Analyst® 1.7 Software

Getting Started Guide

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Foreword

This guide is for operators who are new to the Analyst® software. You can use the procedures to learn how to use the software and the mass spectrometer. Use this guide for the API 4000™ system, the 4000 QTRAP® system, and the API 5000™ system. For any other mass spectrometer, use the *System User Guide* for that mass spectrometer.

> ⚠️ **WARNING! Personal Injury Hazard. Risk of personal injury or instrument damage. If you need to move the system, contact a SCIEX FSE to assist you.**

Related Documentation

To find software product documentation, refer to the release notes or software installation guide that comes with the software. Documentation for the hardware products can be found on the *Customer Reference* DVD that comes with the system or component.

For the latest versions of the documentation, visit the SCIEX website at sciex.com.

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Technical Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the website at sciex.com.

Create Hardware Profiles and Projects 1

In this section, you will learn how to create a hardware profile. You can use this hardware profile to create methods and batches in the following sections. You will also learn about the types of files in the Analyst® software and how to create projects and subprojects.

Hardware Profiles

A hardware profile tells the software how the mass spectrometer and the devices are configured and connected to the computer. Multiple hardware profiles can be set up, but only one profile can be active at any time.

When a hardware profile is created in the Hardware Configuration Editor, the peripheral devices must be configured so that the software can communicate with them. Configuring the peripheral devices requires two procedures: setting up the physical connections and configuring the software to communicate with the peripheral devices. When the software is installed, the driver required for each peripheral device is also installed. After the peripheral devices are physically connected to the computer, set up the appropriate configuration information.

Each hardware profile must include a mass spectrometer. Before creating an acquisition method, make sure that all devices to be used in the method are included in the hardware profile. The devices configured in the active hardware profile and selected in the Add/Remove Device Method dialog are shown as icons in the Acquisition method pane. Only peripheral devices included in the active hardware profile can be used when creating acquisition methods.

For information about setting up the physical connections to the devices, refer to the *Peripheral Devices Setup Guide*. For a list of the supported devices, refer to the *Software Installation Guide* for the software.

Create a Hardware Profile

The user can create multiple hardware profiles, but only one profile can be active at any time.

1. On the Navigation bar, under **Configure**, double-click **Hardware Configuration**.

Figure 1-1 Hardware Configuration Editor Dialog
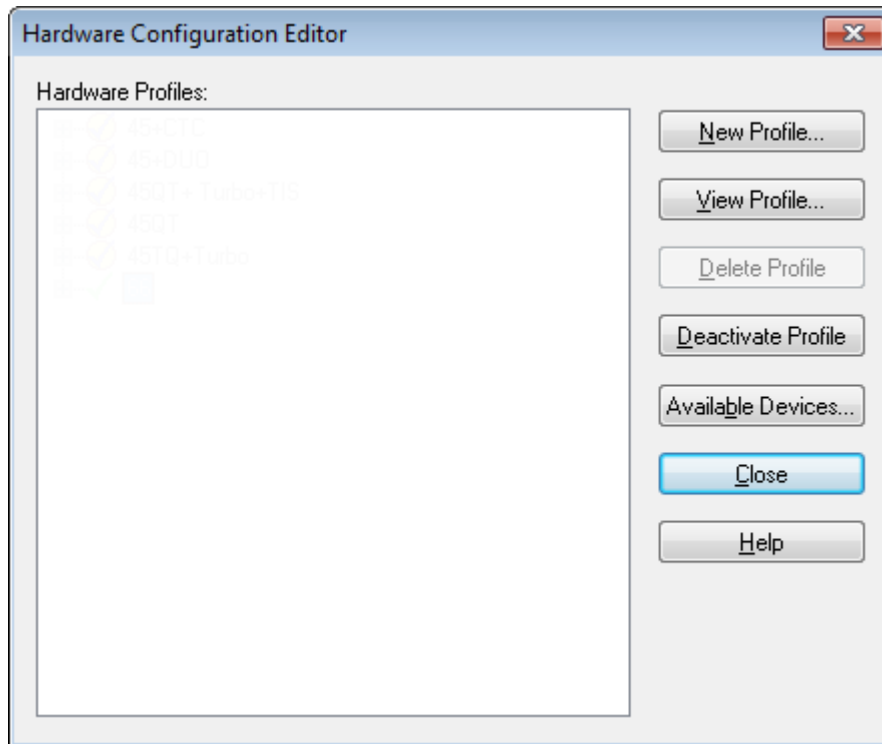
2. In the Hardware Configuration Editor dialog, click **New Profile**.

Figure 1-2 Create New Hardware Profile Dialog
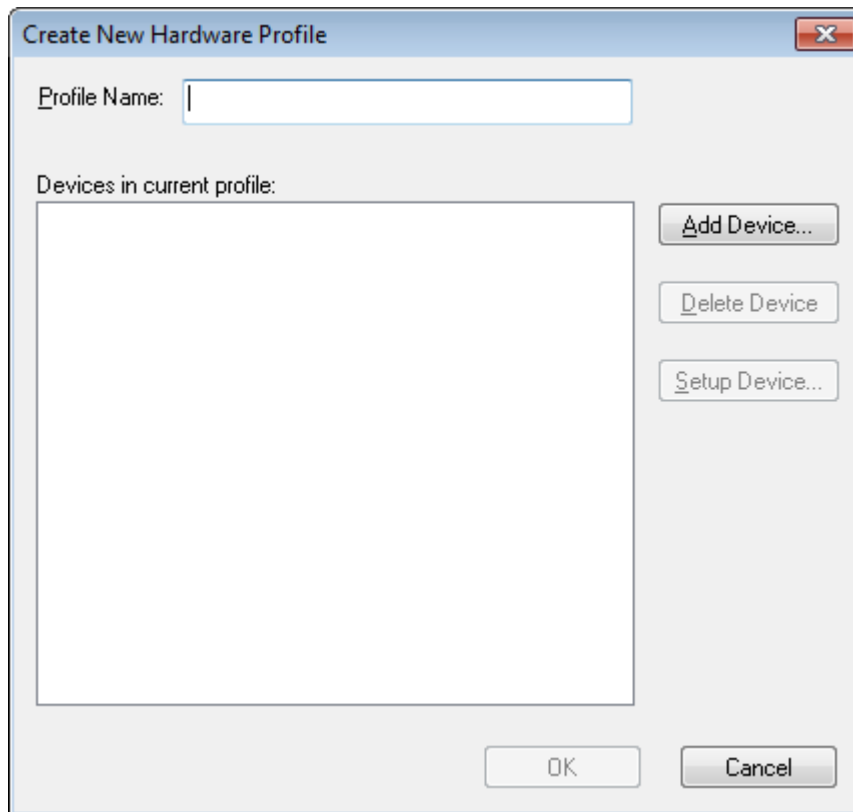
3. Type a name in the **Profile Name** field.

4. Click **Add Device**.

Figure 1-3 Available Devices Dialog
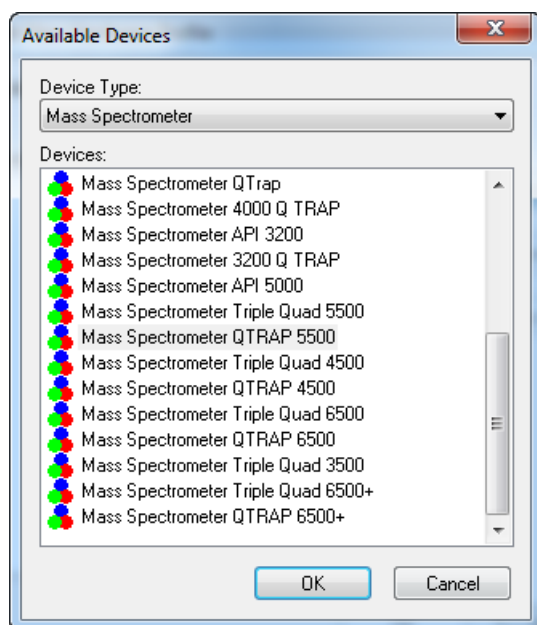
In the Available Devices dialog, in the **Device Type** field, **Mass Spectrometer** is the preset value.

5. In the **Devices** list, select the appropriate mass spectrometer and then click **OK** to return to the Create New Hardware Profile dialog.

6. Click **Setup Device**.

7. (Optional) To configure mass spectrometers that use the integrated syringe pump, on the **Configuration** tab, select the **Use integrated syringe pump** check box.

8. (Optional) Select additional features on the **Configuration** and **Communication** tabs as required.

9. Click **OK** to return to the Create New Hardware Profile dialog.

10. Click **Add Device** and then add and set up each device that is used with the mass spectrometer. Refer to Add Devices to a Hardware Profile on page 12.

11. Click **OK** in the **Create New Hardware Profile** dialog.

12. Click the hardware profile to be activated in the **Hardware Configuration Editor**.

13. Click **Activate Profile**.

The check mark turns green. If a red x is shown, then there is an issue with the hardware profile activation.

Tip! A hardware profile need not be deactivated before another is activated. Click a hardware profile and then click **Activate Profile**. The other profile is deactivated automatically.

14. Click **Close**.

Add Devices to a Hardware Profile

Devices must be configured to enable the software to communicate with them. When the software is installed, the driver required for each device is also installed. After the devices are physically connected to the computer, configure them.

Only the devices configured in the active hardware profile and selected in the Add/Remove Device Method dialog are shown as icons in the Acquisition Method Browser pane.

1. Open the Hardware Configuration Editor.

2. In the **Hardware Profiles** list, deactivate the hardware profile.

3. Click **Edit Profile**.

4. Click **Add Device**.

Note: Remember to add a mass spectrometer. For more information, refer to Create a Hardware Profile on page 8.

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5. In the Available Devices dialog, in the **Device Type** list, select the device and then click **OK**.

Figure 1-4 Available Devices Dialog
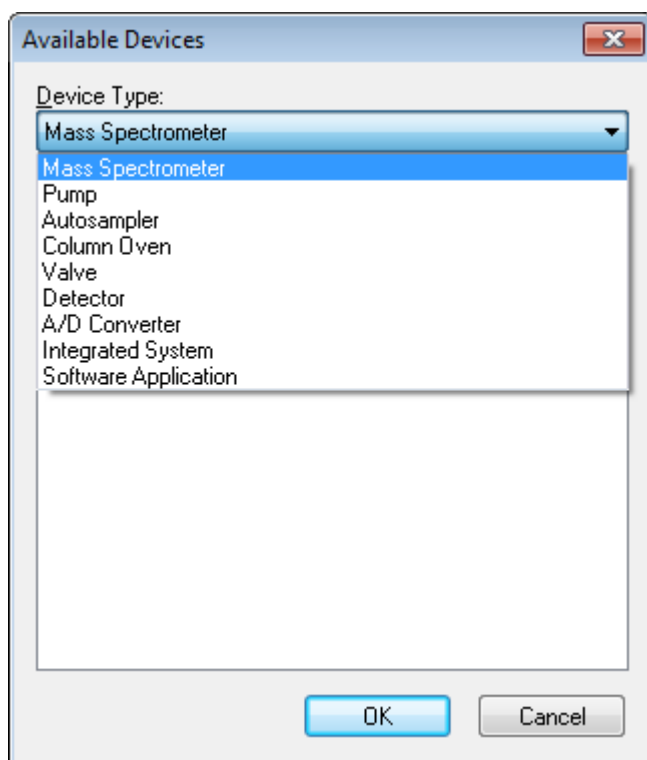
6. Click **OK**.

7. Select the device from the **Devices** list and then click **OK**.

8. Click **Setup Device**.

   A dialog containing configuration values for the device opens.

9. (Optional) On the Communication tab, in the **Alias** field, type a name or other identifier for the device.

Note: For devices using serial communication, make sure that the serial port selected matches the serial port to which the device is physically connected. When the serial expansion cable is used, the number selected in the profile is the number on the cable plus two.

Note: The **Alias** field might also be referred to as the **Name** box and might be found on another tab under **Alias**.

• If the device uses a **Serial Port** as a communication interface, then in the **COM Port Number** list, select the COM port to which the device is connected.

- If the device uses **Ethernet** as a communication interface, then type the **IP Address** assigned to the device by the administrator or use the corresponding **Host Name** for the address.

- If the device uses **GPIB Board** as a communication interface, then do not change the settings for the GPIB board.

The rest of the preset values for the device are probably appropriate. Do not change them. For information about the Configuration and Communication tabs, refer to the Help.

10. To restore the device preset values, on the Communication tab, click **Set Defaults**.

11. To save the configuration, click **OK**.

12. Repeat step 4 to step 11 for each device.

13. Click **OK** in the Create New Hardware Profile dialog.

14. To activate the hardware profile, in the Hardware Configuration Editor, click the hardware profile.

15. Click **Activate Profile**.

The check mark turns green. If a red x is shown, then there is an issue with the hardware profile activation. For more information, refer to Troubleshoot Hardware Profile Activation on page 14.

> **Tip!** An active hardware profile does not have to be deactivated before another one is activated. Click an inactive hardware profile and then click **Activate Profile**. The other profile is deactivated automatically.

16. Click **Close**.

Troubleshoot Hardware Profile Activation

If a hardware profile fails to become active, then a dialog opens indicating which device in the profile failed. A device might fail to activate because of communications errors.

1. Read the error message generated. Depending on the message, there might be an issue with a device or how the communication is set up.

2. Verify that the device has power and is turned on.

3. > **Tip!** On computers with two built-in serial ports, the first port on the serial port expansion card is usually COM3, even though the cable indicates P1.

4. Verify that the communication settings for the device (for example, dip switch settings) are set correctly and match the settings on the Communication tab.

5. Turn off the device.

6. Wait 10 seconds.

7. Turn on the device.

   Wait until all device power-up activities are complete before trying to activate the hardware profile again. Some devices might require 30 seconds or more to complete the power-up activities.

8. Activate the hardware profile.

9. If the issue persists, then delete the failing profile and create a new one.

10. If the issue still persists, then contact technical support.

Projects and Subprojects

Before beginning an experiment, decide where to store the files related to the experiment. Use projects and subprojects for each experiment to better manage data and compare results. For example, use subprojects to store the results for specific dates.

Create Projects and Subprojects

To use a subproject structure within a project, create the subproject structure when the project is created.

1. Click **Tools > Project > Create Project.**

Figure 1-5 Create New Project/Subproject Dialog
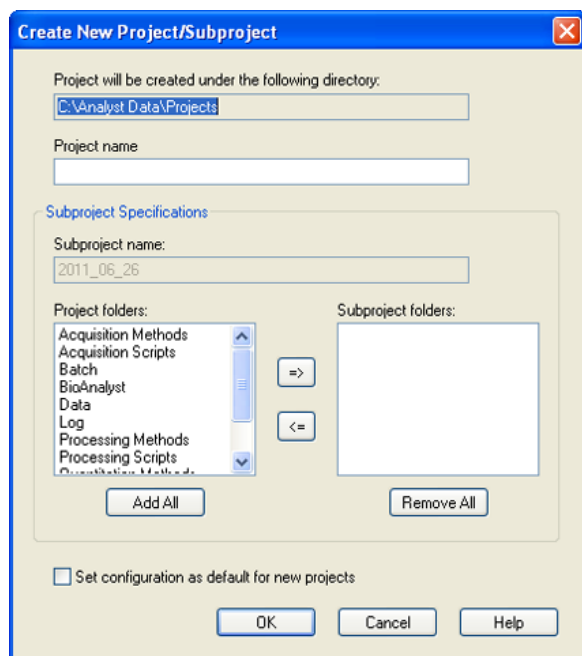
> **Note:** A new subproject cannot be created for a project that was not originally created with a subproject.

2. Type a project name in the **Project name** field.

3. (Optional) To use subprojects, select the required folders and then use the arrow buttons to move them to the **Subproject folders** list.

4. (If subprojects are used) In the **Subproject name** field, type a name for the first subproject or use the existing date.

5. (Optional) To use this project and subproject folder organization for all new projects, select the **Set configuration as default for new projects** check box.

   All new projects are created with this folder configuration.

6. Click **OK**.

Create Subprojects

Subprojects can only be created in a project that has an existing subproject structure.

1. On the **Project** tool bar, from the **Project** list, select the project.

2. Click **Tools > Project > Create Subproject.**

3. In the **Subproject name** box, type a name for the subproject or use the existing date.

4. Click **OK**.

Copy Subprojects

A subproject can be copied from another project that has existing subprojects. If the copied subprojects contain folders that also exist in the project folder, then the software uses the project level folders.

1. Click **Tools > Project > Copy Subproject.**

   The Copy Subproject dialog is shown.

2. Click **Browse** to navigate to the subproject source.

3. Click **OK**.

4. Select the subproject from the **Source Subproject** list.

5. Click **Browse** to navigate to the subproject destination.

6. Type the name in the **Target Subproject** field.

7. Click **OK**.

8. Do one of the following:

- To copy all folders and files from the **Subproject Source** into the **Subproject Destination**, select the **Copy Contents** check box.

- To copy only the folders in the same structure into the **Subproject Destination**, make sure that the **Copy Contents** check box is cleared.

9. Click **Copy**.

Switch Between Projects and Subprojects

- On the software tool bar, from the project list, click the required project or subproject.

Figure 1-6 Project List
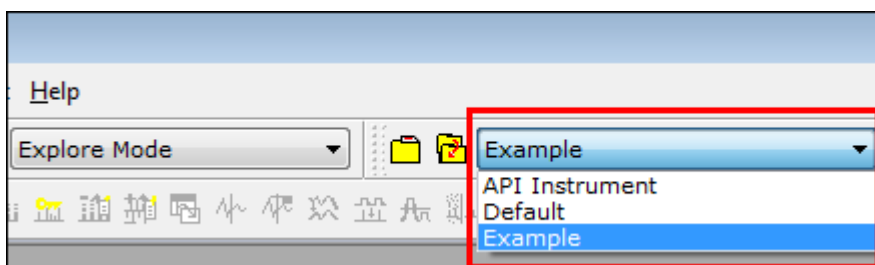
The project list in this figure shows the **API Instrument**, **Default**, and **Example** folders.

Installed Project Folders

Three project folders are installed with the software: **API Instrument**, **Default**, and **Example**.

API Instrument Folder

The API Instrument folder is unique and very important to the correct functioning of the mass spectrometer. The API Instrument folder contains the information required for tuning and calibrating the mass spectrometer. This information includes parameter settings files, reference files, instrument data files that contain calibration and resolution information, and the acquisition methods used during automatic tuning. The API Instrument folder also contains data files for manual tuning runs that were performed using the Start button rather than the Acquire button. These data files are saved automatically in the API Instrument folder in the Tuning Cache folder and named with the date and time they were created. The Tuning Cache folder is automatically cleared periodically.

Default Folder

The Default folder contains folders that are present in new projects and serves as a template for new projects.

Example Folder

The Example folder contains sample methods and data files. Users can practice working with the Explore or Quantitate modes using the example data files. The example files are sorted into subfolders by mass spectrometer type and application area.

Back Up the API Instrument Folder

Back up the API Instrument folder regularly and after routine maintenance has been performed.

- Copy the API Instrument folder, paste it to a different location, preferably to another computer, and then rename the folder. Use the date and a mass spectrometer reference if there is more than one mass spectrometer when the folder is named. For example, API Instrument_4000QTRAP3_010107

Recover the API Instrument Folder

Back up the **API Instrument** folder regularly and after routine maintenance has been performed. To recover the API Instrument folder, do the following:

1. Rename the current **API Instrument** folder.
2. Copy the backup folder into the **Projects** folder.
3. Change the name of the backup folder to **API Instrument**.

Tune and Calibrate 2

Run the Instrument Optimization software to tune, calibrate, and optimize the mass spectrometer to get the best performance.

Run the Verify instrument performance option weekly or after the mass spectrometer is cleaned to confirm that the system is working properly. In general, for triple quadrupole systems, calibration and resolution is maintained for three to six months unless the system loses vacuum. For LIT (linear ion trap) systems, the resolution should also be maintained for three to six months but the system should be calibrated approximately monthly. If the system loses vacuum, then check the calibration and resolution before using the system. For more information about tuning and calibration, refer to the *Advanced User Guide* and the *Manual Tuning Tutorial*.

Note: Perform maintenance tasks regularly to make sure that the mass spectrometer is performing optimally. Refer to the *Qualified Maintenance Person Guide*.

Required Material

Required Materials
• Tuning solutions are supplied in the Standards Chemical Kit shipped with the system. If required, a new kit can be ordered from SCIEX. For information about the appropriate solutions that should be used in a system, refer to Calibration Ions and Solutions on page 90
• 5 mL, 1 mL, and 250 μL serial gas-tight syringes (1.0 ml will be used as reference)
• Red PEEK sample tubing.
• (Optional) Syringe pump, if using an instrument without an integrated syringe pump.

Prerequisites

Prerequisites
• The spray is stable and the correct tuning solution is being used.
• A printer is configured.

Verify Instrument Performance

The following procedure describes how to verify or adjust the performance of the mass spectrometer. For information about using the other instrument performance options, refer to the Help.

1. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning**.

2. Start the syringe pump and then run a calibration method. Confirm that the Total Ion Chromatogram (TIC) is stable and that the peaks of interest are present in the spectrum.

3. On the Navigation bar, under **Tune and Calibrate**, double-click **Instrument Optimization**.

    The **Instrument Optimization** dialog opens.

4. Click **Verify instrument performance**.

5. Click **Next**.

6. Click **Approved Tuning**.

7. Click **Next**.

8. Select a **Tuning Solution** from the list.

    Depending on the solution selected, different modes are available:
    a. Click a polarity.

    b. (If available) Click **Q1** and **Q3** in the **Quad** section.

    c. (If available) Click the required scan speeds.

    d. (If available) Click the scan speeds in the **LIT** section.

    e. (If available) Click **Excitation** in the **MS/MS/MS** section

9. Click **Next**.

10. If the **Select a mode** page opens, then select **Automatic**.

11. Click **Next**.

12. Click **GO**.

    The Verifying or Adjusting Performance dialog opens. After the process has completed, the Results Summary opens. For more information, refer to the Help.

13. If applicable depending on the options selected. Change solutions for different scan types and polarities when prompted.

About the Verifying or Adjusting Performance Dialog

The top left corner shows the part of the instrument that is being tuned.

The Current Spectrum graph shows the spectrum of the current scan, the optimal scan selected by the software, or the scan at the current parameter value when the software results are viewed in interactive mode.

The Instrument Optimization Decision Plots, in the top right graph, dynamically show the intensity versus voltage curves of the parameters that are currently being optimized.

Results Summary

The Results Summary is a record of any instrument settings changes that were made by the Instrument Optimization wizard.

Figure 2-1 Results Summary
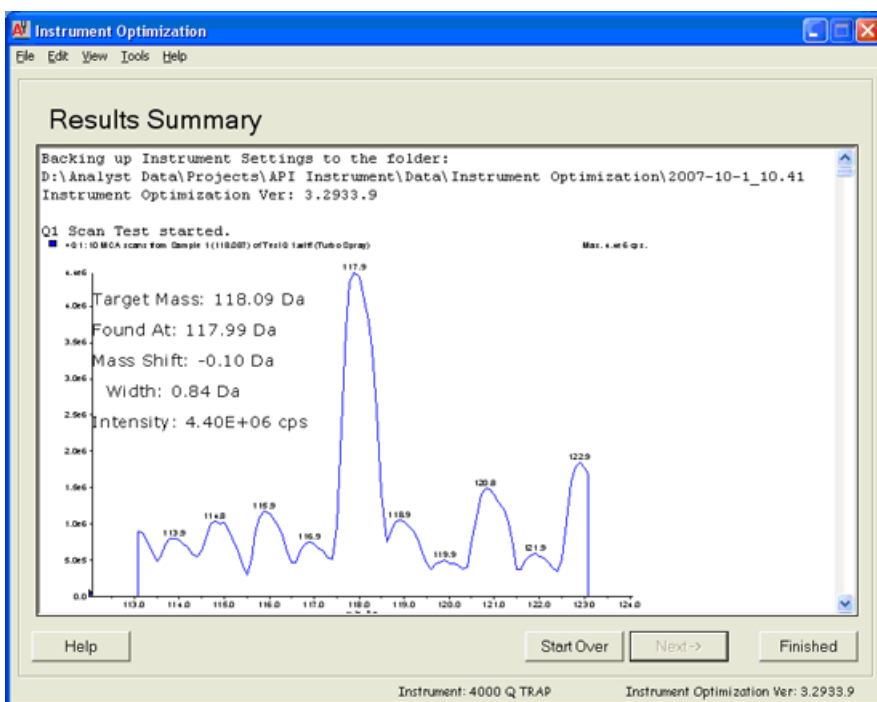
The Results Summary includes the location of data files and instrument settings backups, as well as step-by-step changes and results during optimization.

The Results Summary also shows a verification report. This report contains a snapshot of the mass spectrum for each relevant mass for the scan modes being verified. The spectrum is labeled with the target mass, where the

mass was found, mass shift, peak width, and peak intensity. The spectrum can be used as a visual record of peak shape or scan mode performance. A summary table of results follows the spectra.

The Results Summary is automatically saved in the following path: <drive>:\Analyst Data\Projects\API Instrument\Data\Instrument Optimization\yyyy-mm-dd\results.doc, where *yyyy-mm-dd* is the date on which the report was created. Users can print the Results Summary or open a previously saved Results Summary.

Create Basic Methods 3

Acquisition methods can be created for data acquisition.

About LC Methods

Creating an acquisition method using a peripheral device, such as an LC system, includes providing the operating parameters for that device. If a new acquisition method is created from an existing file, some or all of the peripheral device methods can be used in the acquisition method.

Create Mass Spectrometry Methods

Use the Acquisition Method Editor to create a mass spectrometer acquisition method. Depending on the type of mass spectrometer configured and the scan type selected, different fields and options are available. The Acquisition Method Editor validates the settings as the parameters are typed.
Create one of the following methods and then use it in Create and Submit Batches on page 34 to acquire data:

- Create an Acquisition Method using a Q1 MS Scan Type on page 25
- Create an Acquisition Method using a Q1 MI Scan Type on page 27
- Create an Acquisition Method using an MRM Scan Type on page 29

About Spectral Data Acquisition

Spectral data can be acquired in one of the modes described in Table 3-1.

Table 3-1 Spectral Data

Mode	Description
Profile	The preset value is 0.1 Da. Profile data is the data generated by the mass spectrometer and corresponds to the intensity recorded at a series of evenly spaced discrete mass values. For example, for a mass range from 100 Da to 200 Da and a step size of 0.1 Da, the mass spectrometer scans 99.95 to 100.05 (recorded as value 100), 100.05 to 101.15 (recorded as value 101)…199.95 to 200.05 (recorded as value 200).
Peak Hopping	The preset value is 1.0 Da. Peak Hopping is a mode of operating a mass spectrometer in which large steps (approximately 1 Da) are made. It has the advantage of speed (fewer data steps are made) but with the loss of peak shape information.
Centroid	The mass spectrometer scans as in Profile mode, but creates a centroid of the data, replacing found peaks with the intensity-weighted center of gravity for each peak. Centroid data has the advantage of significantly reducing file size. The disadvantage is that peak shape information is lost and if data has been collected as a centroid then it cannot be altered. We recommend the use of profile mode and centroiding of the data post-acquisition.

Required Material

Required Materials
• Reserpine solution (refer to Table 3-2 on page 24) supplied in the Standards Chemical Kit shipped with the system. If required, a new kit can be ordered from SCIEX.
• 5 mL, 1 mL, and 250 µL serial gas-tight syringes
• Red PEEK sample tubing
• (Optional) Syringe pump, if using an instrument without an integrated syringe pump.
• Required peripheral device, for example an LC.

Reserpine Concentrations

Table 3-2 Reserpine Concentrations

System	Reserpine Concentration
API 4000™ System and 4000 QTRAP® System	0.167 pmol/µl (0.167 µM or 6:1)
API 5000™ System	0.0167 pmol/µl (0.0167 µM or 60:1)

Create an Acquisition Method using a Q1 MS Scan Type

Use the following procedure to create a method using the Q1 MS scan. The ion intensity is returned for every requested mass in the scan range.

Prerequisites
• Make sure that a hardware profile containing the mass spectrometer and syringe pump is active.
• On the software toolbar, make sure that the appropriate project is selected.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.

   The **Method Editor** is shown with a method template based on the active hardware profile.

2. In the Acquisition method pane, click **Acquisition Method**.

3. On the **Acquisition Method Properties** tab, in the **Synchronization Mode** list, make sure that **No Sync** is selected. For more information about synchronization modes, refer to the Help.

4. In the Acquisition method pane, click the **Mass Spec** icon.

5. On the **MS** tab, in the **Scan type** list, select **Q1 MS (Q1)**.

6. In the **Polarity** group, click **Positive**.

7. Clear **Center/Width** and **Parameter Range** check boxes if selected.

8. Type the following values in the mass ranges table:

Figure 3-1 MS Tab Parameter Values

Field	Example Value
Start (Da)	200
Stop (Da)	700
Time (sec)	2.5
Duration (min)	3

9. Click the **Advanced MS** tab. Note that the scan mode is set to **Profile** and the step size is 0.1.

Figure 3-2 Advanced MS tab

In this example, the quadrupole (Q1) is scanning a 500 Da range taking 0.1 Da steps. Therefore, there are 5000 steps across the mass range. If this takes 2.5 seconds to scan, then the dwell time is 0.5 ms per step. This is typically the fastest that Q1 or Q3 should be scanned based on a standard calibration procedure. Proper consideration for mass calibration should be taken if Q1 or Q3 are to be scanned faster.

Note: The step size and the time of the scan control the dwell time per step for the scan. The dwell time is the length of time spent acquiring signal at each step in a scan.

10. On the **MS** tab, click **Edit Parameters**.

11. Click the **Source/Gas** tab in the Parameter table dialog.

12. Type the following values:

Table 3-3 Source/Gas Tab Parameters

Source/Gas parameters	Typical value
Curtain Gas (CUR)	20
IonSpray Voltage (IS)	5000
Temperature (TEM)	0
Ion Source Gas 1 (GS1)	15
Ion Source Gas 2 (GS2)	0

13. Click the **Compound** tab and then set the **Declustering Potential (DP)** to 90 and leave the **Entrance Potential (EP)** at 10.

    A value of 90 might not be optimal for the mass spectrometer but it is a good DP to start with.

14. Click **OK**.

15. In the Acquisition method pane, click the **Harvard Syringe Pump** icon.

16. On the Syringe Pump tab, edit the syringe pump method to include **Syringe Diameter**, **Flow Rate**, and **Unit**.

Figure 3-3 Harvard Syringe Pump Method Properties Tab

17. Save the acquisition method.

    Next steps: Use the acquisition method to acquire data for preliminary analysis. To create and submit batches, refer to Create and Submit a Batch on page 35.

Create an Acquisition Method using a Q1 MI Scan Type

Use the following procedure to create a method using the Q1 MI scan. The ion intensity is returned for only the specified masses.

Prerequisites
• Make sure that a hardware profile containing the mass spectrometer and syringe pump is active.   • On the software toolbar, make sure that the appropriate project is selected.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.

   The **Method Editor** is shown with a method template based on the active hardware profile.

2. In the Acquisition method pane, click **Acquisition Method**.

3. On the **Acquisition Method Properties** tab, in the **Synchronization Mode** list, make sure that **No Sync** is selected. For more information about synchronization modes, refer to the Help.

4. In the Acquisition method pane, click the **Mass Spec** icon.

5. On the **MS** tab, in the **Scan type** list, select **Q1 Multiple Ions (Q1 MI)**.

6. In the **Polarity** group, click **Positive**.

7. Type the following values in the mass ranges table:

Figure 3-4 MS Tab Parameter Values

Field	Value
Q1 Mass (Da)	609
Time (msec)	100

8. Click **Edit Parameters**.

9. Click the **Source/Gas** tab in the Parameter table dialog.

10. Type the following values:

Table 3-4 Source/Gas Tab Parameters

Source/Gas parameters	Typical value
Curtain Gas (CUR)	20
IonSpray Voltage (IS)	5000
Temperature (TEM)	0
Ion Source Gas 1 (GS1)	15
Ion Source Gas 2 (GS2)	0

11. Click the **Compound** tab and then set the **Declustering Potential (DP)** to 90 and leave the **Entrance Potential (EP)** at 10.
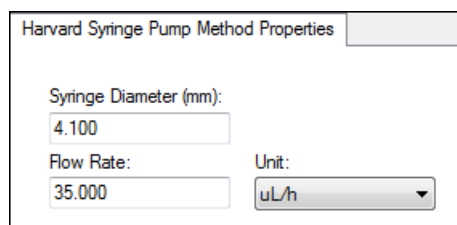
    A value of 90 might not be optimal for the mass spectrometer but it is a good DP to start with.

12. Click **OK**.

13. In the Acquisition method pane, click the **Harvard Syringe Pump** icon.

14. On the Syringe Pump tab, edit the syringe pump method to include **Syringe Diameter**, **Flow Rate**, and **Unit**.

Figure 3-5 Harvard Syringe Pump Method Properties Tab

15. Save the acquisition method.

    Next steps: You have created an acquisition method that you can now use to create and submit a batch. To create and submit batches, refer to .

Create an Acquisition Method using an MRM Scan Type

Use the following procedure to create a method using the MRM scan. This scan is used in quantitative applications. An MRM scan can be used to determine how much of a compound is in a sample. It is now used in pharmacokinetic analysis and increasingly in applied markets and screening applications.

Prerequisites
• Make sure that a hardware profile containing the mass spectrometer and syringe pump is active.   • On the software toolbar, make sure that the appropriate project is selected.

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Method**.

   The **Method Editor** is shown with a method template based on the active hardware profile.

2. In the Acquisition method pane, click **Acquisition Method**.

3. On the **Acquisition Method Properties** tab, in the **Synchronization Mode** list, make sure that **No Sync** is selected. For more information about synchronization modes, refer to the Help.

4. In the Acquisition method pane, click the **Mass Spec** icon.

5. On the **MS** tab, in the **Scan type** list, select **MRM (MRM)**.

6. In the **Polarity** group, click **Positive**.

7. Type the following values in the mass ranges table:

Figure 3-6 MRM Scan Type

Table 3-5 Mass Range and Dwell Time

Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)
609	397.2	100

8. On the **MS** tab, click **Edit Parameters**.

9. Click the **Source/Gas** tab in the Parameter table dialog.

10. Type the following values:

Source/Gas parameters	Typical value
Curtain Gas (CUR)	20
Collision Gas (CAD)	Select **Medium**
IonSpray Voltage (IS)	5000
Temperature (TEM)	0
Ion Source Gas 1 (GS1)	15
Ion Source Gas 2 (GS2)	0

11. Click the **Compound** tab and then set the **Declustering Potential (DP)** to 90 and leave the **Entrance Potential (EP)** at 45.

12. Click **OK**.

13. In the Acquisition method pane, click the **Harvard Syringe Pump** icon.

14. On the Syringe Pump tab, edit the syringe pump method to include **Syringe Diameter**, **Flow Rate**, and **Unit**.

Figure 3-7 Harvard Syringe Pump Method Properties Tab

15. Save the acquisition method.

    Next steps: You have created an acquisition method that you can now use to create and submit a batch. To create and submit batches, refer to .

Add or Remove Devices From Acquisition Methods

Use the Acquisition Method Editor to customize the acquisition method by adding or removing HPLC peripheral device methods. If the required device icon is not in the Acquisition Method Browser pane, then add the peripheral

device only if it is included in the active hardware profile. For more information, refer to the *Advanced User Guide* that gets installed with the Analyst® software.

> **Note:** The available parameters for the LC devices vary depending on the manufacturer.

Change Acquisition Methods

In Acquire mode, users can add or delete periods and experiments to existing acquisition methods.

Add an Experiment

1. In the Acquisition method pane, on the period where the experiment is to be added, right-click and then click **Add experiment**.

   Figure 3-8 Add Experiment
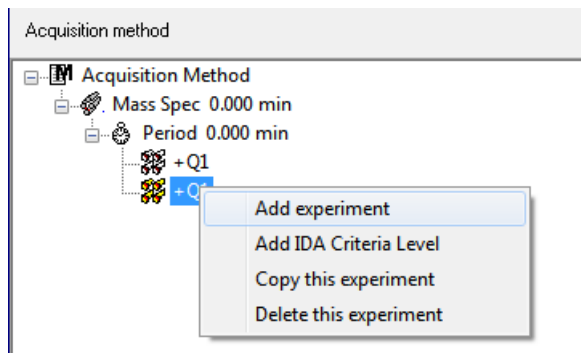
   An experiment is added below the last experiment in the period.

   > **Note:** An experiment cannot be inserted between experiments, IDA criteria, or periods. Users can only add an experiment at the end of the period.

2. In the MS tab, select the appropriate parameters.

Copy an Experiment into a Period

1. Open a multi-period method.

2. In the Acquisition method pane, press **Ctrl**, and then drag the experiment to the period.

   The experiment is copied below the last experiment in the period.

Copy an Experiment within a Period

Use this procedure to add the same or similar experiments to a period if most or all of the parameters are the same.

- Right-click the experiment and then click **Copy this experiment**.

    A copy of the experiment is added below the last experiment created. This is useful when the same or similar experiments are added to an acquisition method.

Add a Period

- In the Acquisition method pane, right-click the **Mass Spec** icon, and then click **Add period**.

    A period is added below the last period created.

    Note: Users cannot use multiple periods in an IDA experiment.

Create and Submit Batches ## 4

A batch is a collection of information about the samples to be analyzed. Batches tell the software the order in which to analyze the samples. For information about importing batches, refer to the *Advanced User Guide*.

Set Queue Options

The queue goes one-by-one through the list, acquiring each sample with the selected acquisition method. After all of the samples have been acquired, the queue stops and the mass spectrometer goes into Standby mode. In Standby mode, the LC pumps and some instrument voltages are turned off.

The user can change the length of time the queue runs after the last acquisition has finished, before the software puts the mass spectrometer into Standby mode. For information about the other fields in the Queue Options dialog, refer to the Help.

1. On the Navigation bar, click **Configure**.

2. Click **Tools > Settings > Queue Options.**

Figure 4-1 Queue Options Dialog
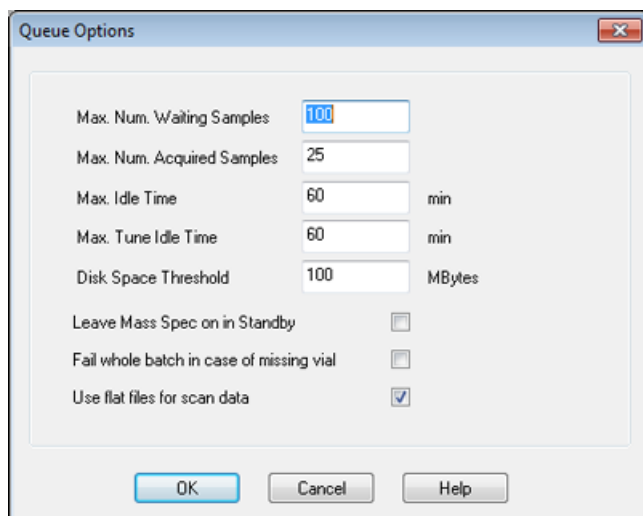
3. In the **Max. Num. Waiting Samples** field, set the maximum number of samples to a value that is greater than the number of samples that will be submitted to the queue

4. In the **Max. Idle Time** field, type the length of time the queue will wait after acquisition is completed before going to Standby mode. The preset value is 60 minutes.

   If gas cylinders are used, then adjust this time to make sure that the gas in the cylinders is not depleted.

   If an LC method is used, then before the run is started, make sure that there is enough solvent in the reservoirs for all of the sample runs at the primary flow rate and the maximum idle time.

5. Select the **Leave Mass Spec on in Standby** check box to keep the mass spectrometer running after analysis has been completed. This feature allows the heaters and gases to continue running, even after devices have entered Idle state, so that the ion source and entrance to the mass spectrometer are kept free of contaminants.

6. Select the **Fail Whole Batch in Case of Missing Vial** check box to fail the entire batch when a missing vial is encountered. If this option is not selected, then only the current sample will fail and the queue will continue to the next sample.

Create and Submit a Batch

Use this workflow to create a batch. In this example, use the MRM scan type that was created previously. Go through the workflow twice more for practice, once using the Q1MS method and the second time using the Q1MI method.

Add Sets and Samples to a Batch

A set can consist of a single sample or multiple samples.

Note: For more information about adding quantitation information to a batch, refer to the *Advanced User Guide*.

---

1. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Batch**.

Figure 4-2 Batch Editor Dialog

2. In the Sample tab, in the **Set** list, type a name.

3. Click **Add Set**.

4. Click **Add Samples** to add samples to the new set.

Figure 4-3 Add Sample Dialog

5. In the **Sample name** section, in the **Prefix** field, type a name for the samples in this set.

6. To add incremental numbering to the end of the sample name, select the **Sample number** check box.

7. If the **Sample number** check box is selected, then in the **Number of digits** field, type the number of digits to include in the sample name.

   For example, if 3 is typed, then the sample names would be samplename001, samplename002, and samplename003.

8. In the **Data file** section, in the **Prefix** field, type a name for the data file that will store the sample information.

9. Select the **Set name** check box to use the set name as part of the data file name.

10. Select the **Auto Increment** check box to increment the data file names automatically.

   > **Note:** The data for each sample can be stored in the same or a separate data file. The names of the data file will have numerical suffixes starting from 1.

11. Type a name in the **Sub Folder** field.

   The folder is stored in the Data folder for the current project. If the **Sub Folder** field is left blank, then the data file is stored in the **Data** folder and a subfolder is not created.

12. In the **New samples** section, in the **Number** field, type the number of new samples to add.

13. Click **OK**.

   The sample table fills with the sample names and data file names.

   > **Tip! Fill Down** and **Auto Increment** options are available in the right-click menu after a single column heading or several rows in a column are selected.

14. On the Sample tab, in the **Acquisition** section, select a method from the list.

   Depending on how the system is set up, specific information for the autosampler must be entered. Even if the injection volume is set in the method, the user can change the injection volume for one or more samples by changing the value in the injection volume column.

   > **Note:** To use different methods for some of the samples in this set, select the **Use Multiple Methods** check box. The **Acquisition Method** column is shown in the **Sample** table. Select the acquisition method for each sample in this column.

15. To change the injection volumes from the volumes listed in the method, in the **Inj. Volume (µL)** column, type the injection volume for each sample.

16. To set sample locations, do one of the following:

- Set Sample Locations in the Batch Editor on page 39
- Select Vial Positions Using the Locations Tab (Optional) on page 39

17. (Optional) To define quantitation details prior to submitting the batch, refer to the *Set Quantitation Details in the Batch Editor (Optional)* section in the *Advanced User Guide.*.

18. Click the **Submit** tab.

Note: The order of samples can be edited before the samples are submitted to the queue. To change the order of samples, on the **Submit** tab, double-click any of the numbers at the far left of the table (a very faint square box is shown), and then drag them to the new location.

---

19. If the **Submit Status** section contains a message about the status of the batch, then do one of the following:

- If the message indicates that the batch is ready for submission, then proceed to step 20.
- If the message indicates that the batch is not ready for submission, then make the changes as indicated by the message.

20. After confirming that all of the batch information is correct, click **Submit**.

The batch is submitted to the queue and can be viewed in the Queue Manager.

21. Save the file.

Acquire Data

The system must not be in Tune and Calibrate mode when sample acquisition is started. Also, if the system has been previously run that day and has not yet been set to Standby mode, then sample acquisition will start automatically.

1. Make sure that the column oven temperature is reached.

2. Make sure that the [T] icon is not pressed in.

3. On the Navigation bar, click **Acquire**.

4. Click **View > Sample Queue.**

The Queue Manager opens with all submitted samples.

Figure 4-4 Queue Manager
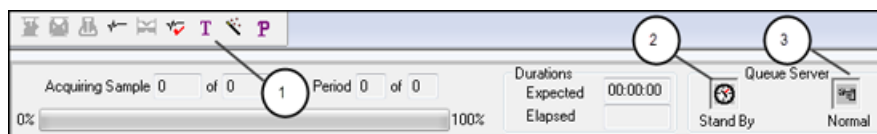
Item	Description
1	The Reserve Instrument for Tuning icon should not be pressed in.
2	Queue status should be in Ready mode.
3	Queue Server should be in Normal. Refer to Queue States on page 43.

5. Click **Acquire > Start Sample.**

Set Sample Locations in the Batch Editor

If an autosampler is used in the acquisition method, then the vial positions of the samples must be defined in the acquisition batch. Define the location in the **Sample** tab or in the **Locations** tab. For more information about creating batches, refer to *Add Sets and Samples to a Batch on page 35*.

1. In the **Sample** tab, from the **Set** list, select the set.

2. For each sample in the set, do the following if applicable:

   • In the **Rack Code** column, select the rack type.

   • In the **Rack Position** column, select the position of the rack in the autosampler.

   • In the **Plate Code** column, select the plate type.

   • In the **Plate Position** column, select the position of the plate on the rack.

   • In the **Vial Position** column, type the position of the vial in the plate or tray.

3. Save the file.

Select Vial Positions Using the Locations Tab (Optional)

1. Click the **Locations** tab in the **Batch Editor**.

2. Select the set from the **Set** list.

3. Select the autosampler from the **Autosampler** list.

4. In the space associated with the rack, right-click and then select the rack type.

   The plates or trays are shown in the rack.

5. Double-click in the white space labeled rack type. A visual sample rack layout is shown.

   The appropriate number of rack spaces for the autosampler is shown in the graphic rack view.

6. Double-click one of the rectangles.

   The circles depicting the wells or vials for the plate or tray are shown.

   Tip! To see the corresponding vial number in the graphical representation, move the cursor over the sample position. Use this information to confirm that the vial positions in the software match the vial positions in the autosampler.

Figure 4-5 Locations Tab
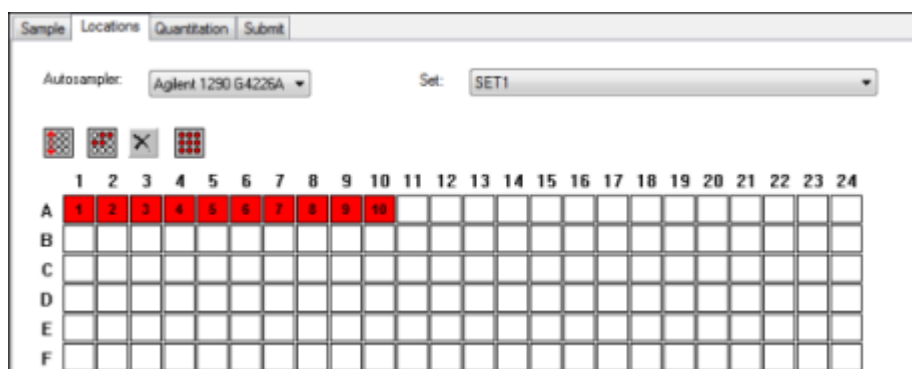
   Note: Depending on the autosampler being used, it might not be necessary to type details in additional columns.

7. To select whether samples are marked by row or column, click the **Row/Column selection** selector button.

   If the button shows a red horizontal line, then the **Batch Editor** marks the samples by row. If the button shows a red vertical line, then the **Batch Editor** marks the samples by column.

8. Click the sample wells or vials in the order to be analyzed.

   Tip! Click a selected well or vial again to clear it.

Tip! To fill in the samples automatically, press the **Shift** key while clicking the first and last vial within a set. To perform multiple injections from the same vial, press the **Ctrl** key while clicking the vial location. The red circle changes to a green circle.

Stop Sample Acquisition

When a sample acquisition is stopped, the current scan finishes before the acquisition is stopped.

1. In the **Queue Manager**, click the sample in the queue after the point where acquisition should stop.

2. On the Navigation bar, click **Acquire**.

3. Click **Acquire > Stop Sample.**

   The queue stops after the current scan in the selected sample is complete. The sample status in the **Queue Manager (Local)** window changes to **Terminated**, and all other samples following in the queue are **Waiting**.

4. To continue processing the batch, click **Acquire > Start Sample.**

Batch and Acquisition Method Editor Tips

Table 4-1 Tips

| To do this...	...do this
To change the values in the table	(For example, to change a sample name) click in a cell and then type the new value.
To change all the values in a column simultaneously	Click a column heading and then right-click. From the menu that is shown, select the **Auto Increment** and **Fill Down** commands to change the values in the column. This also works for multiple cells in the same column.
To change an existing acquisition method	From the list, select the method and then click **Method Editor**. To create a new acquisition method, from the list, select **None** and then click **Method Editor**. Only experienced users should use this feature. Do not use this feature when you are using the Use **Multiple Methods** option.

Table 4-1 Tips (continued)

To do this...	...do this
To apply a previously created quantitation method	Select the method from the **Quantitation** menu.
To select more than one well or vial at a time	Press **Shift** and click the first and last well or vial of the range you want to select.

Batch Editor Right-click Menu

Right-click in the **Batch Editor** table to access the options.

Figure 4-6 Batch Right-Click Menu
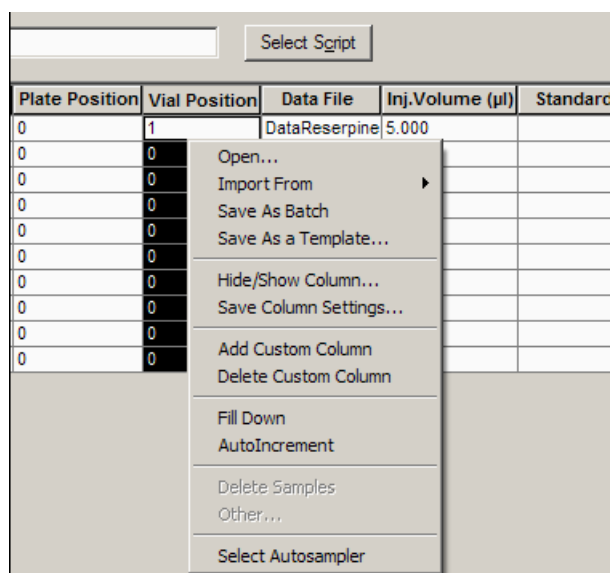
Menu	Function
Open	Opens a batch file.
Import From	Imports a file.
Save As Batch	Saves the batch with a different name.
Save As a Template	Saves the batch as a template.
Hide/Show Column	Hides or shows a column.
Save Column Settings	Saves the batch column settings.
Add Custom Column	Adds a custom column.

Menu	Function
Delete Custom Column	Deletes a custom column.
Fill Down	Copies the same data into the selected cells.
AutoIncrement	Automatically increments data into the selected cells.
Delete Samples	Deletes the selected row.
Select Autosampler	Selects an autosampler.

Queue States and Device Status

The **Queue Manager** shows queue, batch, and sample status. Detailed information about a particular sample in the queue can also be viewed.

Tip! Click to view the queue.

Queue States

The current state of the queue is indicated in the **Queue Server**.

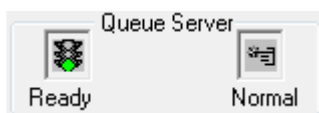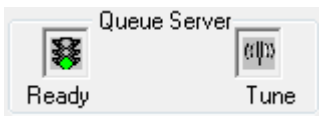**Figure 4-7 Queue Server Indicator Showing Normal Mode**

Figure 4-8 Queue Server Indicator Showing Tune Mode

The first icon indicates the queue state. The second icon indicates whether the queue is in **Tune** mode (for tuning) or **Normal** mode (for running samples). Table 4-2 describes the icons and queue states.

Table 4-2 Queue States

Icons	State	Definition
Queue Server — Not Ready — Normal	Not Ready	The hardware profile is deactivated and the queue is not accepting any sample submissions.
Queue Server — Stand By — Normal	Stand By	The hardware profile has been activated, but all devices are idle. Pumps are not running and gases are turned off.
Queue Server — Warming Up — Normal	Warming Up	The mass spectrometer and devices are equilibrating, columns are being conditioned, the autosampler needle is being washed, and column ovens are reaching temperature. The duration of equilibration is selected by the operator. From this state, the system can go to the **Ready** state.
Queue Server — Ready — Normal	Ready	The system is ready to start running samples and the devices have been equilibrated and are ready to run. In this state, the queue can receive samples and will run after samples are submitted.
Queue Server — Waiting — Normal	Waiting	The system will automatically begin acquisition when the next sample is submitted.
Queue Server — PreRun — Normal	PreRun	The method is being downloaded to each device and device equilibration is occurring. This state occurs before the acquisition of each sample in a batch.
Queue Server — Acquiring — Normal	Acquiring	The method is running and data acquisition is occuring.
Queue Server — Paused — Normal	Paused	The system has been paused during acquisition.

View Instrument and Device Status Icons

Icons representing the mass spectrometer and each device in the active hardware configuration are shown on the status bar in the bottom right corner of the window. The user can view the detailed status of an LC pump to

determine whether the LC pump pressure is appropriate or view the detailed status of the mass spectrometer to confirm the temperature of the ion source.

Note: For each status, the background color can be red. A red background indicates that the device encountered an error while in that state.

- On the status bar, double-click the icon for the device or mass spectrometer.

   The Instrument Status dialog opens.

Table 4-3 Instrument and Device Status Icons

Status	Icon	Background Color	Description
Idle		Green or yellow	The device is not running. If the background color is yellow, then the device should be equilibrated before it is ready to run. If the background color is green, the device is ready to run.
Equilibrating		Green or yellow	The device is equilibrating.
Waiting		Green	The device is waiting for a command from the software or another device, or for some action by the operator.
Running		Green	The device is running a batch.
Aborting		Green	The device is aborting a run.
Downloading		Green	A method is being transferred to the device.
Ready		Green	The device is not running, but is ready to run.
Error		Red	The device has encountered an error that should be investigated.

Queue Right-click Menu

Right-click in the Queue table to access the options.

Figure 4-9 Queue Manager Right-Click Menu
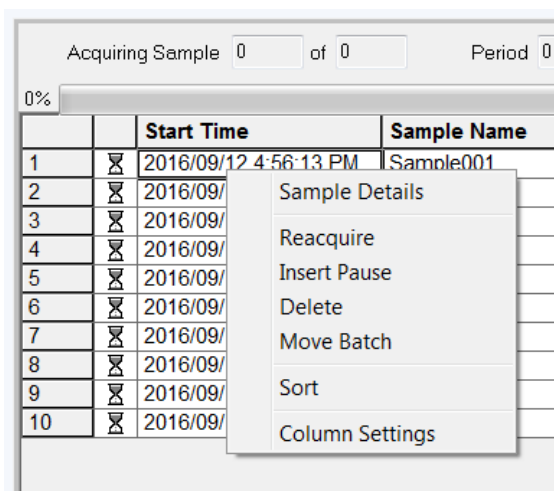
Menu	Function
Sample Details	Opens the Sample Details dialog.
Reacquire	Acquires a sample again.
Insert Pause	Inserts a pause, in seconds, between two samples.
Delete	Deletes either the batch or the selected samples.
Move Batch	Moves the batch within the queue.
Sort	Sorts on the preselected column.
Column Settings	Changes the column settings.

Analyze and Process Data 5

Use the sample files installed in the Example folder to learn how to view and analyze data using the most common analysis and processing tools. For more information about the following topics, refer to the *Advanced User Guide*

- Labeling graphs

- Overlaying and summing spectra or chromatograms

- Performing background subtractions

- Smoothing algorithms

- Working with smoothed data

- Working with centroid data

- Working with contour plots

- Working with the fragment interpretation tool

- Working with library databases and library records

Open Data Files

Tip! To turn off the automatic update on the mass spectrum, right-click the mass spectrum and then click **Show Last Scan**. If there is a check mark beside **Show Last Scan**, then the spectrum will update in real-time.

1. On the Navigation bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog is shown.

2. In the **Data Files** list, navigate to the data file to open, select a sample, and then click **OK**.

   The data acquired from the sample is shown. If data is still being acquired, then the mass spectrum, DAD/UV trace, and TIC continue to update automatically.

Navigate Between Samples in a Data File

Note: If samples were saved in separate data files, then open each file individually.

shows the navigation icons used in this procedure.

- Open a data file that contains multiple samples and then do one of the following:

  - Click the icon with the arrow pointing to the right to skip to the next sample in the data file.

  - Click the icon with the arrow curving to the right to skip to a non-sequential sample.

  - In the Select Sample dialog, from the **Sample** list, select the sample to view.

  - Click the icon with the arrow pointing to the left to go to the previous sample in the data file.

View Experimental Conditions

The experimental conditions used to collect data are stored in the data file with the results. The information contains the details of the acquisition method used: the MS acquisition method (that is, the number of periods, experiments, and cycles) including instrument parameters and the HPLC device method (LC pump flow rate). In addition, it also contains the MS resolution and mass calibration tables used for the sample acquisition. Table 5-1 shows the software functionality available when the user views the file information.

Note: If data is acquired from more than one sample into the same wiff file, then the file information pane does not refresh automatically while scrolling through the samples. Close the file information pane and then reopen it to view the details for the next sample in the wiff file.

- Click **Explore > Show > Show File Information.**

  The File Information pane opens below the graph.

  Tip! To create an acquisition method from the **File Information** pane, right-click the **File Information** pane and then click **Save Acquisition Method**.

Table 5-1 Right-click Menu for Show File Information Pane

Menu	Function
Copy	Copies the selected data.
Paste	Pastes data.
Select All	Selects all the data in the pane.
Save To File	Saves data as an rtf file.
Font	Changes the font.
Save Acquisition Method	Saves the acquisition method as a dam file.

Table 5-1 Right-click Menu for Show File Information Pane (continued)

Menu	Function
Save Acquisition Method to CompoundDB	Opens the Specify Compound Information dialog. Select the IDs and molecular weights to be saved in the compound database.
Delete Pane	Deletes the selected pane.

View the Data in Tables

• With a data file open, click **Explore > Show > Show List Data**.

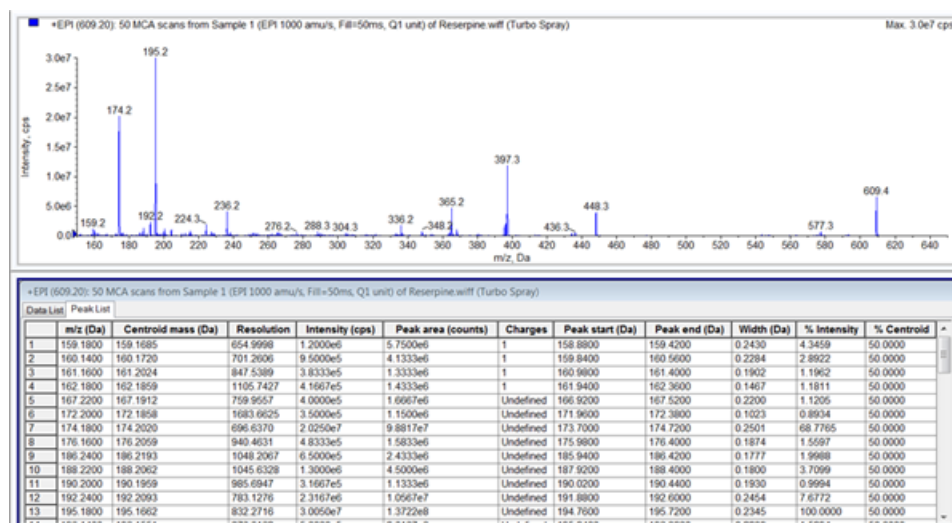The data is shown in a pane below the graph.

Figure 5-1 Data Table

Table 5-2 RIght-Click Menu for the Spectral Peak List Tab

Menu	Function
Column Options	Click to open the Select Columns for Peak List dialog.
Save As Text	Click to save the data as text file.
Delete Pane	Click to delete the pane.

Table 5-3 Right-Click Menu for the Chromatographic Peak List Tab

Menu	Function
Analyst Classic Parameters	Click to open the Analyst Classic dialog.
IntelliQuan Parameters	Click to open the Intelliquan dialog.
Centroid Parameters	Click to open the Centroid Parameters dialog.
Save As Text	Click to save the data as text file.
Delete Pane	Click to delete the pane.

Show ADC Data

ADC (analog-to-digital converter) data is acquired from a secondary detector (for example from a UV detector through an ADC card), and is useful for comparison with mass spectrometer data. To have ADC data available, acquire the data and the mass spectrometer data simultaneously and then save it in the same file.

1. Make sure that the **Example** folder is selected.

2. On the Navigation bar, under **Explore**, double-click **Open Data File**.

   The Select Sample dialog opens.

3. In the **Data Files** field, double-click **Devices** and then click **Adc16chan.wiff**.

4. In the **Samples** list, select a sample, and then click **OK**.

5. Click **Explore > Show > Show ADC Data**.

   Figure 5-2 Select ADC Channel Dialog
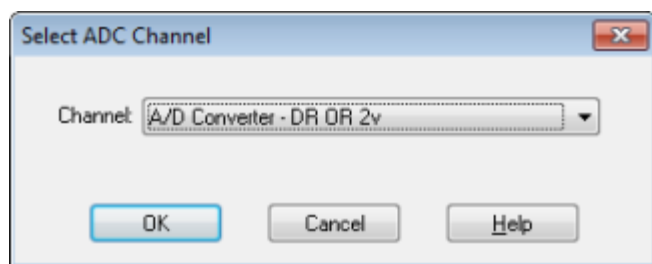
6. In the **Channel** list, select a channel, and then click **OK**.

   The ADC data is shown in a new pane beneath the active pane.

Show Basic Quantitative Data

1. Open a data file.

2. Click **Explore > Show > Show List Data**.

Figure 5-3 List Data
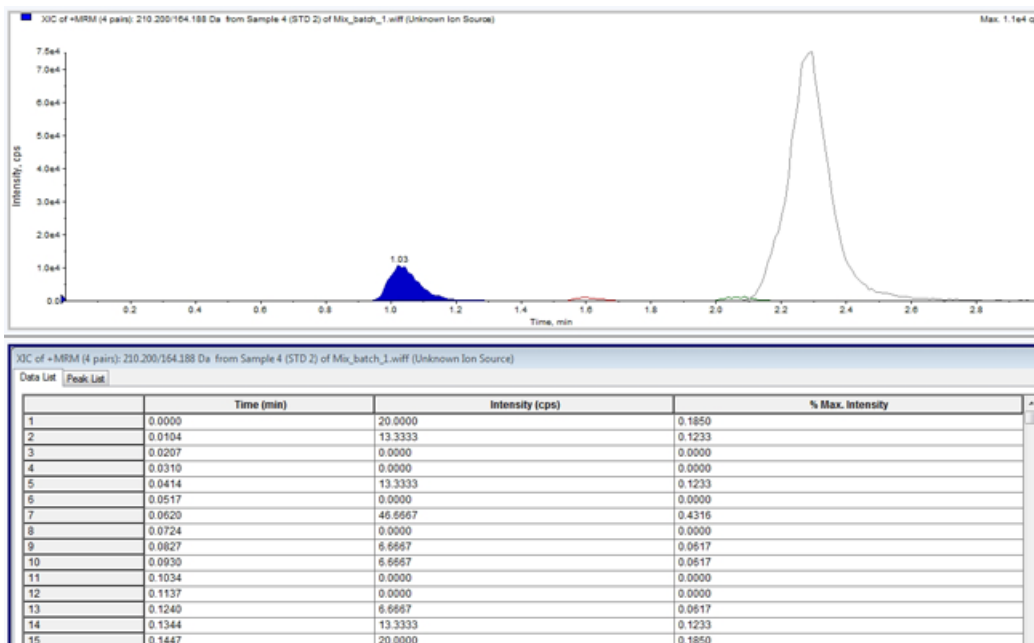
3. In the Peak List tab, right-click and then select **Show Peaks in Graph**.

   Peaks are shown in two colors.

4. To change the peak finding algorithm settings, right-click and then select either **Analyst Classic Parameters** or **Intelliquan Parameters**, whichever is active.

5. (Optional) To remove the colored peaks, right-click in the Peak List tab and then clear **Show Peaks in Graph**.

Chromatograms

A chromatogram is a graphical view of the data obtained from the analysis of a sample. It plots the signal intensity along an axis that shows either time or scan number. For more information about software functionality available for chromatograms, refer to Table 5-6 on page 61.

The software plots intensity, in counts per second (cps), on the Y-axis against time on the X-axis. Peaks above a set threshold are labeled automatically. In the case of LC-MS, the chromatogram is often shown as a function of time. Table 5-4 contains the a description of the types of chromatograms.

Refer to Table 5-8 on page 63 for more information about using the available icons.

Table 5-4 Types of Chromatograms

Types of Chromatograms	Purpose
TIC (Total Ion Chromatogram)	A chromatographic view generated by plotting the intensity of all ions in a scan against time or scan number. When a data file is opened, it is preset to open as a TIC. If the experiment contains only one scan, then it is shown as a spectrum. If the MCA check box is selected during acquisition of the data file, then the data file opens to the mass spectrum. If the MCA check box is not selected, then the data file opens as the TIC.
XIC (Extracted Ion Chromatogram)	An ion chromatogram created by taking intensity values at a single, discrete mass value, or a mass range, from a series of mass spectral scans. It indicates the behavior of a given mass, or mass range, as a function of time.
BPC (Base Peak Chromatogram)	A chromatographic plot that shows the intensity of the most intense ion within a scan versus time or scan number.
TWC (Total Wavelength Chromatogram)	A chromatographic view created by summing all of the absorbance values in the acquired wavelength range and then plotting the values against time. It consists of the summed absorbances of all ions in a scan plotted against time in a chromatographic pane.
XWC (Extracted Wavelength Chromatogram)	A subset of TWC. An XWC shows the absorbance for a single wavelength or the sum of the absorbance for a range of wavelengths.
DAD (Diode Array Detector)	A UV detector that monitors the absorption spectrum of eluting compounds at one or more wavelengths.

Total Ion Chromatogram (TIC)

This is the default view shown when a scan or multi-scan wiff file is opened. The TIC shown corresponds to a chromatogram generated by summing the intensities of all of the ions in each spectrum and then plotting the sum as a function of retention time.

If the sample was acquired using looped experiments, then the TIC shown corresponds to the intensity sums of both experiments and a special arrow indicator is drawn in the x-axis to indicate this. Refer to Figure 5-4. If the indicator is double-clicked, then a new pane, showing overlaid individual TICs for each experiment, is shown.

Figure 5-4 TIC
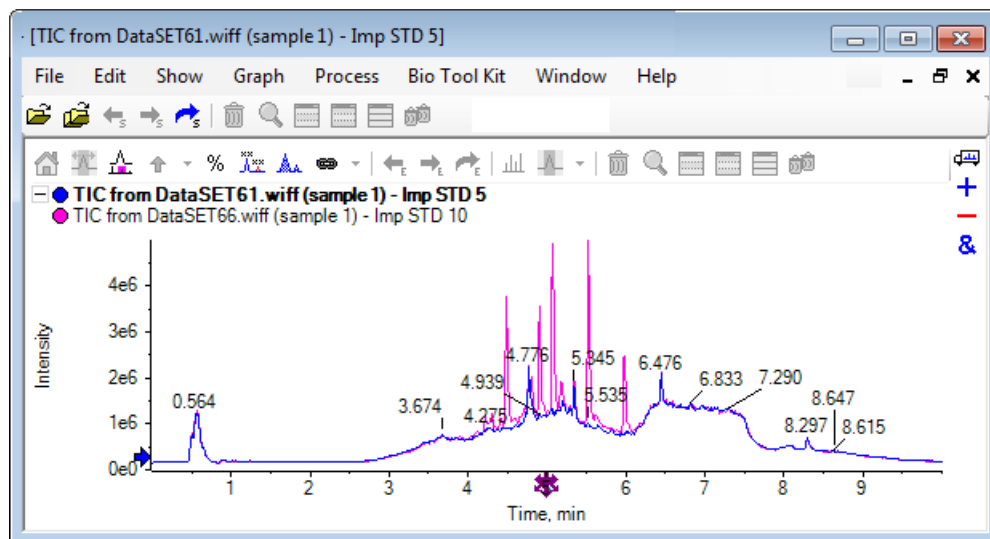
If the sample contains IDA data, then select either the IDA Explorer, which is a graphical way of showing the mass and retention times of selected precursors, or a conventional TIC. If the conventional TIC option is selected, then separate TICs are shown for the IDA survey and the IDA dependent sum.

Show the TIC at any time by clicking **Show > Total Ion Chromatogram (TIC)** to open a dialog that allows the selection of any experiment. Selecting Period 1 shows the TIC for all of the experiments while the other entries correspond to individual TICs. Use **Shift+** or **Ctrl+** click to select more than one.

View a TIC from a Spectrum

1. Make sure that you are in the **Example** project.
2. On the Navigation bar, under **Explore**, double-click **Open Data File**.

    The Select Sample dialog opens.
3. In the **Data Files** field, double-click **LIT** and then click **Reserpine.wiff**.
4. In the **Samples** list, select a sample, and then click **OK**.
5. Click **Explore > Show > Show TIC**.

    The TIC opens in a new pane.

   Tip! You can also right-click inside a pane containing a spectrum and then click **Show TIC**.

Show a Spectrum from a TIC

1. In a pane containing a TIC, select a range.

2. Click **Explore > Show > Show Spectrum.**

   The spectrum opens in a new pane.

   Tip! Double-click in the TIC pane at a particular time to show the spectrum.

Figure 5-5 Example of a TIC
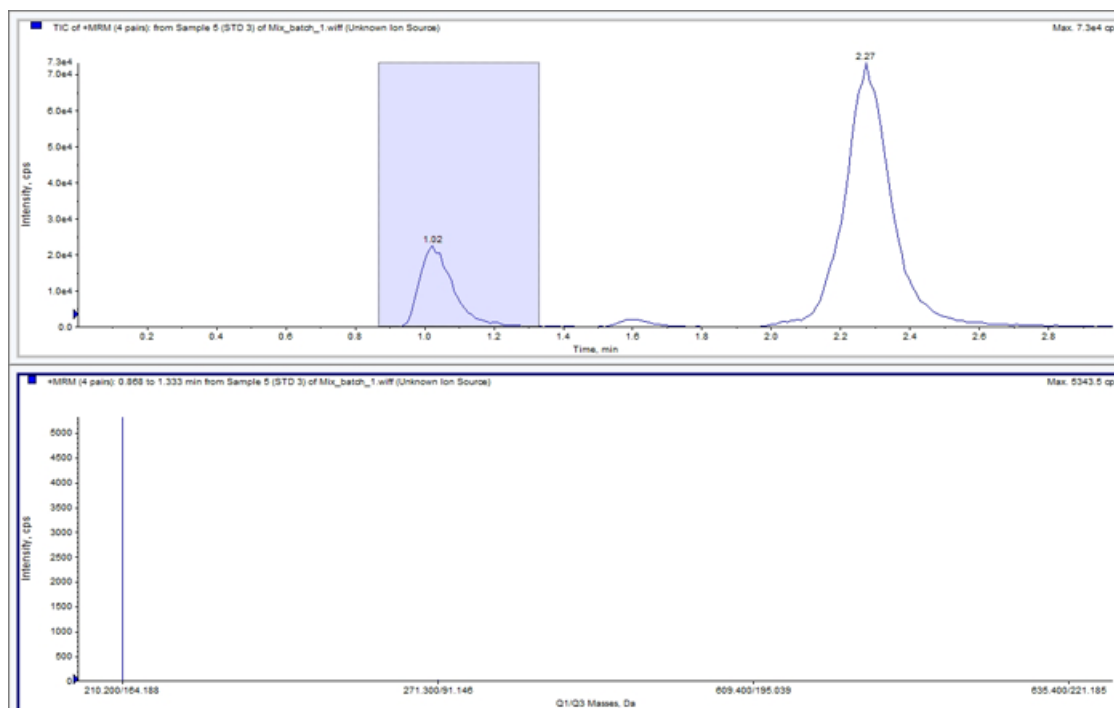
XICs

An XIC is an extracted ion chromatogram created by taking intensity values at a single, discrete mass value, or a mass range from a series of mass spectral scans. It shows the behavior of a given mass, or mass range, as a function of time. The intensity of the ion, or the summed intensities of all ions in a given range, is plotted in a chromatographic pane.

Generate XICs

XICs are generated from only from single period, single experiment chromatograms or spectra. To obtain an XIC from multi-period or multi-experiment data, first split the data into separate panes by clicking the triangle that is shown under the X-axis. For more information about using the available icons, refer to Table 5-8 on page 63.

There are several methods for extracting ions to generate an XIC, depending on whether you are working with chromatographic or spectral data. Table 5-5 contains a summary of methods that can be used with chromatograms and spectra.

Table 5-5 Summary of XIC Generation Methods

Method	Use with chromatogram	Use with spectrum	Extraction
Selected range	No	Yes	The selected range method extracts ions from a selected area in a spectrum.
Maximum	No	Yes	The maximum method extracts ions from a selected area in a spectrum using the most intense peak in the selected area. This creates an XIC using the maximum mass from the selected spectral range.
Base peak masses	Yes	No	The base peak masses method can be used only with BPCs (Base Peak Chromatograms.) Using the Use Base Peak Masses command to extract ions results in an XIC with a different colored trace for each mass. If the selection includes multiple peaks, then the resulting XIC will have an equal number of colored traces representing each mass.
Specified masses	Yes	Yes	The specified masses method extracts ions from any type of spectrum or chromatogram. Select up to 10 start and stop masses for which to generate XICs.

Generate an XIC Using a Selected Range

1. Open a data file containing spectra.

2. Select a range by pressing the left mouse button at the start of the range, dragging the cursor to the stop point, and then releasing the left mouse button.

   The selection is indicated in blue.

3. Click **Explore > Extract Ions > Use Range.**

   An XIC of the selection opens in a pane below the spectrum pane. The experiment information at the top of the pane contains the mass range and the maximum intensity in counts per second.

Generate an XIC Using the Maximum Peak

1. Open a data file containing spectra.

2. Select a range in a spectrum.

   The selection is indicated in blue.

3. Click **Explore > Extract Ions > Use Maximum.**

   An XIC of the maximum peak specified selection opens below the spectrum pane. The experiment information at the top of the pane contains the mass range and the maximum intensity in counts per second.

Generate an XIC Using Base Peak Masses

1. In a BPC, select the peak from which you want to extract ions.

   The selection is highlighted in blue.

2. Click **Explore > Extract Ions > Use Base Peak Masses**.

   An XIC of the specified selection is shown below the spectrum pane. The experiment information at the top of the pane shows the mass range and the maximum intensity in counts per second.

Extract Ion by Selecting Masses

1. Open a spectrum or chromatogram.

2. Click **Explore > Extract Ions > Use Dialog.**

Figure 5-6 Extract Ions Dialog
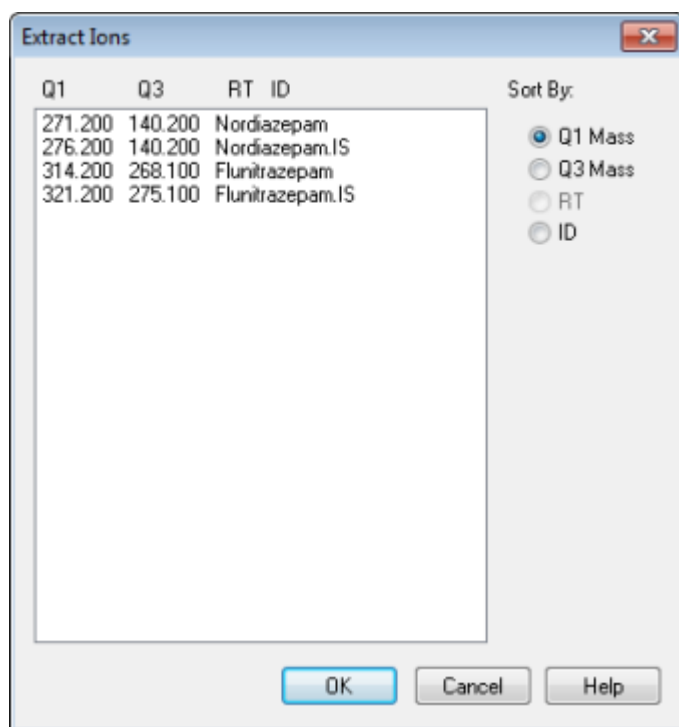
3. Type the values for each XIC to be created. If a stop value is not typed, then the range is defined by the start value.

    • In the **Start** field, type the start value (lower value) for the mass range.

    • In the **Stop** field, type the stop value (higher value) for the mass range.

4. Click **OK**.

    An XIC of the selection opens below the chromatogram pane. The experiment information at the top of the pane includes the masses and the maximum intensity in counts per second.

BPCs

A BPC shows the intensity of the most intense ion in every scan as a function of scan number or retention time. It is useful in instances where the TIC is so dominated by noise that there is a large offset and chromatographic peaks are hard to distinguish. It also helps to distinguish between co-eluting components. BPCs are generated only from single period, single experiment data.

The graph uses two colors, alternating each time the mass of the base peak changes. The color changes are maintained when the data is manipulated by scrolling or zooming. For information about selecting the colors used in the graph, refer to the Help.

Generate BPCs

BPCs can be generated only from single-period, single-experiment data.
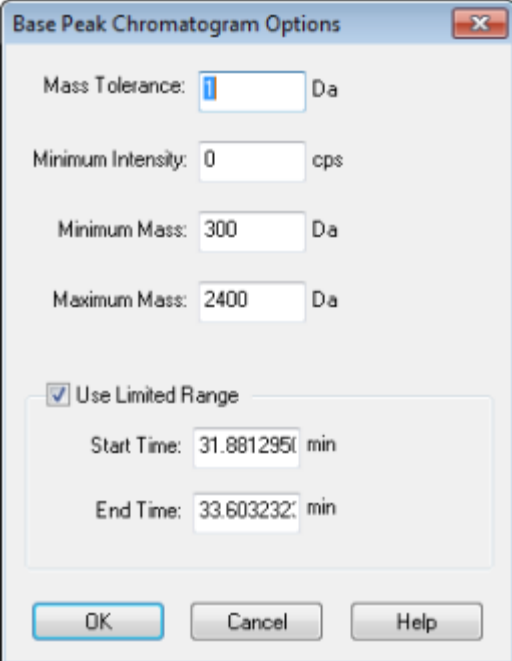
1. Open a data file.

2. Select an area within a TIC.

   The selection is indicated in blue.

3. Click **Explore > Show > Show Base Peak Chromatogram.**

   The selections are shown in the **Start Time** and **End Time** fields.

Figure 5-7 Base Peak Chromatogram Options

4. In the **Mass Tolerance** field, type the value to indicate the mass range used to find a peak. The software finds the peak using a value twice the typed range (± the mass value).

5. Type the intensity below which peaks are ignored by the algorithm in the **Minimum Intensity** field.

6. Type the mass that determines the beginning of the scan range in the **Minimum Mass** field.

7. Type the mass that determines the end of the scan range in the **Maximum Mass** field.

8. To set the start and end times, select the **Use Limited Range** check box and do the following:

   • In the **Start Time** field, type the time that determines the start of the experiment.

- In the **End Time** field, type the time that determines the end of the experiment.

9. Click **OK**.

The BPC is generated in a new pane.

Generate XWCs

An XWC is a wavelength chromatogram created by taking intensity values at a single wavelength, or by the sum of the absorbance for a range of several wavelengths. Up to three ranges can be extracted from a DAD spectrum to generate the XWC. Refer to for more information about using the available icons.

1. Open a data file that contains a DAD spectrum.

2. Right-click anywhere in the pane and then click **Extract Wavelengths**.

Figure 5-8 Extract Wavelengths Dialog
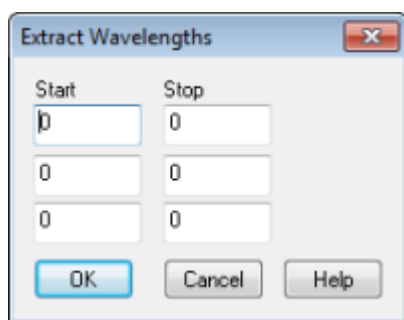
3. Type **Start** and **Stop** values.

4. Click **OK**.

The XWC is shown in a pane below the DAD spectrum.

Show DAD Data

Like mass spectrometer data, DAD data can be viewed in chromatogram or spectrum form. Users can view the DAD spectrum for a single point in time, or for a range of time as a Total Wavelength Chromatogram (TWC).

1. Open a data file containing data acquired with a DAD.

The TWC, which is analogous to a TIC, opens in a pane below the TIC.

2. In the TWC pane, click a point to select a single point in time, or highlight an area of the spectrum to select a range of time.

3. Click **Explore > Show > Show DAD Spectrum.**

The DAD spectrum opens in a pane below the TWC. The Y-axis shows the absorbance and the X-axis shows the wavelength.

> **Tip!** If the pane with the TWC is closed, then click a point anywhere in the TWC to open it again. Click **Explore > Show > Show DAD TWC.**

Generate TWCs

A TWC is a less commonly used chromatogram. It shows the total absorbance (mAU) as a function of time. The TWC provides a way of viewing an entire data set in a single pane. It consists of the summed absorbances of all ions in a scan plotted against time in a chromatographic pane. If the data contains results from multiple experiments, then create individual TWCs for each experiment and another TWC that represents the sum of all experiments.

A TWC shows total absorbance (mAU) on the Y-axis plotted against time on the X-axis. Refer to Table 5-8 on page 63 for more information about using the available icons.

1. Open a data file that contains a DAD spectrum.

2. Click **Explore > Show > Show DAD TWC.**

   The TWC is shown in a pane below the DAD spectrum.

   > **Tip!** Right-click inside the pane containing the DAD spectrum and then click **Show DAD TWC**.

Adjust the Threshold

The threshold is an invisible line drawn parallel to the X-axis of a graph that sets a limit below which the software will not include peaks in a spectrum. The line has a handle, represented by a blue triangle to the left of the Y-axis. Click the blue triangle to view a dotted line that represents the threshold. The threshold can be raised or lowered, but changing the threshold value does not change the data. The software does not label any peaks in the region that lies below the threshold.

1. Open a data file.

2. Do one of the following:

   - To raise the threshold, drag the blue triangle up the Y-axis. To lower the threshold, drag the blue triangle down.

   - Click **Explore > Set Threshold.** In the Threshold Options dialog that opens, type the threshold value and then click **OK**.

   - Click **Explore > Threshold.**

   The graph updates to show the new threshold. Peak labeling and the peak list are also updated.

   > **Tip!** To view the current threshold value, move the pointer over the threshold handle.

Chromatogram Panes

Table 5-6 Right-click Menu for Chromatogram Panes

Menu	Function
List Data	Lists the data points and integrates the peaks found in chromatograms.
Show Spectrum	Generates a new pane containing the spectrum.
Show Contour Plot	Shows a color-coded plot of a data set, where the color represents the intensity of the data at that point. Only certain MS modes are supported.
Extract Ions	Extracts a specific ion or set of ions from a selected pane and then generates a new pane containing a chromatogram for the specific ions.
Show Base Peak Chromatogram	Generates a new pane containing a base peak chromatogram.
Show ADC Data	Generates a new pane containing the ADC data trace, if acquired.
Show UV Detector Data	Generates a new pane containing the UV data trace, if acquired.
Spectral Arithmetic Wizard	Opens the Spectral Arithmetic Wizard.
Save to Text File	Generates a text file containing the data in a pane, which can be opened in Microsoft Excel or other programs.
Save Explore History	Saves information about changes to processing parameters, also called processing options, that were made when a wiff file was processed in Explore mode. The processing history is stored in a file with an eph (Explore Processing History) extension.
Add Caption	Adds a caption at the cursor point in the pane.
Add User Text	Adds a text box at cursor point in the pane.
Set Subtract Range	Sets the subtract range in the pane.
Clear Subtract Range	Clears the subtract range in the pane.
Subtract Range Locked	Locks or unlocks the subtract ranges. If the subtract ranges are not locked, then each subtract range can be moved independently. The subtract ranges are preset to locked.
Delete Pane	Deletes the selected pane.

Spectra

A spectrum is the data that is obtained directly from the mass spectrometer and normally represents the number of ions detected with particular mass-to-charge (m/z) values. It is shown as a graph with the m/z values on the

X-axis and intensity (cps) represented on the Y-axis. For more information on how to work with spectra, refer to Table 5-7.

In the case of MS/MS data, the intensity is associated with two masses, the precursor ion mass (Q1) and the product ion mass or masses (Q3).

Spectra Panes

Table 5-7 Right-click Menu for Spectra Panes

Menu	Function
List Data	Lists the data points and integrates chromatograms.
Show TIC	Generates a new pane containing the TIC.
Extract Ions (Use Range)	Extracts a specific ion or set of ions from a selected pane and then generates a new pane containing a chromatogram for the specific ions.
Extract Ions (Use Maximum)	Extracts ions using the most intense peak in a selected area.
Save to Text File	Generates a text file of the pane, which can be opened in Microsoft Excel or other programs.
Save Explore History	Saves information about changes to processing parameters, also called Processing Options, that were made when a wiff file was processed in Explore mode. The processing history is stored in a file with an eph (Explore Processing History) extension.
Add Caption	Adds a caption at the cursor position in the pane.
Add User Text	Adds a text box at the cursor position in the pane.
Show Last Scan	Shows the scan prior to the selection.
Select Peaks For Label	In this dialog, select the parameters to reduce peak labeling.
Delete Pane	Deletes the selected pane.
Add a Record	Adds records and compound-related data, including spectra, to the library. An active spectrum is required to perform this task.
Search Library	Searches the library without constraints or with previously saved constraints.
Set Search Constraints	Searches the library using the criteria typed in **Search Constraints** dialog.

Graphical Data Processing

Graphical data can be processed many ways. This section provides information and procedures for using some of the most commonly used tools.

Manage Data

Data can be compared or examined in different ways. Users might want to keep the data for comparison purposes before performing processing operations such as smoothing or subtraction.

A window contains one or more panes, arranged in such a way that all the panes are fully visible and they do not overlap.

Panes can be of variable or fixed size. Panes are automatically tiled within the window and are arranged into column and row format. If window size is changed, then the panes within the window change in size to accommodate the resizing. A window cannot be resized to the point where any of the panes would become smaller than its minimum size.

Two or more windows or panes containing similar data can be linked, for example, spectra with similar mass ranges. When the user zooms in one pane or window, the other pane zooms simultaneously. For example, the user can link an XIC to the BPC from which it was extracted. Zooming in the BPC also zooms the XIC, so that both chromatograms are shown with the same magnification.

* Use the following menu options or icons to manage data in graphs.

Table 5-8 Graph Options

To do this...	use this menu option...	...or click this icon
Copy a graph to a new window	Select the graph to copy. Click **Explore > Duplicate Data > In New Window.**	
Rescale a graph to its original size	Select the graph. Click **Explore > Home Graph.**	

Table 5-8 Graph Options (continued)

To do this...	use this menu option...	...or click this icon
Move a pane	• Select the graph. Click **Window > Move Pane.** • Select the pane or window and then drag it to the new position. This position can be inside the same window or within another window. A four-headed arrow is shown when the cursor is on the boundary of the active window or pane. • If the pane is at the top or bottom of the target pane, then the pane moves above or below that pane, respectively. • If the pane is at the left or right of the target pane, then the pane moves to the left or right of that pane, respectively. • If the pane is at any other position, then the pane moves to the target row. The drop shadow of the pane as the pane is moved indicates its new position.	
Link panes	a. With the two graphs open, click one to make that pane active. b. Click **Explore > Link** and then click the other pane.	
Remove linking	Close one of the panes. Click **Explore > Remove Link.**	
Delete a pane	Select the graph. Click **Window > Delete Pane.**	
Lock a pane	Select the graph. Click **Window > Lock Panes.**	
Hide a pane	Select the graph. Click **Window > Hide Pane.**	

Table 5-8 Graph Options (continued)

To do this...	use this menu option...	...or click this icon
Maximize a pane	Select the graph. Click **Window > Maximize Pane.**	
Tile panes	Select the graph. Click **Window > Tile all Panes.**	

Zoom In on the Y-axis

1. Move the pointer to the left of the Y-axis to either side of the area to be expanded and then drag away from the starting point in a vertical direction while holding the left mouse button.

   A box is drawn along the y-axis representing the new scale.

   Note: Take care when zooming in on the baseline. Zoom in too far and the zoom-in box closes.

2. Release the mouse button to draw the graph to the new scale.

Zoom In on the X-axis

Tip! To return the graph to the original scale, double-click either axis. To restore the entire graph to the original scale, click **Explore > Home Graph.**

1. Move the pointer under the X-axis to either side of the area to be expanded and then drag away from the starting point in a horizontal direction while holding the left mouse button.

2. Release the mouse button to draw the graph to the new scale.

Analyze and Process Quantitative Data   6

This section describes how to use the Analyst® software to analyze and process quantitative data. Data can also be processed using the MultiQuant™ software. We suggest that the MultiQuant™ software is used to quantitate data. Refer to the documentation that comes with the MultiQuant™ software.

Use the sample files found in the Example folder to learn how to select samples for quantitation, how to select preset queries and create table-specific queries, and how to analyze the acquired data. For more information about the following topics, refer to the *Advanced User Guide*.

- Metric Plots
- Layout of a Results Table

Quantitative Analysis

Quantitative analysis is used to find the concentration of a particular substance in a sample. By analyzing an unknown sample and comparing it to other samples containing the same substance with known concentrations (standards), the Analyst® software can calculate the concentration of the unknown sample. The process involves creating a calibration curve using the standards and then calculating the concentration for the unknown sample. The calculated concentrations of each sample are then available in a Results Table.

Quantitation Methods

A quantitation method is a set of parameters used to generate peaks in a sample. The quantitation method can include parameters used to locate and integrate peaks, generate standard curves, and calculate unknown concentrations. A previously saved quantitation method can be selected from the Quantitation menu in the batch. The user can create a quantitation method before data acquisition and then apply the method to the quantitative data automatically after the batch is complete. Alternatively, a quantitation method can be created and applied post-acquisition.

Three tools can be used to create a quantitation method: the Quantitation Wizard, the Build Quantitation Method, and Quick Quant.

Build Quantitation Method

The Build Quantitation Method does not generate a quantitation Results Table although the method can subsequently be used in the Quantitation Wizard to create a Results Table. The Build Quantitation Method can also be used to change existing quantitation methods. This is the most flexible way of creating a quantitation method. Refer to Create a Method Using the Quantitation Method Editor on page 67.

Quantitation Wizard

With the Quantitation Wizard, a Results Table is generated at the same time as the quantitation method. Alternatively, an existing quantitation method can be used to quantitate different sets of data.

Quick Quant

Quick Quant is not recommended for quantitation of results.

Quick Quant is part of the Batch Editor. Use Quick Quant to add compound concentrations prior to data acquisition. Because a sample has not been acquired, a representative sample cannot be selected nor can peaks be reviewed. With this process, only the method components are defined.

To use a previously saved quantitation method, select it from the Quantitation menu in the batch. For instructions on creating a batch, refer to Create and Submit a Batch on page 35.

About Results Tables

Results tables summarize the calculated concentration of an analyte in each unknown sample based on the calibration curve. Results tables also include the calibration curves as well as statistics for the results. The user can customize the results tables and view the results tables in layouts.

The data from a results tables can be exported to a txt file for use in other applications, such as Microsoft Excel. The user can also export data in the table or just the data in the visible columns.

Quantitation Methods and Results Tables

For the following procedures, use the sample data that is installed in Example folder. The **Triple Quad folder** contains the data files, Mix_Batch_1 and Mix_Batch_2. These sample files are used to demonstrate the usefulness of metric plots to isolate problematic samples. The ions scanned were reserpine (609.3/195.0), minoxidil (210.2/164.2), tolbutamide (271.1/91.1) and rescinnamine (635.3/221.2), which is the internal standard. Mix_Batch_1 contains no errors in terms of sample preparation, whereas Mix_Batch_2 contains a QC sample where the internal standard was added twice (sample QC2).

Create a Method Using the Quantitation Method Editor

Prerequisites
• Select the project that contains the data to be quantified.
• Switch Between Projects and Subprojects on page 17

1. Make sure that the Example folder is selected.

2. On the Navigation bar, under **Quantitate**, double-click **Build Quantitation Method**.

   The Select Sample dialog opens.

3. Double-click the **Triple Quad** folder in the **Data Files** list.

4. Select **Mix_Batch_2. wiff**.

   The samples in the selected data file are shown in the **Samples** list.

   > **Note:** If the **Compound ID** field was populated for the samples and internal standards in the acquisition method, then in the **Internal Standards** table, when a value is selected in the **Q1/Q3** field, the **Name** field is automatically populated.

5. Select a sample that provides a detectable signal to select integration parameters that fit the entire batch and then click **OK**.

6. In the **Internal Standards** table, in the **Name** column, select **rescinnamine**. In **Q1/Q3** column, select **635.3/221.2**.

7. In the **Analytes** table, do the following:

   a. In the **Name** column, select **minoxidol** for the **Q1/Q3** column masses of **210.2/164.188**, **tolbutamide** for **271.3/91.146**, and **reserpine** for **609.4/195.039**.

   b. In the **Internal Standard** column, from the list, select the **rescinnamine** as internal standard to be associated with each analyte.

   c. Delete **635.4/221.185** from the **Q1/Q3** column in the **Analytes** table.

   > **Note:** If the Compound ID field was populated for the samples and internal standards in the acquisition method, then in the Analytes table, the Name field and Q1/Q3 field are populated.

8. Click the **Integration** tab.

   The preset integration parameters are suitable for most peaks.

9. If the integration is not suitable, then change the algorithm. Refer to .

10. Click the **Show or Hide Parameters** icon to show the additional integration algorithms.

11. Click the **Calibration** tab.

   The preset parameters are suitable for these samples. User can change the fit, weighting, and regression parameter depending on the specific applications.

12. Save the quantitation method.

   The new method can be used when a batch is created in the Batch Editor or when the Quantitation Wizard is used to generate a Results Table.

> **Tip!** The quantitation method can only be used in the current project unless it is copied to another project. To do this, click **Tools > Project > Copy Data**. A new project must be created and selected to be available for use.

Create a Results Table Using the Quantitation Wizard

Prerequisites
• Select the project that contains the data to be quantified.
•

1. On the Navigation bar, under **Quantitate**, double-click **Quantitation Wizard**.

   The Create Quantitation Set - Select Samples page opens.

2. Double-click the **Triple Quad** folder in the **Available Data Files** list.

3. Select **Mix_batch_2. wiff**.

4. Click **Add All**.

   > **Note:** We recommend that users do not process or report results from any sample for which acquisition was abnormally or unexpectedly terminated.

5. Click **Next**.

   The Create Quantitation Set - Select Settings & Query page opens.

6. Click **Select Existing: Query** in the **Default Query** section.

7. Select **Accuracy 15%** from the **Query** list.

   > **Note:** To create a query at the same time, refer to .

   > **Note:** It is the responsibility of the user to evaluate and validate the query to be used for specific applications.

8. Click **Next**.

   The Create Quantitation Set - Select Method page opens.

9. Click **Choose Existing Method**.

10. Select **PK Data_Mix.qmf** from the **Method** list.

11. Click **Finish**.

The Results Table opens.

> **Tip!** To add or remove samples in the Results Table, click **Tools > Results Table > Add/Remove Samples.**

12. Review the sample type, actual concentration, peak integration, calibration curves, statistics pane, metric plot for the internal standard, and other information related to data quantitation.

13. Save the Results Table.

> **Note:** We recommend that users do not change datafile (wiff) names if a Results Table includes samples from that file.

> **Tip!** Well-formatted reports can be created from a Results Table using the Reporter software. We recommend that the user validate the results if a Reporter template that contains a query is used. Refer to Reporter Software on page 85.

Create a Standard Query (Optional)

Advanced users can create a query and a standard query numerous ways. The following is one example. For more information about creating queries, refer to the Help.

1. On the Navigation bar, under **Quantitate**, double-click **Quantitation Wizard**.

2. Select samples in the Create Quantitation Set - Select Samples page.

3. Click **Next**.

4. In the Select Settings & Query page, in the **Default Query** section, select **Create New Standard Query**.

5. Type a query name.

Figure 6-1 Create Quantitation Set — Select Settings & Query Page

6. Click **Next**.

Figure 6-2 Create Quantitation Set — Create Default Query Page

7. In the **Maximum Allowed Accuracy Variation for QCs (%)** table in the **Max. Variation** column, type the maximum allowable percent of variation for each QC, for example 5 is ± 5%, in the same row as the corresponding concentration. If the concentrations were not specified during acquisition, then they are not shown here. In this case, type them in the **Concentration** column.

8. In the **Maximum Allowed Accuracy Variation for Standards (%)** table, in the **Max. Variation** column, type the maximum allowable percent of variation for each standard, for example 10 is ±10%, in the same row as the corresponding concentration. If the concentrations were not specified during acquisition, then they are not shown here. Type the concentrations in the **Concentration** column.

9. Click **Next**.

Figure 6-3 Create Quantitation Set — Select Method Page

10. Select or create a method.

11. Click **Finish**.

The query is applied as a standard query. The query results are shown as a Pass or Fail entry in the **Standard Query Status** column of the Results Table.

Tip! To return to the full view, right-click and then click **Full**.

Results Table Right-click Menu

Right-click in the Results Table to access the options shown in Table 6-1.

Table 6-1 Results Table Right-click Menu

Menu	Function
Full	Shows all the columns.
Summary	Shows specific columns.
Analyte	Shows a specific analyte.

Table 6-1 Results Table Right-click Menu (continued)

Menu	Function
Analyte Group	Creates an analyte group.
Sample Type	Shows samples of a specific type or all samples.
Add Formula Column	Adds a formula column.
	We recommend that the user validate the results if a formula column is used.
Table Settings	Edits or selects a table setting.
Query	Creates or selects a query.
Sort	Creates a sort or sorts by index.
Metric Plot	Creates a metric plot.
Delete Pane	Deletes the active pane.
Fill Down	Copies the same data into the selected cells.
Add Custom Column	Adds a custom column.
Delete Custom Column	Deletes the selected custom column.

Peak Review and Manual Integration of Peaks

Use peak review to survey the peaks that the software has identified and then redefine the peak or the start and end points where required.

After identifying the analytes and internal standards that the software must find, the software searches for the peaks in the samples. When the software identifies a peak, it shows the chromatograms for each analyte and internal standard in the Create Quantitation Method: Define Integration page of the Standard Wizard or on the Integration tab of the Full Method Editor. The user can confirm the peaks that are found or change the quantitation method to better define the peaks. We recommend that users manually review all integration results.

Review Peaks

During peak review, the user might want to view a peak in its entirety or to examine the baseline to find out how well the software found the start and end points of the peak. The automatic zooming feature can be used to do either.

To help the software find a peak, define the exact start and end points of the peak and background manually. These changes will apply only to that individual peak unless the global method is updated.

Note: We recommend that manually integrated results be validated.

Tip! To review an individual peak, right-click on a point on the curve and then click **Show Peak**. The software opens the Peak Review window with the selected peak.

---

1. Right-click in the **Results Table** and then click **Analyte**.

2. Select an analyte.

3. Click **Tools > Peak Review > Pane.**

   The peaks are shown below the **Results Table** with only the peaks listed in the Results Table.

Figure 6-4 Peak Review
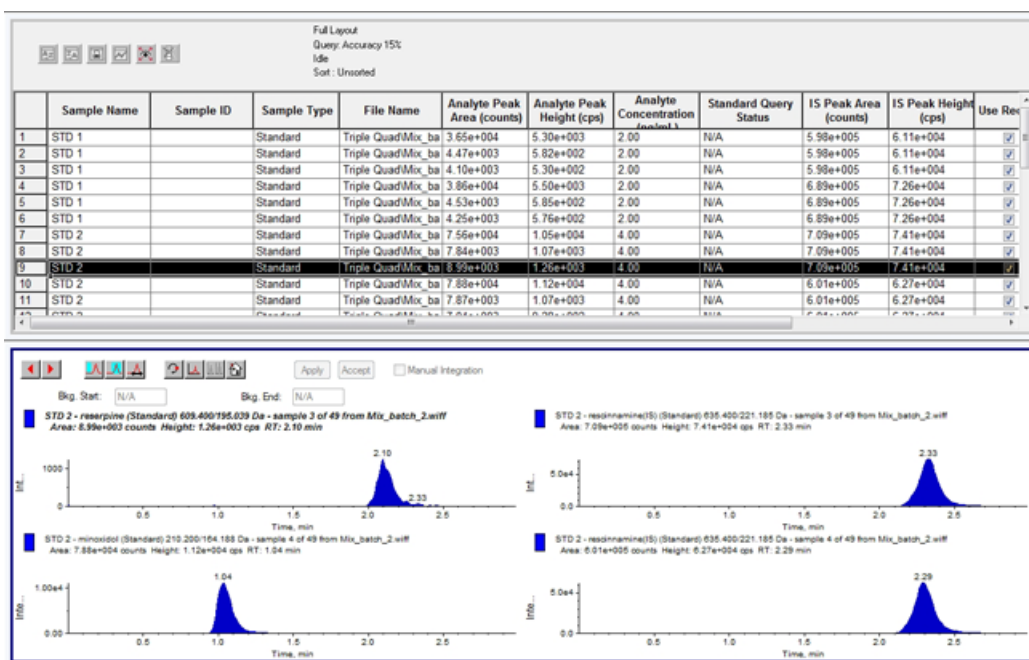
4. Right-click in the pane and then click **Options**.

5. In the **Peak Review Options** dialog, in the **Appearance** section, change **Num. rows** to **1** and **Num. columns** to **2**.

6. In the **Automatic Zooming** section, click **Zoom Y axis to: 100% of largest peak** to show the entire peak.

Figure 6-5 Peak Review Options Dialog
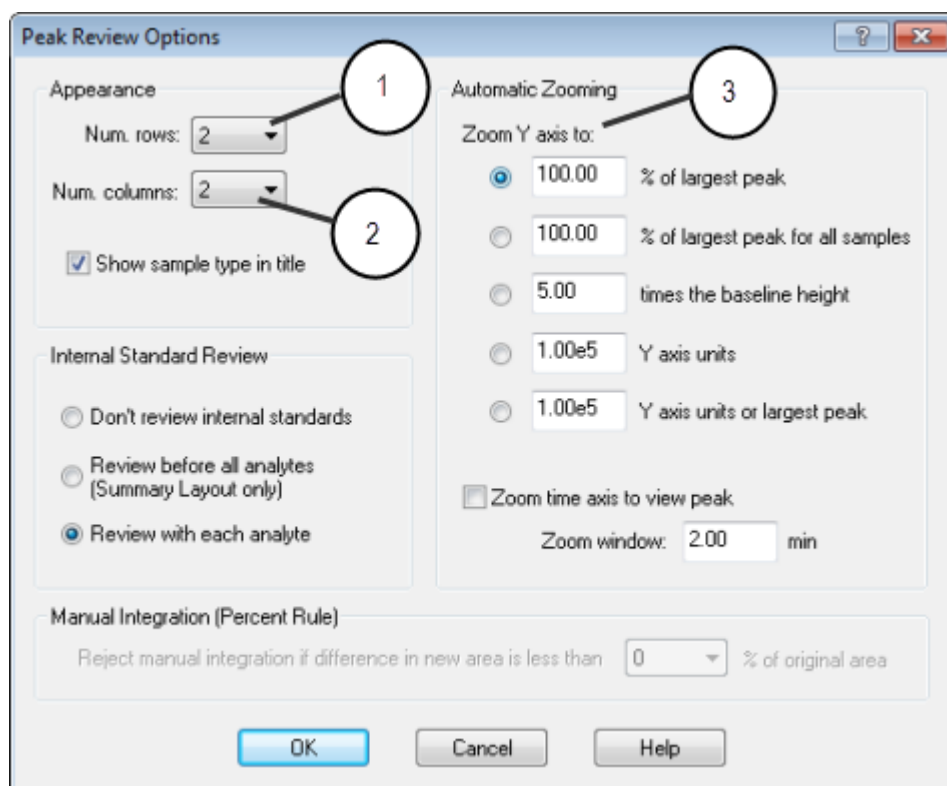
Item	Definition
1	Number of rows
2	Number of columns
3	**Zoom Y-axis to** 100% **of largest peak** to show the entire peak

7. Click **OK**.

8. To move through the peaks, click the right-pointing arrow. Refer to and Figure 6-6 on page 77.

9. Go to the second injection of standard 3.

   In this example, the peak can be integrated closer to the baseline by selecting the **Specify Parameters** option.

   Tip! To move to a specific peak in the Peak Review pane, select the corresponding row in the Results Table.

Figure 6-6 Peak Review Pane
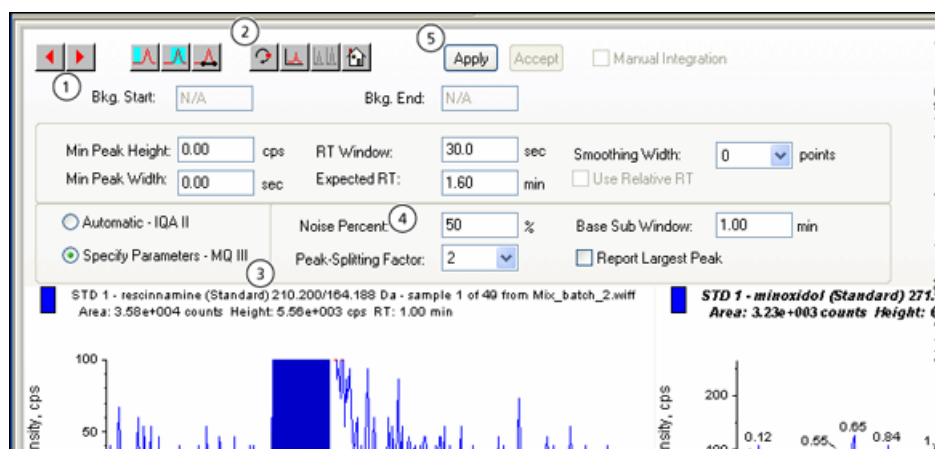
Item	Definition
1	Arrows: Click to move through the peaks.
2	Show or Hide Parameters: Click to show the integration parameters.
3	Integration parameters: Click to change the parameters.
4	Noise Percentage: Type a noise percent.
5	Apply: Click to integrate the parameters.

10. Click **Show or Hide Parameters** twice.

11. Click **Specify Parameters - MQ III**.

12. Change the **Noise Percent** value.

13. Click **Apply**.

    The peak is integrated closer to the baseline.

14. If the change does not improve the peak integration, then adjust the **Noise Percent** parameter until the optimal value is found.

Note: The **Update Method** function only updates the algorithm values for that specific analyte (or internal standard) and not all analytes.

---

15. To update the algorithm for all peaks, right-click in the pane and then click **Update Method**.

Figure 6-7 Update Method
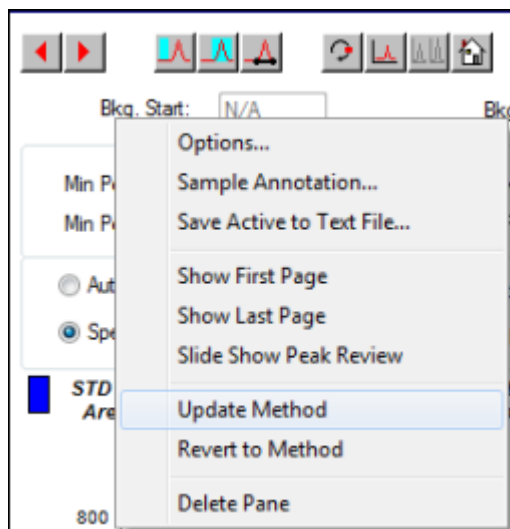
Manually Integrate Peaks

Manually integrating peaks should be done last, to limit person-to-person variability. Manually integrate peaks only if all the peaks have not been found after the algorithm parameters have been adjusted and updated. We recommend that users validate the results to determine whether manual integration is acceptable for specific applications.

Note: Peaks that are manually integrated, or where the algorithm was changed for only that peak, are identified in the Record Modified column of the Results Table, as are peaks that have algorithm parameter changes for a sample that are not applied to the entire analyte group.

1. In the **Peak Review** pane, click **Manual Integration Mode**.

Figure 6-8 Peak Review Pane: Manual Integration

2. Zoom in on the lower 10% of the peak.

Figure 6-9 Peak Review Pane: Zooming in on a Peak
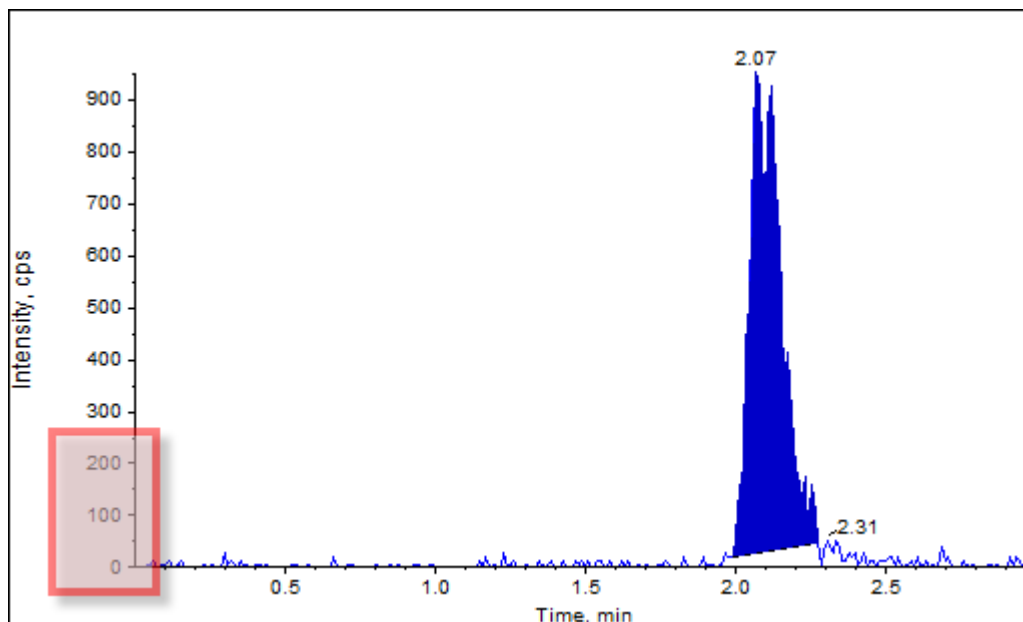
3. Move the cross-hair to where the start of the peak is to be defined and then drag the cross-hair to where the end of the peak is to be defined.

   The software shades the area bounded by the base and sides of the peak. Peak parameters are gray as they are no longer applicable because the peak was drawn manually.

4. Do one of the following:

   • To make this change permanent, click **Accept**.

   • To discard the changes, clear the **Manual Integration** check box.

   Tip! If a peak was correct as originally selected, right-click the peak and then click **Revert to Method**.

Peak Review Right-Click Menu

Right-click in the **Peak Review** window or pane to access the options shown in Table 6-2.

Table 6-2 Peak Review Right-click Menu

Menu	Function
Options	Opens the Peak Review Options dialog.
Sample Annotation	Opens the Sample Annotation dialog.
Save Active to Text File	Saves the selected peak as a text file.
Show First Page	Goes to the first sample.
Show Last Page	Goes to the last sample.
Slide Show Peak Review	Opens the slide show.
Update Method	Updates the algorithm for all peaks.
Revert to Method	Selects a redefined peak based on the current quantitation method.
Delete Pane	Deletes the active pane.

Calibration Curves

Use calibration curves to find the calculated concentration of samples, including quality control (QC) samples. QC samples are added to a batch to estimate the data quality and accuracy of standards in the batch. QC samples have known analyte concentrations but are treated as unknowns so that the measured concentrations can be compared to the actual value.

The calibration curve is generated by plotting the concentration of the standard versus its area or height. If an internal standard is used, then the ratio of the standard concentration or internal standard versus the ratio of the standard peak height or area to the internal standard peak height or area is plotted. The area or height ratio of a sample is then applied to this curve to find the concentration of the sample, as shown in the Results Table. A regression equation is generated by this calibration curve according to the regression that was specified. The regression equation is used to calculate the concentration of the unknown samples.

View Calibration Curves

The user can view the calibration curve and change the regression options in an open Results Table. If two or more Results Tables are open, then the calibration curves can be overlaid. To overlay curves, make sure that the method used to create the tables is the same.

Plot a calibration curve to see the curve used for regression. The Calculated Concentration field in the Results Table reflects any changes resulting from the fit of the curve to the points of the standard.

Note: This option is available only when a Results Table is open in the workspace.

1. Open a Results Table.

2. Click **Tools > Calibration > Pane.**

   The Calibration Curve pane containing the calibration curve opens.

   Figure 6-10 Calibration Curve
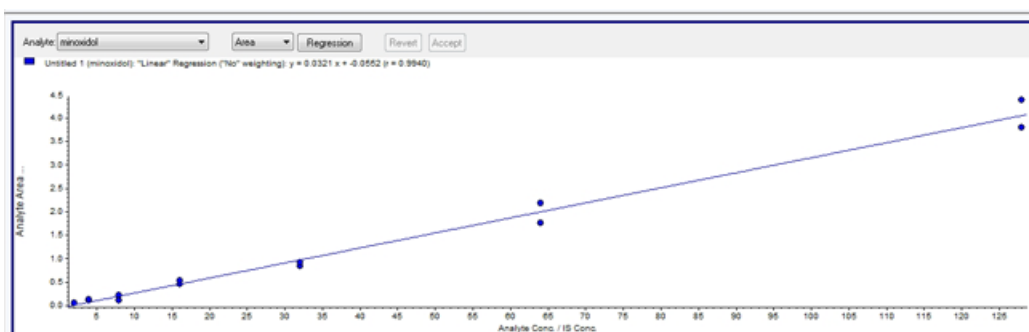
3. If there is more than one analyte, then use the following steps to view the calibration curve for another analyte:

   a. From the **Analyte** list, select an analyte.

   b. If required, from the next list, select **Area** or **Height**.

4. To change the regression options for the calibration curve, do the following:

   a. Click **Regression**.

      Figure 6-11 Regression Options Dialog
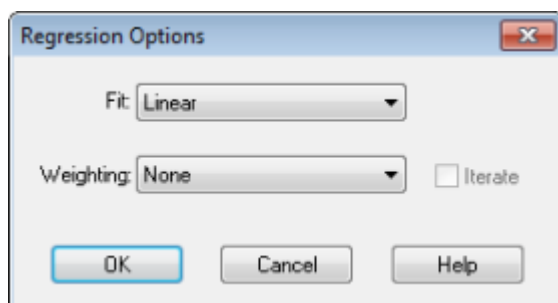
   b. Select **Linear** in the **Fit** list.

   c. Select **1 / x** in the **Weighting** list.

   d. Click **OK**.

The calibration curve opens. The user can review individual peaks on the curve or exclude points from the curve to produce a better curve.

5. If required, repeat these steps to create a more appropriate curve.

6. To save the changes, click **Accept**.

Overlay Calibration Curves

Tip! To examine the curve for one table more closely, right-click the curve and then click **Active Plot**. Select the curve to be plotted on top.

1. With two or more open Results Tables, view a calibration curve for one of the tables.

2. Right-click the calibration curve and then click **Overlay**.

Figure 6-12 Overlay Dialog
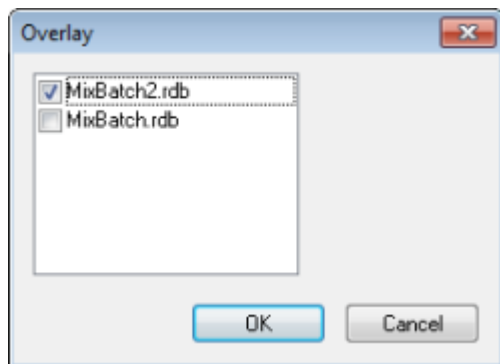
3. Select the tables to overlay with the current curve.

4. Click **OK**.

The software plots the curves for all selected tables on the same graph.

Calibration Curve Right-Click Menu

Right-click in the Calibration window or pane table to access the options shown in Table 6-3.

Table 6-3 Calibration Curve Right-click Menu

Menu	Function
Exclude (Include)	Right-click the point and then click **Exclude** to exclude the point from the curve. Right-click the point and then click **Include** to include the point.
Exclude All Analytes (Include All Analytes)	Right-click a point and then click **Exclude All Analytes** to exclude all the analytes from the curve. Right-click a point and then click **Include All Analytes** to include the points.
Show Peak	Reviews an individual peak.
Overlay	Overlays two graphs.
Active Plot	Determines which plot is active.
Legend	Shows the graph legend.
Log Scale X Axis*	Uses a log scale for the X-axis.
Log Scale Y Axis*	Uses a log scale for the Y-axis.
Delete Pane	Deletes the active pane.
Home Graph	Scales the graph to its original size
* A log scale arranges the data points in a more manageable view so that the effect of all points can be monitored simultaneously. For this view, select **Log Scale Y Axis** versus **Log Scale X** and not just the log of one axis.	

Sample Statistics

Use the Statistics window to view the statistics samples, typically for standards and QCs (quality controls). The data from each available batch in the Results Table opens in tabular form in the grid and a row of data is shown for each standard or QC concentration.

View the Statistics for Standards and QCs

When more than one **Results Table** is open, statistical information about the standards and QCs for additional batches in the **Statistics** window can be obtained. This facilitates comparison of results between batches and identification of trends in the standards or QCs.

1. Open a Results Table.

2. Click **Tools > Statistics.**

3. Select **Concentration** from the **Statistics Metric** list.

4. Select an analyte in the **Analyte Name** field.

5. Select **Standard** in the **Sample Type** field.

   The results are shown.

6. Look at the **%CV** and **Accuracy** columns.

   The **%CV** shows the coefficient of variance between the measurements of a single parameter, for example the area. **Accuracy** shows how close the plotted point is to the interpolated value.

7. If required, select the **Display Low/High values** check box and then examine the **Low**, **High** values, and **Mean** for each row in the grid. Each row represents standards that have the same concentration levels.

8. Select another analyte.

   The results are shown on a per-analyte basis.

9. To check for **Quality Control** variations at the same concentration levels, select **QC** in the **Sample Type** field.

Compare Results Between Batches

When more than one Results Table is shown, obtain statistical information about the standards and QCs for additional batches in the Statistics window. Normally results are compared between batches to look for trends in the standards or QCs or to verify that the method is valid.

For two or more open Results Tables, compare results in the Statistics window. Both sets of statistics are shown in the Statistics window.

The number of analytes and the analyte names must be the same for the data to be combined in the Statistics pane.

1. Open a Results Table.

2. Click **Tools > Statistics.**

3. Do one of the following:

   • To arrange the results by **Results Table**, select **Group By Batch** in the **Conc. as Rows** list.

   • To arrange the results in order of concentration, select **Group By Concentration** in the **Conc. as Rows** list.

   • To arrange the results in order of concentration without a row showing the statistics for each group or batch, select **Group By Concentration (no All)** in the **Conc. as Rows** list.

   The software sorts the results. At the end of each batch or group, one or two additional rows are shown: **All** (statistics for all Results Tables in that group) and **Average** (statistics on the statistics for that batch or group).

Reporter Software 7

The Reporter software extends the reporting functionality available in the Analyst® software.

We recommend that users validate the results if a modified Reporter template or one that contains a query is used.

The Reporter software can be used to create custom reports with Microsoft Word and Excel (2010, 2013, or 2016). The Reporter software has the following features:

- Provides a variety of reports that use the data available in a Results Table, in file information, and in quantitative peak review windows.

- Provides a variety of reports that present MS/MS library search results. The user can configure the Reporter software to search against any MS/MS spectral library that uses the Analyst® (mdb) format.

- Uses Microsoft Word templates to provide the format information needed when generating reports. These templates can be created or modified to provide customized report formats. Refer to the Help for information about creating or editing the Report Template Editor.

- Contains a blank starting template that can be used in the Analyst® software Reporter editing environment to design report templates to meet most reporting requirements.

- Automates report generation through the use of the Autoquan Reporter batch script.

- Automatically prints, exports to Adobe Portable Document Format (pdf), and delivers results by e-mail.

- Attaches processing scripts to report templates to expand both the content and automation level for various workflow requirements.

- Generates reports from custom software applications that use the available Analyst® software programming libraries.

Reporter software can be used as follows:

- Within the Analyst® software to manually generate a report or set of reports.

- By a batch script to automate report generation within a batch. Users can generate reports on a sample-by-sample basis, either during or after batch acquisition.

- By applications that do not use the Analyst® software.

Analyst Reporter User Interface

Figure 7-1 Analyst Reporter

Item	Option	Description
1	**File** > **Exit**	Exits the program and releases all resources.
2	**Settings** > **Select Output Language**	Sets the language dictionary that will be used to replace language tags within a report template. Templates that contain language tags can be used to generate reports in any language. The language tags are replaced with text from a matching tag in the dictionary file for the selected language. These dictionary files are contained in the folder: C:\Program Files\AB SCIEX\AnalystReporter\Resources\Languages on Windows 7, 32- bit operating systems or C:\Program Files (x86)\AB SCIEX\AnalystReporter\Resources\Languages on Windows 7, 64-bit or Windows 10, 64-bit operating systems.
2	**Settings** > **Select Library**	Browse to a spectral library. This library will be used for matching and scoring MS/MS data from Results Tables that contain data from information dependent acquisition (IDA) triggered MS/MS scan types.
2	**Settings** > **Select Template Folder**	Sets the folder from which the available templates will be read. To return to the default template folder, select the **Default** option.
3	**Help** > **About**	Shows information about the version of Reporter software currently installed.
4	Current Output Language	Shows the currently selected language dictionary used for replacing language tags within a report template. The language dictionary can be selected using **Settings** > **Select Output Language.**
5	Current Spectral Library	Shows the currently selected spectral library. The spectral library can be selected using **Settings** > **Select Library.**
6	Available Templates and Description	Shows a list of available report templates. Selecting a template will show a description of the template. To change the folder from which available templates are read, select **Settings** > **Select Template Folder** > **Browse.**

Item	Option	Description
7	Output Format	Shows the output formats that are supported by the Reporter software. Only formats that are compatible with the selected report template are enabled.
		• Word: Microsoft Word document (docx) is produced. This document can be viewed by Microsoft Word 2010 and above.
		• PDF: A report is created directly in PDF format.
		• HTML: Microsoft Word is used to generate an HTML file. Associated image files are stored in a folder with the same name as the HTML file.
		• Excel: A plain text file (csv) is produced. Report templates that contain values separated by commas can be opened in Microsoft Excel, where each value will be shown in a separate cell. Only templates that are specifically marked as text-compatible can be used for this output format.
		• Text: A plain text document (txt) is produced. Only templates that are specifically marked as text compatible can be used for this output format.
8	Print Automatically	After the report has been created it is printed to the selected printer. Select any available printer.
9	Create Report	Creates the report in the selected output format using the selected report template.
10	Close	Exits the program and releases all resources.

Generate Reports

The Reporter software extracts numerical data from the Results Table and sample and graphical information from the wiff file.

Select a template in the Available Template field.

Tip! For reports that can be generated on a sample-by-sample basis, it may be more efficient to generate the reports automatically using a batch script during acquisition to avoid long processing times at the end of the acquisition. For more information about batch scripts, refer to the *Scripts User Guide*.

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1. Open a **Results Table**.

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2. Under **Companion Software**, double-click **Reporter**.

3. In the **Analyst Reporter** dialog, in the **Available Templates** field, select the applicable report template.

4. Click the **PDF** output format.

    The Word option is pre-selected and the report is automatically saved in the current project Results folder. If this option is not selected, then the report is created and opened in Word or printed as selected, but the report is not saved. This lets the user edit the report in Word prior to saving the original report.

5. Select either one document containing all samples or multiple documents with one sample in each.

6. (Optional) Select the **Print Automatically** check box to print the reports automatically on a pre-selected printer.

    The Default Printer set in Windows is used unless a different printer is selected. The Reporter tool retains the selected printer between operations. If the printer is set to a PDF printer driver, then the Reporter generates PDF file versions of the created reports automatically.

7. Click **Create Report**.

    The screen shows various progress indicators as the tool opens the template and populates it with data from the Results Table. Some reports can take seconds to generate, others can take longer. A large data set with many MRM transitions or a large number of graphics might result in reports of several hundred pages that take hours to generate.

Calibration Ions and Solutions $\quad$ **A**

Table A-1 Tuning Frequency

	Calibration		Resolution Optimization	
Scan Type	**Frequency**	**Manual/ Automated**	**Frequency**	**Manual/Automated**
Q1 and Q3	3 months to 6 months	Both	3 months to 6 months	Both
LIT	Every 2 weeks, as required	Both	3 months to 6 months	Automated only

Table A-2 Suggested Tuning Solutions for the 4000 Systems

System	Q1 and Q3		LIT
	Positive	**Negative**	**Positive and Negative**
API 4000™ LC-MS/MS system	POS PPG, 2e-6 M	NEG PPG, 3e-4 M	N/A
4000 QTRAP® LC-MS/MS system	POS PPG, 2e-6 M	NEG PPG, 3e-4 M	Agilent ESI Tuning Mix

Table A-3 Suggested Tuning Solutions for the API 5000™ System

System	**Positive**	**Negative**
API 5000™ LC-MS/MS system	POS PPG, 2e-6 M	NEG PPG, 3e-4 M

Table A-4 Q1 and Q3 PPG Positive Ion Scans

Instrument	Masses							
API 4000™ LC-MS/MS system	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
4000 QTRAP® LC-MS/MS system	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
API 5000™ LC-MS/MS system	59.0	175.1	616.5	906.7	1080.8	1196.9	—	—

Table A-5 Q1 and Q3 PPG Negative Ion Scans

Instrument	Masses							
API 4000™ LC-MS/MS system	45.0	585.4	933.6	1223.8	1572.1	1863.3	2037.4	2211.6
API 5000™ LC-MS/MS system	45.0	411.3	585.4	933.6	1223.8	—	—	—
4000 QTRAP® LC-MS/MS system	45.0	585.4	933.6	1223.8	1572.1	1863.3	2037.4	2211.6

Table A-6 Masses and Polarity for the 4000 QTRAP® LC-MS/MS System (Agilent)

Instrument/ Polarity	Masses						
LIT Positive	118.087	322.049	622.030	922.010	1521.972	2121.934	2721.895
LIT Negative	112.985	431.982	601.978	1033.988	1633.949	2234.911	—

Parameters and Scan Types   B

This section describes the different parameters and scan types that you can use for your analysis.

About Instrument Parameters

Source-dependent parameters, compound-dependent parameters, and detector parameters are all configured in the Analyst® software and applied at specific points to the mass filter rail (ion path). Users should understand what each parameter controls and how it affects resolution, intensity, and peak shape so that optimal results are achieved during sample analysis. Users should also consider how changing the value of one parameter can affect another parameter further along the ion path.

Source-Dependent Parameters

These parameters might change depending on the source you are using.

GS1 (Gas 1): The GS1 parameter controls the nebulizer gas. The nebulizer gas helps generate small droplets of sample flow and affects spray stability and sensitivity.

GS2 (Gas 2): The GS2 parameter controls the auxiliary, or turbo, gas. It is used to help evaporate the solvent to produce gas phase sample ions.

TEM (Temperature): The TEM parameter controls the temperature of the turbo gas in the TurboIonSpray® probe or the temperature of the probe in the heated nebulizer (or APCI) probe.

CUR (Curtain Gas): The CUR parameter controls the gas flow of the Curtain Gas™ interface. The Curtain Gas™ interface is located between the curtain plate and the orifice. It assists in solvent evaporation and prevents solvent droplets from entering and contaminating the ion optics. The gas flow should be maintained as high as possible without losing sensitivity.

IS (IonSpray Voltage): The IS parameter controls the voltage applied to the electrode that ionizes the sample in the ion source. It depends on the polarity and it affects the spray stability and the sensitivity. This parameter can be compound-dependent and should be optimized for each compound.

NC (Needle Current): The NC parameter controls the current applied to the corona discharge needle in the APCI probe, used in the Turbo V™ source. The discharge ionizes solvent molecules, which in turn ionize the sample molecules.

ihe (Interface Heater): The ihe parameter switches the interface heater on and off. Heating the interface helps maximize the ion signal and prevents contamination of the ion optics. This should always stay on. The button controlling the interface heater reads ON when the interface heater is on.

IHT (Interface Heater Temperature): The IHT parameter controls the temperature of the NanoSpray® interface heater and is only available if the NanoSpray® ion source and interface are installed.

Compound-Dependent Parameters

The compound-dependent parameters consist mostly of voltages in the ion path. Optimal values for compound-dependent parameters vary depending on the compound being analyzed.

Quadrupole- and LIT-Mode Scan Parameters

The following parameters are available for optimization if you are running a quadrupole mode scan or an LIT-mode scan.

DP (Declustering Potential): The DP parameter controls the voltage on the orifice, which controls the ability to decluster ions between the orifice and the skimmer (or for systems with a QJet® Ion Guide, between the orifice and QJet® Ion Guide). It is used to minimize the solvent clusters that might remain on the sample ions after they enter the vacuum chamber, and, if required, to fragment ions. The higher the voltage, the higher the energy imparted to the ions. If the DP parameter is too high, unwanted fragmentation might occur.

EP (Entrance Potential): The EP parameter controls the potential difference between the voltage on Q0 and ground. The entrance potential guides and focuses the ions through the high-pressure Q0 region.

CE (Collision Energy): The CE parameter controls the potential difference between Q0 and Q2 (collision cell). It is used only in MS/MS-type scans. This is the amount of energy that the precursor ions receive as they are accelerated into the collision cell, where they collide with gas molecules and fragment.

CAD (CAD Gas): The CAD parameter controls the pressure of collision gas in the collision cell during Q3, MS/MS-type, and LIT scans. For Q3 scans, the collision gas helps to focus the ions as they pass through the collision cell; the preset for the CAD parameter is in fixed mode. For MS/MS-type scans, the collision gas aids in fragmenting the precursor ions. When the precursor ions collide with the collision gas, they can dissociate to form product ions. For LIT scans, the collision gas helps to focus and trap ions in the LIT.

CXP (Collision Cell Exit Potential): The CXP parameter controls the potential difference between RO2 and ST3 (for the API 4000™ system, API 5000™ system, and 4000 QTRAP® system). It is only used in Q3 and MS/MS-type scans, where it transmits the ions into Q3.

IE1 (Ion Energy 1): The IE1 parameter controls the potential difference between Q0 and RO1. Although this parameter does affect the sensitivity, it has a greater impact on peak shape, and it is considered a resolution parameter. IE1 is used in Q1, MS/MS-type, and LIT scans. This parameter should only be used by experienced users.

IE3 (Ion Energy 3): The IE3 parameter controls the potential difference between RO2 and RO3. Although this parameter does affect the sensitivity, it has a greater impact on peak shape, and it is considered a resolution parameter. IE3 is used in Q3 and MS/MS-type scans. This parameter should only be used by experienced users.

LIT Mode Scan Parameters

In addition to the compound-dependent parameters that are available on the Compound tab, several parameters are available on the MS tab or the Advanced MS tab for LIT (linear ion trap) mode scans that will affect your results. Because parameters on the MS tab or the Advanced MS tab cannot be changed in real time, the best method of sample introduction for optimizing these parameters is infusion. The acquisition must be stopped between each parameter change.

Q0 Trapping: The Q0 trapping parameter controls the storage of ions in the Q0 region. It is used to increase sensitivity and duty cycle by trapping ions in the Q0 region while ions are being mass-selectively ejected from the LIT. You must use fixed fill time with this parameter.

CES (Collision Energy Spread): The CES parameter, in conjunction with the Collision Energy (CE), determines which three discreet collision energies will be applied to the precursor mass in an Enhanced Product Ion (EPI) or MS/MS/MS (MS3) experiment when CES is used. By entering a collision energy spread value, CES is automatically turned on.

TDF CE (Time Delayed Fragmentation Collision Energy): The TDF CE parameter controls the potential difference between RO2 and RO3 for TDF (Time Delayed Fragmentation) scans. This is the amount of energy that the precursor ions receive as they are accelerated into Q3, where they collide with gas molecules and fragment.

Q3 Cool Time: The Q3 Cool Time parameter controls the amount of time that the precursor ions are allowed to cool prior to collection of their product ions in TDF (Time Delayed Fragmentation) scans.

Q3 Entry Barrier: The Q3 Entry Barrier parameter controls the potential difference between RO2 and RO3. It is used to transfer the ions from Q2 into the LIT.

AF2 (Excitation Energy): The AF2 parameter is the voltage of the auxiliary frequency (Aux RF) applied to Q3 during MS/MS/MS scans. It is used to fragment the isolated second precursor ion.

MS/MS/MS Fragmentation Time: The MS/MS/MS Fragmentation Time parameter controls the amount of time that the excitation energy is applied in MS/MS/MS scans. It is used in combination with the excitation energy to fragment the isolated second precursor ion.

MCS (Multi-Charge Separation) Barrier: The MCS Barrier parameter controls the voltage used when eliminating the singly-charged ions from the LIT in an EMC (Enhanced Multi-Charge) scan.

Q3 Empty Time: The Q3 Empty Time parameter controls the amount of time that singly-charged ions are removed from the LIT in an EMC (Enhanced Multi-Charge) scan.

Fixed LIT Fill Time: The Fixed LIT Fill Time parameter controls the amount of time that the LIT fills with ions.

DFT (Dynamic Fill Time): DFT will dynamically calculate the length of time that ions are collected in the LIT based on the incoming ion signal. When DFT is turned on the signal is optimized to either increase sensitivity or minimize space-charging.

EXB (Exit Barrier): The EXB parameter controls the voltage on the exit lens. It is used in LIT scans to mass-selectively eject ions from the LIT. It affects the peak width, the peak shape, and the intensity of the ion signal.

AF3 (Trap RF Amplitude): The AF3 parameter controls the zero-to-peak voltage of the auxiliary frequency (Aux RF) applied to Q3 when ejecting ions from the LIT. The AF3 parameter affects the peak width, the peak shape, and the intensity of the ion signal.

Detector Parameters

The following parameters affect the detector.

CEM (CEM): The CEM parameter controls the voltage applied to the detector. The voltage controls the detector response.

DF (Deflector): The DF parameter controls the voltage applied to the deflector. It is used to direct ions into the detector. It is preset to be in fixed mode.

Scan Types

You can perform quadrupole-mode and LIT-mode scans either individually or in combination when analyzing your sample.

Scan Techniques

MS: In MS scans, also referred to as single MS scans, ions are separated according to their mass-to-charge ratio (*m/z*). A single MS scan might be used to determine the molecular weight of a compound. Single MS scans can also be referred to as survey scans. MS scans do not provide any information about the chemical make-up of the ions other than the mass. Perform MS/MS or MS/MS/MS scans to obtain more information about the ions.

MS/MS: MS/MS scans are used to determine a molecular species.

- For MS/MS scans in triple quadrupole systems, precursor ion fragmentation occurs in the collision cell.

- For MS/MS scans in QTRAP® systems, precursor ion fragmentation can occur in the collision cell or the linear ion trap.

If enough energy is used, then the precursor ion fragments to produce characteristic product ions.

MS/MS/MS: The linear ion trap (LIT) system MS/MS/MS scans go one step further than MS/MS scans. A fragment that is produced in the collision cell is fragmented further in the linear ion trap to give more structural information about the molecular ion.

Quadrupole-Mode Scan Types

Triple quadrupole instruments have high-sensitivity Multiple Reaction Monitoring (MRM) capabilities required for quantitation experiments. In addition, they have highly specific scan types, such as precursor ion and neutral loss scans, which allow a more advanced search to be performed on the components of the samples.

Q1 MS (Q1): A full scan type using the first quadrupole (Q1). The ion intensity is returned for every mass in the scan range.

Q1 Multiple Ions (Q1 MI): A zero-width scan type using the Q1 quadrupole. The ion intensity is returned for the specified masses only.

Q3 MS (Q3): A full scan type using the third quadrupole (Q3). The ion intensity is returned for every mass in the scan range.

Q3 Multiple Ions (Q3 MI): A zero-width scan type using the Q3 quadrupole. The ion intensity is returned for the specified masses only.

MRM (MRM): An MS/MS scan in which a user-selected ion is passed through the Q1 quadrupole and then fragmented in the Q2 collision cell. The Q3 quadrupole then selects the fragment ion that enters the detector. This scan mode is used primarily for quantitation.

Product Ion (MS2): An MS/MS full scan where the Q1 quadrupole is fixed to transmit a specific precursor ion and the Q3 quadrupole scans a defined mass range. Used to identify all of the products of a particular precursor ion.

Precursor Ion (Prec): An MS/MS scan where the Q3 quadrupole is fixed at a specified mass-to-charge ratio to transmit a specific product ion and the Q1 quadrupole scans a mass range. Used to confirm the presence of a precursor ion or, more commonly, to identify compounds sharing a common product ion.

Neutral Loss (NL): An MS/MS scan where both the Q1 quadrupole and the Q3 quadrupole scan a mass range, a fixed mass apart. A response is observed if the ion chosen by the Q1 quadrupole fragments by losing the neutral loss (the fixed mass) specified. Used to confirm the presence of a precursor ion or, more commonly, to identify compounds sharing a common neutral loss.

LIT-Mode Scan Types

The LIT-mode scans use the Q3 quadrupole as a linear ion trap. Ions are trapped and stored in the Q3 quadrupole before being scanned out, giving increased sensitivity. In addition, MS/MS/MS analysis can be performed in the linear ion trap, providing more information about the sample. LIT-mode scan types are typically used for qualitative measurements.

Enhanced MS (EMS): Ions are scanned in the Q1 quadrupole to the linear ion trap where they are collected. These ions are scanned out of the Q3 quadrupole to produce single MS type spectra.

Enhanced Multi-Charge (EMC): This scan type is similar to the EMS scan except that before ions are scanned out of the linear ion trap, there is a delay period during which low-charge state ions (primarily singly-charged ions) are allowed to preferentially escape from the linear ion trap. When the retained ions are scanned out, the multiply-charged ion population dominates the resulting spectrum.

Enhanced Product Ion (EPI): This scan type is used to obtain a high quality MS/MS spectrum on a specific ion. The fragmentation is done in the Q2 collision cell and thus provides the information-rich MS/MS spectrum typical of collisionally activated dissociation (CAD) fragmentation. In this scan mode, the precursor ion to be fragmented is first selected in the Q1 quadrupole with a mass window that is 1 Da to 4 Da wide, filtering out all other ions. The precursor ion is fragmented by CAD gas in the Q2 collision cell. The fragment ions generated are

captured in the linear ion trap and then scanned out at one of three scan speeds, depending on the required fragment ion resolution.

For IDA experiments, the **Product Of** field is set to 30 Da by default, and this value should not be changed.

Enhanced Resolution (ER): This scan type is similar to the EMS scan except that a small 30 Da mass around the precursor mass is scanned out of the linear ion trap at the slowest scan rate to produce a narrow window of the best-resolved spectra.

MS/MS/MS (MS3): A precursor ion is selected by the Q1 quadrupole and fragmented with collisionally activated dissociation in the Q2 collision cell. The resulting product ions are all transmitted to the linear ion trap, where a single product ion is then isolated. The isolated ion is further fragmented in the linear ion trap, and the resulting product ions are scanned out of the trap at one of three scan speeds. As with any in-trap Collision Induced Disassociation (CID) technique, there is a low mass cut-off for the second MS/MS step due to the condition that the lowest mass fragment and precursor must be simultaneously stable in the trap. For QTRAP® systems, this results in the loss of ions lower than 28 percent of the mass of the precursor ion during MS3 experiments. This phenomenon is often referred to as the one-third cut-off rule.

Time Delayed Frag (TDF): Product ions are generated and collected in the linear ion trap. During the first part of the collection period, the lower mass ions are not collected. During the second part of the collection period, all masses in the mass range of interest are collected. The resulting enhanced product ion spectra are similar to EPI scan type spectra. The nature of the spectra aids in the interpretation of the structure and fragmentation pathways of the molecule of interest.

Toolbar Icons C

For additional toolbar icons, refer to the *Advanced User Guide*.

Table C-1 Tool Bar Icons

Icon	Name	Description
	New Subproject	Creates a subproject. **Subprojects** can only be created later in the process if the project was originally created with subprojects.
	Copy Subproject	Copies a Subproject folder. Subprojects can be copied only from another project that has existing subprojects. If the same folders exist at both the project and subproject levels, then the software uses the project level folders.

Table C-2 Acquisition Method Editor Icons

Icon	Name	Description
	Mass Spec	Click to show the MS tab in the Acquisition Method editor.
	Period	Right-click to add an experiment, add an **IDA Criteria Level**, or delete the period.
	Autosampler	Click to open the Autosampler Properties tab.
	Syringe Pump	Click to open the Syringe Pump Properties tab.
	Column Oven	Click to open the Column Oven Properties tab.
	Valve	Click to open the Valve Properties tab.
	DAD	Click to open the DAD Method Editor. Refer to Show DAD Data on page 59.
	ADC	Click to open the ADC Properties tab. Refer to Show ADC Data on page 50.

Table C-3 Acquire Mode Icons

Icon	Name	Description
	View Queue	Shows the sample queue.
	Instrument Queue	Shows a remote instrument station.
	Status for Remote Instrument	Shows the status of a remote instrument.
	Start Sample	Starts the sample in the queue.
	Stop Sample	Stops the sample in the queue.
	Abort Sample	Aborts a sample acquisition in the middle of the processing of that sample.
	Stop Queue	Stops the queue before it has completed processing all the samples.
	Pause Sample Now	Inserts a pause in the queue.
	Insert Pause before Selected Sample(s)	Inserts a pause before a specific sample.
	Continue Sample	Continues acquiring the sample.
	Next Period	Starts a new period.
	Extend Period	Extends the current period.
	Next Sample	Stops acquiring the current sample and starts acquiring the next sample.
	Equilibrate	Selects the method to be used to equilibrate the devices. This method should be the same as the method used with the first sample in the queue.
	Standby	Puts the instrument in Standby mode.
	Ready	Puts the instrument in Ready mode.

Table C-3 Acquire Mode Icons (continued)

Icon	Name	Description
T	Reserve Instrument for Tuning	Reserves the mass spectrometer for tuning and calibrating.
	IDA Method Wizard	Starts the IDA Method Wizard.
P	Purge Modifier	Starts the modifier purge from the modifier pump.

Table C-4 Tune and Calibrate Mode Icons

Icon	Name	Description
	Calibrate from spectrum	Opens the Mass Calibration Option dialog and uses the active spectrum to calibrate the mass spectrometer.
	Manual Tune	Opens the Manual Tune Editor.
	Compound Optimization	Optimizes for a compound using infusion by FIA.
A	Instrument Optimization	Verifies the instrument performance, adjusts the mass calibration, or adjusts mass spectrometer settings.
	View Queue	Views the sample queue.
	Instrument Queue	Views a remote instrument.
	Status for Remote Instrument	Views the status of a remote instrument.
T	Reserve Instrument for Tuning	Reserves the instrument for tuning and calibrating.
	IDA Method Wizard	Starts the IDA Method Wizard.

Table C-5 Explore Quick Reference: Chromatograms and Spectrum

Icon	Name	Description
	Open Data File	Opens files.
	Show Next Sample	Goes to the next sample.
	Show Previous Sample	Goes to the previous sample.
	Go To Sample	Opens the Select Sample dialog.
	List Data	Views the data in tables.
	Show TIC	Generates a TIC from a spectrum.
	Extract Using Dialog	Extracts ions by selecting masses.
	Show Base Peak Chromatogram	Generates a BPC.
	Show Spectrum	Generates a spectrum from a TIC.
	Copy Graph to new Window	Copies the active graph to a new window.
	Baseline Subtract	Opens the Baseline Subtract dialog.
	Threshold	Adjusts the threshold.
	Noise Filter	Shows the Noise Filter Options dialog, which can be used define the minimum width of a peak. Signals below this minimum width are regarded as noise.
	Show ADC	Shows ADC data.
	Show File Info	Shows the experimental conditions used to collect the data.
	Add arrows	Adds arrows to the X-axis of the active graph.
	Remove all arrows	Removes arrows from the X-axis of the active graph.
	Offset Graph	Compensates for slight differences in the time during which the ADC data and the mass spectrometer data were recorded. This is useful when overlaying graphs for comparison.

Table C-5 Explore Quick Reference: Chromatograms and Spectrum (continued)

Icon	Name	Description
	Force Peak Labels	Labels all of the peaks.
	Expand Selection By	Sets the expansion factor for a portion of a graph to be viewed in greater detail.
	Clear ranges	Returns the expanded selection to normal view.
	Set Selection	Defines start and stop points for a selection. This feature provides more accurate selection than is possible by selecting the region using the cursor.
	Normalize To Max	Scales a graph to maximum size, so that the most intense peak is scaled to full scale, whether or not it is visible.
	Show History	Shows a summary of data processing operations performed on a particular file, such as smoothing, subtraction, calibration, and noise filtering.
	Open Compound Database	Opens the compound database.
	Set Threshold	Adjusts the threshold.
	Show Contour Plot	Shows selected data as either a spectrum graph or an XIC. Additionally, for data acquired by a DAD, a contour plot can show selected data as either a DAD spectrum or an XWC.
	Show DAD TWC	Generates a TWC of the DAD spectrum.
	Show DAD Spectrum	Generates a DAD spectrum.
	Extract Wavelength	Extracts up to three wavelength ranges from a DAD spectrum to view the XWC.

Table C-6 Explore Toolbar Quick Reference: Overlaying Graphs

Icon	Name	Description
	Home Graph	Click to return the graph to the original scale.
	Overlay	Click to overlay graphs.

Table C-6 Explore Toolbar Quick Reference: Overlaying Graphs (continued)

Icon	Name	Description
	Cycle Overlays	Click to cycle between overlaid graphs.
	Sum Overlays	Click to add the graphs together.

Table C-7 Explore Toolbar Quick Reference: Fragment Interpretation Tool

Icon	Name	Description
	Show Fragment Interpretation Tool	Click to open Fragment Interpretation tool, which calculates the single, non-cyclic bond cleavage fragments from a .mol file.

Table C-8 Navigation Icons on the Explore Toolbar

Icon	Name	Function
	Open File	Click to open files.
	Show Next Sample	Click to navigate to the next sample.
	Show Previous Sample	Click to navigate to the previous sample.
	GoTo Sample	Click to open the Select Sample dialog.
	List Data	Click to view the data in tables.
	Show TIC	Click to generate a TIC from a spectrum.
	Extract Using Dialog	Click to extract ions by selecting masses.
	Show Base Peak Chromatogram	Click to generate a BPC.
	Show Spectrum	Click to generate a spectrum from a TIC.
	Copy Graph to new Window	Click to copy the active graph to a new window.
	Baseline Subtract	Click to open the Baseline Subtract dialog.

Table C-8 Navigation Icons on the Explore Toolbar (continued)

Icon	Name	Function
	Threshold	Click to adjust the threshold.
	Noise Filter	Click to use the Noise Filter Options dialog to define the minimum width of a peak. Signals below this minimum width are regarded as noise.
	Show ADC	Click to view ADC data.
	Show File Info	Click to show the experimental conditions you used to collect your data.
	Add arrows	Click to add arrows to the x-axis of the active graph.
	Remove all arrows	Click to remove arrows from the x-axis of the active graph.
	Offset Graph	Click to compensate for slight differences in the time during which the ADC data and the mass spectrometer data were recorded. This is useful when overlaying graphs for comparison.
	Force Peak Labels	Click to label all the peaks.
	Expand Selection By	Click to set the expansion factor for a portion of a graph that you want to view in greater detail.
	Clear ranges	Click to return the expanded selection to normal view.
	Set Selection	Click to type start and stop points for a selection. This provides more accurate selection than is possible by highlighting the region using the cursor.
	Normalize to Max	Click to scale a graph to maximum, so that the most intense peak is scaled is to full scale, whether or not it is visible.
	Show History	Click to view a summary of data processing operations performed on a particular file, such as smoothing, subtraction, calibration, and noise filtering.
	Open Compound Database	Click to open the compound database.
	Set Threshold	Click to adjust the threshold.
	Show Contour Plot	Click to display selected data as either a spectrum graph or an XIC. Additionally, for data acquired by a DAD, a contour plot can display selected data as either a DAD spectrum or an XWC.
	Show DAD TWC	Click to generate a TWC of the DAD.

Table C-8 Navigation Icons on the Explore Toolbar (continued)

Icon	Name	Function
	Show DAD Spectrum	Click to generate a DAD spectrum.
	Extract Wavelength	Click to extract up to three wavelength ranges from a DAD spectrum to view the XWC.

Table C-9 Integration Tab and Quantitation Wizard Icons

Icon	Name	Description
	Set parameters from Background Region	Uses the selected peak.
	Select Peak	Uses the selected background.
	Manual Integration Mode	Manually integrates peaks.
	Show or Hide Parameters	Toggles the peak-finding parameters between shown and hidden.
	Show Active Graph	Shows the analyte chromatogram only.
	Show Both Analyte and IS	Shows the analyte and its associated chromatogram (available only when an associated internal standard exists).
	Use Default View for Graph	Returns to the preset (view all data) view (if, for example, the user has zoomed in on a chromatogram).

Table C-10 Results Table Icons

Icon	Name	Description
	Sort Ascending by Selection	Sorts the selected column by ascending values.
	Sort Descending by selection	Sorts the selected column by descending values.
	Lock Or Unlock Column	Locks or unlocks the selected column. A locked column cannot be moved.
	Metric Plot By Selection	Creates a metric plot from the selected column.

Table C-10 Results Table Icons (continued)

Icon	Name	Description
	Show all Samples	Shows all the samples in the Results Table.
	Delete Formula Column	Deletes formula columns.
	Report Generator	Opens the Reporter software.

Table C-11 Icon Quick Reference: Quantitate Mode

Icon	Name	Description
	Add/Remove Samples	Adds or removes samples from the Results Table.
	Export as Text	Saves the Results Table as a text file.
	Modify Method	Opens a wiff file.
	Peak Review - Pane	Opens peaks in a pane.
	Peak Review - Window	Opens peaks in a window.
	Calibration - Pane	Opens the calibration curve in a pane.
	Calibration - Window	Opens the calibration curve in a window.
	Show First Peak	Shows the first peak in the pane or window.
	Show Last Peak	Shows the last peak in the pane or window.
	Show Audit Trail	Shows the audit trail for the Results Table.
	Clear Audit Trail	Clears the audit trail for the Results Table. This functionality is not available.
	Statistics	Opens the Statistics window.
	Report Generator	Opens the **Reporter** software.

Revision History

Revision	Reason for Change	Date
A	First release of document.	April 2013
B	• Changed AB SCIEX to SCIEX where required. • Updated the copyright page. • Added reference to 6500+ series of instruments where required. • Added Revision History. • Removed 2007 from chapter 7.	June 2015
C	New templates were applied to the content, which has led to some edit changes in the content and also content reorganization. All the tables listing the various types of toolbars are now moved into one section in the appendix. Added a new tip in the View Experimental Conditions section. Added a new screenshot in Show Basic Quantitative Data section. Total Ion Chromatograms section is updated with new content and a screenshot. Show a Spectrum from a TIC section is updated with additional content and a screenshot. Changed screenshot in the Extract Ion by Selecting Masses section. Data Processing section renamed to Graphical Data Processing. The Work with Graphs in Panes section renamed to Manage Data. Removed information related to 2000 and 3000 systems. Also removed descriptions for Focusing Potential and Collision Cell Entrance Potential. Duplicate content that is already present in the *Advanced User Guide* has been removed from this guide.	October 2017