Analyst® 1.6 Software

Getting Started Guide

Release Date: August 2011

AB SCIEX

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AB SCIEX

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Contents

Foreword

This guide is for instrument operators who are new to the Analyst® software. You can use the procedures to learn how to use the software and the instrument.

WARNING! Risk of personal injury or instrument damage. If you need to move the system, contact an FSE to assist you.

Related Documentation

The guides and tutorials for the instrument and the Analyst software are installed automatically with the software and are available from the Start menu: All Programs > AB SCIEX > Analyst. A complete list of the available documentation can be found in the Help. To view the Analyst software Help, press F1.

Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the Web site at www.absciex.com.


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Creating 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.

Topics in this section:

Hardware Profiles

You can set up multiple hardware profiles, but only one profile can be active at any time. A hardware profile tells the Analyst software what instrument and devices you want to use, and how the instrument and the devices are configured and connected to the computer.

Each hardware profile must include a mass spectrometer. Before creating an acquisition method, make sure that all devices you want to use in the method are included in the hardware profile, including a syringe pump, if your instrument comes with an integrated syringe pump. Only peripheral devices included in the active hardware profile can be used when creating acquisition methods.

When you create a hardware profile in the Hardware Configuration Editor, you must also configure the peripheral devices that you add 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, you can set up the appropriate configuration information.

For information about setting up the physical connections, see the *Peripheral Devices Setup Guide*. For a list of the supported peripheral devices, see the Analyst software *Installation Guide*.

Topics in this section:

Creating a Hardware Profile

You can add peripheral devices to this hardware profile.

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

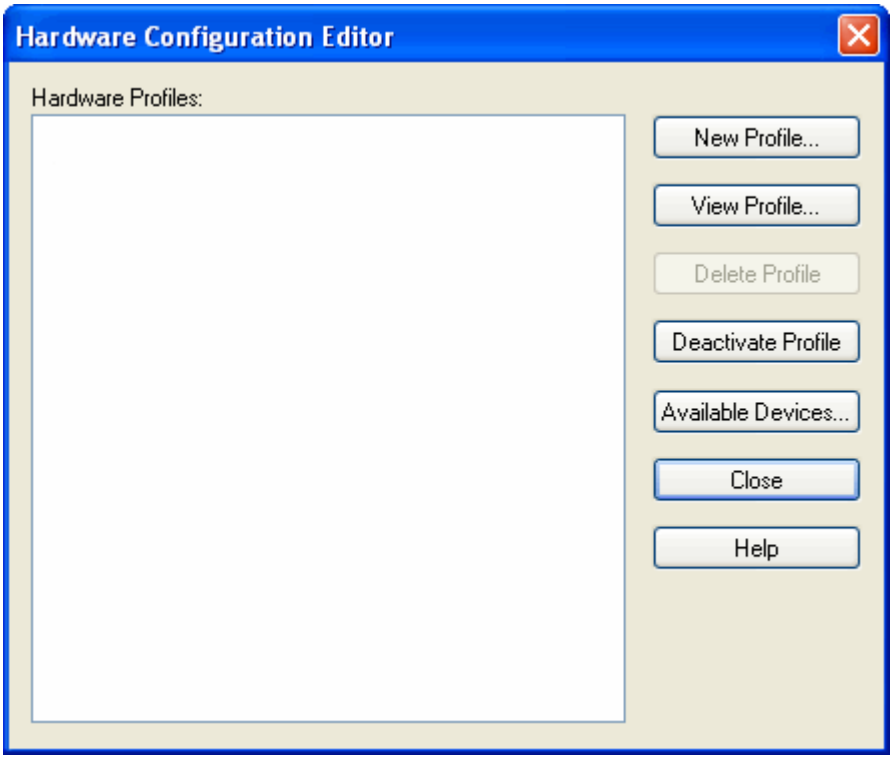


Figure 1-1 Hardware Configuration Editor dialog

2. In the **Hardware Configuration Editor** dialog, click **New Profile**.

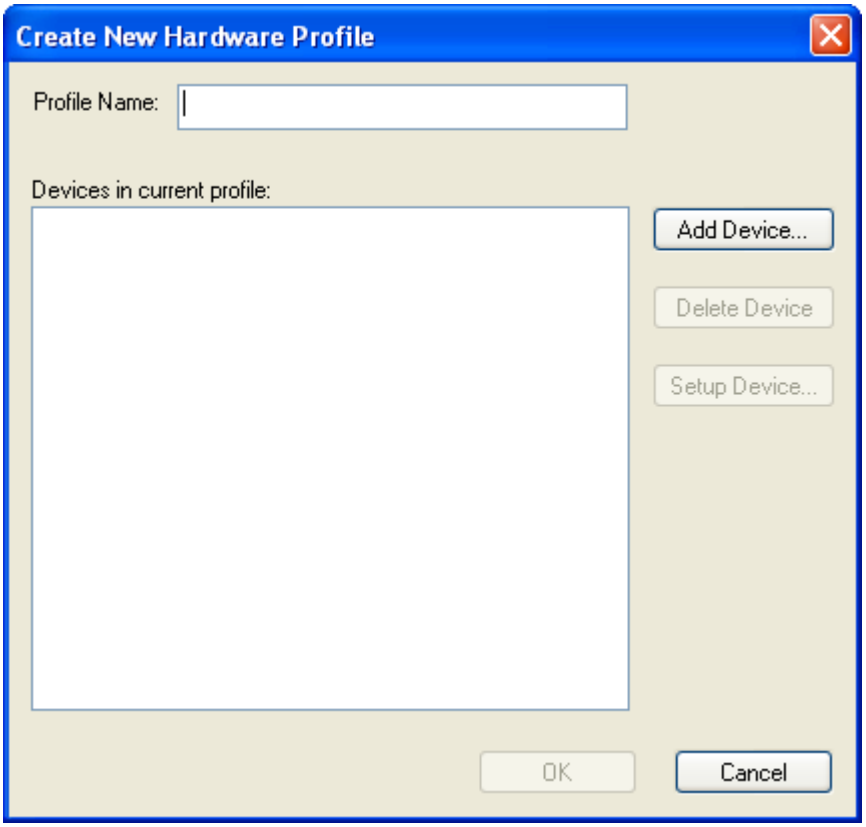


Figure 1-2 Create New Hardware Profile

3. In the **Profile Name** field, type a name for the profile.
4. Click **Add Device**.

   In the Available Devices dialog, in the Device Type field, Mass Spectrometer is the default value (Figure 1-3).

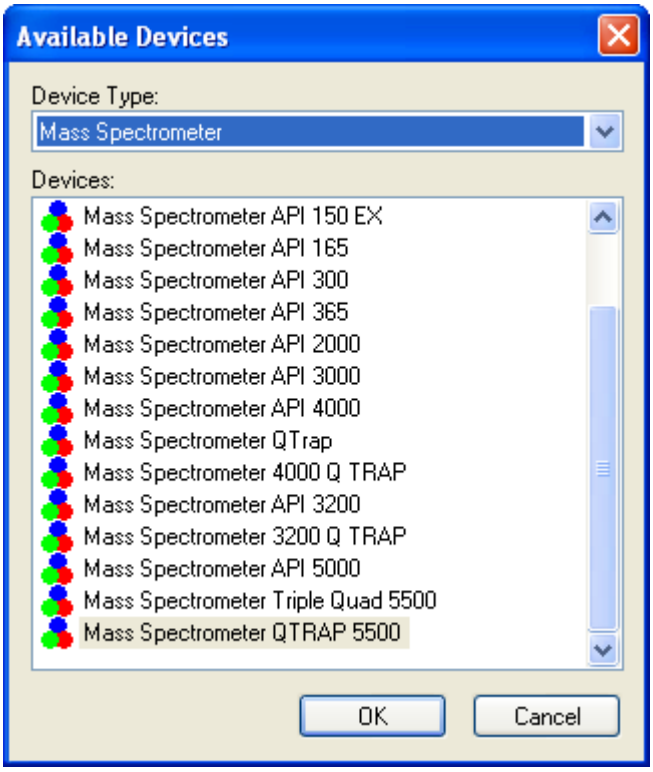


Figure 1-3 Available Devices dialog

5. In the **Devices** list, select the instrument and then click **OK**.
6. In the **Devices in current profile** list, select the instrument and then click **Setup Device**.
7. Select the features on the **Configuration** tab and **Communication** tab as required.
8. Click **OK** to return to the **Create New Hardware Profile** dialog.
9. Click **Add Device** and then add and configure each device that you are using with the instrument. See Adding Devices to Hardware Profiles on page 13.
10. On the **Create New Hardware Profile** dialog, click **OK**.
11. To activate the hardware profile, on the **Hardware Configuration Editor**, click the hardware profile and then click **Activate Profile**.

    A green check mark appears next to the profile.

Tip!: You do not have to deactivate one hardware profile before activating another. Just click the hardware profile that you want to activate and then click **Activate Profile**; the other profile is deactivated automatically.

12. Click **Close**.
13. Next steps: You can either create projects and subprojects or you can optimize the instrument. For more information, see Creating Projects and Subprojects on page 17 or Using the Instrument Optimization Feature on page 22.

Adding Devices to Hardware Profiles

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

1. Open the Hardware Configuration Editor.
2. In the **Hardware Profiles** list, make sure the hardware profile has been deactivated.
3. Click **Edit Profile**.
4. Click **Add Device**.
5. In the **Available Devices** dialog, in the **Device Type** list, select the device and then click **OK**.

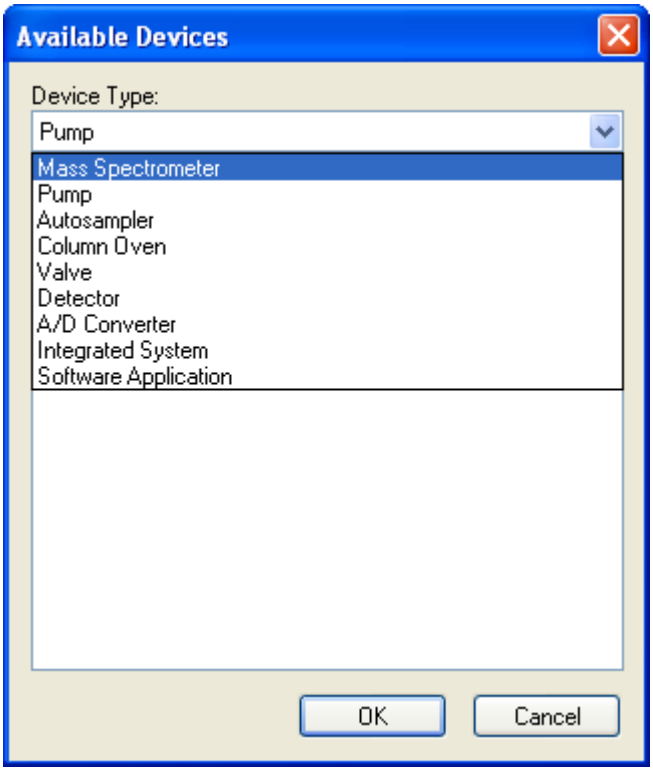


Figure 1-4 Available Devices dialog

Note: Remember to add a mass spectrometer. For more information, see Creating a Hardware Profile on page 10.

6. In the **Devices in current profile** list, select the device, and then click **Setup Device**.

   A dialog containing configuration values for the device appears.

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

7. 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. If you are using the serial expansion cable, the number selected in the profile is the number on the cable plus two.

   - If the device uses Serial Port as a communication interface, in the COM Port Number list, select the COM port that the device is connected to.
   - If the device uses Ethernet as a communication interface, type the IP address assigned to the device by the administrator or use the corresponding host name for the address.
   - If the device uses a GPIB board as a communication interface, do not change the settings for the GPIB board.

   The rest of the preset values for the device are likely appropriate; do not change them. For information about the Configuration and Communication tabs, see the Help.
8. To restore the device preset values, on the **Communication** tab, click **Set Defaults**.
9. To save the configuration, click **OK**.
10. Repeat steps 4 to 9 for each device.
11. On the **Create New Hardware Profile** dialog, click **OK**.
12. To activate the hardware profile, on the **Hardware Configuration Editor**, click the hardware profile and then click **Activate Profile**.

    The check mark should turn green. If a red x appears then there is a problem with the hardware profile activation. For more information see, Troubleshooting Hardware Profile Activation.

Tip!: You do not have to deactivate one hardware profile before activating another. Just click the hardware profile that you want to activate and then click **Activate Profile**; the other profile is deactivated automatically.

13. Click **Close**.

Troubleshooting Hardware Profile Activation

1. Read the error message generated. Depending on the message, there may be an issue with a device or how the communication is set up.
2. Verify that the peripheral device has power and is turned on.
3. Verify that the COM port assigned to the peripheral device is correct.

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 with the peripheral device (for example, dip switch settings) are set correctly and match the settings in the **Communication** tab.

5. Turn off the power to the peripheral device, wait 10 seconds, and then turn it back on. Wait until all peripheral device power-up activities are complete before trying to activate the hardware profile again. Some peripheral devices may require 30 seconds or more to complete their power-up activities.
6. If the issue persists, delete the failing profile and then create a new one.
7. If you are still having problems contact technical support.

Projects and Subprojects

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

Topics in this section:

Project Organization

A project is a folder structure for organizing and storing sample information, data, quantitation information and so forth. Within each project there are folders that can contain different types of files; for example, the Data folder contains acquisition data files. Table 1-1 describes the contents of the different folders.

The software can access a project only if it is stored in a root folder. You cannot create projects in a folder that has not been defined as a root folder.

The preset root folder is Analyst Data on the drive where the Analyst® software is installed. If you want to store projects in other locations, you must create new root folders. For more information about root folders, see the Help system.

Table 1-1 Project Folders

Folder	Contents
\Acquisition Methods	Contains all acquisition methods used. Acquisition methods have the .dam extension.
\Acquisition Scripts	Contains all the acquisition batch scripts available.
\Batch	Contains all the acquisition batch files used. Acquisition batches have the .dab extension. It also contains a subfolder, Templates, that contains acquisition batch templates. Batch templates have the .dat extension.
\BioAnalyst	Contains files used with the BioAnalyst™ software add-on, a protein analysis tool.

Table 1-1 Project Folders (Continued)

Folder	Contents
\Data	Contains the acquisition data files (.wiff extension).
\Log	Contains results of quantitation and compound optimization.
\Processing Methods	Contains all qualitative data processing methods used.
\Processing Scripts	Contains all data processing scripts available. Processing scripts stored in the API Instrument project appear in the Scripts menu.
\Project Information	Contains all project information and settings for the project. This folder cannot be stored in a subproject.
\Quantitation Methods	Contains all quantitation methods used. Quantitation methods have a .qmf extension.
\Results	Contains all quantitation results table files (.rdb extension).
\Templates	Contains report templates (.rpt extension).

About Subprojects

A subproject contains a subset of the folders in the project. All subprojects must contain the same folders. Subprojects can be very useful for organizing your data. For example, if you are running samples of various compounds from different laboratories using the same acquisition method, you could create subprojects to store the results for each laboratory, but leave the acquisition method folder in the project. The acquisition method would then be available for use with subproject or laboratory. Alternatively, if you were running samples over a period of several weeks, you could store the results from each day in a separate subproject.

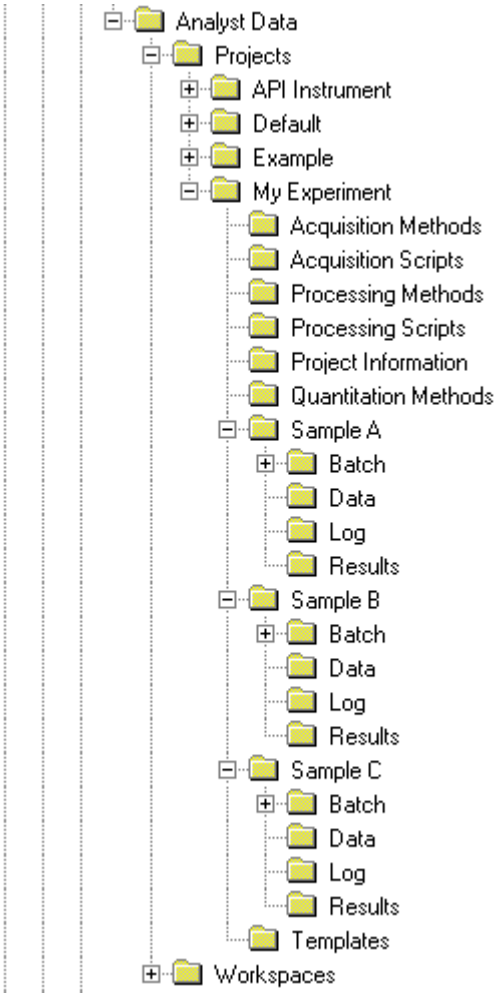


Figure 1-5 Example of a project and subproject folder structure

Creating Projects and Subprojects

If you want to use a subproject structure within a project, you must create a subproject when you first create the project. You cannot create a subproject in an existing project that does not already have a subproject structure.

1. Click **Tools > Project > Create Project**.
2. In the **Project name** field, type a project name.
3. If you want to use subprojects in this project, select the folders that you want to store in the subprojects and then use the arrow buttons to move them to the **Subproject folders** list.

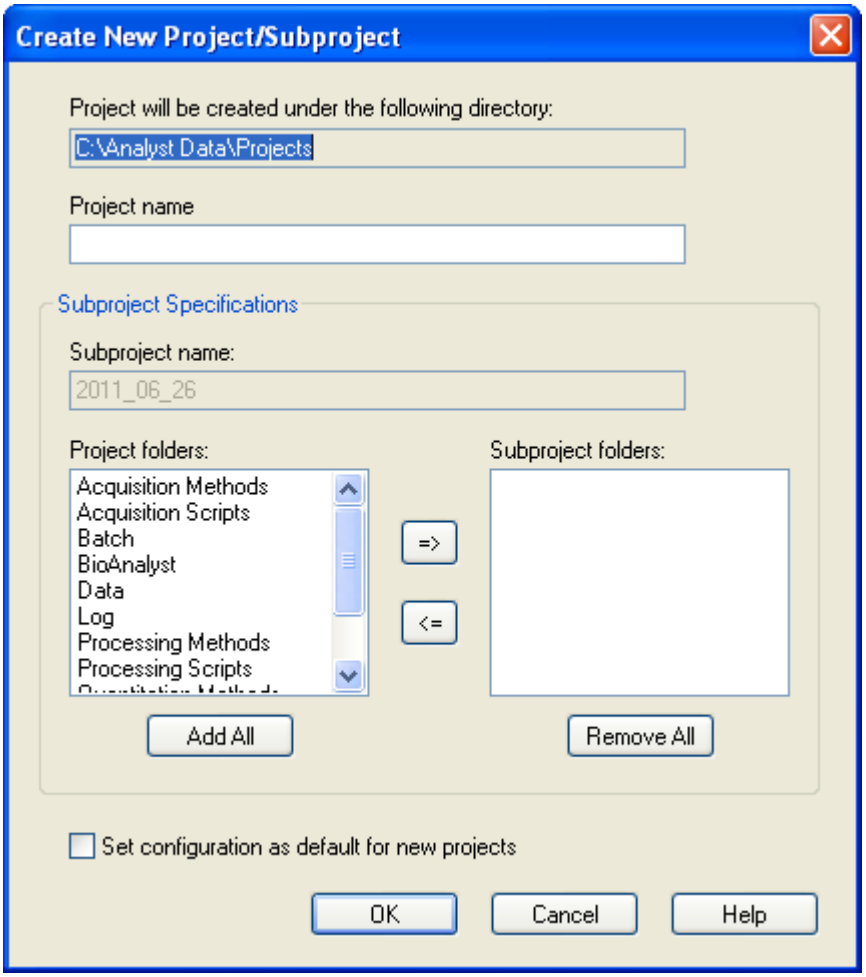


Figure 1-6 Create new Project/Subproject dialog

Note: You cannot create a new subproject for a project that was not originally created with a subproject.

4. If you are using subprojects, in the **Subproject name** field, type a name for the first subproject or use the existing date.
5. If you want 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 will be created with this folder configuration.
6. Click **OK**.

Creating a Subproject

1. On the Project toolbar, in the **Project** list, select the project in which you want to create a subproject.
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**.

Copying a Subproject

Note: You can copy a subproject from another project that has existing subprojects; however, the copied subprojects may contain folders that also exist in the project folder. When the same folders exist at both the project and subproject levels, the software uses the project level folders.

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

   The Copy Subproject dialog appears.
2. Click **Browse** to navigate to the subproject source and then click **OK**.
3. In the **Source Subproject** list, select the subproject to be copied.
4. Click **Browse** to navigate to the subproject destination.
5. In the **Target Subproject** field, type the name for the copied subproject and then click **OK**.
6. 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.
7. Click **Copy**.

To switch between projects and subprojects

- On the Analyst software toolbar, from the project selection list, select the project or subproject.

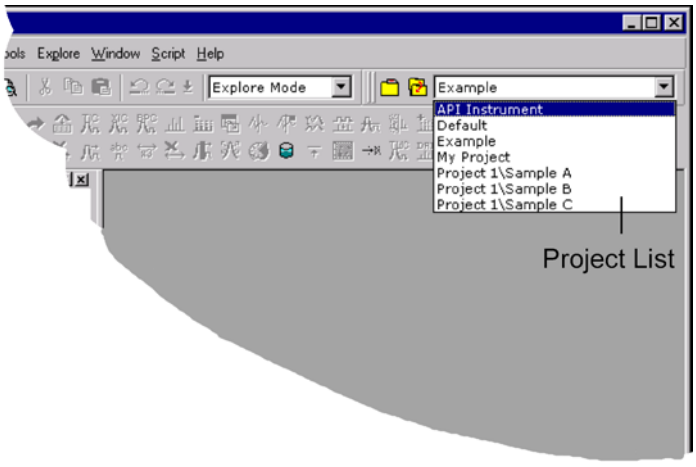


Figure 1-7 Analyst toolbar

Installed Projects

Three projects are installed with the software: API Instrument, Default, and Example.

API Instrument Project

The API Instrument project is unique and very important to the proper functioning of the instrument. The API Instrument project contains the information required for tuning and calibrating your instrument. 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 project 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 project in the Tuning Cache folder and named with the date and time they were created. The Tuning Cache needs to be emptied on a regular basis.

Default Project

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

Example Project

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

Backing up the API Instrument Project

You should routinely back up this project. You should also back up this project after routine maintenance has been done.

1. To create the backup, copy the API Instrument project, paste it to a different location, preferably to another computer, and then rename the folder. You should use the date, and an instrument reference if you have more than one instrument, when you rename the folder; for example, API Instrument__4000QTRAP3_010107
2. To recover the project, rename the current API Instrument folder, and then copy the backup into the Projects folder and then change its name back to API Instrument.

Table 1-2 Icons on the Analyst Software Toolbar

Icon	Name	Function
	New Subproject	Click to create a subproject.You can create subprojects later in the process only if the project was originally created with subprojects.
	Copy Subproject	Click to copy a Subproject folder. You can copy a subproject from another project that has existing subprojects; however, the copied subprojects may contain folders that also exist in the project folder. When the same folders exist at both the project and subproject levels, the software uses the project level folders.

Instrument Tuning and Calibrating 2

In this section, you will learn how to use the Instrument Optimization software to tune, calibrate, and optimize the instrument to get the best performance.

You should run the Verify instrument performance option weekly or after you clean the instrument to confirm that the system is working properly. In general, the calibration and resolution for quadrupole are fine for 3 to 6 months unless the system loses vacuum. For LIT (linear ion trap) systems, the resolution should also be good for 3 to 6 months but the calibration should be done approximately monthly. If the system loses vacuum then you should check the calibration and resolution before using the system.

About Tuning and Calibrating

Tuning the instrument is the process of optimizing the resolution and instrument parameters to attain the best sensitivity and performance of the mass spectrometer. Optimizing the resolution means adjusting the peak width and peak shape. You can tune and calibrate the instrument either automatically or manually.

Automatic tuning: The software performs resolution optimization and mass calibration, using the Instrument Optimization wizard. For LIT instruments, MS3 optimizations are also performed.

Manual tuning: You can perform many of the instrument resolution optimizations and calibrations manually.

Automatically Tuning and Calibrating

Instrument Optimization is automatic instrument tuning software that tunes both quadrupole and LIT modes and performs mass calibration. For quadrupole mode, it adjusts the resolution offsets. For LIT mode, it optimizes AF3 and EXB. For MS3, it adjusts the Excitation and Isolation coefficients. You can select one of the instrument performance options:

- **Verify instrument performance**: Tests the instrument performance but leaves the instrument settings unchanged. A report is generated at the end of the test. You can use this option weekly to check how well the instrument is performing.
- **Adjust mass calibration only**: Automatically checks and adjusts the mass calibration. If the mass calibration has changed, then the software corrects it. You can use this option weekly for LIT instruments, or monthly to check and adjust the mass calibration if required.
- **Adjust instrument settings**: Checks and adjusts the instrument settings and mass calibration. The instrument settings are updated from the current settings to optimal settings. You can use this option if instrument performance is poor or if the peak shape is bad. Only experienced users should adjust the instrument settings.

Note: Old LIT methods must be updated with the new settings. This can be done two ways. The first way is by toggling the LIT speed in the advanced MS tab and then saving the method. The second way is by using the Change All Methods script that is available from AB SCIEX technical support.

- **Reset selected scan modes to default values and adjust instrument settings**: Resets the instrument values to the factory preset values. Select this option if a major component of the instrument is replaced or after the first installation. *Only FSEs should use this feature.*

You can back up your current instrument parameters in case you want to restore them later. The preset location for the instrument parameters is C:\Analyst Data\Projects\API Instrument\Instrument Optimization\Instrument Settings Backups\User Created Backups.

Backing up Instrument Parameters

1. On the Navigation bar, double-click **Instrument Optimization**.
2. Click **File > Backup Instrument Settings**.
3. Type a file name and then click **Save**.

Restoring Instrument Parameters

1. On the Navigation bar, double-click **Instrument Optimization**.
2. Click **File** > **Restore Instrument Settings**.
3. Navigate to the instrument settings that you want to restore and then click **Open**.

Required material

- Tuning solutions that are supplied in the Standards Chemical Kit shipped with the system. If needed, a new Kit can be ordered from AB SCIEX. For information about the appropriate solutions that should be used in a system, see Calibration Ions and Solutions on page 125.
- 5 ml, 1 ml, and 250 µl serial gas-tight syringes (1.0 ml will be used as reference).
- PEEK (red) sample tubing.
- Syringe pump, if using an instrument without an integrated syringe pump.

Prerequisites

- Make that you have a printer configured.
- Make sure that the spray is stable and that the proper tuning solution is being used.

Using the Instrument Optimization Feature

The following procedure describes how to verify the performance of the instrument. For more information on using the other instrument performance options, see the Help.

1. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning**.
2. Run a calibration method and confirm that there is a stable TIC and that the peaks of interest are present in the spectrum.
3. On the Navigation bar, under **Tune and Calibrate**, double-click **Instrument Optimization**.
4. Click **Verify instrument performance** and then click **Next**.

5. Click **Approved Tuning** and then click **Next.**
6. Select a **Tuning Solution** from the list.

   Depending on the solution you choose, different modes are available.

   - Click a polarity.
   - If available, click **Q1** and **Q3** in the **Quad** section. If available, click the required scan speeds.
   - If available, click the scan speeds in the **LIT** section.
   - If available, click MS/MS/MS options and then click **Next**.
7. If the **Select a mode** page appears, select **Automatic** and then click **Next.**
8. Click **GO**. In this example, the preset values are suitable.

   The Verifying or Adjusting Performance screen appears. After the process has completed, the Results Summary appears. For more information, see the Help system.
9. Depending on what you had selected, you will be prompted to change solutions for the various scan types and polarities.

Verifying or Adjusting Performance

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

Current Spectrum: This graph displays the spectrum of the current scan, the optimal scan selected by the software, or the scan at the current parameter value when you are reviewing the software results in interactive mode.

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

Results Summary

The Results Summary, shown in Figure 2-1, is a record of any instrument settings changes that were made by the Instrument Optimization software. This includes the location of data files and instrument settings backups, as well as step-by-step changes and results during optimization. In addition, the Results Summary displays 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 labelled 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 saved as a document in the folder indicated at the top of the report. You can print the Results Summary or you can open a previously saved Results Summary.

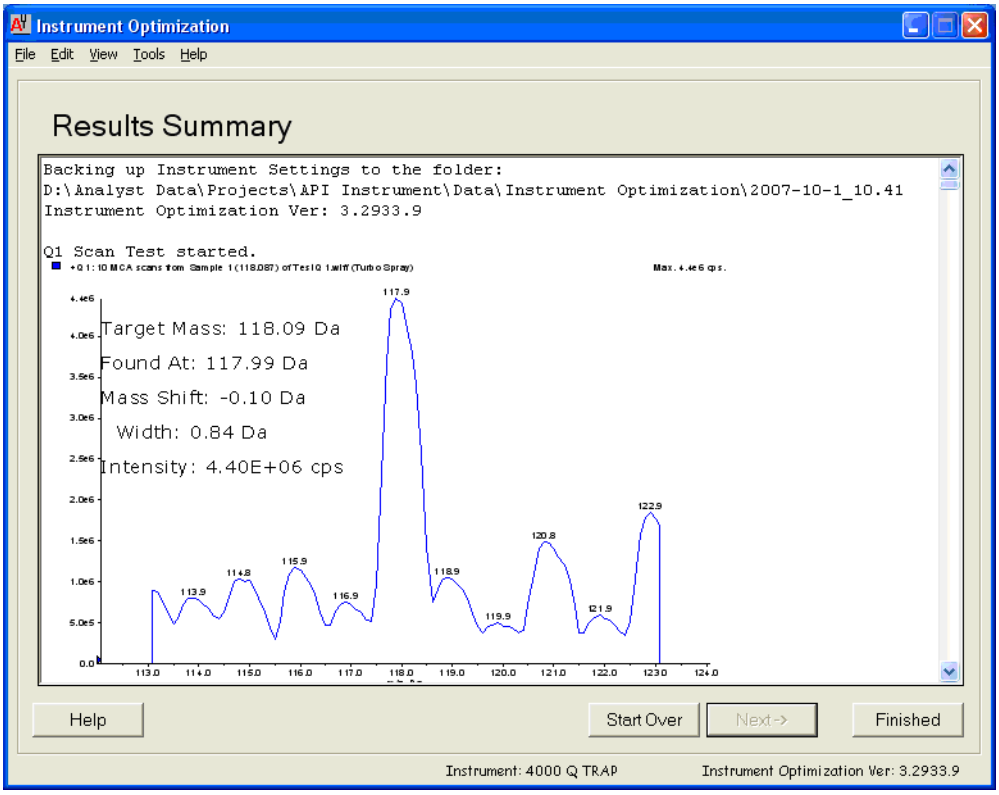


Figure 2-1 Results Summary

Creating Basic Methods

3

In this section, you will learn how to create methods that you can use for data acquisition.

Topics in this section:

About LC Methods

Creating an acquisition method using a peripheral device, such as an HPLC, includes providing the operating parameters for that device. If you are creating a new acquisition method file from an existing file, you may decide to use some or all of the peripheral device methods in the acquisition method.

Creating Mass Spectrometry Methods

You create the mass spectrometer acquisition method using the Acquisition Method Editor. Depending on the type of mass spectrometer configured and the scan type selected, different fields and options are available. As you type the mass spectrometer parameters, the Acquisition Method Editor validates the settings.

Topics in this section:

Spectral data can be acquired in one of three modes, as shown in Table 3-1:

Table 3-1 Spectral Data Acquisition

Mode	Description
Profile	The preset value is 0.1 Da. Profile data is the data generated by the instrument and corresponds to the intensity recorded at a series of evenly spaced discrete mass values. For example, for a mass range 100 Da to 200 Da and step size 0.1, the instrument scans 99.95 to 100.05 (records as value 100), 100.05 to 101.15 (records as value 101)…199.95 to 200.05 (records 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 (less data steps are made) but with the loss of peak shape information.

Table 3-1 Spectral Data Acquisition

Mode	Description
Centroid	The instrument scans as in Profile mode, but centroids the data, replacing found peaks with the intensity-weighted center of gravity for each peak. Centroiding has the advantage of significantly reducing file size with a loss of peak shape information. The disadvantage is that if data has been collected as a centroid it cannot be altered. It is recommended to use Profile mode and centroid the data post-acquisition.

You can create one of the following methods and use it in Creating and Submitting Batches on page 39 to acquire data:

- Creating an Acquisition Method using a Q1 MS Scan Type on page 26
- Creating an Acquisition Method using a Q1 MI Scan Type on page 28
- Creating an Acquisition Method using an MRM Scan Type on page 30

Required equipment

- Reserpine solution (see Table 3-2.) that is supplied in the Standards Chemical Kit shipped with the system. If needed, a new Kit can be ordered from the manufacturer.
- 5 mL, 1 mL, and 250 µL serial gas-tight syringes.
- PEEK (red) tubing transfer line.
- Syringe pump.

Table 3-2 Reserpine Concentrations

System	Reserpine Concentration
API 2000™ System and QTRAP® System	1 pmol/µl (1 µM)
API 3000™ System, API 3200™ System, and 3200 QTRAP System	0.167 pmol/µl (0.167 µM or 6:1)
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)
AB SCIEX QTRAP 5500 System	0.0167 pmol/µl (0.0167 µM or 60:1)
AB SCIEX Triple Quad™ 5500 System	0.0167 pmol/µl (0.0167 µM or 60:1)

Creating 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. You can save the acquisition method in the Tutorial project that you created in Creating Projects and Subprojects on page 17.

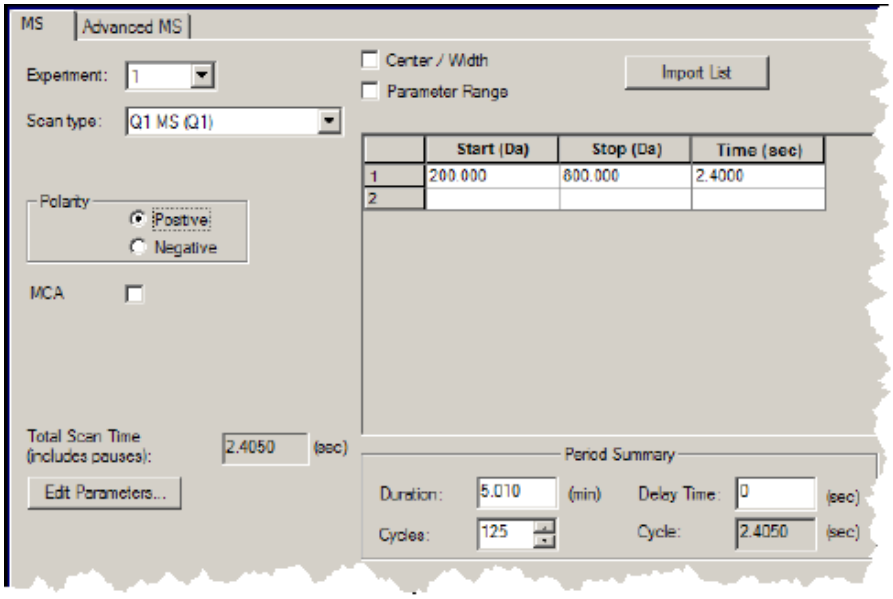
1. Make sure that a hardware profile containing the mass spectrometer and syringe is active.
2. On the software toolbar, make sure that the appropriate project is selected.
3. On the Navigation bar, in **Acquire** mode, double-click **Build Acquisition Method**.

The Method Editor appears with a method template based on the active hardware profile.

4. In the **Acquisition method** pane, click **Acquisition Method**.
5. On the **Acquisition Method Properties** tab, in the **Synchronization Mode** list, make sure that **No Sync** is selected. For more information about synchronization modes, see the Help.
6. In the **Acquisition method** pane, click the **Mass Spec** icon.
7. On the **MS** tab, in the **Scan type** list, select **Q1 MS (Q1)**.
8. In the **Polarity** group, click **Positive**.
9. Clear **Center/Width** and **Parameter Range** check boxes if selected.
10. Type the following values:

Table 3-3 MS Tab Parameter Values

Field	Value
Start (Da)	200
Stop (Da)	700
*Time (sec) (if available)	2.5
*Scan rate (Da/s) (if available)	200
Duration (min)	3
*** These fields are instrument dependent.**	



11. Click the **Advanced MS** tab. Note that the scan mode is set to **Profile** and the step size is 0.1 in the **Step size** field.

    In this example, the quadrupole (Q1) is scanning a 600 amu mass range taking 0.1 Da steps; therefore, there are 6000 steps across the mass range. If this takes 2.4 seconds to scan, the dwell time is 0.4 ms per step. This is typically the fastest that you want to scan a Q1 or Q3 scan based on 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.

12. On the **MS** tab, click **Edit Parameters** and then click the **Source/Gas** tab.
13. On the **Source/Gas** tab, 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

14. Click the **Compound** tab and then set **DP** to 90 and leave **EP** at 10.

    A value of 90 may not be optimal for your instrument but it is a good declustering potential (DP) to start with.
15. In the Acquisition method window on the left, click the syringe pump.
16. On the **Syringe Pump** tab, edit the syringe pump method to include **Syringe Diameter**, **Flow Rate**, and **Unit**.

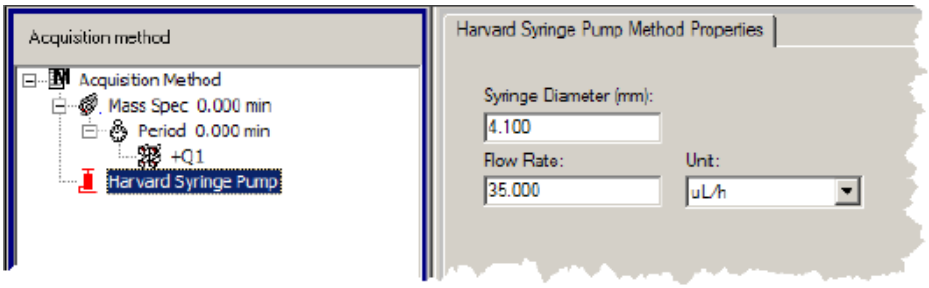


Figure 3-1 Harvard Syringe Pump Method Properties tab

17. Save the acquisition method as Q1MS_tutorial.dam.
18. Next steps: You have created an acquisition method that you can now use to acquire data for preliminary analysis. To create and submit batches, see Creating and Submitting a Batch on page 40.

Creating 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. You can save the acquisition methods in the Tutorial project that you created in Creating Projects and Subprojects on page 17.

1. Make sure that a hardware profile containing the mass spectrometer and syringe is active.
2. On the software toolbar, make sure that the **Tutorial** project is selected.
3. On the Navigation bar, in **Acquire** mode, double-click **Build Acquisition Method**.

   The Method Editor appears with a new method based on the active hardware profile.
4. In the **Acquisition method** pane, click **Acquisition Method**.
5. On the **Acquisition Method Properties** tab, in the **Synchronization Mode** list, make sure that **No Sync** is selected. For more information about synchronization modes, see the Help.
6. In the **Acquisition method** pane, click the **Mass Spec** icon.
7. On the **MS** tab, in the **Scan type** list, select **Q1 Multiple Ions (Q1 MI)**.
8. In the **Polarity** group, click **Positive**.
9. Type the following values:

Table 3-5 MS Tab Parameter Values

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

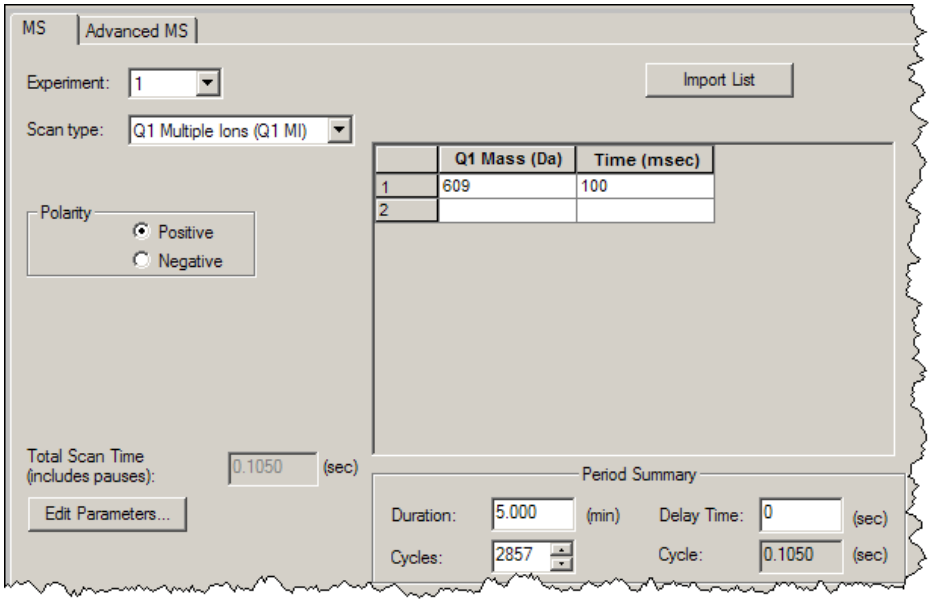


Figure 3-2 MS Tab

10. Click **Edit Parameters** and then click the **Source/Gas** tab.

11. On the **Source/Gas** tab, type the following values:

Table 3-6 Source/Gas Tab Parameter Values

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

12. Click the **Compound** tab and then set **DP** to 90 and leave **EP** at 10.

    A value of 90 may not be optimal for your instrument but it is a good DP to start with.

13. In the Acquisition method window on the left, click the syringe pump.
14. On the **Syringe Pump** tab, edit the syringe pump method to include **Syringe Diameter**, **Flow Rate**, and **Unit**.

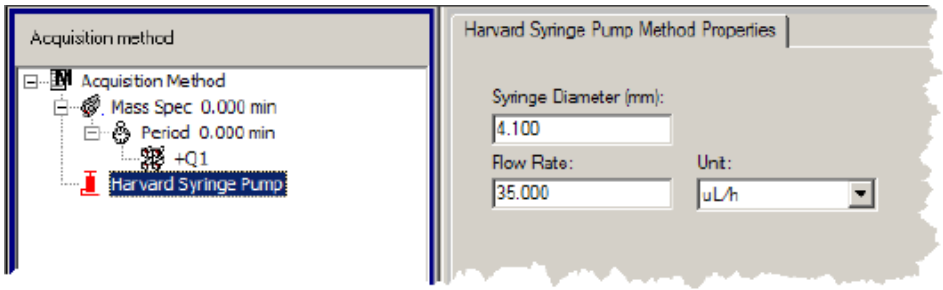


Figure 3-3 Harvard Syringe Pump Method Properties tab

15. Save the acquisition method as Q1MI_tutorial.dam.
16. Next steps: You have created an acquisition method that you can now use to create and submit a batch. To create and submit batches, see Creating and Submitting a Batch on page 40.

Creating 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; and it is now used in pharmacokinetic analysis and increasingly in applied markets and screening applications. You can save the acquisition methods in the Tutorial project that you created in Creating Projects and Subprojects on page 17.

1. Make sure that a hardware profile containing the mass spectrometer and syringe is active.
2. On the software toolbar, make sure that the **Tutorial** project is selected.
3. On the Navigation bar, in **Acquire** mode, double-click **Build Acquisition Method**.

   The Method Editor appears with a new method based on the active hardware profile.

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

5. On the **Acquisition Method Properties** tab, in the **Synchronization Mode** list, make sure that **No Sync** is selected. For more information about synchronization modes, see the online Help.
6. In the **Acquisition method** pane, click the **Mass Spec** icon.
7. On the **MS** tab, in the **Scan type** list, select **MRM (MRM)**.
8. In the **Polarity** group, click **Positive**.
9. In the mass ranges table, type the following values:

Table 3-7 Mass Range and Dwell Time

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

10. Click **Edit Parameters** and then click the **Source/Gas** tab.
11. On the **Source/Gas** tab, type the following values:

Table 3-8 Source/Gas Tab Parameters

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

12. Click the **Compound** tab and then change the **DP** to 90 and the **CE** to 45.
13. In the Acquisition method window on the left, click the syringe pump.
14. On the **Syringe Pump** tab, edit the syringe pump method to include **Syringe Diameter**, **Flow Rate**, and **Unit**.

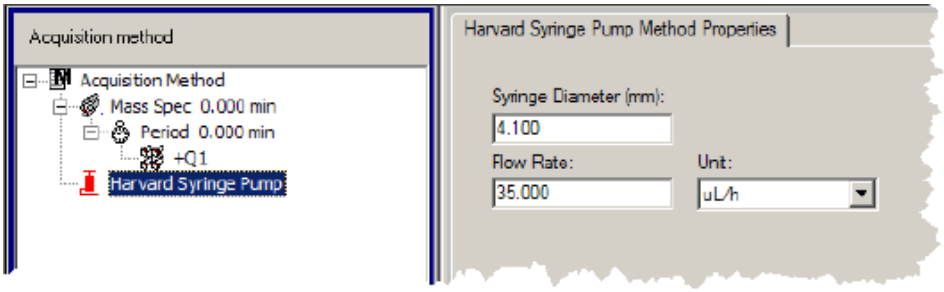


Figure 3-4 Harvard Syringe Pump Method Properties tab

15. Save the acquisition method as MRM_tutorial.dam.
16. Next steps: You have created an acquisition method that you can now use to create and submit a batch. To create and submit batches, see Creating and Submitting a Batch on page 40.

Adding or Removing Devices From Acquisition Methods

With the Acquisition Method Editor, you can 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 you can add the peripheral device only if it is included in the active hardware profile.

Topics in this section:

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

Adding or Removing an LC Device

1. With a method file open in the Acquisition Method Editor, in the **Acquisition method** pane, right-click **Acquisition Method** and then click **Add/Remove Device Method**.

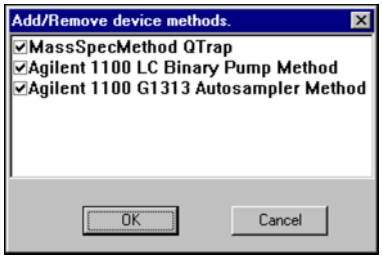


Figure 3-5 Add/Remove Device Method dialog

2. Select or clear the check boxes beside the device method to add or remove the device method.
3. Click **OK**.

Modifying LC Pump Properties

You need to modify each device in the method in order for it to function properly for your method.

1. With an acquisition method file open in the Acquisition Method Editor, in the **Acquisition method** pane, click the **Pump** icon.

The Pump Properties tab appears in the Acquisition Method Editor pane. The following illustration is for the Agilent LC binary pump.

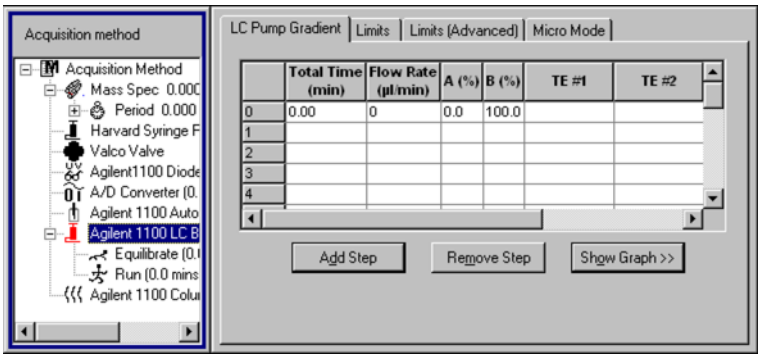


Figure 3-6 LC Pump Gradient tab

2. Edit the LC pump method to include the pump conditions, including the flow rate and sample composition, that you will be using during sample acquisition.
3. If required, on the **Limits (Advanced)** tab, edit the advanced pump parameters.
4. Save the method.

Setting Autosampler Properties

1. Make sure that on the **Acquisition Properties** tab, the **Synchronization Mode** field is set to **LC Sync**. The LC and the mass spectrometer will start simultaneously.
2. With a method file open in the Acquisition Method Editor, in the **Acquisition method** pane, click the **Autosampler** icon.

   The Autosampler Properties tab appears in the Acquisition Method Editor pane. The following illustration shows an Agilent autosampler.

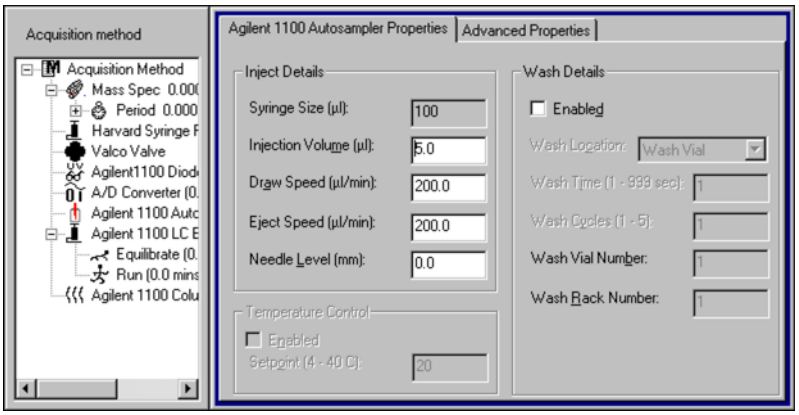


Figure 3-7 Autosampler Properties tab

3. If required, edit the **Inject Details** and **Wash Details**.
4. If required, click the **Advanced Properties** tab, and then type the needle and advanced injection information.
5. Save the file.

Setting Integrated Syringe Pump Properties

This procedure is for systems with built-in syringe pumps. To use this function with an external syringe pump you must have a syringe pump with a serial port and the serial cable.

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

   The Syringe Pump method properties tab appears in the Acquisition Method Editor pane.

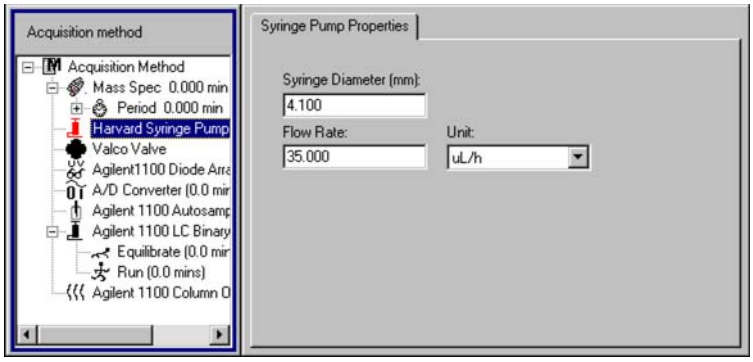


Figure 3-8 Syringe Pump Properties tab

2. In the **Syringe Diameter (mm)** field, type the syringe diameter.
3. In the **Flow Rate** field, type the flow rate.
4. In the **Unit** list, select the units of flow.
5. Save the file.

Setting Column Oven Properties

1. With a method file open in the Acquisition Method Editor, in the **Acquisition method** pane, click the **Column Oven** icon.

   The Column Oven properties tab appears in the Acquisition Method Editor pane.

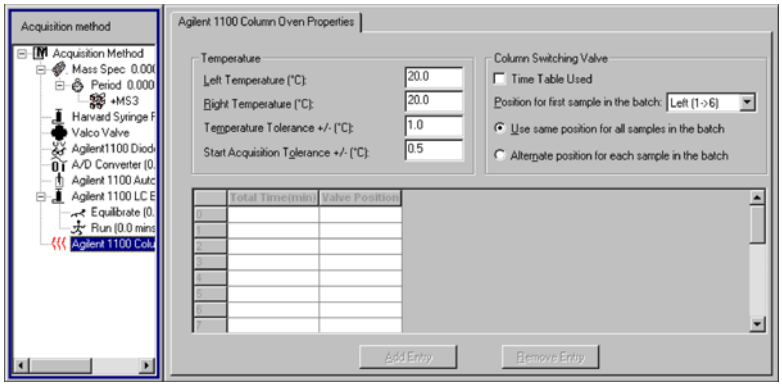


Figure 3-9 Column Oven tab

2. Type the temperature of the column oven or column oven compartments in degrees Celsius.

3. In the **Temperature Tolerance** field, type the temperature tolerance in degrees Celsius.
4. In the **Start Acquisition Tolerance** field, type the start acquisition tolerance in degrees Celsius.
5. If you are using the Agilent column oven, in the **Column Switching Valve** section, select **Time Table Used** check box to add and remove entries from the time table at the bottom of the pane, or select an option from the **Position for the first sample in the batch** list and then click one of the sample positions options.
6. Save the file.

Setting Switching Valve Properties

The switching valve can be used as a diverter or injection valve. Select the Manual Sync with Valve synchronization mode if you are using the valve as an injector; choose any other mode if you are using the valve as a diverter.

1. With a method file open in the Acquisition Method Editor, in the **Acquisition method** pane, click the **Valve** icon.

   The Valve Properties tab appears in the Acquisition Method Editor pane.

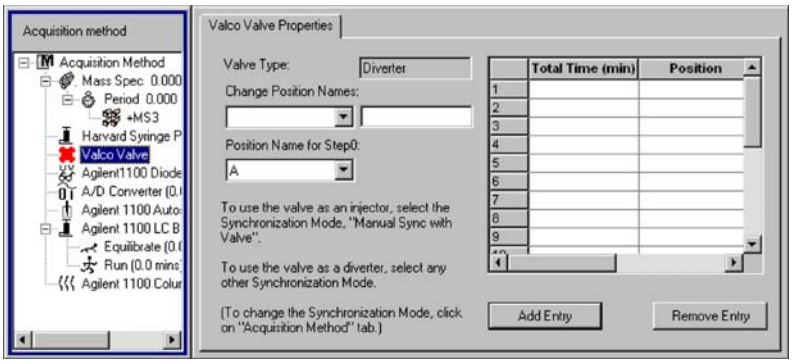


Figure 3-10 Valve Properties tab

2. Change the position names from their preset names, if required. The switching valve is sometimes used to switch the flow of solvent to waste, or to a different column. The preset position names are A and B.
   - In the **Change Position Names** list, select a position.
   - In the **Change Position Names** list, rename the preset position names A and B to Inject and Divert or to Column and Waste, depending on how the valve is plumbed.
3. In the **Total Time (min)** column, click a cell and then type the total time the valve will remain in this position.
4. In the **Position** column, click a cell and then, in the **Position** list, select the valve position.
5. Repeat the steps 3 and 4 for each switch of the valve required during acquisition.
6. Save the file.

Setting Diode Array Detector Properties

1. With a method file open in the Acquisition Method Editor, in the **Acquisition method** pane, click the **Diode Array Detector (DAD)** icon.

   The DAD Method Editor tab appears in the Acquisition Method Editor pane.

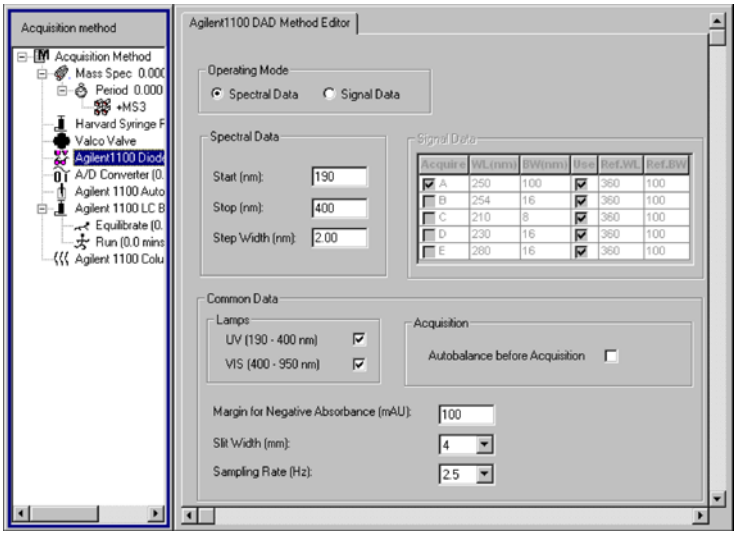


Figure 3-11 DAD Method Editor tab

2. Do one of the following:
   - To scan one to five individual wavelengths, in the **Operating Mode** section, click **Signal Data** and then edit the data requirements.
   - To scan over a wavelength range, in the **Operating Mode** section, click **Spectral Data** and then edit the data requirements.
3. Save the file.

Setting Analog to Digital Converter Properties

1. With a method file open in the Acquisition Method Editor, in the **Acquisition method** pane, click the **Analog to Digital Converter (ADC)** icon.

   The Analog/Digital Convertor Properties tab appears in the Acquisition Method Editor pane.

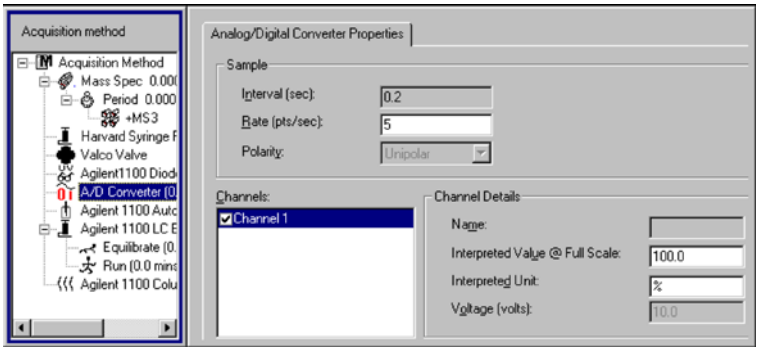


Figure 3-12 Analog to Digital Converter Properties tab

2. In the **Sample** section, in the **Rate (pts/sec)** field, type the rate.

Note: The interval and rate are proportional to each other. When you change the rate, the software automatically re-calculates the interval.

3. Do the following to set the channel details:
   - In the **Channels** field, click the channel name, and then select the check box beside the name to include it in the method.
   - In the **Interpreted Value @ Full Scale** field, type the appropriate value.
   - In the **Interpreted Unit** field, type the appropriate unit.

   The number of available channels is specified when setting up the ADC in the hardware profile.
4. Save the file.

Changing Acquisition Methods

You can add or delete periods and experiments to existing acquisition methods. You must have a method open in Acquire mode to get the view.

Topics in this section:

Adding an Experiment

1. In the period where you want to add an experiment, right-click, and then click **Add experiment**.

   An experiment is added below the last experiment in the period.

Note: You cannot insert an experiment, IDA criteria, or period. You can only add an experiment at the end of the period.

2. In the **Acquisition Method Editor** pane, select the appropriate device or instrument parameters.

Copying an Experiment into a Period

- 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.

Copying an Experiment within a Period

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

  This is useful when you are adding the same or similar experiments and most or all the parameters are the same as in IDA.

Adding 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.

Table 3-9 Icon Quick Reference: Method Editor

Icon	Name	Function
	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. For more information on DAD, see Table 5-5 *Chromatograms* on page 60.
	ADC	Click to open the ADC Properties tab. For more information on ADC, see Viewing ADC Data on page 59.

In this section, you will learn how to create and submit batches and also how to monitor the sample queue.

A batch is a collection of information about the samples that you want to analyze. When you create a batch, you are telling the software the order in which you want to analyze samples.

Queue Options

Before you submit the samples, you can review and edit the queue conditions, such as the maximum number of acquired and waiting samples and the maximum idle time.

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

You can modify the length of time the queue runs after the last acquisition has finished, before it puts the instrument into Standby mode. For more information about the other fields in the Queue Options dialog, see the Help.

Setting Queue Options

1. On the Navigation bar, click **Configure**.
2. Click **Tools > Settings > Queue Options**.

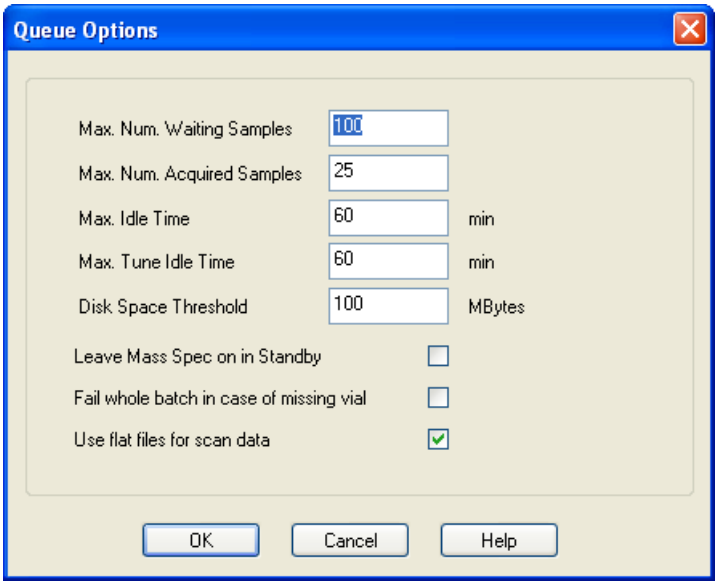


Figure 4-1 Queue Options dialog

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

If you are using gas cylinders, you may want to adjust this time to make sure that you do not deplete the gas in the cylinders.

4. If you are using an LC method, before the run is started, make sure that there is enough solvent in the reservoirs for the primary flow rate for all of the sample runs and the Max. Idle Time.

Creating and Submitting a Batch

Use this workflow to create a batch. In this example, we will use the MRM scan that you created previously. You can also go through the workflow twice more for practice, once using the Q1MS and the second time using Q1MI methods.

Adding Sets and Samples to a Batch

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

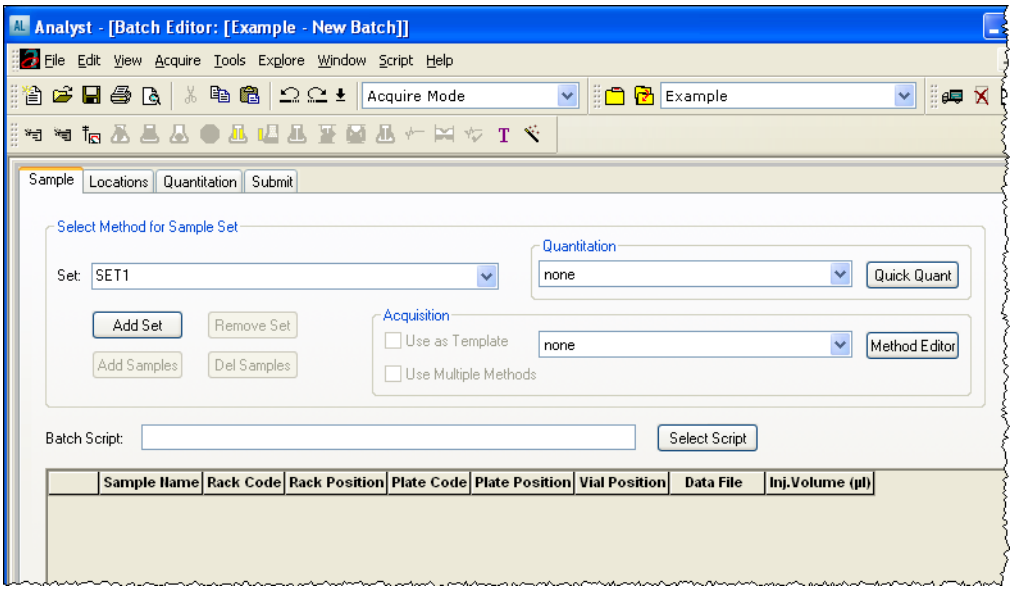


Figure 4-2 Batch Editor

2. On the **Sample** tab, in the **Set** list, type **Test Set**.
3. Click **Add Set**.
4. Click **Add Samples** to add samples to the new set.

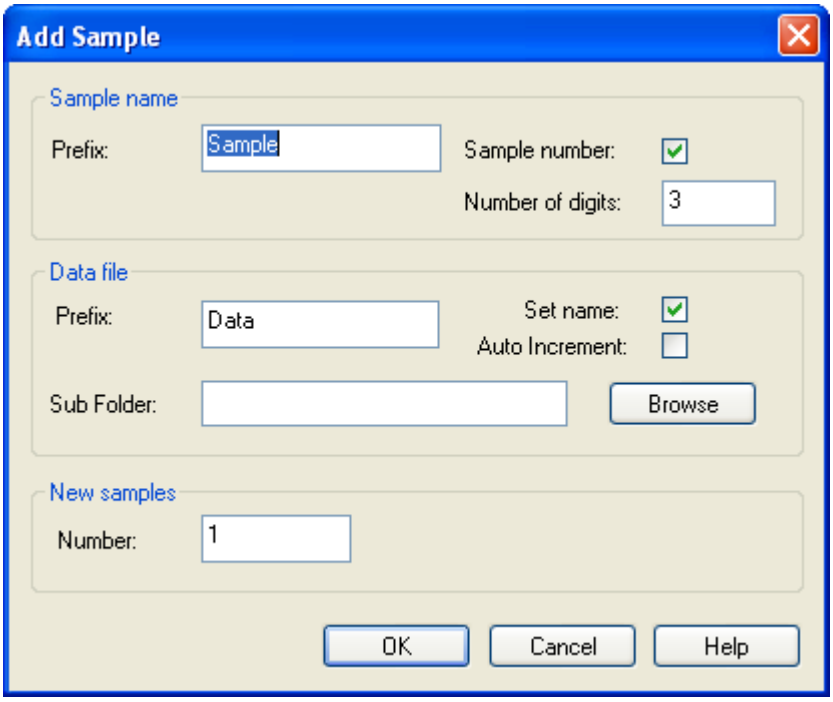


Figure 4-3 Add Sample dialog

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 you have selected the **Sample number** check box, in the **Number of digits** field, type the number of digits to include in the sample name. For example, if you type 3, the sample names would be samplename001, samplename002, 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. If you want the set name to be part of the data file name, select the **Set name** check box.
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 separate data file. The names of the data file will have numerical suffixes starting from 1.

11. In the **Sub Folder** field, type **Test Data**.

    The folder is stored in the Data folder for the current project. If you leave the Sub folder field blank, the data file will be stored in the Data folder and a subfolder will not be created.
12. In the **New samples** section, in the **Number** field, type the number of new samples to add and then click **OK**. For this example, add three samples.

    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 you select a single column heading or several rows in a column.

13. On the **Sample** tab, in the **Acquisition** section, select the MRM method from the list. You can run the batch twice more using the Q1MS and the Q1MI methods.

Tip!: If you want to use different methods for some of the samples in this set, select the Use Multiple Methods check box. The Acquisition Method column appears in the Sample table. You can select the acquisition method for each sample in this column.

    Depending on how your system is set up, you will have to type the information specific for your autosampler. Even if the injection volume is set in the method you can change it for one or more samples by changing the value in Inj. Volume column.

14. If you want 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.
15. To set sample locations, do one of the following:
    - Setting Sample Locations in the Batch Editor on page 43
    - Selecting Vial Positions Graphically using the Locations tab (Optional) on page 44
16. (Optional) If you want to define quantitation details prior to submitting the batch, then see Setting Quantitation Details in the Batch Editor (Optional) on page 45.
17. Click the **Submit** tab.
18. If the **Submit Status** section contains a message about the status of the batch, do one of the following:
    - If the message indicates that the batch is ready for submission, proceed to step 19.
    - If the message indicates that the batch is not ready for submission, make the changes as indicated by the message.
19. Click **Submit**.

    The Acquisition dialog appears.
20. Save the file.

Acquiring Data

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

1. In **Acquire** mode, click **View > Sample Queue**.

   The Queue Manager appears with all submitted samples.

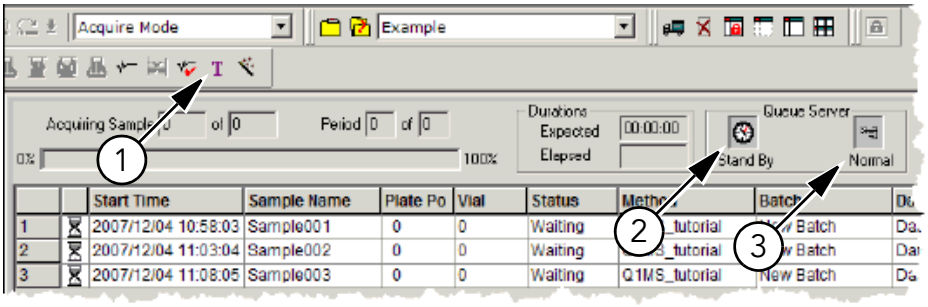


Figure 4-4 Queue Manager

Item	Description
1	The Tune icon should not be pressed in.
2	Queue status.
3	Queue Server should be in Normal mode. For more information, see Queue States on page 49.

2. Click **Acquire > Start Sample**.
3. Next steps: You can now analyze the data that you have just acquired. For more information, see Analyzing and Processing Data on page 55 or Analyzing and Processing Quantitative Data on page 95.

Setting Sample Locations in the Batch Editor

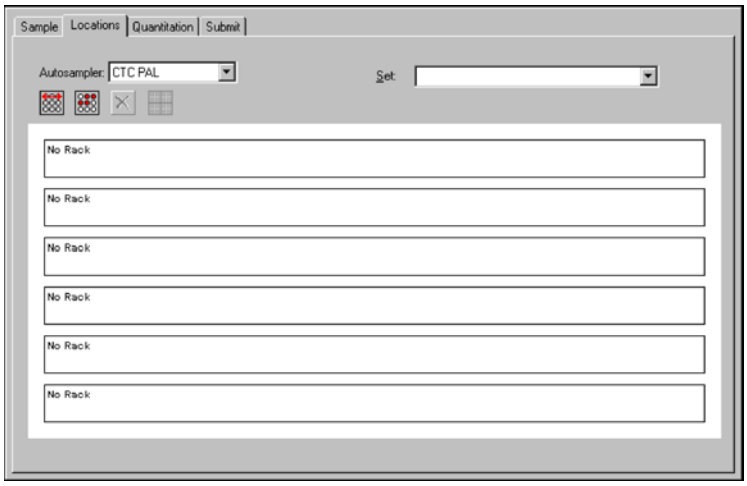
If an autosampler is being used in the acquisition method, then the vial positions of the samples must be defined in the acquisition batch. You can define the location on the Sample tab or on the Locations tab. For more information on creating batches, see Creating and Submitting Batches on page 39.

Note: Depending on the autosampler you are using, it may not be necessary to type details in additional columns.

1. On the **Sample** tab, in the **Set** list, select the set for which you want to select sample locations.
2. For each sample in the set, do the following if applicable:
   - In the **Rack Code** column, select the rack type for the autosampler.
   - In the **Rack Position** column, select the position of the rack in the autosampler.
   - In the **Plate Code** column, select the plate type for the autosampler.
   - 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.

Selecting Vial Positions Graphically using the Locations tab (Optional)

1. In the **Batch Editor**, click the **Locations** tab.



2. In the **Set** list, select the set for which you want to select sample locations.
3. In the **Autosampler** list, select the autosampler that you are using.

   The appropriate number of rack spaces for the autosampler you chose appears in the graphic rack display.
4. In the space associated with the rack you are using, right-click and then select the rack type you are using.

   The plates or trays you selected appear in the rack.
5. Double-click one of the rectangles.

   The circles depicting the wells or vials for the plate or tray appear.
6. To select whether samples are marked by row or column, click **Row/Column Selection**. If the button shows a red horizontal line, the Batch Editor marks the samples by row. If the button shows a red vertical line, the Batch Editor marks the samples by column.
7. Click the sample wells or vials in the order to be analyzed. Click a selected well or vial again to clear it.
8. Save the file.

Tip!: It is also possible to auto fill in the samples by clicking the first and final vial within a set with the Shift key held down. Multiple injections from the same vial can be done by holding down the Ctrl key while clicking the vial location (red circle changes to a green).

Setting Quantitation Details in the Batch Editor (Optional)

If you are using a Quantitation method with a batch and if you do not want to select quantitation details post-acquisition then define the quantitation details prior to submitting a batch.

The appropriate Internal Standard and Standard columns appear in the Quantitation tab according to the quantitation method selected in the Sample tab.

1. With a batch file open in the **Batch Editor** window, click the **Quantitation** tab.
2. Select the set containing the samples that you want to modify.
3. From the list in the cell, select a **Quant Type** for all the samples.
4. If applicable, type the peak concentration in the **Analyte** column.
5. If applicable, type the **Internal Standard**.
6. Repeat the preceding steps for each set in the batch.
7. Save the file.

Tip!: The order of samples can be edited before you submit them to the Queue. To change the order of a sample, when in the Submit tab, double-click any of the numbers on the far left of the table (you'll see a very faint square box), and then drag them to the new location.

Stopping Sample Acquisition

There are three ways to stop a sample after it is in the sample queue and the instrument is running. The following procedures shows you one way to stop sample acquisition. For more information on the other ways to stop sample acquisition, see Table 4-4 on page 52. When you stop a sample acquisition, the current scan finishes before the acquisition is stopped.

1. In the Queue Manager, click the sample in the queue after the point where you want to stop acquisition.
2. Click **Acquire > Stop Sample**.

   The queue stops after the current scan in the sample you selected is complete. The sample status on the Queue Manager (Local) window changes to Terminated, and all others following in the queue are Waiting.
3. When you are ready to continue processing the batch, click **Acquire > Start Sample**.

Importing and Submitting Batch Files

You can import a text file containing batch information instead of creating a batch in the Batch Editor. If you have all the details for the samples you want to process in a spreadsheet, it is faster to rearrange and import the data in the spreadsheet than to manually type the data into the Batch Editor.

Before you import batch information from a text file, make sure the data in the file is organized and formatted correctly. In particular, the column headings in the spreadsheet must match the Batch Editor column headings.

Building a Batch as a Text File

To make sure that your text file includes the proper headings, you must create a batch using the Batch Editor, export it as a text file, type the appropriate values in a spreadsheet editor, and then import the file back into the Batch Editor. You can export a batch only if it contains at least one set with at least one sample. If you save the text file, you can use it again later as a template.

1. Make sure that the active hardware profile includes all the devices you will use to acquire your samples.
2. In the Batch Editor, create a single-set, single-sample batch.
3. Click **File > Export**.

   The Save As dialog appears.
4. In the **File name** field, type the name you want to use for the text file, and then click Save.
5. Open the text file in a spreadsheet program such as Microsoft Excel.
6. Type, or copy and paste, the details for the samples: one sample per row, with the details under the appropriate headings.

   Note: Do not delete any of the columns. The columns in the spreadsheet must match the columns in the Batch Editor.
7. Save the modified text file as a .txt or .csv file and then close the spreadsheet program.

   The text file is now ready to be imported into the Batch Editor.

Importing a Batch as a Text File

1. In the Batch Editor, in the **Sample** tab, right-click, and then click **Import From > File**.

   The Open dialog appears.
2. Click the text file in which you saved the batch details, and then click **Open**.

   If you are using an autosampler, then the Select Autosampler dialog appears.

   Note: If you do not see the text file you saved, in the Files of type list, select Microsoft Text Driver (*.txt; *.csv). Files with the extension .txt appear in the field.

3. In the autosampler list, select the autosampler you are using, and then click **OK**.

   The sample table fills with the details from the text file. You are now ready to submit the batch.

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 appears, use 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.
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	hold down the Shift key and click the first and last well or vial of the range you want to select.

Batch Editor Right-Click Menu

The following options are available if you right-click in the Batch Editor table.

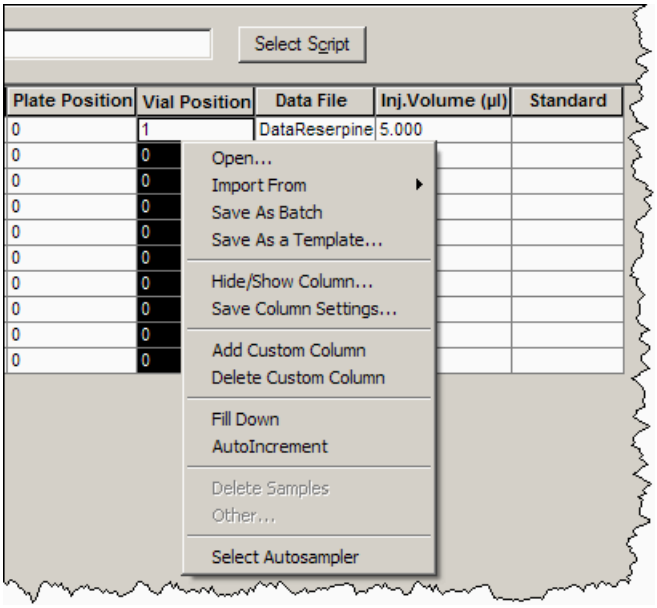


Figure 4-5 Batch Right-Click Menu

Menu	Function
Open	Click to open a batch file.
Import From	Click to import a file.
Save As Batch	Click to save the batch.
Save As a Template	Click to save the batch as a template. Used with the Express View feature.
Hide/Show Column	Click to hide or show a column.
Save Column Settings	Click to save the batch column settings.
Add Custom Column	Click to add a custom column.
Delete Custom Column	Click to delete a custom column.
Fill Down	Click to fill the same data into the selected cells.
Auto Increment	Click to automatically increment data into the selected cells.
Delete Samples	Click to delete the selected row.
Select Autosampler	Click to select an autosampler.

Queue States and Device Status

The Queue Manager shows queue, batch, and sample status, so that you can manage samples and batches in the queue. You can also access detailed information about a particular sample in the queue.

Queue States

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

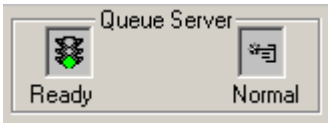


Figure 4-6 Queue Server indicator showing Normal mode

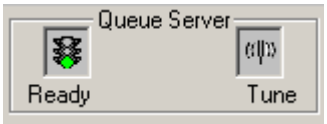


Figure 4-7 Queue Server indicator showing Tune mode

The first icon in Figure 4-6 shows 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 shows the various queue states.

Table 4-2 Queue States

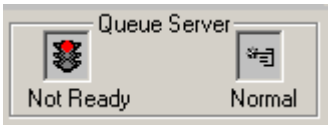
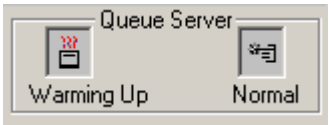
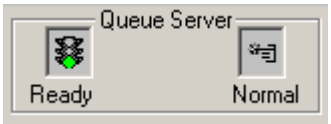
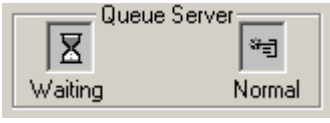
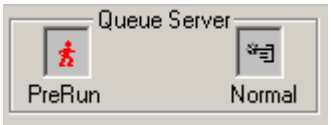
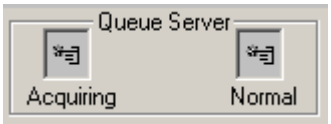
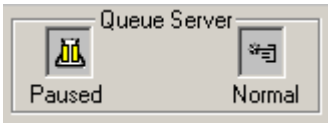
Icons	State	Definition
	Not Ready	In the Not Ready state, the hardware profile is deactivated and the queue is not accepting any sample submissions.
	Stand By	In the Stand By state, the hardware profile has been activated, but all devices are idle. Pumps are not running and gases are turned off.
	Warming Up	In the Warming Up state, the instrument and devices are equilibrating, columns are being conditioned, the autosampler needle is being washed, and column ovens are reaching temperature. The period of equilibration is selected by the operator. From this state, the system can go to the Ready state.
	Ready	In the Ready state, 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.
	Waiting	In the Waiting state, the system will automatically begin acquisition when the next sample is submitted.

Table 4-2 Queue States (Continued)

Icons	State	Definition
	Prerun	In the Prerun state, 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.
	Acquiring	In the Acquiring state, the method is run and data acquisition occurs.
	Paused	In the Paused state, the instrument has been paused during acquisition.

Viewing Instrument and Device Status Icons

Icons representing the instrument and each device in the active hardware configuration appear on the status bar in the bottom right corner of the window.

You might want to view the detailed status of an LC pump to check if the LC pump pressure is appropriate, or view the detailed status of the instrument to check the temperature of the source.

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

  The Instrument Status dialog appears.

Table 4-3 Instrument and Device Status (showing the instrument icon)

Status	Icon	Background Color	Description
Idle		Green or yellow	The device is not running. If the background color is yellow, 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, from another device, or for some action by the operator.
Running		Green	The device is running.

Table 4-3 Instrument and Device Status (showing the instrument icon) (Continued)

Status	Icon	Background Color	Description
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.

Note: For each status the background color can also be red. This situation means that the device encountered an error while in that status.

Queue Right-Click Menu

The following options are available if you right-click in the Queue table.

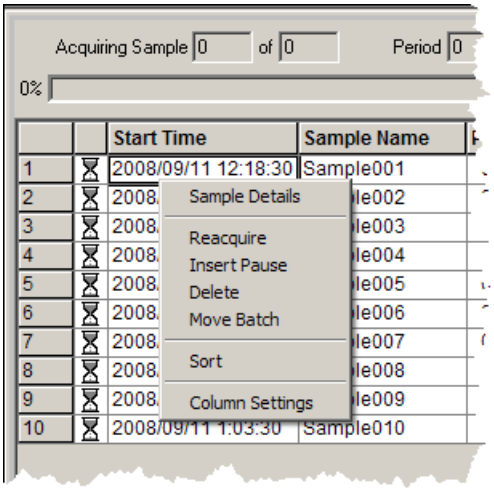


Figure 4-8 Queue Manager Right-Click Menu

Menu	Function
Sample Details	Click to open the Sample Details dialog.
Reacquire	Click to reacquire a sample.
Insert Pause	Click to insert a pause, in seconds, between two samples.
Delete	Click to delete either the batch or the selected samples.
Move Batch	Click to move the batch within the queue.

Figure 4-8 Queue Manager Right-Click Menu (Continued)

Menu	Function
Sort	Click to sort by the preselect column.
Column Settings	Click to change the column settings.

Icon Quick Reference: Acquire Mode

Table 4-4 Icons in Acquire Mode

Icon	Name	Function
	View Queue	Click to view the sample queue.
	Instrument Queue	Click to view a remote instrument station.
	Status for Remote Instrument	Click to view the status of a remote instrument.
	Start Sample	Click to start the sample in the queue.
	Stop Sample	Click to stop the sample in the queue.
	Abort Sample	Click to abort a sample acquisition in the middle of the processing of that sample.
	Stop Queue	Click to stop the queue before it has completed processing all the samples.
	Pause Sample Now	Click to insert a pause in the queue.
	Insert Pause before Selected Sample	Click to insert a pause before a specific sample.
	Continue Sample	Click to continue acquiring the sample.
	Next Period	Click to start a new period.
	Extend Period	Click to extend the current period.
	Next Sample	Click to stop acquiring the current sample and to start acquiring the next sample.
	Equilibrate	Click to select a method to use to equilibrate the devices. This method should be the same as the one you are using with the first sample in the queue.

Table 4-4 Icons in Acquire Mode (Continued)

Icon	Name	Function
	Standby	Click to put the instrument in Standby mode.
	Ready	Click to put the instrument in Ready mode.
	Reserve Instrument for Tuning	Click to reserve the instrument for tuning and calibrating.
	IDA Method Wizard	Click to start the IDA Method Wizard.


AB SCIEX

Analyzing and Processing Data 5

In this section, you will 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.

Overview of Spectral and Chromatographic Data

When you view data as a spectrum, you obtain mass-specific information about a compound. A chromatogram gives you a general view of the data, usually time dependent when using an LC column, but it does not tell you anything about the components of a peak. A spectrum, however, looks at a particular peak and gives you the molecular weight of the corresponding compound, which you can use to find more specific information. For example, while a chromatogram may show only one peak, that peak can represent more than one compound; that is, different masses. A spectrum shows all of the masses that make up a peak, including the intensity of each mass.

Chromatographic data can change in both time and intensity if there is a change in the chromatographic conditions in a given sample. Spectral intensities may change, but the masses are fixed because the mass of a compound does not change.

There are two ways to generate spectral data:

- If only one scan is acquired, by default the data is shown as a spectrum.
- From a chromatogram.

A typical spectrum appears with the molecular weight, labeled with the *m/z* (mass/charge ratio), on the x-axis. The intensity appears on the y-axis.

Analyzing Data

When you open a data file, different panes appear in windows, depending on the type of experiment you performed. The software stores data in files with a .wiff extension. Wiff files can contain data for more than one sample. In addition to .wiff files, the software can open .txt files; however, .txt files contain data for only one sample.

You can view the information contained in a data file in table or graph form. Graphical data is presented either as a chromatogram or as a spectrum. Data from either of these can appear as a table of data points, and you can perform various sorting operations on the data.

You can open files containing existing data or data that is currently being acquired. You can also view all experiment-related data in tabular form. The table pane consists of two tabs, the Data List tab and the Peak List tab.The Data List tab contains experiment-related information, such as acquisition time and scan intensity. The Peak List tab displays peak-related information such as peak height, peak area, and baseline type.

If the data contains results from multiple experiments, you can create individual TICs (Total Ion Chromatograms) for each experiment, and another TIC that represents the sum of all experiments.

The preset TIC that represents the sum of all of the experiments is shown with a splitter tool below the center of the x-axis.

Opening Data Files

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 appears.
3. In the **Data Files** field, double-click **Triple Quad** and then click **QuanData.wiff**.
4. In the **Samples** list, select sample AP13-020, and then click **OK**.

   The data acquired from your sample appears. If you were still acquiring data, the mass spectrum, DAD/UV trace, and TIC would continue to update automatically.

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.

Navigating Between Samples in a Data File

Table 5-1 contains the navigation icons used in this procedure.

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

1. Open a data file that contains multiple samples. For this example, you can use **QuanData.wiff**.
2. To skip to the next sample in the data file, click the icon with the arrow pointing to the right. (See Table 5-1.)
3. To skip to a non-sequential sample, click the icon with the arrow curving to the right.
4. In the **Select Sample** dialog that appears, in the **Sample** list, select the sample you want to view.
5. To go to the previous sample in the data file, click the icon with the arrow pointing to the left.

Table 5-1 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.

Viewing Experimental Conditions

The experimental conditions you used to collect your data are stored in the data file along with your 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 peripheral 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 5-2 shows the software functionality available when you view the file information.

Note: If you have acquired data from more than one sample into the same .wiff file, the file information pane will not refresh automatically as you scroll through the samples. You will need to 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 appears below the graph.

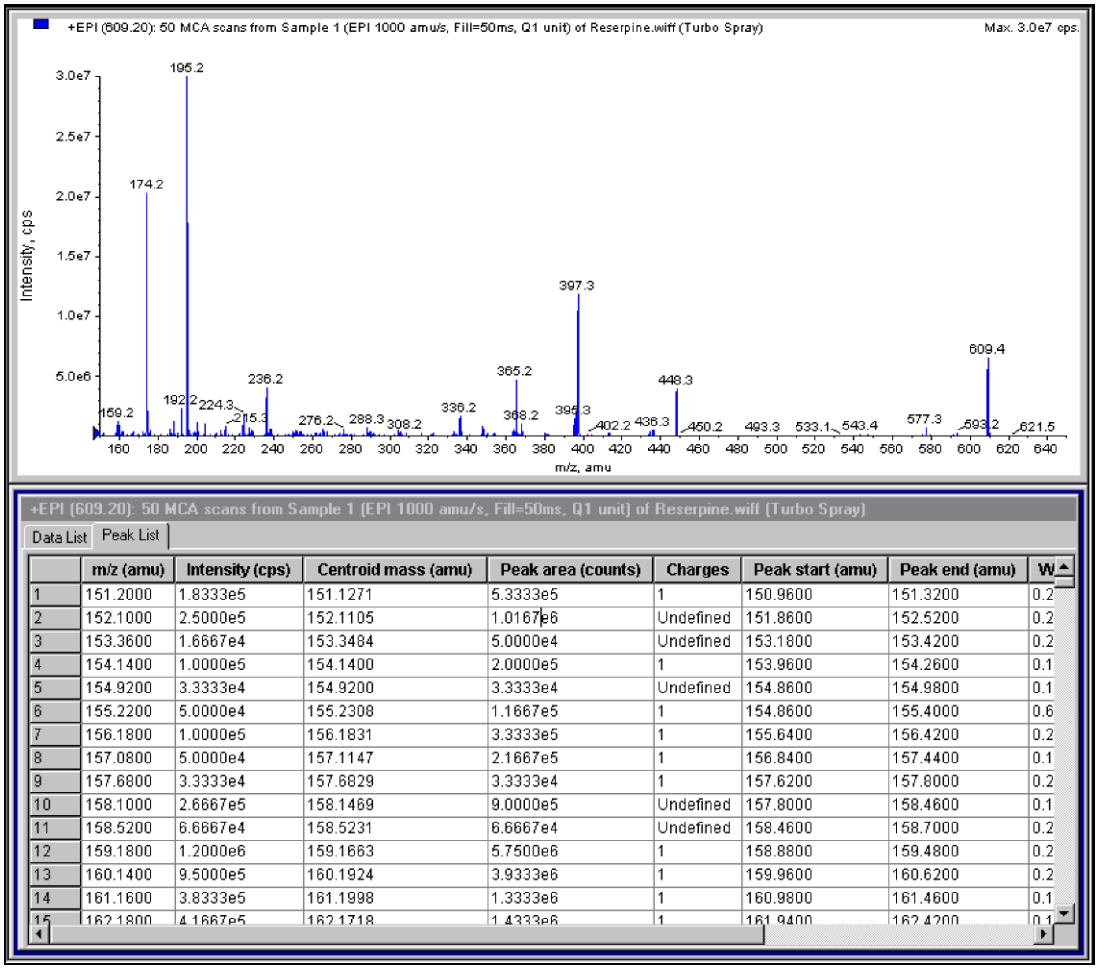
Table 5-2 Right-Click Menu for Show File Information Pane

Menu	Function
Copy	Click to copy the selected data.
Paste	Click to paste data.
Select All	Click to select all the data in the pane.
Save To File	Click to save data in an .rtf file.
Font	Click to change the font.
Save Acquisition Method	Click to save the acquisition method as .dam file.
Save Acquisition Method to CompoundDB	Click to open the Specify Compound Information dialog. Select the IDs and molecular weights to be saved in the compound database.
Delete Pane	Click to delete the pane.

Viewing The Data In Tables

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

  The data appears in a pane below the graph.



	m/z (amu)	Intensity (cps)	Centroid mass (amu)	Peak area (counts)	Charges	Peak start (amu)	Peak end (amu)	W
1	151.2000	1.8333e5	151.1271	5.3333e5	1	150.9600	151.3200	0.2
2	152.1000	2.5000e5	152.1105	1.0167e6	Undefined	151.8600	152.5200	0.2
3	153.3600	1.6667e4	153.3484	5.0000e4	Undefined	153.1800	153.4200	0.2
4	154.1400	1.0000e5	154.1400	2.0000e5	1	153.9600	154.2600	0.1
5	154.9200	3.3333e4	154.9200	3.3333e4	Undefined	154.8600	154.9800	0.1
6	155.2200	5.0000e4	155.2308	1.1667e5	1	154.8600	155.4000	0.6
7	156.1800	1.0000e5	156.1831	3.3333e5	1	155.6400	156.4200	0.2
8	157.0800	5.0000e4	157.1147	2.1667e5	1	156.8400	157.4400	0.1
9	157.6800	3.3333e4	157.6829	3.3333e4	1	157.6200	157.8000	0.2
10	158.1000	2.6667e5	158.1469	9.0000e5	Undefined	157.8000	158.4600	0.1
11	158.5200	6.6667e4	158.5231	6.6667e4	Undefined	158.4600	158.7000	0.2
12	159.1800	1.2000e6	159.1663	5.7500e6	1	158.8800	159.4800	0.2
13	160.1400	9.5000e5	160.1924	3.9333e6	1	159.9600	160.6200	0.2
14	161.1600	3.8333e5	161.1998	1.3333e6	1	160.9800	161.4600	0.1
15	162.1800	4.1667e5	162.1718	1.4333e6	1	161.9400	162.4200	0.1

Table 5-3 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 5-4 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 Intelliquan dialog.
Centroid Parameters	Click to delete the pane.
Save As Text	Click to save the data as text file.
Delete Pane	Click to delete the pane.

Viewing 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. If you want to have ADC data available, you must acquire the data and the mass spectrometer data simultaneously and save it in the same file.

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 appears.
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**.

   The Select ADC Channel dialog appears.

6. In the **Channel** list, select a channel, and then click **OK**.

   The ADC data appears in a new pane beneath the active pane.

Obtaining Basic Quantitative Data

1. In the Peak List tab, right-click and select **Show Peaks in Graph.**

   Peaks appear in two colors.
2. To change the peak finding algorithm settings right-click and then select either **Analyst Classic Parameters** or **Intelliquan Parameters**, which ever is active.
3. To remove the colored peaks, right-click on the Peak List tab and clear **Show Peaks in Graph**.

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 appears as a graph with the *m/z* values on the x-axis and intensity (cps) represented on the y-axis. For a more information about software functionality available when you are working with spectrums, see Table 5-8 *Right-Click Menu for Spectra Panes* on page 68.

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).

Chromatograms

A chromatogram is a graphical display 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 when you are working with chromatograms, see Table 5-7 *Right-Click Menu for Chromatogram Panes* on page 67.

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 often appears as a function of time. Table 5-5 contains the a description of the types of chromatograms.

Table 5-5 Chromatograms

Types of chromatograms	Purpose
TIC (Total Ion Chromatogram)	A chromatographic display generated by plotting the intensity of all ions in a scan against time or scan number.
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)	Chromatographic plot that displays the intensity of the most intense ion within a scan versus time or scan number.
TWC (Total Wavelength Chromatogram)	A chromatographic display 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 displays 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.

TICs

A TIC is created by summing the intensity contributions of all ions from a series of mass scans. You can use the TIC to view an entire data set in a single pane. It consists of the summed intensities of all ions in a scan plotted against time in a chromatographic pane.

If the data contains results from multiple experiments, you can create individual TICs for each experiment and another TIC that represents the sum of all experiments.

Displaying TICs

When you open a data file, it is preset to appear as a TIC. However, if the experiment contains only one scan, it appears as a spectrum. For more information about using the available icons, see Table 5-9 *Working With Graphs* on page 69.

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 with the TIC.

To display 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 appears.
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**.

To display a spectrum from a TIC

1. Select a range in the spectrum.
2. Click **Explore** > **Show** > **Show Spectrum**.

   The spectrum opens in a new pane.

Tip!: You can also double-click in the TIC pane at a particular time to show the spectrum.

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.

Generating XICs

You can generate XICs only from single period, single experiment chromatograms or spectra. To obtain an XIC from multi-period or multi-experiment data you must first split the data into separate panes by clicking the triangle that appear under the x-axis. For more information about using the available icons, see Table 5-9 *Working With Graphs* on page 69.

There are several methods for extracting ions to generate an XIC, depending on whether you are working with chromatographic or spectral data. Table 5-6 contains a summary of methods that can be used with chromatograms and spectra.

Table 5-6 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.

Table 5-6 Summary of XIC Generation Methods (Continued)

Method	Use with chromatogram	Use with spectrum	Extraction
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 your selection includes multiple peaks, 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. You can select up to 10 start and stop masses for which to generate XICs.

To generate an XIC using a selected range

1. To select a range inside the pane, click and hold the left mouse button where you want to start the range and then drag the cursor to the stop point and release.

   The selection is highlighted in blue.

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

   An XIC of the specified selection appears 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.

To generate an XIC using the maximum peak

1. Select a range in a spectrum.

   The selection is highlighted in blue.

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

   An XIC of the maximum peak specified selection appears 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.

To 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 appears below the spectrum pane. The experiment information at the top of the pane displays the mass range and the maximum intensity in counts per second.

To extract ions by selecting masses

1. Select a spectrum or chromatogram.
2. Click **Explore** > **Extract Ions** > **Use Dialog**.

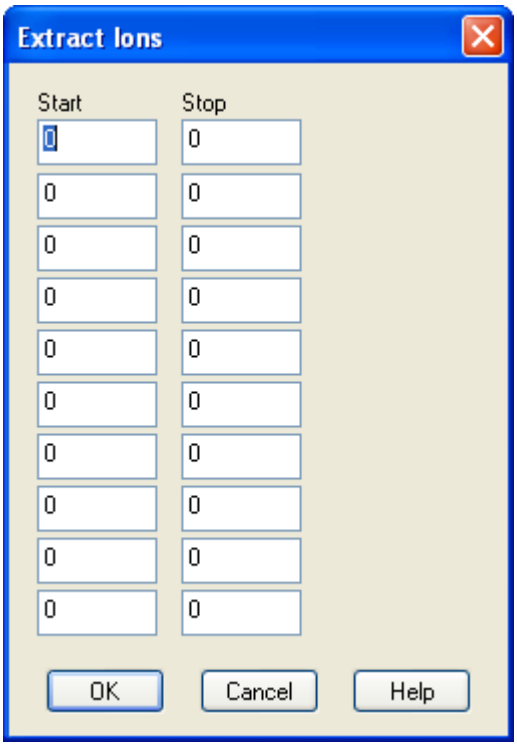


Figure 5-1 Extract Ions dialog

3. Type the following values for each XIC that you want to create. If you do not define a stop value, the range will be defined by the start value that you entered.
   - In the **Start** box, type the start value (lower value) for the mass range you want to extract.
   - In the **Stop** box, type the stop value (higher value) for the mass range you want to extract.
4. Click **OK**.

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

BPCs

A BPC displays 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 is also helps to distinguish between co-eluting components. You can generate BPCs 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 you manipulate the data by scrolling or zooming. For information about selecting the colors used in the graph, see the Help.

Generating BPCs

BPCs can be generated only from single period, single experiment data. For more information about using the available icons, see Table 5-9 *Working With Graphs* on page 69.

1. Select an area within a TIC.

   The selection is highlighted in blue.

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

   The selections that you specified are shown in the Start Time and End Time fields.

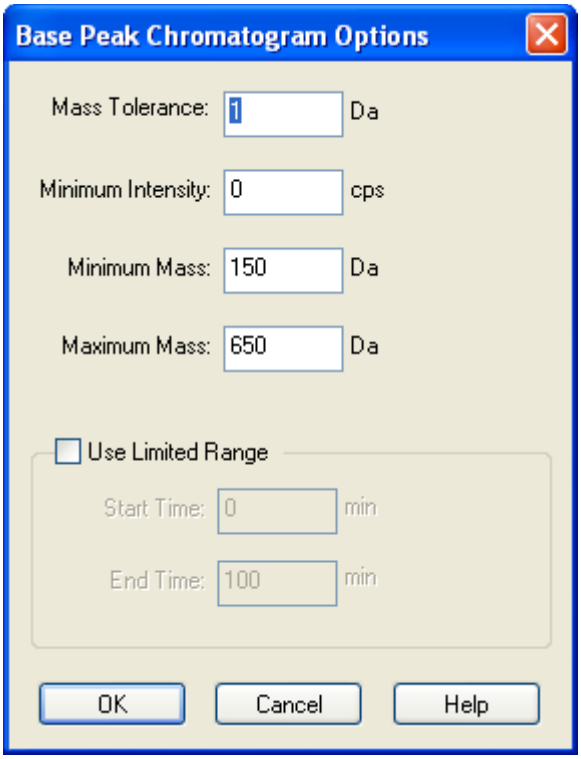


Figure 5-2 Base Peak Chromatogram Options dialog

3. In the **Mass Tolerance** field, type the value to dictate the mass range used to find a peak. The software finds the peak using a value twice the entered range (± the mass value).
4. In the **Minimum Intensity** field, type the intensity below which peaks are ignored by the algorithm.
5. In the **Minimum Mass** field, type the mass that determines the beginning of the scan range.

6. In the **Maximum Mass** field, type the mass that determines the end of the scan range.
7. 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.
8. Click **OK**.

   The BPC is generated in a new pane.

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

Generating XWCs

You can extract up to three ranges from a DAD spectrum to generate the XWC. For more information about using the available icons, see Table 5-9 *Working With Graphs* on page 69.

1. Open a data file that contains a DAD spectrum.
2. Anywhere in the pane, right-click and then click **Extract Wavelengths**.

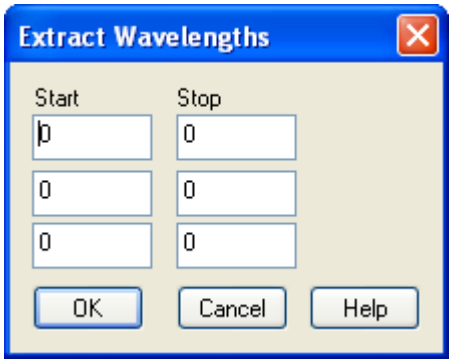


Figure 5-3 Extract Wavelengths dialog

3. Type start and stop values and then click **OK**.

   The XWC appears in a pane below the DAD spectrum.

DAD

You can view the DAD spectrum for a single point in time, or for a range of time as a Total Wavelength Chromatogram. For more information about using the available icons, see Table 5-9 *Working With Graphs* on page 69.

Viewing DAD data

You can view DAD data in chromatogram or spectrum form, the same as mass spectrometer data.

1. Open a data file containing data acquired with a DAD.
2. The TWC, which is analogous to a TIC, appears in a pane underneath the TIC.
3. 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.
4. Click **Explore** > **Show > Show DAD Spectrum**.

   The DAD spectrum appears in a pane below the TWC. The y-axis shows absorbance and the x-axis shows wavelength.

Tip!: If you close the pane with the TWC, you can open it again by clicking a point anywhere in the TWC and then clicking **Explore** > **Show** > **Show DAD TWC**.

TWCs

A TWC is a less commonly used chromatogram. It displays 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, you can create individual TWCs for each experiment, and another TWC that represents the sum of all experiments.

Generating TWCs

A TWC shows total absorbance (mAU) on the y-axis plotted against time on the x-axis. For more information about using the available icons, see Table 5-9 *Working With Graphs* on page 69.

1. Open a data file that contains a DAD spectrum.
2. Click **Explore** > **Show** > **Show DAD TWC**.

   The TWC appears in a pane below the DAD spectrum.

   You can also right-click inside the pane containing the DAD spectrum and then select Show DAD TWC from the shortcut menu.

Adjusting 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. You can raise or lower the threshold; however, changing the threshold value does not alter data. The software does not label any peaks in the region that lies beneath the threshold.

1. Open a data file.
2. You can adjust the threshold in three ways:
   - 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** and then, in the **Threshold Options** dialog that appears, type the threshold value.
   - Click **Explore** > **Threshold**.

   The graph updates to display 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.

Table 5-7 Right-Click Menu for Chromatogram Panes

Menu	Function
List Data	Lists the data points and integrates chromatograms.
Show Spectrum	Generates a new pane.
Show Contour Plot	Displays 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 chromatograph 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 UV data trace, if acquired.
Spectral Arithmetic Wizard	Opens the Spectral Arithmetic Wizard.
Save to Text File	Generates a text file of the pane, which can be opened in Excel or other programs.
Save Explore History	The Explore History File records changes to processing parameters, also called Processing Options, when a .wiff file is 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 the position of the mouse cursor.

Table 5-7 Right-Click Menu for Chromatogram Panes (Continued)

Menu	Function
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. By default, the subtract ranges are locked.
Delete Pane	Deletes the selected pane.

Table 5-8 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	Extracts a specific ion or set of ions from a selected pane and then generates a new pane containing a chromatograph for the specific ions.
Save to Text File	Generates a text file of the pane, which can be opened in Excel or other programs.
Save Explore History	The Explore History File records changes to processing parameters, also called Processing Options, when a .wiff file is 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 the position of the mouse cursor.
Show Last Scan	Shows the scan prior to the selection.
Select Peaks For Label	In this dialog, you can specify parameters to reduce peak labeling.
Delete Pane	Deletes the selected pane.
Add a Record	You may add records and compound-related data including spectra to the library. You must have an active spectrum to perform this task.
Search Library	Click to search the library without constraints or with previously saved constraints.
Search With Constraints	Click to search using the Search Constraints dialog.

Data Processing

You can process graphical data in a variety of ways. This section provides information and procedures for using some of the most commonly used tools.

Working With Graphs in Panes

At some point you will want to compare data or examine the same data different ways. You may want to keep your 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 may be of variable or fixed size. Panes are automatically tiled within the window and are arranged into column and row format. If you change the size of a window, the panes within the window change in size to accommodate the resizing. You cannot resize a window to the point where any of the panes would become smaller than its minimum size.

You can link two or more windows or panes containing similar data, for instance, spectra with similar mass ranges. As you zoom in one pane or window, the other pane zooms simultaneously. For example, you can link an XIC to the BPC from which it was extracted. Zooming in the BPC also zooms the XIC, so that both chromatograms display the same magnification.

Table 5-9 Working With Graphs

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

Move a pane	• Select the graph and then click **Window** > **Move Pane**.
	• Select the pane or window and then drag it to the new position. This position can be within the same window or within another window.
	A four-headed arrow appears 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, the pane moves above or below that pane, respectively.
	  • If the pane is at the left or right of the target pane, the pane moves to the left or right of that pane, respectively.
	  • If the pane is at any other position, the pane moves to the target row. The drop shadow of the pane as you move it around indicates its new position.
		

Link panes	• With the two graphs displayed, click one to make that pane active. • Click **Explore > Link**, and then click the pane to which you want to link.	
Remove linking	• Close one of the panes and then click **Explore > Remove Link**	

Table 5-9 Working With Graphs (Continued)

To do this...	use this menu option...	...or click this icon
Delete a pane	• Select the graph and then click **Window > Delete Pane**.	
Lock a pane	• Select the graph and then click **Window > Lock Panes**.	
Hide a pane	• Select the graph and then click **Window > Hide Pane**.	
Maximize a pane	• Select the graph and then click **Window > Maximize Pane**.	
Tile panes	• Select the graph and then click **Window** > **Tile all Panes**.	

Zooming in on a Graph

You can zoom in on part of a graph to view a particular peak or area in greater detail in both spectra and chromatograms. You can zoom repeatedly to view smaller peaks.

To zoom on the y-axis

1. Position the pointer to the left of the y-axis and then drag vertically away from your starting point.

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

Note: Take care when zooming in on the baseline. If you go too low the zoom-in box disappears.

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

To zoom on the x-axis

1. Position the pointer under the x-axis to either side of the area that you want to expand and then drag away from the starting point in a horizontal direction to expand the area of interest.
2. Release the mouse button to redraw the graph to the new scale.

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

Labeling Graphs

You can customize the preset style for labels on graphs and chromatograms. You can select the fonts to use for peak and axis labels, and the colors to use for your traces. You can add axis labels and the type of label and precision for your peaks.

To add captions to a graph

You can use captions to label peaks of interest or significant points on the graph. When you place a caption beside a peak, the caption stays with the peak when you zoom in or out. Captions also stay with the original sample when you navigate between samples in a data file. A caption contains one line of text, with a maximum of 128 characters.

1. On the spectrum, right-click, and then click **Add Caption**.

   The Add Caption dialog appears.
2. In the **Caption** box, type the text.
3. To change the size and style of the caption, click **Font**.
4. To place the caption, click **OK**.

Tip!: If you are not satisfied with the position of the caption, you can drag it to a different position. The caption stays in the same place relative to the x- and y-axes when you zoom in or out. To edit or delete the caption, right-click the caption and then click the appropriate command.

To add text to a graph

You can use text to add multiple lines of information to a graph. Unlike captions, which are associated with a specific peak and move with it as you zoom, text labels remain in their original location as you zoom. They do not stay with the original sample when you navigate between samples in a data file.

1. On the graph, right-click and then click **Add User Text**.

   The Add User Text dialog appears.
2. In the **User Text** field, type the text.
3. If you want the text to be centered, select the **Center Text** check box.
4. If you want to change the size and style of the caption, click **Font**.
5. To insert the text, click **OK**.

Tip!: If you are not satisfied with the position of the text, you can drag the text to a different position. To edit or delete the text, right-click the text and then choose the appropriate command.

Overlaying and Summing Spectra or Chromatograms

You can visually compare two or more sets of data by overlaying graphs created by similar methods. Each individual spectrum is distinguished by the color of its trace. For full scan data, this allows you to visualize the differences between several sample spectra.

After you have two or more graphs overlaid, you can sum the graphs to get a new trace. Each point on the new trace is the sum of the points from the graphs. Summing several overlays of similar data type can make subsequent processing operations easier and faster. For example, you can overlay several XICs, sum them, and then smooth the summed overlay to remove noise.

Summing overlays is similar to generating a TIC with the benefit of being able to choose which graphs to overlay. For example, if you were looking at ten experiments, the TIC will add all ten experiments together. If you sum overlays, you have the option of adding only nine of the ten overlaid graphs. You might want to do this if the data collected in the one experiment is just noise.

To overlay graphs

If you choose one or more panes, then each XIC will appear in a separate pane.

Tip!: To overlay fewer than four graphs in the same pane, press **Ctrl + right-click** in a pane and then click **Appearance Options**. In the Appearance Options dialog, Multiple Graph Options tab, select **Yes** for the **Overlay Multiple Panes** fields for **Spectrum** and **Chromatogram**.

1. Select the first pane that you want to overlay.
2. Click **Explore** > **Overlay**.
3. Click in the pane that you want to overlay.

   The graphs are overlaid showing the two traces in different colors.

Tip!: To view a color-coded list of the overlaid graphs, right-click the title bar of the pane.

To cycle between overlaid graphs

1. Select a pane that contains overlaid graphs.
2. Click **Explore** > **Cycle Overlays**.

   The display changes so that the next graph in sequence appears in the foreground.

To sum overlays

1. Overlay the graphs that you want to sum.
2. Click **Explore** > **Sum Overlays**.

   The overlaid graphs are added together.

Table 5-10 Explore Toolbar Quick Reference: Overlaying Graphs

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

Table 5-10 Explore Toolbar Quick Reference: Overlaying Graphs (Continued)

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

Performing Background Subtractions

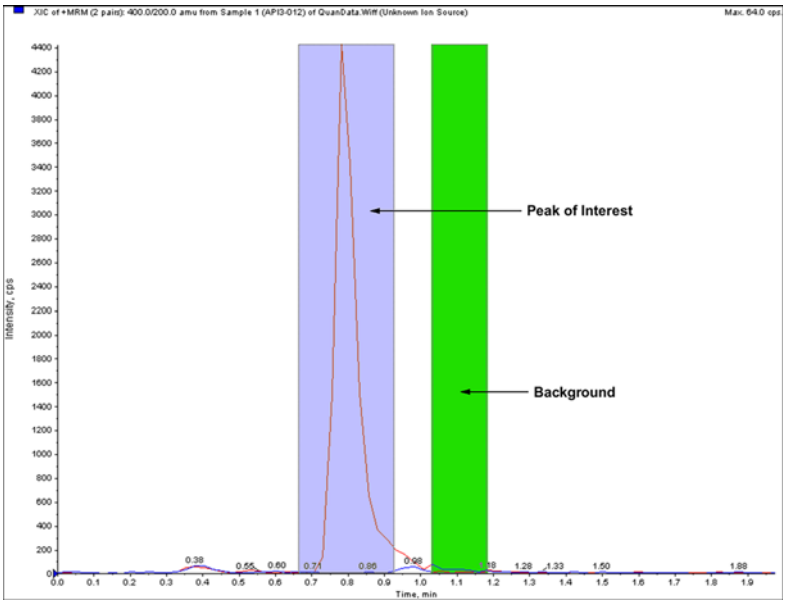
Background subtraction reduces the amount of noise in a spectrum by subtracting either one or two ranges that contain noise from a range that contains a peak. You can move the ranges independently, or lock them and move them as a single entity within the graph. Locked Background Subtraction is the preset setting. The software offers different methods of background subtraction.

Background Subtract: You can use background subtract to isolate a peak of interest. You can highlight and subtract up to two selected ranges from your peak. You can also lock your ranges and move them within the graph to optimize peak isolation, or to isolate another peak.

Background Subtract to File: You can use Background Subtract to File to save a new file that has a defined noise region subtracted from each scan. When a range is selected in the TIC, all the scans from that selection are internally averaged, and the resulting spectrum is subtracted from all the scans. In some cases, the resulting file might look close to the original scan.

To perform a background subtraction from a spectrum

1. Open a data file.
2. Select a background range.



3. Hold down the Shift key and then select another background range.

4. To set the subtract range, click **Explore** > **Background Subtract** > **Set Subtract Range**.
5. Select the peak of interest.
6. Click **Explore** > **Background Subtract** > **Perform Background Subtract**.

   The background is subtracted from the peak and a new spectrum is generated.
7. To isolate another peak, drag the locked ranges in the chromatogram and repeat the background subtract.

Tip!: To clear the background subtract region, click **Explore** > **Background Subtract** > **Clear Subtract Range**.

8. To save your background subtracted spectrum as a processed data file, click **File** > **Save**.

To unlock the ranges

The selected subtraction range is set to locked.

- Click **Explore** > **Background Subtract** > **Subtract Range Locked**.

  The ranges are unlocked and you can move each one independently.

To perform a background subtraction to file

Use the Background Subtract to File function to save a new file that has a defined noise region subtracted from each scan. You first select a representative background range in the TIC; all the scans in that region are averaged internally, and the resulting spectrum is then subtracted from all the scans. In some cases, the results might look like the original scan.

1. Open a data file.
2. Select the background range within the TIC and set it as background.
3. Click **Explore** > **Background Subtract to File**.

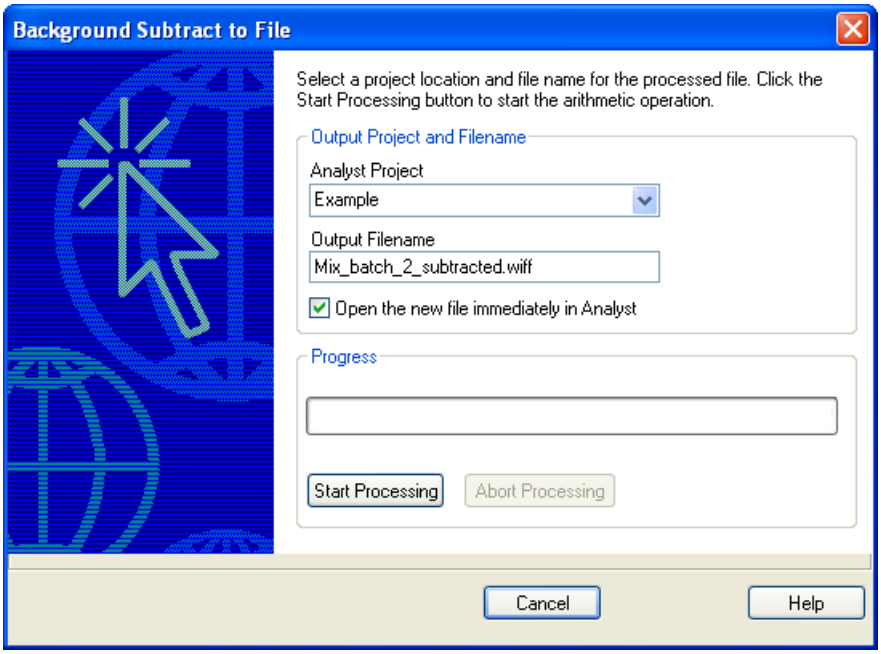


Figure 5-4 Background Subtract to File dialog

4. In the **Output Project and Filename** section, type the project and file names for the resulting file.
5. Click **Start Processing**.

   The progress bar displays the progress of the subtraction process. If you select the **Open the new file immediately in Analyst** check box, when the subtraction is complete, the file will appear.
6. If the check box is cleared, when the subtraction is complete, click **Finish**.

Table 5-11 Explore Toolbar Quick Reference: Background Subtract

Icon	Name	Function
	Perform Background Subtract	Click to perform a background subtract after you have selected the background ranges.
	Subtract Range Locked	Click to lock the selected background ranges. If you unlock the background ranges you can move each range independently.
	Background Subtract to File	Click to save a new file that has a defined noise region subtracted from each scan.
	Spectral Arithmetic Wizard	Click to use the Spectral Arithmetic Wizard to perform arithmetic operations using two complete chromatograms to create a new chromatogram, which you can save as a new data file.

Smoothing Algorithms

Smoothing a data set removes local variations that are most likely due to noise. You may apply several smoothing cycles to the data, but you can undo only the most recent smooth. Smoothing is not available for MI/MRM spectra. You can choose the smooth algorithm or the Gaussian smoothing algorithm as your preset smoothing method.

Smooth Algorithm

When you smooth data, you set the point weighting values for three data points; the current point, the preceding point, and the following data point. The smooth algorithm multiplies the data points by the assigned weighting values, sums these values, and then divides the total by the sum of the point weight values. It is a gentler smooth than the Gaussian algorithm, and it takes a long time to smooth very noisy data.

Gaussian Smoothing Algorithm

Gaussian smoothing involves replacing each data point with the weighted average of a number of data points on either side of it. The weighting for each new data point is calculated on the basis of a Gaussian curve. It is a coarser smooth than the smooth algorithm, but it is good for smoothing very noisy data.

You set two values when using the Gaussian smoothing method:

Gaussian filter width (% of minimal distance between points): This value shows the width used to calculate the weighting of neighboring points. The width is described in terms of percentages of the distance between two points in the scan, where the preset width of 100% gives a distribution that is as wide as the distance between data points.

Limit of Gaussian filter (number of minimal distance between points): This value corresponds to the limits of the Gaussian curve, shown in multiples of the distance between points. For example, the preset value of 10 creates a Gaussian curve that truncates after ten data point widths on either side of the center.

Smoothing Data

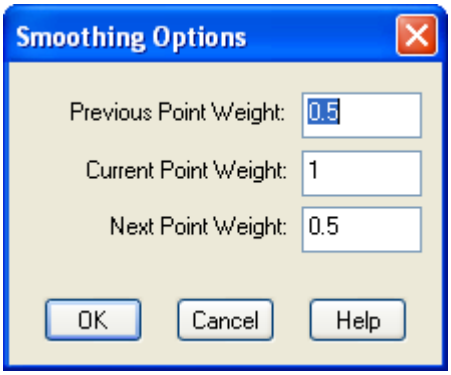
You can choose the Analyst® software smoothing method or the Gaussian smoothing method.

Tip!: To undo smoothing, click **Edit** > **Undo**. The software supports one level of undo.

To smooth data using the Smooth algorithm

1. Select a pane containing a chromatogram or spectrum.
2. Click **Explore** > **Smooth**.

   The Smoothing Options dialog appears.



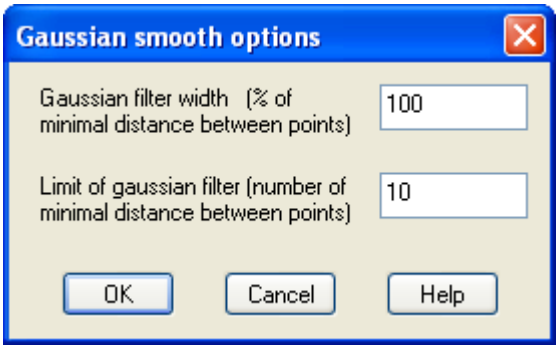
3. In the **Previous Point Weight** field, type the weighting factor to be applied to the previous data point.
4. In the **Current Point Weight** field, type the weighting factor to be applied to the center data point.
5. In the **Next Point Weight** field, type the weighting factor to be applied to the following data point.
6. To smooth the data, click **OK**.

   The data set is smoothed, replacing the current data set in the pane.

To smooth data using Gaussian smoothing

1. Select a pane containing a chromatogram or spectrum.
2. Click **Explore** > **Gaussian Smooth**.

   The Gaussian smooth options dialog appears.



3. In the **Gaussian filter width** field, type the width used to find the weighting of neighboring points as a percentage of the distance between the two points.
4. In the **Limit of gaussian filter** field, type the limit of the Gaussian curve, given in multiples of the distance between points.
5. To smooth the data, click **OK**.

   The data set is smoothed, replacing the current data set in the pane.

Table 5-12 Explore Toolbar Quick Reference: Smoothing Data

Icon	Name	Function
	Smooth	Click to smooth data using the smooth algorithm.
	Gaussian smooth	Click to smooth data using Gaussian smoothing.

Centroiding Data

Centroiding converts peak distribution values into a single value of *m/z* and intensity that represents the peak. Centroiding data collected in profile mode simplifies the data and reduces the file size. Centroiding provides more accurate peak assignment and reduces the amount of data, but it also removes the information about the peak shape.

The centroiding algorithm converts peaks to single values by using an intensity weighted average to calculate the center of gravity of the peak. The output of the algorithm is a list of peaks with parameters, as shown in Table 5-13.

Table 5-13 Peak Parameters

Parameter	Definition
Centroid Value	The value of the centroided data in units of mass or time.
Intensity	The intensity of each peak in cps.
Width	The width of the centroided peak in amu.

Data is automatically centroided when added to a library or when a search is conducted.

To centroid data

1. Select a pane containing a spectrum.

   Centroiding data changes the appearance of the existing graph. To compare the result with the original data, make a copy of the graph before centroiding.

2. Click **Explore** > **Centroid**.

   The data is centroided.

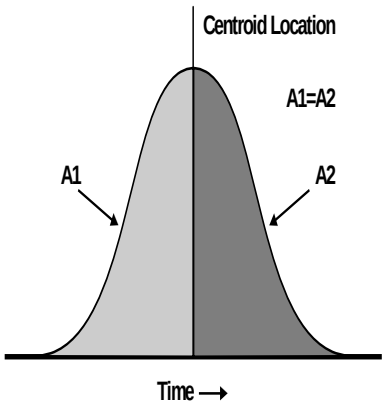


Figure 5-5 Analyte Centroid Location

Table 5-14 Explore Toolbar Quick Reference: Centroiding Data

Icon	Name	Function
	Centroid	Click to centroid data.

Saving and Opening Processed Data Files

You can save processed data, such as specific layouts and captions, that can be re-opened in Explore mode only. These files also contain relevant history information and are similar to data files except they will contain only the data from the active pane in Explore. These files have the .pdt extension and are stored in the Data folder in your current project.

To save a processed data file

1. Select the pane of data that you want to save.
2. Click **File** > **Save Processed Data File**.

   The Save Processed Data File dialog appears.
3. In the **File name** field, type the name of the processed data file and then click **Save**.

To open a processed data file

1. In **Explore** mode, click **File** > **Open Processed Data File**.

   The Load Processed Data File dialog appears.
2. Select a file and then click **Open**.

Contour Plots

A Contour Plot is a color-coded plot of a complete data set that uses color to represent a third dimension in the plot. In a Contour Plot of a TIC, the x-axis represents retention time or scan number, the y-axis represents mass, and the color represents the intensity of the data at that point. In a Contour Plot of a TWC for DAD data, the x-axis represents retention time or scan number, the y-axis represents wavelength, and the color represents absorbance. Contour Plot is a post-acquisition tool that does not function in a real-time scan acquisition.

Note: Contour Plot does not support MI or MRM scans, but it does support DAD scans.

Working with Contour Plots

Color is the third axis in Contour Plot, and it represents either intensity or absorbance. You can change the high and low intensity or absorbance values in Contour Plot using the control triangles on the color bar above the Contour Plot. The percentage parameters at the top of the Contour Plot pane indicate the values held by the low and high sliders. The actual values are based on a percentage of the maximum intensity or absorbance within the selected area. The value appears in the top right corner of the Contour Plot pane.

The controls shown in Figure 5-6 change the colors in a Contour Plot.

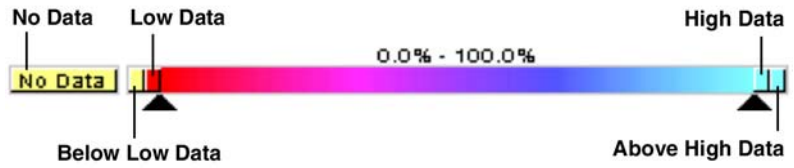


Figure 5-6 Buttons Controlling Contour Plot Colors

You can define the colors on a Contour Plot graph to provide better contrast and display data specifications according to your needs. For example, setting the intensity/wavelength and changing the color of the values for Below Low Data and Above High Data can eliminate background noise in Contour Plot.

The Below Low Data and Above High Data buttons shrink and expand on the color bar if you move the slider controls. When you change the contour plot colors, the new colors become the preset colors for all subsequent graphs.

To view a Contour Plot

You can view a Contour Plot only after acquisition. You can view a Contour Plot from TIC, XIC, TWC, or XWC graphs. TICs and XICs are available for all .wiff data files. TWCs and XWCs are available only for data acquired by a DAD.

1. In Explore mode, open a data file as a TIC, XIC, TWC, or XWC graph.
2. Highlight the range you want to view in the Contour Plot. If you do not make a selection, you will view the entire range.
3. Click **Explore** > **Show** > **Show Contour Plot**.

   A Contour Plot of the selected area appears in a separate pane.

To select an area in a Contour Plot

You may wish to zoom in on a particular selection, or view the corresponding mass spectrum for that selection.

- Do one of the following:
  - To select a standard area within a box, drag the pointer to create a box around an area in the Contour Plot.
  - To make a vertical selection, press Ctrl and drag the pointer vertically.
  - To make a horizontal selection, press the space bar and drag the pointer horizontally.

To set the intensity and absorbance in a Contour Plot

- Do one of the following:
  - To set the low intensity/absorbance value in Contour Plot, from the color bar above the Contour Plot, drag the left triangular slider to the required position.

    Contour Plot automatically adjusts the color of values below the setting to indicate they are outside the range.
  - To set the high intensity/absorbance value in Contour Plot, from the color bar above the Contour Plot, drag the right triangular slider to the required position.

  Contour Plot automatically adjusts the color of values above the setting to indicate they are outside the range.

To change colors in a Contour Plot

1. In the Contour Plot pane, click one of the color buttons.

   The Color dialog appears.
2. Click a color, and then click **OK**.

   The graph changes to reflect the color change.

Tip!: By using the Define Custom Colors palette, you can create customized colors for use in a Contour Plot.

Table 5-15 Right-Click Menu for Contour Plot Panes

Menu	Function
Show DAD Spectrum	Opens a new pane with the DAD spectrum.
Extract Wavelengths (Use Range)	Extracts up to three wavelength ranges from a DAD spectrum to display the XWC.
Extract Wavelengths (Use Maximum)	Extracts wavelength ranges using the maximum wavelengths.
Zoom to selection	Zooms in on the selected area.
Add User Text	Adds a text box at the position of the cursor.
Undo Zoom	Returns the graph to the original scale
Delete Pane	Deletes the selected pane

Table 5-15 Right-Click Menu for Contour Plot Panes (Continued)

Menu	Function
Show Cross-Hair	Shows the Cross-Hair (nm/min)

Fragment Interpretation

The Fragment Interpretation Tool generates a list of theoretical fragment masses from single, non-cyclic bond cleavage of a molecular structure. You create the molecular structure in a third-party drawing program and then save it as a .mol file. Fragment Interpretation displays the theoretical fragments in the fragment list and compares the fragment masses to peaks in the mass spectrum. Peaks above the threshold intensity and within the user-defined mass tolerance (maximum 2 amu) of fragment masses are considered matched and appear in bold text in the fragment list.

Note: The Fragment Interpretation tool cannot be used with the following scan types:

- Precursor Ion
- Neutral Loss
- Q1 Multiple Ion
- Q3 Multiple Ion
- Multiple Reaction Monitoring (MRM)

Working with the Fragment Interpretation Tool

If you are viewing multiple spectrum panes, then the Fragment Interpretation tool connects to the active spectrum. If the data file contains more than one sample, then the Fragment Interpretation tool connects to the active spectrum.

The tool automatically calculates the single, non-cyclic bond cleavage fragments from a .mol file. When the Fragment Interpretation tool is connected to a spectrum, theoretical fragments in bold text, indicate a matching peak in the spectrum within the specified mass tolerance and intensity threshold.

When you select a single, non-cyclic bond in the molecular structure, the Fragment Interpretation tool highlights the two fragments created when the bond is cleaved, and displays matching peaks in the connected spectrum.

To connect the Fragment Interpretation tool to a spectrum

If you have a spectrum open when you open the Fragment Interpretation tool, then the active panel links to the open spectrum automatically.

1. Click **Explore** > **Show** > **Show Fragment Interpretation Tool**.
2. From the lower right corner of the Fragment Interpretation pane, click the connect button.

   The pointer changes to the connecting tool.

3. Click the spectrum graph that you want to connect to the Fragment Interpretation tool.

   The connected graph indicator in the lower left corner contains the name of the graph connected to the Fragment Interpretation pane. The connection is broken when either the graph or Fragment Interpretation is closed. If the connected .wiff file has more than one sample, the Fragment Interpretation pane updates automatically as you scroll through the samples.

To match fragments with peaks

1. Click **Explore** > **Show** > **Show Fragment Interpretation Tool**.
2. With a .mol file in the Fragment Interpretation pane, select a cell in the Fragment List that appears in bold.

   In the spectrum, the software highlights the matching spectral peak in the color selected under the Options tab. In the molecular structure, the bond is highlighted.
3. If you click a row that has more than one matching fragment, the spectral peak that is closest to its monoisotopic mass is highlighted in the mass spectrum in the color specified in the Options tab.

To select a bond in a molecular structure

1. Click **Explore** > **Show** > **Show Fragment Interpretation Tool**.
2. With a .mol file opened in the Fragment Interpretation pane, click a single, non-cyclic bond in the molecular structure.

   The two resulting fragments appear as highlights in the fragment list. The masses of the two fragments appear on either side of the bond.

   If a spectrum is connected, then the Fragment Interpretation tool displays any matching peaks in the graph. If you select a fragment in the list and the fragment is matched to a peak, then the Fragment Interpretation window zooms in on that peak.

To view isotopes

The Fragment Interpretation tool can display the theoretical isotopic distribution for a peak matching a fragment in the fragment list.

1. Click **Explore** > **Show** > **Show Fragment Interpretation Tool**.
2. In the **Fragment Interpretation** pane, click the **Options** tab.
3. Select the **Show Isotopes** check box.
4. Click **Apply**.
5. From the fragment list, select a fragment that matches a peak.

   The isotopic distribution for matched peaks appears in the spectrum.

Displaying Formula Differences for Fragments

You can display the formula and monoisotopic mass difference between two related hypothetical fragments. The formula difference appears when you select two peaks. The formula and monoisotopic mass difference appears when you select two fragments, or two single, non-cyclic bonds.

To display a formula difference in a spectrum

1. Click a fragment peak.
2. Press the Shift key and then click another fragment peak.

   If the formula difference is equal to a fragment from the fragment list, the fragment highlights in the list. Otherwise, the formula difference between the peaks' matching fragments appears in a message box.

To display a formula difference in the fragment list

1. Click the row number for one fragment.
2. Press the Ctrl key and then click another fragment.

   The formula and monoisotopic mass difference appears in a message box if the fragments are related.

To display a formula difference in a molecular structure

1. Click a single, non-cyclic bond. The default fragment (of the two highlighted fragments) is selected. If you want to select the other fragment of the cleaved bond, CTRL+click the bond.
2. Select a second non-cyclic bond. To select the default fragment, press the Shift key and then click the bond. To select the other fragment of the cleaved bond, press Ctrl+Shift+click the bond.

   Fragment Interpretation calculates the formula and monoisotopic mass difference between the fragment you selected in step 1 and the fragment selected in step 2, if the fragments are related. The formula and monoisotopic mass difference appears in a message box.

Table 5-16 Explore Toolbar Quick Reference: Fragment Interpretation Tool

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

Library Databases

The Library Search feature compares unknown spectra to known MS spectra contained in the library database and generates a list of possible matches.

With Library Search you can:

- Compare library contents against an unknown spectrum.
- Add records to the library.
- Edit existing records.

You can store library data in the following locations:

- MS Access on a local database
- MS SQL Server

Connecting to a Library Database

Before you can use the Library Search feature, you must find where the library data is stored and connect your computer to that location. Library databases can be stored locally on your computer or on a server and accessed over a network.

To switch between existing library databases

You can connect to any databases that have aliases that are already set up.

1. Click **Tools** > **Settings** > **Optimization Options**.

   The Optimization Options dialog appears.

2. Click the **Library Manager** tab.

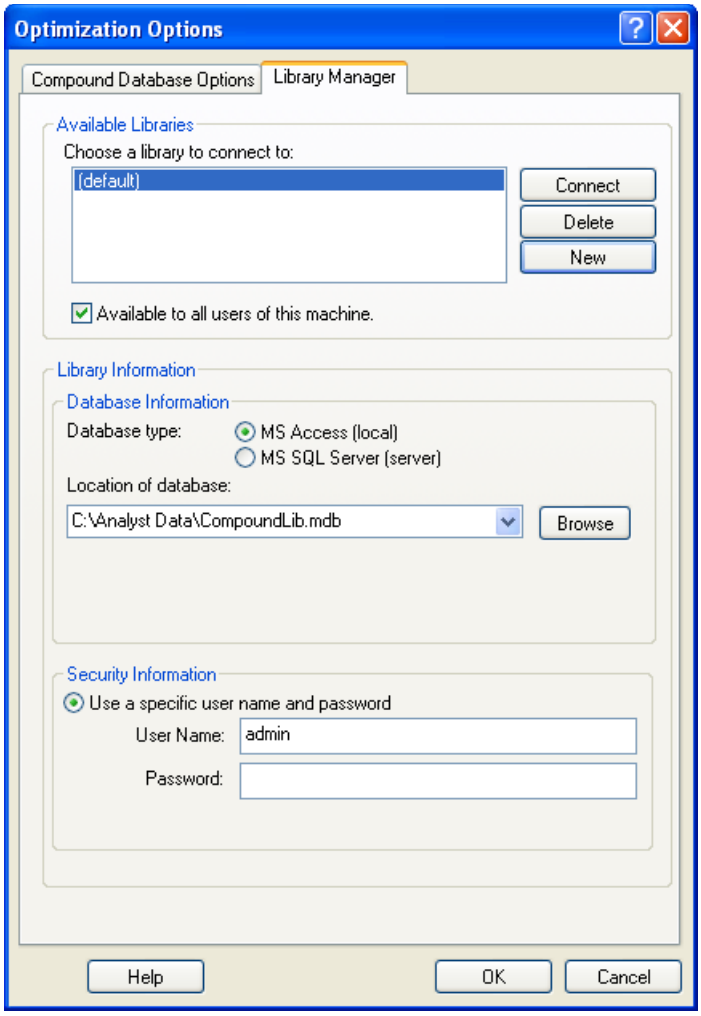


Figure 5-7 Library Manager tab

3. In the **Available Libraries** section, click the alias of the database to connect to and then click **Connect**.
4. To allow other users to access the database, select the **Available to all users of this machine** check box.

5. Click **OK**.

To connect to a local library database

1. Click **Tools** > **Settings** > **Optimization Options**.

   The Optimization Options dialog appears.
2. Click the **Library Manager** tab.
3. In the **Available Libraries** section, click **New**.

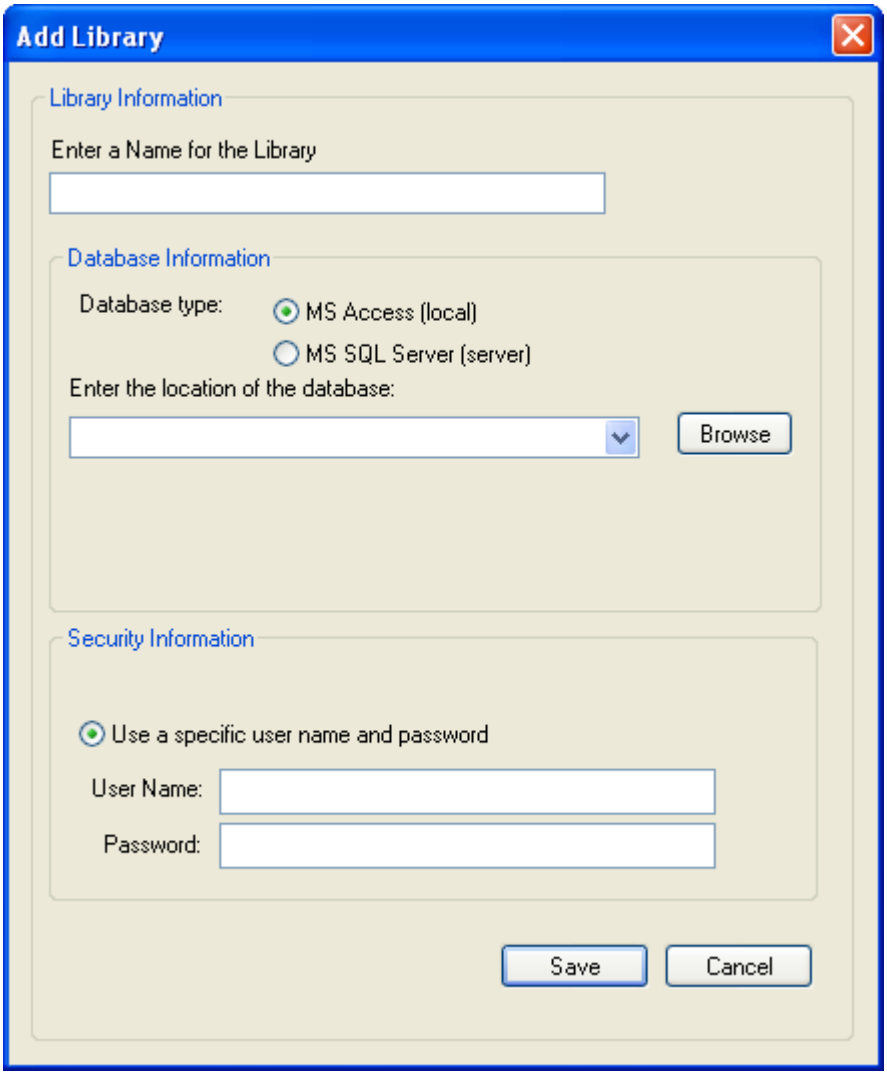


Figure 5-8 Add Library dialog

4. Type a name for the library.
5. In the **Database Information** section, select **MS Access (local)**.
6. Type the database location.
7. In the **Security Information** section, if a user name and password are required to access this database, then type your user name and password.
8. Click **Save**.

To connect to a server library database

1. Click **Tools** > **Settings** > **Optimization Options**.

   The Optimization Options dialog appears.
2. Click the **Library Manager** tab.
3. In the **Available Libraries** section, click **New**.
4. Type a name for the library.
5. In the **Database Information** section, select **MS SQL Server (server)**.

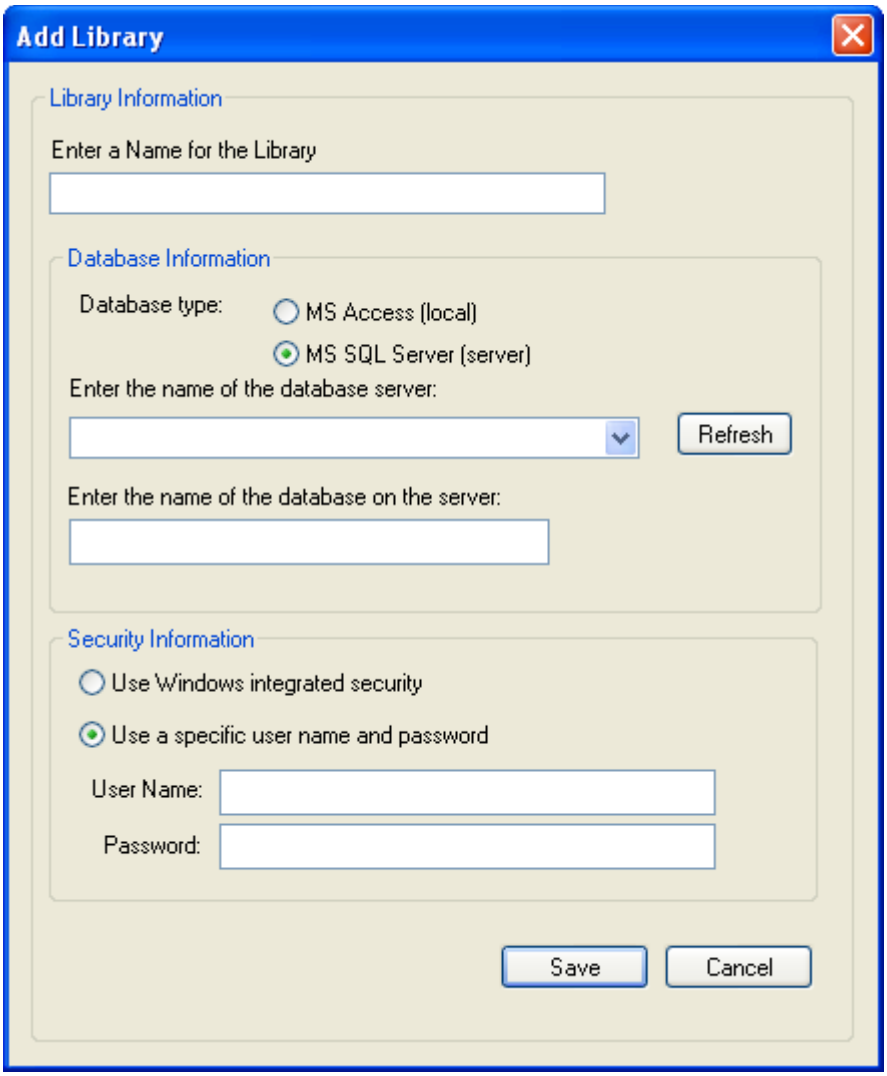


Figure 5-9 Add Library dialog

6. Type the name of the database server.
7. Type the name of the database.
8. Do one of the following:
   - If a specific user name and password are required to access this database, then type your user name and password.

- If you want to use Windows security, then in the **Security Information** section, select the **Use Windows integrated security** option.

9. Click **Save**.

Working with Library Records

If you request the library contents without constraints, all records will be listed. When you request records from the library with constraints, only those records that match the constraints you specified will appear. The number of records displayed depends on the number of constraints you select. If you select many restraints, few records appear.

To view all library records

- Click **Explore** > **Library Search** > **List**.

  The Librarian dialog appears with all records in the database.

To search library records with constraints

1. Click **Explore** > **Library Search** > **List With Constraints**.

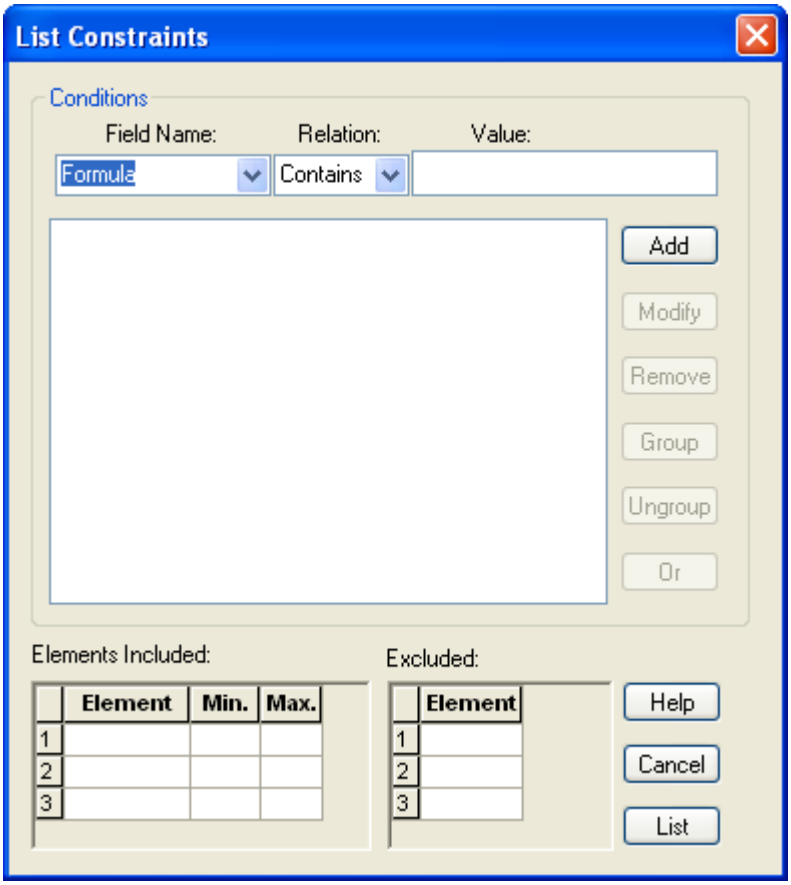


Figure 5-10 List Constraints dialog

2. In the **Field Name** list, select a field on which you want to base a constraint.
3. In the **Relation** list, select the relation (operator) that applies to the field name.

4. In the **Value** field, type the value of the field name based on the relation.
5. To add the selected constraint to the **Conditions** list, click **Add**.
6. Continue adding constraints to the conditions list as required.
7. Coupling distinct constraints within the **Conditions** list creates more specific conditions that enhance your search. To group constraints, select the constraints and then click **Group**. To separate grouped constraints, click the group, and then click **Ungroup**.
8. To change the relationship between constraints, click the relationship, and then click **And** or **Or**.
9. To exclude compounds containing a certain number of atoms of specific elements, select or type the elements in the **Elements Included** table, and then type a minimum and maximum number of atoms of the element.

Note: Element symbols are case-sensitive. For example, Hydrogen is H, not h and Sodium is Na, not NA or na.

10. To exclude compounds containing certain elements, select or type the elements in the **Excluded** table.
11. To search for compounds fitting your criteria, click **List**.

    Records that match all the constraints appear in the Records table. Listing constraints are saved.

To add a record to the library

1. Right-click an active spectrum, and then click **Add a Record**.

   The spectrum will be centroided automatically if it has not been centroided already. The Add a Record dialog appears with data from the spectrum.
2. On the **Mass Spectral Information** tab, type a name in the **Compound Name** field. The compound name is mandatory and must uniquely identify the compound within the library.
3. Edit any of the other fields. Many of the fields are filled in automatically from the data associated with the spectrum.
4. Click the **General Information** tab, edit the fields as required, and then click **OK**.

Searching for a Similar Spectrum

You can search the library for a spectrum (and its related compound information) that matches (or is similar to) an active spectrum. Searches can be performed with or without constraints. When you search with constraints, only those records that match all the criteria appear. The results appear in a ranked list; the first item on the list is the best fit to the active spectrum. Entries lower in the list do not match as well.

The more constraints you select, the more precise the list becomes and fewer, more relevant matches appear. Once you define a set of constraints they will apply to all subsequent searches, unless you edit them.

Only peaks above the threshold are used in the search. When selecting search constraints, you can also add or subtract peaks from the active spectrum. For example, if you think a peak is

actually a background or noise spike, you would not want to use it for the search because it could produce inaccurate results.

When you search without constraints you see a much larger list of suggested spectra because the library makes fewer specific matches to the spectral data.

To search for a similar spectrum

1. Right-click on an active spectrum and then click **Search With Constraints**.

   The software will centroid the spectrum automatically if you have not already done so.

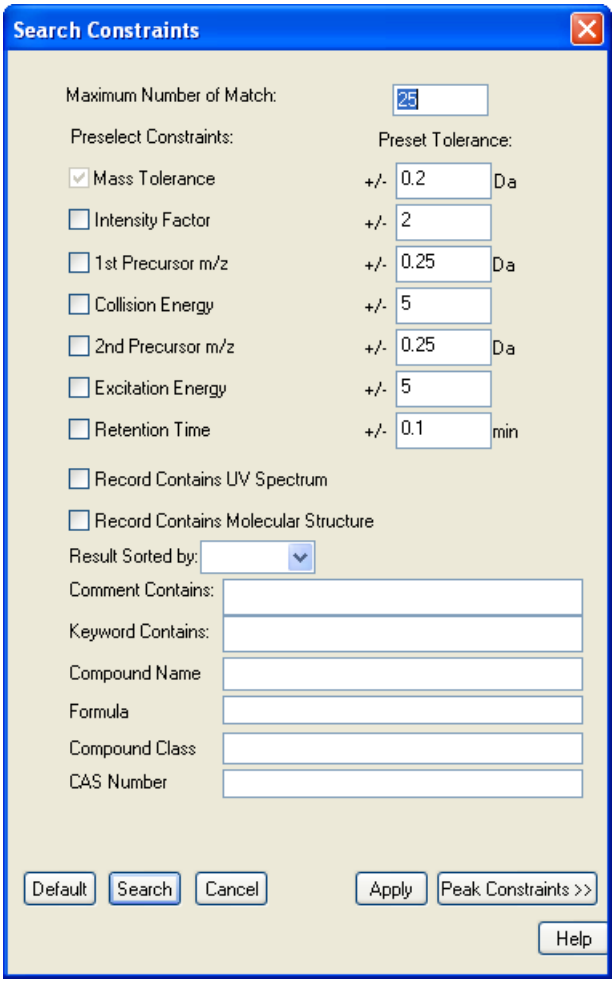


Figure 5-11 Search Constraints dialog

2. In the **Maximum Number of Match** field, type the maximum number of compounds you want returned by the search.
3. In the **Preselect Constraints** section, select the check boxes for the constraints to apply.
4. For each constraint selected, in the **Preset Tolerance** section, type the tolerance.
5. If required, select a method of sorting records from the **Result Sorted by** list.
6. If required, type text in the **Comment Contains** field.

7. If required, type text in the **Keyword Contains** field.
8. To apply peak constraints by adding and removing peaks, click **Peak Constraints**.

   The Peaks Included table appears.
9. To add peaks to the list you want to search against, click **Add** and then type the *m/z* and the corresponding intensity in the empty cell.
10. To remove peaks so they will not be included in the search, select the peaks that you do not want to search against and then click **Remove**.
11. Click **Search** to save the constraints and begin the search.

To view a compound from the search results

If several spectra match the unknown spectrum, you may want to view other spectra and compare them to the unknown.

1. In the S**earch Results** dialog, in the list of compounds, select the row number of the compound.
2. Click the spectrum pane of one of the known compounds.

   The spectrum of the selected compound appears.

Table 5-17 Tips

To do this...	... do this
Library searches: To group conditions	Select the conditions to group and then click **Group**. This function behaves like parentheses in formulas.
Library searches: To search without using constraints	Right-click an active spectrum, and then click **Search Library**. The Search Results dialog appears.
Table-specific queries: To view the entire table again	Right-click anywhere in the Results Table and then click **Query** > **Show All**. You can also apply the query again or edit the query.
Peak Integration: To review peaks	To review all peaks, make sure that all samples are listed in the Results Table. The Peak Review window contains the peaks listed in the Results table. If some samples are hidden in the table (for example, if you applied a query), then they are also hidden in peak review.
Peak Integration: To move to the first peak in the batch	Right-click anywhere in the Peak Review pane and then click **Show First Page**. To move to the last peak in the batch, right-click anywhere in the Peak Review pane and then click **Show Last Page**.
To examine calibration curves	Right-click anywhere in the curve, click **Active Plot**, and select the curve to be plotted on top.
Sample statistic review: To review an individual peak	Select the **Display the Data Set(s)** check box, and then, in the **Data Point** column, double-click the data point that represents the peak. The software displays the Peak Review window with the peak you chose.
Results Tables: To return the Results Table to its original order	Right-click on the Results Table and click **Sort** > **Sort By Index**.

Table 5-17 Tips

To do this...	... do this
Acquisition Methods: To create an acquisition method from the file information pane	Right-click the file information pane and then click **Save Acquisition Method**.

Table 5-18 Explore Quick Reference: Chromatograms and Spectrum

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.
	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.

Table 5-18 Explore Quick Reference: Chromatograms and Spectrum (Continued)

Icon	Name	Function
	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.
	Show DAD	Click to generate a DAD.

Table 5-18 Explore Quick Reference: Chromatograms and Spectrum (Continued)

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

Analyzing and Processing Quantitative Data 6

In this section, you will use the sample files found in the Example folder to learn how to select samples for quantitation, how to choose preset queries and create table-specific queries, and how to analyze the acquired data.

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.

About 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.

You can create a quantitation method before data acquisition and then apply it to the quantitative data automatically upon completion of the batch. Alternatively, a quantitation method can be created and applied post-acquisition.

You can use three tools to create a quantitation method: the Quantitation Wizard, the Build Quantitation Method, and Quick Quant.

Build Quantitation Method

If you use the Build Quantitation Method you will 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 modify existing quantitation methods. This is the most flexible way of creating a quantitation method.

Quantitation Wizard

If you use the Quantitation Wizard, a Results Table is generated at the same time as the quantitation method. Alternatively, you can use a pre-existing quantitation method to create quantitate different sets of data. This is the most common way of creating a quantitation method.

Quick Quant

Quick Quant is part of the Batch Editor. You can use Quick Quant to add compound concentrations prior to data acquisition. Because you have not yet acquired a sample, you cannot choose a representative sample or review any peaks. With this process, you are only defining the method components.

We do not recommend that you use the Quick Quant method that is generated. This quantitation method does not have the compound and sample-specific algorithm parameters that you need for peak selection.

If you have a previously saved quantitation method that you would like to use, you can select it from the Quantitation menu in your batch. For instructions on creating a batch, see Creating and Submitting a Batch on page 40.

About Results Tables

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

Using the software, you can export the data from a Results Table to a .txt file for use in other applications, such as Microsoft Excel. You can also export data in the table or just the data in the visible columns.

Creating Quantitation Methods and Generating Results Tables

For the following procedures, you will use previously acquired sample data that is installed with the Analyst software.

PK Data contains the batches Mix_Batch1 and Mix_Batch2. These sample batches are used to demonstrate the usefulness of metric plots to isolate problematic samples. The ions scanned were reserpine (609.4/195.0), minoxidil (210.2/164.2), tolbutamide (271.3/91.1) and rescinnamine (635.4/221.2), which is the internal standard. Batch 1 contains no errors in terms of sample preparation, whereas Batch 2 contains a QC sample where the internal standard was added twice (sample QC2).

To create a method using the Quantitation Method Editor

1. Make sure that you have the **Example** folder selected.
2. On the Navigation bar, under **Quantitate**, double-click **Build Quantitation Method**.

   The Select Sample dialog appears.
3. In the **Data Files** list, navigate to **Triple Quad > Mix_Batch_2**.

   The samples in the selected data file appear in the Samples list.
4. In the **Samples** list, select the samples and then click **OK**.
5. In the **Internal Standards** table, do the following:
   - In the **Name** column, select resinamine from the list.
   - In the **Q1/Q3** column, from the list of masses, select 635.400/221.185 for each standard.

   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 you select a value in Q1/Q3 field, the Name field is automatically populated.
6. In the **Analytes** table, do the following:
   - In the **Name** column, select reserpine.
   - In the **Internal Standard** column, from the list, select the internal standard to be associated with each analyte.

- In the **Q1/Q3 column,** from the list of masses, select 609.400/195.039.
- If required, you can add one or more of the other compounds for a more complex analysis.

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.

7. Click the **Integration** tab.
8. In general, the preset integration parameters are suitable for most peaks. However, if the integration is not suitable, you can change the algorithm. Click the **Show or Hide Parameters** icon to show the additional integration algorithms available.
9. Click the **Calibration** tab. The preset parameters are suitable for these samples.
10. Save the quantitation method as TutorialQuantMethod.qmf.

    The new method can now be used when you create a batch in the Batch Editor or when you can use the Quantitation Wizard to generate a Results Table.

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

To use the Quantitation Wizard to create a Results Table

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

   The Create Quantitation Set - Select Samples page appears.
2. In the **Available Data Files** list, navigate to **Triple Quad > Mix_Batch_2**, add all the samples, and then click **Next**.

   The Create Quantitation Set - Select Settings & Query page appears.
3. In the **Default Query** section, click **Select Existing: Query** and then select **Accuracy 15%**. Click **Next**.

Note: If you want to create a query at the same time, see To create a new standard query on page 98.

   The Create Quantitation Set - Select Method page appears.
4. Click **Choose Existing Method**, select TutorialQuantMethod.qmf, and then click **Finish**.

   The Results Table appears.

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

5. Save the Results Table as Tutorial Results Table.rdb.

Tip!: You can create well-formatted reports from a Results Table using the Reporter software. See Using Reporter Software on page 121.

To create a new standard query

You can create a query and a standard query numerous ways. The following is one example. For more information on creating queries, see the Help.

1. On the Navigation bar, in **Quantitate** mode, double-click **Quantitation Wizard**.
2. On the **Create Quantitation Set - Select Samples** page, select samples and then click **Next**.
3. On the **Select Settings & Query** page, in the **Default Query** section, select **Create New Standard Query** and then type a query name.

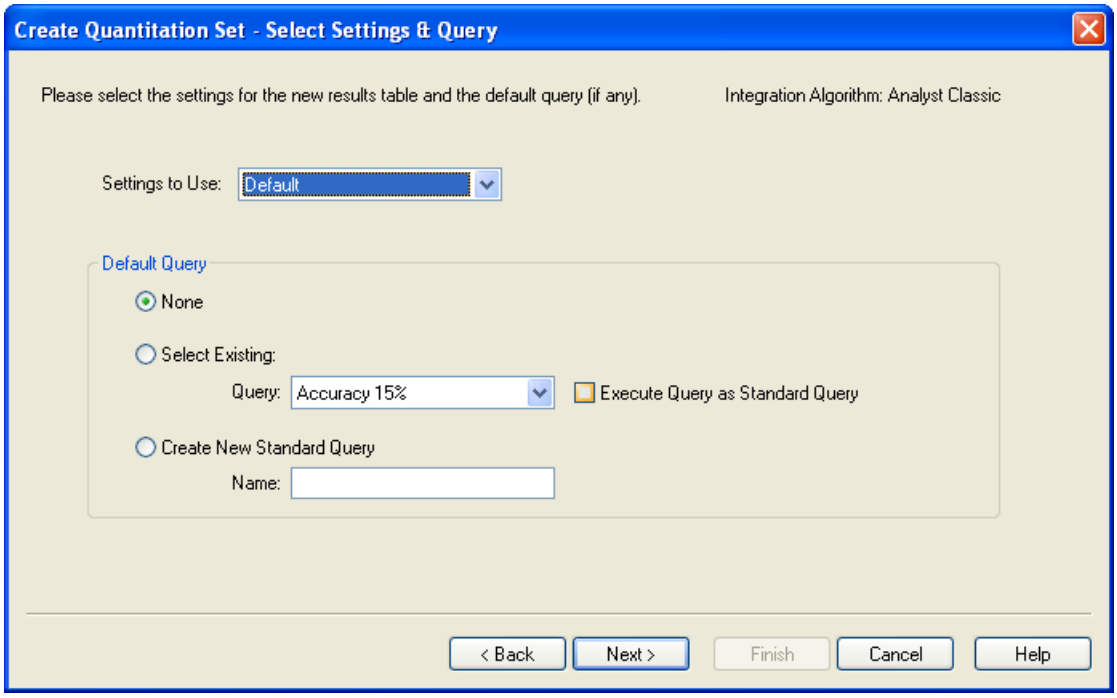


Figure 6-1 Create Quantitation Set - Select Settings & Query page

4. Click **Next**.

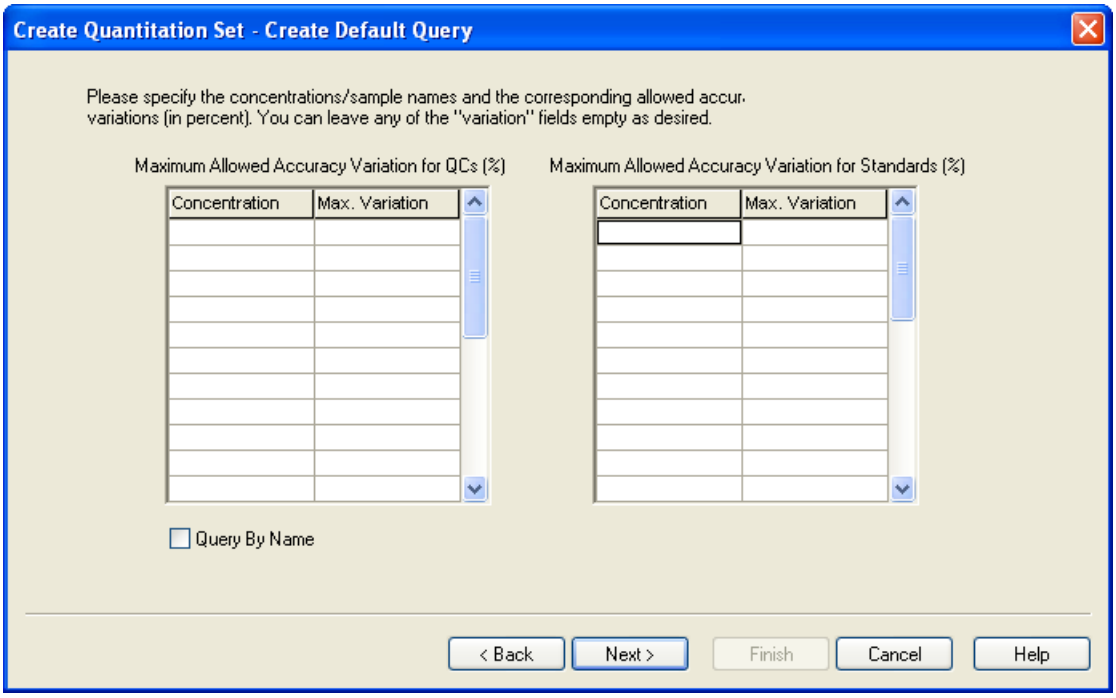


Figure 6-2 Create Default Query page

5. 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, they do not appear here. In that case, you will have to type them in the **Concentration** column.
6. 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, they do not appear here. In that case, you will have to type them in the **Concentration** column.
7. Click **Next**.

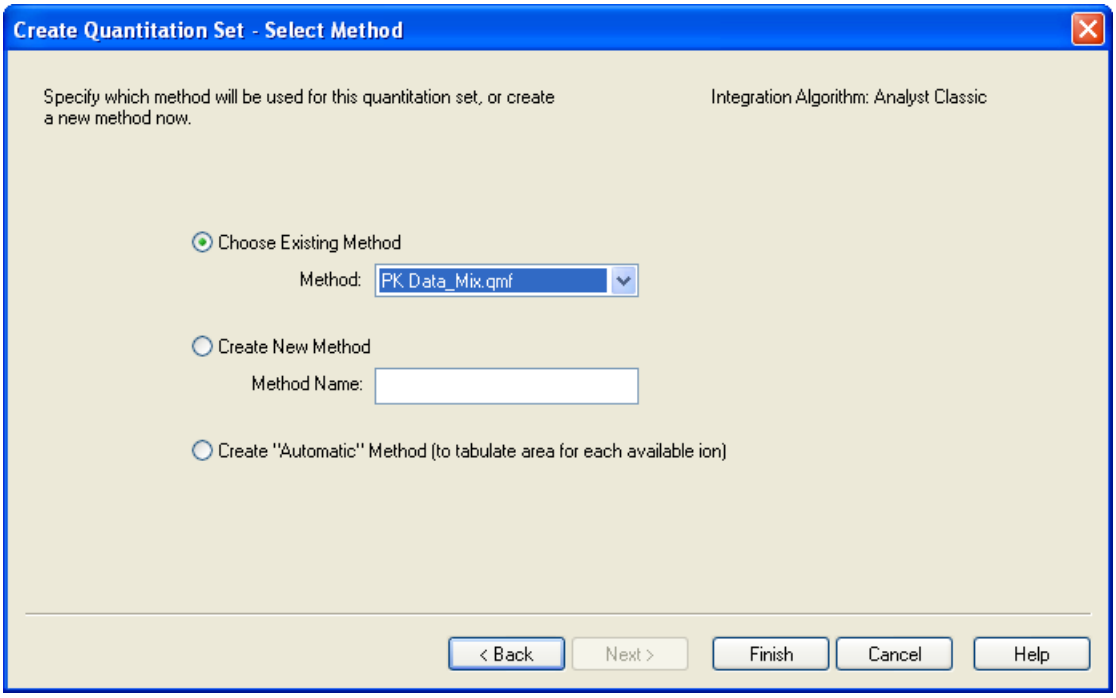


Figure 6-3 Create Quantitation Set - Select Method page

8. Select or create a method.
9. Click **Finish**.

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

Defining the Layout of Results Tables

Four predefined views of the Results Table are available.

- Right-click in the Results Table and then click one of the following:
  - To view the Full layout, click **Full**.

    All the analytes are displayed.
  - To view the Summary layout, click **Summary** and then click a field name.
  - To view the Analyte layout, click **Analyte** and then click a single analyte if more than one analyte exists.
  - To view the Analyte Group layout, click **Analyte Group** and then click an analyte group.

Tip!: A new analyte group must be created first. To do this, right-click in the Results Table and then click **Analyte Group** > **New**.

The table appears with the selected layout.

Tip!: To go back to the full view, right-click and click **Full**.

Sorting Data in Results Tables

You can sort the data in a Results Table in three different ways:

- Quickly sort the table on one to three columns, using one of the Sort buttons. This sort criteria cannot be saved.
- Create a table-specific sort to save the sort criteria with the current table. Table-specific sorts allow you to sort the current table on one to three columns and save the criterion for use with that table.
- Use a previously created preset sort. You can create and save a sort and later apply it to a Results Table.

Tip!: To save a sort or any other table setting, right-click in the table and then click **Table Settings** > **Export To New Table Settings**. The sort and other parameters can be used in the current project. To use the table settings in a different project you must copy it to another project. Click **Tools** > **Project** > **Copy Data**. A new project must be created and selected to be available for use.

To sort a Results Table

1. Select up to three columns in the Results Table in the order that you want to sort them.
2. Do one of the following:
   - To sort in ascending order, click **A-Z**.
   - To sort in descending order, click **Z-A**.

To sort a Results Table and save the sort criteria

1. Right-click in the Results Table and then click **Sort** > **New**.

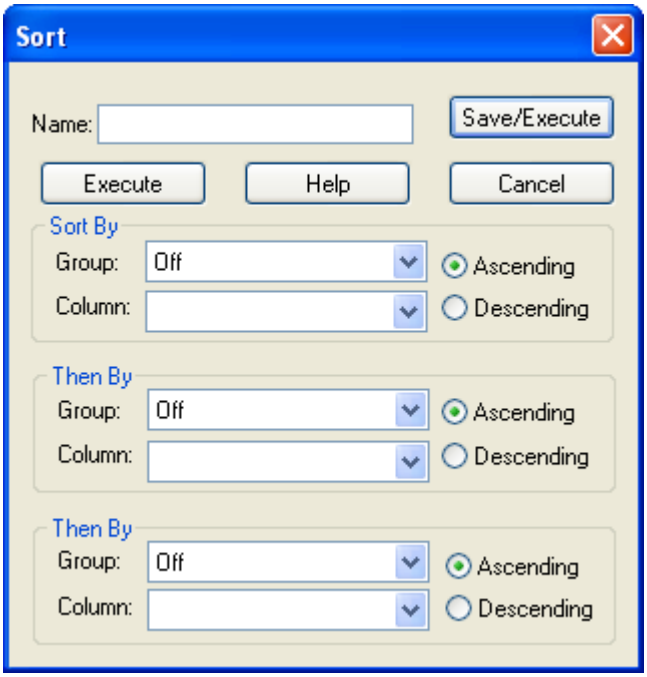


Figure 6-4 Sort dialog

2. In the **Name** field, type the name for the new sort.
3. For each sorting rule you want to set, in the **Sort By** section, do the following:
   - In the **Group** list, select the type of column you want to sort on.
   - In the **Column** list, select the column you want to sort on.
   - Select the direction of the sort: **Ascending** or **Descending**.
4. Do one of the following:
   - To perform the sort, save the sort criteria, and close the **Sort** dialog, click **Save/Execute**.
   - To perform the sort and close the **Sort** dialog without saving the sort criteria, click **Execute**.

To save default sort criteria for future Results Tables

1. Click **Tools** > **Settings** > **New Quantitation Results Table Settings**.

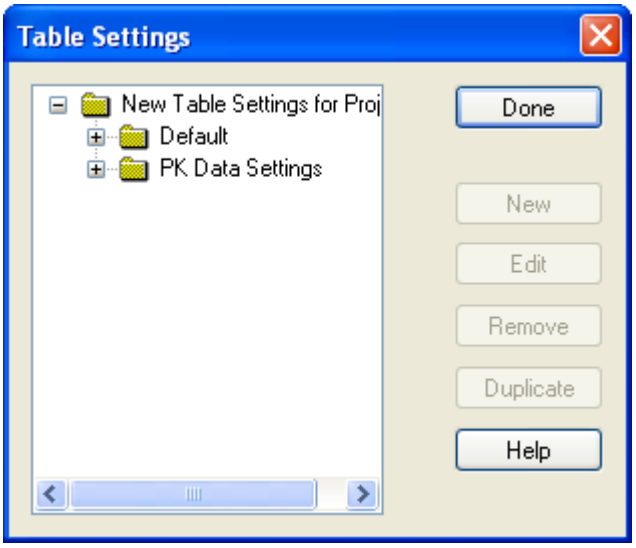


Figure 6-5 Table Settings dialog

2. Expand the **Table Settings** folder and then double-click the **Default** folder.
3. From the expanded **Default** folder, select the **Sorts** folder, and then click **New**.

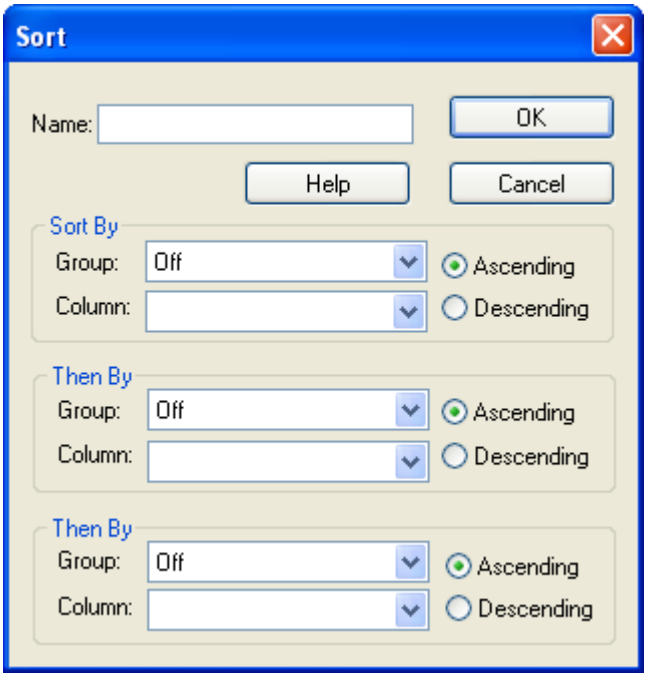


Figure 6-6 Sort dialog

4. In the **Name** field, type a name for the new sort.
5. For each sorting rule you want to set, in the **Sort By** section, do the following:
   - In the **Group** list, select the type of column you want to sort on.
   - In the **Column** list, select the column you want to sort on.
   - Select the direction of the sort: **Ascending** or **Descending**.

6. To save the criteria and close the **Sort** dialog, click **OK**.
7. To close the **Table Settings** dialog, click **Done**.

To sort a Results Table using preset sort criteria

- Right-click in the Results Table, click **Sort** and then select the name of the sort that you want to use.

Results Table Right-Click Menu

The following options are available if you right-click in the Results Table.

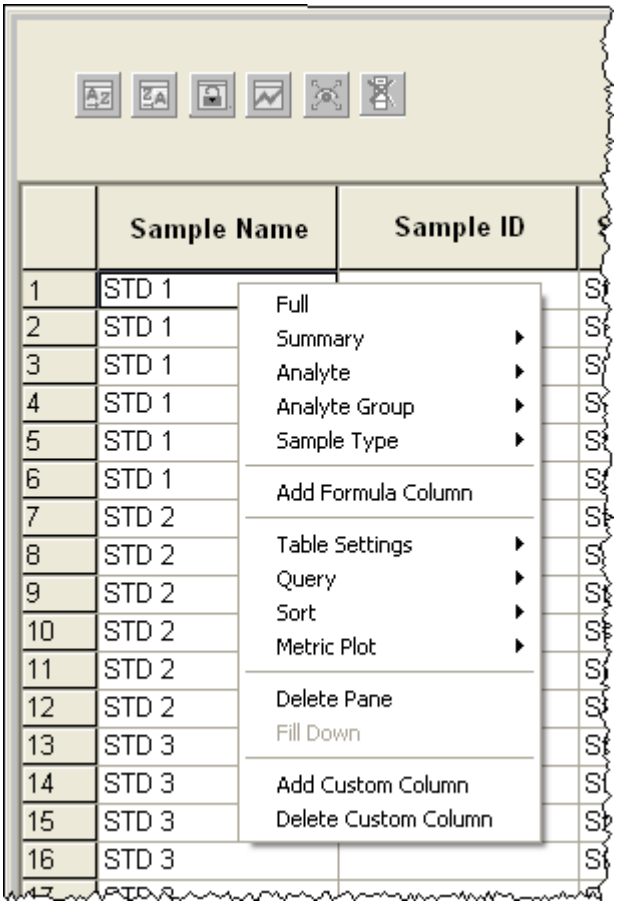


Figure 6-7 Results Table Right-Click Menu

Menu	Function
Full	Click to show all the columns.
Summary	Click to show specific columns.
Analyte	Click to show a specific analyte.
Analyte Group	Click to create an analyte group.
Sample Type	Click to show samples of a specific type or all samples.

Figure 6-7 Results Table Right-Click Menu (Continued)

Menu	Function
Add Formula Column	Click to add a formula column.
Table Settings	Click to edit or select a table setting.
Query	Click to create or select a query.
Sort	Click to create a sort or to sort by index.
Metric Plot	Click to create a metric plot.
Delete Pane	Click to delete the active pane.
Fill Down	Click to fill the same data into the selected cells.
Add Custom Column	Click to add a custom column.
Delete Custom Column	Click to delete the selected custom column.

Peak Review and Manual Integration of Peaks

You can use peak review to survey the peaks that the software has identified, and then redefine the peak or the start and end points where necessary.

After you have identified 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 displays 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. At this point, you confirm the peaks that are found or modify the quantitation method to better define your peaks.

Integrating Peaks Manually

During peak review, you may want to view a peak in its entirety—or you may want to examine the baseline to find out how well the software found the start and end points of the peak. Use the automatic zooming feature to help you do either.

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

To review peaks

Tip!: To review an individual peak, right-click on a point on the curve and then click Show Peak. The software displays the Peak Review window with the peak you chose.

1. Right-click in the **Results Table**, click **Analyte** and then select a sample.
2. Click **Tools** > **Peak Review** > **Pane**.

   The peaks appear below the Results Table with only the peaks listed in the Results Table.

3. Right-click in the pane and then click **Options**.
4. In the **Peak Review Options** dialog, in the **Appearance** section, change **Num. rows** to **1** and **Num. columns** to **2**.
5. In the **Automatic Zooming** section, click **Zoom Y axis to**: **100% of largest peak** to show the entire peak.

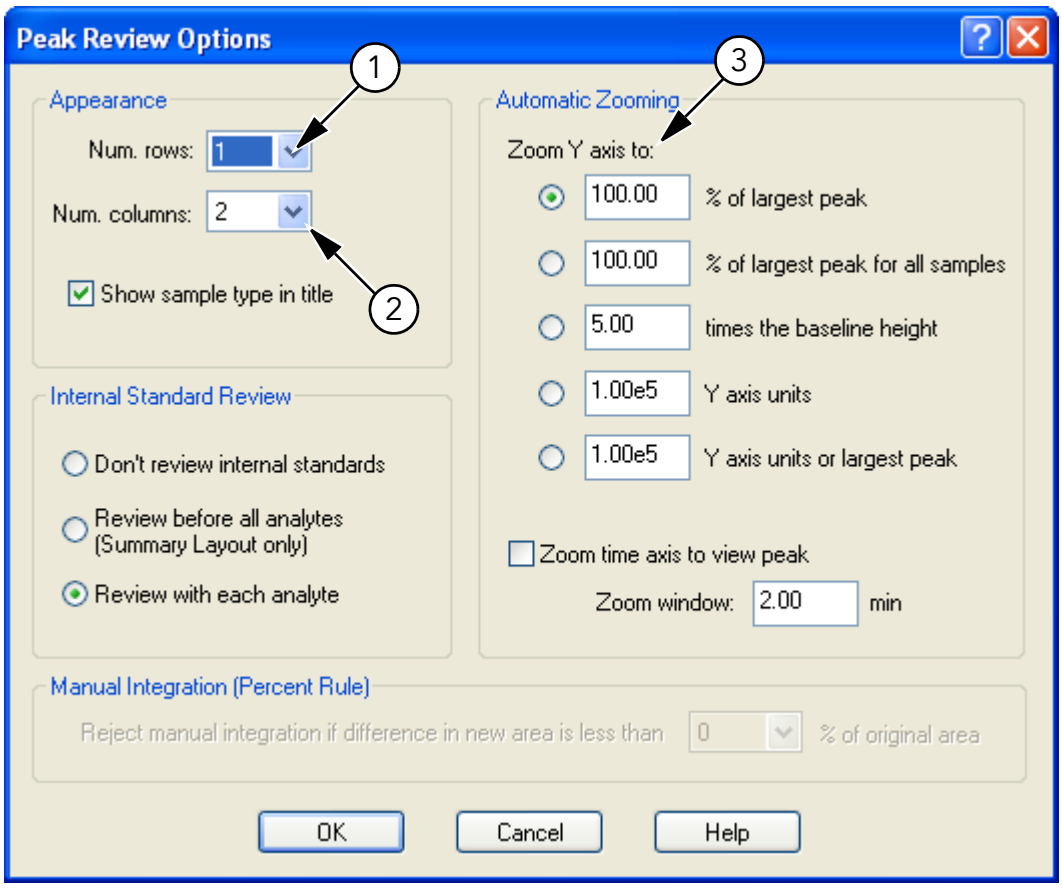


Figure 6-8 Peak Review Options dialog

Item	Definition
1	Num. rows
2	Num. columns
3	Zoom Y axis to 100% of largest peak

6. Click **OK**.
7. To move through the peaks, click the right-pointing arrow (for more information, see Figure 6-9 *Peak Review Pane* on page 107.) Go to the second injection of standard 3. In this example, you could integrate the peak 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.

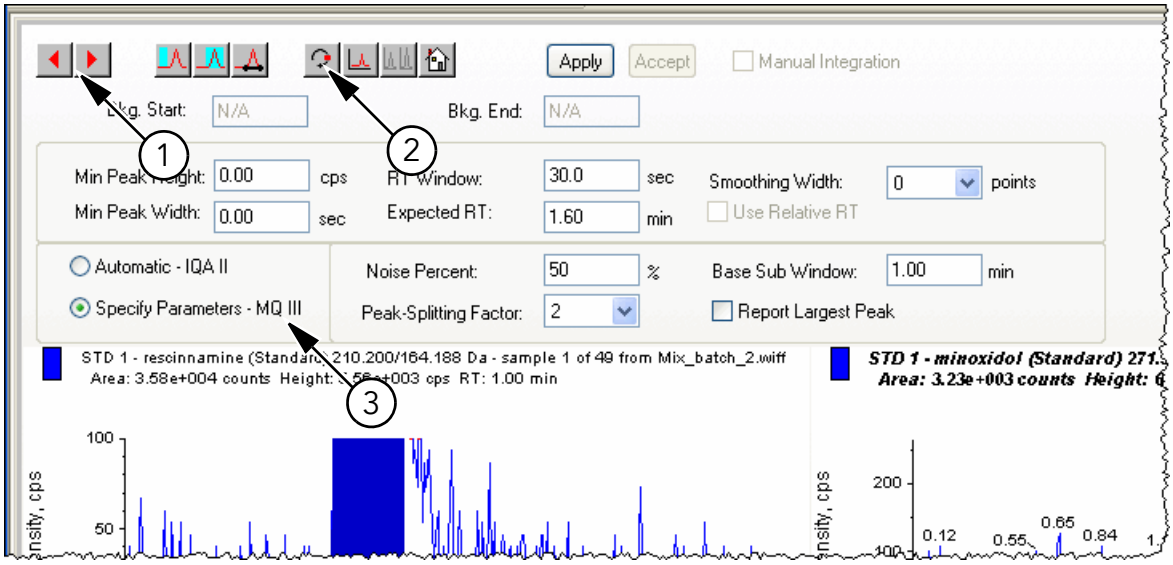


Figure 6-9 Peak Review Pane

Item	Description
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.

8. Click **Show or Hide Parameters** twice, and then click **Specify Parameters -MQ III**.
9. Change the **Noise Percent** value and click **Apply**.

   You will see the peak integrated closer to the baseline.
10. If the change does not improve the peak integration, then adjust the **Noise Percent** parameter until you find the optimal value.
11. To update the algorithm for all peaks, right-click in the pane and then click **Update Method**.

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

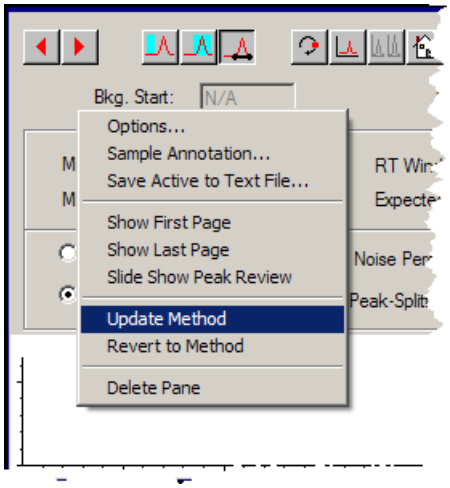


Figure 6-10 Update Method

To manually integrate peaks

Manually integrating peaks should be done last. You should manually integrate peaks only if you have been unsuccessful in finding all the peaks after adjusting and updating the algorithm parameters. This is done to limit person-to-person variability.

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

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

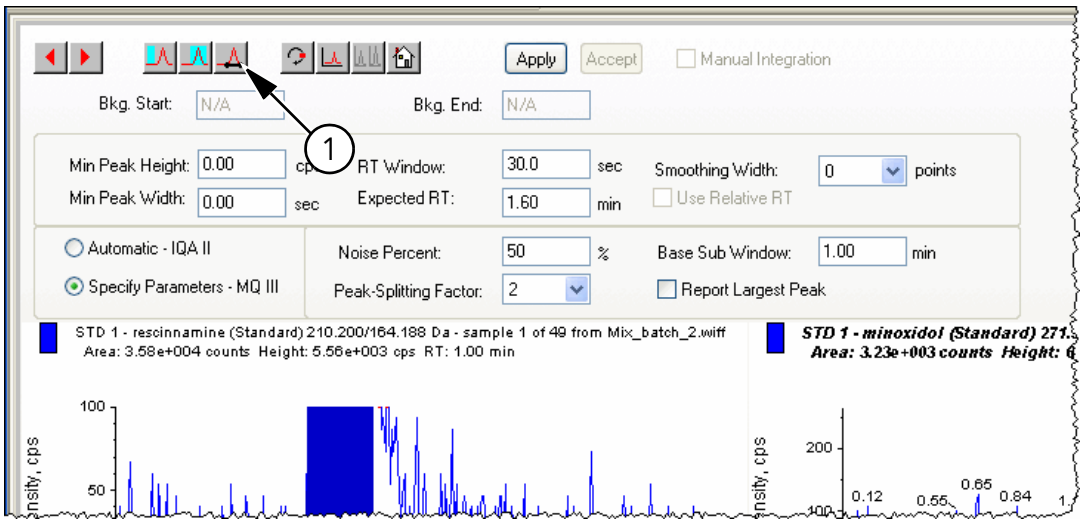


Figure 6-11 Peak Review pane: Manual integration

Item	Description
1	Manual Integration Mode

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

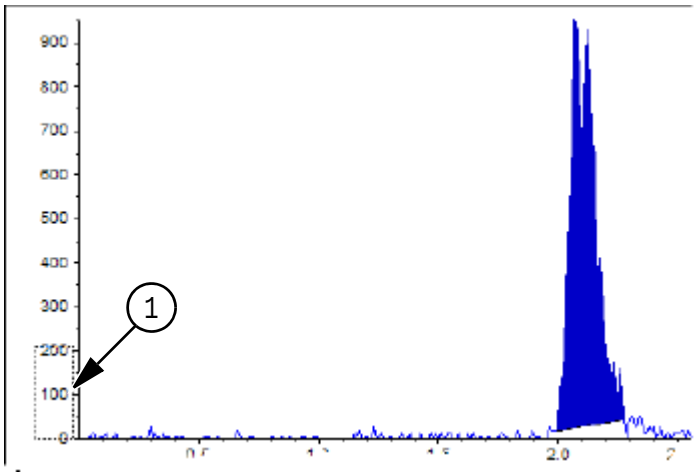


Figure 6-12 Peak Review pane: Zooming in on a peak

Item	Description
1	Lower 10% of the peak

3. Position the cross-hair where you want to define the start of the peak and then drag the cross-hair to where you want to define the end of the peak.

   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 your changes, clear the **Manual Integration** check box.

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

Peak Review Right-Click Menu

The following options are available if you right-click in the Peak Review window or pane.

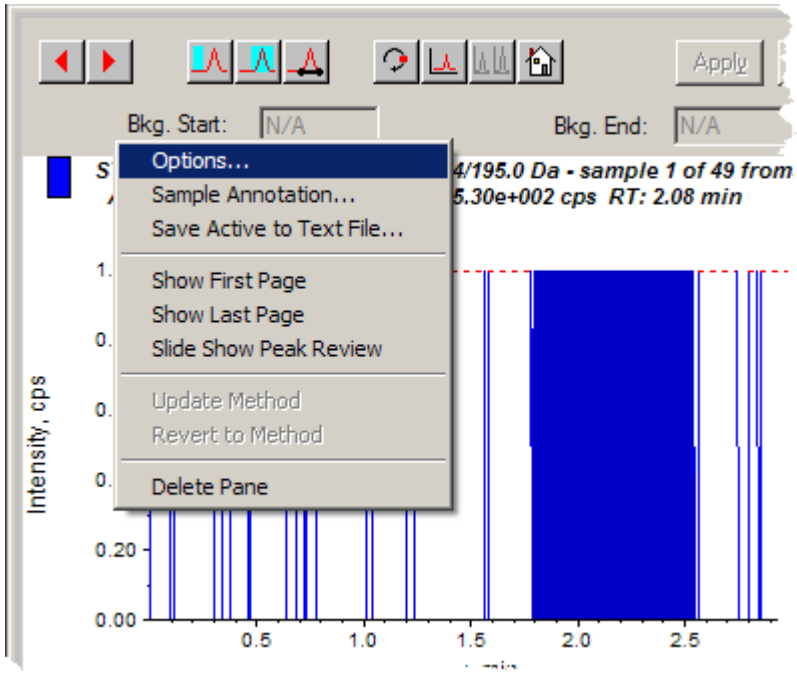


Figure 6-13Peak Review Right-Click Menu

Menu	Function
Options	Click to open the Peak Review Options dialog.
Sample Annotation	Click to open the Sample Annotation dialog.
Save Active to Text File	Click to save the selected peak as a text file.
Show First Page	Click to go to the first sample.
Show Last Page	Click to go to the last sample.
Slide Show Peak Review	Click to open the slide show.
Update Method	Click to update the algorithm for all peaks.
Revert to Method	Click to have a redefined peak reselected based on the current quantitation method.
Delete Pane	Click to delete the active pane.

Calibration Curves

A calibration curve is used to find the calculated concentration of samples, including QC (quality control) 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 you are using an internal standard, the ratio of the standard concentration/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 you specified. The regression equation is used to calculate the concentration of the unknown samples.

Working with Calibration Curves

If you have a Results Table open, you can view the calibration curve and change the regression options. If you have two or more Results Tables open, you can overlay their calibration curves. To overlay curves, the method used to create the tables must be the same.

To display calibration curves

Plot a calibration curve to see the curve used for regression. If two or more curves are open, you can overlay them. The Calculated Concentration field in the Results Table reflects any changes resulting from the fit of the curve to the standard's points

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

1. With a Results Table open, click **Tools** > **Calibration** > **Pane**.

   The Calibration Curve pane containing the calibration curve appears.

2. If you have more than one analyte you can view the calibration curve for another analyte:
   - From the **Analyte** list, select an analyte.
   - From the next list, select **Area** or **Height**, if needed.
3. To change the regression options for the calibration curve, do the following:
   - Click **Regression**.

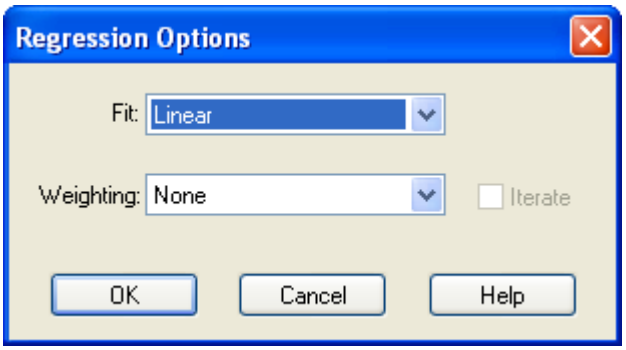


Figure 6-14 Regression Options dialog

   - In the **Fit** list, select **Linear**.
   - In the **Weight** list, select **1 / x**.
   - Click **OK**.

The calibration curve reappears. The Calculated Concentration field reflects any changes resulting from the fit of the curve to the standard's points. You can now review individual peaks on the curve. You can also exclude points from the curve to produce a better curve.

4. If necessary, repeat these steps, to create a more appropriate curve.
5. To save the changes, click **Accept**.

To overlay calibration curves

Note: If you want to examine the curve for one table more closely, right-click on the curve and click Active Plot. Choose 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**.

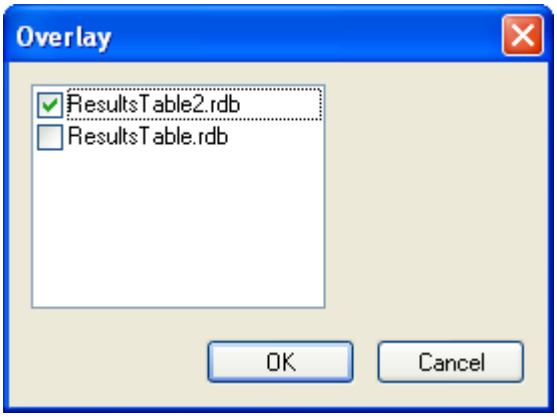


Figure 6-15 Overlay dialog

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

The following options are available if you right-click in the Calibration window or pane.

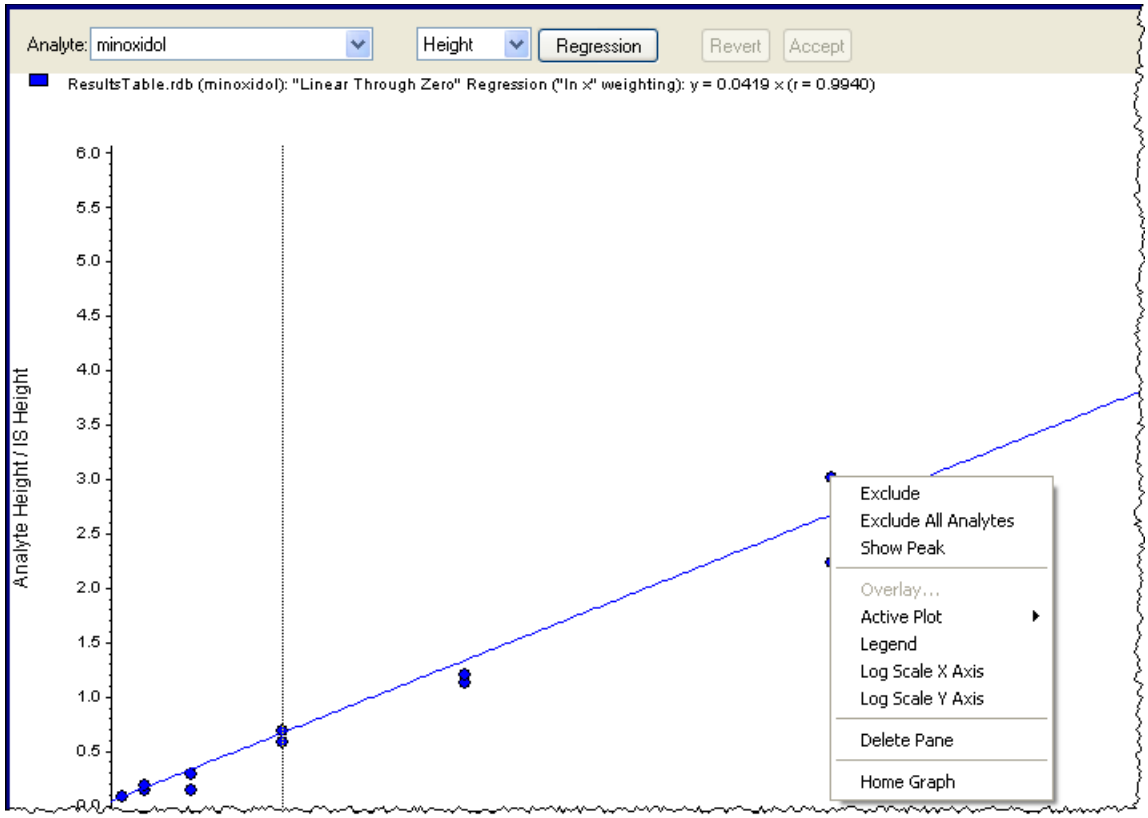


Figure 6-16 Calibration Curve Right-Click Menu

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 dropped 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	Click to review an individual peak.
Overlay	Click to overlay two graphs.
*** 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.**	

Figure 6-16 Calibration Curve Right-Click Menu (Continued)

Menu	Function
Active Plot	Click to see which plot is active.
Legend	Click to display the graph legend.
Log Scale X Axis*	Click to use a log scale for the X axis.
Log Scale Y Axis*	Click to use a log scale for the Y axis.
Delete Pane	Click to delete the active pane.
Home Graph	Click to rescale 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 appears in tabular form in the grid and a row of data appears for each standard or QC concentration.

Reviewing Sample Statistics

When you are viewing more than one Results Table, you can obtain statistical information on the standards and QCs for additional batches in the Statistics window. This allows you to compare results between batches and look for trends in the standards or QCs.

To view the statistics for standards and QCs

1. With a Results Table open, click **Tools** > **Statistics**.

   The Statistics window appears.
2. In the **Statistics Metric** list, select **Concentration**.
3. In the **Analyte Name** field, select an analyte.
4. In the **Sample Type** field, select **Standard**.

   The results appear.
5. Look at the **%CV** and **Accuracy** columns.

   The %CV shows you the coefficient of variance between the measurements of a single parameter, for example the area. Accuracy shows you how close the plotted point is to the interperlated value.
6. If required, select the **Display Low/High values** check box and then examine the **Low**, **High**, and **Mean** for each row in the grid. Each row represents standards that have the same concentration levels.
7. Choose another analyte.

   The results appear on a per-analyte basis.

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

To compare results between batches

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

1. Open the Results Tables that you want to compare.
2. Click **Tools** > **Statistics**.

   The Statistics window appears.
3. Do one of the following:
   - To arrange the results by Results Table, in the **Conc. as Rows** list, select **Group By Batch**.
   - To arrange the results in order of concentration, in the **Conc. as Rows** list, select **Group By Concentration**.
   - To arrange the results in order of concentration, but without a row showing the statistics for each group or batch, in the **Conc. as Rows** list, select **Group By Concentration (no All)**.

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

Metric Plots

A metric plot graphically shows the data in a Results Table column plotted against the x-axis or the y-axis, or the data in two columns plotted against each other. This section describes how to generate and work with metric plots.

A few predefined metric plots are also included:

- Int_Std_Response (to locate problem sample)
- Analyte_Area versus Height (to verify chromatography behavior)
- PK profile (conc. versus time point, to run after Sample query)

Generating Metric Plots

You can use metric plots to plot a given column, such as Analyte Peak Area, Accuracy, or Calculated Concentration, from the Results Table. You can also plot two Results Table fields against each other. You can then investigate points that appear outside the normal range. Metric plots are often used with queries. For more information about queries, see the Help.

You can generate metric plots in the following ways:

- Use the Plot button to plot a column or columns of the current Results Table, but not save the plotting criteria.
- Create a table-specific plot to save the plot criteria with the current table.
- Create a global plot to save the plotting criteria for use with future Results Tables.

You cannot see QC, unknown, blanks, double blanks, or solvents on the calibration curve, but you can generate metric plots of them.

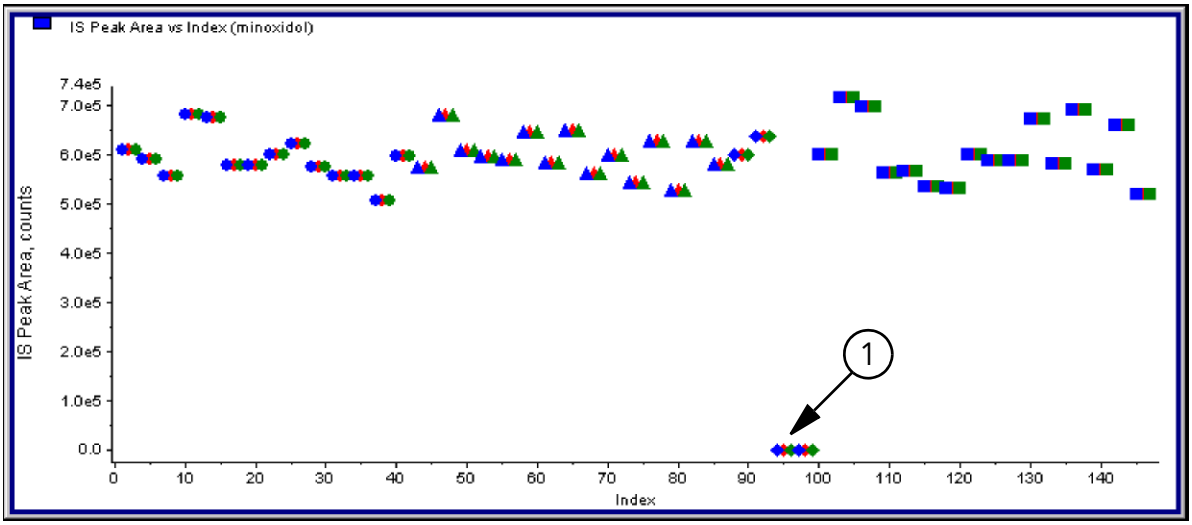


Figure 6-17 Example of a metric plot

Item	Description
1	Double blanks

To generate a metric temporary plot

1. With a Results Table open, do one of the following:
   - To plot the data on the y-axis with the x-axis as an index, click the heading of the column for the data you want to plot.
   - To plot the data from the first selected column on the x-axis and the second selected column on the y-axis, select two columns by pressing the Ctrl key as you click the column headings.
2. Above the Results Table, click the **Metric Plot by Selection** icon. (See Table 6-2 *Results Table Icons* on page 119.)

   The metric plot appears.
3. Right-click in the plot pane and then click **Data Legend** to view an explanation of the colors used by the plot.
4. Right-click in the plot pane and then click **Point Legend** to view an explanation of the symbols used by the plot.

To generate a metric plot and save the plot criteria

1. Open the **Tutorial Results Table.rdb** Results Table.
2. Right-click in the Results Table and then click **Metric Plot** > **New**.

Figure 6-18 Metric Plot dialog

3. In the **Name** field, type the name for the new plot criteria.
4. In the **X-Axis** section, in the **Group** list, to plot a field in the y-axis using the x-axis as an index, select **Index** and leave the **Column** list blank.
5. If you want to plot two columns against each other, in the **Y-axis** section, in the **Group** list, select **Internal Standard**, and then, in the **Column** list, select **IS Peak Area**.
6. If required, in the **Regression** list, select the type of regression you want to use, and then select the appropriate regression settings.
7. To generate the plot and save the plot criteria, click **Save/Execute**.

   The metric plot appears. For more information, see Figure 6-17 *Example of a metric plot* on page 116.
8. Right-click in the plot pane and then click **Data Legend** to view an explanation of the colors used by the plot.
9. Right-click in the plot pane and then click **Point Legend** to view an explanation of the symbols used by the plot.

   This set of criteria is now available for future plots of this Results Table when you right-click in the Results Table. You can also edit the plotting criteria.
10. To view the problem sample, try plotting the concentration of the unknown against time or plotting the area of the internal standard against the index.

To save default plot criteria for future Results Tables

1. Right-click in the Results Table and then click **Table Settings > Export To New Table Settings**.

   This will export the table settings from the .rdb so that it can be reused in other quantitation runs within the project.

2. To export table settings to another project, on the **Tools** menu click **Project** > **Copy Data**.

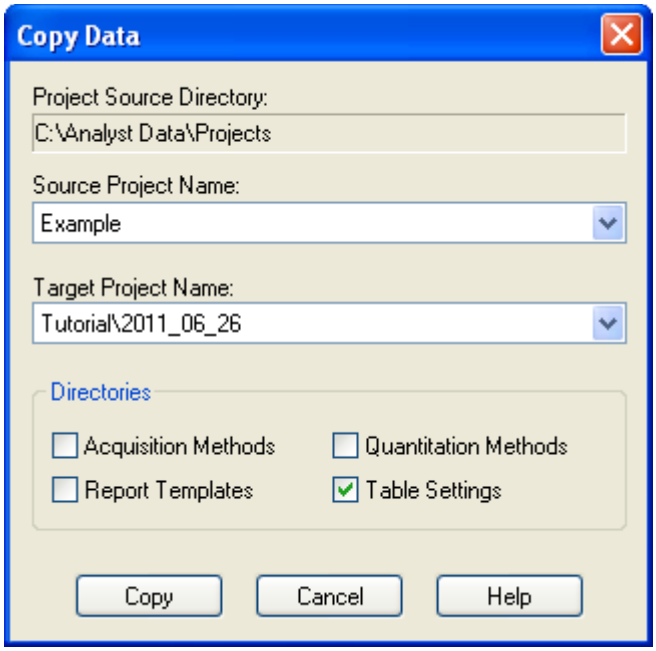


Figure 6-19 Copy Data dialog

Table 6-1 Integration Tab and Quantitation Wizard Icons

Icon	Name	Function
	Set parameters from Background Region	Click to use the peak that you selected.
	Select Peak	Click to use the background that you selected.
	Manual Integration Mode	Click to manually integrate peaks.
	Show or Hide Parameters	Click to toggle the peak-finding parameters between shown and hidden.
	Show Active Graph	Click to show the analyte chromatogram only.
	Show Both Analyte and IS	Click to show the analyte and its associated chromatogram (available only when an associated internal standard exists).

Table 6-1 Integration Tab and Quantitation Wizard Icons (Continued)

Icon	Name	Function
	Use Default View for Graph	Click to return to the preset (view all data) view (if, for example, you have zoomed in on a chromatogram).

Table 6-2 Results Table Icons

Icon	Name	Function
	Sort Ascending by Selection	Click to sort the selected column by ascending values.
	Sort Descending by Selection	Click to sort the selected column by descending values.
	Lock or Unlock Column	Click to lock or unlock the selected column. You cannot move a locked column.
	Metric Plot by Selection	Click to create a metric plot from the selected column.
	Show all Samples	Click to show all the samples in the Results Table.
	Delete Formula Column	Click to delete formula columns.

Table 6-3 Icon Quick Reference: Quantitate Mode

Icon	Name	Function
	Add/Remove Samples	Click to add or remove samples from the Results Table.
	Export as Text	Click to save the Results Table as a text file.
	Modify Method	Click to open a .wiff file.
	Peak Review - Pane	Click to review peaks in a pane.
	Peak Review - Window	Click to review peaks in a window.
	Calibration - Pane	Click to open the calibration curve in a pane.
	Calibration - Window	Click to open the calibration curve in a window.

Table 6-3 Icon Quick Reference: Quantitate Mode (Continued)

Icon	Name	Function
	Show First Peak	Click to show the first peak in the pane or window.
	Show Last Peak	Click to show the last peak in the pane or window.
	Show Audit Trail	Click to show the audit trail for the Results Table.
	Clear Audit Trail	Click to clear the audit trail for the Results Table.
	Statistics	Click to open the Statistics window.
	Report Generator	Click to open the Reporter software.

Using Reporter Software 7

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

Analyst Software Reporter Overview

The Reporter software can be used to create custom reports with Microsoft Word and Excel (2007 or 2010). 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.
- 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. See the Help if you want to create or edit templates using 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 report requirement.
- Automates report generation through the use of the Autoquan Reporter batch script.
- Automatically prints, exports to Adobe Portable Document Format (pdf), and delivers results via email. This functionality requires the Save as PDF (Office 2007) addin that is installed by the Analyst software.
- 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 in three ways:

- Within the Analyst software to manually generate a report or set of reports.
- By a batch script to automate report generation within a batch. You may generate reports on a sample-by-sample basis, either during or after batch acquisition.
- By applications that do not use the Analyst software.

Reporter User Interface

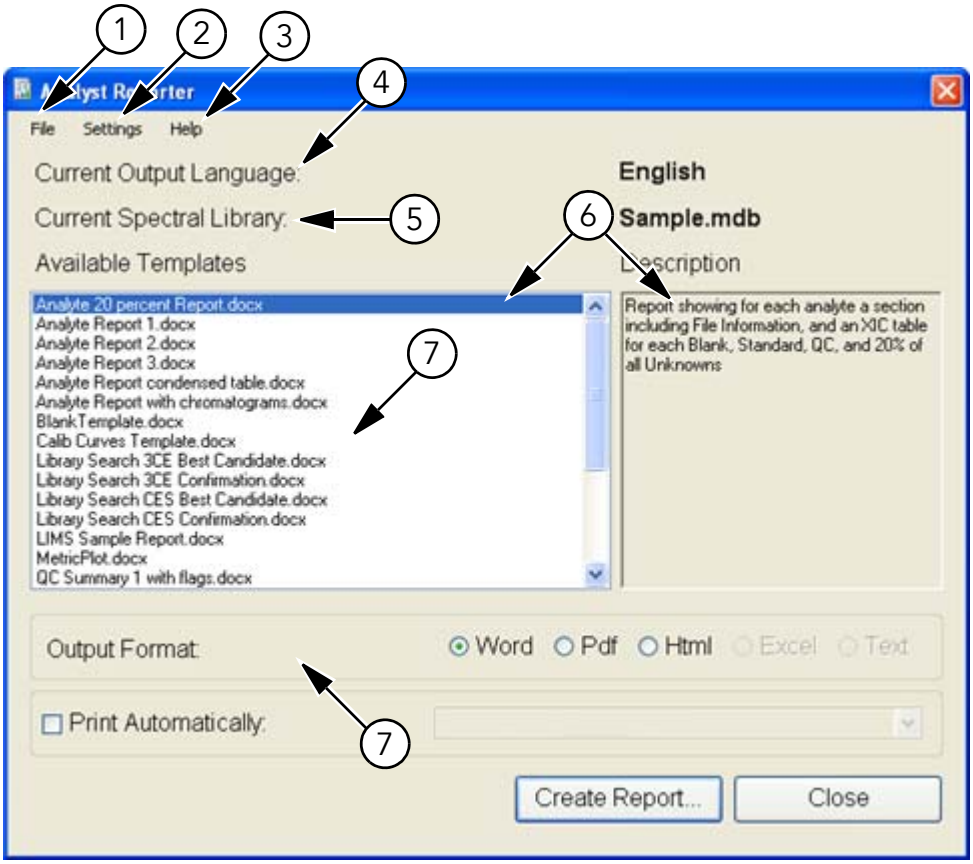


Figure 7-1 User Interface

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 within them 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.
2	Settings > Select Library	Browse to an spectral library. This library will be used for matching and scoring MS/MS from Results Tables that contain data from information dependent acquisition (IDA) triggered MS/MS.
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.

Figure 7-1 User Interface (Continued)

Item	Option	Description
4	Current Output Language	Displays 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	Displays the currently selected spectral library. The spectral library can be selected using **Settings > Select Library**.
6	Available Templates and Description	Displays a list of available report templates. Selecting a template will show a description of the template. To change the folder where available templates are read from, select **Settings > Select Template Folder > Browse**.
7	Output Format	The Reporter software supports several output formats. 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 2007 and above. • PDF: The PDF option creates a report 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 displayed 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 have been specifically marked as text-compatible can be used for this output format. • Print Automatically: If selected, after the report has been created it is printed to the selected printer. Select from any available printer.

Generating Reports

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

You can 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, see the *Scripts User Guide*.

1. Open a Results Table.
2. Under **Companion Software**, double-click **Reporter 3.2**.
3. In the Analyst Reporter dialog, in the **Available Templates** field, select a template.
4. Click an 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 you 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. Select the **Print Automatically** check box if you want your reports to print automatically to a pre-selected printer.

   The Default Printer set in Windows is used unless you select a different printer. The Reporter tool retains the selected printer between operations. If the printer is set to a .pdf printer driver, then you can use the Analyst Reporter to generate .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 may take seconds to generate, others may take longer. A large data set with many MRM transitions or a large number of graphics could result in reports of several hundred pages and could take hours to generate.

Table 7-1 Icons in Quantitate Mode

Icon	Name	Function
	Report Generator	Click to open the Reporter software.

Calibration Ions and Solutions

A

Table A-1 Tuning Frequency

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

Table A-2 Suggested Tuning Solutions for Quadrupole Systems

System	Positive	Negative
API 3200™ system and API 3000™ System	1×10^{-5} M PPG (1:10)	3×10^{-4} NEG PPG
API 4000™ system	2×10^{-6} M PPG (1:50)	3×10^{-4} NEG PPG
API 5000™ system	2×10^{-7} M PPG (1:500)	3×10^{-5} NEG PPG (1:10)
AB SCIEX Triple Quad™ 5500 system	2×10^{-7} M PPG (1:500)	3×10^{-5} NEG PPG (1:10)

Table A-3 Suggested Tuning Solutions for LIT Systems

	Q1 and Q3		LIT
Instrument	Positive	Negative	Positive and Negative
3200 QTRAP® system	1×10^{-5} M PPG (1:10)	3×10^{-4} NEG PPG	3×10^{-4} NEG PPG
4000 QTRAP system	2×10^{-6} M PPG (1:50)	3×10^{-4} NEG PPG	1:100 Agilent mix
AB SCIEX QTRAP 5500 system	2×10^{-7} M PPG (1:500)	3×10^{-5} NEG PPG (1:10)	1:100 Agilent mix

Table A-4 Q1 and Q3 PPG Positive Ion Scans

Instrument	Masses							
API 2000™ system	59.0	175.1	616.5	906.7	1254.9	1545.1	–	–
API 3000™ system	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
API 3200 system	59.0	175.1	616.5	906.7	1254.9	1545.1	–	–
API 4000 system	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
API 5000 system	59.0	175.1	616.5	906.7	1080.8	1196.9	–	–
AB SCIEX Triple Quad 5500 system	59.0	175.1	616.5	906.7	1080.8	1196.9	–	–
QTRAP system	59.0	175.1	616.5	906.7	1254.9	1545.1	–	–

Table A-4 Q1 and Q3 PPG Positive Ion Scans (Continued)

Instrument	Masses							
3200 QTRAP system	59.0	175.1	616.5	906.7	1254.9	1545.1	–	–
4000 QTRAP system	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
AB SCIEX QTRAP 5500 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 2000 system	45.0	585.4	933.6	1223.8	1572	–	–	–
API 3000 system	45.0	585.4	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
API 3200 system	45.0	585.4	933.6	1223.8	1572.1	1863.3	–	–
API 4000 system	45.0	585.4	933.6	1223.8	1572.1	1863.3	2037.4	2211.6
API 5000 system	45.0	411.3	585.4	933.6	1223.8	–	–	–
AB SCIEX Triple Quad 5500 system	45.0	411.3	585.4	933.6	1223.8	–	–	–
QTRAP system	45.0	585.4	933.6	1223.8	1572.1	–	–	–
3200 QTRAP system	45.0	585.4	933.6	1223.8	1572.1	–	–	–
4000 QTRAP system	45.0	585.4	933.6	1223.8	1572.1	1863.3	2037.4	2211.6
QTRAP 5500 system	45.0	411.3	585.4	933.6	1223.8	–	–	–

Table A-6 Masses and Polarity for the 3200 QTRAP System (PPG 3000)

Instrument/Polarity	Masses			
LIT Positive	115.1	500.4	1080.8	1661.2
LIT Negative	121.1	585.4	991.8	1630.1

Table A-7 Masses and Polarity for the 4000 QTRAP 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	–

Table A-8 Masses and Polarity for the AB SCIEX QTRAP 5500 System (Agilent)

Instrument/ Polarity	Masses						
LIT Positive	118.087	322.049	622.030	922.010	–	–	–
LIT Negative	112.985	431.982	601.978	–	–	–	–

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). You should understand what each parameter controls and how it affects resolution, intensity, and peak shape so that you can achieve optimal results during sample analysis. You should also consider how changing the value of one parameter can affect another parameter further along the ion path.

Source-Dependent Parameters

These parameters may 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.

IS (Ion Transfer Voltage): For the PhotoSpray® source, the IS parameter controls the voltage that transfers the ions from the primary ionization region towards the curtain plate orifice.

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.

svp (Multi-source Selector): The svp parameter controls the selection of the DuoSpray™ ion source probes: TurboIonSpray probe or APCI probe.

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 may 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 may 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.

FP (Focusing Potential): (For API 2000™ and API 3000™ systems only.) The FP parameter controls the voltage applied to the focusing ring lens. The focusing potential helps focus the ions through the skimmer region of the mass spectrometer interface. It can induce fragmentation in the interface area, similar to the declustering potential.

CEP (Collision Cell Entrance Potential): (For the API 2000 system, API 3200™ system, and the 3200 QTRAP® system only.) The CEP parameter controls the collision cell entrance potential, which is the potential difference between Q0 and IQ2. It focuses ions into Q2 (collision cell). CEP is used in Q1, MS/MS-type, and LIT scans. Note that for Q3 scans, this voltage is called IQ2 and is preset to fixed mode.

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 IQ3 (for the API 2000 system, API 3200 system, QTRAP system, and 3200 QTRAP system) or between RO2 and ST3 (for the API 3000 system, 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 instrument operators.

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 instrument operators.

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. A single MS scan may be used to find or confirm the molecular weight of a compound. Single MS scans can also be referred to as survey scans. MS scans do not provide any information as to the chemical make-up of the ions other than the mass. To obtain more information about your ions, you need to perform MS/MS or MS/MS/MS.

MS/MS: MS/MS scans are used to help identify or confirm a molecular species. In MS/MS, a precursor ion can be fragmented in one of two locations.

- For triple quadrupole instruments, fragmentation occurs in the collision cell.
- For LIT instruments, fragmentation can occur in the collision cell or the linear ion trap.

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

MS/MS/MS: The LIT instrument 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 trap to give more structural information about the molecular ion.

Quadrupole-Mode Scan Types

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

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

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

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

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

MRM (MRM): Mode of operating a triple quadrupole instrument so that an ion of given mass in Q1 must fragment or dissociate to give a product ion of specific mass in Q3 in order for a response to be detected. Used primarily for quantitation.

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

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

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

LIT (Linear Ion Trap)-Mode Scan Types

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

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

Enhanced Multi-Charge (EMC): This scan is similar to the EMS scan except that before scanning the ions out of the linear ion trap, there is a delay period in 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): Product ions are generated in the Q2 collision cell by the precursor ions from Q1 colliding with the collision (CAD) gas in Q2. These characteristic product ions are transmitted and collected in Q3. The ions are scanned out of the linear ion trap to produce product ion spectra. Use the EPI scan mode to achieve good resolution and intensity.

Enhanced Resolution (ER): This scan 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): In MS/MS/MS, product ions are generated in the Q2 collision cell. These product ions are transmitted and then collected in linear ion trap. The linear ion trap isolates a specific product ion and removes all other ions from the trap. By applying a different voltage in the trap, the selected product ion collides with the residual nitrogen in Q3, further fragmenting the ion producing MSn fragments.

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 in the linear ion trap. During the second part of the collection period, all masses over the mass range of interest are collected. The resulting enhanced product ion spectra are simplified compared 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.

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