Analyst® TF 1.7.1

Advanced User Guide

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Contents

Foreword 1

Audience

The Advanced User Guide provides information about the Analyst® TF software features.

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

Access System Documentation

The Help, guides, and tutorials for the mass spectrometer and the software are installed automatically.

- Click **Start > All Programs > SCIEX > Analyst TF.** A complete list of the available documentation can be found in the Help.
- Press **F1** to open the Help.

Technical Support

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

General Information 2

Analyst® TF Software Events

The system log contains reports of system events including errors, warnings, and messages. Use the Windows Event Viewer to display information that may be helpful in troubleshooting and performing system diagnostics. To effectively use the information in the system log, filter the information to display only the items relevant to the software.

Filter System Log Information Relevant to the Analyst® Software

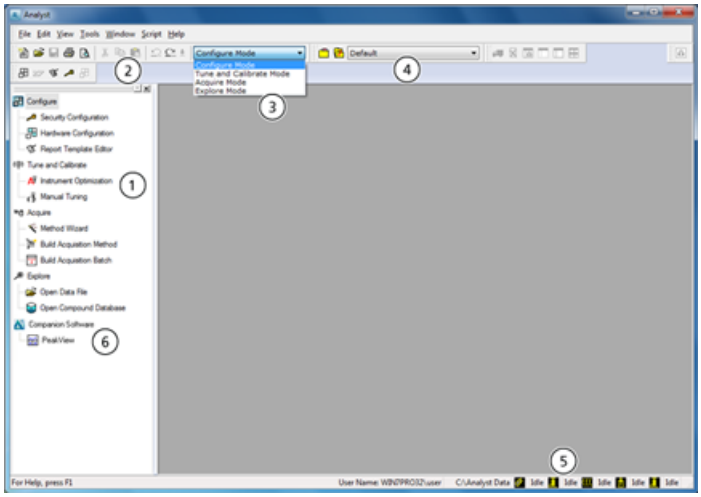
The system log contains reports of system events including errors, warnings, and messages. Use the Windows Event Viewer to display information that may be helpful in troubleshooting and performing system diagnostics. To effectively use the information in the system log, filter the information to display only the items relevant to the software.

1. Click **View > Event Log** .
2. Double-click the **Windows Logs** folder in the **Event Viewer**.
3. Click **Application**.
4. Click **Action > Filter Current Log** .
5. In the **Filter Current Log** dialog, select **Analyst** in the **Event Sources** field.
6. Click **OK**.

   The Event Viewer now displays only the filtered Analyst software events.

Analyst Software Window

Figure 2-1 Analyst TF Software Window



Item	Description
1	**Navigation bar**: The Navigation bar allows access to the software's various modes. Users can customize some elements of the Navigation bar to suit their preferences. For example, users can resize it, move it, or fix it in place. To hide the Navigation bar, click the x in the top right corner. To view the Navigation bar, click View > Navigation Bar. The top level of the navigation tree has an icon that represents each software mode. Double-click the icon for a particular mode to expand or collapse the tree. This shows or hides the icons for the available functionality within the selected mode.
2	**Menu bar**: Changes depending on the mode. Some options, such as Cut, Copy, and Paste, are the same in every mode. Other options are specific to certain modes and are unavailable in other modes.
3	**Mode list**: Click to change modes. Different modes have different toolbar icons available.
4	**Project list**: Click to change the project in which data is saved.
5	**Instrument and peripheral device status**: The Status bar displays information about current activities. It depicts the status of the instrument by color: green (ready), yellow (warning), red (error), or white (no local instrument workstation). An icon indicates the status of the remote instrument. Double-click an icon to open the device status window.
6	**Companion Software**: Any installed companion software that is launched from the software appears in this section.

Analyst Software Modes

The software is divided into modes, which are discrete functional areas where users can perform a range of activities related to a main task. Users can access modes through the Navigation Bar or the Mode list in the toolbar and can switch from one mode to another without losing any work.

Table 2-1 Modes in the Analyst Software

Name	Description
Configure	Use Configure mode to configure devices and system settings. Set various options and parameters for the software, including hardware configuration and report template settings.
Tune and Calibrate	Use Tune and Calibrate mode to set options for tuning the instruments to ensure optimal results. In Tune and Calibrate mode, users can: • Perform instrument optimization. • Perform manual tuning. • Change the appearance of graphical displays, find what file information is displayed, and set linking options and other appearance options. • Change processing options.
Acquire	Use Acquire mode to set options to decide how samples should be acquired. In Acquire mode, users can: • Create an acquisition method using the Method Wizard. • Create an acquisition method with the Acquisition Method Editor. • Create a batch with the Batch Editor. • View the queue with Queue Manager. • Monitor the acquisition status.
Explore	Use Explore mode to perform qualitative analysis on samples. In Explore mode, users can: • View a graph. • View a chromatogram. • View a spectrum. • Display data in real time during batch acquisition.
Companion Software	Any installed companion software that is launched from the Analyst software appears here.

Analyst Service

The Analyst Service is the communication path between the instrument and attached devices. The Analyst Service is started each time the Analyst software is started. In general the Analyst Service starts automatically when the user logs on to Windows. If the service is not running when the Analyst software is started, then the Analyst Service will start automatically.

Start the Analyst Service

If the Startup Type for the service is set to Manual, manually start the Analyst Service before starting the Analyst software. Do not change the Startup Type.

1. Open the **Administrative Tools**.
2. Double-click **Services** and then click **Analyst Service**.
3. Click **Start**.

Stop the Analyst Service

Stop the Analyst service if there are issues communicating with the instrument or if there are communication issues between the instrument and the peripheral devices.

1. Open the **Administrative Tools**.
2. Double-click **Services** and then click **Analyst Service**.
3. Click **Stop**.

API Instrument Project Folders

The API Instrument project folder is important for the instrument to function properly. The API Instrument project contains the information required to tune and calibrate the system. The API Instrument project folder also contains data files for a manual tune that was performed using the Start button instead of the Acquire button. These data files are saved automatically in the API Instrument project folder in the Tuning Cache folder and named with the date and time they were created. Empty the Tuning Cache folder regularly.

The following are some of the folders found in the API Instrument project:

- **Configuration**: Contains all the hardware profiles (.hwpf files).
- **Example Scripts**: Contains some of the scripts that are used with the software.
- **Instrument Data**: Contains a file called *InstrumentData.ins*. The file stores all the critical calibration information and more.
- **Parameter Settings**: Contains all the instrument parameters and linkages. Instrument parameters are saved as *ParamSettingsdef.psf* files.

- **Preferences**: Contains the *Tunedata.tun* file. All settings (parameter, tuning, instrument, processing, appearances, and queue) are saved as *Tunedata.tun* in this folder. The *Tunedata.tun* file also contains all reference files, which are used for calibrating the instrument.
- **Processing Scripts**: Contains the scripts for data processing in Explore mode. Scripts are found in the Script menu.
- **Queue Data**: Contains information from the queue.
- **Tuning Cache**: Contains all the data created in Manual Tuning by clicking Start instead of Acquire. Files are saved with a generic time and date stamp for their names. The Tuning Cache folder holds a limited number of files and will overwrite files as needed. Save the files with a new name and move the files immediately if they need to be saved.

Program Files

The following folders are found within the Program Files\Analyst folder.

- **bin**: Contains the software program files. Contents of this folder should not be changed as this will affect the software functionality.
- **Firmware**: Contains the instrument firmware files. Use these files to download new firmware to the instrument when required. For more information, refer to the software installation guide included with the software.
- **Help**: Contains the help files, guides, tutorials, release notes and software installation guide.
- **Scripts**: Contains all the scripts that can be installed. Research-grade scripts are available to extend the functionality of the software. Some scripts are installed automatically and some scripts can be installed individually. For more information, refer to the *Scripts User Guide*.
- **Simulation**: Contains the instrument data files required to run the software in simulation mode.

Projects and Subprojects

Decide where to store the files related to an experiment before starting the experiment. Use projects and subprojects for each experiment to manage the data better and compare the results. For example, subprojects can be used to store the results for specific dates.

Note: To use a subproject structure within a project, create at least one subproject when first creating the project. Users cannot create a subproject in an existing project that does not already have a subproject structure.

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 the data.

For example, if samples of various compounds from different laboratories are run using the same acquisition method, then create subprojects to store the results for each laboratory, but leave the acquisition method folder in the project. The acquisition method is then available for use in the subproject or laboratory. Alternatively, if samples are being analyzed over a period of several weeks, then the results from each day can be stored in a separate subproject.

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 2-2* describes the contents of different folders.

The software can access a project only if it is stored in a root folder. Users 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 software is installed. To store projects in other locations, create new root folders. For more information about root folders, refer to the *Help*.

Table 2-2 Project Folders

Folder	Contents
\Acquisition Methods	Contains all acquisition methods used. Acquisition method files have the .dam extension.
\Acquisition Scripts	Contains all the acquisition batch scripts available.
\Batch	Contains all the acquisition batch files used. Acquisition batch files have the .dab extension. It also contains a subfolder, Templates, that contains acquisition batch templates. Batch template files have the .dat extension.
\Data	Contains the acquisition data files: .wiff and .wiff.scan.
\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 are shown in the Scripts menu.
\Project Information	Contains all project information and settings. This folder cannot be stored in a subproject.
\Results	Contains all quantitation results table files (.rdb extension).

Table 2-2 Project Folders (continued)

Folder	Contents
\Templates	Contains report template files (.rpt extension).
\Templates\Method	Contains the factory acquisition method templates for the Method Wizard.

Software Security

The software has a number of functions for configuring and managing security. The software administrator can:

- Choose a security mode to best suit the needs of the operating environment.
- Add and delete users and roles.
- Set access rights to users and roles as required.
- Control access to remote instrument stations.
- Control access to project files.

For more information about software security, refer to the *Laboratory Director's Guide*.

Workspaces

A workspace is a particular arrangement of windows and panes, including any associated file or files. For example, while working on a particular data set, users can open and size various windows to help with the analysis. This arrangement, or workspace, can be saved so that the next time users look at the data, the window arrangement is identical.

Users can customize a workspace by selecting which windows and panes they want each workspace to contain. Users can resize and reposition the windows and panes, lock panes together, and hide or show certain panes and windows in it. In this way, users can customize a workspace to suit the tasks at hand.

In Explore mode, users can have multiple workspaces per session. This means that different workspaces can be designed to suit different tasks within this modes, and then save them for future use. When in this mode, a particular workspace can be opened without exiting that mode.

Tune and Calibrate 3

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 includes adjusting the peak width and peak shape. Users 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.

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

Instrument Optimization

Instrument Optimization is an automatic process that tunes both the quadrupole and TOF modes and performs mass calibration. For quadrupole mode, it adjusts the resolution offsets. Users can verify the instrument performance or select one or more scan types to optimize the instrument components.

Before starting, the system backs up the current instrument parameters. The backed up instrument parameters can be accessed in the Instrument Optimization dialog using the File menu.

(Optional) Manually Back up Instrument Parameters

Back up the current instrument parameters in case they must be restored later. The preset location for the manually backed up instrument parameters is <drive>:\Analyst Data\Projects\API Instrument\Instrument Optimization\Instrument Settings Backups\User Created Backups.

1. On the navigation bar, under **Tune and Calibrate**, double-click **Instrument Optimization**.
2. Click **File > Backup Instrument Settings.**
3. Type a file name.
4. Click **Save**.

(Optional) Restore Instrument Parameters

1. On the navigation bar, under **Tune and Calibrate**, double-click **Instrument Optimization**.
2. Click **File > Restore Instrument Settings File.**
3. Navigate to the instrument settings to restore.
4. Click **Open**.

Infusion

Infusion is the continuous flow of the sample at low flow rates into the ion source using a syringe pump.

Acquisition Methods 4

If creating an acquisition method file from an existing file, the user can use some or all of the device methods in the acquisition method. Use the Acquisition Method Editor to customize the acquisition method by adding or removing device methods. If the required device icon is not in the Acquisition Method Browser pane, then users can add the device only if it is included in the active hardware profile.

Devices in Acquisition Methods

Create an acquisition method for a peripheral device by selecting the operating parameters for that device. Acquisition methods can be created for any of the following devices if they are configured in the active hardware profile:

- Pumps
- Autosamplers
- Syringe pumps
- Column ovens
- Switching valves
- Diode array detector
- Analog-to-digital converters
- Integrated systems

For information about setting properties for devices, refer to the *Peripheral Devices Setup Guide*.

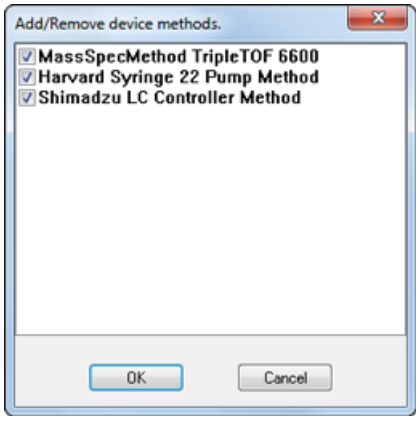
Note: The available parameters for the LC devices vary depending on the manufacturer.

Add or Remove a Peripheral 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**.

   The **Add/Remove Device Method** dialog opens, showing the devices configured in the active hardware profile.

Figure 4-1 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**.

Set the LC Pump Properties

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 opens in the **Acquisition Method Editor** pane.
2. Edit the fields as required.
3. Save the file.

Set the Autosampler Properties

1. Make sure that on the **Acquisition Properties** tab, the **Synchronization Mode** field is set to **LC Sync**. The device and the instrument 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 opens in the **Acquisition Method Editor** pane.
3. Edit the fields as required.
4. Save the file.

Set the Integrated Syringe Pump Properties

This procedure is for systems with built-in syringe pumps.

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

   The **Syringe Pump Method Properties** tab appears in the **Acquisition Method Editor** pane.
2. Edit the fields as required.
3. Save the file.

Set the 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 opens in the **Acquisition Method Editor** pane.
2. Edit the fields as required.
3. Save the file.

Set the Switching Valve Properties

The switching valve can be used as a diverter or injection valve. Select the Manual Sync with Valve synchronization mode if using the valve as an injector; choose any other mode if 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 opens in the **Acquisition Method Editor** pane.
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. If the valve is being used as diverter, rename the preset position names A and B to Divert and Inject or to Waste and Column.
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.

Set the Diode Array Detector Parameters

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 opens in the **Acquisition Method Editor** pane.

2. Set the desired acquisition parameters and save the file.

Set the 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 opens in the **Acquisition Method Editor** pane.

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 the rate is changed, the software automatically calculates the interval again.

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.

Experiments and Periods

The mass spectrometer acquisition method consists of experiments and periods. In the Acquisition Method Browser pane, create a sequence of acquisition periods and experiments for the instrument. Users can also open a method previously created in the Tune Method Editor.

Experiments

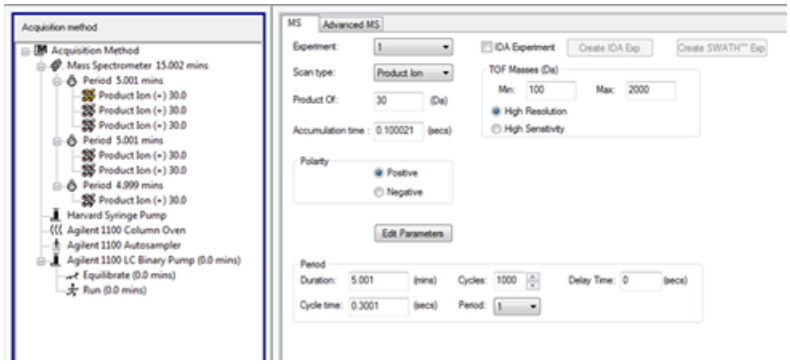
An experiment includes the instrument settings and the scan type during an MS scan. A set of MS scans performed for a specific amount of time is called a period. An acquisition method in which the MS parameters and actions are the same through the entire duration is called a single-period, single-experiment method.

In looped experiments, MS settings are changed on a scan-by-scan basis. For example, if the sample contains two compounds, A and B, users may want to loop an MS/MS experiment of compound A with an MS/MS experiment of compound B to obtain information about both compounds in the same run. The mass spectrometer method will alternate between the two scan types. An example of looped experiments is an Information Dependent Acquisition (IDA) method.

Periods

A period can contain one or more looped experiments. In a multi-period acquisition method, experiments are performed for a specified amount of time and then switch to another set of experiments. Periods are useful when the elution time of the compounds in an LC run is known. The instrument can perform different experiments according to when the compounds elute to obtain as much information as possible in the same run. *Figure 4-2* shows a three-period method.

Figure 4-2 Example of a Multi-period Experiment



SWATH™ Acquisition

The SWATH acquisition allows MS/MS analysis of all precursors across a wide mass range on a LC timescale. The Q1 quadrupole is set to a wider selection window width (typically 10 to 50 Da) than that used for conventional Product Ion acquisitions. By stepping through multiple, sequential selection windows, a wide mass range is covered rapidly. The resulting mass spectra are a composite of the fragments of all of the precursor ions that passed through the respective Q1 selection window. This technique allows for non-targeted MS/MS analysis of all species in a sample.

The SWATH acquisition feature is supported for the TripleTOF® 4600, TripleTOF 5600/5600+, and TripleTOF 6600 systems.

Create a SWATH Acquisition Method Using the Quick Method in the Acquisition Method Editor

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

   The **Acquisition Method Editor** opens.
2. Select the **Mass Spectrometer** option in the Acquisition method pane.
3. On the **MS** tab, select **TOF MS** and then type values in the fields as required.

   The **Create SWATH Exp** button is shown on the MS tab page.
4. Click **Create SWATH Exp**.

   The **Create SWATH Experiments** dialog opens.
5. To create SWATH experiments using the quick method, either accept the default values for all the parameters on the Quick tab or provide different values and then click **OK**. For more information, refer to the Analyst TF Help.

   The note at the bottom of the Quick tab indicates that the acquisition method will contain a specific number of SWATH windows at specific width.

   The **Create SWATH Experiments** dialog closes and the specified number of SWATH experiments of specified width are created and displayed in the Acquisition method pane. Each SWATH experiment is a window. The first window starts at the specified SWATH Scan Start Mass and is as wide as the SWATH width specified in the note on the Quick tab. Each subsequent SWATH window starts at the mass where the previous SWATH window ends minus 1. The last SWATH window ends at the specified SWATH Scan Stop Mass
6. Click **File > Save.**

Note: If the TOF MS experiment is deleted from a SWATH acquisition method, the Create SWATH Exp button is no longer available. Additionally, a message is shown to inform the user that the method does not meet the requirements for a SWATH method and, therefore, the SWATH feature is no longer available.

Note: If an experiment is manually added to a SWATH acquisition method, the Create SWATH Exp button is no longer available. Additionally, a message is shown to inform the user that the method does not meet the requirements for a SWATH method and, therefore, the SWATH feature is no longer available.

Create a SWATH Acquisition Method Using the Manual Method in the Acquisition Method Editor

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

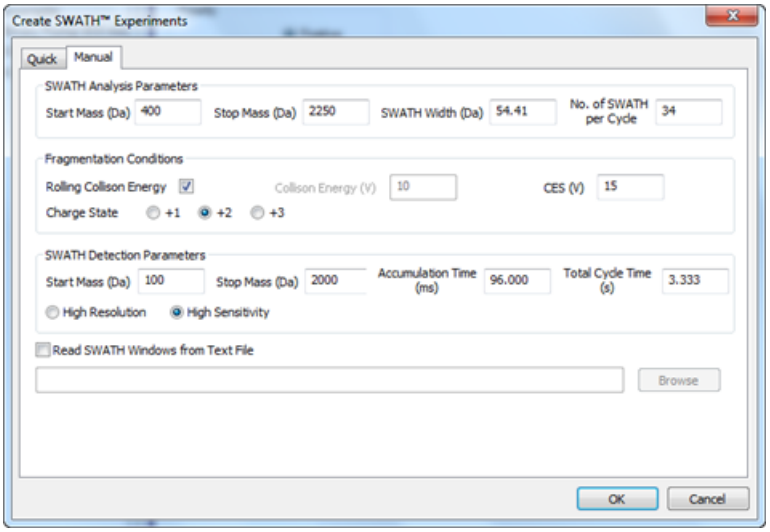
   The **Acquisition Method Editor** opens.

2. Select the **Mass Spectrometer** option in the Acquisition method pane.
3. On the **MS** tab, select **TOF MS** and then type values in the fields as required.

   The **Create SWATH Exp** button is shown on the MS tab page.
4. Click **Create SWATH Exp**.

   The **Create SWATH Experiments** dialog opens.
5. Click the **Manual** tab.

Figure 4-3 Manual Tab



For information about the parameters on the Manual tab, refer to the *Create SWATH Experiments Dialog — Manual Tab* topic in the Analyst TF Help.

6. Either accept the default values for all the parameters on the **Manual** tab or provide different values and then click **OK**.

   The Create SWATH Experiments dialog closes and the specified number of SWATH experiments of specified width are created and shown in the Acquisition method pane. Each SWATH experiment is a window. The first window starts at the specified SWATH Scan Start Mass and is as wide as the SWATH width specified on the Manual tab. Each subsequent SWATH window starts at the mass where the previous SWATH window ends minus 1. The last SWATH window ends at the specified SWATH Scan Stop Mass.
7. Click **File > Save.**

Create a SWATH Acquisition Method Using the Method Wizard

1. On the navigation bar, under **Acquire**, double-click **Method Wizard**.

   The Method Wizard opens.

2. In the **Choose MS Method** field, select either **SWATH (+)** or **SWATH (-)** from the list.

   The Ion Source Parameters and SWATH tabs are shown in the Method Wizard.
3. In the **Choose LC Method** field, select the required method either from the list or using the **Browse** button.
4. In the **Save Method As** field, type a name for the SWATH acquisition method and then press **Enter**.
5. Click **Next**.
6. On the **Ion Source Parameters** tab, modify the values of the ion source parameters if required.
7. Click **Next**.
8. On the **SWATH** tab, modify the values of the parameters if required. For more information about the various parameters on SWATH tab, refer to the *Parameters on SWATH Tab* topic in the Analyst TF Help.

   Based on the values provided for the parameters on the SWATH tab page, the software updates the note at the bottom of the page informing the user how many SWATH windows are created as well as the width of the SWATH windows. If the user uses the default values for all the parameters, the software informs the user that the default parameter settings create a SWATH acquisition method with 34 SWATH windows at the width of 25 Da each using the TOF mass range (only applicable to TripleTOF 4600 and TripleTOF 5600/5600+ systems). For TripleTOF 6600 system, if the user uses the default values for all the parameters, the software informs the user that the default parameter settings create a SWATH acquisition method with 34 SWATH windows at the width of 54.41 Da each using the TOF mass range.
9. Click **Finish**.

   The Method Wizard shows a message indicating that the new SWATH acquisition method was created successfully.

Create a SWATH Acquisition Method with Variable SWATH Acquisition Windows Using the Acquisition Method Editor

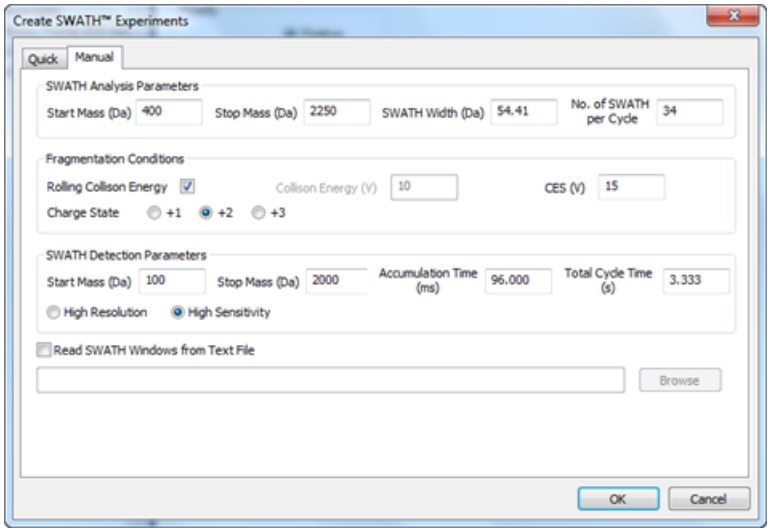
1. On the navigation bar, under **Acquire**, double-click **Build Acquisition Method**.

   The **Acquisition Method Editor** opens.
2. Select the **Mass Spectrometer** option in the Acquisition method pane.
3. On the **MS** tab, select **TOF MS** and then type values in the fields as required.

   The **Create SWATH Exp** button is shown on the MS tab page.
4. Click **Create SWATH Exp**.

   The **Create SWATH Experiments** dialog opens.
5. Click the **Manual** tab.

Figure 4-4 Create SWATH Experiments Dialog — Manual Tab

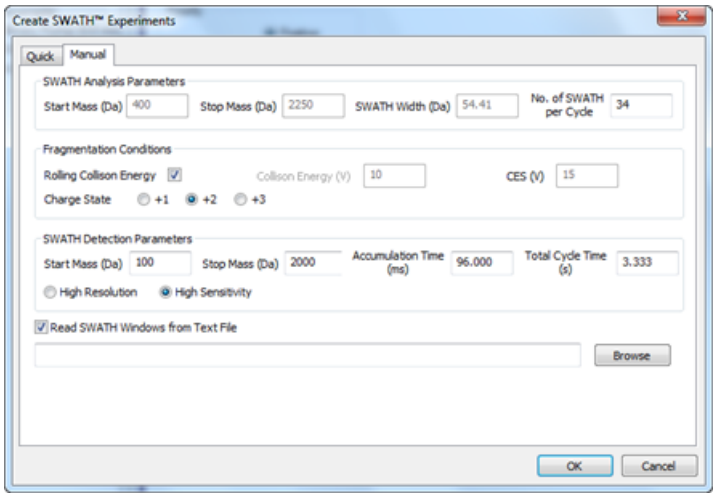


6. Click the **Read SWATH Windows from Text File** check box. Selecting this option enables the creation of a SWATH acquisition method with variable SWATH acquisition windows by reading the start mass, stop mass, and CES value for each SWATH acquisition window from a text file.

   The text file must list the start masses, the stop masses, and the CES values in the following order: Start Mass, Stop Mass, and CES Value separated by a tab. Each set of Start Mass, Stop Mass, and CES value must be listed on a new line. Refer to the sample text file in *Figure 4-11*.

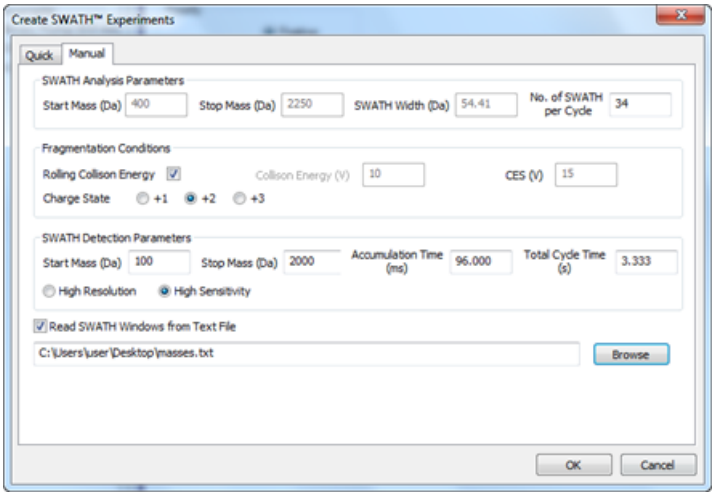
   When the Read SWATH Windows from Text File option is selected, the Start Mass, Stop Mass, SWATH Width, and CES fields are grayed out.

Figure 4-5 Read SWATH Windows from Text File Selected



7. Click **Browse**.
8. From the **Open** dialog, select the text file to use to create the variable SWATH acquisition method.

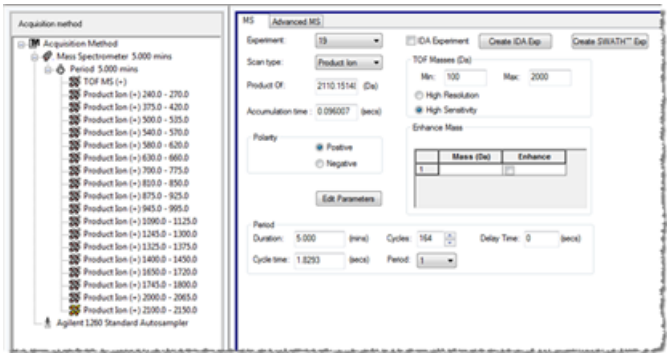
Figure 4-6 Manual Tab — Text File Selected



9. If required, modify the parameters in the SWATH Detection Parameters group.
10. Click **OK**.

    The **Create SWATH Experiments** dialog closes and the specified number of SWATH experiments of specified width are created and shown in the Acquisition method pane. Each SWATH experiment is a window. Each window corresponds to the start mass and stop mass that was specified in the text file selected in step 8.

Figure 4-7 SWATH Acquisition Method with Variable SWATH Windows Created



11. Click **File > Save.**

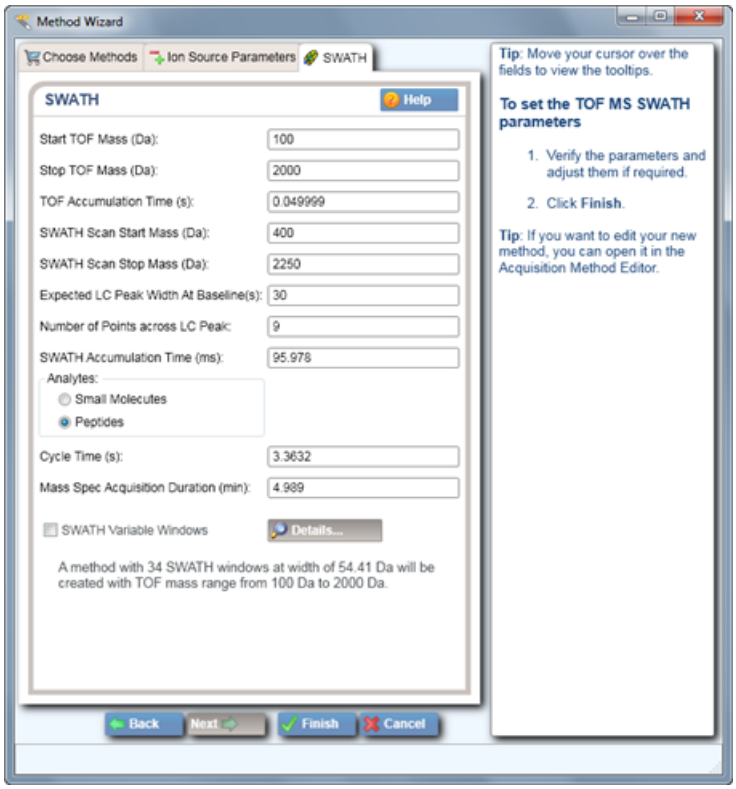
Create a SWATH Acquisition Method with Variable SWATH Acquisition Windows Using the Method Wizard

1. On the navigation bar, under **Acquire**, double-click **Method Wizard**.

   The Method Wizard opens.
2. In the **Choose MS Method** field, select either **SWATH (+)** or **SWATH (-)** from the list.

   The Ion Source Parameters and SWATH tabs are shown in the Method Wizard.
3. In the **Choose LC Method** field, select the required method either from the list or using the **Browse** button.
4. In the **Save Method As** field, type a name for the SWATH acquisition method and then press **Enter**.
5. Click **Next**.
6. On the **Ion Source Parameters** tab, modify the values of the ion source parameters if required.
7. Click **Next**.

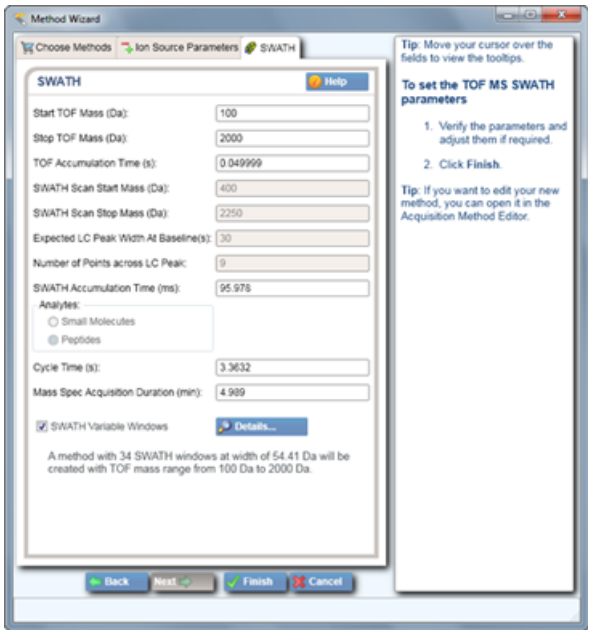
Figure 4-8 SWATH Tab in Method Wizard



8. On the **SWATH** tab, select the **SWATH Variable Windows** check box.

The **SWATH Scan Start Mass**, **SWATH Scan Stop Mass**, **Expected LC Peak Width At Baseline(s)**, **Number of Points across LC Peak**, and **Analytes** parameters are grayed out.

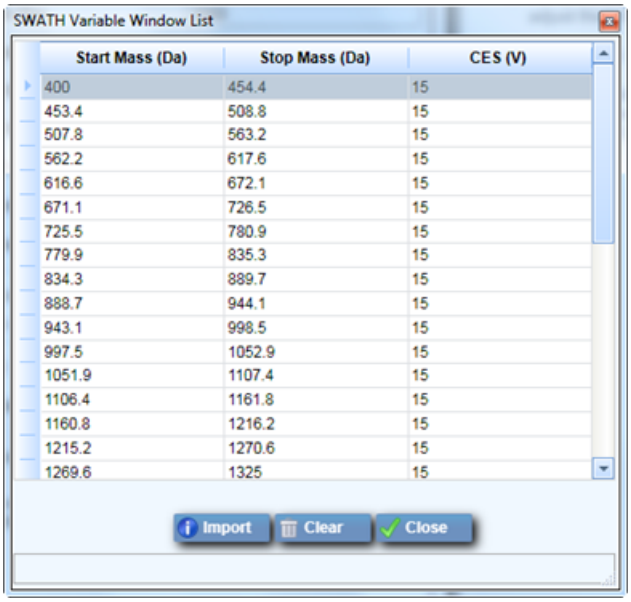
Figure 4-9 SWATH Variable Windows Option Selected



9. Click **Details**.

   The SWATH Variable Window List dialog opens. By default, the dialog shows the start mass and the stop mass for 34 SWATH windows with the width of 54.41 Da based on the default values on the SWATH tab. By default, the dialog shows the start mass and the stop mass for 34 SWATH windows with the width of 25 Da based on the default values on the SWATH tab. The CES value for each window is also shown.

Figure 4-10 SWATH Variable Window List Dialog



10. Click **Import**.
11. In the **Open** dialog, select the text file to use to create the variable SWATH acquisition method.

    The start masses, the stop masses, and the CES values from the text file replace the default values in the SWATH Variable Window List dialog. The text file must list the start masses, the stop masses, and the CES values in the same order as the SWATH variable Window List dialog — Start Mass, Stop Mass, and CES Value separated by a tab. Refer to a sample text file in *Figure 4-11*.

Figure 4-11 Sample Text File with Start Masses, Stop Masses, and CES values

```
240     270     10
375     420     10
500     535     10
540     570     10
580     620     10
630     660     10
700     775     10
810     850     10
875     925     10
945     995     10
1090    1125    10
1245    1300    10
1325    1375    10
1400    1450    10
1650    1720    10
1745    1800    10
2000    2065    10
2100    2150    10
```

12. Click **Close**.

    The SWATH Variable Window List dialog closes. Based on the Start masses, Stop masses, the width of the SWATH windows, and the number of SWATH windows specified in the SWATH Variable Window List dialog,

a message is displayed on the SWATH tab. The message indicates that a method will be created with the specified number of variable SWATH windows. The values in the SWATH Accumulation Time, Cycle Time, and Mass Spec Acquisition Duration fields also change.

13. If required, modify the values of the parameters on the SWATH tab. For more information about the various parameters on SWATH tab, refer to the *Parameters on SWATH Tab* topic in the Analyst TF Help.
14. Click **Finish**.

    A message indicates that the SWATH acquiescing method was successfully saved. For information about the SWATH acquisition, refer to the Analyst TF Help and the Method Wizard Help.

Information Dependent Acquisition Methods

Information Dependent Acquisition (IDA) is an acquisition method that analyzes data during acquisition. IDA is used to change the experimental conditions depending on the analysis results. These real-time changes are controlled by criteria set in the acquisition method, including:

- Ion intensity and charge state.
- Inclusion and exclusion lists.
- Isotope pattern.

Optimizing data acquisition settings while the data is being acquired allows users to conserve both the sample and working time on an instrument.

An IDA method automatically runs experiments based on results obtained from previous experiments. Use IDA criteria to optimize data acquisition settings while acquiring data to reduce the sample acquisition time in a single injection. With IDA, users can conserve both the amount of sample required and valuable working time.

For TripleTOF® 5600/5600+ systems, users can create an IDA method with up to two survey scans and 100 dependent scans in a single experiment. For TripleTOF® 5600/5600+ and TripleTOF 6600 systems, users can create an IDA method with up to two survey scans and 100 dependent scans in a single experiment. For TripleTOF 4600 system, an IDA method can be created with up to two survey scans and 50 dependant scans in a single experiment. A survey scan is used in IDA to trigger additional experiments. The TOF MS scan type is used as a survey scan and the MS/MS scan type is used as a dependent scan.

In an IDA experiment, the instrument actions are varied from scan type to scan type based on the data acquired in a previous scan. The software analyzes data as it is being acquired and then determines the masses on which to perform dependent scans. Users can set the criteria that will activate an IDA experiment and the method parameters to be used.

IDA experiments modify experiments and improve results based on the following user-defined criteria:

- Ion intensity and charge state
- Inclusion and exclusion lists
- Isotope pattern

- Mass defect
- Neutral loss
- Dynamic exclusion
- Rate of change in ion intensity (Refer to *Dynamic Background Subtraction Algorithm*.)

Solvent Compressibility Values

Table 4-1 Solvent Compressibility Values

Solvent	Compressibility (10^{-6}/bar)
Acetone	126
Acetonitrile	115
Benzene	95
Carbon Tetrachloride	110
Chloroform	100
Cyclohexane	118
Ethanol	114
Ethyl acetate	104
Heptane	120
Hexane	150
Isobutanol	100
Isopropanol	100
Methanol	120
1-Propanol	100
Toluene	87
Water	46

Syringe Size Versus Flow Rate

The flow rate of a syringe pump depends on the syringe installed in the pump. The following tables show the relationship between flow rate and syringe size.

Table 4-2 Syringe Size and Flow Rate at L/hour

Syringe size (µL)	L/hour Minimum	L/hour Maximum
0.5	.002	23.8
1.0	.003	47.8
2.0	.006	95.2
5.0	.015	238.0
10.0	.029	474.0
25.0	.073	1193.0

Table 4-3 Syringe Size and Flow Rate at µL/minute

Syringe size (µL)	µL/minute Minimum	µL/minute Maximum
50	.002	39.7
100	.005	79.7
250	.012	197.8
500	.024	397.0
1000	.048	795.0
1.0	.049	805.0

Table 4-4 Syringe Size and Flow Rate at mL/hour

Syringe size (mL)	mL/hour Minimum	mL/hour Maximum
2.0	.011	186.8
2.5	.010	168.2
3.0	.011	181.4
5.0	.019	317.0
10.0	.028	461.0
20.0	.050	821.0
30.0	.074	1208.0

Table 4-5 Syringe Size and Flow Rate at mL/minute

Syringe size (mL)	mL/minute Minimum	mL/minute Maximum
50.0	.002	28.40
100.0	.003	47.60
140.0	.004	55.10

Batches 5

A batch is a collection of information about the samples to be analyzed. Samples are usually grouped into sets to make it easier to submit them. Grouping the samples into a set also reduces the amount of data that must be typed manually. A set can consist of a single sample or multiple samples. All of the sets in a batch use the same hardware profile, however, samples in a set can have different acquisition methods. A batch can be submitted only from an acquisition station.

Batches link together:

- Sample information, such as name, ID, and comment.
- Autosampler location (rack information).
- Acquisition methods.
- Processing method or script (optional).
- Quantitation information (optional).
- Custom sample data (optional).
- Set information.

Batch Editor

Use the Batch Editor to create or modify batches and to create batch templates. To run samples, each using different acquisition methods, select multiple acquisition methods in the same set.

An acquisition method can also be used as a template. In this case, the same method is used for each sample, but the user can select different masses or mass ranges for each sample. The Batch Editor can also be used to import sample lists created in external programs, such as Microsoft Excel.

The user can modify every detail of the batch before submitting it for processing. When a batch is submitted for analysis, the user can submit the entire batch, specific sets within the batch, or specific samples within a set.

For example, to analyze ten samples, five using one acquisition method and five using a different acquisition method, create a batch of two sets, one for each method used.

Table 5-1 Batch Editor Tabs

Tab	Description
Sample	Used to create the sample list and to select sample details such as the sample name and the acquisition method to be used to acquire the sample.
Locations	Used to select the positions of samples in the autosampler. Sample locations can be specified numerically in the Sample tab. However, the Locations tab provides a graphical interface for selecting sample locations.
Calibration	Used to select the set that users want to calibrate. Mass calibration is performed on each set in a batch, which means that a different calibrant can be used on multiple sets in a single batch.
Quantitation	Used to select the sample types and quantitation information.
Submit	Used to verify sample information and to submit samples to the acquisition queue. The Queue Manager shows queue, batch, and sample status and allows users to manage samples in the queue.

Batch Files

Users can import a text file containing batch information instead of creating a batch in the Batch Editor. If users have all the details for the samples they 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.

To make sure that the text file includes the proper headings, 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.

For examples of correctly formatted files, refer to the Batch folder in the Example project.

The information in a batch file can also be exported for use with other applications, such as Microsoft Excel, Microsoft Access, and certain LIMS (Laboratory Information Management System) software.

Build a Batch as a Text File

Users can export a batch only if it contains at least one set with at least one sample. If the text file is saved, it can be used again later as a template.

1. Make sure that the active hardware profile includes all the devices needed to acquire the samples.
2. In the navigation bar, under **Acquire**, double-click **Build Acquisition Batch**.
3. Create a single-set, single-sample batch.
4. Click **File > Export.**
5. Name the file.

6. Click **Save**.
7. Open the text file in a spreadsheet program.
8. 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.

9. Save the modified text file as a .txt or .csv file.
10. Close the spreadsheet program.

    The text file can now be imported into the Batch Editor.

Import a Batch as a Text File

Before batch information is imported 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.

1. In the navigation bar, under **Acquire**, double-click **Build Acquisition Batch**.
2. In the **Sample** tab, right-click and then click **Import From > File.**
3. Click the text file containing the batch information.

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

4. Click **Open**.

   If an autosampler is being used, then the **Select Autosampler** dialog opens.
5. In the autosampler list, select the autosampler.
6. Click **OK**.

   The sample table fills with the details from the text file.

Set Quantitation Details in the Batch Editor (Optional)

If users do not want to select quantitation details post-acquisition, then Quantitation methods can be included with a batch and used to define the quantitation details prior to submitting a batch.

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

Use the following steps to include quantitation information in the batch:

1. Click **Quick Quant** and then enter the analyte and internal standard names and the mass ranges to be used for quantitation.

   Users can use Quick Quant to populate quantitative information, such as sample type and sample concentration on the Quantitation tab of the Batch Editor, prior to data acquisition.

2. Click OK and then save the method.
3. Click the **Quantitation** tab and complete the table as required.

Tip! Quantitation type and concentration information can be copied and pasted into the table.

Data Analysis and Processing 6

Users 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 displays can be viewed as a table of data points and various sorting operations can be performed on the data.

The software stores data in files with a .wiff extension. Wiff files can contain data for more than one sample. To see spectral data, users also need the .wiff.scan files. In addition to .wiff files, the software can open .txt files; .txt files contain data for only one sample. When a data file is opened in the software, different panes appear depending on the type of experiment that was performed.

If the MCA check box is selected during the acquisition of the data file, the data file will open to the MS (mass spectrum). If the MCA check box is not selected, then the data file opens with the Total Ion Chromatogram (TIC). Users can select a range and then double-click in the TIC pane at a particular time to show the MS for this range.

Chromatograms

A chromatogram displays the variation of some quantity with respect to time in a repetitive experiment; for example, when the instrument is programmed to repeat a given set of mass spectral scans several times. Chromatographic data is contiguous, even if the intensity of the data is zero. Chromatograms are not generated directly by the instrument, but are generated from mass spectra.

In the chromatogram display, the intensity, in counts per second (cps), is shown on the y-axis versus time on the x-axis. Peaks are automatically labeled.

In the case of LC/MS, the chromatogram is often displayed as a function of time, the time at which a particular scan was obtained, which can be derived from the scan number.

A chromatogram provides a general view of the data, usually time dependent when using an LC column, but it does provide information about the components of a peak. For example, while a chromatogram may show only one peak, that peak can represent more than one compound; that is, different masses.

Chromatographic data can change in both time and intensity if there is a change in the chromatographic conditions in a given sample.

Spectra

A spectrum is the data that is obtained directly from the instrument and normally represents the number of ions detected with particular mass-to-charge (*m/z*) values. It is displayed as a graph with the *m/z* values on the x-axis and intensity (cps) represented on the y-axis.

For MS/MS data, the intensity is associated with all masses, precursor and fragment ions.

When data is viewed as a spectrum, mass-specific information about a compound is obtained. A spectrum looks at a particular peak and provides *m/z* values of the corresponding compound, which can be used to find more specific information. For example, a spectrum shows all of the masses that make up a peak, including the intensity of each mass.

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, then the data is shown as a spectrum.
- From a chromatogram.

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

Background Subtraction

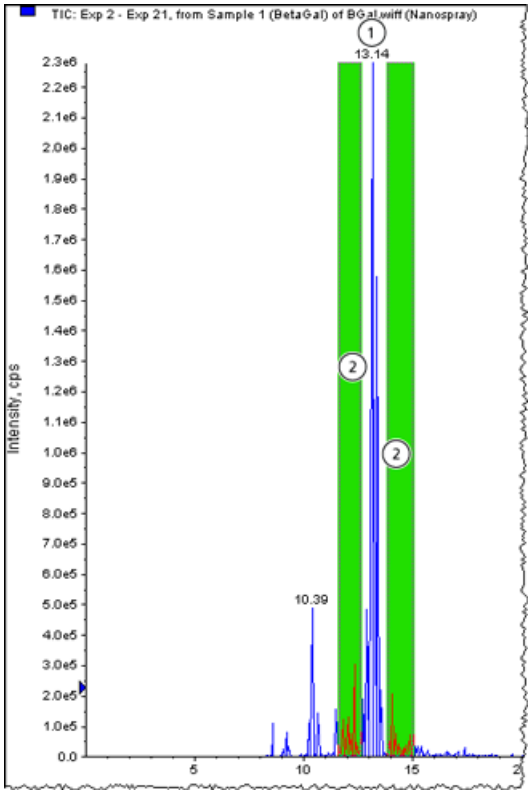
Background subtraction reduces the amount of noise in a spectrum by subtracting either one or two ranges that contain noise from a range that contains a peak. Users can move the ranges independently or lock them and move them as a single entity within the graph to optimize peak isolation, or to isolate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

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

Perform a Background Subtraction for a Chromatogram

1. Open a data file.
2. Select a background range in the chromatogram.
3. Press the Shift key and then select another background range.

Figure 6-1 Chromatogram



Item	Description
1	Peak of interest
2	Background

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 the background subtracted spectrum as a processed data file, click **File > Save Processed Data File.**

Unlock the Ranges

The selected subtraction range is set to locked.

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

  The ranges are unlocked and each one can be moved independently.

Baseline Subtract

Baseline subtract removes a constant or slowly varying offset from a set of data. This is useful in locating small peaks that are obscured by noise. The software uses the following algorithm in performing a baseline subtraction.

- Every data point in the data set is considered as the center of a window (in mass or time) with a user-definable width measured in amu or minutes.
- The minimum values on either side of the current data point (minima) within the window are located.
- A straight line is fitted between the two minima and the height (intensity) of the current data point above the line is calculated. The end points of the data are regarded as minima.
- The data point is replaced with the new calculated value.

Calculators

Use a calculator to perform calculations on the basis of collected data. Although the calculator is a separate window, it is connected to the active graph within the software.

The following calculators are available.

- *Elemental Composition Calculator*
- *Hypermass Calculator*
- *Elemental Targeting Calculator*
- *Mass Property Calculator*
- *Isotopic Distribution Calculator*

Users can cut and paste from one text box to another between the different windows in the calculators. Data from any of the calculators can be printed by clicking the Print icon in the top left corner of the window. For more information about using calculators, refer to the Help.

Data from the Elemental Composition, Mass Property, and Isotopic Distribution calculators can be exported to a separate file. Use the Elemental Targeting calculator to modify the data within the active graph. Data from the HyperMass and Isotopic Distribution calculators can be overlaid on the active spectrum.

Note: Set the precision of calculator data in the **Calculators** tab of the **Appearance Options** dialog. To open the dialog, click **Tools > Settings > Appearance Options.**

Elemental Composition Calculator

The Elemental Composition calculator determines potential molecular or amino acid compositions based on a target mass-to-charge ratio. Type this ratio manually or select it from an active spectrum. This calculator creates a table with the possible element or amino acid combinations making up the mass of interest and the characteristics of each.

Type or select values for such parameters as tolerance, electron state, and number of charges. Users can also type a list of possible elements and put a limit on the number of each.

Hypermass Calculator

The Hypermass calculator determines the distribution of a multiply charged envelope based on an uncharged mass. Users can select the uncharged mass, including the adduct and its polarity.

The calculator displays a graphical representation of the Hypermass series, which can be overlaid onto the active spectrum. A list of the Hypermass data is also available.

Elemental Targeting Calculator

The Elemental Targeting calculator reduces the data spectrum based on a specific pattern, primarily one corresponding to isotopic distributions. It can also search an MS data spectrum for a specific pattern of peaks, which can be entered either as a formula or as an isotopic distribution.

If the calculator finds a match, it creates a reduced plot containing only data pertaining to the specified pattern. For a spectrum, the calculator removes all unmatched data. For a chromatogram, the calculator calculates the elemental target for each of the underlying spectra and regenerates each point in the chromatogram on the basis of these new spectra.

Mass Property Calculator

The Mass Property calculator determines various properties such as exact mass, the average mass, the mass accuracy, and the mass defect of a mass of interest. The results generated by this calculator depend upon the number of input fields completed.

Isotopic Distribution Calculator

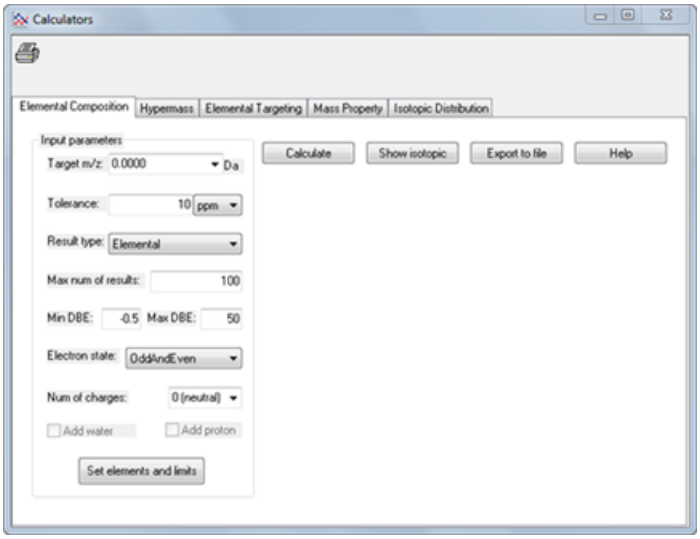
The Isotopic Distribution calculator determines the isotopic distribution based on an entered formula. This allows users to distinguish between compounds with the same mass based on relative intensities of isotopes.

The calculated isotopic distribution can be displayed in graphical or text format on the Isotopic Distribution pane, overlaid on the active spectrum, or exported to a separate file.

Access the Calculators

- Click **Tools > Calculators.**

Figure 6-2 Calculators dialog



The **Calculators** dialog opens.

Centroided Peaks

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

The centroid 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 6-1*.

Table 6-1 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 calculated as a centroid when added to a library or when a search is conducted.

Calculate the Centroid of a Peak

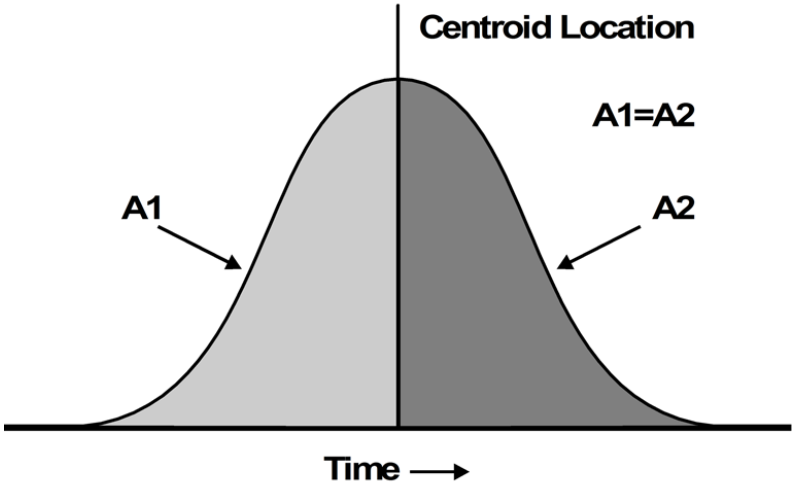
1. Select a pane containing a spectrum.

   Calculating the centroid of the peak changes the appearance of the existing graph. To compare the result with the original data, make a copy of the graph before calculating the centroid.

2. Click **Explore > Centroid.**

   The data is centroided.

   Figure 6-3 Analyte Centroid Location



Noise and Area Threshold Parameters

To identify peaks, the software requires a set of noise and area threshold parameters. The software sets these parameters initially, but users can change them later. It sets the parameters as follows:

1. The software calculates the largest intensity difference between any two sequential data points. This number represents the difference between two intensities, not the actual intensity itself.
2. For each sequential pair with an intensity difference of less than 5% of the value calculated in step 1, it calculates the standard deviation (using a mean of zero) of the intensity differences. (The software does not use those pairs of points with an intensity difference larger than 5% of the maximum.)

   It sets the noise and area thresholds as follows:
3. The noise threshold is equal to the standard deviation calculated in step 4.
4. The area threshold is equal to five times the noise threshold.

Note: The minimum value for both the noise and area thresholds is 0.000001. If the preceding calculations produce a value that is lower than this minimum, the software resets the value of that threshold at 0.000001.

Recalculate the Noise and Area Threshold

If a new background area is defined, the software recalculates the noise and area thresholds as follows. For each sequential pair of data points, the software calculates the standard deviation, using a mean of zero, of the intensity difference. The Analyst software uses all points within the selected range because it is explicitly being told that the selected area is background noise.

It sets the noise and area thresholds as follows:

1. The noise threshold is equal to the standard deviation calculated for the selected range.
2. The area threshold is equal to five times the noise threshold.

Note: The minimum value for both the noise and area thresholds is 0.000001. If the preceding calculations produce a value that is lower than this minimum, the software resets the value of that threshold at 0.000001.

Noise Filter

Use the noise filter to discriminate between baseline noise and peaks by setting a minimum peak width. Peaks with width below this minimum threshold are considered noise. The lower the noise threshold, the more peaks are detected by the algorithm. Raising the threshold decreases the number of peaks found. In chromatograms the width is measured in minutes, and in spectra the width is measured in Da. For more information, refer to the Help.

Peak Review

During peak review users can survey the peaks that the software selected and then redefine the peak or the start and end points where necessary.

In general, the software is adept at accurately identifying analyte and internal standard peaks. For a variety of reasons, including sample acquisition and quantitation method definition, sometimes the software misses the correct peak, chooses the wrong one, or is unable to locate a peak at all. Other times, although the software may correctly identify the peak, users may not agree with the start or end points selected.

Detect Peaks

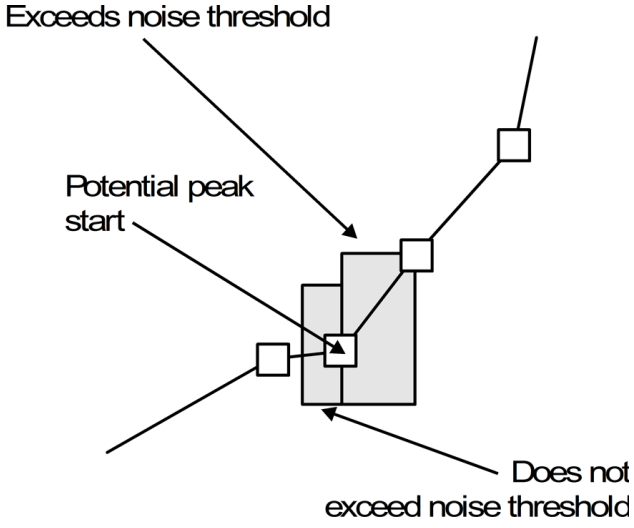
The software detects peaks in four stages.

1. First, it finds the potential peak start by examining the distance between each bunched point and the preceding one. When the distance exceeds the current noise threshold, a potential peak start has been found.
2. Then it confirms the peak start by making sure that enough points exist in a row to exceed the area threshold.
3. Next, it finds the peak top by searching for a point that is lower than the previous point.
4. Finally, it finds the end of the peak by identifying the place where the distance between one bunched point and the next falls below the noise threshold. If necessary, it then separates peaks.

Find the Potential Peak Start

To find the potential start of a peak, the software measures the intensity difference between sequential pairs of bunched points, starting at the first point. When it finds a difference that exceeds the current noise threshold, the software declares the first point a potential peak start.

Figure 6-4 Find the Potential Peak Start



Confirm the Peak Start

To make sure that it has found a real peak, the software moves along the curve, adding the intensity difference between each bunched data point from the intensity at the potential peak start to a total sum. This process stops when the intensity difference between successive points is less than the noise threshold. This sum is an approximation of the area of the leading edge of the peak. If this sum exceeds the area threshold, then the software confirms the peak start.

Next, the software determines the actual start of the peak by moving backward from the potential peak start until it finds the lowest point in the peak. It moves back through five bunches of raw data. This point is the actual peak start.

Figure 6-5 Confirm the Peak Start

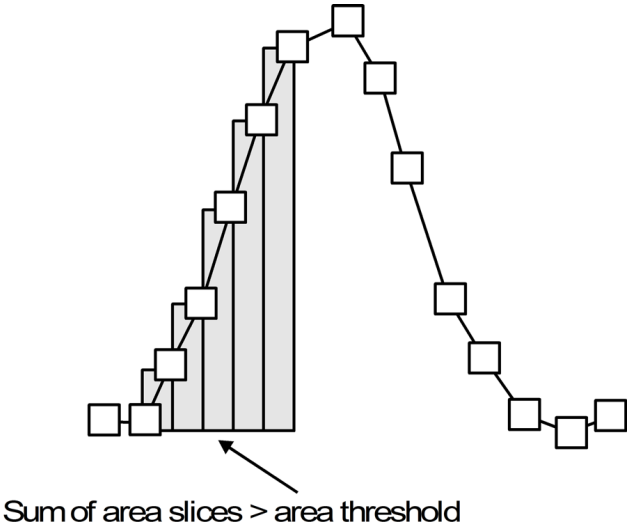
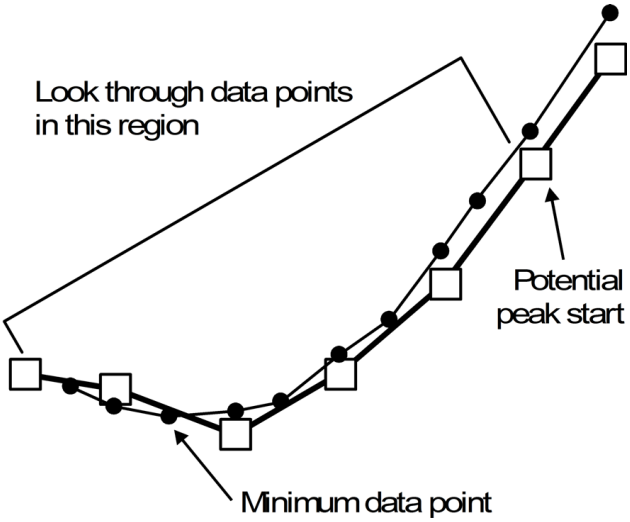


Figure 6-6 Confirm the Actual Peak Start



Find the Peak Top

To find the peak top, the software first looks for a point that is lower than the preceding point. Then, to confirm that it has found the top correctly, it sums the intensity differences between the potential top and subsequent bunched points until it reaches the end of the peak. If the total distance between points exceeds two-thirds of the area threshold, then the peak top is confirmed. That is, the software makes sure it has a peak first, and then works backward to find the top of it.

If, however, the software finds a higher bunched point before the area test has been passed, then it identifies a new top and restarts the area test.

Note: The actual retention time for a peak is not simply the point identified as described above. Instead, it is determined from a quadratic fit based on the three highest data points.

Figure 6-7 Find the Peak Top

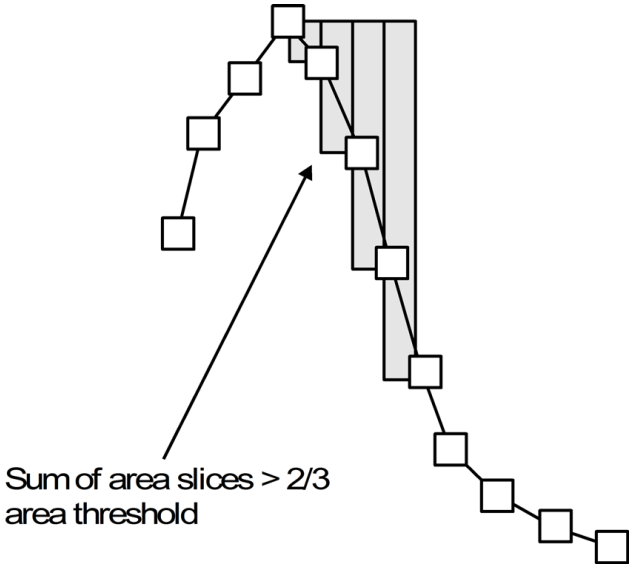
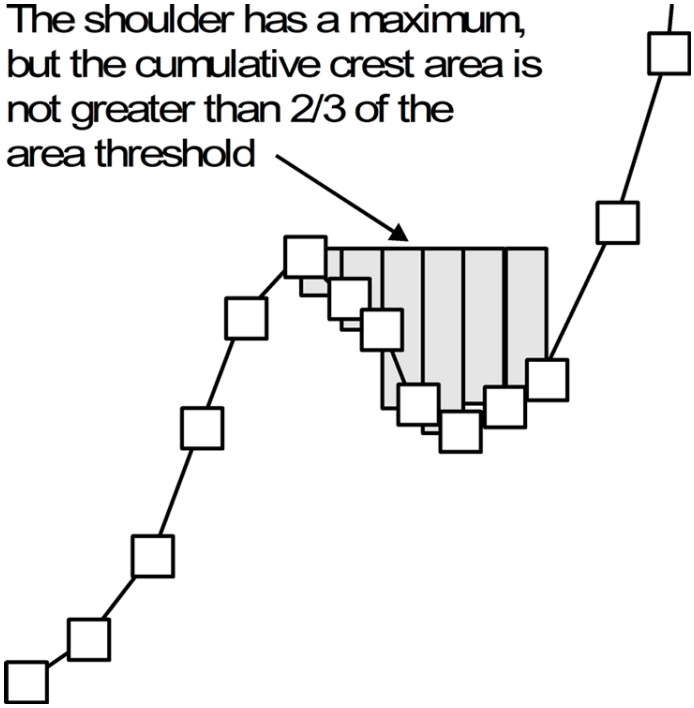


Figure 6-8 Identify a New Peak Top



Find the Peak End

The software declares a peak end point when one of the following occurs:

- The difference between two consecutive points fails the noise threshold test.
- The software detects the start of a new peak.

In either case, the lowest bunched point from the last five bunches is considered to be the actual end point of the peak.

In general, the software finds several peaks for each chromatogram. The peak it selects is the one whose retention time is the closest to the expected retention time, specified in the method. If no peak has a retention time within the specifications, the software marks the peak as not found.

Figure 6-9 Find Peaks

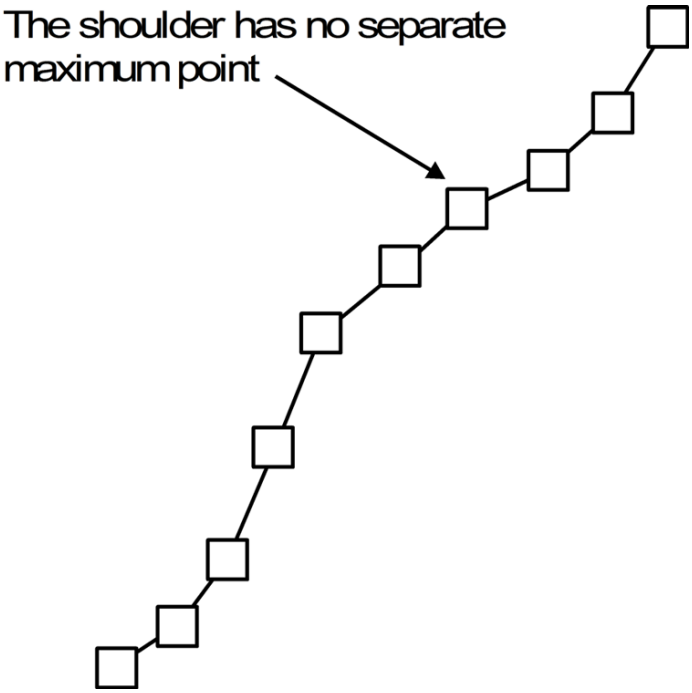


Figure 6-10 Find the Peak End

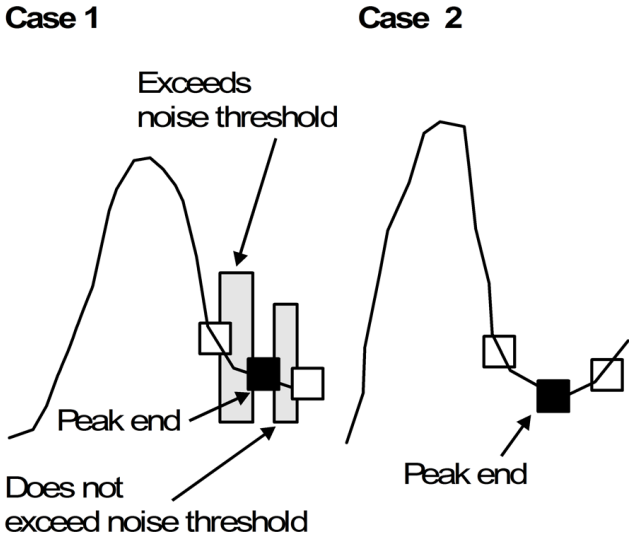


Figure 6-11 Find the Peak End: Case 2

Item	Description
1	Peak end

Separate Peaks

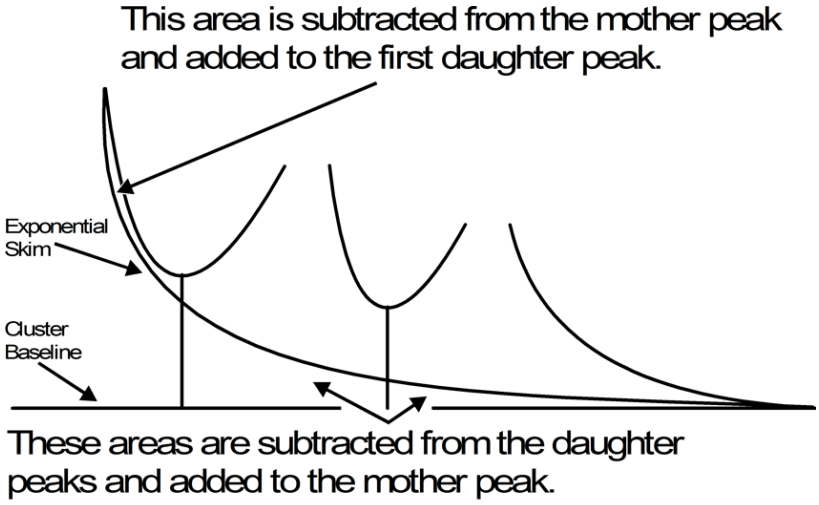
If a new peak begins before the current peak hits the baseline, the software decides, based on the following criteria, whether to resolve the baseline by using exponential skims. The skim passes under one or more peaks that follow the precursor. These peaks are called product peaks.

When the software performs an exponential skim, the software subtracts the area underneath the skim from the product peaks and gives it to the precursor peak. It then subtracts the small area above the skim from the precursor peak and gives it to the first product peak.

The software uses the following criteria to determine whether it will use exponential skimming:

- Exponential Peak Ratio
- Exponential Adjusted Ratio
- Exponential Valley Ratio

Figure 6-12 Separate Peaks: An Exponential Skim



An exponential skim

Data Analysis

Users can open files containing existing data or data that is currently being acquired. All experiment-related data can also be viewed 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.

Total Ion Chromatogram

A Total Ion Chromatogram (TIC) is created by summing the intensity contributions of all ions from a series of mass scans. Users 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, individual TICs (Total Ion Chromatograms) for each experiment and another TIC that represents the sum of all experiments can be created. 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.

Extracted Ion Chromatogram

An Extracted Ion Chromatogram (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.

Base Peak Chromatogram

A Base Peak Chromatogram (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 also helps to distinguish between co-eluting components. BPCs can only be generated from single period, single experiment data.

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

Extracted Wavelength Chromatogram

An Extracted Wavelength Chromatogram (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.

Diode Array Detector

Users can view the Diode Array Detector (DAD) spectrum for a single point in time, or for a range of time as a Total Wavelength Chromatogram.

Total Wavelength Chromatogram

A Total Wavelength Chromatogram (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, individual TWCs for each experiment and another TWC that represents the sum of all experiments can be created.

Overlay Graphs

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

If one or more panes are chosen, 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 to be overlaid.
2. Click **Explore > Overlay.**
3. Click in the second pane.

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.

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 is shown in the foreground.

Sum Overlays

If two or more graphs are overlaid, users 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, users 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 ten experiments are being viewed, the TIC will add all ten experiments together. If overlays are summed, then users have the option of adding only nine of the ten overlaid graphs. This procedure can be used if the data collected in the one experiment is just noise.

1. Overlay the graphs that are to be summed.
2. Click **Explore > Sum Overlays.**

   The overlaid graphs are added together.

Graphs Labels

Graphs can be customized using the preset style for labels on graphs and chromatograms. Users can select the fonts to use for peak and axis labels, and the colors to use for the traces. Users can also add axis labels and the type of label and precision for the peaks.

Add Captions to a Graph

Use captions to label peaks of interest or significant points on the graph. When a caption is placed beside a peak, the caption stays with the peak when the graph is zoomed in or out. Captions also stay with the original sample when users navigate between samples in a data file. A caption contains one line of text, with a maximum of 128 characters.

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

   The **Add Caption** dialog opens.

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 the position of the caption is not satisfactory, then drag the caption to a different position. The caption stays in the same place relative to the x- and y-axes when the graph is zoomed in or out. To edit or delete the caption, right-click the caption and then click the appropriate command.

Add Text to a Graph

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 the graph is zoomed, text labels remain in their original location as the graph is zoomed. They do not stay with the original sample when users navigate between samples in a data file.

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

   The **Add User Text** dialog opens.
2. In the **User Text** field, type the text.
3. To center the text, select the **Center Text** check box.
4. To change the size and style of the caption, click **Font**.
5. To insert the text, click **OK**.

Tip! If the position of the text is not satisfactory, then drag the text to a different position. To edit or delete the text, right-click the text and then choose the appropriate command.

Compound Database

The compound database stores information about compounds, including optimization specifications. Use the compound database when there is a large numbers of samples and a large number of compounds need to be optimized quickly. The Compound Database window stores optimized conditions for compounds that can be retrieved to run samples. For more information, refer to the Help.

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

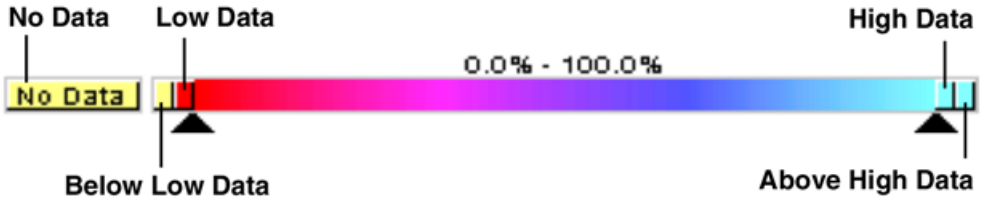
represents retention time or scan number, the y-axis represents wavelength, and the color represents absorbance. The Contour Plot is a post-acquisition tool that does not function in a real-time scan acquisition.

Note: The Contour Plot does not support DAD scans.

Color is the third axis in Contour Plot, and it represents either intensity or absorbance. Users 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 is shown in the top right corner of the Contour Plot pane.

The controls shown in *Figure 6-13* change the colors in a Contour Plot.

Figure 6-13 Buttons Controlling Contour Plot Colors



Users can define the colors on a Contour Plot graph to provide better contrast and display data specifications according to their 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 a Contour Plot.

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

Table 6-2 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

Table 6-2 Right-Click Menu for Contour Plot Panes (continued)

Menu	Function
Delete Pane	Deletes the selected pane
Show Cross-Hair	Shows the Cross-Hair (nm/min)

View a Contour Plot

A Contour Plot can be viewed only after acquisition. Users 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 to be viewed in the Contour Plot. If a selection is not made, the entire range is viewed.
3. Click **Explore > Show > Show Contour Plot.**

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

Select an Area in a Contour Plot

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.

Set the Intensity and Absorbance in a Contour Plot

Do one of the following:

- To set the low intensity/absorbance value in a 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 a Contour Plot, from the color bar above the Contour Plot, drag the right triangular slider to the required position.

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

Change Colors in a Contour Plot

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

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

   The Color dialog opens.

2. Click a color.
3. Click **OK**.

   The graph changes to reflect the color change.

Dynamic Background Subtraction Algorithm

Dynamic Background Subtraction™ algorithm improves detection of precursor ions in an IDA (Information Dependant Acquisition) experiment. The algorithm creates an XIC of the candidate ion over the last 30 data points. It takes the first derivative and determines whether the candidate ion is at the apex of the first derivative. If the candidate ion is at the apex, MS/MS will be triggered. The Dynamic Background Subtraction algorithm works best for small molecule applications as it ensures that the Product Ion scan is acquired close to the top of an LC peak and minimizes redundant MS/MS collection.

Fragment Interpretation

The Fragment Interpretation tool helps the user interpret MS/MS data. Given the chemical structure of a molecule, this tool can generate a list of theoretical fragment masses from single non-cyclic bond cleavage of that molecular structure. The tool can then match the theoretical list with peaks in the current mass spectrum.

The Fragment Interpretation Tool generates a list of theoretical fragment masses from single, non-cyclic bond cleavage of a molecular structure. The molecular structure can be created in a third-party drawing program and then saved 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

Connect the Fragment Interpretation Tool to a Spectrum

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

If multiple spectrum panes are being viewed, 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.

If a spectrum is open when the Fragment Interpretation tool is opened, 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 is to be connected 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 users scroll through the samples.

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 is shown 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 a row that has more than one matching fragment is clicked, the spectral peak that is closest to its monoisotopic mass is highlighted in the mass spectrum in the color specified in the Options tab.

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 a fragment in the list is selected and the fragment is matched to a peak, then the Fragment Interpretation window zooms in on that peak.

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. Click the **Show Isotopes** check box.
4. Click **Apply**.
5. In the fragment list, select a fragment that matches a peak.

   The isotopic distribution for matched peaks is shown in the spectrum.

Display a Formula Difference in a Spectrum

The formula and monoisotopic mass difference between two related hypothetical fragments can be displayed. The formula difference is shown when two peaks are selected. The formula and monoisotopic mass difference is shown when two fragments are selected, or two single, noncyclic bonds.

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 matching fragments of the peaks is shown in a message box.

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 is shown in a message box if the fragments are related.

Display a Formula Difference in a Molecular Structure

1. Click a single, non-cyclic bond. The preset fragment (of the two highlighted fragments) is selected. To select the other fragment of the cleaved bond, CTRL+click the bond.
2. Select a second non-cyclic bond. To select the preset 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 selected in step 1 and the fragment selected in step 2, if the fragments are related. The formula and monoisotopic mass difference is shown in a message box.

IDA Explorer

The Information Dependent Acquisition (IDA) Explorer is used to display data acquired through an IDA method.

The IDA Explorer can be turned off and on in the IDA Explorer tab in the Appearance Options dialog. Columns present in the List View can be defined in this tab as well.

The left side of the viewer shown in Figure 5-13 displays the masses on which a product ion scan was performed. In this area, users can examine the mass, intensity, time, and collision energy of ions on which product ion scans were performed in either a list view or a tree view. In list view, the list can be sorted by double-clicking on any column header. Use the Appearance Options dialog to customize the columns in the list view.

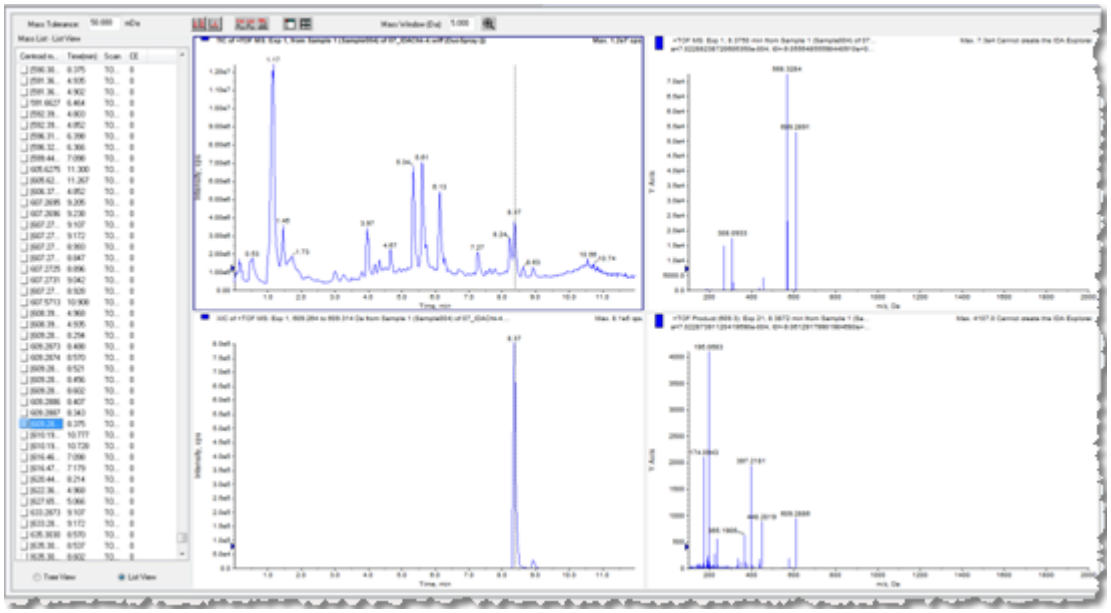
On the right side, the viewer is split into four panes. The top left pane displays the survey TIC data. The bottom left pane shows the XIC of the mass. The top and bottom right panes show the survey and product scans, respectively.

The IDA viewer lists all the masses on which Enhanced Product Ion scans or Enhanced Resolution scan types were performed. In the IDA viewer, users can do the following:

- Click a mass in the list or tree view to display plots relevant to that mass.
- View the survey spectrum from which the mass was identified and the product spectrum of that mass.
- Display the TIC of the survey scan and the XIC for each mass.

Note: Brackets around a mass indicate that the mass is merged. A merged mass is contiguous across a number of cycles. When a merged mass is displayed, it indicates an averaged spectrum, containing the average of all contiguous spectra.

Figure 6-14 IDA Viewer



Library Databases

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

With Library Search users can:

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

Library data can be stored in the following locations:

- MS Access on a local database.
- MS SQL Server.

Before using the Library Search feature, determine where the library database is stored and connect the computer to that location. Library databases can be stored locally on a computer or on a server.

Use an alias to connect to a database. In this case, the alias specifies a connection to a specific database and may include the user name and password required to access the database. For example, a user may have a small library database of identified compounds on a computer and the company may have a central database that is used occasionally by the users. Creating aliases for each database allows the user to switch between them quickly. For information about creating aliases and connecting to databases, refer to the Help.

Switch Between Existing Library Databases

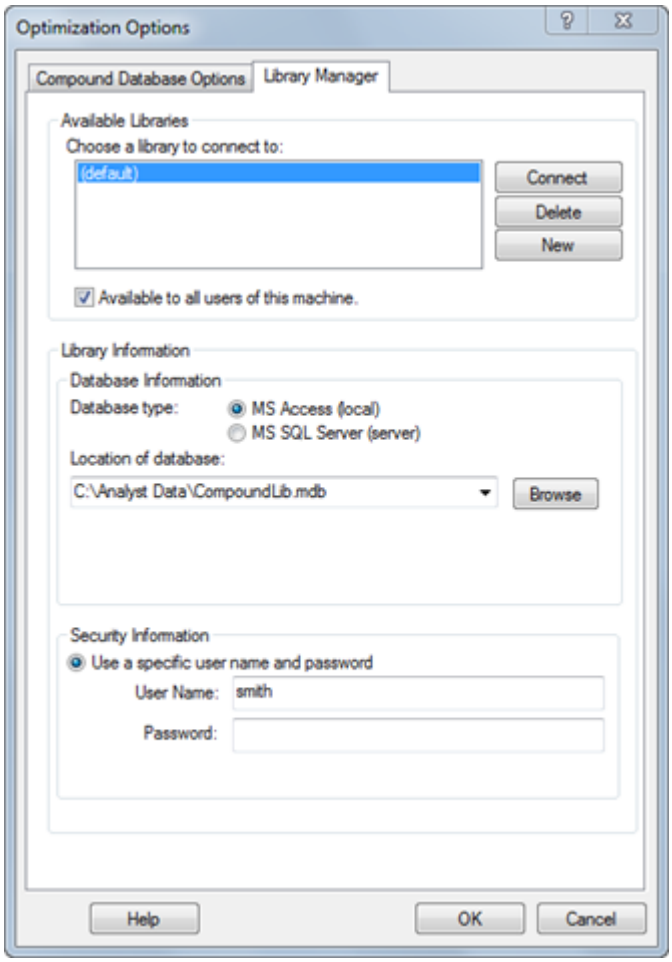
Users can connect to any databases that have aliases that are already set up.

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

   The **Optimization Options** dialog opens.

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

Figure 6-15 Library Manager tab



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

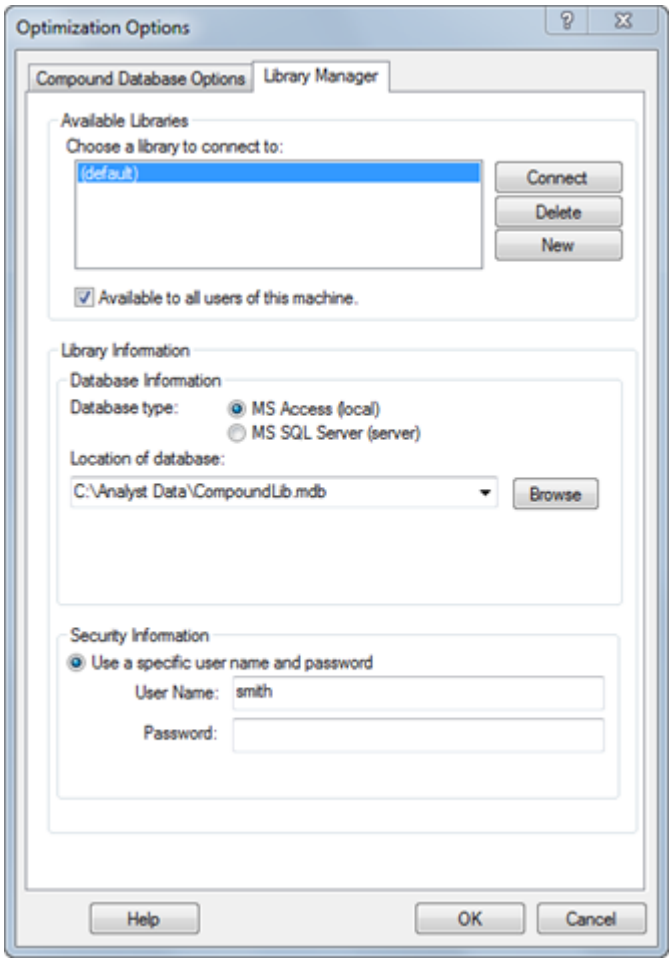
Create a Local Library Database

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

   The **Optimization Options** dialog opens.

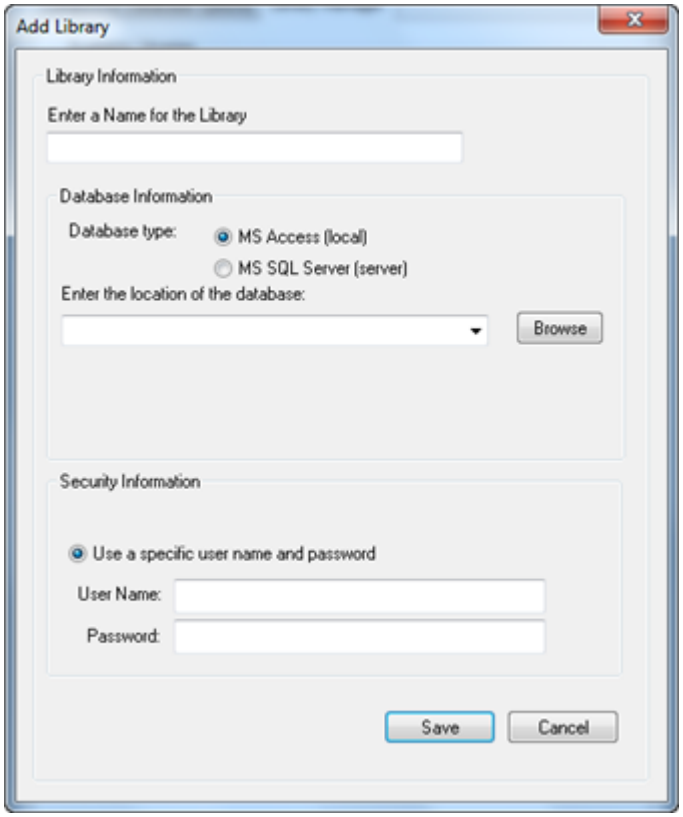
2. Click the **Library Manager** tab.

Figure 6-16 Optimization Options Dialog



3. In the **Available Libraries** section, click **New**.

Figure 6-17 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 required, type a user name and password to access the database.
8. Click **Save**.

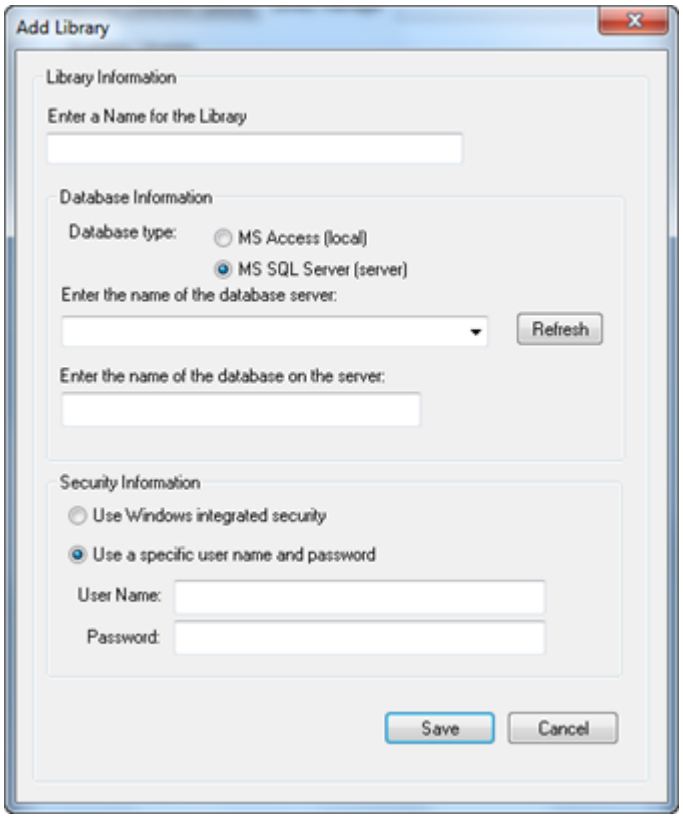
Connect to a Server Library Database

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

   The **Optimization Options** dialog opens.
2. Click the **Library Manager** tab.
3. In the **Available Libraries** section, click **New**.
4. In the **Add Library** dialog, type a name for the library.

5. In the Database Information section, select MS SQL Server (server).

Figure 6-18 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 the user name and password.
   - If Windows security is used, then in the Security Information section, select the Use Windows integrated security option.
9. Click Save.

View All Library Records

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

The **Librarian** dialog opens with all records in the database.

Add a Record to the Library

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

   The spectrum is automatically calculated as a centroid. The **Add a Record** dialog opens with data from the spectrum.

2. In 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.

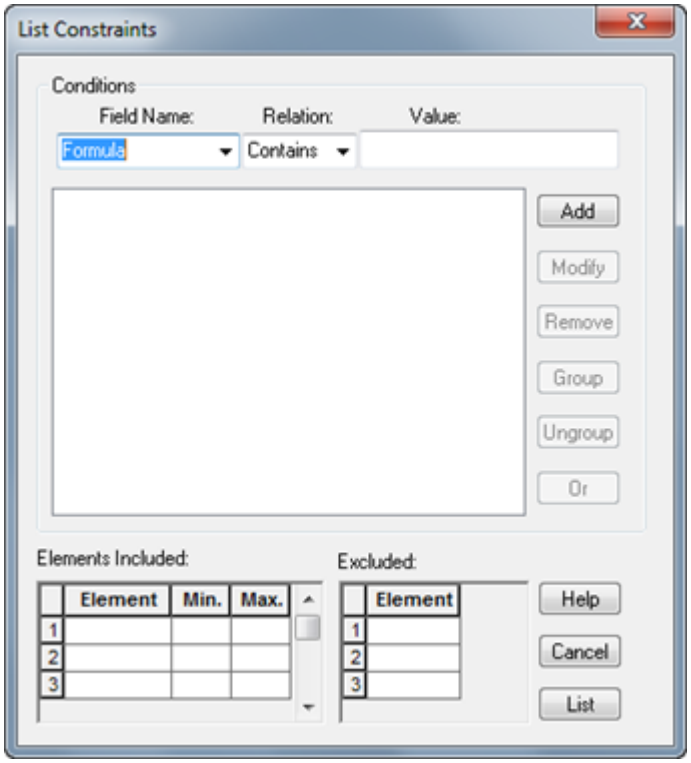
3. Edit any of the other fields. Many of the fields are filled in automatically from the data associated with the spectrum.
4. Click the **General Information** tab.
5. Edit the fields as required.
6. Click **OK**.

Search Library Records with Constraints

Use List with Constraints to narrow results. Once defined, constraints are used for all searches.

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

Figure 6-19 List Constraints Dialog



The **List Constraints** dialog opens.

2. In the **Field Name** list, select a field on which 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 to add constraints to the conditions list as required.
7. Coupling distinct constraints within the **Conditions** list creates more specific conditions that enhance the 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 include 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 the criteria, click List.

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

Library Search Tips

To do this	do this
Group conditions	Select the conditions to group and then click **Group**. This function behaves like parentheses in formulas.
Search without using constraints	Right-click an active spectrum, and then click **Search Library**. The **Search Results** dialog opens.

Search for a Similar Spectrum

The user 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 the user searches 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 that are selected, the more precise the list becomes and fewer, more relevant matches appear. After a set of constraints is defined, they will apply to all subsequent searches, unless they are edited. When a user searches without constraints, there is a much larger list of suggested spectra because the library makes fewer specific matches to the spectral data.

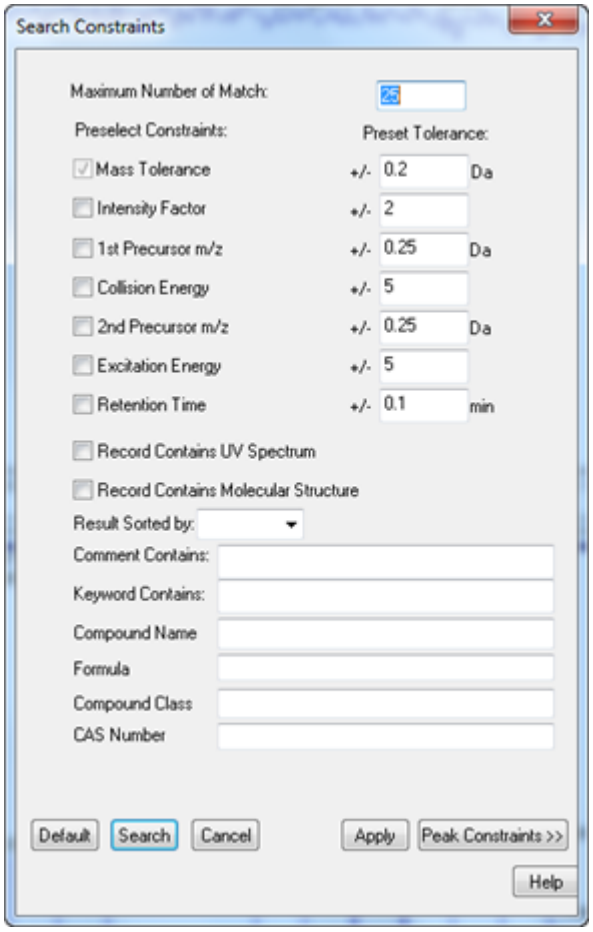
Only peaks above the threshold are used in the search. When selecting search constraints, the user can also add or subtract peaks from the active spectrum.

For example, if the user thinks a peak is actually a background or noise spike, the peak should not be used for the search because it could produce inaccurate results.

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

   The software calculates the centroid of the spectrum automatically.
2. In the **Maximum Number of Match** field, type the maximum number of compounds to be returned by the search.

Figure 6-20 Search Constraints Dialog



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

View a Compound from the Search Results

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

1. In the **Search Results** dialog, in the list of compounds, select the row number of the compound to be viewed.
2. Click the spectrum pane of one of the known compounds.

   The spectrum of the selected compound is shown.

Processed Data Files

The user can save processed data, such as specific layouts and captions, that can be opened in the Explore mode only. These files also contain history information and are similar to data files except that 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 the current project.

Save a Processed Data File

1. Select the pane of data to be saved.
2. Click **File > Save Processed Data File.**
3. In the **File name** field, type a name.
4. Click **Save**.

Open a Processed Data File

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

   The **Load Processed Data File** dialog opens.
2. Select a file.
3. Click **Open**.

Qualitative Data

The user can view the information contained in a data file in table or graph form. Graphical data is shown as a chromatogram or as a spectrum. Data in a table is shown as data points. The user can perform various sorting operations on the data.

When the user opens a data file, different panes appear depending on the type of experiment performed.

If the MCA check box is selected in the Tune Method Editor, the data file opens with the mass spectrum. If the MCA check box is not selected, the data file opens the TIC. Select a range and then double-click in the TIC pane at a particular time to show the MS for this range.

The software stores data in files with a .wiff extension. A wiff file can contain data for more than one sample. In addition to .wiff files, the software can open .txt files. A .txt file contains data for only one sample.

Report Templates

The following information can be added to report headers and footers.

Note: Make a backup of the existing report templates before editing them.

Table 6-3 Basic Design Elements

Element	Definition
Printing Date	Date the document was printed.
Printing Time	Time the document was printed.
Operator	Operator who printed the document.
Workstation	Workstation that the document was printed from.
Page n of N	Page number of total number of pages.
Custom Field	Create customized text here.
Analyst Version	Version of the Analyst software.
User Type	User type (Security).
Electronic Signature	Indicates whether the electronic signature feature (security) is enabled or disabled.

Table 6-4 Acquisition Elements

Element	Definition
Acquisition File	The name of the data file with the sample acquisition information.
Acquisition Date	Date of the sample acquisition.
Acquisition Time	Time of the sample acquisition.
Operator	Name of the operator who ran the sample batch.
Batch Name	Name of the batch.

Table 6-4 Acquisition Elements (continued)

Element	Definition
Sample Number	Number related to the sample.
Sample Name	Name of the sample.
Sample Comment	Comment about the sample entered through the Acquisition Method Editor.
Sample ID	Identification number of the sample.
Scan Mode	The method in which the system calculates the mass points for a scan for a full mass range scan.
Scan Type and Polarity	Acquisition scan type (TOF MS, Q1, MS/MS, or Precursor Ion) and acquisition method polarity (positive or negative).
Scan Mass(es)	Ions or ion fragments to be scanned.
Accumulation Time	The time the system takes to acquire a spectrum.
Pause Time	A pause between the scanning of mass ranges or between experiments.
Ion Energy	Ion energy comes from the acquisition method and is related to the IonSpray™ ion source voltage or the collision energy.
Collision Energy	Collision energy comes from the acquisition method and is related to the IonSpray ion source voltage.
Period and Experiment	A period contains a collection of experiments; an experiment contains a number of properties such as Scan Type, Scan Mode, Resolution, Ion Source Parameters, and a collection of mass ranges or masses.
State Table Parameters	The instrument parameters used in a particular experiment.
Pump	Name of the pump used for the experiment.
Autosampler	Name of the autosampler used for the experiment.
Custom Annotation	Custom text added through the Batch Editor.
Collected By	Name of the person who collected the data.

Customize Reports

The Report Template Editor provides a way to customize reports by setting up headers, footers, and page layouts. Users can use report templates with both printed output and data exported to another application.

Printed output includes several types of elements:

- **Window**: Windows in the Analyst software appear in the working area of the software window, below the toolbar, and to the right of the Navigation bar. When users print a window, they are printing everything that appears in that space.
- **Pane**: Panes are parts of a window arranged in such a way that they do not overlap and are always fully visible. For example, the Method Editor window contains two panes: the Browser pane and the Method Editor pane. Users can print information from each pane in the window.
- **Report**: Reports are structured sets of information created in the software. Some reports can be directly printed, such as calibration reports; other information must be exported, such as batches and quantitation Results Tables.
- **Workspace**: A workspace is a particular arrangement of windows and panes along with an associated file or files. When users print a workspace, they print each open window and pane in the current mode.

Preview, Print, and Export Reports

Acquisition methods and batches can be exported as reports. Other forms of information, such as calculator data, can be exported but cannot be customized with a report template.

Users can print most areas seen on the screen. Using the Print Preview feature, users can preview, scale, or copy graphs.

When a report is exported, the data is saved in a file format that is appropriate for programs such as Notepad, Word, Excel, or SCIEX SQL*LIMS (Laboratory Information Management System) software.

Depending on the information being exported, users can export reports in the following formats:

- .csv
- .doc
- .pdf
- .txt

The formats available at any one time depend on the information being exported. For example, a graph can be exported as a .pdf; a table of data can be exported as a .txt file.

To include additional information in the header and footer of the report, print the report using an appropriate report template.

Table 6-5 Preview, Print, and Export Reports

To do this	Do this
To preview a graph	Click **File > Print Preview > Pane** .
To print a report without a template	Click **File > Print** , and then click the report to print.

Table 6-5 Preview, Print, and Export Reports (continued)

To do this	Do this
To print a report with a template	1. Click **File > Print & Report Setup** . 2. In the **Report Template** section, select the template to use and then click **OK**.
To export a report	1. Click **File > Export** . 2. In the **File** field, type the name of the file. 3. In the **Save as type** list, select the file type.

Scripts

Research-grade scripts are available to extend the functionality of the software. Some scripts are installed automatically with the software. The remaining scripts can be installed individually. For more information, see the *Scripts User Guide* for the software.

Signal-to-Noise Ratio

The signal-to-noise ratio is the peak height divided by the noise.

To calculate the noise, the software uses the standard deviation (using a mean of zero) of all data points in the chromatogram from the Background Start to Background End time. These times are set when a new background range is defined.

Smoothing Algorithms

The user can select either the smoothing algorithm or the Gaussian smoothing algorithm as the smoothing method. The smoothing operation involves replacing each data point with the average of the data point before and after it. The smoothed data set replaces the old set. Smoothing a data set removes local variations that are most likely due to noise.

Data can be smoothed more than once, but the software can undo only the last smooth.

Smooth Algorithm

When smoothing data, the user sets 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.

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.

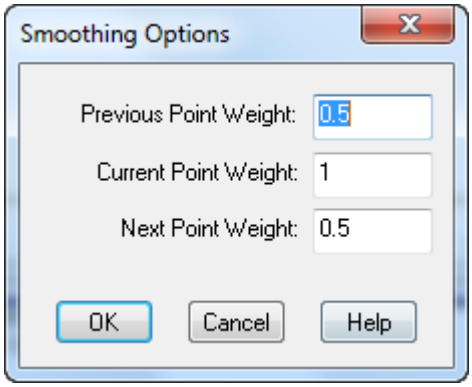
Smooth Data using the Smooth Algorithm

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

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

   The **Smoothing Options** dialog opens.

   Figure 6-21 Smoothing Options Dialog



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. Click **OK**.

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

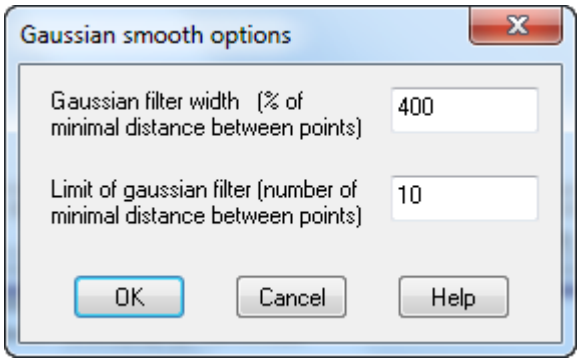
Smooth Data using Gaussian Smoothing

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

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

   The **Gaussian smooth options** dialog opens.

   Figure 6-22 Gaussian Smooth Options Dialog



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. Click **OK**.

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

System Logs

To understand the information in the system logs and troubleshoot errors, refer to the Windows Application Event Log. It contains relevant troubleshooting information.

Save the System Log and Forward to Support

1. Click **View > Event Log** .
2. Click the plus sign to the right of the **Windows Logs** folder.

3. Right-click **Application**.
4. Select the **Save All Events As** option.
5. Type a file name in the **Save As** dialog and click **Save**.
6. In the **Display Information** dialog, click the **Display information for these languages** option.
7. Make sure **English (United States)** option is selected.
8. Click **OK**.
9. Attach the file to an email and then send it to SCIEX.

Note: For additional login features for troubleshooting issues, contact Technical Support using http://sciex.com/support/request-support.

Toolbars Icon

Icon	Name	Function
	Background Subtract	Performs a background subtract after the background ranges have been selected.
	Subtract Range Locked	Locks the selected background ranges. If the background ranges are unlocked, then users can move each range independently.
	Centroid	Calculates the centroid of the data.
	Home Graph	Returns the graph to the original scale.
	Overlay	Overlays graphs.
	Cycle Overlays	Cycles between overlaid graphs.
	Sum Overlays	Adds the graphs together.
	Show Fragment Interpretation Tool	Opens Fragment Interpretation tool, which calculates the single, non-cyclic bond cleavage fragments from a .mol file.
	Smooth	Smooths data using the smooth algorithm.
	Gaussian smooth	Smooths data using Gaussian smoothing.

Exact Masses and Chemical Formulas

A

PPG

Table A-1 contains the exact monoisotopic masses and charged species (positive and negative) observed with the PPG (polypropylene glycol) calibration solutions. The masses and ions were calculated using the formula $M = H[OC_3H_6]_nOH$, while the positive ion MSMS fragments used the formula, $[OC_3H_6]_n(H^+)$. In all calculations, H = 1.007825, O = 15.99491, C = 12.00000, and N = 14.00307.

Note: When performing calibrations with the PPG solutions, use the correct isotope peak.

Table A-1 PPG Exact Masses

n	Exact Mass (M)	$(M + NH4)^+$	MSMS fragments	$(M + NH4)^{2+}$	$(M + COOH)^-$
1	76.05242	94.08624	59.04914	56.06003	121.05061
2	134.09428	152.12810	117.09100	85.08096	179.09247
3	192.13614	210.16996	175.13286	114.10189	237.13433
4	250.17800	268.21182	233.17472	143.12282	295.17619
5	308.21986	326.25368	291.21658	172.14375	353.21805
6	366.26172	384.29554	349.25844	201.16468	411.25991
7	424.30358	442.33740	407.30030	230.18561	469.30177
8	482.34544	500.37926	465.34216	259.20654	527.34363
9	540.38730	558.42112	523.38402	288.22747	585.38549
10	598.42916	616.46298	581.42588	317.24840	643.42735
11	656.47102	674.50484	639.46774	346.26933	701.46921
12	714.51288	732.54670	697.50960	375.29026	759.51107
13	772.55474	790.58856	755.55146	404.31119	817.55293
14	830.59660	848.63042	813.59332	433.33212	875.59479

Table A-1 PPG Exact Masses (continued)

n	Exact Mass (M)	$(M + NH4)^+$	MSMS fragments	$(M + NH4)^{2+}$	$(M + COOH)^-$
15	888.63846	906.67228	871.63518	462.35305	933.63665
16	946.68032	964.71414	929.67704	491.37398	991.67851
17	1004.72218	1022.75600	987.71890	520.39491	1049.72037
18	1062.76404	1080.79786	1045.76076	549.41584	1107.76223
19	1120.80590	1138.83972	1103.80262	578.43677	1165.80409
20	1178.84776	1196.88158	1161.84448	607.45770	1223.84595
21	1236.88962	1254.92344	1219.88634	636.47863	1281.88781
22	1294.93148	1312.96530	1277.92820	665.49956	1339.92967

Reserpine ($C_{33}H_{40}N_2O_9$)

Table A-2 Reserpine Exact Masses

Description	Mass
Molecular Ion $C_{33}H_{41}N_2O_9$	609.28066
Fragment $C_{23}H_{30}NO_8$	448.19659
Fragment $C_{23}H_{29}N_2O_4$	397.21218
Fragment $C_{22}H_{25}N_2O_3$	365.18597
Fragment $C_{13}H_{18}NO_3$	236.12812
Fragment $C_{10}H_{11}O_4$	195.06519
Fragment $C_{11}H_{12}NO$	174.09134

Taurocholic Acid ($C_{26}H_{45}NO_7S$)

Table A-3 Taurocholic Acid Exact Masses

Description	Mass
Molecular Ion $C_{26}H_{44}NO_7S$	514.28440
Fragment $C_2H_3O_3S$	106.98084
Fragment $C_2H_6NO_3S$	124.00739
Fragment SO_3	79.95736

TOF Calibration Solution

Table A-4 TOF Calibration Solution Exact Masses

Description	Mass
Molecular Ion Cs^+	132.90488
Molecular Ion Peptide ALILTLVS	829.5393

Revision History

Revision	Description	Date
A	First release of document.	July 2014
B	Changed Analyst® TF Software version to 1.7.1. Changed release date. Updated the copyright page. Changed AB SCIEX to SCIEX where required.	May 2015