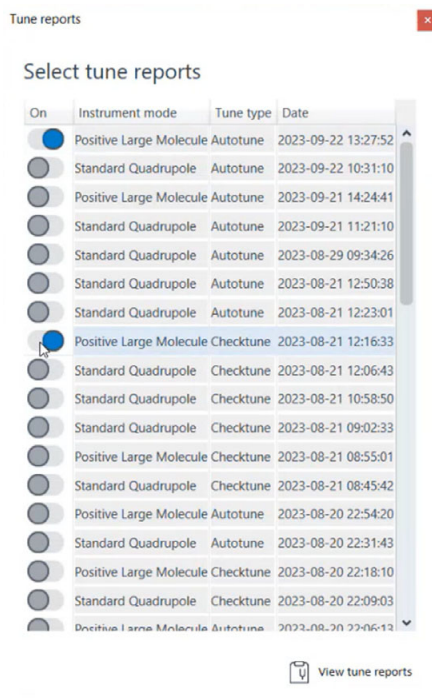


7. When the tune completes, review the report.
8. Click  **Tune control** **Tune control** to release control of the LC/TOF instrument.

Generating a Detailed Tune Report

Generate a detailed tune report after running an Autotune, Checktune, or Mass Calibration.

1. In the Method Editor window, click the **MS TOF/Q-TOF** tab.
2. Click **Tune > Autotune** in the left pane.
3. Click  **Tune control**.
4. Click  **Tune Reports**. If this button is not available, run an autotune, checktune, or mass calibration first.
5. Use the toggles to select reports for viewing.



Tuning

Generating a Detailed Tune Report

6. Click  **Tune Reports** to generate PDFs for viewing in a PDF viewer.
7. Click  **Tune control** to release control of the TOF instrument.



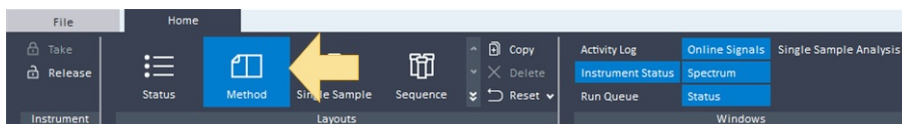
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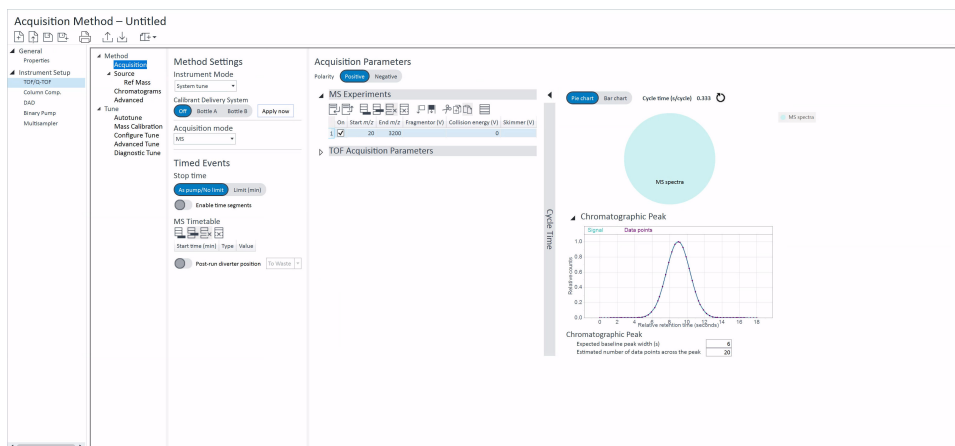
OpenLab Acquisition methods include the parameters for each component associated with your instrument.

The Method Editor Window

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

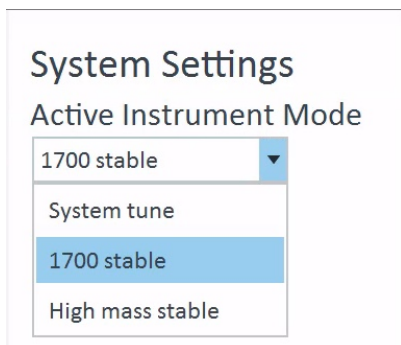


The Method Editor opens in the Main Window.

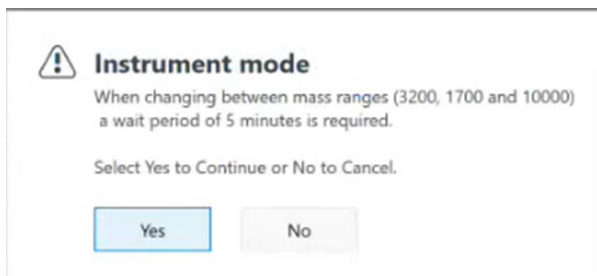


Set the Active Instrument Mode

1. Click **Tune > Autotune** in the left pane.
2. Click  **Tune control**.
3. From the **Active Instrument Mode** drop-down list, select **1700 Stable**.



4. Select **Yes**. Wait for the system to stabilize.



5. Click  **Release tune control** in the toolbar to release control of the TOF instrument.

Set Up and Run an Acquisition Method

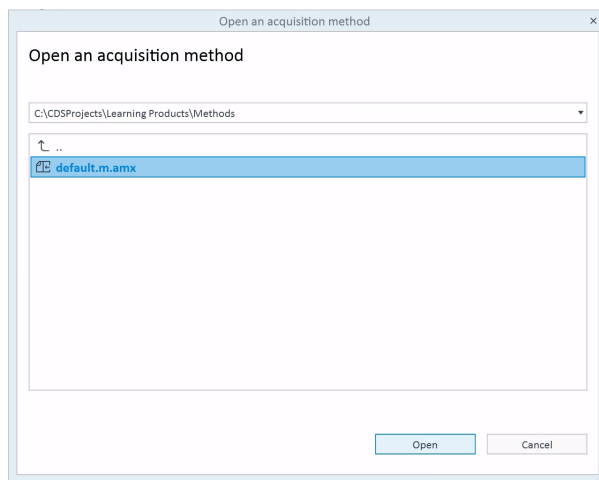


Working with the Default Method

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  **Open Method** to review available methods.
2. Select **default.m** and click **Open**.

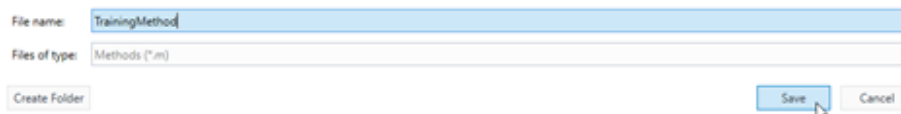


3. Under Method, review all the default Method subsections.
 - a. Acquisition – Set TOF acquisition parameters.
 - b. Source – Set source parameters for the TOF.
 - c. Chromatograms – Specify plots to display in the Chromatogram Plot window during the run.

Using Methods

Set Up and Run an Acquisition Method

- d. Advanced – Set advanced parameters such as Data Storage Thresholds, Data Storage Mode, and Experiment Defaults.
4. Click  **Save As Method**.
5. Enter a **File name**—for this example, *Training Method*—then click **Save**.



File name: TrainingMethod

Files of type: Methods (*.m)

Create Folder Save Cancel

NOTE

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

6. Click  **Apply**.

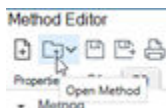
Load an Acquisition Method



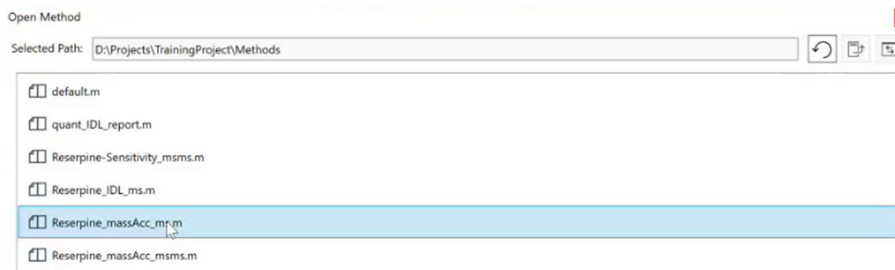
Load an existing method and Save As new method

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

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



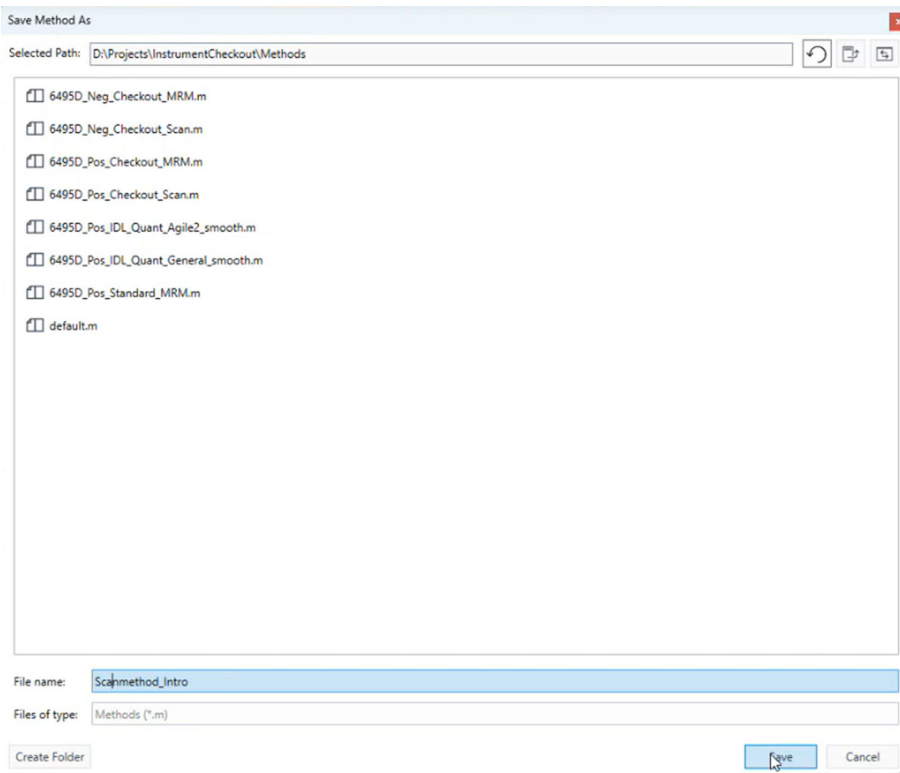
2. Select the checkout method used in installation (for example, Reserpine_massAcc_ms.m) and click **Open**. The method loads into the Method Editor.



Using Methods

Load an Acquisition Method

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



Using Methods

Load an Acquisition Method

4. Select **Method > Acquisition**, then under Acquisition Parameters, review the acquisition settings, noting Start m/z and End m/z. Under TOF Acquisition Parameters, set the Rate to 3.

Acquisition Parameters

Polarity **Positive** Negative

MS Experiments

On	Start m/z	End m/z	Fragmentor (V)	Collision energy (V)	Skimmer (V)
1 ☒	100	1700	180	0	45

TOF Acquisition Parameters

MS-only Acquisition

Rate (spectra/s)

Time (ms/spectrum)

Transients/spectrum 5692

5. Under MS Timetable, click **Add a row** at the end twice.

MS Timetable

6. Set the Start time (min) and Value as shown below.

Start time (min)	Type	Value
1	0 LC Stream Valve	To Waste
2	1 LC Stream Valve	To MS

7. Click to enable **Post-run diverter position**.

☒ Post-run diverter position

Using Methods

Load an Acquisition Method

- Click **Pump/Sampler/Column Comp settings** to review the settings programmed for the LC pump, noting the flow rate.

Properties DA Multisampler Multisampler Pretreatment **Binary Pump** Column Comp MS Q-TOF

Flow
0.400 mL/min

Solvents

Channel	Percentage	Solvent	Flow
1	50.00 %	100.0 % Water V.03	0.1 % F.I.
2		100.0 % Water V.03	
1	50.00 %	100.0 % Acetonitrile V.03	
2		100.0 % Acetonitrile V.03	

Pressure Limits
Min: 0.00 bar Max: 600.00 bar

Stoptime
☐ As Injector/No Limit
☒ 4.35 min

Posttime
☒ Off
☐ 1.00 min

Advanced
 ▲ Timetable (8/100 events)

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	50.00	50.00	0.400	600.00
0.20	50.00	50.00	0.400	---
0.85	10.00	90.00	0.400	---
2.85	10.00	90.00	0.400	---
2.86	50.00	50.00	0.400	---

ISFT

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

Run Methods



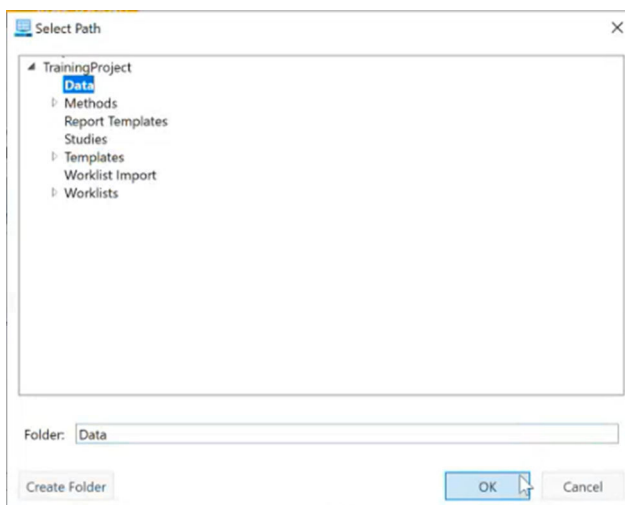
Run an acquisition method

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

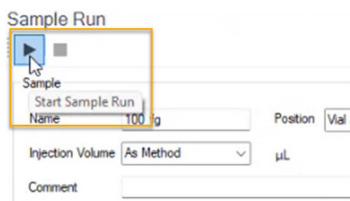
1. Place the Vial 4 100 pg/μl checkout sample, prepared during the system checkout, into a vial location of the sampler and note the location.
2. In the main window, click the **Sample Run** tab to display the Sample Run window.
3. In the Sample Run window, specify the following information:
 - a. Sample Name: Sample 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_001.sirslr
(optional) Select **Auto Increment** to automatically increment the file name if that file exists.

NOTE

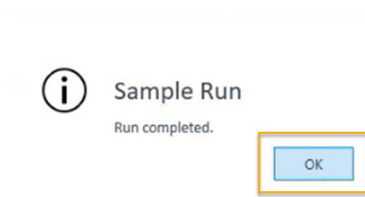
- d. Data File Path: Set to **D:\Projects\InstrumentCheckout\Data\Introduction**. Create a folder if necessary.



4. Click **Start Sample Run**.



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



**Monitor the status windows**

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

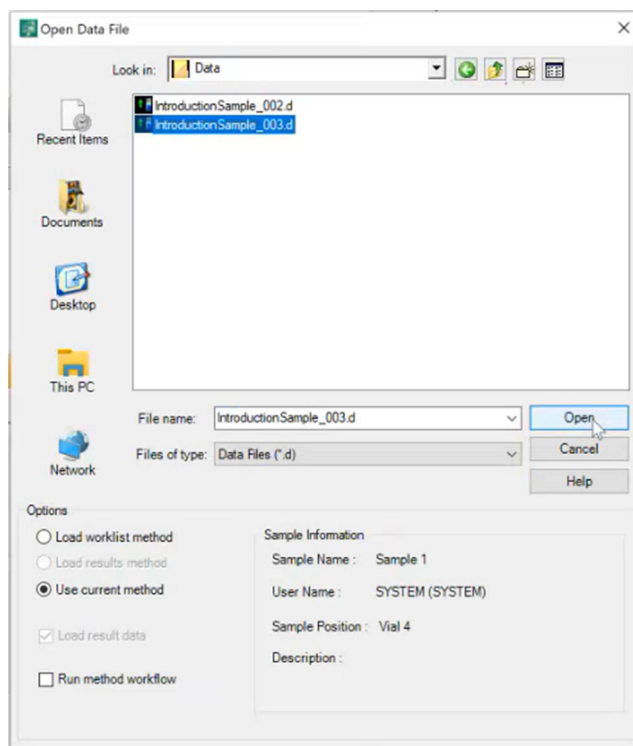
1. View the Chromatogram Plot and note the retention time for Reserpine.
2. Observe the Spectrum window while the samples runs. Discuss with your Agilent-certified service professional the changes observed over time.

Review the Data using MassHunter Qualitative Analysis

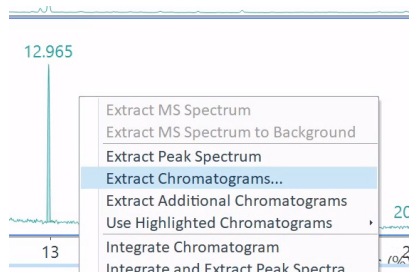
1. From the desktop, double-click the MassHunter Qualitative Analysis icon.



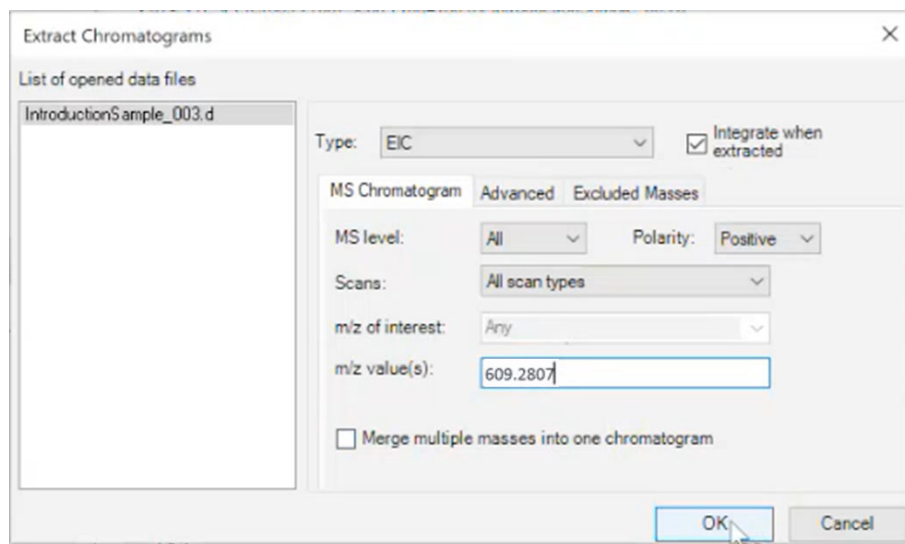
2. In the Open Data File window, browse to the data file directory created earlier (Introduction), select the data file to review, and click **Open**.



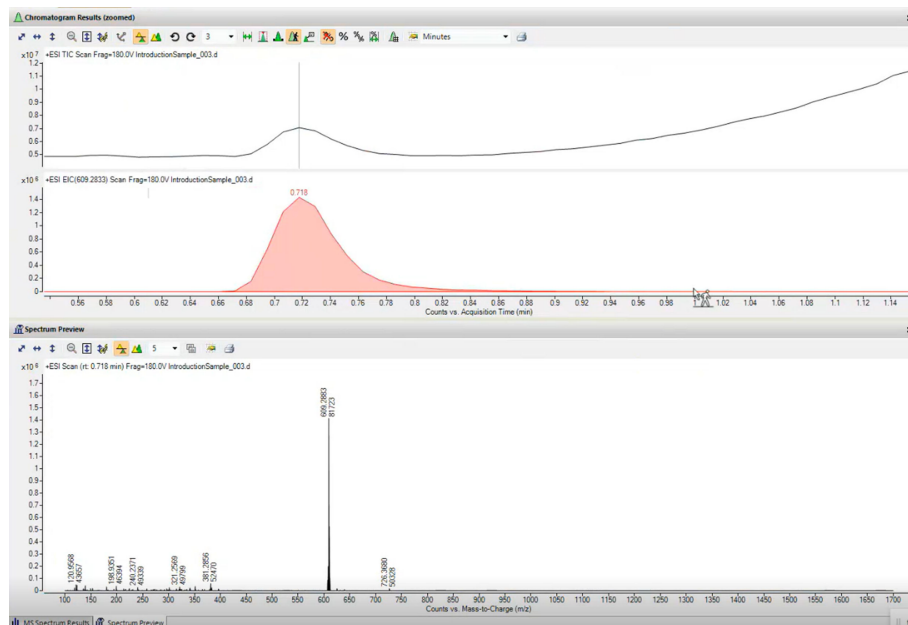
3. In the main window, in the Navigator View, right-click a chromatogram and select **Extract Chromatograms** from the shortcut menu.



4. In the Extract Chromatograms dialog box, click **Type:** and select **EIC**.
5. Enter the m/z value: 609.2807, then click **OK**.



6. Review the results.

**Review the Results**

1. What is the retention time for Reserpine?

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

6 Setting Up and Running a Sequence

A sequence can only be run after an acquisition method has been developed.

1. Click the **Sequence** layout.
2. In the Sequence window, enter the **Properties** information.
 - a. In the navigation pane at the left of the window, click **General > Properties**.
 - b. Select or clear **Audit Trail Activated**. Audit Trail cannot be turned off once it has been enabled.
 - c. Enter the **Description** for the sequence.

The screenshot shows the 'Sequence Properties' dialog box with the 'General > Properties' tab selected. The left navigation pane shows 'General' expanded, with 'Properties' selected. The main area is divided into 'Location' and 'Description' sections. The 'Description' section is a large text area. At the top right, there is a 'Version' field, a 'Show Audit Trail' button, and an 'Audit Trail Activated' checkbox. At the bottom, there are fields for 'Created at', 'Modified at', 'Created by', and 'Modified by'. The 'Created by' field is populated with 'SYSTEM (SYSTEM)'.

Location	Version	Show Audit Trail	Audit Trail Activated
			☐

Description

Created at

Modified at

Created by SYSTEM (SYSTEM)

Modified by

3. Enter the **Run Options** information:
 - a. In the navigation pane at the left of the window, click **General > Run Options**.
 - b. Select or clear **Use Barcode Reader Before Injection**.

- c. Click either **Inject anyway** or **Abort current injection**.

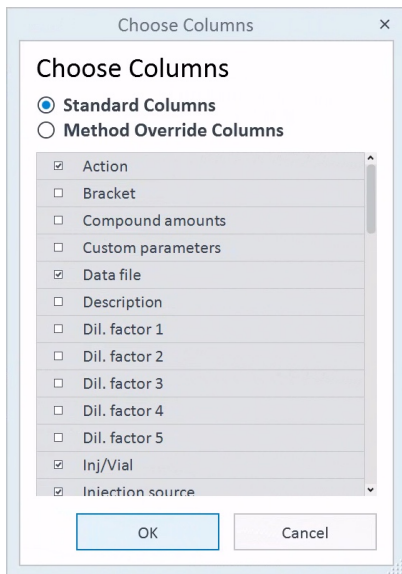
The screenshot shows the 'Run Options' dialog box. On the left is a navigation pane with three items: 'General Properties', 'Run Options' (which is highlighted in blue), and 'Injections Table'. The main area of the dialog is titled 'Injection report printer:' with a dropdown menu showing '[Printer specified in processing method]'. Below this is a section titled 'Barcode Reader' which contains a checkbox 'Use Barcode Reader Before Injection' and a sub-section 'On Barcode Mismatch' with two radio buttons: 'Inject anyway' (selected) and 'Abort current injection'. At the bottom is a section titled 'Data processing error handling for High Resolution Mass Spectra Data' which contains a checkbox 'Use Data processing error handling' and a sub-section 'On Data processing error' with two radio buttons: 'Ignore error and continue' (selected) and 'On error, pause after current injection'.

4. *Optional.* Add a column to the Sequence table.
- a. In the navigation pane at the left of the window, click **Injections > Table**.

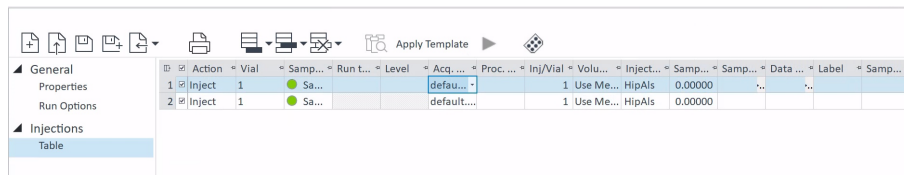
Setting Up and Running a Sequence

Setting Up and Running a Sequence

- b. In the upper left corner of the Sequence table, click . The Choose Columns dialog opens.



- c. Select the desired column (or columns).
- d. Click **Method Override Columns**.
- e. Select the desired override column (or columns).
- f. Click **OK**.
5. Enter the **Table** information:
- a. In the navigation pane at the left of the window, click **Injections > Table**.
- b. Enter the information for each injection.



Setting Up and Running a Sequence

Setting Up and Running a Sequence

6. Select the rows to submit as part of the run. By default, all rows are selected.
7. To save your results to a sub-folder inside your Results folder, change the **Result Path** by clicking browse and creating a new folder. Results cannot be saved to an existing .srlt folder.

Result path	E:\CDSPProjects\Learning Products\Results	>	...	Save result as	Separate single injections ▼
Result name		>			

8. The **Result name** defaults to the last filename used for this instrument and project. Enter a filename by typing in the field or using tokens. If left blank, the instrument name and local date & time is used for the filename.
9. Select to **Save result as**:
 - **Separate single injections** to save each sample injection in the sequence as an individual result set. When using this option, result set names will be based on the value of the **data file** column for each sequence row. For example:
 - DataFileName: A1
 - DataFileName: B1
 - DataFileName: A2
 - DataFileName: B2
10. *Optional.* Click Validate to validate the sequence against the specified acquisition method. The columns validated are **Acq. method**, **Volume**, and any override parameters. If invalid entries are found, correct those entries.
11. Click Run. If this is a new "Untitled" sequence, the sequence file is saved as the instrument name + the local date and time.
Submitted sequences are placed in the **Run Queue**, where runs can be stopped or paused, and sequences can be edited. A result folder (.srlt) is created when the run is added to the Run Queue.



7 Maintenance

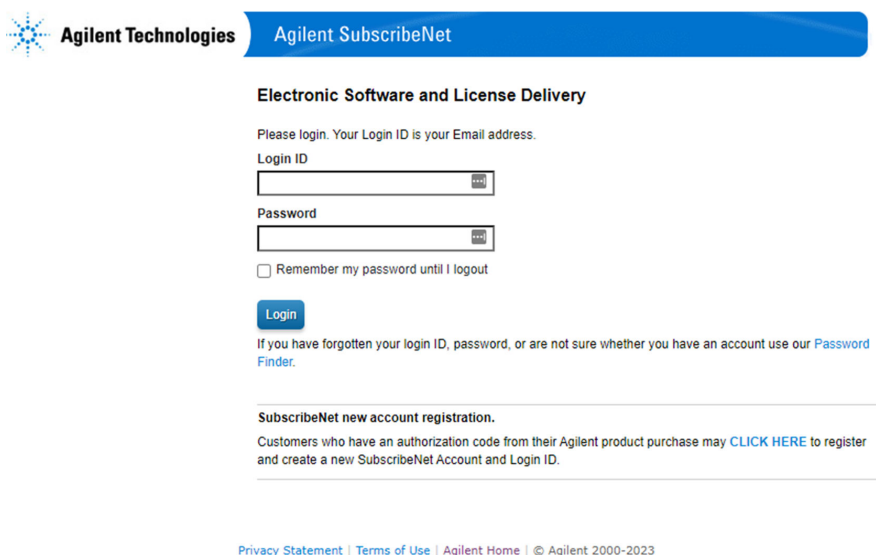
Register on Agilent SubscribeNet (New Account Registration)	60
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Register on Agilent SubscribeNet (New Account Registration)

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.subscribe.net.com/>.



The screenshot shows the Agilent SubscribeNet website. At the top, there is a blue header with the Agilent logo and the text "Agilent Technologies" and "Agilent SubscribeNet". Below the header, the main content area is titled "Electronic Software and License Delivery". Under this title, there is a message: "Please login. Your Login ID is your Email address." Below this message are two input fields: "Login ID" and "Password". Below the "Password" field is a checkbox labeled "Remember my password until I logout". Below the checkbox is a blue "Login" button. Below the "Login" button is a link: "If you have forgotten your login ID, password, or are not sure whether you have an account use our [Password Finder](#)." Below this link is a section titled "SubscribeNet new account registration." followed by the text: "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." At the bottom of the page, there is a footer with links: "Privacy Statement | Terms of Use | Agilent Home" and copyright information: "© Agilent 2000-2023".

2. Click **CLICK HERE** 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

Maintenance**Register on Agilent SubscribeNet (New Account Registration)**

- e. First Name
 - f. Last Name
 - g. Phone
 - h. Address
 - i. City
 - j. State/Province
 - k. Country
4. Click **Submit**.
You'll receive an email to activate your account.

Perform Back Up and Platform Best Practices

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



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.

Maintenance

Perform Back Up and Platform Best Practices



Review routine procedures in the user guide

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



Remove, clean, and replace the capillary

Review the steps in the user guide.



Add a new user defined EMF counter

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

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G1960-90155

May 2026

