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# SCIEX OS Software

**For X500 QTOF and ZenoTOF 7600 Systems**

Software User Guide



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## Software Overview

The SCIEX OS software contains instrument control, data acquisition, data processing, and reporting functionality, all in one package.

## Open the Software

1. Select the software from the Start menu:
  - Windows 7: **Start > All Programs > SCIEX > SCIEX OS > SCIEX OS**
  - Windows 10: **Start > SCIEX OS > SCIEX OS**

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**Note:** If the **LibraryViewServiceHost** service is not running, then the User Account Control dialog is shown. Click **Yes** to start the service.

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If the software is configured for Integrated mode, then the Home page opens.

If the software is configured for Mixed mode, then the Logon dialog opens. Continue with the following step.

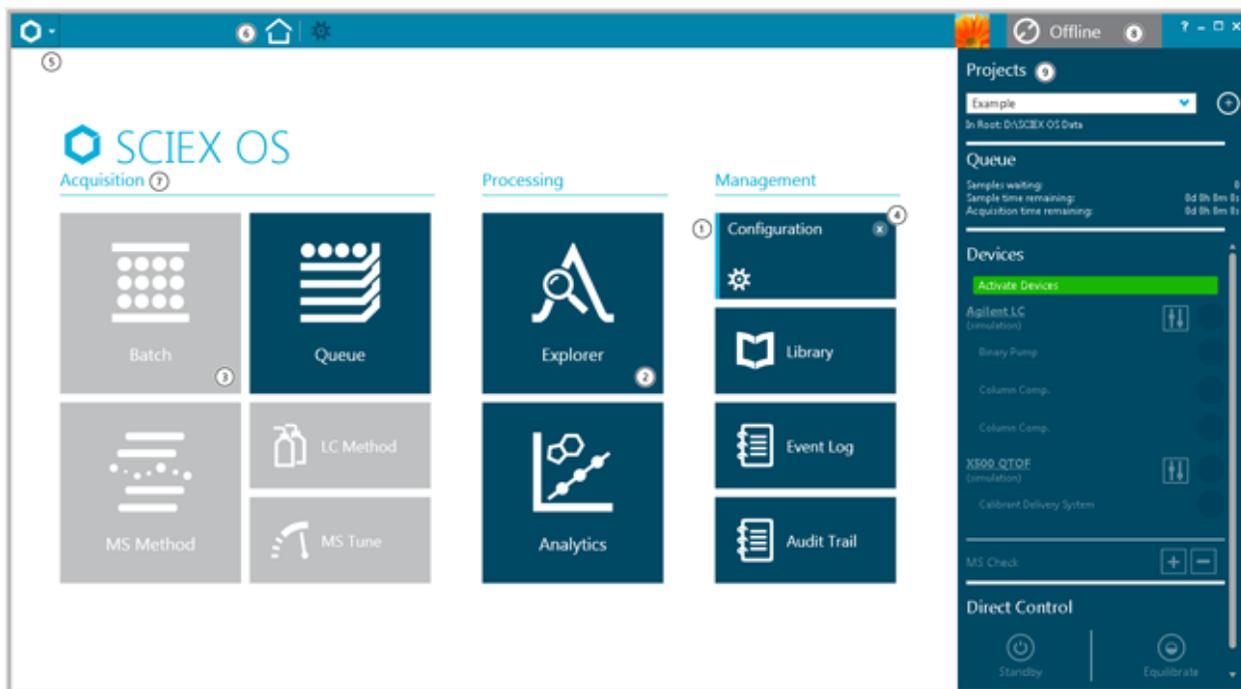
2. If the Central Administrator Console (CAC) software is being used, and SCIEX OS is configured for centralized administration, then select the workgroup to log on to.
3. If the Logon dialog opens, type the user name and password of a user who is authorized to use the software, and then click **OK**.  
The Home page opens.

## About the Home Page

The Home page consists of workspace tiles, grouped by function, the status panel, the ribbon, and the launcher. Access to workspaces is determined by the role assigned to the user, as well as the license.

## Introduction

Figure 1-1 Home Page



Item	Description
1	A light blue vertical line at the left side of a dark blue tile indicates that the workspace is open, that work is in progress, and that the user has access to the functionality. The status of the open workspace is shown on the tile.
2	A dark blue tile indicates that the workspace is closed.
3	A gray tile indicates that the workspace is not enabled.
4	The close icon (×) is shown in the top right corner of the tile when the workspace is open.
5	Access to the launcher. The launcher contains a list of all of the workspaces. Click ▾ to the right of the icon to open the launcher.
6	The ribbon. Refer to the section: <a href="#">About the Ribbon and Launcher</a> . To navigate to another workspace, click a workspace in the list. The currently open workspace remains active and the workspace icon is shown in the ribbon. To close the active workspace, click ×. To return to the Home page, click  .
7	Functions: Acquisition, Processing, and Management. Access is dependent on the role assigned to the user and the licensing.

Item	Description
8	Status of the system. Click the title bar to show or hide the status panel.
9	The status panel. Refer to the section: <a href="#">About the Status Panel</a> .

Table 1-1 Functions

Label	Description
<b>Acquisition</b>	Use the functions in the Acquisition group to create methods and batches, and to submit samples for acquisition. Users can also tune the mass spectrometer using MS Tune.
<b>Processing</b>	Use the functions in the Processing group to quantitatively or qualitatively process data.
<b>Management</b>	Use the functions in the Management group to configure devices, configure access to the software, and view the event log.

Table 1-2 Tiles

Label	Description
<b>Batch</b>	Use the Batch workspace to create batches and submit them to the queue. Refer to the section: <a href="#">Batch Workspace</a> .
<b>Queue</b>	Use the Queue workspace to monitor acquisition and processing status, and to manage samples in the queue. Refer to the section: <a href="#">Queue Workspace</a> .
<b>MS Method</b>	Use the MS Method workspace to create and edit MS methods. Refer to the section: <a href="#">MS Method Workspace</a> .
<b>LC Method</b>	Use the LC Method workspace to create and edit LC methods. Refer to the section: <a href="#">LC Method Workspace</a> .
<b>MS Tune</b>	Use the MS Tune workspace to optimize the mass spectrometer. Refer to the section: <a href="#">MS Tune Workspace</a> .
<b>Explorer</b>	Use the Explorer workspace to examine acquired data. Refer to the section: <a href="#">Explorer Workspace</a> .
<b>Analytics</b>	Use the Analytics workspace to process and review acquired data. Refer to the section: <a href="#">Analytics Workspace</a> .
<b>Configuration</b>	Use the Configuration workspace to configure the software, add and activate devices, assign user roles, and create and assign audit maps. Refer to the document: <i>Help System</i> .

## Introduction

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Table 1-2 Tiles (continued)

Label	Description
Library	Use the <b>Library</b> workspace to manage compound libraries.
Event Log	Use the Event Log workspace to view system events, including errors and warnings. Refer to the document: <i>Laboratory Director Guide</i> .
Audit Trail	Use the Audit Trail workspace to view records of software events, such as configuration changes and data processing. Refer to the document: <i>Laboratory Director Guide</i> .

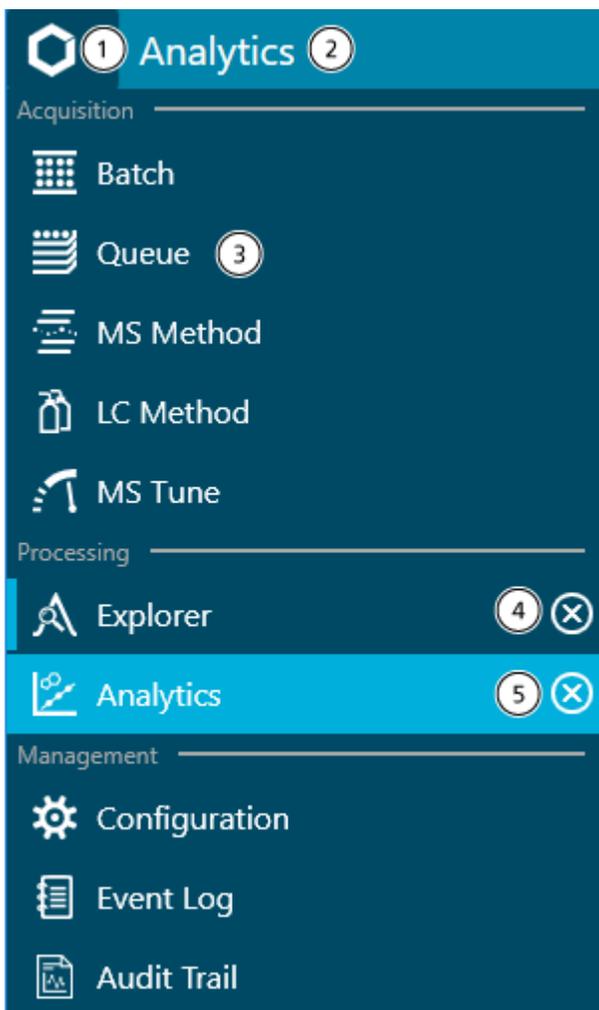
## About the Ribbon and Launcher

Figure 1-2 Ribbon



Item	Description
1	Allows the user to open another workspace by selecting it from the list. This workspace becomes the active workspace. The previously active workspace remains open. Refer to the figure: <a href="#">Figure 1-3</a> .
2	Shows the name of the active workspace.
3	Opens the Home page.
4	Shows the open workspaces. The active workspace is shown in white. To make an open workspace active, click the workspace icon.
5	Shows the currently logged in user.
6	Shows the system status. Refer to the section: <a href="#">About the Status Panel</a> .
7	Opens the Help System. Click ?.

Figure 1-3 Launcher



Item	Description
1	Shows the list of workspaces. Click  .
2	Shows the name of the active workspace.
3	Shows the status of the workspaces. A dark blue background indicates that the workspace is closed. A light blue vertical bar on the left indicates that the workspace is open. A light blue background indicates that the workspace is active.
4	Closes an open workspace. Click  .
5	Closes the active workspace. Click  .

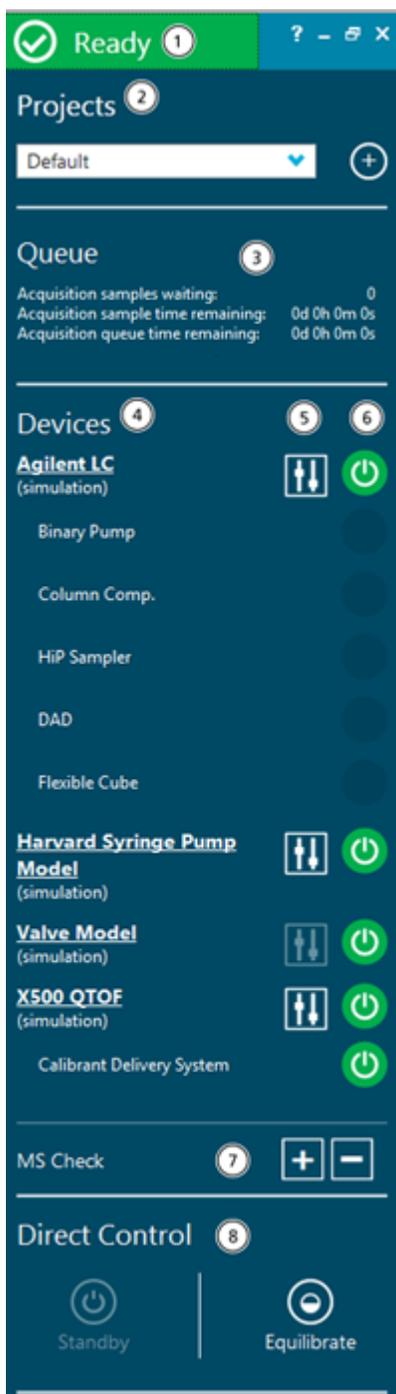
### About the Status Panel

To open this panel, click the status panel title bar. Refer to the figure: [Figure 1-2](#).

The icon, text, and color of the status title bar change to indicate the status of the system. Use the status panel to do the following:

- Add or select a project.
- View the samples remaining in the queue and the estimated time remaining for the batch to be acquired.
- View the number of samples remaining in the queue and the estimated time remaining for the queue to be completed.
- View the system status or status of the individual devices that have been activated in the Devices list in the Configuration workspace.
- Access direct device control to start or stop devices.
- View device details.
- Put the mass spectrometer or LC system in Standby state.
- Verify and calibrate the TOF MS and TOF MS/MS modes.
- Equilibrate the system.

Figure 1-4 SCIEX OS Status Panel



## Introduction

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Item	Description
1	Shows the status of the system. Click the title bar to show or hide the status panel. <ul style="list-style-type: none"> <li>• Ready is indicated by green</li> <li>• Offline is indicated by gray.</li> <li>• Equilibrating, running, and loading are indicated by blue.</li> <li>• Stopped and stopping are indicated by yellow.</li> <li>• Fault is indicated by red.</li> </ul>
2	Shows the current project. To change to an existing project, select it from the list. To add a project, click <b>Create Project</b> (  ) , type the project name, and then click <b>OK</b> .
3	Shows the status of the samples in the queue.
4	Shows the status of the devices. Click the title of the device to open the Device Details dialog and view the details. If devices are inactive, then the <b>Activate Devices</b> button is shown in this section of the Status Panel. Click this button to activate the devices.
5	Click the <b>Direct Device Control</b> icon to access controls for the device. The optional syringe can be started or stop on the Device Control dialog.
6	Shows the status of the device. The icon is a view-only indicator of the status of the device.
7	Click to access MS Tune procedures.
8	Click the appropriate button to equilibrate the system or go to Standby state. Refer to the section: <a href="#">Equilibrate the System</a> .

**Table 1-3 Status Panel Sections**

Label	Description
Projects	Shows the current project. Click <b>Create Project</b> (  ) to create a project. Refer to the section: <a href="#">Add a Project</a> .

Table 1-3 Status Panel Sections (continued)

Label	Description
<b>Queue</b>	Shows the status of the samples in the queue. Information is provided for: <ul style="list-style-type: none"> <li>• <b>Samples waiting</b></li> <li>• <b>Sample time remaining</b></li> <li>• <b>Acquisition time remaining</b></li> </ul> Refer to the section: <a href="#">Manage the Queue</a> .
<b>Devices</b>	Lists the devices in the active configuration. From this list, the devices can be managed in the following ways: <ul style="list-style-type: none"> <li>• Click the device name to open and view the Device Details dialog.</li> <li>• View the status of the icon or move the cursor over the status icon to show the status of the device.</li> <li>• Click <b>Direct device control</b> () to open the Device Control dialog.</li> </ul>
<b>MS Check</b>	Performs the MS tuning procedure in Positive (+) or Negative (-) mode.
<b>Direct Control</b>	Allows the user to control the device manually. Click <b>Standby</b> to put the system in Standby state. Click <b>Equilibrate</b> to open the Equilibrate dialog. Refer to the section: <a href="#">Equilibrate the System</a> .

Table 1-4 Status Panel Functions

To Do This	Do This
Show the status panel	Click the status panel title bar, at the top of the minimized status panel. Refer to the figure: <a href="#">Figure 1-2</a> .
Hide the status panel	Click the title bar of the status panel when it is showing.
Change the active project	Select a project from the <b>Projects</b> list on the status panel.  <b>Tip!</b> Click <b>Create Project</b> (  ) to create a project. Type the project name and then click <b>OK</b> .

## Introduction

**Table 1-4 Status Panel Functions (continued)**

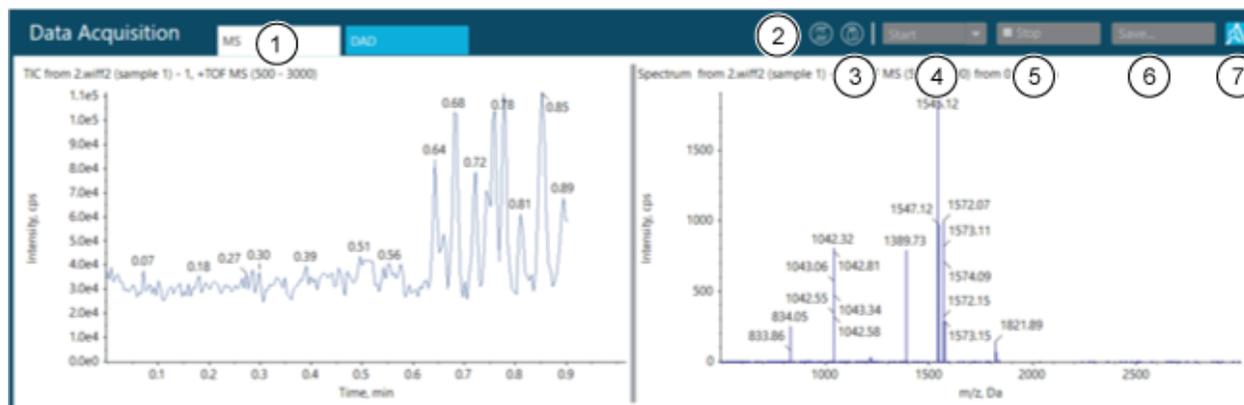
To Do This	Do This
Control the device status	<ol style="list-style-type: none"> <li>On the status panel, click <b>Direct device control</b> (  ) at the right of the device title. The Device Control dialog opens.</li> <li>Start, stop, or update the device, as required.</li> <li>Click <b>OK</b>.</li> </ol> <p>Use this procedure to obtain detailed feedback on the status of a device. For example, temperatures, pressures, and voltages. To monitor the device status, click the icon at the far right of the device title.</p>

## Data Acquisition Panel

Use the Data Acquisition panel to start and monitor real-time data acquisition. Users can also edit the acquisition method parameters during real-time data acquisition, as well as save data or open data in the Explorer workspace.

**Tip!** Click the top of the Data Acquisition panel and then drag it up or down to resize the contents.

**Figure 1-5 Data Acquisition Panel**



Item	Description
1	Shows the TIC and spectrum or XIC. If a detector is active, then DAD or UV data is also shown.

Item	Description
2	MS method. Hover to show the name of the MS method that is running.
3	LC method. Hover to show the name of the LC method that is running.
4	Click <b>Start</b> to start manual acquisition. Click <b>Start &gt; Start with LC</b> to open the Start with LC dialog.
5	Click to stop manual acquisition.
6	Click to save data.
7	Click to explore data in real time.

## Lock the Screen

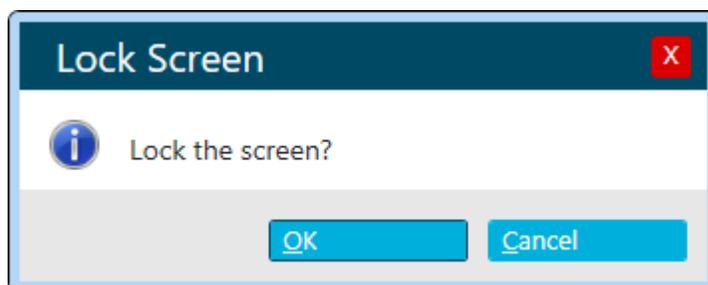
To prevent unauthorized access to the software when the workstation is unattended, lock the software. While the software is locked, any acquisition or processing that is in progress continues.

When the Auto logoff time expires, the user is logged off. Acquisition continues.

**Note:** Logoff does not occur if processing is in progress, or if the Results Table has not been saved.

1. Press **Ctrl+Q**.

**Figure 1-6 Lock Screen Dialog**



2. Click **OK**.  
The SCIEX OS is Locked dialog opens.

## Unlock the Software

If the software is locked, then the user who is currently logged on can unlock it.

**Note:** Other users cannot unlock the software, but a user with the **Force User Logoff** permission can log off the current user.

## Introduction

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In the SCIEX OS is Locked dialog, type the password for the current user, and then click **Unlock**.

## Electronic Laboratory Notebook Support

SCIEX does not support any one specific electronic laboratory notebook (ELN) solution, but SCIEX does offer products, tools and services to facilitate data import and export for integration with ELN systems:

- **Batch Creation:** SCIEX OS can import batch files in csv and txt format. Refer to [Batch Workspace](#).
- **Results Upload:** SCIEX OS can export data to a txt file for use in a LIMS system. Refer to [Analytics Workspace](#).

## Documentation Symbols and Conventions

The following symbols and conventions are used throughout the guide.



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**DANGER!** Danger signifies an action that leads to severe injury or death.

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**WARNING!** Warning signifies an action that could cause personal injury if precautions are not followed.

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**CAUTION:** Caution signifies an operation that could cause damage to the system or corruption or loss of data if precautions are not followed.

---

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**Note:** Note emphasizes significant information in a procedure or description.

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**Tip!** Tip provides useful information that helps apply the techniques and procedures in the text for a specific need and provides shortcuts, but is not essential to the completion of a procedure.

---

# Operating Instructions—Device Configuration

# 2

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Use the Configuration workspace to:

- Activate and deactivate devices
- Add and delete devices
- Edit device settings
- Test the devices

## Add Devices

---

**Note:** To avoid any activation issues, always add the mass spectrometer before adding any other devices.

---

1. Open the Configuration workspace.
2. Click **Devices**.
3. If any devices are active, then click **Deactivate**.
4. Click **Add**.  
The Device dialog opens.
5. In the **Type** list, select the required type.
6. In the **Model** list, select the required model.
7. Click **Settings** to edit settings or restore default values.
8. Click **Test Device** to verify that the device is configured correctly and available for use.
9. Click **Save**.
10. Repeat step 4 to step 9 as required.
11. Select the **Activate** check box beside each device to be activated, and then click **Activate Devices**.  
All of the selected devices are activated.
12. To edit or delete devices, refer to the Help System.

## Delete Devices

---

**Note:** If the device that is being deleted is part of an integrated system, then all of the devices in the integrated system are deleted. Users cannot delete one device in an integrated system.

---

1. Open the Configuration workspace.
2. Click **Devices**.
3. Click **Deactivate**.
4. Select a device.
5. Click **Delete**.
6. Select the **Activate** check box beside each device to be activated, and then click **Activate Devices**.  
All of the selected devices are activated.

## Edit Device Settings

1. Open the Configuration workspace.
2. Click **Devices**.
3. If the devices are active, then click **Deactivate**.
4. Select the device to be edited.
5. Click **Edit**.  
The Device dialog opens.
6. (Optional) Edit the device properties in the **Device Display Names** section. For information about the properties, refer to the document: *Help System*.
7. (Optional) Click **Settings** to view and change additional device information. Use the Settings dialog to perform these tasks:
  - Click **Restore Defaults** to restore the default settings for the device.
  - Click **Test Device** to verify that the device is configured correctly and available for use. If the test is successful, then the Settings dialog closes.
8. Click **Test Device** to verify that the device is configured correctly and available for use. If the test is successful, then a green message is shown. Otherwise, a message indicates that the configuration is not valid and requires updates.
9. Click **Save**.
10. Select the **Activate** check box beside each device to be activated, and then click **Activate Devices**.  
All of the selected devices are activated.

# Operating Instructions—Software Configuration

# 3

For information about configuring users and roles, refer to the document: *Laboratory Director Guide*.

## About Projects and Root Directories

A root directory is a folder that contains one or more projects. It is the folder in which the software looks for project data. The predefined root directory is `C:\SCIEX OS Data`.

To make sure that project information is stored safely, create projects using SCIEX OS. Refer to the section: [Add a Project](#).

Project data can be organized in subfolders. Create the subfolders with SCIEX OS. Refer to the section: [Add a Subfolder](#).

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**Note:** For workgroups administered by the Central Administrator Console (CAC) software, the configuration of the CAC software controls the ability to manage projects with SCIEX OS. If the **Use central settings for projects** option is selected in the CAC software, then the Projects page is read-only.

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## Add a Root Directory

A root directory is the folder in which one or more projects are stored.

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**Note:** The software saves up to ten root directories.

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1. Open the Configuration workspace.
2. Click **Projects**.
3. In the **Advanced** section, click **Create Root** (  ) beside the **Current root directory** field.
4. Type the full path to the root directory folder.  
The folder is created.

---

**Tip!** Instead of typing the path, click **Browse**, and then select the folder in which the root directory will be created. Type "\" and the name of the root directory folder at the end of the path.

---

## Operating Instructions—Software Configuration

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---

**Tip!** Alternatively, create a folder in File Explorer, and then browse to and select the folder.

---

**Note:** For SCIEX OS installations with a processing license, the root directory can be an Analyst software `Analyst Data\Projects` folder.

---

5. Click **OK**.  
The new root directory becomes the root directory for the current project.

## Remove a Root Directory

The software maintains a list of the last ten root directories that were used. The user can remove root directories from this list.

---

**Note:** The **Current root directory** cannot be deleted.

---

1. Open the Configuration workspace.
2. Click **Projects**.
3. In the **Advanced** section, click  beside the **Current root directory** field.  
The Clear Root Directory dialog opens.
4. Select the folders to be removed from the list of root directories, and then click **OK**.

## Specify a Secure Network Account

If projects are stored on a network resource, then an SNA can be specified, to make sure that all users of the workstation have the required access to the network resource.

1. Open the Configuration workspace.
2. Click **Projects**.
3. In the **Advanced** section, click **Credentials for Secure Network Account**.
4. Type the user name, password, and domain of the secure network account defined on the network resource.
5. Click **OK**.

## Add a Project

The project stores acquisition methods, data, batches, processing methods, processing results, and so on. We recommend the use of separate project folders for each project.

---

**Tip!** Projects can also be created by clicking **Create Project** (  ) on the Status panel.

---

Do not create projects or copy or paste files outside of SCIEX OS.

1. Open the Configuration workspace.
2. Click **Projects**.
3. Click **Create Project** (  ) beside the **Current Project** field.  
The New Project dialog opens.
4. Type the project name.
5. Click **OK**.

### Add a Subfolder

Within projects, data can be further organized in subfolders.

1. Open the Configuration workspace.
2. Click **Projects**.
3. Click **Add Data Sub-Folders to any Project**.  
The Add Data Sub-Folders dialog opens.
4. In the **SCIEX OS Project** field, select the project to which the subfolder is to be added.
5. Click **Add a new data sub-folder** (  ) above the box in the **Project Data Sub-Folders** section.  
The Data Sub-Folder Name dialog opens.
6. Type the name of the subfolder.
7. Click **Save**.
8. Close the Add Data Sub-Folders dialog.

### Select Queue Options

The software processes the submitted samples in the list sequentially, running each sample with the selected acquisition method. After all of the samples have been acquired, the queue stops and the system goes to the Ready state. After the time set in the Instrument Idle Time field has elapsed, the system goes to the Standby state. In the Standby state, the LC pumps and column oven are turned off and some mass spectrometer voltages are turned off. The autosampler temperature control stays on to prevent sample degradation.

Only a user who has been assigned permissions to manage the queue can modify the length of time the queue runs after the last acquisition has finished, before it puts the instrument in the Standby state.

1. Open the Configuration workspace.
2. Click **Queue**.
3. Select the queue options as required. For descriptions of the options, refer to the document: *Help System*.
4. Click **Save**.

## Select Laboratory Information Management System (LIMS) Settings

Use this feature to connect to a LIMS server. Users can import batch information from, as well as export results to, a LIMS.

---

**Note:** This procedure is not required for connection to a Watson LIMS.

---

1. Open the Configuration workspace.
2. Click **LIMS Communication**.
3. To communicate with a LIMS, type the URL of the LIMS server in the **LIMS Server** field and then select **Enable import from the specified LIMS server**.

---

**Note:** The customer IT department or the middleware provider is responsible for configuring the LIMS server. Contact them for the URL or location of the server.

---

4. Click **Save**.

## Enable Full Screen Mode

Select this feature to use SCIEX OS as the primary application. Users cannot close the software or access other software programs.

1. Open the Configuration workspace.
2. Click **General**.
3. Under **General**, select the **Enabled** check box to enable **Full Screen Mode**.
4. Click **Save**.

## Select Regional Settings

This feature applies the region and language settings selected in Control Panel. Only a period “.” or comma “,” can be used as a decimal separator. Digit grouping is not supported.

1. Open the Configuration workspace.
2. Click **General**.

3. Under **Regional Settings**, click **Apply**.  
The regional settings configured in the Windows operating system are applied to the software after the computer is started again.
4. Click **Save**.
5. Start the computer again.

## Manage the Compound Libraries

### Import a LibraryView Software Package

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Click the **Import** icon.
4. Click **LibraryView Package (\*.lbp)** on the Library Importer dialog.
5. Navigate to the appropriate file on the Open dialog.
6. Select the file and then click **Open**.
7. Do one of the following on the Library Importer dialog:
  - Click **All** above the **Compound** column to import all of the compounds.
  - Click inside the appropriate row to import individual compounds.

---

**Tip!** To help locate compounds, use the **Search** field. As the search criteria is typed, the visible columns are searched and refreshed to show only the information that matches the specified criteria.

---

8. Do one of the following to add the compounds to a library:
  - Select the appropriate library from the **Add to Compound Library** list.
  - Type the name of the library in the **Add to Compound Library** list field.
9. Click **Next**.

---

**Note:** If the user cancels the import before all of the compounds have been copied to the database, then any compounds that have already been imported remain in the database. The software does not revert the database to the pre-import state.

---

10. Resolve any conflicts, if required.
11. Click **Finish**.

### Import a Compound Database

1. Expand the **Compounds** list in the Manage pane.

## Operating Instructions—Software Configuration

---

2. Click **All Compounds**.
3. Click the **Import** icon.
4. Do one of the following on the Library Importer dialog:
  - Click **DiscoveryQuant Compound Database (\*.mdb)**.
  - Click **Analyst Compound Database (\*.mdb)**.
5. Navigate to the appropriate file on the Open dialog.
6. Select the file and then click **Open**.
7. Do one of the following on the Library Importer dialog:
  - Click **All** above the **Compound** column to import all of the compounds.
  - Click inside the appropriate row to import individual compounds.

---

**Tip!** To help locate compounds, use the **Search** field. As the search criteria is typed, the visible columns are searched and refreshed to show only the information that matches the specified criteria.

---

8. Do one of the following to add the compounds to a library:
  - Select the appropriate library from the **Add to Compound Library** list.
  - Type the name of the library in the **Add to Compound Library** list field.
9. Click **Next**.

---

**Note:** If the user cancels the import before all of the compounds have been copied to the database, then any compounds that have already been imported remain in the database. The software does not revert the database to the pre-import state.

---

10. Resolve any conflicts, if required.
11. Click **Finish**.

## Import a Cliquant Software Package

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Click the **Import** icon.
4. Click **Cliquant Package (\*.clq)** on the Library Importer dialog.
5. Navigate to the appropriate file on the Open dialog.
6. Select the file and then click **Open**.
7. Do one of the following on the Library Importer dialog:

- Click **All** above the **Compound** column to import all of the compounds.
- Click inside the appropriate row to import individual compounds.

---

**Tip!** To help locate compounds, use the **Search** field. As the search criteria is typed, the visible columns are searched and refreshed to show only the information that matches the specified criteria.

---

8. Do one of the following to add the compounds to a library:
  - Select the appropriate library from the **Add to Compound Library** list.
  - Type the name of the library in the **Add to Compound Library** list field.
9. Click **Next**.
10. Type the name of the mass spectrometer in the **Instrument Name** field, if required, on the Instrument Name dialog.
11. Click **OK**.

---

**Note:** If the user cancels the import before all of the compounds have been copied to the database, then any compounds that have already been imported remain in the database. The software does not revert the database to the pre-import state.

---

12. Resolve any conflicts, if required.
13. Click **Finish**.

## Import an Excel File

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Click the **Import** icon.
4. Click **Excel file (\*.xls)** on the Library Importer dialog.
5. Navigate to the appropriate file on the Open dialog.
6. Select the file and then click **Open**.
7. Select the appropriate **Excel worksheet to import** on the Library Importer dialog.
8. If the worksheet contains column headers, then select the check box beside **Selected Excel Worksheet has headers**.
9. Type the name of the mass spectrometer in the **Instrument Name** field, if required, on the Instrument Name dialog.
10. Select the appropriate heading for each column of information.

## Operating Instructions—Software Configuration

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**Tip! Compound:CompoundId** and **Compound:Name** are mandatory selections. Select **---[not used]---** for information that is not required.

---

11. Click **Next**.
12. Do one of the following on the Library Importer dialog:
  - Click **All** above the **Compound** column to import all of the compounds.
  - Click inside the appropriate row to import individual compounds.

**Tip!** To help locate compounds, use the **Search** field. As the search criteria is typed, the visible columns are searched and refreshed to show only the information that matches the specified criteria.

---

13. Do one of the following to add the compounds to a library:
  - Select the appropriate library from the **Add to Compound Library** list.
  - Type the name of the library in the **Add to Compound Library** list field.
14. Click **Next**.

**Note:** If the user cancels the import before all of the compounds have been copied to the database, then any compounds that have already been imported remain in the database. The software does not revert the database to the pre-import state.

---

15. Resolve any conflicts, if required.
16. Click **Finish**.

## Import a Library Database Snapshot

---

**CAUTION: Potential Data Loss.** Back up the current LibraryView software database before performing this procedure. The information in this package overwrites all of the existing data in the LibraryView software database. The Cancel option is not available after the import begins.

---

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Click the **Import** icon.
4. Click **Overwrite Database with Library Snapshot (\*.lbp)** on the Library Importer dialog.
5. Click **Yes** on the Warning dialog.
6. Navigate to the appropriate file on the Open dialog.
7. Select the file and then click **Open**.

8. Click **Finish**.

## Import a Library Package from a Third Party

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Click the **Import** icon.
4. Click **Third Party Library Package (\*.tplp)** on the Library Importer dialog.
5. Navigate to the appropriate file on the Open dialog.
6. Select the file and then click **Open**.
7. Do one of the following on the Library Importer dialog:
  - Click **All** above the **Compound** column to import all of the compounds.
  - Click inside the appropriate row to import individual compounds.

---

**Tip!** To help locate compounds, use the **Search** field. As the search criteria is typed, the visible columns are searched and refreshed to show only the information that matches the specified criteria.

---

8. Do one of the following to add the compounds to a library:
  - Select the appropriate library from the **Add to Compound Library** list.
  - Type the name of the library in the **Add to Compound Library** list field.
9. Click **Next**.

---

**Note:** If the user cancels the import before all of the compounds have been copied to the database, then any compounds that have already been imported remain in the database. The software does not revert the database to the pre-import state.

---

10. Resolve any conflicts, if required.
11. Click **Finish**.

## Install a Licensed LibraryView Software Package

---

**Note:** The LibraryView software must be installed.

---

**Note:** Internet access is required to obtain the LibraryView software license. If a computer does not have Internet access, then make a copy of the generated computer ID. On a computer with Internet access, go to the licensing page of the SCIEX website and then follow the instructions to obtain the license.

---

## Operating Instructions—Software Configuration

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A licensed library can be installed from a DVD or from a zip application file downloaded from the SCIEX website. The application file can include compound names, compound transition information, and compound library spectra.

1. Log on to the computer as a Windows user with administrator privileges.
2. Do one of the following:
  - If the library is being installed from a DVD, then load the DVD in the DVD drive and continue with step 5.
  - If the library is being installed from a downloaded file, then continue with step 3.
3. Download the required zip file from the SCIEX website.

---

**Tip!** To prevent potential installation issues, save the file to a location other than the computer desktop.

---

4. After the download is complete, right-click the downloaded file and then click **Extract All**.
5. Browse to the extracted files or the DVD and then double-click **Library.exe**.

---

**Tip!** If the User Account Control dialog opens, then click **Yes**.

---

**Tip!** If the LibraryView Setup (Not Responding) message dialog opens, then close the message dialog, right-click the **Library.exe** file, and select the **Run as administrator** option to start the installation again.

---

6. Click **Software Activation** on the LibraryViewPackages Feature Unavailable dialog. The LibraryViewPackages Activation dialog opens.
7. Type the license key, exactly as shown, in the appropriate field.  
If a license key is not available, then contact [sciex.com/request-support](http://sciex.com/request-support).
8. Click **Generate Computer ID**.  
A unique identifier is created for the workstation.
9. Click **Copy ID to Clipboard**.
10. Follow the instructions to obtain the license.  
  
After the required information is submitted, a license file is sent to all of the e-mail addresses provided.
11. Close the browser window.
12. When the e-mail containing the license file is received, copy the license file to the workstation desktop.
13. Click **Install License File** on the LibraryViewPackages Activation dialog.
14. Browse to and then select the license file on the Select the new license file to be installed dialog.

15. Click **Open**.

Both the Select the new license file to be installed and the LibraryViewPackage Activation dialogs close.

16. Do one of the following:

- Click **All** above the **Compound** column on the Library Importer dialog to import all of the compounds.
- Click inside the appropriate row on the Library Importer dialog to import individual compounds.

---

**Tip!** To help locate compounds, use the **Search** field. As the search criteria is typed, the visible columns are searched and refreshed to show only the information that matches the criteria specified.

---

17. Click **Next**.

---

**Note:** If the user cancels the import before all of the compounds have been copied to the database, then any compounds that have already been imported remain in the database. The software does not revert the database to the pre-import state.

---

18. Resolve any conflicts, if required.

19. Click **Finish**.

## Compound Conflicts

When installing a library containing a group of compounds or installing individual compounds, the software searches the database for compounds with the same name or formula as a compound in the package. If compounds are found, then the software flags the corresponding compounds in the package and waits for user input to continue.

Users have the option to:

- Merge the compound information. New spectra, transitions, and retention times from the compound in the package are added to the compound information stored in the database.
- Overwrite the compound information. Compound information from the package replaces the compound information stored in the database.
- Keep compound information. Compound information in the database is kept and the compound information from the package is discarded.

Conflict information is available to help the user make the correct choice.

## View Compound Conflicts

1. Click **Resolve** beside the compound on the Library Importer dialog to view the details of the conflict.

## Operating Instructions—Software Configuration

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2. Do one of the following:
  - Click **Keep Original** to keep the existing compound information and discard the new information.
  - Click **Use New** to replace the existing compound information with the new information.
3. Repeat steps 1 and 2 for each compound.
4. After all of the conflicts are resolved, click **Finish**.

### Merge Compounds

1. On the Library Importer dialog, do one of the following:
  - Click **Merge** to merge new spectra, transitions, and retention times from individual compounds in the import package with the corresponding compound information stored in the database.
  - Click **Merge All** to merge new spectra, transitions, and retention times from all of the compounds in the import package with the corresponding compound information stored in the database.
2. After all of the conflicts are resolved, click **Finish**.

### Overwrite Compounds

1. Do one of the following on the Library Importer dialog:
  - Click **Overwrite All** to overwrite all of the compound information stored in the database with the corresponding compound information from the import package.
  - Click **Resolve** beside the appropriate compound and then click **Use New** to overwrite the compound information stored in the database with the corresponding compound information from the import package.
2. Click **Finish** after all of the conflicts are resolved.

### Keep Original Compounds

1. Do one of the following on the Library Importer dialog:
  - Click **Keep All Original** to keep all of the compound information stored in the database and discard the compound information from the import package.
  - Click **Keep Original** beside the appropriate compound to keep the individual compound information stored in the database and discard the compound information from the import package.
2. Click **Finish** after all of the conflicts are resolved.

## Add a Compound

---

**Note:** Compounds can also be added to a library using the **Edit Library** option.

---

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Click the **Add** icon.

---

**Note:** The compound name is mandatory. All of the other information is optional.

---

4. Type the appropriate information in the fields on the Details tab.
5. Click **Save**.

## Add a Mass Spectrum to a Compound

1. Expand the **Compounds** list in the Manage pane.
2. Click **All Compounds**.
3. Double-click the appropriate compound.
4. Click the **MS Spectra** tab.
5. Click the **Edit Mode** icon.
6. Click the **Add Spectra** icon.
7. Click **Open \*.wiff file** on the Add Mass Spectrum from \*.wiff file to Compound dialog.
8. Browse to and then select the appropriate wiff or wiff2 file on the Open dialog.
9. Click **Open**.
10. Do one of the following to add the compounds to a library:
  - For IDA data, expand the sample and then select the appropriate compound in the navigation pane on the left.
  - For EMS, MRM, and looped data, select the appropriate sample.
11. Do one of the following to add spectrum to the compound:
  - For IDA data, click **Add Spectrum** in the Acquired Spectrum pane.
  - For EMS, MRM, and looped data, double-click the TIC and then click **Add Spectrum** in the Acquired Spectrum pane.
12. Repeat steps 7 through 11 for each spectrum to be added.
13. Click **Save**.
14. Click **Save** on the MS Spectra tab.

# Operating Instructions—User Workflows

# 4

## Analysts

Task	Refer to
View the main screen and status panel to check the system status.	<a href="#">About the Home Page</a> and <a href="#">About the Status Panel</a> .
Create and submit a batch either using a Microsoft Excel spreadsheet or LIMS, or manually. LC and MS methods must be locked by Method Developers before batches are created and submitted by Analysts.	<a href="#">Batch Workspace</a> .
View and manage samples in the queue.	<a href="#">Queue Workspace</a> .
Process and review data in Results Tables.	<a href="#">Analytics Workspace</a> .
Explore data.	<a href="#">Explorer Workspace</a> .

## Method Developers

Task	Refer to
Configure the system.	<ul style="list-style-type: none"><li>• <a href="#">Operating Instructions—Device Configuration</a>.</li><li>• <a href="#">Define the Default Processing Parameters for the Project</a>.</li><li>• <a href="#">Customize the Results Table</a>.</li></ul>
Tune the mass spectrometer.	<a href="#">MS Tune Workspace</a> .
Configure the liquid chromatography (LC) devices.	The documentation for the LC device.
Create LC methods.	<a href="#">Create an LC Method</a> .
Create mass spectrometer (MS) methods.	<a href="#">MS Method Workspace</a> .

Task	Refer to
Develop processing methods.	<a href="#">Create a Processing Method.</a>

## Administrators

Task	Refer to
Set the Windows file permissions.	<i>Laboratory Director Guide.</i>
Configure the LIMS.	<a href="#">Select Laboratory Information Management System (LIMS) Settings.</a>
Add users to the software and assign roles.	<i>Laboratory Director Guide.</i>
Archive logs.	<a href="#">Archive Logs.</a>

## Reviewers

Task	Refer to
Review processed results.	<a href="#">Analytics Workspace.</a>
Explore data.	<a href="#">Explorer Workspace.</a>
Review logs.	<a href="#">View Logs.</a>

# Operating Instructions—Acquisition 5

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Use the following workspaces to perform acquisition tasks:

- [MS Method Workspace](#): Create and manage MS methods
- [LC Method Workspace](#): Create and manage LC methods
- [Batch Workspace](#): Create batches and submit them to the queue
- [Queue Workspace](#): Manage samples in the queue

---

**Note:** To avoid performance issues or data corruption, do not do computer maintenance procedures, such as defragmentation or disk cleanup, virus scans, or Windows Updates, during sample acquisition.

---

## MS Method Workspace

Use this workspace to create and manage mass spectrometer (MS) methods.

Multiple methods can be open in the MS Method workspace. Using the **Views** menu, the user can change the arrangement of the method windows to tabbed, vertical tiled, horizontal tiled, or floating views. In floating view, windows can be resized, maximized, or minimized, moved outside of the SCIEX OS window, and moved to a different monitor.

The title bar of the method window contains the method and project names. In the tiled and floating views, the title bar of the active method is blue and the title bars of the other methods are gray. In the tabbed view, the tab for the active method is white, and the tabs for the other methods are blue.

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

## Create an MS Method

Refer to the following as required:

- [MS Method Experiments](#)
- [About MS Methods](#)
- [Calculate the Dynamic Collision Energy for MS Methods](#)

1. Open the **MS Methods** workspace.
2. Click **New** and then click a method.
3. (Optional) Click **Options** and then select the following as required:

Table 5-1 Options Menu

Parameter	Description
<b>Apply experiment scheduling</b>	Select to apply retention time window when the experiments will be executed. For the looped experiments, one of the starting run times must be 0 and one of the stopping run times must be equal to the method duration time.
<b>Apply ionization scheduling</b>	Select to show the <b>ionization start time</b> and <b>ionization stop time</b> .
<b>Show EAD parameters</b>	(ZenoTOF 7600 systems) Select to show the EAD parameters. The following fields are enabled when the EAD fragmentation mode is being used and this option is selected: <ul style="list-style-type: none"> <li>• <b>Fragmentation mode: EAD</b></li> <li>• <b>Electron KE</b></li> <li>• <b>ETC</b></li> <li>• <b>Electron beam current</b></li> <li>• <b>Load time</b></li> <li>• <b>EAD RF</b></li> <li>• <b>Reaction time</b></li> </ul>
<b>Apply intact protein mode</b>	(X500 QTOF systems) Select to show the Intact Protein mode fields.
<b>Ramp</b>	Select to ramp parameters. The Ramp Compound Parameters dialog opens.  Ramping can be used to optimize parameter for ions.  Ramping a parameter consists of automatically running an experiment while increasing or decreasing the value of a parameter. Only one parameter can be ramped at a time, and the steps must be in the same direction, either increasing or decreasing within the start and stop values. Users can set the start and stop voltages, and the size of the steps in between.  For TOF MS methods, users can ramp the DP parameter. For TOF MSMS methods, users can ramp either the DP or CE parameter. Ramping can be enabled by selecting Apply ramping to the compound parameter.

**Table 5-1 Options Menu (continued)**

Parameter	Description
<b>Calibrate</b>	Select to calibrate the spectrum and the mass spectrometer while acquiring. The Calibrate dialog opens. This dialog enables the user to select the appropriate ion reference table for calibration.  The calibrate feature is usually used with the calibrant delivery system (CDS). To view calibration results, users can go to the Queue workspace and then double-click the acquisition status icon of the calibration run. Calibration takes 1.25 mins.
<b>Dynamic collision energy</b>	Click to open the Dynamic Collision Energy dialog.
<b>Dynamic ETC</b>	(ZenoTOF 7600 systems) Click to open the Dynamic ETC dialog.

4. Type values in the fields, as required. For descriptions of the parameters, refer to the document: *Help System*.
5. (Optional) Click **Add Experiment**.

---

**Tip!** Use the list next to the **Experiment** field to change or delete the experiment.

---

6. Do one of the following:
  - Click **Save > Lock Method** to save and lock the MS Method.
  - Click **Save > Save**.
  - Click **Save > Save as**.

## Create an MRM HR Method Using Guided MRM HR

Use the **Guided** option if greater control over the start and stop voltages is required.

1. Open the MS Method workspace.
2. Click **New > Guided MRM HR**.  
The Preparation page opens.
3. Select the mode:
  - **Guided**: For greater control over the start and stop voltages.
  - **Automatic**: To allow the software to automatically select the voltage start and stop values.
4. Select a **Polarity**.
5. To use known transitions, do this:

- a. Click **Use known transitions**.
  - b. Type the **Compound ID**, **Precursor Ion (Da)**, and **Fragment to Use (Da)**.
6. To use unknown transitions, do this:
- a. Click **Find transitions automatically**.
  - b. Specify the **Compound Name**, **Charge**, **Precursor Ion**, and **Number of Fragments to Use** in the table for each compound.
7. Click **Continue**.  
The Initial Conditions page opens.
8. If required, adjust the **Source and Gas Parameters**.
9. If processing does not occur automatically, then click **Start**.
10. On the Optimize DP page, click **Ramp**.  
The software automatically ramps the DP parameter and finds the most intense DP value for each transition.
11. (Automatic mode) Wait until the optimal DP and the optimal CE are identified for each of the product ions, and the Review Report page is shown. Then go to step 13.
12. On the Optimize DP page, click **Ramp**.  
The software automatically ramps the DP parameter and finds the most intense DP value for each transition.
13. (Optional) Save the report by following these steps:
- a. On the Report page, click **Save report as**.
  - b. Navigate to the folder where the report will to be saved, type a **File name**, and then click **Save**.
14. Click **Continue** to open the optimized method in the MS Method workspace.
15. Type the required method duration time in the **Method Duration** field.
16. Do one of the following to save the MS method:
- Click **Save > Save** to save the method in the same project with the same name.
  - Click **Save > Save As** to save the method with a new name or in a different project.
  - Click **Save > Lock Method** to lock the method if it is ready for routine analysis.

---

**Note:** Lock the method to prevent unauthorized users from editing it. Only users with the **Lock/Unlock methods** permission can edit locked methods. Other users can only submit them.

---

The Save As MS Method dialog opens.

17. Type a name in the **File Name** field.

18. Click **Save**.

### MS Method Experiments

Use the MS Method workspace to create or edit MS methods. An MS method can contain one or more experiments. By default, a new TOF MS method contains one experiment.

The types of MS experiments available are as follows:

- Three basic method experiments: TOF MS, TOF MSMS, and Q1
- Three combined method experiments: IDA, SWATH, and MRM<sup>HR</sup>

In addition, a step-by-step procedure is available to guide the creation of an MRM<sup>HR</sup> experiment. After the procedure is completed, the parameters are used to populate the MRM<sup>HR</sup> method.

**Table 5-2 Basic Method Experiment**

Type	Definition
TOF MS	Mass analysis using the TOF region. The <i>m/z</i> values of the ions are retained based on their flight time in the TOF region.
TOF MSMS	The precursor ion is selected using the quadrupole mass filter. Then <i>m/z</i> value of the fragment ions are returned based on their flight time in the TOF MS regions. This experiment is used to determine the structure of the compounds.
Q1	A data acquisition using the quadrupole mass filter. The ion intensity is returned for masses in the scan range.

**Table 5-3 Combined Method Experiments**

Type	Definition
IDA	An IDA (Information Dependent Acquisition) experiment analyzes data as it is being acquired and changes experiment conditions according to the results of the analysis. Analysis of the results determine the masses on which to perform dependent scans. The user has total control over the criteria that activates an IDA experiment and the parameters of the IDA experiment that are activated.

**Table 5-3 Combined Method Experiments (continued)**

Type	Definition
SWATH	SWATH acquisition enables the MS/MS analysis of all precursor ions across a wide mass range on an LC timescale. The Q1 quadrupole is set to a wider selection window width (typically 10 Da 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.
MRM HR	The MRM <sup>HR</sup> experiment helps acquire high quality MS/MS data from compounds with known masses and retention times. This acquisition can also be used to extract fragment masses with narrow widths (0.02 Da) from TOF MSMS spectra. The narrow extraction gives much better selectivity.
Guided MRM HR	A step by step procedure to guide the creation of an MRM <sup>HR</sup> method. After the procedure steps are completed, the parameters are used to populate the MRM <sup>HR</sup> method type.

## About MS Methods

An MS method is comprised of the following elements:

- Parameters that pertain to the entire method, including **Source and Gas** parameters.
- One or more experiments.
  - Each method must contain at least one experiment
  - Any method can contain more than one experiment. This is referred to as looped experiments.
  - The TOF MS and TOF MSMS experiments can be looped within a method, up to a maximum of 10 experiments. Q1 experiments cannot be looped.
  - The IDA, SWATH, MRM<sup>HR</sup> experiments can be looped within a method, up to a maximum of 2 experiments.

---

**Note:** Only specific combinations of experiments can be used, for example, IDA + IDA, IDA + MRM<sup>HR</sup>, IDA + SWATH, and SWATH + MRM<sup>HR</sup>.

---

- Each experiment has specific advanced settings.
- Individual scans within each experiment

**Table 5-4 MS Methods Workspace Features**

To Do This	Do This
Create a method with more than one experiment, that is, a looped experiments.	Click <b>Add Experiment</b> and then click an experiment type.
Switch the experiment within an existing MS method.	Click the list next to <b>Experiment</b> and then click an experiment types.
Convert a TOF MSMS experiment to an IDA experiment.	Click the list next to <b>Experiment</b> and then click <b>Add IDA criteria</b> .
In an MRM <sup>HR</sup> experiment, remove the TOF MS from the method.	Click the list next to <b>Experiment</b> and then click <b>Delete TOF MS (of MRM HR)</b> . <hr/> <b>Note:</b> Only applied to the looped experiments.
Delete an experiment when there are multiple experiments within a method.	Click the list next to <b>Experiment</b> and then click <b>Delete experiment</b> .
To view the following method structures: <ul style="list-style-type: none"> <li>• The number of experiments within a method.</li> <li>• The scheduling duration of each experiment within the method.</li> <li>• The number of TOF MSMS scans for multiple experiments.</li> </ul>	Expand or collapse the <b>Method Overview</b> panel on the left side of the workspace.

## Calculate the Dynamic Collision Energy for MS Methods

1. Open the MS Method workspace.
2. Create or open an MS method that contains IDA criteria or SWATH acquisition criteria.
3. Click **Options > Dynamic collision energy**.
4. Modify the information in the fields, as required.
5. Do one of the following:

- To use previously saved default values to calculate the dynamic CE, click **Load Default Settings**.
- To save the current values as the default values to be used to calculate the dynamic CE in new methods, click **Save as Default Settings**.
- To apply the current values to the current method to calculate the dynamic CE, click **Apply**.
- To close the dialog and abandon any changes, click **Cancel**.

## Open an MS Method

Use this procedure to open an MS method created with SCIEX OS.

1. Open the MS Method workspace.
2. Click **Open**.  
The Open MS Method dialog opens. It contains the list of MS methods in the current project.
3. (Optional) If the method to be opened is not in the current project, then select the project that contains the method to be opened.
4. Select the MS method to open, and then click **Open**.

---

**Tip!** To select multiple methods, use the **Shift** or **Ctrl** key.

---

## Run an MS Method Manually

### Prerequisite Procedures

- In the MS Method workspace, create an MS method or open an existing method. Refer to the section: [MS Method Workspace](#) or [Open an MS Method](#).

Use this procedure to run the active method in the MS Method workspace.

1. Click the down arrow on the **Start** button in the Data Acquisition panel and then click one of the following:
  - **Start**: This option runs the MS method without an LC.
  - **Start with LC**

Refer to the section: [Data Acquisition Panel](#).



**WARNING! Fire Hazard.** Do not direct more than 3 mL/min of solvent in the ion source. Although the LC components can provide a flow rate up to 5 mL/min, directing more than 3 mL/min of solvent could result in solvent accumulating in the ion source. Flow can be split with a tee to make sure that the maximum flow rate provided to the ion source does not exceed 3 mL/min.

---

If the user clicks **Start with LC**, then the Start with LC dialog opens. For information about the fields on this dialog, refer to the document: *Help System*.

---

**Note:** The LC system must be activated and an LC method must have been created and saved.

---

**Figure 5-1 Start with LC Dialog**

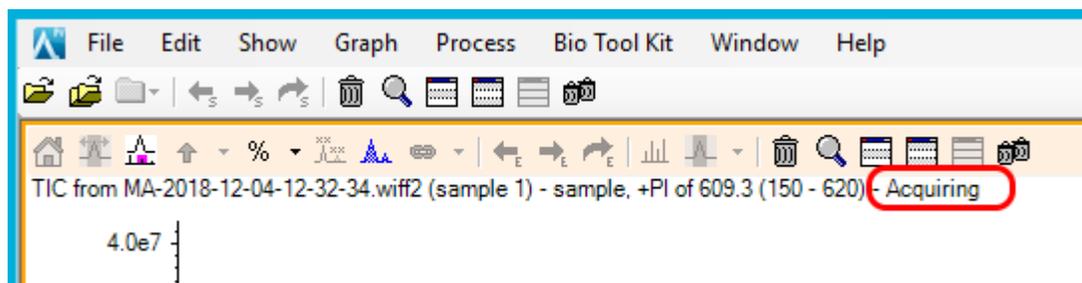
The screenshot shows a dialog box titled "Start with LC". The fields and their values are as follows:

Field	Value
Project Name:	Default
MS Method:	Untitled 1
LC Method:	new-method
Rack Type:	
Rack Position:	
Plate Type:	
Plate Position:	
Vial Position:	

Buttons: OK, Cancel

- (Optional) To view the data in the Explorer workspace, click **Open data exploration to view real-time data** () in the Data Acquisition panel. Real-time acquisition is indicated in the Explore pane by the words **Acquiring**, **Finished**, or **Aborted** in the sample title.

Figure 5-2 Real-time Acquisition—Acquiring



3. (Optional) Optimize the MS parameters, as required. For a description of the parameters, refer to the document: *Help System*.
4. Click **Stop** in the Data Acquisition panel.
5. (Optional) To save the data, follow these steps:
  - a. Click **Save** to save the data.  
The Save Data dialog opens.
  - b. (Optional) Select the project and subfolder, if applicable, in which to save the data.
  - c. Type a name in the **File Name** field.
  - d. Click **Save**.
6. Do one of the following to save the MS method:
  - Click **Save > Save** to save the method in the same project with the same name.
  - Click **Save > Save As** to save the method with a new name or in a different project.
  - Click **Save > Lock Method** to lock the method if it is ready for routine analysis.

---

**Note:** Lock the method to prevent unauthorized users from editing it. Only users with the **Lock/Unlock methods** permission can edit locked methods. Other users can only submit them.

---

The Save As MS Method dialog opens.

7. Type a name in the **File Name** field.
8. Click **Save**.

## LC Method Workspace

Use this workspace to create and manage LC methods.

Multiple methods can be open in the LC Method workspace. Using the **Views** menu, the user can change the arrangement of the method windows to tabbed, vertical tiled, horizontal tiled,

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or floating views. In floating view, windows can be resized, maximized, or minimized, moved outside of the SCIEX OS window, and moved to a different monitor.

The title bar of the method window contains the method and project names. In the tiled and floating views, the title bar of the active method is blue and the title bars of the other methods are gray. In the tabbed view, the tab for the active method is white, and the tabs for the other methods are blue.

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

## Create an LC Method

Refer to the documentation that comes with the LC device.

1. Open the LC Method workspace.
2. Click **New**.
3. Click a device in the left panel and then edit the fields, as required.
4. Save and optionally lock the LC method by clicking one of the following commands:
  - **Save**: To save the LC method.
  - **Save > Lock Method**: To save and lock the LC method.

The Save As LC Method dialog opens.

5. Type a name for the LC method in the **File Name** field, and then click **Save**.

## Batch Workspace

The Batch workspace contains information about a set of samples to be acquired and, optionally, processed. Batches tell the software the order in which to acquire and process the samples.

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

---

**Note:** For the selected autosampler, the rack type, rack position, plate type, plate position, and vial position are all dependent on each other and only certain values are valid.

---

**Table 5-5 Batch Workspace Columns**

Column Name	Definition	Field Value Requirements
Sample and method information (  )		
Sample Name	Name of the sample.	Less than 252 characters.

Table 5-5 Batch Workspace Columns (continued)

Column Name	Definition	Field Value Requirements
<b>Sample ID</b>	A custom number or other identifier for the sample.	Less than 252 characters. The <b>Sample ID</b> field cannot contain any of these invalid characters: \ / : ; * ? " < >   =
<b>Barcode ID</b>	Unique ID from a sample.	Less than 250 characters.
<b>MS Method</b>	Name of the method.	The MS method must exist in the current project. The field is not case-sensitive.
<b>LC Method</b>	Name of the liquid chromatography method.	The LC method must exist in the current project. The field is not case-sensitive.
<b>Rack Type</b>	The rack type for the autosampler.	Must be one of the valid choices for the autosampler specified in the LC method.
<b>Rack Position</b>	The position of the rack on the tray.	Numerical value.
<b>Plate Type</b>	The type of well-plate in the autosampler. <hr/> <b>Note:</b> This column is unavailable if the <b>Rack Type</b> describes vials. <hr/>	Must be one of the valid choices for the autosampler specified in the LC method.
<b>Plate Position</b>	The position of the plate on the rack.	Must match one of the predefined autosampler plate positions.
<b>Vial Position</b>	(LC methods) The position of the vial in a rack or on a plate.	Numerical value. The largest value must not be larger than the number of vials in the rack.

Table 5-5 Batch Workspace Columns (continued)

Column Name	Definition	Field Value Requirements
<p><b>Injection Volume (µL)</b></p>	<p>The amount of sample to be injected.</p> <hr/> <p><b>Note:</b></p> <p>For LC methods only, the injection volume is taken from the LC method. The user can override this injection volume in the Batch workspace or in the imported batch file. When the batch is submitted, the injection volume is validated against the range supported by the LC device.</p> <p>To revert to the injection volume specified in the LC method, delete the contents of this field, and then select the LC method again in the <b>LC Method</b> field.</p> <hr/>	<p>Numerical value.</p>
<p><b>Sample Type</b></p>	<p>The type of sample.</p>	<p>Make sure that the sample type matches one of the predefined sample types. Any type that does not match is automatically replaced with Unknown.</p>
<p><b>Dilution Factor</b></p>	<p>The dilution factor for individual samples.</p>	<p>For SCIEX-developed methods, the value must be 1.000000.</p> <p>Must be a value greater than zero and with six decimal places. The default value is 1.000000. Do not leave the field blank.</p>

Table 5-5 Batch Workspace Columns (continued)

Column Name	Definition	Field Value Requirements
<b>Data File</b>	<p>The name of the file to which the acquired data is saved. Include the full path to the subfolder in which the file will be stored.</p>	<p>Must be less than 252 characters. The total number of characters includes the number of characters in the data subfolder path. The data file cannot contain any of these invalid characters: \ / : ; * ? " &lt; &gt;   =</p> <hr/> <p><b>Tip!</b> Click the arrow to select a subfolder from the list or type the name of a new subfolder. Make sure to include a backslash (\) between the subfolder and file name. If the subfolder does not exist, then it will be created when the batch runs.</p>
<b>Processing Method</b>	<p>Name of the method. If an existing <b>Results File</b> will be used, then leave this field blank. When an existing <b>Results File</b> is selected, the value <i>*Embedded Method*</i> is automatically shown in this field.</p> <hr/> <p><b>Note:</b> The processing method must be compatible with the MS method specified for the sample.</p>	<p>Select a processing method from the list of processing methods in the project.</p>

Table 5-5 Batch Workspace Columns (continued)

Column Name	Definition	Field Value Requirements
<b>Results File</b>	<p>The name of the file to which the processed results are saved. If a valid <b>Results File</b> is specified, then the sample data will be processed automatically after acquisition is complete. If the file name is invalid, then the batch submission process cannot be completed.</p> <hr/> <p><b>Note:</b> If an existing <b>Results File</b> is selected, then the embedded method for the selected Results file is used for processing, and the text in the <b>Processing Method</b> cell is replaced with <i>*Embedded Method*</i>.</p>	<p>The file name cannot contain any of these invalid characters: \ / ; : * ? " &lt; &gt;   =</p> <p>The file path, including the file name and subfolders, must be less than 252 characters.</p> <hr/> <p><b>Tip!</b> Click the arrow to select an existing Results File from the list. To create a file, type the file name. The file will be created when the first sample in the submitted batch is processed.</p>
<b>Comment</b>	Text	<p>Must be less than 50 characters. The <b>Comment</b> field cannot contain any of these invalid characters: \ / ; : * ? " &lt; &gt;   =</p>
<b>Custom columns</b>	(Optional) User-defined columns, in text, integer, or real number format.	Requirements depend on the format.
<b>Component Concentrations</b> (  )		

**Table 5-5 Batch Workspace Columns (continued)**

Column Name	Definition	Field Value Requirements
<b>Component</b>	<p>The name of a component defined in the MS method, processing method or Results Table.</p> <p>The batch can contain up to 4,000 component rows.</p>	<p>Component names are taken from the MS method, for MRM scans, the processing method, or the Results Table. The name is validated during method creation.</p> <p>Components can also be added to the table manually. Refer to the section: <a href="#">Add a Component Concentration</a>.</p> <hr/> <p><b>Note:</b> If the import file contains a data column that does not match any of the columns in the batch grid, then the column is treated as a Compound or Component Name column. A concentration column is added and it is populated with the values from this data column.</p>
<b>Component concentration</b>	Analyte or internal standard concentration for standard and QC sample types. The table contains a column for each sample. The sample name is used as the column name.	Numerical value greater than or equal to zero.

## Manage the Batch

**Note:** Make sure that the correct project name is selected in the status panel.

In the Batch workspace, use the following features to manage the batch.

**Table 5-6 Batch Workspace Features**

To Do This	Do This
Show or hide columns	Click <b>View</b> . Refer to the section: <a href="#">Show or Hide Columns</a> .
Cut rows	Click <b>Manage Samples &gt; Cut</b> .
Copy rows	Click <b>Manage Samples &gt; Copy</b> .
Paste rows	Click <b>Manage Samples &gt; Paste</b> .

**Table 5-6 Batch Workspace Features (continued)**

To Do This	Do This
Insert a row	Click <b>Manage Samples &gt; Insert sample.</b>
Delete a row	Click <b>Manage Samples &gt; Delete sample.</b>
Select columns	Click <b>View</b> . Refer to the section: <a href="#">Show or Hide Columns</a> .
Add a subfolders to a project	Click <b>Manage Samples &gt; Add data sub-folders</b> . Refer to the document: <i>Help System</i> .
Print the batch	Click <b>Print</b> .
Save the batch to the current project	Click <b>Save &gt; Save</b> or <b>Save &gt; Save As</b> .
Export the batch as a txt or csv file	Click <b>Save &gt; Export</b> .

### Show or Hide Columns

1. Open the Batch workspace.
2. Click **View**.
3. Select or clear the column check boxes, as required, in the View dialog. For descriptions of the columns, refer to the table: [Table 5-5](#).

Figure 5-3 View Dialog

**View**

Configure the samples for display and printing

Maximum number of sample rows to use

Select the columns to use

**Predefined Columns**

<input checked="" type="checkbox"/> Sample Name	<input type="checkbox"/> Sample ID	<input type="checkbox"/> Barcode ID
<input checked="" type="checkbox"/> MS Method	<input checked="" type="checkbox"/> LC Method	<input checked="" type="checkbox"/> Rack Type
<input checked="" type="checkbox"/> Rack Position	<input checked="" type="checkbox"/> Plate Type	<input checked="" type="checkbox"/> Plate Position
<input checked="" type="checkbox"/> Vial Position	<input checked="" type="checkbox"/> Injection Volume (ul)	<input checked="" type="checkbox"/> Sample Type
<input type="checkbox"/> Dilution Factor	<input checked="" type="checkbox"/> Data File	<input checked="" type="checkbox"/> Processing Method
<input checked="" type="checkbox"/> Results File	<input type="checkbox"/> Comment	

**Custom Columns**

Configure the component concentrations for printing

Include the concentrations in the printed version

4. Click **OK**.

## Add a Custom Column

Use this procedure to add columns to the batch to store extra information about the sample, such as dry weight, so that it can be used in processing, for example, in formulas and calculated columns.

1. Open the Batch workspace.
2. Right-click in the batch grid, and then click **Add Custom Column**. The Add Custom Column dialog opens.
3. In the **Column name** field, type a name for the column. The name must be unique. It cannot be the same as the name of any predefined column.

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4. In the **Column type** field, select one of these types:
  - **Integer**: The column contains whole numbers. Decimal values will be rounded to the nearest whole number.
  - **Real**: The column contains real numbers, up to six decimal places.
  - **Text**: The column contains text, up to 128 characters.
5. Click **Add**.

The new column is added at the right of the Batch workspace.

## Change the Name of a Custom Column

---

**Note:** The **Column type** cannot be changed.

---

1. Open the Batch workspace.
2. Right-click in the column to be changed, and then click **Edit Custom Column**.

The Edit Custom Column dialog opens.
3. In the **Name** field, type the new name for the column.

The name must be unique. It cannot be the same as the name of any predefined column.
4. Click **Apply**.

## Remove Custom Columns

1. Open the Batch workspace.
2. Right-click in the batch grid, and then click **Delete Custom Column**.

The Delete Custom Column dialog opens.
3. Select the check box beside the names of the columns to be deleted.
4. Click **Delete**.

---

## Import a Batch from a File

### Prerequisite Procedures

- Create a batch file. For a description of the fields to be included in the file, refer to the table: [Table 5-5](#).

---

**Note:** In the Microsoft Excel file being imported, the predefined columns must be first, followed by the custom columns. The column headers for the predefined columns must match the column names in SCIEX OS. If the column headers for the predefined columns are not correct, then the information will not be imported. Only a period is supported as a decimal separator in csv or xsl files.

---

**Note:** Close the batch file before importing it. The batch file cannot be imported if it is open in Microsoft Excel.

---

- (Optional for import from a Watson LIMS) To automatically populate the **LC Method** field, make sure that the name of the LC method is the same as the name of the MS method.

---

**Note:** The Watson LIMS does not have an LC method field. If the name of the LC method is not the same as the name of the MS method, then the LC method column must be populated manually.

---

Review the batch contents before submitting the samples.

---

**Tip!** To access the cut, copy, paste, add rows, and remove rows features, click **Manage Samples**.

---

1. Open the Batch workspace.
2. (Optional) Click **View** to select the columns that will be shown in the Batch workspace.
3. Click **Open > Import from file**.  
The Batch Import dialog opens.
4. Click **Browse**.
5. Navigate to the required file.
6. Click **Open**.
7. (Optional) Select or clear the **Append to current batch** check box, as required.

---

**Note:** Any existing data in the grid is overwritten if the user does not select the **Append to current batch** option.

---

8. Click **Import**.

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---

9. (Optional) To use the plate layout as a reference for selecting or confirming a sample location, click **Plate Layout**.  
The plate layout automatically provides well and vial positions for unassigned samples.
10. Make sure that the column oven temperature is reached before submitting the batch.
11. Save the batch:
  - a. Click **Save As**.  
The Save As Batch dialog opens.
  - b. Type a **File Name** and then click **Save**.
12. Submit the batch. Refer to the section: [Submit a Batch](#).

## Import a Batch from a LIMS

Prerequisite Procedures
-------------------------

- |   |
|---|
| <ul style="list-style-type: none"><li>• Configure the LIMS in the Configuration workspace. Refer to the document: <i>Help System</i>.</li></ul> |
|---|

---

**Note:** To import a batch from a Watson LIMS, refer to the section: [Import a Batch from a File](#).

---

1. Open the Batch workspace.
2. (Optional) Click **View** to select the columns that will be shown in the Batch workspace.
3. Click **Open > Import from LIMS**.  
The Import a Batch File dialog opens.
4. Type the file location or file name.
5. Type the batch identifier in the **Batch Identifier** field.
6. (Optional) Select or clear the **Append to current batch** check box, as required.

---

**Note:** Any existing data in the grid is overwritten if the user does not select the **Append to current batch** option.

---

7. Click **Import**.
8. (Optional) To use the plate layout as a reference for selecting or confirming a sample location, click **Plate Layout**.  
The plate layout automatically provides well and vial positions for unassigned samples.
9. (Optional) To include calibration samples in the batch, do the following:
  - a. To open the Batch-Automatic Calibration Editor dialog, click **Auto-Calibrate**.
  - b. Select the ion reference and calibrant delivery settings to be applied automatically, at the specified frequency.

- c. Click **OK**.
  - d. Select the check box to the left of the **Auto-Calibrate** button.
10. Make sure that the column oven temperature is reached before submitting the batch.
11. Save the batch:
  - a. Click **Save As**.  
The Save As Batch dialog opens.
  - b. Type a **File Name** and then click **Save**.
12. Submit the batch. Refer to the section: [Submit a Batch](#).

## Create a Batch Manually

Review the batch contents before submitting the samples.

---

**Note:** If the mass spectrometer is using contact closure to communicate with an external device, then follow these guidelines:

- Make sure that the sample sequence defined in the batch matches the sequence defined on the external device.
- Make sure that the method duration is less than or equal to the interval between injections, as defined on the external device.

---

**Tip!** To access the cut, copy, paste, add rows, and remove rows features, click **Manage Samples**.

---

1. Open the Batch workspace.
2. (Optional) Click **View** to select the columns that will be shown in the Batch workspace.

---

**Tip!** To use an existing batch, click **Open > Open**.

---

3. Click **New**.
4. (Optional) To use the plate layout as a reference for selecting or confirming a sample location, click **Plate Layout**.  
The plate layout automatically provides well and vial positions for unassigned samples.
5. Type the batch information in the grid.  
For a description of the columns in the grid, refer to the table: [Table 5-5](#).

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**Tip!** The Batch workspace provides the following features to make creating batches easier:

- The content for some cells, such as the **Sample Type** cell, can be selected from a list in the cell. Click the right side of the cell to show the list.
- The second and subsequent rows added to a batch automatically populate with the values from the preceding row.
- The user can copy a single cell by selecting the cell, clicking the bottom right corner of the cell, and then dragging to the last row to which the cell content is to be copied.
- The user can copy a group of cells in the same row by selecting the cells, clicking the bottom corner of the right-most cell, and then dragging to the last row to which the cell content is to be copied.
- The user can copy a series of values by typing sequential values in two rows, selecting both cells, clicking the bottom right corner of the bottom cell, and dragging to the last row in the series.
- The user can use the Copy (**Ctrl+C**) and Paste (**Ctrl+V**) commands to copy the content of a cell or group of cells and then paste them in a new location.

---

**Note:** LC columns are not available until an LC method is selected.

---

**Tip!** To configure the batch to process the sample automatically after it is acquired, use one of the following methods:

- To use an embedded processing method, select an existing **Results File**. The sample will be processed with the embedded method of the corresponding Results file.
- To use a new processing method, clear the **Results File** field. When the **Results File** field is cleared, the **Processing Method** field becomes available. Select a **Processing Method** and then type a new **Results File** name. The sample will be processed with the selected processing method.

When processing in the non-targeted screening workflow, a comparison sample cannot be selected for automatic processing. For processing methods that use the AutoPeak algorithm, the software always builds the integration model with the samples used to create the method.

- 
6. (Optional) Define component concentrations. Refer to the section: [Add a Component Concentration](#)
  7. (Optional) To apply decision rules to the batch, follow these steps:
    - a. Select the **Decision Rules** check box.
    - b. Click **Decision Rules** and then select **Apply** for each decision rule to be applied to the batch. To add decision rules, refer to the section: [Add a Decision Rule](#).

- c. Click **Save**.

---

**Note:** If the **Decision Rules** option is selected and at least one decision rule is active for a batch, then **Decision Rules: Active** is shown beside the batch name in the Queue workspace. If the active project is located on the network and the network is unavailable, then the text is **Decision Rules: Disabled** is shown.

---

8. Save the batch:
  - a. Click **Save As**.  
The Save As Batch dialog opens.
  - b. Type a **File Name** and then click **Save**.
9. Make sure that the column oven temperature is reached before submitting the batch.
10. Make sure that the system has been equilibrated with the MS and LC method that is used in the batch.
11. Submit the batch. Refer to the section: [Submit a Batch](#).

## Use the Plate Layout Feature to Create a Batch

The plate layout feature provides a graphical representation of the rack and plate structures that can be used to populate the grid in the Batch workspace.

1. Open the Batch workspace.
2. Select an **MS Method**.
3. Select an **LC Method**.  
The LC system must be active.
4. Type the name of the **Data File** in which the acquired data will be saved.
5. Select the **Processing Method** that will be used to process the data after it is acquired.
6. Type the name of the **Results File** in which the processed data will be saved.
7. Click **Plate Layout**.  
The Plate Layout window opens and, by default, shows a graphical representation of the plate.
8. Set the properties for the plate.  
The window updates to show a graphical representation of the selected plate type.
9. On the graphical representation, click a sample position.  
The selected sample position is fully highlighted in the graphical representation. The Batch workspace is updated, starting with the first row that does not have the sample position defined completely, that is, a row that does not include the **Rack Type**, **Plate Type**, if wells are used, and **Vial Position** values. The grid shows the sample positions accordingly.

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- Continue to click sample positions as needed in the graphical representation to populate the grid in the Batch workspace.

If sample positions are typed in the grid in the Batch workspace, then the graphical representation is updated accordingly.

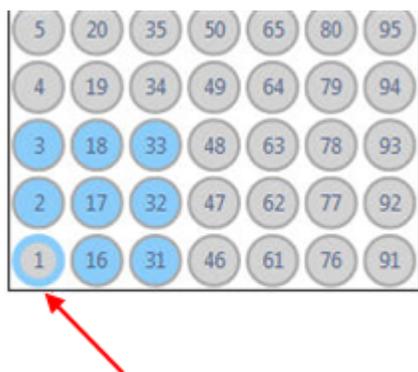
---

**Tip!** To remove all of the data associated with a specified rack type, click **Clear All**. If the selected rack type identifies a plate, then the menu under **Clear All** includes **Clear Front** and **Clear Back**.

---

- To specify a replicate selected sample position, click the sample position in the graphical representation.  
The graphical representation of the plate layout shows the replicate sample position with a colored outline and the grid in the Batch workspace shows the data accordingly.

**Figure 5-4 Plate Layout—Replicate Sample Position (Position 1)**



---

**Note:** Unselected positions are shown in gray, and positions that have been selected once are shown in blue with a gray border.

---

- To see the sample index in the graphical representation, hover the cursor over the highlighted sample position.  
A tooltip shows the sample index.
- When all of the positions are assigned and reviewed, click **Close** in the Plate Layout window and then click **Save** in the Batch workspace.

## Create an Ion Reference Table

- Open the Batch workspace.
- Click **Auto-Calibrate**.  
The Batch - Automatic Calibration Editor dialog opens.
- Click **Edit**.  
The Ion Reference Table Editor dialog opens.

4. Click **New**.

---

**Tip!** Use the Tab key to move between cells and press Enter to add a row.

---

5. In the **Reference Ions for TOF MS Calibration** grid, type a precursor mass. The **Compound Name** field is optional.
6. Add rows as required.
7. In the **Use** column, select the ions to use.
8. Select the **Use for MS/MS** radio button for the precursor mass to be used for MS/MS.
9. Type values in the **CE for MS/MS** and **DP for MS/MS** fields for the precursor mass selected in step 8.
10. In the **Reference Ions for MS/MS Calibration** grid, add and then select at least two fragment masses. The **Fragment Name** field is optional.
11. Click **OK**.
12. Type a name in the Save Reference Table dialog and then click **OK**.

---

**Note:** If users select an LC Method as the calibrant delivery method, then the Retention Time and the Retention Time Tolerance must be specified in the Reference Ions table.

---

## Calibrate the System Using the CDS

---

**Note:** If the mass spectrometer is configured with the contact closure option, then refer to the section: [Calibrate a System Configured with Contact Closure](#).

---

1. Open the **Batch** workspace.
2. Click **Auto-Calibrate**. The Batch - Automatic Calibration Editor opens.
3. Select an ion reference table.

---

**Note:** For TOF MSMS methods, make sure that the precursor mass selected in the reference table is greater than the smallest precursor mass in the method.

---

4. Type the number of samples to be acquired between calibrations.
5. Select **CDS** as the calibrant delivery method. By default, CDS channel 1 is selected. Use channel 1 for positive solutions and use channel 2 for negative solutions.
6. Click **OK** to close the dialog.
7. Make sure that the check box to the left of the **Auto-Calibrate** button is selected.

8. Create and submit a batch.

## Calibrate the System Using an LC Method

---

**Note:** If the mass spectrometer is configured with the contact closure option, then refer to the section: [Calibrate a System Configured with Contact Closure](#).

---

1. Open the **Batch** workspace.
2. Click **Auto-Calibrate**.  
The Batch - Automatic Calibration Editor opens.
3. Select an ion reference table.
4. Type the number of samples to be acquired between calibration.
5. Select an LC Method as the calibrant delivery method.  
The autosampler rack, plate, and vial fields as well as the MS method field are shown on the right of the dialog.
6. Select an MS method and then select the appropriate rack, plate, and vial information.
7. Click **OK** to close the dialog.
8. Make sure that the check box to the left of the **Auto-Calibrate** button is selected.
9. Create and submit a batch.

## Manage Component Concentrations

### Add a Component Concentration

The batch contains component concentrations defined in the MS method, processing method, or Results Table. Use this procedure to add additional component concentrations.

---

**Note:** Component concentrations added using this procedure are editable for samples of type QualityControl and Standard. Component concentrations are also added to a batch when a processing method that contains components is defined for a sample. The component concentrations added by the processing method are only editable for samples with processing methods that contain the component.

---

1. In the Batch workspace, click **Component Concentrations** ()
2. Click **Manage Components > Add Component**.
3. Type the name of the **Component**.
4. Click **OK**.  
The new component concentration is added to the current batch.

## Delete a Component Concentration

Use this procedure to remove a component concentration from the batch.

1. In the Batch workspace, click **Component Concentrations** ()
2. Click **Manage Components > Remove Component**.  
A list of components is shown. It contains all components added with the **Add Component Concentration** command, or when a MRM method or processing method was added to the batch.
3. Select the component from the list.
4. Click **OK**.

## Manage Decision Rules

### Add a Decision Rule

Use this procedure to add a decision rule.

1. In the Batch workspace, click **Decision Rules**.  
The Decision Rules dialog opens.
2. Click **Add Rule**.  
The Decision Rule Configuration dialog opens.
3. Type a name for the decision rule.
4. Define the properties for the decision rule, including the flagging rule, when the decision rule will be evaluated, and the response. Refer to the document: *Help System*.
5. Click **Save** to save the decision rule.
6. Click **Save** to close the dialog.

---

**Note:** If the user does not click **Save** on the Decision Rules dialog, then the new decision rule is not saved.

---

### Change a Decision Rule

1. In the Batch workspace, click **Decision Rules**.  
The Decision Rules dialog opens.
2. Click the **Decision Rule Name** of the decision rule to be changed.  
The Decision Rule Configuration dialog opens.
3. Change the **Decision rule name** and the settings for the decision rule, including the flagging rule, when the decision rule will be evaluated, and the response. Refer to the document: *Help System*.
4. Click **Save** to save the decision rule.

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---

5. Click **Save** to close the dialog.

---

**Note:** If the user does not click **Save** on the Decision Rules dialog, then the new decision rule is not saved.

---

### Delete a Decision Rule

Use this procedure to delete a decision rule.

1. In the Batch workspace, click **Decision Rules**.  
The Decision Rules dialog opens.
2. Click the **Flagging Rule Used**.
3. Click **Delete Rule** to delete the decision rule.
4. Click **Save**.

### Create a Duplicate Rule

Use this procedure to create a duplicate rule.

1. In the Batch workspace, click **Decision Rules**.  
The Decision Rules dialog opens.
2. Click the decision rule to be duplicated.
3. Click **Duplicate Rule**.
4. Click **Save**.

### Import Decision Rules

Use this procedure to import decision rules.

1. In the Batch workspace, click **Decision Rules**.  
The Decision Rules dialog opens.
2. Click **Import List**.
3. Navigate to and select the text file to be imported, and then click **Open**.
4. Click **Save**.

### Export Decision Rules

1. In the Batch workspace, click **Decision Rules**.  
The Decision Rules dialog opens.
2. Click **Export List**.
3. Navigate to the folder where the text file will be saved, type a file name, and then click **Save**.
4. Click **Cancel**.

## Equilibrate the System

Equilibrate the system at the start of the day, before a new method is run, or before submitting a batch. Equilibration warms up and prepares the mass spectrometer and LC system for the next sample or batch.

1. Click **Equilibrate** on the status panel.  
The Equilibrate dialog opens.
2. Select an MS Method from the **MS Method** list.
3. Select an LC method from the **LC Method** list.
4. Type the equilibration time in the **Time (min)** field, in minutes.
5. Click **OK**.  
When equilibration is complete, the system status in the status panel is Ready.

---

**Tip!** Open the Queue workspace to monitor the progress of the equilibration. The Queue workspace indicates how much time is required for the equilibration to complete. To stop equilibration before it finishes, click **Stop** in the Queue workspace.

---

## Submit a Batch

Prerequisite Procedures
<ul style="list-style-type: none"> <li>• <a href="#">Equilibrate the System</a>.</li> <li>• Open a batch in the Batch workspace.</li> </ul>

- [Equilibrate the System](#).
- Open a batch in the Batch workspace.

1. Click **Submit**.  
The Submit Samples dialog opens.
2. Click **OK** to continue.

---

**Note:** If the **Auto-Calibrate** option is selected, and the mass spectrometer is configured with the contact closure option, then the first calibration run is performed automatically. Then, the system goes into Loading state until the user starts an injection on the external device.

---

If errors are shown at the top of the screen, resolve them, and then click **Submit** again. The batch is not added to the queue until all of the errors are resolved.

---

**Tip!** If the queue is not started, navigate to the Queue workspace, and then click **Start** on the menu bar.

---

## Submit a Single Sample to the Queue from the Batch Workspace

Prerequisite Procedures
<ul style="list-style-type: none"><li>• <a href="#">Equilibrate the System.</a></li><li>• Open a batch in the Batch workspace.</li></ul>

- [Equilibrate the System.](#)
- Open a batch in the Batch workspace.

1. Select the row index number of the sample.
2. Click **Submit**.  
The Submit Samples dialog opens.
3. Click **OK** to continue.

---

**Note:** If the **Auto-Calibrate** option is selected, and the mass spectrometer is configured with the contact closure option, then the first calibration run is performed automatically. Then, the system goes into Loading state until the user starts an injection on the external device.

---

If errors are shown at the top of the screen, resolve them, and then click **Submit** again. The batch is not added to the queue until all of the errors are resolved.

---

**Tip!** If the queue is not started, navigate to the Queue workspace, and then click **Start** on the menu bar.

---

## Submit Multiple Samples to the Queue from the Batch Workspace

Prerequisite Procedures
<ul style="list-style-type: none"><li>• <a href="#">Equilibrate the System.</a></li><li>• Open a batch in the Batch workspace.</li></ul>

- [Equilibrate the System.](#)
- Open a batch in the Batch workspace.

1. Do one of the following:
  - Press **Ctrl** while clicking the sample row index number of each sample.
  - Drag up or down the list of index numbers.

---

**Note:** Samples are submitted in the order that they are selected and not in the order that they are shown in the batch.

---

2. Click **Submit**.
-

The Submit Samples dialog opens.

3. Click **OK** to continue.

**Note:** If the **Auto-Calibrate** option is selected, and the mass spectrometer is configured with the contact closure option, then the first calibration run is performed automatically. Then, the system goes into Loading state until the user starts an injection on the external device.

If errors are shown at the top of the screen, resolve them, and then click **Submit** again. The batch is not added to the queue until all of the errors are resolved.

**Tip!** If the queue is not started, navigate to the Queue workspace, and then click **Start** on the menu bar.

## Queue Workspace

The Queue workspace shows:

- Queue status
- Batch status
- Sample acquisition and processing status

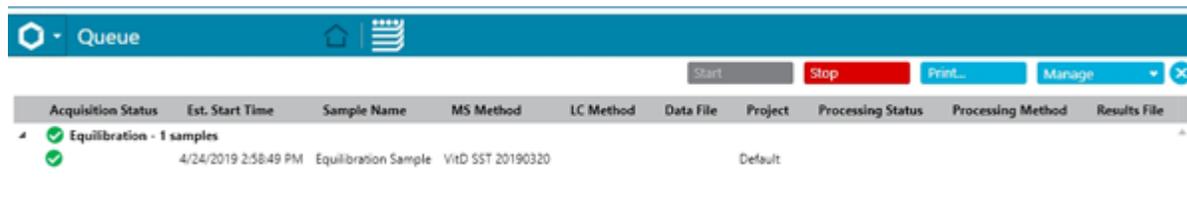
In this workspace, the user can manage batches and samples in the queue.

By default, the samples are not shown in the queue. Sample information is collapsed under the batch name. The batch status, the batch name, the number of samples in the batch, and the time remaining to acquire the current batch are shown. The calibration sample included in the batch is shown as Cal in the queue in the Sample Name column.

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

**Note:** Do not manually change the integrated diverter valve position during sample acquisition.

**Figure 5-5 Queue Workspace**



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---

**Table 5-7 Queue Workspace Columns**

<b>Label</b>	<b>Description</b>
<i>Batch Name</i>	<p>The name of the batch that was submitted to the queue, the number of samples in the batch, and the decision rule processing status. The queue contains a row for each batch. By default, the batch is collapsed, but it can be expanded to show all of the samples in the batch. For each sample, information is shown in the following columns.</p> <hr/> <p><b>Note:</b> For batches with decision rule processing, the software delays the acquisition of the next sample to allow processing of the current sample to finish. If processing does not finish within the allowed time, then decision rules are disabled. The delay time is 1.5 times the acquisition time.</p> <hr/>
<b>Acquisition Status</b>	The status of the data acquisition. For information on the status icons, refer to the section: <a href="#">Queue Icons</a> .
<b>Est. Start Time</b>	The time that acquisition of this sample started.
<b>Acquisition Time</b>	How long it took to acquire this sample.
<b>Sample Name</b>	The name of the sample, as specified in the batch.
<b>Sample ID</b>	The identifier for the sample, as specified in the batch.
<b>Barcode</b>	The barcode number of the sample vial, as specified in the batch.
<b>Rack Code</b>	The identifier for the LC rack, as specified in the batch.
<b>Rack Position</b>	The installed location of the LC rack, as specified in the batch.
<b>Plate Code</b>	The identifier for the LC plate, as specified in the batch.
<b>Plate Position</b>	The installed location of the LC plate, as specified in the batch.
<b>Vial Position</b>	The location of the sample in the LC plate or rack.
<b>MS Method</b>	The MS method, as specified in the batch.
<b>LC Method</b>	The LC method, as specified in the batch.
<b>Injection Volume</b>	The amount of sample injected.
<b>Data File</b>	The name of the data file to which the data will be acquired.
<b>Scanned Barcode</b>	The identifier for the vial.
<b>User</b>	The name of the user who submitted the batch.
<b>Project</b>	The project in which the data file will be saved.
<b>Data File Status</b>	The status of the data file.

**Table 5-7 Queue Workspace Columns (continued)**

Label	Description
<b>Auto Processing Status</b>	The status of the data processing. For information on the status icons, refer to the section: <a href="#">Queue Icons</a> .
<b>Processing Method</b>	The processing method that will be used to process the acquired data. If an existing Results file is being used, then this column contains the text <i>*Embedded Method*</i> .
<b>Results File</b>	The file to which the processed data will be written.
<b>Decision Rule Status</b>	The flagging state of a sample and the action take by the decision rule.
<b>Decision Rule Summary</b>	The name of the decision rule that is triggered.

## Manage the Queue

Acquisition begins after the samples have been submitted from the Batch workspace. Make sure that the system is equilibrated prior to submitting a batch. Refer to the section: [Equilibrate the System](#).

**Note:** Run the sample again if an abnormal termination occurs during sample acquisition. If the abnormal termination is caused by a power failure, then the temperature of the autosampler tray is not maintained and sample integrity might be compromised.

Use the features in the following table to manage the samples and batches in the queue.

**Table 5-8 Queue Workspace Features**

To Do This	Do This
Show or hide columns.	Click <b>Manage &gt; Display Columns</b> . Refer to the section: <a href="#">Show or Hide Columns</a> .
View all of the samples in the batch.	Click  .
Collapse all of the samples in the batch.	Click  .
Start acquisition.	Click <b>Start</b> . Equilibrate the system before running any samples.
View the status of the submitted samples.	Double-click the batch header.

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**Table 5-8 Queue Workspace Features (continued)**

To Do This	Do This
Reacquire the selected samples.	<ol style="list-style-type: none"> <li>Click the samples.</li> <li>Click <b>Manage &gt; Reacquire samples</b>.</li> </ol>
Delete the selected samples.	<ol style="list-style-type: none"> <li>Click the samples.</li> <li>Click <b>Manage &gt; Delete samples</b>.</li> </ol>
Delete all of the samples below the selected sample.	<ol style="list-style-type: none"> <li>Click the sample.</li> <li>Click <b>Manage &gt; Delete samples below row selection</b>.</li> </ol>
Clear the queue of all of the acquired batches or samples.	Click <b>Manage &gt; Clear queue</b> .
Remove the focus from a selected row.	Click <b>Manage &gt; Clear all selections</b> .
Move the selected batch or sample to the top of the queue.	<ol style="list-style-type: none"> <li>Click the batch header.</li> <li>Click <b>Manage &gt; Move row to top</b>.</li> </ol> <hr/> <p><b>Note:</b> Only single batches or samples that have not been acquired can be moved.</p> <hr/>
Move the selected sample up in the queue.	<ol style="list-style-type: none"> <li>Click the sample.</li> <li>Click <b>Manage &gt; Move row up</b>.</li> </ol> <hr/> <p><b>Note:</b> Only single samples that have not been acquired can be moved.</p> <hr/>
Move the selected sample down in the queue.	<ol style="list-style-type: none"> <li>Click the sample.</li> <li>Click <b>Manage &gt; Move row down</b>.</li> </ol> <hr/> <p><b>Note:</b> Only single samples that have not been acquired can be moved.</p> <hr/>
Collapse all of the samples and batches.	Click <b>Manage &gt; Collapse all rows</b> .
Show all of the samples and batches.	Click <b>Manage &gt; Expand all rows</b> .

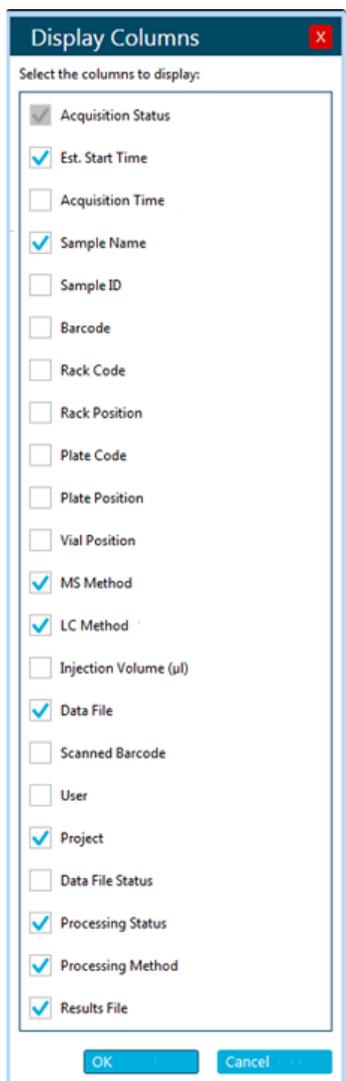
**Table 5-8 Queue Workspace Features (continued)**

To Do This	Do This
View data from a sample that is in the process of being acquired.	<p>Do one of the following:</p> <ul style="list-style-type: none"> <li>• Double-click the sample that is in the process of being acquired.</li> </ul> <hr/> <p><b>Note:</b> Double-click one of the columns to the left of the <b>Processing Status</b> column.</p> <hr/> <ul style="list-style-type: none"> <li>• Click <b>Open data exploration to view real time data</b> () in the Data Acquisition panel.</li> </ul>
View data from a sample that has been acquired.	Double-click the green check mark (  ) in the <b>Acquisition Status</b> column.
View the Results file that was created.	Double-click the green check mark (  ) in the <b>Processing Status</b> column.
View the barcode vials that are being scanned.	<ol style="list-style-type: none"> <li>1. Click <b>Manage &gt; Display Columns</b>.</li> <li>2. Select the <b>Barcode</b> or <b>Scanned Barcode</b> check box, or both, in the Select Columns dialog. Refer to the section: <a href="#">Show or Hide Columns</a>.</li> <li>3. Click <b>OK</b>.</li> </ol>
Stop the queue.	<ol style="list-style-type: none"> <li>1. Click <b>Stop</b>.</li> <li>2. Select <b>Stop now</b> or <b>Stop after the current tasks are completed</b>.</li> <li>3. Click <b>OK</b>.</li> </ol>
Stop processing of all of the remaining queued samples.	<ol style="list-style-type: none"> <li>1. Click <b>Cancel remaining processing</b>.</li> <li>2. Click <b>Yes</b>.</li> </ol>
Print the queue.	Click <b>Print</b> from the workspace menu.

## Show or Hide Columns

1. In the Queue workspace, click **Manage > Display Columns**.
2. Select or clear the column check boxes, as required, in the Display Columns dialog. For a description of the columns, refer to the table: [Table 5-7](#).

Figure 5-6 Display Columns Dialog



3. Click **OK**.

## Queue Icons

Table 5-9 Queue Icons

Icon	Name	Description
▶	Expand arrow	Shows the samples in the batch.
◀	Collapse arrow	Hides the samples in the batch.

Table 5-10 Acquisition Status Icons

Icon <sup>1</sup>	Name	Description
	<b>Completed</b>	The sample or entire batch was acquired successfully. Double-click this icon to open the sample in the Explorer workspace.
	<b>Warning</b>	The sample was acquired, but the user stopped or extended the acquisition.
	<b>Failed</b>	The sample or any sample within the batch was not acquired successfully.
	<b>Failed</b>	The calibration sample did not meet the acceptance criteria. Double-click the icon to view the status report.
	<b>In Progress</b>	The sample or batch is being acquired.
	<b>Waiting</b>	The sample or batch has not been acquired yet or is not in the process of being acquired.
	<b>Barcode Warning</b>	There was a barcode reading error or a mismatch of the barcode scan and the sample.

Table 5-11 Processing Status Icons

Icon <sup>2</sup>	Name	Description
	<b>Completed</b>	The sample was processed successfully. Double-click this icon to open the Results file in the Analytics workspace.
	<b>Warning</b>	Processing was stopped by the user.
	<b>Failed</b>	The sample was not processed successfully.
	<b>In Progress</b>	The sample is being processed.

<sup>1</sup> If decision rules are used, then the acquisition status might be affected by the decision rule. For example, the decision rule might abort a sample or stop the queue. The decision rule takes into account all samples in the batch and, if the samples are being processed into different Results files, then their associated Results files. Even samples that are no longer visible in the queue are taken into account.

<sup>2</sup> If the **Processing Status** column is empty, then no processing method or Results file was selected for the sample.

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---

**Table 5-11 Processing Status Icons (continued)**

Icon	Name	Description
	Waiting	The sample has not yet been processed.

**Table 5-12 Decision Rule Status Icons**

Icon <sup>3 4</sup>	Name	Description
	Flagging rule passed	The sample meets the pass criteria for the flagging rule configured in the decision rule.
	Flagging rule marginal	The sample meets the marginal criteria for the flagging rule configured in the decision rule.
	Flagging rule failed	The sample meets the fail criteria for the flagging rule configured in the decision rule.
	Queue stopped	The queue is stopped based on a decision rule. This icon is also shown when the queue is stopped and the next batch is acquired.
	Sample injected	The sample is reinjected based on a decision rule or the sample is injected from a vial configured in the decision rule.

**Table 5-13 Data File Status Icons**

Icon	Name	Description
	Transfer Complete	The sample has been successfully transferred to the network project.
	Transfer in Process	The sample is being transferred to the network project.
	Transfer Failed	The sample transfer failed. SCIEX OS will try to transfer the sample again.

---

<sup>3</sup> The flagging state icons and their tooltips are shown when the user hovers over the decision rule name, flagging rule name, and action taken.

<sup>4</sup> If the user selects to evaluate the rule after all standards are acquired, then the statuses of the flagged samples are updated retroactively.

---

## MS Tune Workspace

A dat file is created by the software when the instrument data is saved. Use this file to restore earlier parameter states. The dat backup file is named using the time that the file was created, not the time that the file was backed up.

---

**Note:** When the APCI probe is being used, only the Quick Status Check and Advanced Troubleshooting functions are available. To perform any other tuning procedures, install the ESI probe.

---

Each time the user loads the MS Tune procedure, all of the mass spectrometer parameters are backed up.

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

### Perform a Quick Status Check

Prerequisite Procedures
<ul style="list-style-type: none"><li>• Make sure that the correct probe is installed</li></ul>



Use this procedure to calibrate the system and to quickly verify the resolution in TOF MS and MS/MS modes. If the channel alignment mass accuracy does not meet the specification, then the user can repeat the steps and calibrate the system. If the resolution does not meet the specification, then the user can perform the TOF Tuning procedure to optimize the system.

---

**Tip!** Users can assess the this procedure by clicking **MS Check** on the status panel.

---

**Note:** If the mass spectrometer is configured with a CDS, then the software automatically starts the CDS at the beginning of the Achieve Stable Spray step. The software stops the CDS when the user closes the MS Tune workspace.

---

1. Open the MS Tune workspace.
2. Select **Positive Quick Status Check** or **Negative Quick Status Check** from the **Tuning Procedures** list.
3. Click **Next**.
4. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
5. (Optional ) Review the report to verify the results of each step.
6. (Optional) Save the report.
7. Click **Save Tuning Settings** if the results are satisfactory. If the results are not satisfactory, then do one of the following:

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- Repeat the steps.
- Run the TOF MS tuning procedure. Refer to the section: [Tune TOF](#).
- Discard the results by closing the **MS Tune** workspace.
- Restore the previous settings by selecting the appropriate backup file from the **Restore Instrument Data** menu.

## Optimize the Detector

When the system sensitivity is low, use this procedure to verify that the detector voltage is optimized. During the procedure, the software can adjust the detector voltage to provide the optimum sensitivity. When the optimization is completed, the user can save the optimized value or discard the changes.

---

**Note:** Make sure to perform this procedure in both High Mass and Low Mass modes.

---

We recommend optimizing the detector once a month. The detector should also be optimized if there is a significant drop in sensitivity and after venting and cleaning the instrument.

---

**Note:** Detector aging is a function of ion exposure, so more frequent optimization might be necessary when highly concentrated samples are used.

---

---

**Note:** If the mass spectrometer is configured with a CDS, then the software automatically starts the CDS at the beginning of the Achieve Stable Spray step. The software stops the CDS when the user closes the MS Tune workspace.

---

1. Open the MS Tune workspace.
2. From the **Tuning Procedures** list, do one of the following:
  - (ZenoTOF systems) Select **Positive Detector Optimization** or **Negative Detector Optimization**.
  - (X500 QTOF systems) Select **Detector Optimization**.

The Introduction page is shown. It describes the purpose of the optimization process, any prerequisites, and the instructions.

3. Make sure that the syringe pump is properly configured. Refer to the document: *System User Guide*. Then click **Next**.
4. Make sure that the spray is stable and then click **Next**.
5. Follow the on-screen instructions. Refer to the document: *Help System*. The optimization report is shown.
6. (Optional) Save the report by following these steps:
  - a. On the Report page, click **Save report as**.

- b. Navigate to the folder where the report will to be saved, type a **File name**, and then click **Save**.
7. Click **Next**.
8. Click **Save Settings**.

---

**Note:** If the detector optimizes at 2650 V or higher, then contact [sciex.com/request-support](https://sciex.com/request-support) to replace the detector.

---

The following message is shown: "Tuning settings were saved".

## Tune Q1 Unit

In MS/MS experiments, the Q1 region is used to select a precursor ion for fragmentation. Q1 Unit tuning optimizes the peak width and calibrates the Q1 mass. Q1 Unit represents the width of the precursor ion selection window at unit resolution. Q1 Low or Open represents the width of the precursor ion selection window at Low resolution (wider window ) or Open resolution (open window ). After the Q1 Unit is tuned, Q1 Low and Open settings are calculated based on the Q1 Unit values.

---

**Note:** If the mass spectrometer is configured with a CDS, then the software automatically starts the CDS at the beginning of the Achieve Stable Spray step. The software stops the CDS when the user closes the MS Tune workspace.

---

1. Open the MS Tune workspace.
2. Select **Positive Q1 Unit Tuning** or **Negative Q1 Unit Tuning** from the **Tuning Procedures** list.
3. Click **Next**.
4. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
5. (Optional) Click **Edit Method** to adjust the parameters.
6. If calibration was performed, then click **Confirm** to run a confirmation acquisition.
7. Click **Next**.
8. (Optional) Save the report.
9. Click **Next**.
10. Click **Save Settings**.

## Tune TOF

The TOF MS Tuning procedure optimizes the parameters for resolution and sensitivity in TOF MS and MS/MS modes. The optimization starts verifying the system performance before tuning, and then ramps various parameters for maximum intensity and resolution. After channel alignment, the system is calibrated and the system performance is determined. If the

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performance is satisfactory, then the user can save the tuning settings to the system or discard the settings.

TOF MS tuning can be performed in Automatic or Manual mode. In Manual mode, users can select the optimized parameter values or pause at the end of the tuning steps.

---

**Note:** If the mass spectrometer is configured with a CDS, then the software automatically starts the CDS at the beginning of the Achieve Stable Spray step. The software stops the CDS when the user closes the MS Tune workspace.

---

1. Open the MS Tune workspace.
2. From the **Tuning Procedures** list, do one of the following:
  - (X500 QTOF systems) Select **Positive TOF MS Tuning** or **Negative TOF MS Tuning**.
  - (ZenoTOF systems) Select **Positive TOF Tuning** or **Negative TOF Tuning**.
3. Make sure that the spray is stable.
4. Click **Next**.
5. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
6. Click **Next**.
7. (Optional) Save the report.
8. Click **Save Settings** if the results are satisfactory. If the results are not satisfactory, then do one of the following:
  - Repeat the steps.
  - Discard the results by closing the **MS Tune** workspace.
  - Restore the previous settings by selecting the appropriate backup file from the **Restore Instrument Data** menu.
  - Contact [sciex.com/request-support](http://sciex.com/request-support).

## Tune Q1 High

In MS/MS experiments, the Q1 region is used to select a precursor ion for fragmentation. Q1 High tuning optimizes the peak width and calibrates the Q1 mass. Q1 High represents a narrower precursor ion selection window.

---

**Note:** If the mass spectrometer is configured with a CDS, then the software automatically starts the CDS at the beginning of the Achieve Stable Spray step. The software stops the CDS when the user closes the MS Tune workspace.

---

1. Open the MS Tune workspace.

2. Select **Positive Q1 High Tuning** or **Negative Q1 High Tuning** from the **Tuning Procedures** list.

---

**Note:** If the positive Q1 High procedure has not been run for a period of time, then click **Copy** to use the Positive Q1 Unit settings as a starting point.

---

3. Make sure that the spray is stable.
4. Click **Next**.
5. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
6. (Optional) Click **Edit Method** to adjust the parameters.
7. If calibration was performed, then click **Confirm** to run a confirmation acquisition.
8. Click **Next**.
9. (Optional) Save the report.
10. Click **Next**.
11. Click **Save Settings**.

## Calibrate Zeno (ZenoTOF Systems)

1. Open the MS Tune workspace.
2. Select **Positive Zeno Calibration** or **Negative Zeno Calibration** from the **Tuning Procedures** list.  
The Introduction page is shown. It describes the purpose and prerequisites for the calibration process.

3. Make sure that the spray is stable and then click **Next**.

---

**Note:** The user can manually adjust the **Source and Gas Parameters** on the Achieve Stable Spray/Modify page.

---

4. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
5. Click **Next**.
6. (Optional) Save the report by following these steps:
  - a. On the Report page, click **Save report as**.
  - b. Navigate to the folder where the report will to be saved, type a **File name**, and then click **Save**.
7. Click **Next**.
8. Click **Save Tuning Settings** if the results are satisfactory. If the results are not satisfactory, then do one of the following:
  - Repeat the steps.

- Discard the results by closing the **MS Tune** workspace.
- Restore the previous settings by selecting the appropriate backup file from the **Restore Instrument Data** menu.

### Perform EAD Optimization (ZenoTOF Systems)

1. Open the MS Tune workspace.
2. Select **EAD Optimization** from the **Tuning Procedures** list.  
The Introduction page is shown. It describes the purpose and prerequisites for the optimization process.
3. Select the **Tuning process** and then click **Next**.
4. On the Filament Calibration Verification page, select the **Filament** and then click **Calibrate Filament**.

---

**Tip!** To change the selected filament, click the list in the **Filament** field, and then select the required filament.

---

5. Click **Next**.
6. Make sure that the spray is stable and then click **Next**.

---

**Note:** The user can manually adjust the **Source and Gas Parameters** on the Achieve Stable Spray/Modify page.

---

7. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
8. Click **Next**.
9. (Optional) Save the report by following these steps:
  - a. On the Report page, click **Save report as**.
  - b. Navigate to the folder where the report will to be saved, type a **File name**, and then click **Save**.
10. Click **Next**.
11. Click **Save Settings**.

### Perform EAD EI Background Reduction (ZenoTOF Systems)

1. Open the MS Tune workspace.
2. Select **EAD EI Background Reduction** from the **Tuning Procedures** list.  
The Introduction page is shown. It describes the purpose and prerequisites of the tuning procedure.
3. Click **Next**.

4. Follow the on-screen instructions for each step. Refer to the document: *Help System*.
5. Click **Next**.
6. (Optional) Save the report by following these steps:
  - a. On the Report page, click **Save report as**.
  - b. Navigate to the folder where the report will to be saved, type a **File name**, and then click **Save**.

## Perform EAD Diagnostics (ZenoTOF Systems)

1. Open the MS Tune workspace.
2. Select **EAD Diagnostics** from the **Tuning Procedures** list.  
The Introduction page is shown. It describes the purpose and prerequisites for the EAD Diagnostic.
3. Click **Next**.
4. Follow the on-screen instructions for each step. Refer to the document: *Help System*.

## Perform ADC Initialization (ZenoTOF Systems)

1. Open the MS Tune workspace.
2. Select **Tuning Procedures > ADC Initialization**.  
The Introduction page is shown. It describes the purpose of the initialization.
3. Click **Next**.  
The ADC Initialization page is shown. Refer to the document: *Help System*.

## Perform Advanced Troubleshooting

<b>Prerequisite Procedures</b>
<ul style="list-style-type: none"> <li>• Make sure that the correct probe is installed</li> </ul>

If the tuning procedure results are not satisfactory, then use this advanced troubleshooting procedure to optimize the parameters related to the mass spectrometer. Users can also view the TDC channel statistics and spectra during acquisition.

---

**Tip!** The Live Method window can be used to view the optimized parameters after tuning is performed.

---

1. Open the MS Tune workspace.
2. Select **Advanced Troubleshooting** from the **Tuning Procedures** list.
3. Select a scan type.

4. Click **Edit Method** and then edit the parameters in the Live Window window, as required.
5. Click **Start/Restart Method**.
6. View the data and then adjust the parameters, as required.
7. Click **Stop** and then save the detector parameters or the TOF MS parameters, as required.

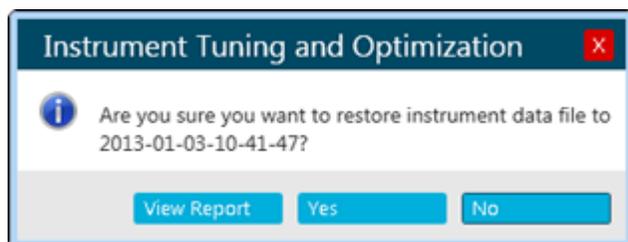
## Restore Instrument Data

The software generates a copy of the instrument data file (dat) and then updates the current dat file whenever the user saves the tuning settings at the end of each tuning procedure. Previously saved settings can be restored using the **Restore Instrument Data** feature.

When each tuning procedure is performed, the report and data files are generated to track the optimized results. By default, the wiff2 data file and report can be found at D:\SCIEX OS Data\Optimization.

1. Open the MS Tune workspace.
2. From the **Restore Instrument Data** menu, select a dat file with an earlier timestamp to be restored.

**Figure 5-7 Instrument Tuning and Optimization Dialog**



3. (Optional) View the report for the dat file to be restored by following these steps:
  - a. Click **View Report**.
  - b. If a report was generated for the selected instrument data file, then navigate to and double-click the report file to open it.
4. Click **Yes**.

# Operating Instructions—Processing 6

---

## Explorer Workspace

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

### Open Samples

Before performing data review tasks in the Explorer workspace, open the samples to review.

1. Open the Explorer workspace.
2. To open a single sample, follow these steps:
  - a. Click **File > Open Sample**.  
The Select Sample dialog opens.
  - b. Browse to and then select the sample to be opened.
  - c. Click **OK**.
3. To open multiple samples, follow these steps:
  - a. Click **File > Open Multiple Samples**.
  - b. In the Select Samples dialog, select the samples from the **Available** list and then click the arrow to move the files to the **Selected** list.

---

**Tip!** To select one sample, expand the file, click the sample, and then click the arrow.

---

- c. Click **OK**.

### Verify the Presence of an Analyte

Prerequisite Procedures
-------------------------

- |   |
|---|
| <ul style="list-style-type: none"><li>• <a href="#">Open Samples</a>.</li></ul> |
|---|

1. Extract ions. Refer to the section: [Extract Ions](#).
2. (Optional) Show the Data and Peaks table. Refer to section: [Show the Data and Peaks Table](#).
3. Review the peak area, intensity, masses, and charge states of the compounds.  
For SCIEX Triple Quad Systems, charge state is only available for full scan data types.

## Extract Ions

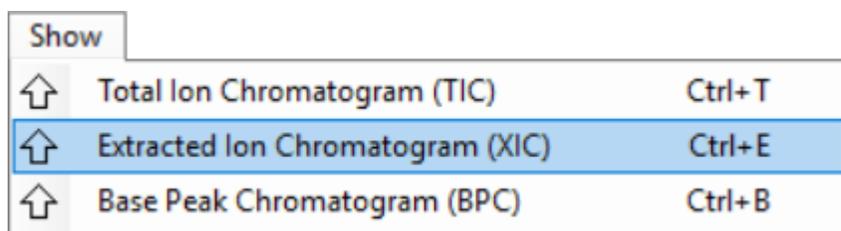
### Prerequisite Procedures

- [Open Samples](#).

Used to calculate one or more overlaid extracted ion chromatograms (XICs), which is the plot of the intensity sum over a given mass range as a function of retention time.

1. Click **Show > Extract Ion Chromatogram (XIC)**.

**Figure 6-1 Show Menu: Extracted Ion Chromatogram (XIC)**



2. If the Specify XIC Ranges dialog opens, then perform these steps:

- a. Type the **Center**, **Width**, and **Compound** values or import the values.

---

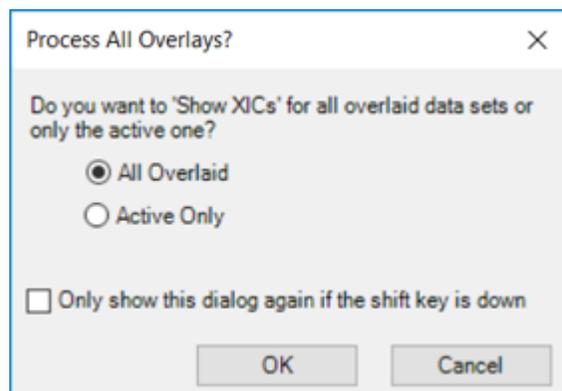
**Note:** The default title of the XIC includes the compound names shown in the cells for a given row.

---

**Tip!** When the **Center/Width** mode is used, a chemical formula rather than a mass can be specified for the **Center** value. When a neutral composition, such as H<sub>2</sub>O, is used, a proton is automatically added for Positive mode or subtracted for Negative mode. For example, the *m/z* ratio of H<sub>3</sub>O<sup>+</sup> is used for Positive mode. Specify an explicit charge state by ending the composition with '+*n*' or '-*n*' where *n* is the charge state. If the *n* is omitted, then it is assumed to be one. For example, if H<sub>2</sub>ONa<sup>+</sup> is specified, then the *m/z* ratio of H<sub>2</sub>ONa<sup>+</sup> is used as-is.

---

- b. (Optional) Use the features in the right-click menu to customize the options for ion extraction. For more information, refer to the document: *Help System*.
- c. Click **OK**.  
If the active graph contains overlaid series from different samples, then the Process All Overlays? dialog opens.

**Figure 6-2 Process All Overlays? Dialog**

3. If the Select MRMs dialog opens, then select the MRMs to include in the XIC, and then click **OK**.
4. If the Process All Overlays? dialog opens, then follow these steps:
  - a. Do one of the following:
    - Select **All Overlaid** to generate overlaid XICs for all of the available samples.
    - Select **Active Only** to generate XICs only from the currently active sample.
  - b. Click **OK**.

If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.

## Open a Total Ion Chromatogram

### Prerequisite Procedures

- [Open Samples](#).

A total ion chromatogram (TIC) is created by summing the intensity contributions of all of the ions from a series of mass scans. Use the TIC to view an entire data set in a single pane. The TIC consists of the summed intensities of all of the ions in a scan plotted against time in a chromatographic pane.

1. Click **Show > Total Ion Chromatogram (TIC)**.  
If the active graph contains overlaid series from different samples, then the Process All Overlays? dialog opens.
2. If the Process All Overlays? dialog opens, then follow these steps:
  - a. Do one of the following:

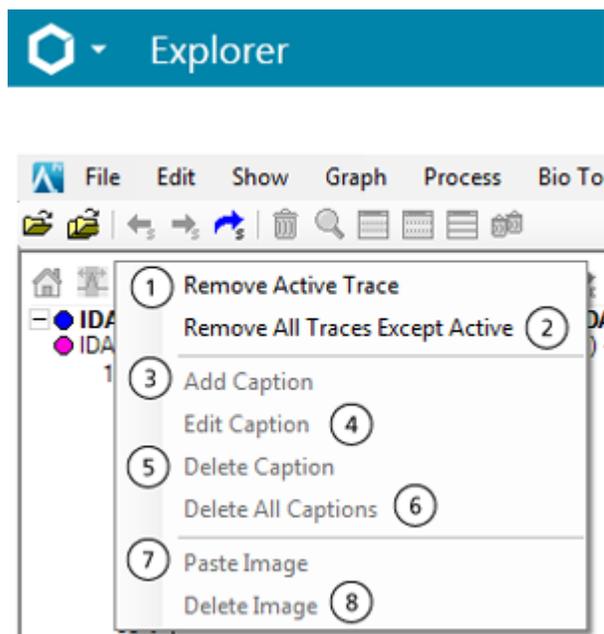
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- Select **All Overlaid** to generate overlaid TICs for all of the available samples.
  - Select **Active Only** to generate TICs only from the currently active sample.
- b. Click **OK**.

If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.

3. Right-click in the TIC and then use the features in the right-click menu.

**Figure 6-3 Total Ion Chromatogram Right-Click Menu**



Item	Description
1	Available when there is more than one overlaid trace. Removes the currently active trace from the graph. To remove a trace that is not currently active, activate it and then select the feature.
2	Available when there is more than one overlaid trace. Removes all of the traces except the currently active trace. If the trace to be kept is not currently active, then activate it and select the feature.

Item	Description
3	<p>Adds text to a graph.</p> <p>If required, click <b>Font</b> to adjust the font properties and then click <b>OK</b>. The caption is added at the (x, y) position where the user right-clicked to open the menu.</p> <p>After the caption has been added, the user can drag it to a new location. If the user drags it to the X- or Y-axis, then this cancels the drag operation.</p> <p>The character sequences '\d' and '\u' are treated in a special way. In the former case, the one character immediately following is drawn as a subscript and in the latter case as a superscript. In both cases, the special characters are not visible. This is particularly useful for chemical formulae. For example, 'H\d3O\u+' is shown as H<sub>3</sub>O<sup>+</sup>.</p>
4	Edits the selected caption. The user can also open this dialog by double-clicking a caption.
5	Deletes the selected caption. Alternatively, drag the caption outside the graph to delete it.
6	Available if the graph contains at least one caption. Removes all of the captions at once.
7	Pastes an image in the graph.
8	Deletes the selected image from the graph.

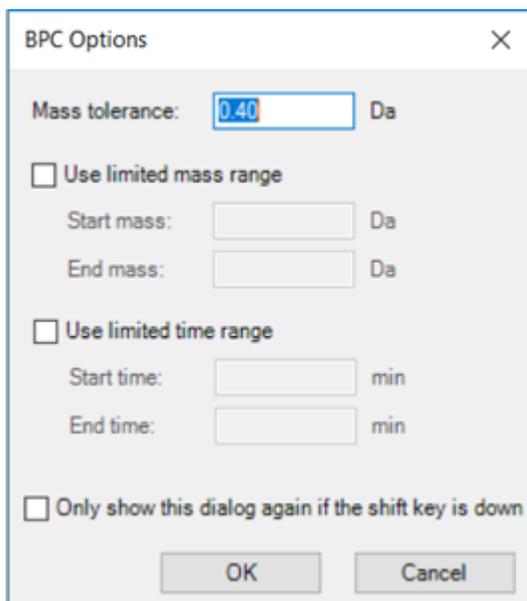
## Open a Base Peak Chromatogram

Prerequisite Procedures
<ul style="list-style-type: none"> <li>• <a href="#">Open Samples</a>.</li> </ul>

Generates a plot of the intensity of the largest peak in each spectrum as a function of time.

1. Click **Show > Base Peak Chromatogram (BPC)**.

**Figure 6-4 BPC Options Dialog**



2. Complete the fields on the BPC Options dialog. For information about the fields, refer to the document: *Help System*.

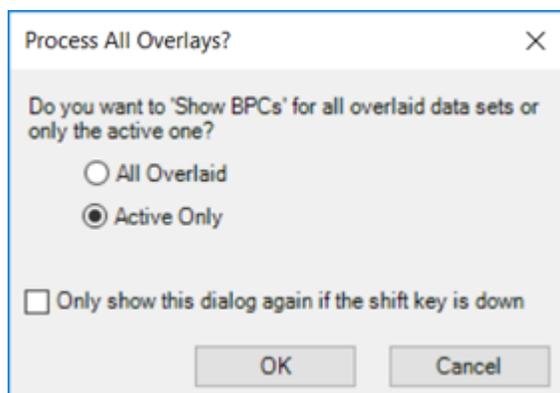
---

**Note:** If a chromatogram with a single selection spanning more than 1.0 minutes is active when the base peak chromatogram is being generated, then the time range defaults to the time range for the selection. Otherwise, the last time range is used. The limited time range saves the user from manually typing the range.

---

If the active graph contains overlaid series from different samples, then the Process All Overlays? dialog opens.

**Figure 6-5 Process All Overlays? Dialog**



3. If the Process All Overlays? dialog opens, then follow these steps:

- a. Do one of the following:
  - Select **All Overlaid** to generate overlaid BPCs for all of the available samples.
  - Select **Active Only** to generate BPCs only from the currently active sample.
- b. Click **OK**.

If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.

## Show the Data and Peaks Table

<b>Prerequisite Procedures</b>
<ul style="list-style-type: none"><li>• <a href="#">Open Samples</a>.</li></ul>



The Data and Peaks Table contains two different tables. The Data table shows the raw (X, Y) values comprising a data set and the Peaks table shows information about the peaks themselves. The table is generated when a graph is active.

---

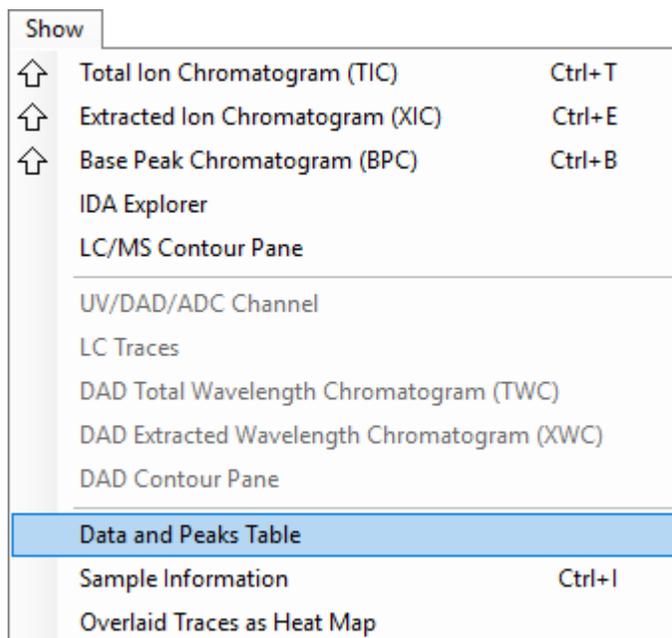
**Note:** Only peaks that are above the current threshold in the graph, set using the blue arrow on the Y-axis of the graph, are present. Refer to the section: [Work with Data in Graphs](#).

---

This feature is used to show a pane containing two tables for the currently active data: one table for the raw (X, Y) values and one for the peak list.

1. Click **Show > Data and Peaks Table**.

Figure 6-6 Show Menu: Data and Peaks Table



2. Use the features in the following table.

Table 6-1 Data and Peaks Table Features

To Do This	Do This
Sort the table based on that field.	Click the column heading.
Copy the currently selected cells.	Right-click in the table and then click <b>Copy</b> . If the Data tab is active, then the selected X- and Y-values are copied. If the Peaks tab is active, then the selected peak information is copied.
Copy only selected rows.	First select the rows by dragging in the row-selector column or by using the <b>Shift</b> or <b>Ctrl</b> keys to select multiple rows. Then right-click in the table and click <b>Copy</b> .
Select multiple columns.	Hold the <b>Ctrl</b> key and then click the column headings. If the user just clicks a column heading, then the column is sorted.
Copy the entire table.	Click <b>Edit &gt; Select All</b> and then click <b>Edit &gt; Copy</b> .

Table 6-1 Data and Peaks Table Features (continued)

To Do This	Do This
Export data as text.	Right-click in the pane and then click <b>Export Data as Text</b> .  Saves the entire data list to the specified file. The X- and Y-values are separated with a tab and there is a hard return after each (X, Y) pair.
Export peak list data as text.	Right-click in the pane and then click <b>Export Peak List as Text</b> .  Saves the entire peak list to the specified file. This does not include peaks that are below the current threshold set in the Y-axis of the associated graph. The peak metrics are separated with a tab and there is a hard return after each peak.

- Review the peak area, intensity, masses, and charge states of the compounds.

## Show Sample Information

Prerequisite Procedures
<ul style="list-style-type: none"> <li><a href="#">Open Samples</a>.</li> </ul>

The Sample Information pane shows a textual description of the experiment used to acquire the active data. This information includes sample-specific information, including the sample name and information about the data acquisition, such as the number and type of the experiments.

If two or more Sample Information panes, associated with different samples from the same data file, are visible, then clicking an item in the tree view for any one of the panes causes all of the other panes to scroll to the corresponding section. This assumes that sections with the same names exist in all of the panes. This feature is useful if the user wants to compare two similar, but not identical, Sample Information panes.

Click **Show > Sample Information**.

## Show the Graph Selection Information

Prerequisite Procedures
<ul style="list-style-type: none"> <li><a href="#">Open Samples</a>.</li> </ul>

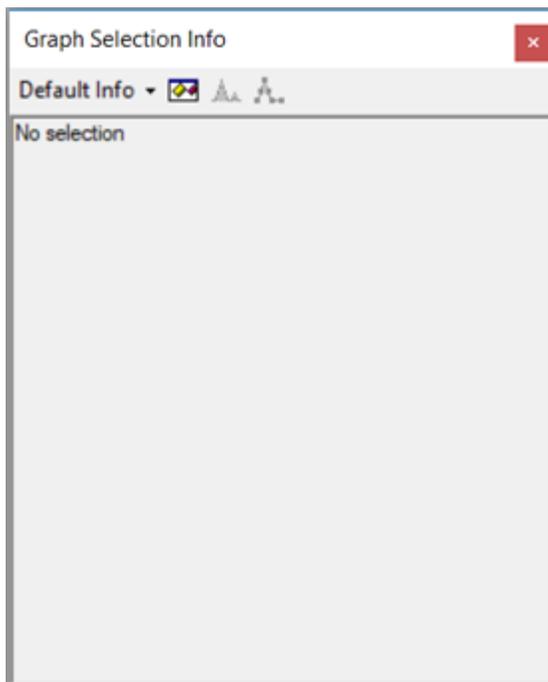
## Operating Instructions—Processing

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The Graph Selection Information dialog shows information about the selected region in a chromatogram or spectrum and is generated when one of those panes is active.

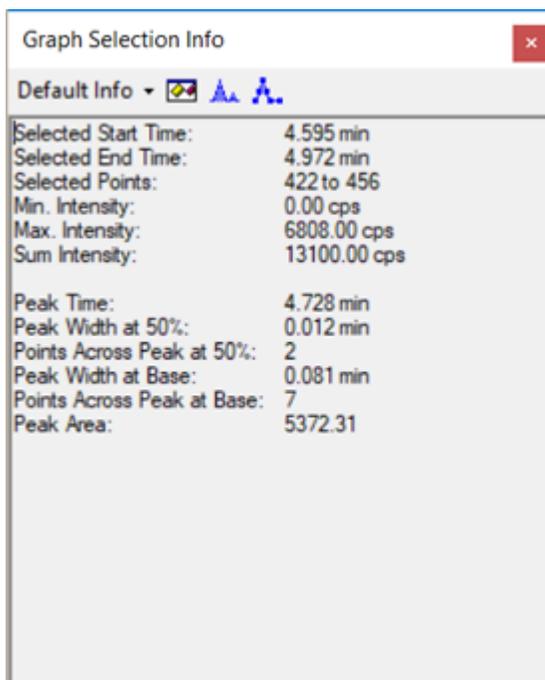
1. Click **Window > Graph Selection Window**.

**Figure 6-7 Graph Selection Info Dialog**



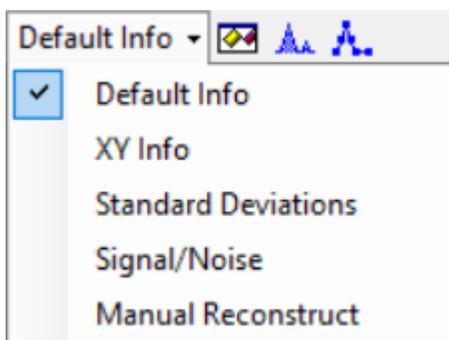
2. Make one or more selections in the chromatogram or spectrum graph.

**Figure 6-8 Graph Selection Info Dialog**



3. Select an option from the list: **Default Info**, **XY Info**, **Standard Deviations**, **Signal/Noise**, or **Manual Reconstruct**, if applicable.

**Figure 6-9 Selection Information Options**



For a description of the fields on the dialog, refer to the document: *Help System*.

4. (Optional) Calculate the signal-to-noise ratio manually.
  - a. Select a chromatograph or, in the Mass Reconstruction workflow, a reconstruction graph.
  - b. Select both the noise region and the target peak, using the **Shift** key to make multiple selections.

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- c. Select **Default Info > Signal/Noise**.
5. (Optional) Click **Options** (🔧), set the Graph Info options, and then click **OK**. For a description of the options, refer to the document: *Help System*.  
For example, to use 3 Sigma as the noise multiplier, set **Noise multiplier for S/N to 3**.
6. (Optional) Click **Fill Peaks** (📊).  
The active graph switches between a mode in which peaks are filled using alternating dark and light fills and a mode in which they are not. This feature is useful if the user wants to see the peak extent that corresponds to the **Peak Width at Base**.
7. (Optional) Click **Show Point Symbols** (📍).  
All spectra in the active pane switch between a mode in which data points are indicated with point symbols and a mode in which they are not. This feature is useful if the user is closely examining a peak and wants to see how many data points it comprises instead of using only the textual information shown in the main window.

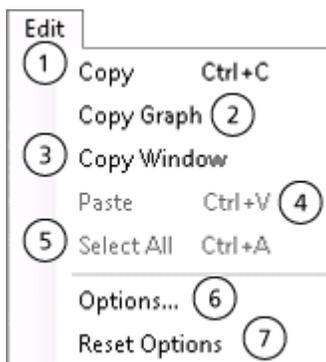
## Edit Settings in Graphs

### Prerequisite Procedures

- [Open Samples](#).

Click **Edit** and then use the features in the **Edit** menu.

**Figure 6-10 Edit Menu: Options**



Item	Description
1	Copies the current data to the clipboard. When a spectrum or chromatogram is active, a picture of this active graph is copied.
2	When a spectrum or chromatogram is active, copies the current graph to the clipboard as a picture.

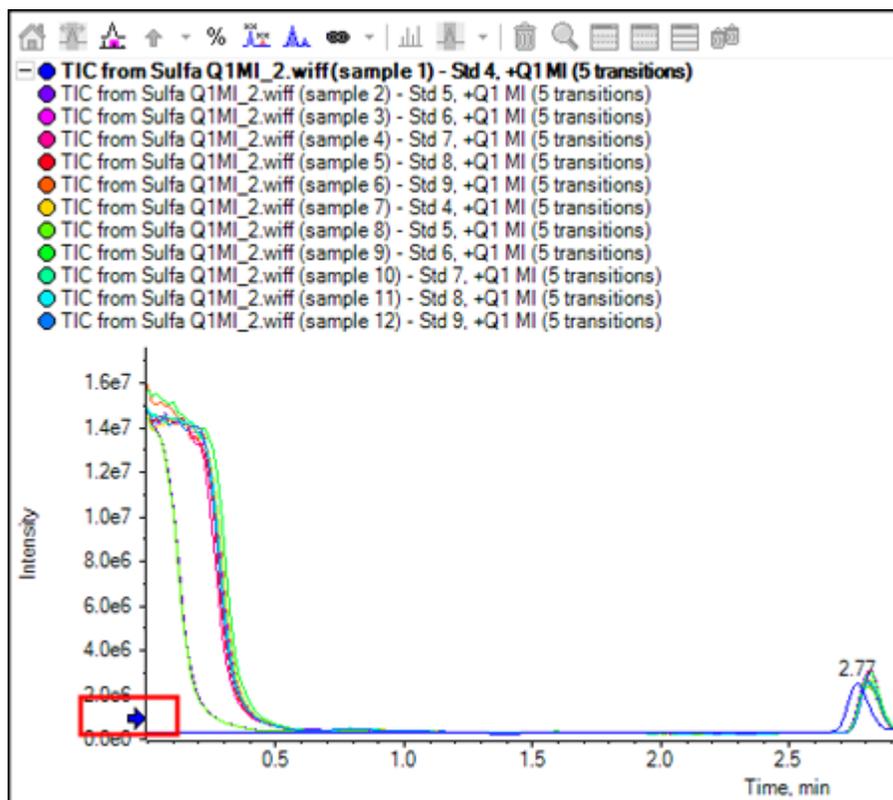
Item	Description
3	Copies an image of the entire active window to the clipboard. The title bar of the window and the toolbars of its various panes are not included.
4	Pastes data from the clipboard in the current view.
5	When a table is active, selects all of the rows in the table. When a text pane is active, selects all of the text.
6	Allows the user to set options for the graph appearance, peak labeling and finding, auto processing, and calculating the XIC ranges. Refer to the section: <a href="#">Set Options</a> .
7	Restores the default Explorer options. Refer to the section: <a href="#">Reset Options</a> .

## Work with Data in Graphs

Prerequisite Procedures
<ul style="list-style-type: none"> <li>• <a href="#">Open Samples</a>.</li> </ul>

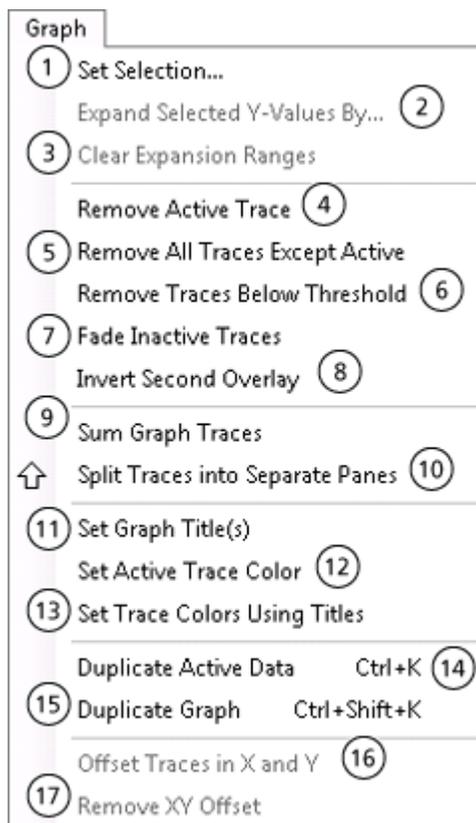
1. To set the threshold for labeling peaks and subsequent features such as the **Data and Peaks** table, drag the blue arrow that is shown on the Y-axis of the graphs.

Figure 6-11 Blue Arrow on the Y-axis



2. Use the features in the **Graph** menu.

Figure 6-12 Graph Menu: Options



Item	Description
1	<p>Selects portions of graphs to be processed in subsequent operations. For example, select an area in a chromatogram and then double-click to obtain an averaged spectrum. Use the <b>Set Selection</b> feature to type specific X-ranges so that selections can be set more accurately than is possible using the cursor.</p> <ol style="list-style-type: none"> <li>Click <b>Graph &gt; Set Selection</b>. The Set Selection dialog opens.</li> <li>Type the <b>Center</b> and <b>Width</b> values.</li> <li>Click <b>OK</b>.</li> </ol> <hr/> <p><b>Tip!</b> To set selections in a graph manually, drag the cursor in the plotting region to make a selection. If the <b>Shift</b> key is held, then any current selections are kept.</p>

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Item	Description
2	<p>Expands the Y-values within a range by a specified factor for plotting purposes.</p> <ol style="list-style-type: none"><li>Open a sample or multiple samples.</li><li>Select a portion of the graph.</li><li>Click <b>Graph &gt; Expand Selected Y-Values by</b>. The Expand Selection dialog opens.</li><li>Type the expansion factor.</li><li>Click <b>OK</b>.</li></ol>
3	<p>Removes all of the expansion ranges.</p> <ul style="list-style-type: none"><li>In a graph that has expanded ranges, click <b>Graph &gt; Clear Expansion Ranges</b>.</li></ul>
4	<p>Removes the currently active trace from the graph. This feature is available when there is more than one overlaid trace.</p> <ul style="list-style-type: none"><li>In a graph that has more than one overlaid trace, click <b>Graph &gt; Remove Active Trace</b>.</li></ul>
5	<p>Removes all of the traces except the currently active one. This feature is available when there is more than one overlaid trace.</p> <ul style="list-style-type: none"><li>In a graph that has more than one overlaid trace, click <b>Graph &gt; Remove All Traces Except Active</b>.</li></ul>
6	<p>Removes overlaid traces from the graph for which all of the data points are below the current threshold setting.</p> <p>If the user zoomed the graph so that only a portion of the X-range is currently visible, then a dialog opens. The user can select whether to remove traces that are below the threshold using the entire range or using only the currently visible portion.</p> <ul style="list-style-type: none"><li>In a graph that has more than one overlaid trace, click <b>Graph &gt; Remove Traces Below Threshold</b>.</li></ul>
7	<p>When the active graph contains more than one overlaid trace, draws all of the traces except for the currently active one using a fainter, less intense, color than normal. Use this feature to focus on the active trace. The inactive traces are less distracting. To return to the original style, select the feature again.</p> <ul style="list-style-type: none"><li>In a graph that has more than one overlaid trace, click <b>Graph &gt; Fade Inactive Trace</b>.</li></ul>

Item	Description
8	When the active graph contains more than one overlaid trace, inverts the second trace. This can make it easier to visually compare two similar traces. Select <b>Invert Second Overlay</b> again to return to the original view.
9	Replaces the graphs with a single trace that is the sum of all of the individual traces. <ul style="list-style-type: none"> <li>• In an active graph containing more than one overlaid trace, click <b>Graph &gt; Sum Graph Traces</b>.</li> </ul>
10	Creates a graph for each separate overlay. For example, if the user begins with a graph containing three overlaid traces and then selects this feature, the final result contains four panes: the original graph with the overlays and one graph for each of the individual data sets. <ol style="list-style-type: none"> <li>a. In an active graph containing more than one overlaid trace, click <b>Graph &gt; Split Traces into Separate Panes</b>. The Number of Columns dialog opens.</li> <li>b. Select the number of columns in the output. The number of rows required is determined based on the number of rows and the number of overlaid traces.</li> <li>c. Select the check box to open the panes in a new window. If the check box is not selected, then the panes are opened in the same window.</li> </ol>
11	Opens the Set Titles dialog. Use this option to manually change the titles of the traces.
12	Opens the Color dialog. Use this option to set the color for the currently active graph trace.
13	Opens the Set Trace Colors Using Titles dialog. When multiple graph traces are overlaid, the software uses default colors for the overlays. Use this option to set specific colors for traces for which the title contains specific text.
14	Creates a copy of the currently active graph data and then adds it to that graph. Use this feature to see the effect of a particular data processing operation. For example, if the user duplicates the data using this feature and then smooths one of the two traces, the resulting graph contains overlaid before and after views. <ul style="list-style-type: none"> <li>• In an active graph, click <b>Graph &gt; Duplicate Active Data</b>.</li> </ul>

## Operating Instructions—Processing

Item	Description
15	<p>Creates a copy of the currently active graph. Use this feature to see the effect of a particular data processing operation. For example, if the user duplicates the data using this feature and then smooths one of the two traces, before and after views in two separate graphs are visible. Link the X-axes so that zooming one graph causes the other to zoom automatically.</p> <ul style="list-style-type: none"><li>• In an active graph, click <b>Graph &gt; Duplicate Graph</b>.</li></ul>
16	Opens the Offset Traces dialog. Use this option to create a three-dimensional stacked graph from a series of overlaid graph traces.
17	Removes the generated offsets from the TIC.

## Use the Two-Pane Operation Tools

Prerequisite Procedures
<ul style="list-style-type: none"><li>• Open the Explorer workspace.</li></ul>

Use the icons along the right edge of panes to perform operations on two panes, the source pane and the target pane. Refer to the section: [Table 6-2](#). In all cases, click the icon in the source pane and then drag it to the target pane.

**Table 6-2 Two-Pane Tools**

Icon	Name	Description
	Move Pane	<p>Changes the relative positions of the panes. Shown in the top right corner of each pane. Click the icon in one pane and then drag it to the top, bottom, left, or right portion of a second pane. Depending on where the cursor is released, the first pane changes positions relative to the second. As the user drags the pane, one side of the second pane is highlighted in red to indicate where the first pane will be placed.</p> <p><b>Note:</b> The user can also drag panes from one window to another.</p>

Table 6-2 Two-Pane Tools (continued)

Icon	Name	Description
	Add Data	<p>Sums two data sets together, point-by-point. The data from the source pane that was originally clicked is added to the target pane, the pane over which the dragged icon is released. The title of the modified pane updates to indicate that it has been modified.</p> <hr/> <p><b>Note:</b> Only two data sets of the same type can be added. For example, the user cannot add a spectrum to a chromatogram.</p> <hr/> <hr/> <p><b>Tip!</b> If the target graph contains more than one overlaid trace, then by default, the source data is added to the active target data only. Hold the <b>Ctrl</b> key to add the source to all of the data sets in the target pane.</p> <hr/>
	Subtract Data	<p>Subtracts the background from a mass spectrum. Similar to the <b>Add Data</b> icon except that the source data is subtracted from the target data.</p> <hr/> <p><b>Tip!</b> If the target graph contains more than one overlaid trace, then by default, the source data is subtracted from the active target data only. Hold the <b>Ctrl</b> key to add the source to all of the data sets in the target.</p> <hr/> <hr/> <p><b>Tip!</b> Normally any data points for which the intensity in the source is greater than in the target are not kept. That is, negative Y-values are discarded. Hold the <b>Shift</b> key to keep the points with negative intensity.</p> <hr/>
	Overlay Data	<p>Overlays the active data in the source graph on the target graph. After the operation is completed, the target graph contains a new series with a copy of the target data.</p> <hr/> <p><b>Tip!</b> If the source graph contains more than one overlaid trace, then by default, only a copy of its active data is moved to the target graph. Hold the <b>Ctrl</b> key to overlay a copy of all of the data sets in the source graph on the target graph.</p> <hr/>

## Move Panes or Windows

<b>Prerequisite Procedures</b>
<ul style="list-style-type: none"> <li>• <a href="#">Open Samples.</a></li> </ul>

Click **Window** and then use the features in the **Window** menu.

**Figure 6-13 Window Menu: Options**



Item	Description
1	Opens a window showing information for the selected region in the active graph. For example, the X-range of the selection, the intensity range of the selected points, and so on. If this window is already visible, then selecting the menu item closes it. Refer to the section: <a href="#">Show the Graph Selection Information.</a>
2	Changes the layout of the information in the window from row format to column format.
3	Removes the currently active pane from its window and places it by itself in a new window.
4	Arranges any open windows that have not been minimized so that they are all beside one another in one row.
5	Arranges any open windows that have not been minimized so that they are all above or below one another in one column.

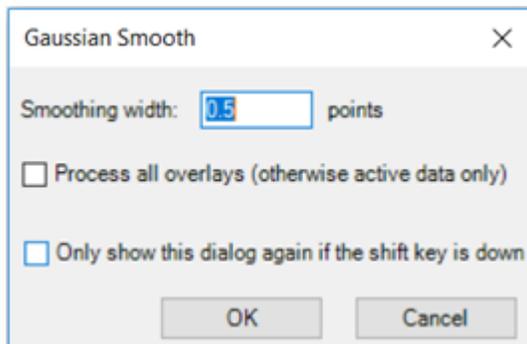
## Perform a Gaussian Smooth

<b>Prerequisite Procedures</b>
<ul style="list-style-type: none"> <li>• <a href="#">Open Samples.</a></li> </ul>

Applies a Gaussian smoothing algorithm. This is a filter of a specified width where the weighting factors follow a Gaussian, or normal, function.

1. Click **Process > Gaussian Smooth**.

**Figure 6-14 Gaussian Smooth Dialog**



2. Type a value in the **Smoothing width** field.  
This is actually the width of the Gaussian function at half of its maximum height. The total width is larger because the calculation is carried out in the wings of the Gaussian. Fractional values are allowed in which case the half width of the Gaussian is less than one point.
3. If there are multiple traces in the active graph, then select **Process all overlays (otherwise active data only)** to apply the operation to all of the traces.  
If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.
4. Click **OK**.

## Threshold Data

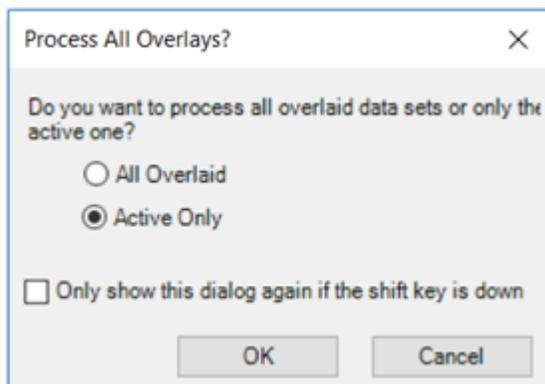
### Prerequisite Procedures

- [Open Samples](#).

Removes any data points that have an intensity below the current threshold setting. Sets the threshold by dragging the blue arrow that is shown in the Y-axes of graphs.

1. Click **Process > Threshold Data**.  
If the active graph contains overlaid series from different samples, then the Process All Overlays? dialog opens.

**Figure 6-15 Process All Overlays? Dialog**



2. If the Process All Overlays? dialog opens, then follow these steps:
  - a. Do one of the following:
    - Select **All Overlaid** to generate overlaid TICs for all of the available samples.
    - Select **Active Only** to generate TICs only from the currently active sample.
  - b. Click **OK**.

If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.

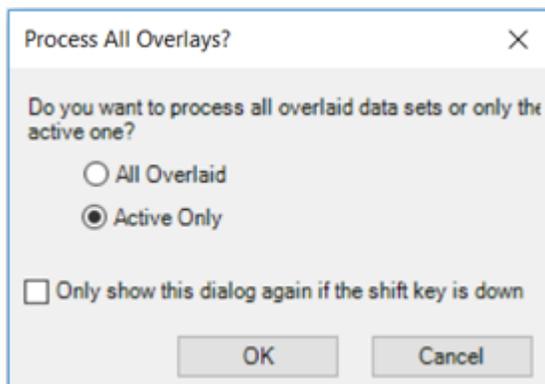
## Subset Data Using Graph Selection

Prerequisite Procedures
<ul style="list-style-type: none"><li>• <a href="#">Open Samples</a>.</li></ul>



This feature is only available when a graph with exactly one selected region is active. Removes data points lying outside of the selected region. Use this feature to focus data processing on a subset of the entire data.

1. Make a selection in the graph.
2. Click **Process > Subset Data (using graph selection)**.  
If the active graph contains overlaid series from different samples, then the Process All Overlays? dialog opens.

**Figure 6-16 Process All Overlays? Dialog**

3. If the Process All Overlays? dialog opens, then follow these steps:
  - a. Do one of the following:
    - Select **All Overlaid** to generate overlaid XICs or TICs for all of the available samples.
    - Select **Active Only** to generate XICs or TICs only from the currently active sample.
  - b. Click **OK**.

If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.

## Baseline Subtract Chromatogram

### Prerequisite Procedures

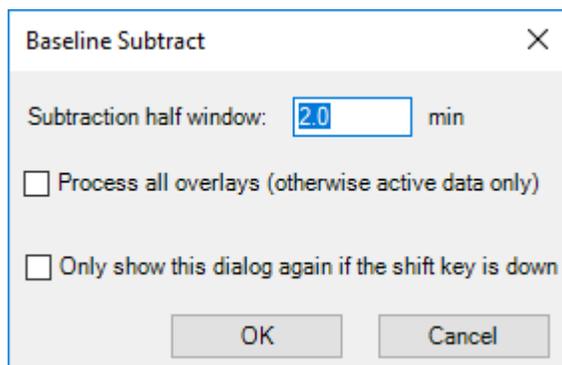
- [Open Samples](#).

Removes a relatively slowly varying background from a chromatogram.

For each data point in the chromatogram, a window is centered at the corresponding X-value and the points with minimum intensity within the window to the left and right are found. A straight line is joined between these two points and the Y-value is calculated at the center of the window. This is the baseline that is removed from the data at that point.

1. Click **Process > Baseline Subtract Chromatogram**.

**Figure 6-17 Baseline Subtract Dialog**



2. Type a value, in minutes, in the **Subtraction half window** field.
3. If there are multiple traces in the active graph, then select **Process all overlays (otherwise active data only)** to apply the operation to all of the traces.  
If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.
4. Click **OK**.

## Offset Chromatogram

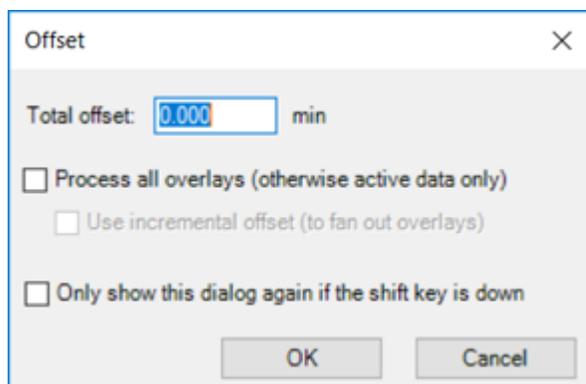
### Prerequisite Procedures

- [Open Samples](#).

Used to offset the time values of a chromatogram.

1. Click **Process > Offset Chromatogram**.

**Figure 6-18 Offset Dialog**



2. Type a value, in minutes, in the **Total offset** field.
3. If there are multiple traces in the active graph, then select **Process all overlays (otherwise active data only)** to apply the operation to all of the traces.  
If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.
4. Select **Use incremental offset (to fan out overlays)** to spread the overlays apart in the time direction.
5. Click **OK**.

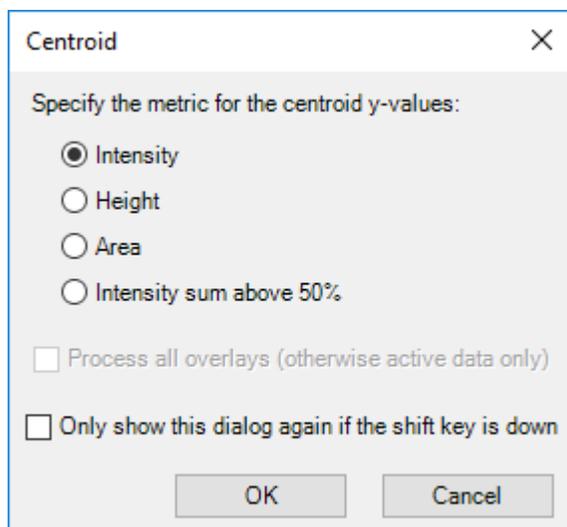
## Centroid a Spectrum

<b>Prerequisite Procedures</b>
<ul style="list-style-type: none"> <li>• <a href="#">Open Samples</a>.</li> </ul>

Creates a centroid of a mass spectrum, that is, replaces a profile spectrum with mass and intensity points for the detected peaks only.

1. Click **Process > Centroid Spectrum**.

**Figure 6-19 Centroid Dialog**



2. Select the metric to be used for the centroid process:
  - **Intensity**: For each peak, the centroid Y-value is the intensity of the largest data point comprising the peak.
  - **Height** : This metric is similar to the Intensity metric except that the intensity is subtracted by the baseline intensity when there is a baseline offset.

## Operating Instructions—Processing

---

- **Area:** For each peak, the centroid Y-value is the total area of the peak. This is a true integral because the reported value depends on both the intensity profile and the width of the peak.
  - **Intensity sum above 50%:** For each peak, the Y-value is the sum of the portion of the intensities comprising the peak which are above 50% of the peak apex intensity. This value is useful because it does not depend only on the intensity of a single data point, as the Intensity and Height metrics do, and it is not influenced by the edges of the peak which might be noisy or which might have interference.
3. If there are multiple traces in the active graph, then select **Process all overlays (otherwise active data only)** to apply the operation to all of the traces.  
If the **Only show this dialog again if the shift key is down** check box is selected, then the selected action is always used unless the user holds the **Shift** key to change the option.
  4. Click **OK**.

## Export Data as Text

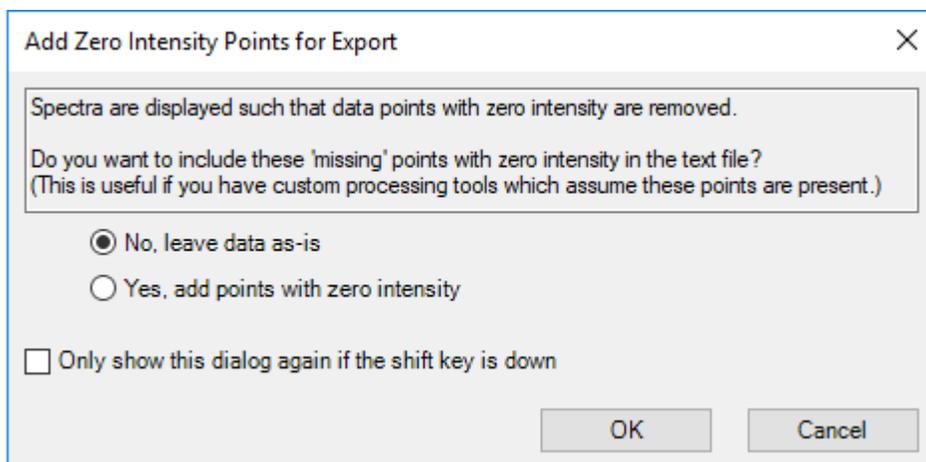
### Prerequisite Procedures

- [Open Samples.](#)

Currently active spectrum or chromatogram is saved to a tab-delimited text file.

1. Click **File > Export > Data as Text**.  
If spectral data is exported, then the Add Zero Intensity Points for Export dialog opens.

**Figure 6-20 Add Zero Intensity Points for Export Dialog**



2. If the Add Zero Intensity Points for Export dialog is open, then do one of the following:
  - Click **No, leave data as-is** to exclude points with zero intensity from the exported file.

- Click **Yes, add points with zero intensity** to include points with zero intensity in the exported file.

Then click **OK**.

3. Type a file name for the exported file.
4. Click **Save**.

## Export the Peak List as Text

<b>Prerequisite Procedures</b>
--------------------------------

- |   |
|---|
| <ul style="list-style-type: none"> <li>• <a href="#">Open Samples</a>.</li> </ul> |
|---|

The user can save the peak list for the currently active spectrum or chromatogram to a tab-delimited text file. This file contains information such as the centroid X-value (mass or time), peak area, height, and so on.

1. Click **File > Export > Peak List as Text**.
2. Type a file name for the exported file.
3. Click **Save**.

## Print Data

<b>Prerequisite Procedures</b>
--------------------------------

- |   |
|---|
| <ul style="list-style-type: none"> <li>• <a href="#">Open Samples</a>.</li> </ul> |
|---|

1. Click **File > Print** and then select the required option.  
The Print dialog opens.
2. Select a printer, and then click **Print**.

## Reset Options

<b>Prerequisite Procedures</b>
--------------------------------

- |  |
|--|
| <ul style="list-style-type: none"> <li>• Open the Explorer workspace.</li> </ul> |
|--|

The user can reset all of the options in the Explorer workspace to the default values. This includes the options described in the previous section, as well as processing options. Resetting the options only affects the currently logged-in Windows user, not other users of the same computer.

## Operating Instructions—Processing

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1. Click **Edit > Reset Options**.  
A confirmation dialog is shown.
2. Click **OK**.

## Set Options

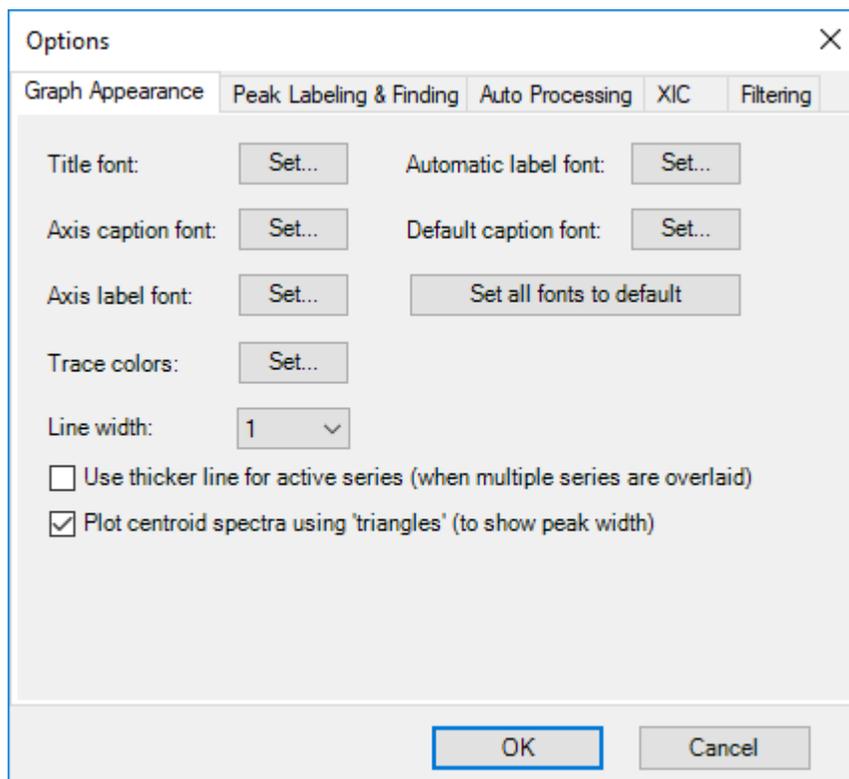
### Prerequisite Procedures

- Open the Explorer workspace.

Use the features on each tab as required.

1. Click **Edit > Options**.

**Figure 6-21 Options Dialog: Graph Appearance Tab**



2. Set the options on each tab, as applicable. For descriptions of the options, refer to the document: *Help System*.
3. Click **OK**.

---

## Analytics Workspace

Access to features in this workspace is controlled by the role assigned to the user. Refer to the document: *Laboratory Director Guide*.

---

**Note:** The controlled ways to output data from the Analytics workspace are: exporting Results Tables, transferring data to a LIMS, and reporting. The other sources of output data, such as copying and pasting from Results Tables, are not controlled. Do not use uncontrolled output methods for regulated purposes.

---

The grouping of numbers is not supported in the Analytics workspace. Do not group numbers in text boxes, for example, in the integration parameters, or grid such as a Results Table.

Processing methods include the criteria used to quantitate the peaks selected for integration.

Reviewers should review the data according to the criteria of peak integration and data acceptance in the laboratory standard operating procedures (SOPs).

SCIEX OS can process data while it is being acquired by SCIEX OS or the Analyst software. Any acquired samples can be added to the Results Table. To add samples that are being acquired, wait until acquisition is complete and then add them to the Results Table.

### Define the Default Processing Parameters for the Project

This option sets the default peak-finding parameters that are used when creating a processing method. If there are more than a few components, then set the default values based on the chromatography so that they do not need to be adjusted individually for every component. However, no one set of parameters is likely to be ideal for all of the components, so it might be necessary to adjust some of the parameters individually for some of the components.

1. In the Analytics workspace, click **Projects > Project default settings**.

---

**Note:** Make sure that the correct project name is selected in the status panel.

---

The Project Default Settings dialog opens.

2. On the Quantitative Processing page, perform these steps:
  - a. Select a signal-to-noise algorithm from the **Signal to Noise Algorithm** list.
  - b. Select an integration algorithm from the **Integration Algorithm** list and then set the default parameters for quantitative processing.

For descriptions of the parameters, refer to the document: *Help System*.

3. On the Qualitative Processing page, select a library search algorithm from the **Library Search Algorithm** list and then set the default parameters for qualitative processing.  
For information about the algorithms, refer to the document: *Help System*.

## Operating Instructions—Processing

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4. On the Mass Reconstruction Processing page, select an integration algorithm from the **Integration Algorithm** list and then set the default integration parameters for mass reconstruction.

For descriptions of the parameters, refer to the document: *Help System*.

---

**Note:** Only the MQ4 and Summation algorithms are available.

---

5. Click **Save**.
6. Click **Close**.

## Work with Workspace Layouts

Use the workspace layouts feature to save customized workspace layouts in the Analytics workspace. The customized layout is saved with the Results file, and automatically applied when the file is opened. This saves users time when they are analyzing results. A saved workspace layout can be applied to other Results files. It can also be set as the default workspace layout for a project, which is applied whenever a Results file in that project is opened. Workspace layouts can be saved anywhere, including on local networks.

Users can change between different saved layouts to perform different types of data analysis on their Results files.

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**Note:** All workspace layouts are saved with the qlayout file name extension.

---

---

**Note:** No settings that change or alter data directly are preserved in a workspace layout.

---

The following table lists the UI elements that are saved with workspace layouts.

**Table 6-3 UI Elements Saved with Workspace Layouts**

Pane	Saved UI Elements
Results Table	<ul style="list-style-type: none"><li>• The <b>Qualify for Rules Filters</b> check box.</li><li>• Qualifying row filters.</li><li>• Table sort choice.</li><li>• Highlighted rows and columns.</li><li>• <b>Table display settings</b>.</li><li>• Column filters.</li></ul> <hr/> <p><b>Note:</b> When the workspace layout is applied to a different Results Table, column filter settings are applied, if possible. If a filtered column does not exist in a Results Table, or if a filtering option is not applicable, then the setting is not applied.</p> <hr/>

**Table 6-3 UI Elements Saved with Workspace Layouts (continued)**

Pane	Saved UI Elements
Views Menu	<ul style="list-style-type: none"> <li>• The <b>Show hidden pane</b> setting.</li> <li>• Whether the <b>Tabbed view</b> option is selected.</li> </ul>
Samples or Components and Groups	<ul style="list-style-type: none"> <li>• Whether the Samples or Components and Groups list is open.</li> <li>• Whether specific samples or components are selected to be shown in a Results Table.</li> <li>• In the Samples list, the setting for <b>Options &gt; Synchronize Sample Selection</b>.</li> <li>• In the Components and Groups list, selection of <b>All Internal Standards, All Analytes, All Components, and Groups (where applicable)</b> options.</li> <li>• In the Components and Groups list, the setting for <b>Options &gt; Show IS</b></li> </ul>
Peak Review	<ul style="list-style-type: none"> <li>• Whether the Peak Review pane is open and if it is docked.</li> <li>• The current <b>View</b>.</li> <li>• Any selected <b>Options</b>, including the Peak review display settings options and the XIC Graph Title option.</li> </ul>
Calibration Curve	<ul style="list-style-type: none"> <li>• Whether the Calibration Curve pane is open.</li> <li>• The <b>Show excluded standards, Show quality controls, Show legend, Use percent Y-axis, and Log-log plot</b> settings in the <b>Options</b> menu.</li> </ul>
Metric Plot	<ul style="list-style-type: none"> <li>• Whether the Metric Plot pane is open.</li> <li>• <b>Link</b> menu settings.</li> <li>• Regression dialog settings.</li> <li>• The <b>Display "N/A" as 0.0, Show sample names, Show legend, Use percent Y-axis, Start Y-axis at 0, and Connect with lines</b> settings in the <b>Options</b> menu.</li> </ul>
Statistics Pane	<ul style="list-style-type: none"> <li>• Whether the Statistics pane is open.</li> <li>• Active <b>Sample grouping</b> selections.</li> <li>• Active <b>Metric</b> selections.</li> </ul>

### Save the Current Workspace Layout

1. Open the Analytics workspace.
2. Open a Results Table.
3. Customize the workspace layout as required.
4. Click **Views > Save current layout**.  
The Save Workspace Layout As dialog opens.
5. Type a name for the workspace layout and then click **Save**.

### Apply Another Workspace Layout to the Current Project

Applying different workspace layouts to the current Results file allows the user to quickly perform different types of results analyses on the same data.

1. Open the Analytics workspace.
2. Open a Results file.
3. Click **Views > Apply different layout to current results**.  
The Apply a Workspace Layout dialog opens.
4. Click **Browse**, select a layout, and then click **Open**.  
The Apply a Workspace Layout dialog shows a preview of the selected workspace layout.
5. Click **OK**.

---

**Tip!** Apply recently used workspace layouts by clicking **Views > Recent layouts** and selecting a layout.

---

### Set the Current Workspace Layout as Project Default

Setting a project default workspace layout preserves a layout between multiple sessions or users. It also makes any new Results files created within the project open with the project default workspace layout.

1. Open the Analytics workspace.
2. Open a Results file.
3. Customize the workspace layout to suit the project.
4. Click **Views > Set current layout as project default**.  
The Default Workspace Layout for the Project dialog opens.
5. Type a name for the layout in the **Default layout name** field and then click **OK**.
6. Click **Results > Save**.

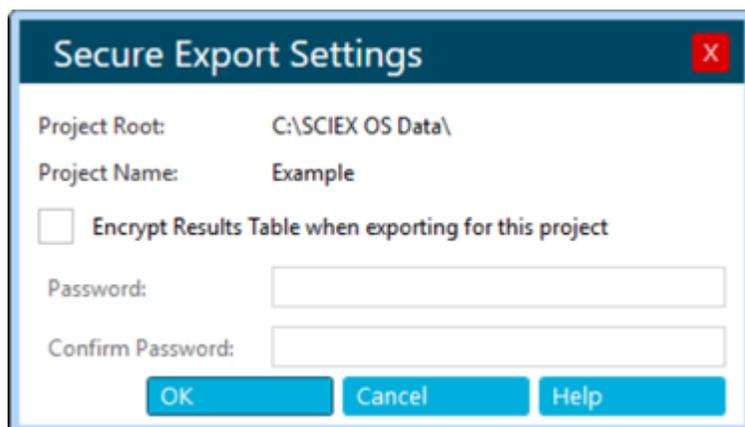
## Set Project Secure Export Settings

Only a user who has been assigned to the Administrator role can perform this task.

If this option is selected, then data in the text file is encrypted during export. Set a password to enable encryption.

1. In the Analytics workspace, click **Projects** > **Project secure export settings**.

**Figure 6-22 Secure Export Settings Dialog**



2. Select the **Encrypt Results Table when exporting for this project** check box.
3. Type a password in the **Password** field.
4. Type the password again in the **Confirm Password** field.
5. Click **OK**.

## Enable the Project Modified Peak Warning

By default, this option is not selected. When it is selected, if a user changes a chromatogram in a Results Table and then saves the changes, a warning message indicates that a change has been made. The user can choose to continue saving or return to the Results Table.

In the Analytics workspace, click **Projects** > **Enable project modified peak warning**.

## Create a Processing Method

Processing methods contain quantitative and qualitative settings for data processing. The Non-targeted workflow is used for unknown components.

---

**Tip!** To edit an existing processing method, click **Process Method** > **Open**.

---

1. Open the Analytics workspace.

## Operating Instructions—Processing

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2. Click **Process Method > New**.

---

**Tip!** To edit the processing method for the current Results Table, click **Process Method > Edit embedded method** and then continue with step 3.

---

3. On the Workflow page select at least one workflow and the reference samples. For descriptions of the fields on this page, refer to the document: *Help System*.

---

**Tip!** To use the mass reconstruction workflow, select **Quantitation** only.

---

4. Select the Components page and then perform these steps:
  - a. If applicable, select the mass reconstruction workflow by clicking **Options > Mass Reconstruction** and then clicking **Yes** in the confirmation dialog.
  - b. Complete the components table. For descriptions of the fields in this table, refer to the document: *Help System*.

---

**Note:** The Mass Reconstruction workflow is only available when the integration algorithm is set to **MQ4** or **Summation**.

---

---

**Tip!** If a group is defined in the Components table, then the user can choose to sum the ions in the group, even if the precursor ion and the experimental index are different for the transitions. The summed ions are not shown in the table but are shown on the Integration page and in the Results Table as **group name > Sum**. This feature is useful for the quantitation of proteins and peptides.

---

---

**Tip!** If the retention time of the components is not known, then set the **Retention Time Mode** for a mass or chemical formula to **Find *n* peaks**, where *n* is 1, 2, 5, 10, or all. The software identifies the specified number of features with the greatest peak area, assigns the appropriate retention time, and then performs a targeted peak processing workflow. When processing is complete, the embedded method for the Results Table can be saved as a targeted method.

---

---

**Tip!** To import components or components and integration parameters from a text file, use the appropriate command on the **Import** menu. If the component information does not contain concentration units, then the software uses the **Concentration units** defined on the Project Default Settings dialog.

---

---

**Note:** Integration parameters cannot be imported from processing methods that use the AutoPeak integration algorithm.

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---

**Note:** Integration parameters can be imported from Analyst software quantitation methods. Analyst software parameters are mapped to the corresponding SCIEX OS parameters, and the project default settings are used for any parameters that cannot be mapped.

---

---

**Note:** Integration parameters can be imported from MultiQuant software quantitation methods that do not use the SignalFinder algorithm. For MQ4 methods, the **S/N Integration Threshold** is changed from 0, the default in the MultiQuant software, to the project default. The parameters for the MultiQuant software are mapped to the corresponding parameters for SCIEX OS.

---

5. Select the Integration page and then perform these steps:
  - a. Select the integration parameters for each component. For descriptions of the fields on this page, refer to the document: *Help System*.

---

**Tip!** To define the rules for automatic outlier removal, click **Options > Remove Outliers Automatically**. Refer to the document: *Help System*.

---

- b. (Optional) To view the noise region, click **Options > Show Noise Regions**. Refer to the section: [Work With Noise Regions](#).

---

**Note:** **Show Noise Regions** is only shown when signal to noise algorithm is set to **Standard Deviation** or **Peak to Peak**.

---

6. (If applicable) Select the Library Search page and then define the library search parameters. For descriptions of the fields on this page, refer to the *Help System*.
7. Select the Calculated Columns page and then define any custom formulas to be used in custom calculated columns. For descriptions of the fields on this page, refer to the *Help System*.

---

**Note:** For more information about calculated columns, refer to the section: [Calculated Columns](#).

---

8. Select the Flagging Rules page and then select the rules to be used to flag results in the Results Table. For descriptions of the fields on this page, refer to the document: *Help System*.

Optionally, create custom flagging rules, or customize the following values for the predefined rules:

- Acceptance criteria for the following:
  - Accuracy of standards and quality controls
  - Calculated concentration range for unknown samples
  - Peak integration
- Traffic light settings for mass accuracy, retention time confidence, isotope match, library score, and formula finder score
- Traffic light settings for ion ratio acceptance

## Operating Instructions—Processing

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Ion ratio is the peak response ratio, that is, the area or height of the qualifier and quantifier.

---

**Tip!** To import flagging rules from a text file, click **Import**.

---

9. Select the Formula Finder page and then select the formula finder settings. For descriptions of the fields on this page, refer to the document: *Help System*.
10. (If the Non-targeted workflow is selected) Select the Non-targeted Peaks page and then define the Non-targeted search parameters. For descriptions of the fields on this page, refer to the document: *Help System*.
11. Click **Save**.

---

**Tip!** If a Non-targeted method is created, then the current project default parameters are used for peak integration, and those parameters are saved in the processing method file. If the processing method contains the targeted analytes, then the customized integration parameters for the targeted components will not affect the Non-targeted peak integration. If the user changes the project default parameter later, then the changed parameter will not impact the existing Non-targeted method, which still contains the parameters at the time the method was created. Only the newly created non-targeted method uses the changed parameters.

---

## Process Data

1. Open the Analytics workspace.
2. Click **Results > New**.
3. On the Process New Results dialog, use the arrows (  and  ) to select samples to be processed.
4. Select a processing method in one of the following ways:
  - Click **Browse** and then select a processing method and click **Open**.
  - Click **New** and then create the new processing method. Refer to the section: [Create a Processing Method](#).
5. (Optional) Click **Edit** to edit the processing method. Refer to the section: [Create a Processing Method](#).
6. Select a comparison sample for non-targeted workflows.
7. Click **Process**.

**Note:** In Non-targeted analysis, automatic grouping by adduct is performed. The grouping algorithm assigns adduct modifiers for compounds with the same retention time if the mass difference between them is associated with a common adduct. This feature helps prevent the investigation of duplicate compounds with different charge adducts.

---

If the data contains custom batch columns that have the same name as predefined Results Table columns or existing formulas, then a warning message is shown. Click **OK** to continue. An underscore ( \_ ) is added at the beginning of these column names.

8. To show or hide sample types, click the filter icon (  ) on the **Sample Type** column and then select or clear the required check boxes.
  9. To set the acceptance filters, click the filter icon (  ) on any of the acceptance columns, select **Filter by Flag**, and then select **Pass** or **Fail**.
- 

**Note:** The Acceptance columns include **Accuracy**, **Accuracy Acceptance**, **Asymmetry Factor**, **Calculated Concentration**, **Concentration Acceptance**, **Integration Acceptance**, **Quality Retention Time Delta (min)**, **Retention Time Error (%)**, and **Total Width**.

---

10. To select qualitative confidence filters, click the **Confidence** traffic light and then select or clear the required check boxes.
- 

**Note:** After the Results Table is generated using the AutoPeak algorithm, if the user changes the XIC width and the expected RT, then the data will be reprocessed using the previous algorithm model unless the user updates the model using the new XIC width and expected RT values.

---

11. To filter based on individual values for a Results Table column, click the filter icon (  ) on the column header and then select the check boxes for the values to be shown in the Results Table.
- 

**Tip!** To apply additional custom filters, select **Text Filters**.

---

**Tip!** To reapply the filter after a change to the Results Table, such as a change to the area count, click **Reapply Filter** (  ).

---

12. Save the Results file in one of the following ways:
  - Click **Results > Save**.
  - To prevent changes to the Results Table, click **Results > Lock results file and save**.

### Add Samples

#### Prerequisites

- In the Analytics workspace, a Results Table is open.

This option adds additional samples to a currently active Results Table.

1. Click **More > Add samples**.
2. In the Select Samples dialog, select the required samples.
  - The Available pane shows the subfolders, wiff2 files, and samples available in the **Data** folder for the current project.
  - Expand individual folders to see any subfolders or wiff2 files. If the wiff2 file is expanded, then it opens to show the available samples.

- Use the arrows to add (  ) or remove (  ) samples.

- Select samples in the following ways:

- Double-click an individual sample.

- Select a sample or data file and then click .

- Drag a sample or data file from the left pane to the right pane.

Press **Shift** or **Ctrl** to select multiple files or samples before moving them.

3. Click **OK**.

A progress bar is shown while the new samples are integrated and added to the existing table.

### Customize the Results Table

#### Prerequisites

- In the Analytics workspace, a Results Table is open.

Select the numeric format and the columns to be shown in the Results Table. The column settings can be applied to all of the Results Tables in the project.

---

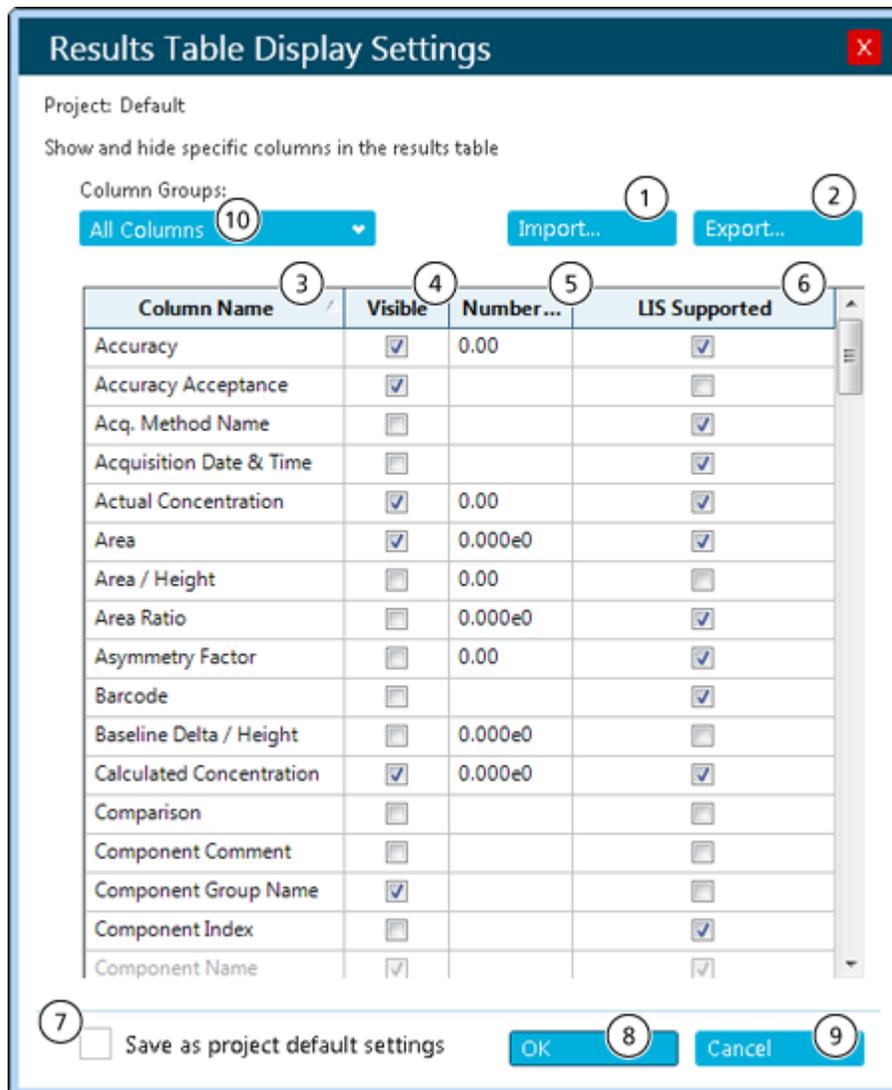
**Note:** Some critical columns, such as **Sample Name**, **Sample ID**, **Barcode**, and so on, should not be hidden when users customize the Results Table column settings.

---

**Tip!** If column names are truncated, then move the cursor over the field to show the column name in a tooltip.

1. Click **More > Table display settings**.  
The Results Table Display Settings dialog opens. For a description of the columns in the Results Table, refer to the section: [Results Table Columns](#).

**Figure 6-23 Results Table Display Settings Dialog**



## Operating Instructions—Processing

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Item	Description
1	Click to select a column settings file previously saved using the Export button. The dialog fields are updated to use the information from the selected file.
2	Click to save the current dialog settings to a file. Use the Import button to import and use these settings. This option lets the user switch between different column layouts.
3	The name of the columns, shown in alphabetical order. <b>Note:</b> This list also includes any calculated columns defined in the processing method that was used to create the Results Table.
4	A checkmark indicates that the column is visible.
5	For numerical fields, use the format 0.00 for non-scientific notations and use the format 0.00e0 for scientific notations. Change the decimal points to indicate the precision of the numbers that are shown. Only a period "." can be used as a decimal separator. <b>Note:</b> Grouping of numbers is not supported.
6	The selected <b>LIS Supported</b> rows are predefined by the LIMS and the column selections cannot be changed.
7	Click to use the column settings for future Results Tables.
8	Click to apply the changes and then close the dialog.
9	Click to abandon the changes and then close the dialog.
10	Select a category of Results Table columns. Users can filter the columns shown in the Results Table based on the selection. Selecting a category helps the user to easily find a column in the Results Table.

2. Select or clear the check box in the **Visible** column, as required.

---

**Note:** In addition to the columns described in the section: [Results Table Columns](#), the Results Table can contain custom calculated and text columns. Calculated columns are identified with an asterisk.

---

3. (Optional) In the **Number Format** column, change the format to integer or scientific notation.
4. (Optional) In the **Number Format** column, change the number of decimal points to be shown.
5. Click **OK**.

The new settings are applied to the Results Table. The settings are also saved and applied when a new Results Table is created or a previously saved Results Table is opened again.

**Tip!** Use the header row of the Results Table to adjust the column widths and column order. Drag the header border to change the width. Drag the column header to another location in the Results Table to change the column order. Click the filter icon (▼) on a column header to apply a filter to the column. When the **Export** button is used to export a Results Table, the column width, order, and filter settings are saved in the exported file.

---

## Create a Report

### Prerequisites

- In the Analytics workspace, a Results Table is open.
- 

**Tip!** To select the analytes to be included in a report, use the **Reportable** column in the Results Table. Refer to the section: [Results Table Columns](#).

---

1. Click **Reporting > Create Report and Save Results Table**.  
The Create Report dialog opens.
  2. Select a template from the **Template name** list.
  3. Select a report format.
  4. To change the file name and location, click **Browse**, navigate to a different location, type a **File name**, and then click **Save**.
- 

**Note:** By default, reports are saved in  
`ProgramData\SCIEX\Analytics\Reporter\Reports`.

---

5. Click the **Create an individual report for each sample** check box, if required.
  6. (Optional) Select a different logo for the report:
    - a. Click **Replace Logo**.
    - b. Use the options in the Replace Logo dialog to modify the logo as required.
    - c. Click **Save**.
    - d. Click **Cancel**.
  7. Click **View Pages** to view the report layout.
  8. Click **Create**.
-

## Operating Instructions—Processing

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**Tip!** To report the selected results using a template such as Per Sample Quant, Per Sample Qual, Per Sample Visible Rows Using Visible Analytes, or Positive Hits Qual, use filters or hide the unwanted rows in the Results Table.

---

**Tip!** Click the example in the **Template View** in the Create Report dialog to view the report template layout. To view a specific template, the user must have a jpg file with the same name as the template in addition to the suffix [Snapshot\_X], where X is the snapshot number in the sequence. Do not use spaces between the file name and the suffix.

For example, All Peaks Qual.docx template would be named: All Peaks Qual[Snapshot\_1].jpg All Peaks Qual[Snapshot\_2].JPG All Peaks Qual[Snapshot\_3].jpg

---

## Export and Save a Results Table

Prerequisites
<ul style="list-style-type: none"><li>In the Analytics workspace, a Results Table is open.</li></ul>



---

**Tip!** To select the analytes to be exported, use the **Reportable** column in the Results Table. Refer to the section: [Results Table Columns](#).

---

1. Click **Reporting > Export results > Export and save Results Table**.

The Export dialog opens.

2. Select the options as required.

For descriptions of the options, refer to the document: *Help System*.

3. Click **OK**.

## Export Results Table – Metric

Prerequisites
<ul style="list-style-type: none"><li>In the Analytics workspace, a Results Table is open.</li></ul>



---

**Note:** The manufacturer assumes no responsibility or contingent liability, including indirect or consequential damages, after data has been exported from the Analytics workspace.

---

Exporting Results Tables is one of the controlled methods for data output in the Analytics workspace.

This feature is used to create a tab-delimited text file containing the information from the active Results Table. Information is exported for all samples and either all components or just the visible components for the one selected metric or field.

1. Click **Reporting > Export results > Results Table - Metric**.

The Export Metric dialog opens.

2. Select the column to export in the **Metric** field, and then set the options. Refer to the document: *Help System*.
3. Click **OK**.

### Transfer Results to a Watson LIMS

Prerequisites
<ul style="list-style-type: none"><li>• A Results Table is open and locked.</li><li>• The Watson LIMS software is open.</li></ul>



---

**Note:** A subset of the columns in the Results Table is transferred, including some hidden columns, and some that are not designated as **Reportable**.

---

1. Click **Reporting > Initiate Transfer to Watson LIMS**.  
The transfer dialog opens.
2. In the Watson LIMS software, import the data.
3. In the transfer dialog in SCIEX OS, do one of the following:
  - If the transfer was successfully completed, then click **Confirm**.
  - If the transfer was not successfully completed, then click **Decline**.

### Transfer Results to Another LIMS

Prerequisite Procedures
<ul style="list-style-type: none"><li>• Configure the LIMS in the Configuration workspace. Refer to the section: <a href="#">Select Laboratory Information Management System (LIMS) Settings</a>.</li><li>• Open a locked Results Table.</li></ul>



---

**Tip!** To select the analytes to be exported, use the **Reportable** column in the Results Table. Refer to the section: [Results Table Columns](#).

---

1. Click **Reporting > Transfer Results to LIMS**.

## Operating Instructions—Processing

---

The LIMS Transfer dialog opens.

2. Select a template from the **Template** list.
3. Click **Transfer**.

## Work With Results Tables

Results Tables summarize the calculated concentration of an analyte, as well as the qualitative analysis results such as library hits, formula finder results, and so on, in each unknown sample based on the calibration curve. Results Tables also include the calibration curves, as well as statistics for the results. The user can customize the Results Tables and view the Results Tables in different layouts.

---

**Note:** Results Table columns with an asterisk (\*) are custom text or calculated columns.

---

The data from a Results Tables can be exported to a txt file for use in other applications, such as Microsoft Excel. The user can export all of the data in the Results Table or just the data in the visible columns.

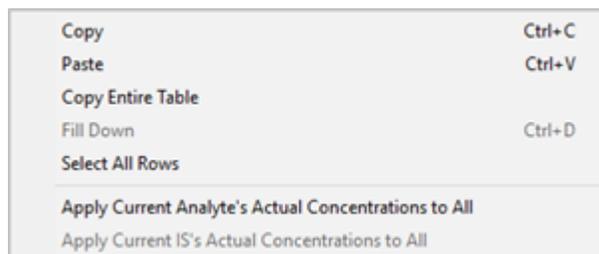
---

**Tip!** If multiple sessions of Results Tables have been tiled either vertically or horizontally, then click **Views > Reset layout** to return the Results Tables to their original layout.

---

Use the right-click menu to edit the Results Table rows. To show this menu, click the right mouse button anywhere in the Results Table.

**Figure 6-24 Right-Click Menu**



**Table 6-4 Right-Click Menu Commands**

Label	Description
<b>Copy</b>	Use this option to copy the current data to the clipboard.
<b>Paste</b>	Use this option to paste data from the clipboard in the current view.
<b>Copy Entire Table</b>	Use this option to copy the entire table to the clipboard.
<b>Fill Down</b>	(Components) Use this option to replicate the information in the first selected row to all of the subsequent selected rows.

Table 6-4 Right-Click Menu Commands (continued)

Label	Description
<b>Select All Rows</b>	Use this option to select all of the rows in the currently active Results Table. This is useful if the user subsequently wants to apply a command, such as <b>Copy</b> , that operates on the selected rows.
<b>Apply Current Analyte's Actual Concentrations to All</b>	<p>(Analytes) If there is more than one analyte and all of the analytes are present in these samples at the same concentration, then use this option to provide a shortcut for setting the actual concentration field for all of the analytes for the standard samples. To use this feature:</p> <ol style="list-style-type: none"> <li>1. Use the <b>Components and Groups List</b> to show only one specific analyte in the table. Refer to the section: <a href="#">Components and Groups List</a>.</li> <li>2. (Optional) Filter the <b>Sample Type</b> column to view only standard samples.</li> <li>3. Specify the actual concentration for the analyte, either by typing in the cells or by selecting the column and pasting text in it.</li> <li>4. Select <b>Apply Current Analyte's Actual Concentrations to All</b>.</li> </ol> <p>Return to viewing all of the components and sample types, as required.</p>

Table 6-4 Right-Click Menu Commands (continued)

Label	Description
<b>Apply Current IS's Actual Concentrations to All</b>	<p>(Internal standards) If there is more than one internal standard and all of the internal standards are present in these samples at the same concentration, then use this option to provide a shortcut for setting the actual concentration field for all of the internal standards for the standard samples. To use this feature:</p> <ol style="list-style-type: none"> <li>1. Use the <b>Components and Groups List</b> to show only one specific internal standard in the table. Refer to the section: <a href="#">Components and Groups List</a>.</li> <li>2. (Optional) Filter the <b>Sample Type</b> column to view only standard samples.</li> <li>3. Specify the actual concentration for the internal standard, either by typing in the cells or by selecting the column and pasting text in it.</li> <li>4. Select <b>Apply Current IS's Actual Concentrations to All</b>.</li> </ol> <p>Return to viewing all of the components and sample types, as required.</p>

## Results Table Filters

Use the fields at the top of the Results Table to view and filter content.

Figure 6-25 Filtering Controls



Table 6-5 Results Table Filters

Label	Description
<b>x of y rows</b>	Shows the number of visible rows (x) out of the total number of rows (y).
<b>Filters</b>	Shows the number of columns to which filters are applied.

**Table 6-5 Results Table Filters (continued)**

Label	Description
<b>Qualify for Rules Filters</b>	Toggles the view of the Results Table between the rows that match the acceptance criteria filters or confidence traffic filters and those that do not. Acceptance criteria and confidence traffic lights are applied in the processing method.
<b>Reapply Filter</b>	Reapplies the filter after a change to the Results Table, such as a change to the area count.  <b>Note:</b> All filters are automatically reapplied when another filter is added or changed.
<b>Clear</b>	Clears all filters.

## Results Table Columns

**Note:**

- Columns with an asterisk (\*) are custom text columns, calculated columns, or columns created as a results of a combined flagging rule.
- Columns with names that begin with an underscore ( \_ ) are custom batch columns that have the same name as a predefined Results Table column or formula.
- The **Format** column indicates how the field is validated in formulas.
- In columns that contain numbers, users can change the number format and the number of significant digits. Choose between **Decimal**, **Significant Digits**, or **Scientific Notation** in the **Number Format** column and then type the number of significant digits in the **Number Format Precision** column in the Results Table Display Settings dialog.

Label	Description	Format	LIS Supported
<b>Accuracy</b>	Shows the accuracy of standards and quality control (QC) samples. For other sample types, this value is set to <b>N/A</b> .  For standards of known concentration, the accuracy of standards and QC samples is defined as $100\% \times (\text{Calculated Concentration})/(\text{Actual Concentration})$ .	Number	Y

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Label	Description	Format	LIS Supported
<b>Accuracy Acceptance</b>	Shows the acceptance status of the accuracy.	Text	N
<b>Acq. Method Name</b>	Shows the name of the acquisition method used to acquire the sample.	Text	Y
<b>Acquisition Date &amp; Time</b>	Shows the date and time at which the sample was acquired.	Text	Y
<b>Actual Concentration</b>	For standards and QC samples, shows the expected known concentration.	Number	Y
<b>Adduct/ Charge</b>	Shows the adduct or charge state of the compound. In the targeted workflow, this value is set by the user. In the non-targeted workflow, this value is automatically set by the software if grouping by adduct is enabled.	Text	N
<b>Area</b>	Shows the detected peak area. If no peak was detected, then this value is set to <b>N/A</b> .	Number	Y
<b>Area / Height</b>	Shows the detected peak area divided by the height. If a peak was not detected, then this value is set to <b>N/A</b> .	Number	N
<b>Area Ratio</b>	For analytes that use an internal standard, shows the ratio of the analyte <b>Area</b> to the <b>IS Area</b> . For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	Y

Label	Description	Format	LIS Supported
<b>Area Ratio of comparison</b>	<p>Shows the area ratio of the sample/control sample.</p> <ul style="list-style-type: none"> <li>• If no peak is found in the control, then the value is <b>N/A</b>.</li> <li>• If no peak is found in the sample, then the value is 0.</li> <li>• If every peak in the sample is below the <b>Area Ratio Threshold</b>, then the value is <b>N/A</b>.</li> <li>• If a comparison sample is not used, then the value is <b>No control sample</b>.</li> <li>• For the control sample, the area ratio for found peaks is always 1.</li> </ul> <p>Applicable to qualitative workflows only.</p>	Number	N
<b>Asymmetry Factor</b>	<p>Shows the distance from the center line of the peak to the back slope, divided by the distance from the center line of the peak to the front slope, with all of the measurements made at 10% of the maximum peak height.</p>	Number	Y
<b>AutoPeak Asymmetry</b>	<p>Shows the ratio of the asymmetry of the integrated peak to the symmetry expected based on the model. A ratio of 1 indicates a good fit. If the value is not 1, then the ion source might be saturated, or the integration might not be correct.</p> <p>Applicable only to processing methods that use the AutoPeak integration algorithm.</p>	Number	N

## Operating Instructions—Processing

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Label	Description	Format	LIS Supported
<b>AutoPeak Candidate Model Quality</b>	<p>Shows the suitability of the peak for use in the creation of a peak model. If the value is significantly greater than 1, then the sample used to create the quantitation method is unsuitable. Use a peak with a larger response to create the model, and then apply that peak to all samples.</p> <p>Applicable only to processing methods that use the AutoPeak integration algorithm.</p>	Number	N
<b>AutoPeak Group Confidence</b>	<p>Shows the probability that the group of real peaks is integrated and that the integration does not include a false positive noise peak.</p> <p>Applicable only to processing methods that use the AutoPeak integration algorithm.</p>	Number	N
<b>AutoPeak Integration Quality</b>	<p>Shows the quality of the data. The quality is represented as a value from 0 to 1. If the quality is less than 0.6, then investigate the integration further.</p> <p>Applicable only to processing methods that use the AutoPeak integration algorithm.</p>	Number	N
<b>AutoPeak Model Source</b>	<p>Shows the names of the samples and components that were used for peak modeling. If the component used for modeling is not the same as the component that was integrated, then review the model to determine whether it is appropriate.</p> <p>Applicable only to processing methods that use the AutoPeak integration algorithm.</p>	Number	N

Label	Description	Format	LIS Supported
<b>AutoPeak Num Peaks</b>	Shows the number of adjacent convoluted peaks that were detected by the algorithm.  Applicable only to processing methods that use the AutoPeak integration algorithm.	Number	N
<b>AutoPeak Peak Width Confidence</b>	Shows the level of confidence in the peak width. A value of 1 indicates that the actual peak width and the expected peak width are equal. A value greater than 1 indicates that the actual peak width is greater than the expected peak width. A value less than 1 indicates that the actual peak width is less than the expected peak width, or that the peak is broader because of a change in chromatographic conditions.  Applicable only to processing methods that use the AutoPeak integration algorithm.	Number	N
<b>AutoPeak Saturated acc</b>	If the <b>Saturation correction</b> option was used and the corresponding peak was saturated, so that the fitted model extends above the peak, then this field shows <b>Yes</b> . Otherwise, the column is blank. If the accuracy and %CVs for samples at higher concentrations are not within acceptable ranges, then adjust the <b>Saturation correction</b> .  Applicable only to processing methods that use the AutoPeak integration algorithm.	Text	N

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Label	Description	Format	LIS Supported
<b>Barcode</b>	Shows the unique ID for a sample. The unique ID is initialized from the value originally specified in the batch used to acquire the data.  The <b>Barcode</b> can contain up to 20 characters. The <b>Barcode</b> cannot contain any of these invalid characters: \ / : * ? " < >  = or characters 0 to 31 from the ASCII table.	Text	Y
<b>Baseline Delta/Height</b>	Shows the absolute value of the difference between the height of the baseline, at the start of the peak and the end of the peak, and the actual peak height. Values greater than 0.1 indicate that the baseline might not have been integrated correctly and that the peak should be reviewed.	Number	N
<b>Calculated Concentration</b>	For standards of known concentration, shows the value of the back-calculated concentration from the calibration curve. Regression equations describe how the regression is performed for the various regression types and weighting.	Number	Y
<b>Combined Score</b>	(Optional) Shows a single number score that can be used for relative comparison purposes.  Applicable to qualitative workflows only.	Number	N
<b>Comparison</b>	Shows the components in the comparison sample.	Number	N
<b>Component Comment</b>	Shows an arbitrary comment for the analyte or internal standard that applies to all of the samples.	Text	N
<b>Component Group Name</b>	Shows any group name associated with the analyte or internal standard.	Text	N
<b>Component Index</b>	Shows the index of the analyte or internal standard in the original processing method.	Number	Y

Label	Description	Format	LIS Supported
<b>Component Name</b>	<p>Shows the name of the analyte or internal standard.</p> <p>This column is always visible in the Results Table. In the Column Settings dialog, the check box is not available.</p> <p>The <b>Component Name</b> can contain up to 50 characters.</p> <hr/> <p><b>Note:</b></p> <ul style="list-style-type: none"> <li>• The <b>Component Name</b> can only be changed in the processing method and not in the Results Table.</li> <li>• This column is mandatory for a Laboratory Information Management System (LIMS) transfer.</li> </ul>	Text	Y
<b>Component Type</b>	Shows the analyte type: <b>Quantifier</b> , <b>Qualifier</b> , or <b>Internal Standard</b> .	Text	N
<b>Conc. Units</b>	Shows the concentration units.	Text	Y
<b>Concentration Acceptance</b>	Shows the acceptance status of the calculated concentration.	Number	N
<b>Concentration Ratio</b>	For analytes that use an internal standard, shows the ratio of the <b>Actual Concentration</b> to the <b>IS Actual Concentration</b> . For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>Difference from Average Sample Time</b>	Shows the difference between the analysis time for this sample and the average analysis time for all samples.	Number	N
<b>Dilution Factor</b>	Shows the factor by which the sample has been diluted. This factor is used in the calculation of the calibration curve.	Number	Y
<b>End Time</b>	Shows the ending retention time of the detected peak, in minutes.	Number	Y

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>End Time at 10%</b>	Shows the time, in minutes, along the back side of the peak where the intensity is at 10% of the peak height.	Number	N
<b>End Time at 5%</b>	Shows the time, in minutes, along the back side of the peak where the intensity is at 5% of the peak height.	Number	N
<b>Expected Ion Ratio</b>	Shows the expected ion ratio for unknown, QC, and standard samples.  For each component in a group, the <b>Expected Ion Ratio</b> is the average of the ion ratios of its standards. A standard is not included in the calculation of the <b>Expected Ion Ratio</b> of the component if these conditions apply:  1. The peak area is N/A. 2. The Use column is not selected.	Number	Y
<b>Expected RT</b>	Shows the original expected retention time from the processing method, in minutes.	Number	Y
<b>Expected MW</b>	Shows the original expected molecular weight, from the processing method, in Da.  Applicable to mass reconstruction workflows only.	Number	Y
<b>Formula</b>	(Optional) Shows a valid chemical formula. If the chemical formula is invalid, then it is not retained by the software. If the chemical formula is valid, then the <b>Mass (Da)</b> and <b>Isotope</b> columns are auto-populated.	Text	Y

Label	Description	Format	LIS Supported
<b>Formula Confidence</b>	<p>Shows, the level of confidence in the <b>Formula Finder Score</b>, as a percentage. It is calculated based on:</p> <ul style="list-style-type: none"> <li>• How well the current MS spectrum fits the theoretical spectrum for the compound, based on mass.</li> <li>• How well the acquired MS/MS spectrum fits the MS/MS spectrum found in the LibraryView software database.</li> </ul> <p>The MS spectrum score has twice the weight of the MS/MS spectrum score.</p> <p>Applicable to qualitative workflows only.</p>	Text	N
<b>Formula Finder</b>	<p>Shows the single number score that can be used for relative comparison purposes. The value is can be updated using data from the Peak Review Formula Finder Results Table.</p> <p>Applicable to qualitative workflows only.</p>	Number	N
<b>Formula Finder Results</b>	<p>(Optional) Shows the best match of the formula finder results.</p> <p>Applicable to qualitative workflows only.</p>	Text	N
<b>Formula Finder Score</b>	<p>(Optional) Shows a single number score that can be used for relative comparison purposes.</p>	Number	Y
<b>Found at Fragment</b>	<p>(Optional) Shows the best requested Fragment Mass (Da) at which matching spectra were found.</p> <p>Applicable to qualitative workflows only.</p>	Number	Y

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>Found at Mass</b>	(Optional) Shows the best requested Extraction Mass (Da) at which the matching spectra were found.  Applicable to qualitative workflows only.	Number	Y
<b>Fragment Mass</b>	(Optional) Shows the fragment mass, as specified in the method. The precursor of the fragment is extracted from the MS/MS in the <b>Extraction Mass (Da)</b> column. When provided, this value must be numeric.	Number	Y
<b>Fragment Mass Error (ppm)</b>	(Optional) Shows the difference between the Found at Fragment and the Fragment Mass, in ppm.	Number	Y
<b>Fragment Mass Error (mDa)</b>	(Optional) Shows the difference between the Found at Fragment and the Fragment Mass, in mDa.	Number	Y
<b>Fragment Mass Error Confidence</b>	(Optional) Shows the level of confidence in the fragment mass error.	Text	Y
<b>Height</b>	Shows the detected peak height. If a peak was not detected, then this value is set to <b>N/A</b> .	Number	Y
<b>Height Ratio</b>	For analytes that use an internal standard, shows the ratio of the <b>Height</b> to the <b>IS Height</b> . For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	Y
<b>Index</b>	Shows the index of the row in the original, unsorted order. If the table is sorted based on another column, then it can be returned to the original order by sorting on this column.	Number	N
<b>Injection Volume</b>	Shows the volume of the sample stored in the method and injected by the autosampler.	Number	Y

Label	Description	Format	LIS Supported
<b>Integration Acceptance</b>	<p>Shows how closely the peak integration meets the acceptance criteria. It is calculated based on these factors, as configured in the flagging rules:</p> <ul style="list-style-type: none"> <li>• Integration quality</li> <li>• Assymetry factor</li> <li>• Total peak width, in minutes</li> <li>• Retention time error, measured as a percentage or in minutes</li> </ul>	Number	N
<b>Integration Type</b>	<p>Shows the type of integration.</p> <ul style="list-style-type: none"> <li>• <b>Baseline:</b> A standalone peak that was integrated in the usual way.</li> <li>• <b>Valley:</b> Indicates that there were two adjacent peaks and that the signal did not return to the baseline value between them.</li> <li>• <b>N/A:</b> Indicates that a peak was not detected.</li> <li>• <b>Manual:</b> Indicates that the peak was manually integrated.</li> </ul>	Text	Y

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>Ion Ratio</b>	<p>Shows the ion ratio. Ion ratios are determined when at least two MRM transitions from a single analyte have been collected in a group.</p> <p>All of the analytes in a group constitute an analyte subgroup. All of the internal standards in a group constitute an IS subgroup. The first component in a subgroup is used as a quantifier ion. The remainder of the components in the subgroup are used as qualifier ions.</p> <p><i><math display="block">\text{Ion Ratio} = \frac{\text{Peak Area or Height of Qualifier}}{\text{Peak Area or Height of Quantifier}}</math></i></p> <p>The ion ratio can be calculated for either the peak area or the peak height. If the processing method uses the area for the regression of the first component, that is, the component for which the component index is 1, in the Results Table, then the peak area is used to calculate the ion ratio for the entire Results Table. If the height is used for the regression of the first component, then the peak height is used for the calculation.</p> <ul style="list-style-type: none"> <li>• If a component is not a member of a group, then the <b>Ion Ratio</b> value is set to <b>N/A</b>.</li> <li>• If a peak is not found, then the <b>Ion Ratio</b> value is set to <b>N/A</b>.</li> <li>• If the ion ratio is applied to all of the components in both of the analyte and IS subgroups, then the qualifier is the quantifier.</li> <li>• If the integration changes for either of the quantifier or the qualifier peaks, then the ion ratio is calculated again.</li> </ul>	Number	Y

Label	Description	Format	LIS Supported
	<hr/> <p><b>Note:</b> The user can define flagging rules for the ion ratio in the processing method.</p> <hr/>		
<b>Ion Ratio Acceptance</b>	Shows the acceptance status of the ion ratio.	Number	N
<b>Ion Ratio Confidence</b>	Shows the level of confidence in the ion ratio.  Applicable to qualitative workflows only.	Text	N
<b>IS</b>	Shows whether the row is an internal standard. A selected check box indicates that the component for the row is an internal standard, not an analyte.  <hr/> <b>Note:</b> The <b>IS</b> check box is automatically selected for sample names containing .heavy or -cis because these samples are defined as internal standards in proteomics workflows. For other workflows, they are not internal standards, so the <b>IS</b> check box should be cleared.	Number	N
<b>IS Actual Concentration</b>	Shows the actual concentration of the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Area</b>	Shows the area for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Area / Height</b>	Shows the ratio of the <b>IS Area</b> to the <b>IS Height</b> for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N

## Operating Instructions—Processing

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Label	Description	Format	LIS Supported
<b>IS Baseline Delta / Height</b>	Shows the absolute value of the height difference between the baseline, at the start of the peak and the end of the peak, and the actual peak height for the internal standard. Values greater than 0.1 indicate that the baseline might not have been integrated correctly and that the peak should be reviewed.	Number	N
<b>IS Comment</b>	Shows an arbitrary comment for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Text	N
<b>IS End Time</b>	Shows the time that the acquisition ends for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Expected MW</b>	Shows the expected molecular weight for the internal standard associated with the current analyte, in Da. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .  Applicable to mass reconstruction workflows only.	Number	Y
<b>IS Expected RT</b>	Shows the expected retention time for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Height</b>	Shows the height for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N

Label	Description	Format	LIS Supported
<b>IS Integration Type</b>	Shows the type of integration for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Text	N
<b>IS Mass Info</b>	Shows the mass information for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Text	N
<b>IS MW</b>	Shows the found molecular weight for the internal standard associated with the current analyte, in Da. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .  Applicable to mass reconstruction workflows only.	Number	N
<b>IS MW Delta (Da)</b>	Shows the difference between the expected and found molecular weight for the internal standard, in Da.  Applicable to mass reconstruction workflows only.	Number	Y
<b>IS MW Delta (ppm)</b>	Shows the difference between the expected and found molecular weight for the internal standard, in ppm.  Applicable to mass reconstruction workflows only.	Number	Y
<b>IS Name</b>	Shows the name of the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Text	N

## Operating Instructions—Processing

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Label	Description	Format	LIS Supported
<b>IS Peak Comment</b>	Shows the peak comment for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Text	N
<b>IS Quality</b>	Shows the quality of the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Region Height</b>	Shows the height for the internal standard region. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Retention Time</b>	Shows the retention time for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Signal / Noise</b>	Shows the signal-to-noise ratio for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Start Time</b>	Shows the start time for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>IS Total Width</b>	Shows the total width for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N

Label	Description	Format	LIS Supported
<b>IS Width at 50%</b>	Shows the width at 50% for the internal standard associated with the current analyte. For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	N
<b>Isotope Confidence</b>	Shows the level of confidence in the isotope ratio.  Applicable to qualitative workflows only.	Text	N
<b>Isotope Ratio Difference</b>	Identifies the difference between the theoretical isotope pattern, based on the formula, and isotope pattern from the acquired spectra.  Applicable to qualitative workflows only.	Number	N
<b>LC Method</b>	Shows the name of the LC method used to acquire the data.	Text	N
<b>Library Confidence</b>	Shows the level of confidence in the <b>Library Hit</b> based on the <b>Library Score</b> of the hit.  Applicable to qualitative workflows only.	Text	N
<b>Library Hit</b>	Shows the compound name of the best library match, that is, the compound with the highest purity score and the formula matching the requested formula.  The value is can be updated using data from the Peak Review Library Search Results grid. Applicable to qualitative workflows only.	Text	N
<b>Library Score</b>	Shows how well the library match fits the found mass.  Applicable to qualitative workflows only.	Number	N

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>Mass Error (ppm)</b>	Shows the difference between the found mass and the extraction mass, expressed in parts per million.  Applicable to qualitative workflows only.	Number	N
<b>Mass Error (mDa)</b>	Show the difference between the found mass and the extraction mass, expressed in milliDaltons.  Applicable to qualitative workflows only.	Number	N
<b>Mass Error Confidence</b>	Shows the level of confidence in mass error.  Applicable to qualitative workflows only.	Text	N
<b>Mass Info</b>	Shows the mass information associated with the component.  <ul style="list-style-type: none"> <li>• For MRM experiments, this is Q1/Q3 and for profile, or full scan, experiments it is Start - Stop.</li> <li>• For UV, ADC, or DAD experiments, it is the wavelength (DAD) or channel information (UV/ADC).</li> </ul> <p>If the fragment mass exists it will be used for XIC extraction.</p> <p>If there is no fragment mass, then the precursor mass should be used for XIC extraction.</p>	Text	Y
<b>Modified</b>	Shows whether the peak-finding parameters have been modified. A selected check box indicates that the peak-finding parameters in the processing method have been modified, using the Peak Review pane.	Number	Y
<b>MS Method</b>	Shows the name of the MS method used to acquire the data.	Text	N

Label	Description	Format	LIS Supported
<b>MW</b>	Shows the found molecular weight of the analyte, from the reconstructed graph, in Da.  Applicable to mass reconstruction workflows only.	Number	Y
<b>MW Delta (Da)</b>	Shows the difference between the expected and found molecular weight, in Da.  Applicable to mass reconstruction workflows only.	Number	Y
<b>MW Delta (ppm)</b>	Shows the difference between the expected and found molecular weight, in ppm.  Applicable to mass reconstruction workflows only.	Number	Y
<b>Non-Targeted Peak</b>	Indicates whether the peak was found by the Enhanced Peak Finder.  Applicable to qualitative workflows only.	Number	N
<b>Operator Name</b>	Shows the name of the instrument operator who acquired the sample.	Text	Y
<b>Original Filename</b>	Shows the name of the file.	Text	Y

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>Outlier Reasons</b>	<p>When the automatic removal of outliers has been enabled in the quantitation method, shows which criterion was found to be outside of the predetermined limits for the component.</p> <p>The <b>Outlier Reasons</b> column is linked to the rules for automatic removal of outliers in the quantitation method. It is a preset column in the Results Table.</p> <p>The reason the outlier is flagged:</p> <ul style="list-style-type: none"> <li>• Accuracy</li> <li>• Concentration</li> <li>• Ion ratio</li> </ul> <p>If there is a peak for only one of the quantifier or qualifier, then the ion ratio is flagged for both components. If neither of these components have peaks, then the ion ratio is not flagged for either of the components.</p> <ul style="list-style-type: none"> <li>• Cannot calculate the expected ion ratio</li> <li>• A custom flagging rule created by the user has failed</li> </ul>	Text	N
<b>Peak Comment</b>	Shows an arbitrary comment for the row.	Text	N
<b>Plate Number</b>	Shows the plate number of the autosampler used to acquire the data, as indicated in the Batch Editor.	Text	Y
<b>Points Across Baseline</b>	Shows the number of scans across the peak.	Number	N
<b>Points Across Half Height</b>	Shows the number of scans across the peak, at approximately 50% of the height.	Number	N
<b>Polarity</b>	Shows the polarity of the experiment used to acquire the sample.	Text	N

Label	Description	Format	LIS Supported
<b>Precursor Mass</b>	Shows the processing input parameters taken from the processing method.  This column is always visible in the Results Table. In the Column Settings dialog, the check box is not available.	Number	N
<b>Proc. Method Name</b>	Shows the name of the processing method that created the Results Table.	Text	Y
<b>Quality</b>	Shows the quality of the integrated peak. The area of the integrated peak and the area of a larger RT window are compared. A value of 0 indicates that the peak is poorly integrated, or that a peak is not present. A value of 1.0 indicates a well-integrated peak that need not be reviewed.	Number	N
<b>Rack Number</b>	Shows the rack number of the autosampler used to acquire data, as specified in the Batch Editor.	Text	Y
<b>Region Height</b>	Shows the peak height of the largest peak in the vicinity of the detected peak. This is useful in conjunction with the <b>Quality</b> field. Peaks with a low quality that also have a reasonable <b>Region Height</b> must be reviewed. If the <b>Region Height</b> is small, then a significant peak is not present.	Number	N
<b>Relative RT</b>	For analytes that are using an internal standard, shows the ratio of the <b>Retention Time</b> to the <b>IS Retention Time</b> . For internal standards, or for analytes without an internal standard, this value is set to <b>N/A</b> .	Number	Y
<b>Reportable</b>	Shows whether the result is included in reports, exports, and LIMS transfers.	Number	Y
<b>Retention Time</b>	Shows the actual retention time of the detected peak, in minutes.	Number	Y
<b>Retention Time Delta (min)</b>	Shows the difference between the retention time defined for the mass and the actual retention time.	Number	N

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>Retention Time Error (%)</b>	Shows the percent error found between "Found at RT" and "Expected RT".  Applicable to qualitative workflows only.	Number	N
<b>RT Confidence</b>	Shows the confidence in the retention time.  Applicable to qualitative workflows only.	Text	N
<b>Sample Comment</b>	Shows a user-specified comment for the sample.	Text	Y
<b>Sample ID</b>	Shows a user-specified identifier for the sample. The <b>Sample ID</b> is specified in the Batch Editor prior to sample submission for acquisition.  If the standard addition workflow is enabled in the processing method, then the <b>Sample ID</b> is used as a group identifier for each standard addition group. SCIEX OS links each sample with an unknown analyte concentration to samples to which known and varying concentrations of the same analyte have been added.  The <b>Sample ID</b> can contain up to 252 characters. The <b>Sample ID</b> cannot contain any of these invalid characters: \ / : * ? " < >   = or characters 0 to 31 from the ASCII table.	Text	Y
<b>Sample Index</b>	Shows the index of the current sample.  <b>Note:</b> This column is locked and always displayed at the left side of the Results Table.	Number	Y

Label	Description	Format	LIS Supported
<b>Sample Name</b>	<p>Shows a user-specified name for the sample. The <b>Sample Name</b> is specified in the Batch Editor prior to sample submission for acquisition.</p> <p>The <b>Sample Name</b> must contain from 1 to 252 characters. The <b>Sample Name</b> cannot contain any of these invalid characters: \ / : * ? " &lt; &gt;   = or characters 0 to 31 from the ASCII table.</p> <hr/> <p><b>Note:</b> This column is locked and always displayed at the left side of the Results Table.</p> <hr/>	Text	Y
<b>Sample Type</b>	Shows the type of sample.	Text	Y
<b>Scanned Barcode</b>	Shows the barcode scanned prior to the injection.	Text	Y
<b>Signal / Noise</b>	<p>Shows an estimate of the ratio of the peak height for the detected peak to the noise present in the chromatogram.</p> <p>For the AutoPeak integration algorithm, noise is estimated using the calculated relative noise and the baseline at the peak apex position. The MQ4 algorithm uses a similar approach, except that the baseline is estimated using the entire chromatogram.</p>	Number	Y
<b>Slope of Baseline</b>	<p>Shows the slope of the integrated peak from the baseline:</p> $((intensity\ at\ peak\ stop) - (intensity\ at\ peak\ start)) \div peak\ width$	Number	N
<b>Start Time</b>	Shows the starting retention time of the detected peak, in minutes.	Number	Y
<b>Start Time at 10%</b>	Shows the time, in minutes, along the front side of the peak where the intensity is at 10% of the peak height.	Number	N

## Operating Instructions—Processing

Label	Description	Format	LIS Supported
<b>Start Time at 5%</b>	Shows the time, in minutes, along the front side of the peak where the intensity is at 5% of the peak height.	Number	N
<b>Std Addition Accuracy</b>	Shows the accuracy of samples of known concentrations that are quantified by the addition of standards of varying concentration. When the standard addition workflow is enabled in the processing method, the <b>Sample Type</b> for all samples is automatically set to <b>Standard</b> . If the <b>Sample Type</b> is changed to another type, or if the standard addition workflow is not enabled, then this value is set to <b>N/A</b> . For samples of known concentration, such as a quality control sample in a batch, the <b>Std Addition Accuracy</b> is defined as:  $100\% \times (\text{Std Addition Calculated Concentration}) / (\text{Std Addition Actual Concentration})$	Number	N
<b>Std Addition Actual Concentration</b>	Shows the user-specified expected known concentration for samples that are quantified by standard addition. For example, a quality control sample in a batch. If the <b>Sample Type</b> is not <b>Standard</b> , then this value is set to <b>N/A</b> .	Number	N

Label	Description	Format	LIS Supported
<b>Std Addition Calculated Concentration</b>	<p>Shows the value of the back-calculated concentration by extrapolating the standard addition curve to the X-intercept using linear regression and no weighting. For samples that are quantified by standard addition, the <b>Std Addition Calculated Concentration</b> is defined as:</p> $\text{Intercept/Slope}$ <p>If the <b>Sample Type</b> is not <b>Standard</b>, if the standard addition workflow is not enabled in the processing method, or if a peak is not found in the unspiked samples of a standard addition group, then this value is set to <b>N/A</b>.</p>	Number	N
<b>Tailing Factor</b>	<p>Shows the distance from the front slope of the peak to the back slope, divided by twice the distance from the center line of the peak to the front slope. All of the measurements are made at 5% of the maximum peak height.</p>	Number	N
<b>Time Since First Sample (min)</b>	<p>Shows the amount of time, in minutes, since the acquisition of the first sample started.</p>	Number	N
<b>Time Since Last Sample (sec)</b>	<p>Shows the amount of time, in seconds, since the acquisition of the last sample started.</p>	Number	N
<b>Total Width</b>	<p>Shows the chromatographic peak width, in minutes, at the baseline.</p>	Number	Y

## Operating Instructions—Processing

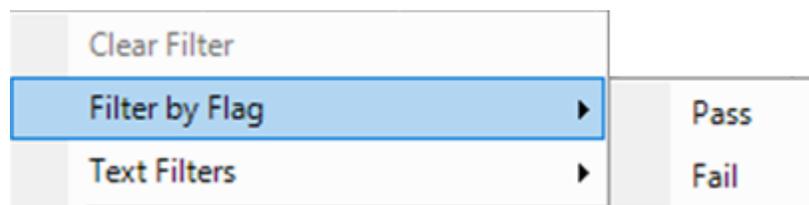
Label	Description	Format	LIS Supported
<b>Used</b>	Shows whether the result is used. <ul style="list-style-type: none"> <li>For all samples, a selected check box indicates that the result is used in the calculation of reference values and execution of flagging rules.</li> <li>For standard samples, a selected check box indicates that the result is used in the construction of the calibration curve, regression, and statistics calculations.</li> <li>For QC samples, a selected check box indicates that the result is used for the calculation of the quality control statistics.</li> <li>For other sample types, a selected check box indicates that the result is used in calculations.</li> </ul>	Number	Y
<b>Vial Number</b>	Shows the vial number in the autosampler used to acquire data, as originally specified in the batch.	Text	Y
<b>Width at 10%</b>	Shows the width of the peak, measured at 10% of the peak height.	Number	N
<b>Width at 5%</b>	Shows the width of the peak, measured at 5% of the peak height.	Number	N
<b>Width at 50%</b>	Shows the chromatographic peak width, in minutes, of the detected peak measured at half of the apex intensity.	Number	Y
<b>XIC Width (Da)</b>	Shows the width of the extracted ion chromatogram, in Daltons.	Number	Y
<b>XIC Width (ppm)</b>	Shows the width of the extracted ion chromatogram, in ppm (parts per million).	Number	Y

## Acceptance Filters

Use the **Filter by Flag** option in the Filter menu for a Results Table column to choose whether to filter the column based on the acceptance criteria. The Results Table can be filtered on acceptance criteria, as follows:

- **Pass:** Shows the rows that match the criteria that were defined in the processing method.
- **Fail:** Shows the rows that do not match the criteria that were defined in the processing method.

**Figure 6-26 Filter By Flag**



Acceptance filters can be selected for any column to which a flagging rule was applied, as well as the following acceptance criteria:

- Accuracy
- Accuracy Acceptance
- Asymmetry Factor
- Calculated Concentration
- Concentration Acceptance
- Integration Acceptance
- Quality
- Retention Time Delta (min)
- Retention Time Error (%)
- Total Width

## Confidence Traffic Lights

Use the acceptance criteria to define qualifying rows. A qualifying row is a row in which the acceptance criteria match the criteria defined in the processing method.

Figure 6-27 Qualifying Rows

Define a qualifying row:

Ion ratio	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Mass error	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Frag. mass error	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
RT	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<sup>14</sup> C Isotope	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Library	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
C <sub>n</sub> H <sub>n</sub> Formula	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

The traffic lights show the confidence status for each row to which a Qualitative Rule or Ion Ratio Acceptance rule is applied. For information on flagging rules, refer to the document: *Help System*.

**Tip!** The Results Table can be filtered using the confidence traffic light filters. Select the **Qualify for Rules Filters** check box to toggle the view of the Results Table between the rows that match the confidence filters and those that do not. Confidence filters include: Pass, Marginal, Fail, and N/A.

Table 6-6 Confidence Traffic Lights

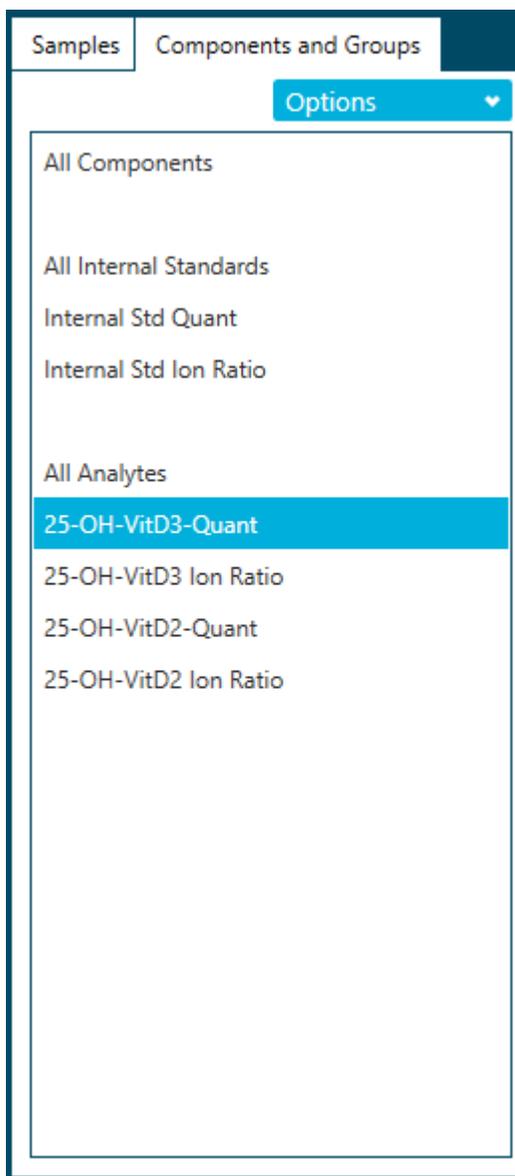
Traffic Light Icon	Description
	Shows which components meet the confidence levels defined in the processing method.
	Shows which components meet the marginal percent difference level defined in the processing method.
	Shows which components meet the unacceptable percent difference level defined in the processing method.
	Shows which confidence parameters are not applicable for the component.

## Components and Groups List

When a Results Table is open, a list of the current components and groups is shown on the left side of the main window. Use this list to change which components are visible in the results, as

well as in any linked Peak Review or Calibration Curve panes. All of the information is shown as it was defined in the processing method.

**Figure 6-28 Components and Groups**



Click an individual item in the list to show only the components for that item. Use **Shift+click** or **Ctrl+click** to select multiple items, for example, two specific analytes.

**Tip!** Change the width of the list by dragging the right edge of the pane to the left or right.

## Operating Instructions—Processing

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The order of the rows in the Results Table is not affected by filtering. The table is preset to be ordered first by sample and then by component, in the order indicated in the processing method.

**Table 6-7 Options**

Label	Description
<b>Show IS</b>	Click to show the rows in the Results Table for both the currently selected analyte and the corresponding internal standard. This is equivalent to clicking the analyte and then clicking the internal standard while pressing <b>Ctrl</b> , so that both are selected.
<b>Find</b>	Click to find the items in the list that match the specified text.

## Review Peaks

Prerequisite Procedures
<ul style="list-style-type: none"><li>• Open a Results Table.</li></ul>

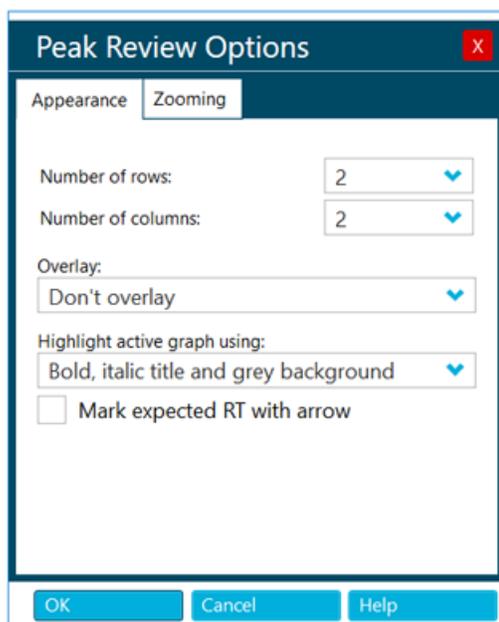
Use the Peak Review pane to:

- Visually inspect the raw chromatograms so that the quality of the peak-finding process can be determined.
- Correct chromatograms that did not integrate properly either by adjusting the peak-finding parameters or by manually selecting the starting and ending points for integration. After a chromatogram is re-integrated, the Results Table is automatically updated with the new peak area and other parameters.
- Visually inspect the MS and MS/MS spectra for the integrated XIC.
- Review the Formula Finding results and Library Search results and, if necessary, manually update the results in the Results Table.
- (Mass Reconstruction workflow) Visually inspect the Average spectrum and Reconstruction spectrum.
- (Mass Reconstruction workflow) Correct chromatograms in which the XIC region was not selected properly, either by adjusting the peak-finding parameters or by manually selecting the XIC region. After a new XIC region is selected, the Average spectrum and Reconstruction spectrum are generated again.
- (Mass Reconstruction workflow) Correct mass peaks that were not selected properly by adjusting the Mass Peak Selection parameters or by manually selecting the mass peak. After the mass peak is changed, the Results Table is automatically updated with the new peak and other parameters.

1. Click **Displays the peak review** ()

2. In the **Components and Group** list in the left pane, select a component.
3. (Optional) Customize the layout of the Peak Review pane with the **View** menu. For a description of the **View** options, refer to the document: *Help System*.
4. (Optional) Click **Options > Peak review display settings** to change the appearance of the Peak Review pane. For example, select the number of chromatograms to be viewed at one time. For descriptions of the options, refer to the document: *Help System*.

**Figure 6-29 Peak Review Options Dialog**



5. (Optional) To zoom in on a peak, use one of these methods:
  - Click **Options > Peak review display settings** and then click **Zooming** to change the zooming parameters of the peaks.
  - Drag the cursor over the region to be zoomed on the X-axis or Y-axis.
6. (Optional) To expand a peak to fill the entire Peak Review pane, select the peak and then click **Peak magnifier** ().

---

**Tip!** When an icon in the Peak Review pane is black, the corresponding feature is enabled. To disable it, click the icon again.

---

7. (Optional) To view and adjust the noise region on the graph, click **Options > Show Noise Regions** and then adjust the noise region, if applicable. Refer to the section: [Work With Noise Regions](#).

## Operating Instructions—Processing

---

**Note:** Noise regions can be adjusted only if the **Peak to Peak** or **Standard Deviation** signal-to-noise algorithm is being used.

---

8. If a chromatogram or a reconstruction graph contains multiple peaks and an incorrect peak is integrated, then drag across the correct peak to set a new expected retention time or expected molecular weight. If required, adjust the peak finding and integration parameters.
  9. (Optional) To apply the new parameters to all samples of the sample component or group, use the right-click menu options. For more information, refer to the section: [Work With Peaks in the Peak Review Pane](#).
- 

**Tip!** To view integrated peaks, click **Displays the peak review** (). In the Peak Review pane, select **Options > Show navigation controls**. Then click the navigation icons. For a description of the icons, refer to the document: *Help System*.

---

**Tip!** Clear the integration by clicking **Set peak to "not found"** (). The user can see the raw data before manually integrating the peak. The integration parameters cannot be edited.

---

10. Click **Enable manual integration mode** () in the Peak Review pane to use the Manual Integration mode.
  11. Drag the cursor from the base of one side of the peak of interest to the other. The peak is now manually integrated and the integration parameters used previously are unavailable.
- 

**Tip!** If the peak has just been modified, then the peak can be reverted to the original method by right-clicking and then clicking **Revert Peak to Original Method**.

---

**Tip!** To clear the manual integration and enable the integration parameter fields, clear the **Manual Integration** check box and then click **Enable manual integration mode** () again.

---

12. (Optional) To show the current peak in the Explorer workspace, click **Open data exploration** ().

The current zoom level is preserved.

---

**Note:** Manual integration of a peak persists until the user changes the integration for that peak in the Peak Review pane or edits the embedded method to change the component.

---

**Note:** In the mass reconstruction workflow, if a reconstruction mass peak is integrated manually, then the corresponding XIC region and average spectrum persist until the user changes the integration for that peak in the Peak Review pane or edits the embedded method to change the component.

## Work With Peaks in the Peak Review Pane

Table 6-8 Peak Review Features

To Do This	Do This
Copy integration parameters	<p>Use this command in conjunction with <b>Paste Integration Parameters</b> to copy the peak-finding parameters from one chromatogram to another. This command can be used if the same adjustment to the parameters needs to be made for several chromatograms.</p> <ol style="list-style-type: none"> <li data-bbox="600 814 1453 882">1. In a graph with an active chromatogram open, right-click, and then click <b>Copy Integration Parameters</b>.</li> <li data-bbox="600 903 1453 1003">2. To apply the change to all of the chromatograms for the component, use the <b>Update Processing Method for Component</b> command.</li> <li data-bbox="600 1024 1453 1125">3. To apply the change to all of the chromatograms for the group, use the <b>Update Processing Method for Group</b> command.</li> </ol>
Paste integration parameters	<p>Use this command in conjunction with <b>Copy Integration Parameters</b> to copy the peak-finding parameters from one chromatogram to another.</p> <ol style="list-style-type: none"> <li data-bbox="600 1291 1453 1358">1. In a graph with an active chromatogram open, right-click, and then click <b>Copy Integration Parameters</b>.</li> <li data-bbox="600 1379 1453 1446">2. Right-click in a different chromatogram and then click <b>Paste Integration Parameters</b>.</li> </ol>

**Table 6-8 Peak Review Features (continued)**

To Do This	Do This
Update the processing method for a component	<p>After adjusting the peak-finding parameters for a specific chromatogram, use this command to modify the copy of the processing method saved with the Results Table to use those parameters for the component.</p> <ul style="list-style-type: none"> <li>Adjust the peak-finding parameters, right-click, and then select <b>Update Processing Method for Component</b>.</li> </ul> <p>For the specific component, all samples are automatically integrated to use the new parameters and the Peak Review pane and Results Table are updated. If any peaks have been manually integrated, then the user is asked if the re-integration should apply to all peaks or only to those that were not manually integrated.</p>
Update the processing method for a group	<p>(Not applicable to the mass reconstruction workflow) Similar to the <b>Update Processing Method for Component</b> option, except that the integration applies to all components that belong to the same group as the component for the currently active chromatogram. If the user has assigned the various components to groups, and if the components assigned to any given group are expected to have the same retention time, then this command is useful because it allows the user to reset the parameters, including the expected retention time, for all components in the group at once. This command is not useful if the components for the groups do not have the same retention times.</p> <ul style="list-style-type: none"> <li>Adjust the peak-finding parameters, right-click, and then select <b>Update Processing Method for Group</b>.</li> </ul>

Table 6-8 Peak Review Features (continued)

To Do This	Do This
<p>Update the processing method for a group, excluding <b>Expected MW</b></p>	<p>(Mass reconstruction workflows only) Similar to the <b>Update Processing Method for Component</b> option, except that the integration applies to all components that belong to the same group as the component for the currently active chromatogram and reconstruction graph. If the user has assigned the various components to groups, and if the components assigned to any given group are expected to have the same retention time and integration parameters, then this command is useful because it allows the user to reset the parameters, including the expected retention time, for all components in the group at once. This command is not useful if the components for the groups do not have the same retention times. This command is not applied to <b>Expected MW</b>.</p> <ul style="list-style-type: none"> <li>Adjust the peak-finding and integration parameters, right-click, and then select <b>Update Processing Method for Group, Excluding Expected MW</b>.</li> </ul>
<p>Apply integration parameters to a sample within a group</p>	<p>(Not applicable to the mass reconstruction workflow) After adjusting the peak-finding parameters for a specific chromatogram, use this command to apply the parameters to all compounds in a sample that belong to the same group as the compound that was changed.</p> <ul style="list-style-type: none"> <li>Adjust the peak-finding parameters for the chromatogram, right-click, and then select <b>Apply integration parameters to sample within a group</b>.</li> </ul>
<p>Apply integration parameters to a sample within a group, excluding <b>Expected MW</b></p>	<p>(Mass reconstruction workflows only) After adjusting the peak-finding parameters for a specific chromatogram and the integration parameters for a reconstruction graph, use this command to apply the parameters to all compounds in a sample that belong to the same group as the compound that was changed. This command is not applied to <b>Expected MW</b>.</p> <ul style="list-style-type: none"> <li>Adjust the peak-finding for the chromatogram and the integration parameters for the deconvoluted mass peak, right-click, and then select <b>Apply integration parameters to sample within a group, excluding Expected MW</b>.</li> </ul>

**Table 6-8 Peak Review Features (continued)**

To Do This	Do This
Revert a peak to the original method	<p>After adjusting the peak-finding parameters for a specific chromatogram, use this command to apply the original parameters from the copy of the processing method saved with the Results Table to the chromatogram.</p> <ul style="list-style-type: none"><li>In a graph with an active chromatogram open, right-click, and then select <b>Revert Peak to Original Method</b>.</li></ul>
Revert all peaks for a component	<p>After adjusting the peak-finding parameters for some chromatograms, use this command to apply the original parameters from the copy of the processing method saved with the Results Table to all chromatograms for the same component as the active chromatogram. If any peaks have been manually integrated, then the user is asked if the re-integration should apply to all peaks or only to those that were not manually integrated.</p> <ul style="list-style-type: none"><li>In a graph with an active chromatogram open, right-click, and then select <b>Revert All Peaks for Component</b>.</li></ul>

### Work With Noise Regions

If the **Peak to Peak** or **Standard Deviation** signal-to-noise algorithm is used, then the noise regions can be adjusted interactively in the Integration page of the processing method and the Peak Review pane.

- Click the noise region on the graph and move it to the required location.
- Move the cursor over the left or right edge of the noise region until the double-headed arrow is shown. Then drag the edge to the required position to adjust the size of the noise region.

### Analyze Peaks Using Library Search or Formula Finder Results

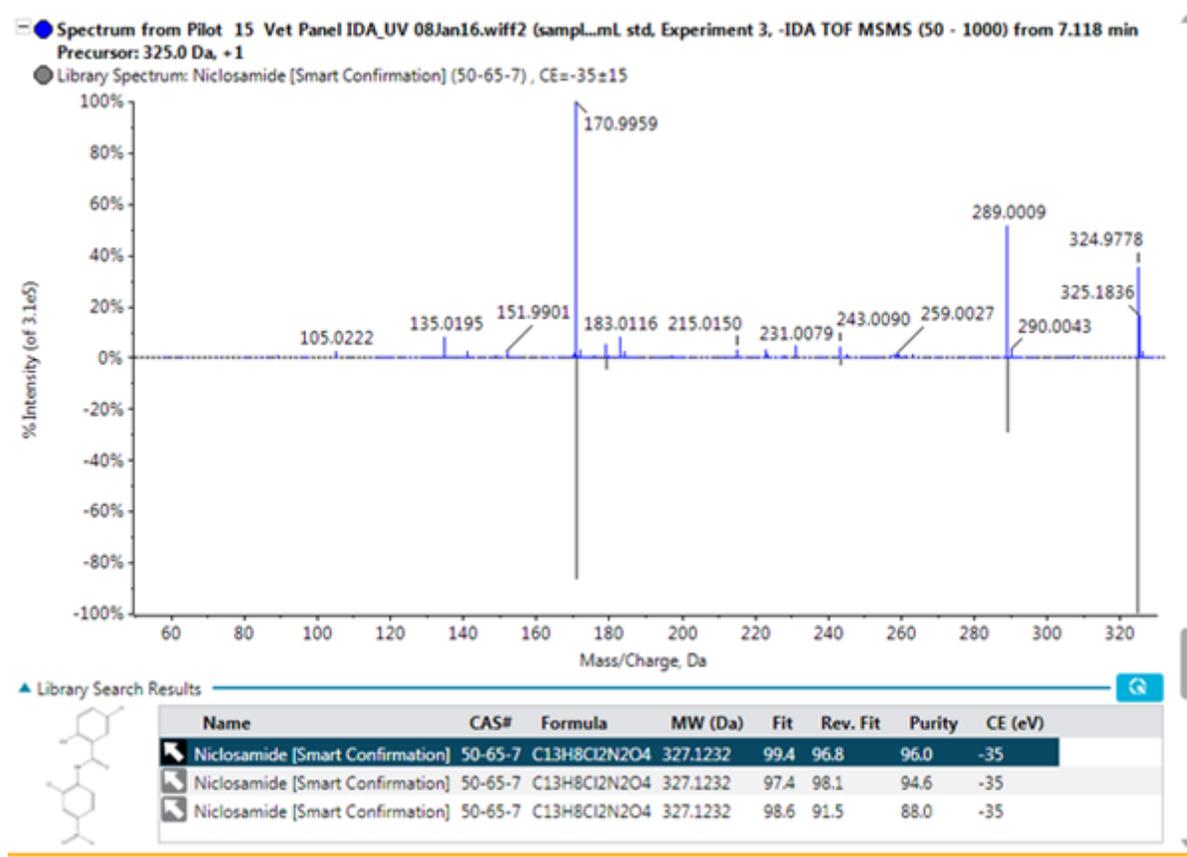
---

**Tip!** Click **Options > Peak review display settings** to change the number of rows shown in the pane. Users can also drag the top of the pane up to increase the size of the Peak Review pane.

---

- In a Peak Review pane, click **View** and then click **XIC + MS**, **XIC + MS/MS**, or **XIC + MS + MS/MS**.  
The search results are shown below the graphs.

Figure 6-30 Library Search Results

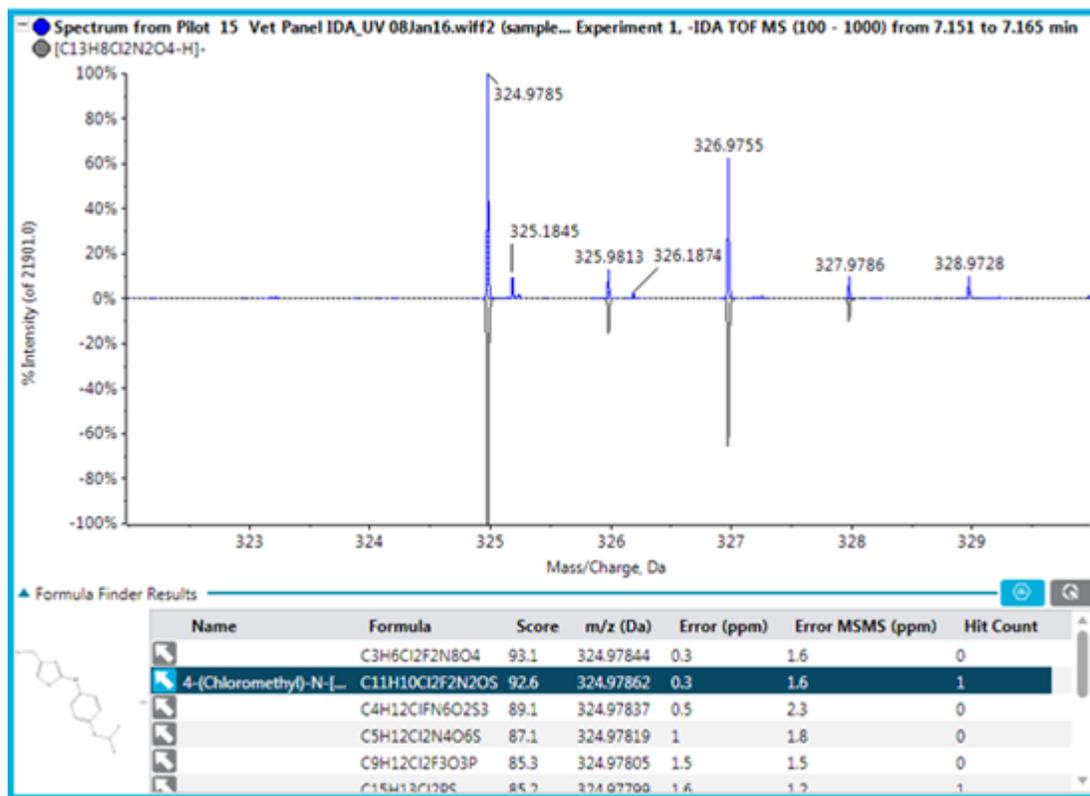


- Click the blue arrow to expand the **Library Search Results** to show more possible library hits.  
The chemical structure of selected library hit is also shown in the table.
- Click the arrow again to collapse the table.  
The results shown in the collapsed table are also shown in the Results Table.
- (Optional) Select a row in the table and then click  to update the results in the Results Table to use that specific library hit in the analysis.
- (Optional) Click  to update the processing method with the information for the selected compound.
- To add a spectrum to the library database, follow these steps:
  - Right-click the spectrum and then click **Add spectrum to library**.  
The Add spectrum to library dialog opens.
  - Update the **Compound Name**, **Library**, and **Precursor m/z** fields.

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- c. Click **OK**.
7. Click the blue arrow to expand the **Formula Finder Results** to show more possible results.

**Figure 6-31 Formula Finder Results**



The chemical structure of the selected formula finder results is also shown in the table if the compound has been updated from ChemSpider.

8. Click the arrow again to collapse the table.  
The results shown in the collapsed table are also shown in the Results Table.
9. Click  to update the **Formula Finder Results** column in the Results Table with the selected compound.
10. Click  to update the processing method with the information of the selected compound.

**Tip!** Click **Options > Get Chemspider hit count** to show the **ChemSpider Hit Count** column in the table below the graph.

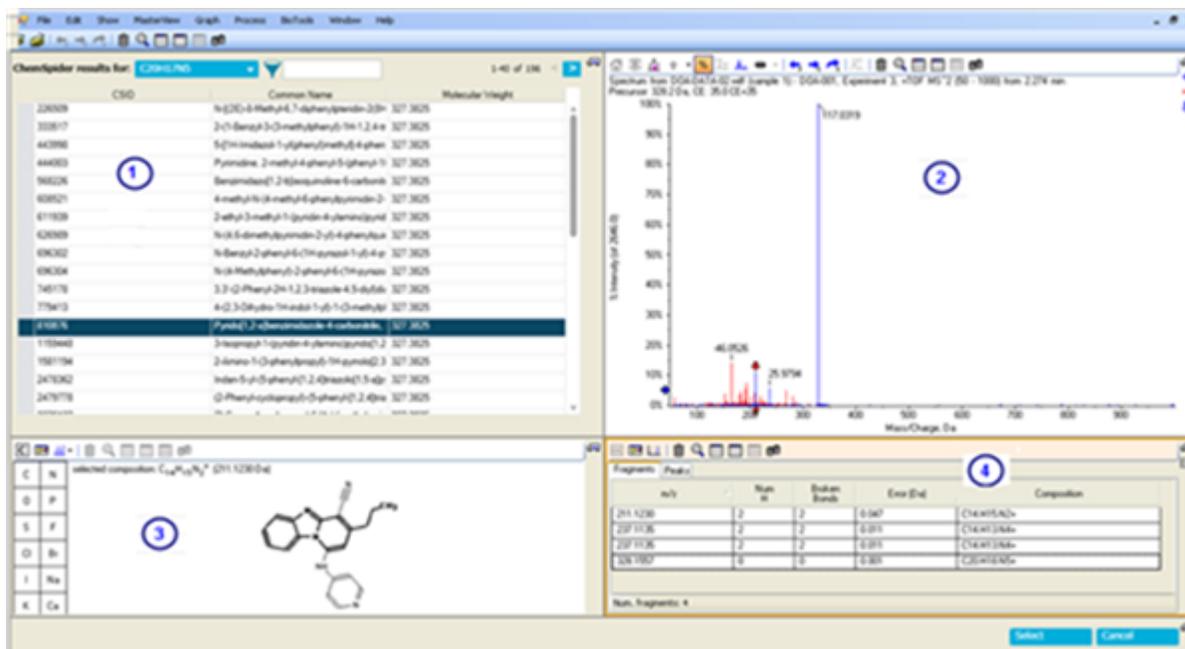
11. Click  to open the ChemSpider application.  
Refer to the section: [ChemSpider](#).

## ChemSpider

**Note:** The workstation must contain a valid license file to access the ChemSpider database.

**Note:** Information in the following image is for example purposes only.

**Figure 6-32 ChemSpider Session**



Item	Description
1	Results pane: Shows a list of suggested compounds that match the selected formula. The results are shown in groups of 40 compounds. Use the right arrow to advanced to the next group in the list. Use the left arrow to return to the previous group in the list.
2	Spectra pane: Shows the acquired spectra (in red) and the matching fragments (in blue). More blue fragments indicate a better match.
3	Structure pane: Shows the chemical structure of the compound selected in the results pane.
4	Fragment table pane, Fragments tab: Shows the total number of matching fragments for the selected compound.

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Item	Description
4	Fragment table pane, Peaks tab: Shows the total number of peaks, the number of matching peaks, and the % of total intensity for the selected compound. The check box in the <b>Assigned</b> column is automatically selected for the matching peaks.

**Table 6-9 ChemSpider Features**

When you do this ...	... this occurs
Type information in the field beside the <b>Filter XIC List</b> icon.	The results pane is refreshed and contains only the results that match the criteria entered.
Click through the entries in the results pane	The remaining panes refresh, showing the information associated with the selection.
Click through the entries on the Fragments tab of the fragment table pane	The remaining panes refresh. In the spectra pane, red arrows appear at the top and bottom of the matching fragment (in blue). In the structure pane, the components of the chemical structure that match the fragment are highlighted (bold).
Click through the <b>Assigned</b> entries on the Peaks tab of the fragment table pane	The remaining panes refresh. In the spectra pane, red arrows appear at the top and bottom of the matching fragment (in blue). In the structure pane, the components of the chemical structure that match the fragment are highlighted (bold).
Click the down arrow to the right of the <b>ChemSpider results for</b> field and select the <b>ChemSpider web site</b> option	The ChemSpider Web site ( <a href="http://www.chemspider.com">www.chemspider.com</a> ) opens in a browser window. Refer to the ChemSpider Help for information on accessing information.
Click the down arrow to the right of the <b>ChemSpider results for</b> field and select the <b>Refresh</b> option	All changes are discarded and the session reverts to the original search results.
Click <b>Select</b>	The selected information in the ChemSpider session is copied to the Formula Finder Results pane in the software session. The ChemSpider session closes.

### Peak Review Pane Tips

- Sort the Results Table on a particular column and review only those chromatograms that sort to the top or bottom of the table.

- The Peak Review pane is always synchronized with its corresponding Results Table and shows the chromatograms for the same peaks, in the same order, as in the table. Any changes, such as sorting rows, filtering sample types, or selecting any components, that are made to the Results Table are automatically reflected in the Peak Review pane.
- Use the scroll bar at the right of the pane to scroll through the available chromatograms. When the Peak Review pane is active, use the up and down arrow keys on the keyboard or the scroll wheel on the mouse to move through the chromatograms.
- Select a row in the Results Table by clicking in the light-blue region to the left of the first column to show the corresponding peak in the Peak Review pane. If the user scrolls to a particular chromatogram in the Peak Review pane, then the Results Table highlights the corresponding row and then brings it in to view.
- The grouping of numbers is not supported in the Analytics workspace. Users should not group numbers in any text box, for example, integration parameters, and grid, for example, Results Tables.
- At any one time, one chromatogram is considered to be active and is indicated by the title in bold. Make a specific chromatogram active by clicking anywhere within it.

---

**CAUTION: Potential Data Loss. Be careful not to drag the cursor within a chromatogram because doing so adjusts the expected retention time and causes the integration to change.**

---

- If the user drags across a specific peak in a chromatogram, then the **Expected RT** integration parameter is updated with the actual retention time of the peak. The new retention time is then automatically applied and the software integrates the peak again, updating the Results Table accordingly.
- (Mass Reconstruction workflow) If the user drags across a specific peak in a reconstruction graph, then the **Expected MW** parameter is updated with the actual molecular weight of the peak. The new molecular weight is then automatically applied and the software integrates the peak again, updating the Results Table accordingly.
- (Mass Reconstruction workflow) If **Recentered on the largest XIC Peak** is not selected, then the user can manually select the desired XIC region. The time at the center of the XIC region becomes the expected RT, and the RT of the largest peak within XIC region becomes the found RT.
- If the user is reviewing peaks in Manual Integration mode, then dragging the cursor across the peak manually integrates the selected peak. Holding **Shift** while dragging helps keep the line straight.
- When a chromatogram becomes active, the integration parameters shown at the left of the pane are updated to reflect the newly active chromatogram. If the user adjusts the peak integration parameters and then clicks **Apply**, then the currently active chromatogram is affected.

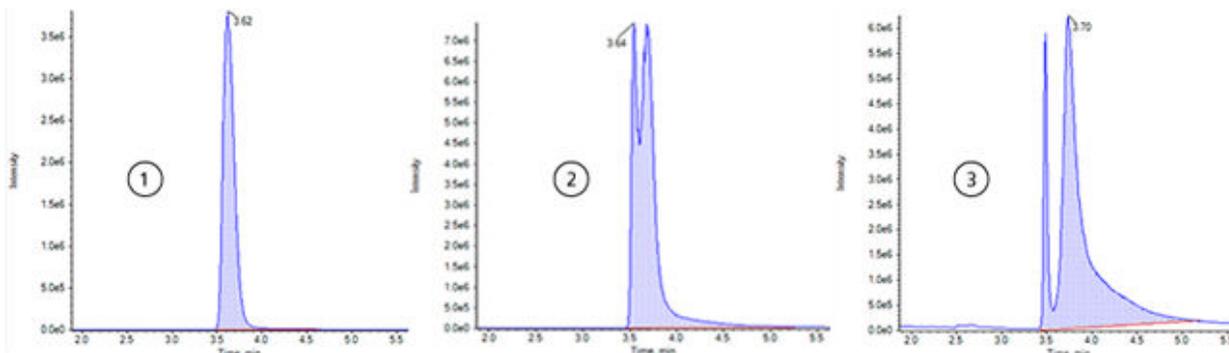
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- Users must inspect the peak shape during peak review to identify potential saturated peaks and to make sure that partial or incorrect integration does not erroneously result in incorrectly reported concentrations.
- Users must inspect the chromatograms during peak review for the presence of excessive noise spikes that might indicate system issues.
- Double-click within the Y-axis to scale the axis to the most intense peak within the entire data set. Zoom in by dragging within the axis to select an intensity range.
- Double-click within the X-axis to return the graph to the home view in which all of the data is visible. Zoom in by dragging within the axis to select a time range.
- When navigating sample by sample in the Results Table, click **Results > Cache all chromatograms for faster peak review** to improve the performance.

Samples of extremely high concentrations, well above the upper limit of quantitation, or ULOQ, might result in increasingly broader, saturated peaks with distorted or split shapes.

The following figure shows the maximum concentration that can be quantified using linear regression.

**Figure 6-33 Examples of Non-Saturated and Saturated Peaks**



Item	Description
1	Shows an acceptable peak that can be used for quantitation.
2	Shows a peak that is saturated. The concentration of the sample that generated this peak is well above the ULOQ. As the peak becomes saturated, the peak becomes wider and the top of the peak is inverted due to gain suppression. Such a peak should be excluded from quantitation because partial integration could result in incorrectly reported concentrations.
3	Shows the extreme saturation that results in the LC peak separating in to two peaks. Such a peak should be excluded from quantitation because partial integration could result in incorrectly reported concentrations.

## Analyze Data Using Statistics

### Prerequisite Procedures

- Open a Results Table.

Use the Statistics pane to view information related to the reproducibility of an analysis. Each row of the table summarizes information, such as the average and standard deviation, for a group of related peaks from the same analyte that would be expected to have the same response.

Review the peak integration, the calibration curve, and the sample statistics using an iterative process. The precision set for the **Actual Concentration** field in the Results Table is used in the statistics table as well.

**Note:** Refer to the laboratory standard operating procedures for information about accepted values for the statistics, including %CV and Accuracy.

Open a Results Table and then click **Views > Statistics pane**.

### Statistics Pane Columns

Label	Description
Row	Shows the row number.
Component Name	Shows the name of the analyte.
Sample Name/ Actual Concentration	When samples are grouped by actual concentration, shows the concentration. When samples are grouped by sample name, shows the sample name.
Num. Values	Shows $m$ of $n$ where $n$ is the total number of samples at the actual concentration, or with the same sample name, and $m$ is the number of these samples used for the calculations. Samples are not used if the corresponding peak could not be integrated, or if the <b>Used</b> field has been manually cleared.
Mean	Shows the average of the used samples.
Standard Deviation	Shows the standard deviation of the used samples.
Percent CV	Shows the co-efficient of variance expressed as a percentage: $100 * \frac{\text{Standard Deviation}}{\text{Mean}}$ .

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Label	Description
<b>Accuracy</b>	Shows the mean value divided by the actual concentration expressed as a percentage: $100 * \text{Mean} / \text{Actual Concentration}$ . This field is shown only when grouping by actual concentration, not when grouping by sample name.
<b>Values</b>	Shows the individual values for the samples in additional columns. If the corresponding sample could not be integrated, then <b>N/A</b> is shown. If the <b>Used</b> field has been manually cleared, then the value is shown with a strikethrough.
<b>Group by</b>	<p>Specifies how the sample for a given analyte should be grouped for the calculation of the statistics. The following options are available:</p> <ul style="list-style-type: none"> <li>• <b>Group by Concentration for Standards:</b> Standard samples are grouped by actual concentration.</li> <li>• <b>Group by Concentration for QCs:</b> Quality control samples are grouped by actual concentration.</li> <li>• <b>Group by Sample Name for Standards:</b> Replicate standard samples are grouped by the <b>Sample Name</b> field.</li> <li>• <b>Group by Sample Name for QCs:</b> Replicate quality control samples are grouped by the <b>Sample Name</b> field.</li> <li>• <b>Group by Sample Name for All Samples:</b> All replicate samples are grouped by the <b>Sample Name</b> field.</li> </ul>
<b>Metric</b>	<p>Specifies the actual metric that is used for the calculation of the statistics. The following options are available:</p> <ul style="list-style-type: none"> <li>• <b>Calculated Concentration:</b> The <b>Calculated Concentration</b> field of the Results Table is used.</li> <li>• <b>Area:</b> The <b>Area</b> field of the Results Table is used.</li> <li>• <b>Height:</b> The <b>Height</b> field of the Results Table is used.</li> <li>• <b>Calibration Y-Value:</b> The regression parameter specified for the analyte is used. This is either <b>Area</b> or <b>Height</b> for an analyte without a corresponding internal standard, or <b>Area Ratio</b> or <b>Height Ratio</b> for an analyte that uses an internal standard.</li> </ul>
<b>Save Results and Export</b>	Click to save the results and export the statistics table. The Export Statistics dialog opens.

## Statistics Pane Tips

- In the **Components and Groups** list, select **All Components** to view the entries for all of the analytes in the Statistics table. Select an individual component to view the entries for that analyte only. If the user selects an individual internal standard from the list, then the Statistics table is empty. Refer to the section: [Components and Groups List](#).
- Click one of the **Value** cells for a row that is visible in the Statistics pane to select the corresponding row in the Results Table for the analyte and sample. If the Peak Review pane is visible, then it links to the Results Table and it is updated when the corresponding cell is clicked.
- Sort the statistics by clicking one of the column headers.
- Copy the whole Statistics table or just the rows of interest by selecting the rows and then pressing **Ctrl+C**.
- Use the **Group by** list to specify how the sample, for a given analyte, should be grouped for the calculation of the statistics.
- Use the **Metric** list to specify the metric that is used for calculation of statistics, calculated concentration, area, and so on.
- Adjust the column widths to optimize the display. These widths are preserved the next time the Statistics pane is shown.
- To change the format and precision for the Statistics table, change them in the Results Table. Refer to the section: [Customize the Results Table](#).
- To change the **Use Peak** option for an individual value, right-click in the cell in the Statistics pane, and select **Use Peak**. The **Use Peak** column in the Results Table is updated.

## View the Calibration Curve

Prerequisite Procedures
<ul style="list-style-type: none"><li>• Open a Results Table.</li></ul>



Use the calibration curve to determine the concentration of a substance in an unknown sample by comparing the unknown sample to a set of standard samples of known concentration. Refer to the section: [Calibration Curves](#).

1. Click **Displays the Calibration Curve** ()
2. To set the regression options, click **Regression**. Refer to the document: *Help System*.

### Export Calibration

Use Export Calibration to save a copy of the calibration equation for all of the analytes associated with the active Results Table to an external file (mqcal). This allows the user to apply the calibration from one set of standard samples to other samples that are not part of the same Results Table.

The typical workflow is:

1. Create a Results Table containing only the standard.
2. Use the Peak Review pane to make sure that the integration was successful.
3. In the Calibration Curve pane, click **Options > Export calibration (and save results)** to save a copy of the calibration.
4. Create a new Results Table containing samples of unknown concentration.
5. In the Calibration Curve pane, click **Options > Assign external calibration** to apply the exported calibration equation to the new Results Table.

---

**Note:** Users can also specify the calibration file (mqcal) to apply to the new Results Table.

---

If changes are made to the original Results Table, with the standard samples, then the Results Table must be exported again to save the updated calibration equation. Previously exported calibrations are not automatically updated.

### Analyze Data Using Metric Plots

<b>Prerequisite Procedures</b>
--------------------------------

- |   |
|---|
| <ul style="list-style-type: none"><li>• Open a Results Table.</li></ul> |
|---|

Use a Metric Plot to plot the values in a Results Table column against either the row number or another column. These plots are a valuable aid for visual data review.

If one column is selected, then the resulting plot shows the values from the column as a function of the row number in the table. If two columns are selected, then the values from the columns are plotted against one another. The first of the two columns to be selected contains the X values and the second contains the Y values.

1. Select one or two columns in the Results Table.

---

**Tip!** To select a second column, press **Ctrl** while clicking the column header.

---

2. Click **More > Create Metric Plot with new settings**.
3. In the Metric Plot, click **Link** and then click **Link to results table columns** or **Link to results table rows** to link the scrolling in the Results Table to the Metric Plot.

For more information about the **Link** menu, refer to the document: *Help System*.

4. To update the Metric Plot, select the rows of interest in the Results Table and then, in the Metric Plot pane, click **Link > Plot selected rows only**.

---

**Tip!** To select multiple rows, press **Ctrl** while selecting the rows.

---

5. (Optional) Customize the Metric Plot options by selecting options from the **Options** menu. For descriptions of the options, refer to the document: *Help System*.

### Metric Plot Tips

- If users left-click on a data point, then the corresponding row of the Results Table is automatically selected and scrolled in to view. If the Peak Review pane is open, then it also updates to show the corresponding chromatogram. This provides a convenient method of performing peak review for outliers.
- The title region always shows the name of the active trace. If traces for multiple components are overlaid, then toggle the title between showing information for all of the traces or just the active one by clicking the plus sign (+) to the left of the title. Activate a specific trace by clicking the title or the color spot to the left of the corresponding title or by selecting a data point in the Metric Plot.
- The Metric Plot can be used to plot peak areas for internal standard or QC samples to monitor possible deviations or trends.

## Edit Report Templates

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**CAUTION: Potential Data Loss.** To prevent users from modifying templates, make sure that the Reporter templates are located in secured, read-only folders that are accessible for writing only by system administrators.

---

The user is responsible for validating the custom template.

1. Open the docx template.

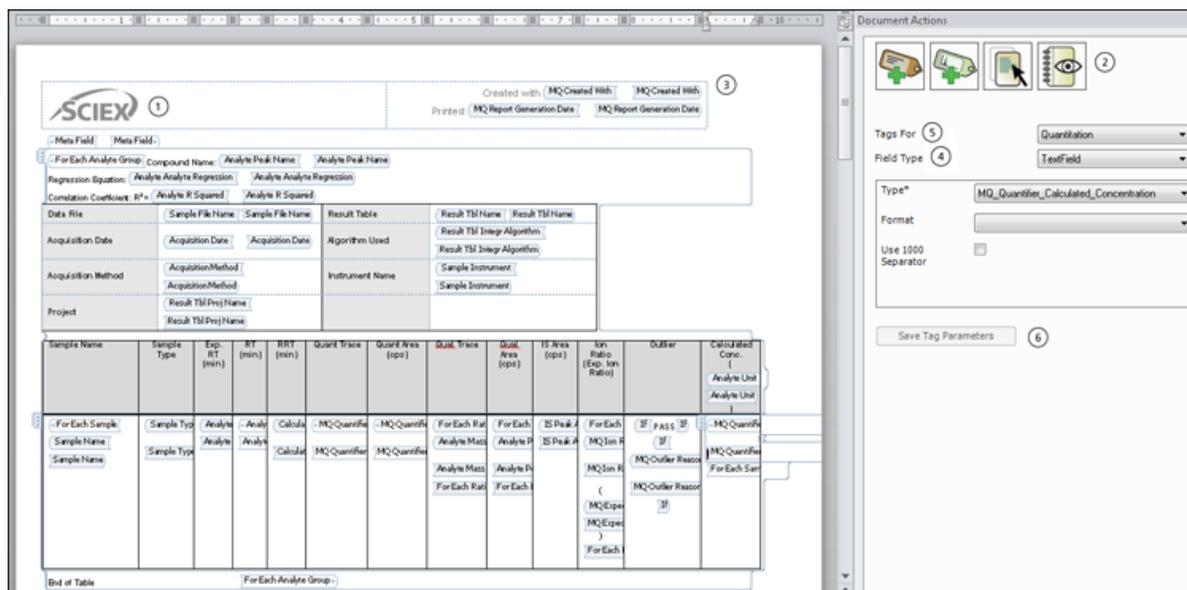
---

**Tip!** The templates are located in `C:\ProgramData\SCIEX\Analytics\Reporter`.

---

When an area is selected, the Reporter template editor opens on the right. The template editor is automatically populated with the tag information.

Figure 6-34 Reporter Template Editor



Item	Description
1	Report template showing the current tags.
2	Icons: <ul style="list-style-type: none"> <li>• Add new tag.</li> <li>• Add picture tag.</li> <li>• Show content area.</li> <li>• View document change log.</li> </ul>
3	<b>Created with:</b> Shows the name of the software providing the tag information.
4	<b>Field Type:</b> Shows the field types applicable to the software.
5	Shows a list of available attributes based on the selected field type. For example, tag name and number format.
6	<b>Save Tag Parameters:</b> Click to save changes. If changes are not saved, then a message is shown prompting the user to save the changes.

2. Use the procedures in the following table.

**Table 6-10 Reporter Functions**

To Do This	Do This
Change the field type.	Click inside the tag, select a new field type, and then select the attributes.
Change the attributes of the field type.	Click inside the tab and then change the attributes, as required.
Add a tag.	Click the <b>Add new tag</b> icon, select the Field Type, and then select the attributes.
Add a picture.	Click the <b>Add picture tag</b> icon and then select the attributes.
Show where a tag starts and ends.	Click the <b>Show content area</b> icon.
Show the document change log.	Click the <b>View document change log</b> icon.
Copy and paste tags.	Copy the selected tags and then paste them in the new location. Update the field type attributes.  The attributes are not copied and must be selected.
Navigate between the tags.	Use the left and right arrow keys to move between the tags.
Delete tags.	Do one of the following: <ul style="list-style-type: none"> <li>• If the cursor is to the left of the tag, then press <b>Delete</b>.</li> <li>• If the cursor is to the right of the tag, then press <b>Backspace</b>.</li> </ul>

3. Click **Save Tag Parameters** after any changes are made.

---

**Tip!** Mandatory information is indicated by a flashing red exclamation sign at the left of the field.

---

## Reporter Templates

It is the responsibility of the user to validate the custom report template.

Some report templates use queries. Users can create queries using Microsoft Excel-based formulas to evaluate, manipulate, and present the data from the Results Table in a report. The Metafield tag in the report template tells the report the name of the query file that it should use. To use queries, the name of the query file must be specified in the MetaField tag in the

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report template. Queries must also have the extension ".query" to be recognized as a query. The queries must be stored in the Reporter folder where the report templates are stored.

We recommend that the user validate the generated results when a Reporter template is used, especially when queries are used in a template. If any modifications are made to the report template after validation, then the report template should be re-validated. Changes to the report template include any modification to Reporter tags or queries.

**Table 6-11 Default Templates**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
All Peaks Qual	A report showing, for each sample, a section including the File Information, Sample Information, Analyte Results Table, and overlaid chromatograms of all of the analytes and internal standard. The Analyte Results Table is printed as shown in the Results Table. All the qualitative confidence traffic lights are listed at the beginning of the table.	N/A
Analyte 20 percent Report	A report showing, for each analyte, a section including File Information, and an XIC table for each Blank, Standard, QC, and 20% of all Unknowns.	This is an example report template that has a Query attached - Analyte20percent.Query.
Analyte Summary	Table of results showing Sample Name, Calculated Concentrations and Outliers for all samples in the batch for the specific analyte and the associated Internal Standard.	N/A
Calibration Curve	A report showing the File Information, Statistics Table (standards), and Calibration Curve for analytes, one page per analyte.	<ul style="list-style-type: none"><li>Standards for which the Reportable check box is cleared will not be reported in the data table. Statistics will not be affected by the Reportable status.</li><li>The report will show the regression equation and graph, as shown and calculated in the Calibration Curve pane in the Analytics workspace, based on the status of the <b>Used</b> column.</li></ul>

**Table 6-11 Default Templates (continued)**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
Intact Quant All Peaks and Graphs	A report showing the Results Table entries for each sample. All columns visible in the Results Table are shown in the report. The report also includes the XIC chromatograph, average spectrum, and reconstruction spectrum, for each sample and analyte.	This report is specific to the Mass Reconstruction workflow.
Intact Quant Analyte Summary and Calibration Curve	A report showing the Results Table entries, the calibration curve, and the statistics data for each analyte. The Results Table includes Sample Name, Sample Type, Analyte name, Actual Concentration, Area, Height, Expected MW, MW, MW Delta, Calculated Concentration, and Accuracy.	This report is specific to the Mass Reconstruction workflow.
Intact Quant Sample Summary	A report showing Results Table entries for all samples. The Results Table includes Sample Name, Sample Type, Analyte Name, Actual Concentration, Area, Height, Expected MW, MW, MW Delta, Calculated Concentration, Accuracy and Accuracy acceptance.	This report is specific to the Mass Reconstruction workflow.
Metric Plot	A report showing, for each analyte, a section including the File Information and a metric plot of the analyte peak area.	The state of the <b>Reportable</b> check box does not affect the report content. All data points are included even if the check boxes are cleared.
MQ Analyte Report 1	A report showing, for each analyte, a section including File Information, Sample Results Table, and XIC table for each sample - WILL GENERALLY PRINT 2 PAGES PER ANALYTE FOR < 8 SAMPLES	N/A

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**Table 6-11 Default Templates (continued)**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
MQ Analyte Report 2	A report showing, for each analyte, a section including File Information and XIC table for each unknown sample - WILL GENERALLY PRINT 2 PAGES PER ANALYTE FOR < 8 SAMPLES	Only unknowns are reported.
MQ Analyte Report 3	A report showing, for each analyte, a section including File Information, and Unknown Samples Summary Table.	Only unknowns are reported.
MQ Analyte Report condensed table	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table. The table is shown as 2 columns to fit more samples per page.	Only unknowns are reported.
MQ Analyte Report with chromatograms	A report showing, for each analyte, a section including File Information, Sample Results Table, and a small chromatogram for each sample.	Only unknowns are reported.
MQ Blank Template	N/A	Only header information, the logo, and page numbers are shown in the report.
MQ Pep Quant	N/A	For use with the Peptide Quantitation dataset. Refer to the the second example, the absolute quantitation example, in the <i>User Guide</i> for the MultiQuant Software.
MQ QC Summary 1 with flags	A report showing File Information, QC Summary Table per analyte (values with a CV higher than 20% are highlighted), and QC Detailed Results Table (values with an accuracy outside of 80-120% are highlighted).	Quality Controls that have the <b>Reportable</b> check box cleared will not be included in the report, nor will they be used in the calculations.

**Table 6-11 Default Templates (continued)**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
MQ Sample Report 1	A report showing, for each sample, a section including File Information, Sample info, IS info, Analyte Results Table, XIC table including IS and each analyte - WILL GENERALLY PRINT 2 PAGES PER SAMPLE FOR < 8 SAMPLES	N/A
MQ Sample Report 2	A report showing, for each unknown sample, a section including File Information, TIC, Sample Details, Analyte XIC, and results in table form - WILL GENERALLY PRINT 2 PAGES PER SAMPLE FOR < 8 SAMPLES	Only unknowns are reported.
MQ Sample Report 3	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table.	Only unknowns are reported.
MQ Sample Report condensed table	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table. The table is shown as 2 columns to fit more analytes per page.	Only unknowns are reported.
MQ Sample Report with chromatograms	A report showing, for each sample, a section including File Information, Sample info, Analyte Results Table, and a small chromatogram for each analyte.	Only unknowns are reported.

Table 6-11 Default Templates (continued)

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
MQ Sample Report with Concentration Threshold	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Sum	<ul style="list-style-type: none"> <li>• The associated query file is Sample Report with Concentration Threshold.query.</li> <li>• Components must be named "Cmpd X #", where X is any character from A to F, and # is any numerical value. <b>Example:</b> In the report, a component named "Cmpd A 1" will be shown under the heading <b>Compound Group A</b>; a component named "Cmpd B 1" will be shown under <b>Compound Group B</b>, and so on.</li> <li>• If components are in the same group, then only the first component, alphabetically, in the group will be included in the report. <b>Example 1:</b> If "Cmpd B 25" and "Cmpd C 1" both belong to the group "Grp", then "Cmpd C 1" will not be in the report. <b>Example 2:</b> If "Cmpd A 1", "Cmpd A 2", and Cmpd A 3" are not assigned to groups, then "Cmpd A 2" and "Cmpd A 3" will not be in the report. <b>Example 3:</b> If "Cmpd A 1", "Cmpd A 2", and Cmpd A 3" are assigned to groups 1, 2, and 3, respectively, then all 3 components will be in the report under the heading <b>Compound Group A</b>.</li> </ul>

**Table 6-11 Default Templates (continued)**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
MQ Sample Report with MRM ratios 2	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table, overlay of all XIC. Expected Ion ratios are calculated automatically using any available standards. Ratio values are placed in custom columns within the Results Table. Any values outside 20% of expected are flagged. Quantifier analyte names must end in a blank space followed by the number 1. Ratio ion analyte names must end in a blank space followed a number between 2 and 9.	N/A
MQ Sample Report with MRM ratios EU	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table. Expected Ion ratios are calculated automatically using any available standards. Ratio values are placed in custom columns within the Results Table. Any values outside of expected are flagged (using EU guidelines for ratio tolerances). Quantifier analyte names must end in a blank space followed by the number 1. Ratio ion analyte names must end in a blank space followed a number between 2 and 9.	The associated query file is <code>MRM ratios EU.query</code> .

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**Table 6-11 Default Templates (continued)**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
MQ Sample Report with MRM ratios MQ EFAB 03	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table. Expected Ion ratios are calculated automatically using any available standards. Ratio values are placed in custom columns within the Results Table. Any values outside 20% of expected are flagged. Quantifier analyte names must end in a blank space followed by the number 1. Ratio ion analyte names must end in a blank space followed a number between 2 and 9.	N/A
MQ Sample Report with MRM ratios	A report showing, for each unknown sample, a section including File Information, Sample info, and Results Summary Table. Expected Ion ratios are calculated automatically using any available standards. Ratio values are placed in custom columns within the Results Table. Any values outside 20% of expected are flagged. Quantifier analyte names must end in a blank space followed by the number 1. Ratio ion analyte names must end in a blank space followed a number between 2 and 9.	The associated query file is <code>MRM ratios.query</code> .

Table 6-11 Default Templates (continued)

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
MQ Sample Report with standards, QC, and blanks	A report showing, for each sample, a section including File Information, Standards Summary Table, QC Summary Table, Blanks Results Table; then for each unknown sample a section including File Information, Sample info, IS info, Analyte Results Table, XIC table including IS and each analyte - WILL GENERALLY PRINT 2 PAGES PER SAMPLE FOR < 8 ANALYTES.	Standards and Quality Controls that have the <b>Reportable</b> check box cleared will not be shown in their respective summary tables in the report, nor will they be used in the statistical calculations.
MQ Tutorial Dataset Heavy Light	N/A	This report is intended for use with the Tutorial Dataset Heavy Light dataset. Refer to the second example, the relative quantitation example, in the <i>User Guide</i> for the MultiQuant Software.
Per Sample Quant-Qual	A report showing, for each selected sample, a section including the File Information, Sample Information, and Analyte Results Table for the selected analytes. The Analyte Results Table is printed as shown in the Results Table. All the qualitative confidence traffic lights are listed at the beginning of the table.	N/A
Per Sample Quant-Qual Visible Rows Using Visible Analyte	A report showing, for each selected sample, a section including the File Information, Sample Information, and Analyte Results Table for the selected analytes. The Analyte Results Table is printed as shown in the Results Table. All the qualitative confidence traffic lights are listed at the beginning of the table.	The hidden state of a row takes precedence over the state of the <b>Reportable</b> check box. If the <b>Reportable</b> check box is selected but the row is hidden, then the row is not reported.

Table 6-11 Default Templates (continued)

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
Per sample Quant-Qual with statistics	A report showing components for each sample with a WYSIWYG table. XIC, MS, and MS/MS are shown. A statistics summary table for area is shown at the end of the report.	<ul style="list-style-type: none"> <li>• If the component table has UV components, then the UV trace is reported under XIC graph in the report.</li> </ul> <hr/> <p><b>Note:</b> If the name of the UV component is in the format [<i>compound_nameuv</i>] or [<i>uv</i>], then no UV traces are reported, because the <i>uv</i> suffix is associated with the UV MS Qual report.</p> <hr/> <ul style="list-style-type: none"> <li>• If a sample is labeled as a QC and there are 2 or more samples, then the mean, STDEV, and %CV will be calculated and included in a QC summary table at the end of the report.</li> <li>• If the <b>Reportable</b> check box is cleared for a QC row, then that row will not be used for any calculations in the QC summary table.</li> </ul>
Per Analyte Quant-Qual	A report showing, for each analyte, a section including the File Information, Results Table, Calibration Curves, and chromatograms including the internal standard and each analyte. This template is suitable for a Results Table with a group defined in it.	N/A

**Table 6-11 Default Templates (continued)**

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
Positive Hits Qual	A report showing, for each selected sample, a section including the File Information; Sample Information; Analyte Results Table for the selected analytes; overlaid chromatograms of all of the analytes, internal standard, and the XIC; the Acquired/Theoretical MS spectra; and the Acquired/Library MS/MS spectra for each selected analyte. The Analyte Results Table is printed as shown in the Results Table. All the qualitative confidence traffic lights are listed at the beginning of the table.	N/A
Qual CSV report	A report in a csv format showing, for each sample, a section including the File Information, Sample Information, and Analyte Results Table.	Recommended to use CSV option for Report format.
Sample Summary	A report showing, for each sample, a section of Analytes Summary Table. This report template is suitable for a Results Table with groups.	N/A

Table 6-11 Default Templates (continued)

Template	Template Description (as shown in the Create Report dialog)	Additional Notes
UV MS Qual report	A report showing, for each sample, the components of that sample and their corresponding UV component with a WYSIWYG table. XIC, MS, and MS/MS are shown along with UV data. A statistics summary table for area is shown at the end of the report.	<ul style="list-style-type: none"> <li>• UVMS data should be processed with the naming convention <i>compound 1</i> (any string) for the mass spectrometer (MS) component and <i>compound 1uv</i> (any string plus uv) for the corresponding UV component.</li> <li>• Only the Mass error, Fragment Mass Error, RT confidence, Istotope confidence and Library confidence traffic lights are shown.</li> <li>• A graph table is created to shown the individual components of the Results Table, including the XIC, MS1 trace, MS/MS trace, and header information from compound 1, and the UV trace from compound 1uv. Refer to <a href="#">Figure 6-35</a>.</li> <li>• Analyte graphs are only repeated for the MS experiments, not for the not the UV experiments.</li> <li>• If a sample is labeled as a QC and there are 2 or more samples, then the mean, STDEV, and %CV are calculated and included in a QC summary table at the end of the report. Refer to <a href="#">Figure 6-36</a>.</li> <li>• If the <b>Reportable</b> check box is cleared for a QC row, then that row in not used for any calculations in the QC summary table.</li> </ul>

Figure 6-35 Graph Table

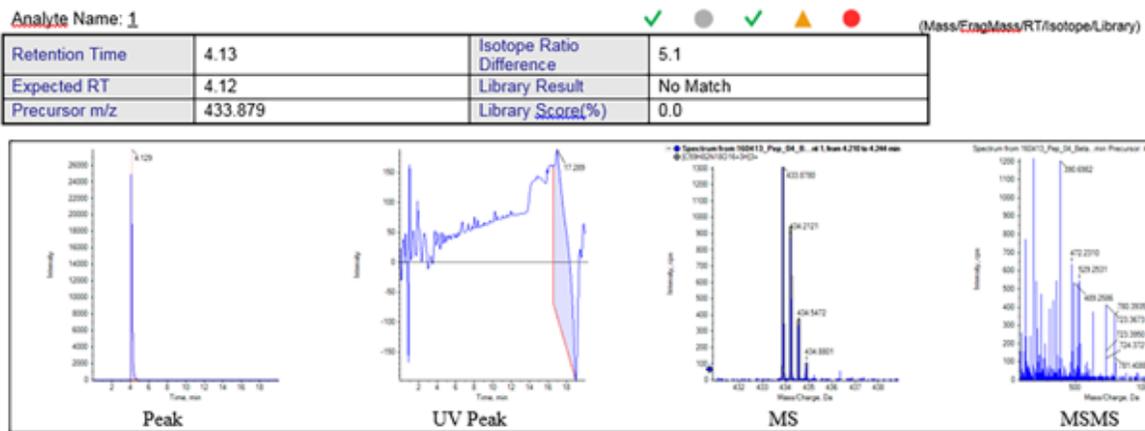


Figure 6-36 Statistics Table

Statistics (Grouped by Concentration for QCs - Area)

Analyte Peak Name (MRM Transition)	Mean	Std. Deviation	% CV	Number of Values Used
1 (723.3573 - 723.3773)	1.062e4	7.367e2	6.93	2 of 2
2 (753.3091 - 753.3291)	2.215e4	6.858e2	3.10	2 of 2
3 (760.3353 - 760.3553)	9.332e3	1.955e1	0.21	2 of 2
4 (631.3450 - 631.3650)	3.244e4	1.110e3	3.42	2 of 2
5 (636.3373 - 636.3573)	1.144e5	3.962e2	0.35	2 of 2
6 (871.4354 - 871.4554)	6.479e4	1.198e3	1.85	2 of 2
7 (932.4493 - 932.4693)	2.183e4	7.301e2	3.34	2 of 2
8 (1000.5743 - 1000.5943)	2.553e4	5.007e2	1.96	2 of 2
9 (755.4352 - 755.4552)	1.127e5	8.422e3	7.48	2 of 2
10 (1184.5929 - 1184.6129)	3.576e4	7.231e2	2.02	2 of 2
11 (884.4871 - 884.5071)	5.183e4	1.512e3	2.92	2 of 2
12 (1176.5468 - 1176.5668)	1.670e4	1.848e2	1.11	2 of 2
13 (871.9418 - 871.9618)	1.597e5	5.501e2	0.34	2 of 2
14 (879.4236 - 879.4436)	1.868e5	5.182e3	2.77	2 of 2

When an issue occurs, the Central Administrator Console (CAC) software logs error reports and warnings that are shown on the screen. The Event Log workspace contains logs of system events, including errors, warnings, and messages.

To open this workspace, click the Event Log tile on the Home page.

**Table 7-1 Event Log Workspace Columns**

Label	Description
Current	A list of the current events for each subsystem.
Severity	The type of event: information, error, or warning.
Time	The time that the event occurred.
Subsystem	The subsystem in which the event occurred.
Event	A description of the event. This information can be used to troubleshoot the system.
User	The name of the user and the system where the event occurred.  <b>Note:</b> For events triggered by a decision rule, this is the user who submitted the batch.

## Event Logs

The following logs are available:

- All
- Device
  - LC
  - Mass Spectrometer
- Workspace
  - Batch
  - Explorer
  - Devices
  - General

- **LC Method**
- **MS Method**
- **MS Tune**
- **Analytics**
- **Queue**
- **Users**
- **Configuration**

After an event log contains 20,000 records, SCIEX OS automatically archives the records and begins a new event log. For more information, refer to the section: [Event Log Archives](#).

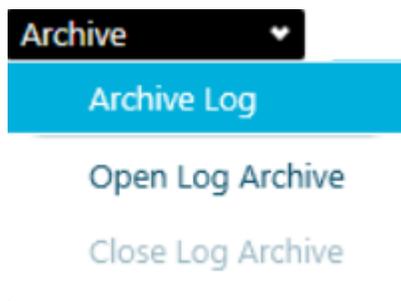
## View Logs

1. Open the Event Log workspace.
2. Click an item from the list in the left panel to view the logs.

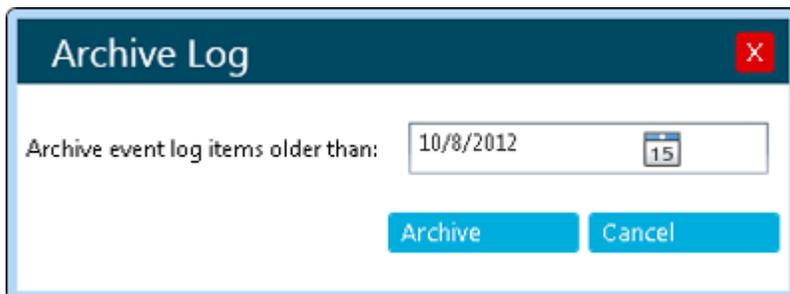
## Archive Logs

1. Open the Event Log workspace.
2. Click **Archive > Archive Log**.

**Figure 7-1 Archive Menu: Archive Log**



**Figure 7-2 Archive Log Dialog**

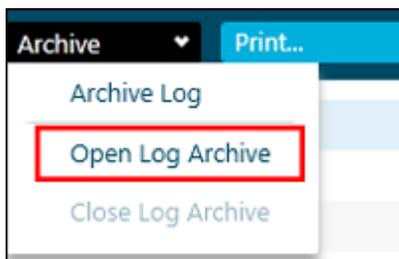


3. In the **Archive event log items older than** field, click the date icon and then select a date.
4. Click **Archive**.

## View Archived Logs

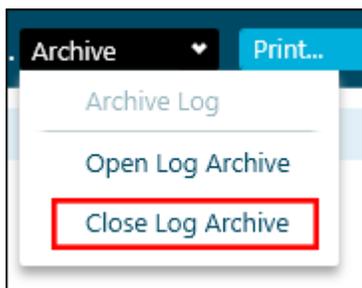
1. Open the Event Log workspace.
2. Click **Archive > Open Log Archive**.

**Figure 7-3 Archive Menu: Open Log Archive**



3. Open the required file.
4. Click **Archive > Close Log Archive**.

**Figure 7-4 Archive Menu: Close Log Archive**



## Print Logs

1. Open the Event Log workspace.
2. (Optional) Open an archived log. Refer to the section: [View Archived Logs](#).
3. Click **Print**.  
The Print dialog opens.
4. Select a printer and then click **Print**.

## Event Log Archives

Event records accumulate in the event logs and can create large files that are difficult to navigate and manage.

When an event log reaches 20,000 records, it is archived. A final event record is added to the event log, and then the event log is saved with a file name indicating the type of event log and the date and time. A new event log is created. The first record in the new event log states that the event log has been archived.

Event log archives are stored in the `C:\ProgramData\SCIEX\Clearcore2.Acquisition` folder. The file names are in the format `<logfile>Archive_<YYYYMMDD>_<HHMMSS>.data`. For example, `CustomerLogArchive_20220427_172915.data`.

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This section explains how to use the auditing functionality.

## View the Audit Trail Records

1. Open the Audit Trail workspace.
2. To view the audit trail for the workstation, click **Workstation** in the left pane.
3. To view the audit trail for a project, select the project in the left pane. Then select one of the following:
  - **General Events:** To show audit records that apply to the whole project, such as audit map changes and sample acquisition.
  - **Analytics:** To show the audit records for a Results Table.
  - **All Project Events:** To show audit records for both general events and processing events.

## Filter Audited Events Using a Keyword Search

The user can filter the audited events in the audit trail using a keyword search. The search highlights every occurrence of the text.

1. Open the Audit Trail workspace.
2. Select the audit trail to be searched. Refer to the section: [View the Audit Trail Records](#). The Audit Trail records are shown.
3. Type the word to find in the **Find in Page** field. All occurrences of the word on the page are highlighted.
4. Use the Next (▼) and Previous (▲) buttons to move through the matches.

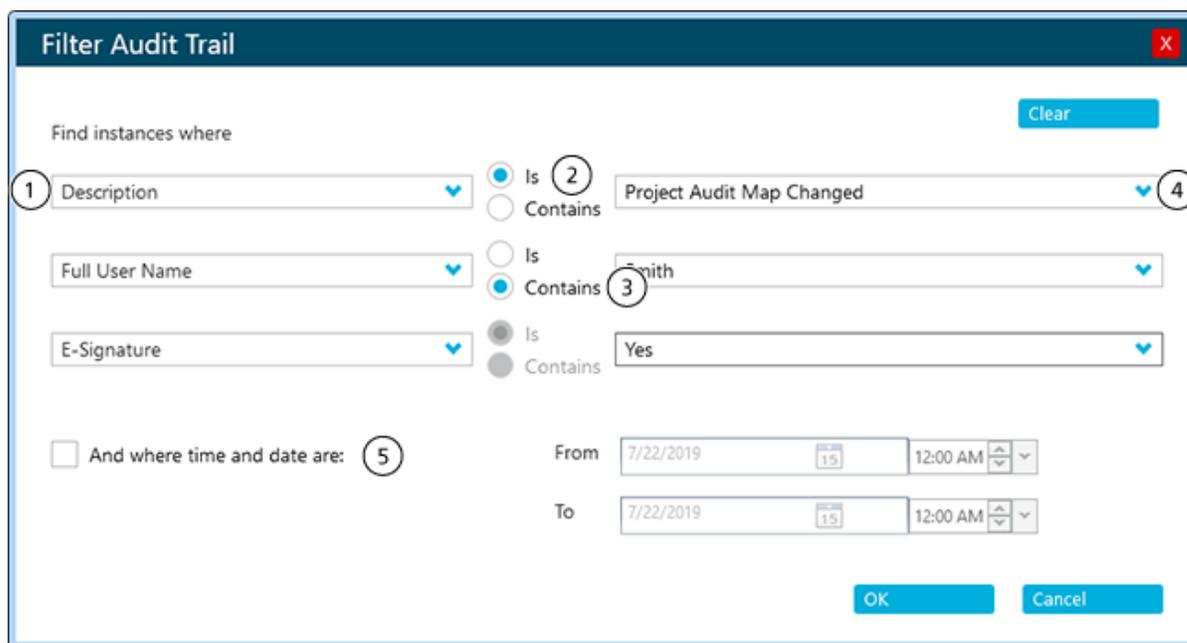
## Filter Audited Events Using a Set of Specified Criteria

The user can filter the audited events in the audit trail using a set of specified criteria.

1. Open the Audit Trail workspace.
2. Select the audit trail to be filtered. Refer to the section: [View the Audit Trail Records](#). The Audit Trail records are shown.

3. Click **Filter** (  ).  
The Filter Audit Trail dialog opens.
4. Use the lists to define the required filter criteria.

**Figure 8-1 Filter Audit Trail Dialog**



Item	Description
1	In the <b>&lt;No Filter&gt;</b> list, select the field to filter on. The following fields are available for filtering: <ul style="list-style-type: none"> <li>• <b>Description</b></li> <li>• <b>Sample Name</b></li> <li>• <b>Full User Name</b></li> <li>• <b>E-Signature</b></li> <li>• <b>Reason</b></li> </ul>
2	Select to filter on an exact word or phrase.
3	Select to filter on a partial word or phrase.

## Auditing

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Item	Description
4	Specify the text to filter on, as follows: <ul style="list-style-type: none"><li>• Type the full text string. Select <b>Is</b> (item 2).</li><li>• Type a partial text string. Select <b>Contains</b> (item 3).</li><li>• Select <b>Yes</b> or <b>No</b>.</li></ul>
5	Use to filter on events that occurred during a specific date and time.

5. To clear the filter, follow these steps:
  - a. Click **Filter** ()
  - b. Click **Clear** to reset all of the filter criteria to **No Filter**.
  - c. Click **OK**.

## Print the Audit Trail

1. Open the Audit Trail workspace.
2. Select the audit trail to be printed. Refer to the section: [View the Audit Trail Records](#).
3. Click **Print**.  
The Print dialog opens.
4. Select a printer and then click **Print**.

This section describes concepts used in the software.

## Data Handling

The SCIEX OS software requires a computer running the Windows 7, 64-bit or Windows 10, 64-bit operating system. The computer and the associated system software work with the system controller and the associated firmware to control the system and data acquisition. During system operation, the acquired data is sent to the SCIEX OS software where it can be shown as either full mass spectra, intensity of single or multiple ions over time, or total ion current over time.

## Scan Techniques

The system is a versatile and reliable system for performing liquid chromatography mass spectrometry analysis on liquid sample streams to identify, quantify, and examine compounds.

The system uses the following mass spectrometry techniques to analyze samples:

- Two modes of single mass spectrometry (MS):
  - Quadrupole-based single mass spectrometry (for Q1 calibration only)
  - Time-of-flight-based single mass spectrometry
- One mode of tandem mass spectrometry (MS/MS):
  - Product ion mass spectrometry

## Different Data View

### Chromatograms

A chromatogram shows 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.

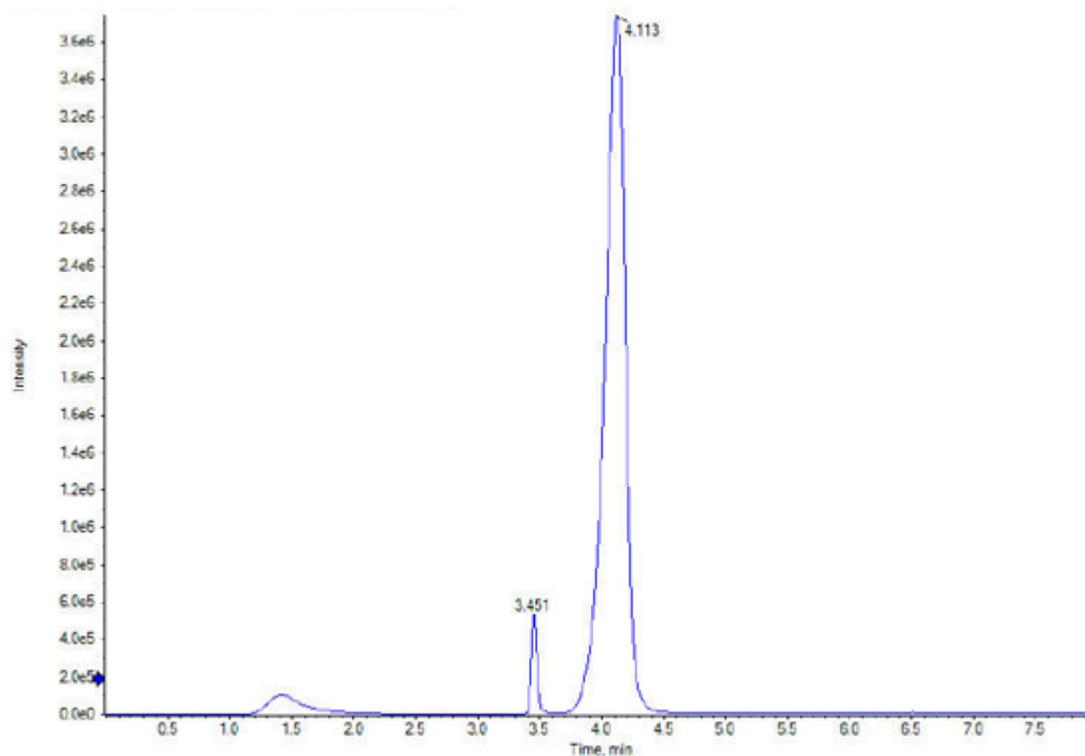
In the chromatogram graph, the intensity, in counts per second (cps), is shown on the Y-axis versus time on the X-axis. Peaks are automatically labeled.

Chromatographic peaks can change in retention time and intensity based on changes in chromatographic conditions for a given sample.

The software show the following types of chromatograms:

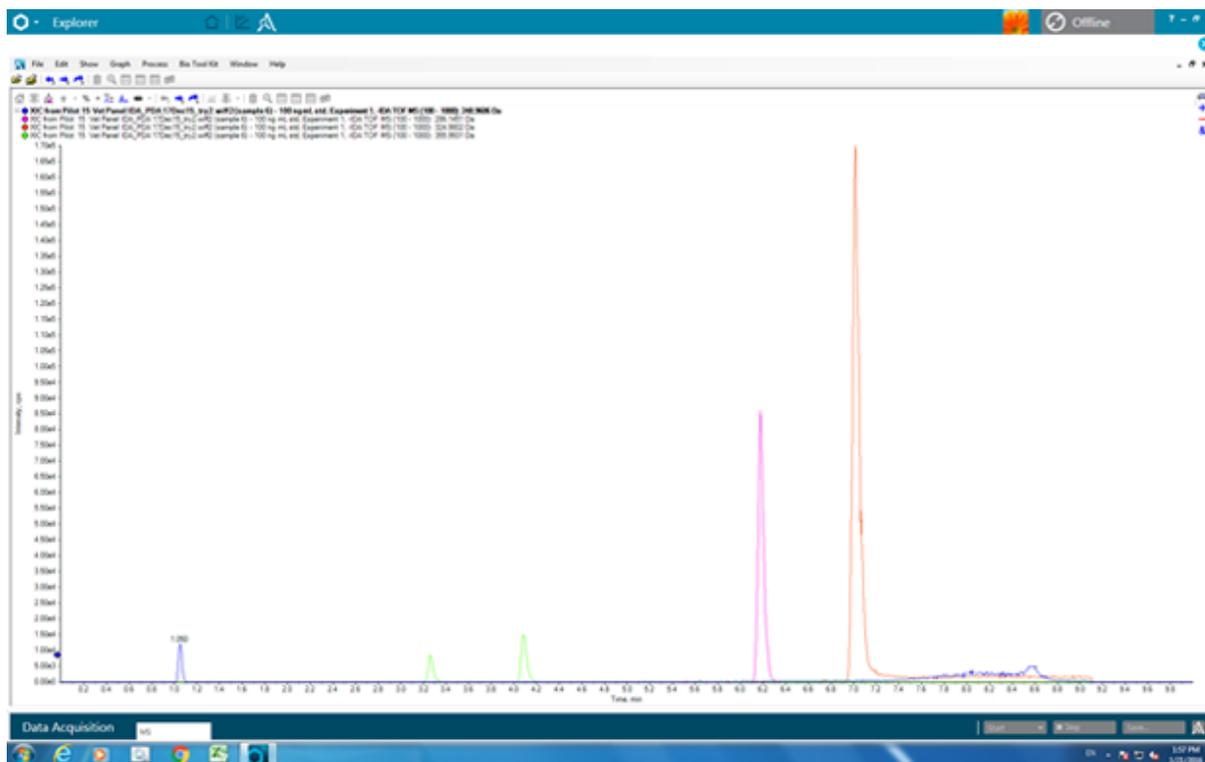
- **TIC:** The plot of the total ion current as a function of time.

**Figure A-1 Example TIC**



- **XIC:** An ion chromatogram created by taking intensity values, at a single, discrete mass value or a mass range, from a series of mass spectral scans. An XIC indicates the behavior of a given mass or mass range as a function of time.

Figure A-2 Example XIC



## Spectra

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

When data is viewed as a spectrum, mass-specific information about a compound is obtained. A spectrum provides the  $m/z$  values for the ions corresponding to a particular chromatographic peak. These ions 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 might change, but the  $m/z$  value is 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.

### Reconstruction Spectra

A processed spectrum is generated by applying a deconvolution algorithm to an MS or MS/MS spectrum. Reconstruction spectra consist of zero-charged or neutral masses with corresponding intensity. The spectrum usually provides the molecular weight information for a compound. Spectral intensity might change, but the molecular weight information does not change.

A typical reconstruction spectrum is shown with mass (Da) on the X-axis and intensity on the Y-axis.

### Decision Rules

While a batch is being processed in the Queue workspace, the software can perform select corrective actions in response to specified analysis results. For example, if a sample fails to meet the acceptance criteria defined in the processing method (analysis result), then the software can be instructed to reinject the sample (corrective action).

This feature is implemented with decision rules. A decision rule consists of two main parts:

- A flagging rule, which defines the analysis result  
Flagging rules are defined in processing methods.
- A corrective action, which is applied if the processing results do not satisfy the criteria for the analysis result

Corrective actions include the following:

- Stopping the queue
- Aborting the batch
- Injecting a different sample
- Reinjecting the flagged sample

When creating a batch, the user can activate decision rules for the batch, and then select the decision rules to be used.

### Dynamic Background Subtraction Algorithm

The Dynamic Background Subtraction algorithm improves detection of precursor ions in an Information Dependent Acquisition (IDA) experiment. When the algorithm is activated, IDA uses a spectrum that has been background subtracted to select the precursor ion of interest for MS/MS analysis, instead of selecting the precursor from the survey spectrum directly. Because this process takes place during LC analysis, the algorithm enables detection of species as their signal increases in intensity. As a result, this algorithm focuses on detection and analysis of the precursor ions on the rising portion of the LC peak, up to or slightly over the top of the LC peaks.

## Quantitative Analysis

Quantitative analysis is used to find the concentration of a specific substance in a sample. By analyzing an unknown sample and comparing it to standard samples, that is, samples containing the same substance with known concentrations, the software can calculate the concentration of the unknown sample. The process involves creating a calibration curve using the signal response or response ratio of the standards and then calculating the concentrations of the unknown samples. The calculated concentrations of all of the samples are added to a Results Table.

Quantitative analysis is most commonly performed using a Multiple Reactions Monitoring (MRM) scan. In an MRM scan, a precursor ion and a characteristic product ion are used to define an MRM transition that is highly specific to the analyte. The MRM transition, coupled with the retention time associated with the analyte during liquid chromatography, provides the specificity required for quantitation.

Quantitation is accomplished through the use of validated MRM LC-MS/MS acquisition methods, acquisition of calibration standard curves, and the subsequent integration of the peaks associated with the compounds of interest. The calibration curve relationship between signal response and concentration is used to determine the quantity of a particular analyte in an unknown sample.

## Standard Addition

Standard addition can be used to determine the concentration of a compound in a sample in which a known matrix effect prevents the use of a traditional calibration curve.

This feature allows the user to perform standard addition calculations directly in the software. If the standard addition feature is enabled in the quantitation workflow, then the standard addition calculation is performed during integration, and results are shown in the Results Table.

If this feature is enabled, then these regression parameters are disabled:

- Regression Type
- Weighting Type
- Automatic Outlier Removal

## Enable the Standard Addition Feature

1. Open the Analytics workspace.
2. Click **Process Method > New**.

---

**Tip!** To edit an existing processing method, click **Process Method > Edit embedded method** and then use the following steps.

---

3. Select the Workflow page and then select at least one workflow and the reference samples.

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4. Select the Components page and then define the component names, masses, internal standards, groups, and so on.

---

**Tip!** If the group is defined in the Components table, then the user can choose to sum the ions in the group, even if the precursor ion and the experimental index are different for the transitions. The summed ions are not shown in the table but are shown on the Integration page and in the Results Table as <group name>\_Sum. This feature is useful for the quantitation of proteins and peptides.

---

**Tip!** Where the retention time of the components is not known, set the **Retention Time Mode** for a mass or chemical formula to **Find  $n$  peaks**, where  $n$  is 1, 2, 5, 10, or all. The software identifies the specified number of features with the greatest peak area, assigns the appropriate retention time, and then performs a targeted peak processing workflow. When processing is complete, the embedded method for the Results Table can be saved as a normal targeted method.

---

5. Select the Integration page and then select the integration parameters for each component.
6. Click **Options > Quantitate by standard addition**.

This feature has specific requirements for the following batch fields:

- **Sample ID:** All samples belonging to the same standard addition group must have the same sample ID.
- **Sample Type:** All samples to be quantitated using standard addition must have the sample type, **Standard**.
- **Actual Concentration:** This field must contain the known concentration of standard added to each sample in the standard addition group. For example, for samples with no standard added, it is **0**. Data from this column is plotted as the X-axis on the Calibration Curve.

If this feature is enabled, then the Results Table contains a new **Standard Addition Accuracy** field that compares the **Standard Addition Calculated Concentration** for a sample to the **Standard Addition Actual Concentration**.

A dynamic view of the calibration curve for a specific sample is shown in the Calibration Curve.

## Mass Reconstruction

For large molecules, a charge state spread is typically observed in an MS full scan spectrum. The mass reconstruction feature allows the user to perform spectrum deconvolution directly in the software, and then to perform quantitation based on the deconvoluted or zero-charged mass peaks. If the mass reconstruction feature is enabled in the quantitation workflow, then peak finding, spectrum deconvolution, mass peak finding, and integration are performed during processing, and results are shown in the Results Table.

---

## Enable Mass Reconstruction Feature

---

**Note:** Mass Reconstruction is only supported in Quantitation workflow.

---

**Note:** Mass Reconstruction is only supported for the MQ4 and Summation integration algorithms.

---

**Note:** If Mass Reconstruction is enabled, then **Options > Sum Multiple Ion** is disabled.

---

1. Open the Analytics workspace.
2. Click **Process Method > New**.

---

**Tip!** To edit an existing processing method, click **Process Method > Edit embedded method** and then use the following steps.

---

3. Select the Workflow page and then select the **Quantitation** workflow and the reference samples.
4. Select the Components page.
5. Click **Options > Mass Reconstruction**.
6. Add the components, typing information in the required fields.

---

**Note:** The **Expected MW** field is optional.

---

7. Click **Integration** to view the integration page, and review the XIC chromatograph, average spectrum, and reconstruction spectrum, and to select the target mass.
8. Save the method.

If this feature is enabled, then the Results Table contains the following new columns: **Expected MW**, **MW**, **MW Delta (Da)**, **MW Delta (ppm)**, **IS Expected MW**, **IS MW**, **IS MW Delta (Da)**, and **IS MW Delta (ppm)**.

## Qualitative Analysis

Qualitative analysis is the identification of a target or unknown compound. In mass spectrometry, determining which compound is present is accomplished using mass accuracy, retention time, isotope pattern, library searching, and formula finding. Using all of these tools together can increase the confidence in identifying both targeted and non-targeted compounds in unknown samples.

## Mass Accuracy

When trying to identify a known target compound in a sample, it is useful to look at the mass accuracy of that compound and determine whether a potential hit for that compound has a

mass accuracy within a certain tolerance. For example, imazalil has a chemical formula of  $C_{14}H_{14}Cl_2N_2O$ , which gives it a monoisotopic mass of 296.0483, to four decimal places. A protonated adduct is an ion with a positive charge that is normally detected using a mass spectrometer. The protonated adduct of imazalil has a mass-to-charge ratio ( $m/z$ ) of 297.0556. If imazalil is suspected to be in a sample, then compare the  $m/z$  of the found compound to the  $m/z$  of the protonated imazalil and determine how closely they match. The smaller the difference, in ppm or Da, the more likely it is that the found compound is a match.

## Retention Time

Most mass spectrometers use some type of chromatography. The retention time for a compound is determined by injection of a known standard of the compound. Retention time can be used to help identify target compounds in a sample. If the suspected compound is in an unknown sample, then the closer the retention time is to the retention time of the standard, the more likely the unknown compound can be identified. Retention times can change and must be routinely confirmed using a known standard.

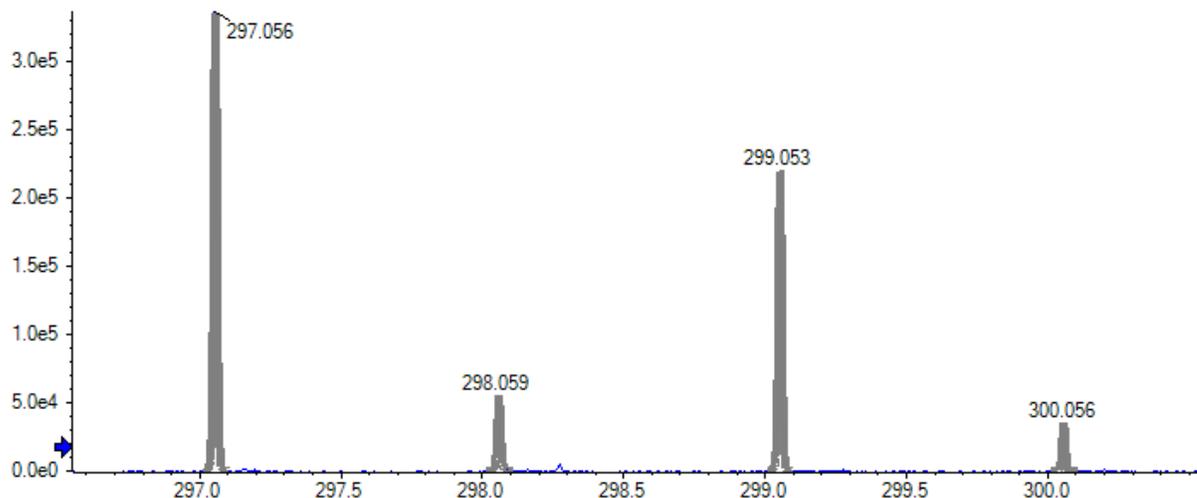
## Isotope Pattern

The full scan mass spectrum from a compound in a mass spectrometer has a distinct isotope pattern based on its molecular formula.

For the isotope pattern for imazalil, refer to the following figure.

**Figure A-3 Isotope Pattern (Imazilil)**

●  $C_{14}H_{14}Cl_2N_2O +H$



This isotope pattern for imazalil is comprised of the different mass isotopes for the elements. The isotope pattern is calculated theoretically and then compared to what was actually acquired for the compound in the unknown. The closer the match between the theoretical and the actual isotope pattern, the more likely it is that the compound has been identified.

---

## Library Searching

Comparing acquired MS/MS spectra from unknown samples to a database of compounds with reference spectra is one of the most powerful tools in qualitative analysis. Library search algorithms compare the unknown spectra from the sample and then try to match the spectra to the known compounds and spectra in the database. The closer the match and the higher the reported score are, the more likely it is that the compound was identified.

The purity, fit and reverse fit are calculated as follows:

- If there is a peak at a given mass in both the (reduced) library spectrum and the (reduced) unknown spectrum whose intensity ratio is within the limits specified by the user, the intensity of the peak in the library spectrum is set equal to that of the unknown spectrum

- The purity is calculated as:

$$100.0 (UL_{\text{total}})^2 / (U_{\text{total}} \cdot L_{\text{total}}) \text{ where:}$$

$$U_{\text{total}} = \sum U_m \cdot U_m$$

$$L_{\text{total}} = \sum L_m \cdot L_m$$

$$UL_{\text{total}} = \sum U_m \cdot L_m$$

The sums include all masses where the intensities  $U_m$  and  $L_m$  are the square roots of the mass-weighted, that is reduced; unknown; and library entries. The purity is guaranteed to fall in the range between 0 to 100 and is a measure of the similarity of the library spectrum and the unknown spectrum.

- The fit is calculated in exactly the same manner as the purity, except that only masses which occur in the library spectrum are included in the sums. This has no effect on  $L_{\text{total}}$  or  $UL_{\text{total}}$  because no terms are deleted from these sums. The fit is a measure of the degree to which the library spectrum is contained in the unknown spectrum. A high fit and a low purity indicates that the unknown spectrum is likely impure, but contains the library compound.
- The reverse fit is also calculated in the same manner as the purity, except that only masses that occur in the unknown spectrum are included in the sums. The reverse fit is a measure of the degree to which the unknown spectrum is contained in the library spectrum.

## Formula Finding

Using a mass number, the formula finding algorithm tries to predict the chemical formula for the compound, based on the MS and MS/MS spectra generated by a mass spectrometer. A high formula finding score does not necessarily mean that the compound in the sample is the one identified by formula finding algorithm, because several formula often match within the mass error. Care must be taken and other confirmatory testing must be done before a compound is identified using formula finding.

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**Note:** It is not recommended to perform formula finding with nominal mass systems.

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The formula finding algorithm uses the traffic light settings for mass accuracy. A red ppm error earns a score of 0 and a perfect match earns a score of 100.

The MS spectrum contributes 67% to the final formula finding score and the MS/MS spectrum contributes 33%. As a result, the ability of the formula to predict the MS mass is the primary influence on the score. However, the matching of the MS/MS fragments also influences the score.

The isotope pattern is used to generate the list of found formula, but it is not used to generate the final score. Therefore a formula with the wrong isotope pattern will probably not be included in the list.

A list of possible formulas is determined using precursor mass accuracy, isotopic pattern, and MS/MS fragmentation. Proposed formulas are scored based on precursor mass accuracy and average MS/MS mass accuracy of matching fragments.

## Integration

In quantitative or qualitative analysis, integration refers to the generation of chromatographic peak areas or heights for the compounds of interest. A processing method contains all of the information needed to process the data.

The compilation of quantitative or qualitative information for a given set of samples is called a Results Table. Refer to [Results Tables](#).

The software has three integration algorithms that can be used:

- **MQ4:** Selects a low concentration, but not the lowest concentration, standard or quality control sample by default as the representative sample of the analytical run.
- **AutoPeak:** Selects a high concentration, but not saturated, standard or quality control sample by default as the representative sample of the analytical run.
- **Summation:** Does not perform a normal peak search, but assumes that a peak is present close to the expected retention time.

It is also possible to manually integrate peaks that were missed by the algorithms.

## AutoPeak Integration Algorithm Parameters

The following parameters are used to identify and report the peak of interest.

For a complete list of available parameters, refer to the Help System.

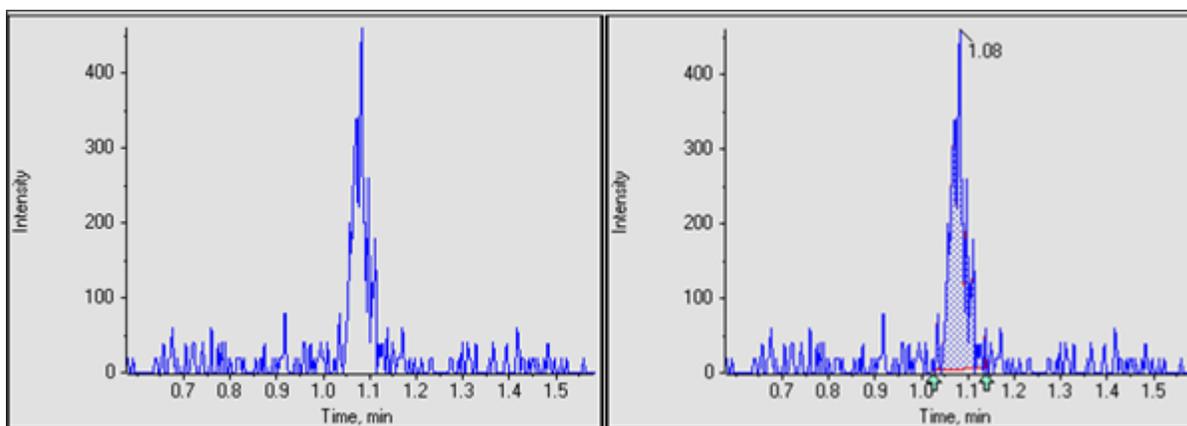
- **Local peak baseline:** The software assesses changes to the baseline locally around the peak as opposed to calculating the baseline with respect to the entire chromatogram.
- **Linear peak baseline:** The software fits a line between the points at the beginning and at the end of that specific group of peaks as opposed to the possibility of having a non-linear baseline below the peak.

**Saturation correction:** When the algorithm detects that a peak is saturated, it uses the model to predict how the peak might look if the detector had not saturated. This causes the profile to extend above the top of the peak to approximate the response that would have been obtained. This can extend the linear dynamic range of calibration curves. This option is only available when setting the overall algorithm default values and not during processing method creation or individual peak review, because it is not useful to use this setting for only some peaks.

## Minimum Signal/Noise

If the minimum signal to noise is set to seven, as shown in the left graph in the following figure, then the peak is not reported. If the minimum signal to noise is set to two, as shown in the right graph, then the peak is reported. This parameter does not affect integration.

Figure A-4 S/N Threshold



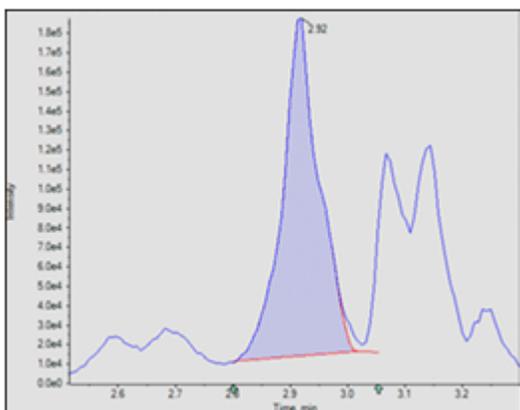
## Confidence Threshold

This parameter is used to filter potential peaks that are false positives. The default value is 50%, which is usually suitable. However, the user might want to use a larger value for very noisy data or for data for which the peak width has considerable variation from sample-to-sample.

The following two figures show how the **Confidence Threshold** affects the number of peaks identified. If the **Confidence Threshold** is set to 50%, then the peak with a little shoulder is identified as one peak. If the **Confidence Threshold** is lowered to 16%, then the SignalFinder algorithm finds two peaks. Drag across the two peak regions to view the two peaks.

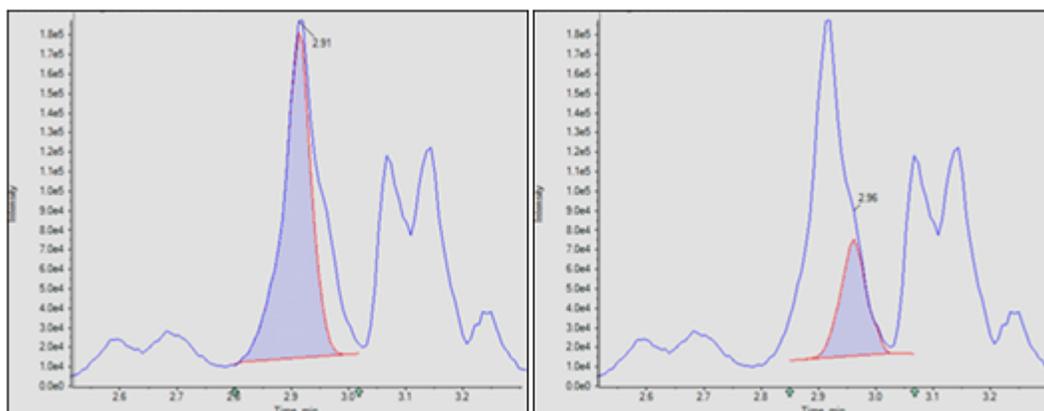
To determine which other peaks are potentially present in this single peak, and if the correct **Confidence Threshold** is not known, press **Ctrl** and then drag across the peak region of interest. This automatically lowers the **Confidence Threshold** to reveal the second peak of interest that is not present when the **Confidence Threshold** is set to 50%.

## Figure A-5 50% Confidence

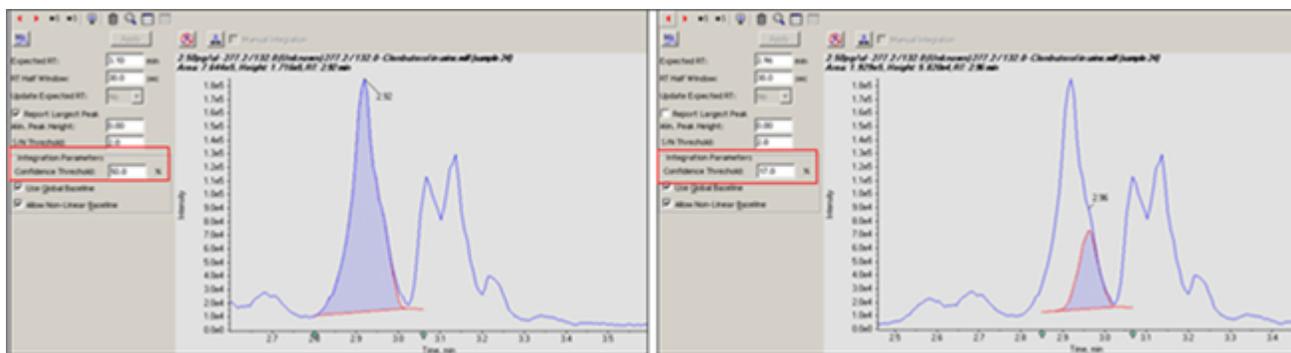


At 16% confidence, two peaks are found. Drag across the peak area to identify the two peaks.

## Figure A-6 16% Confidence



## Figure A-7 Confidence Threshold Parameter

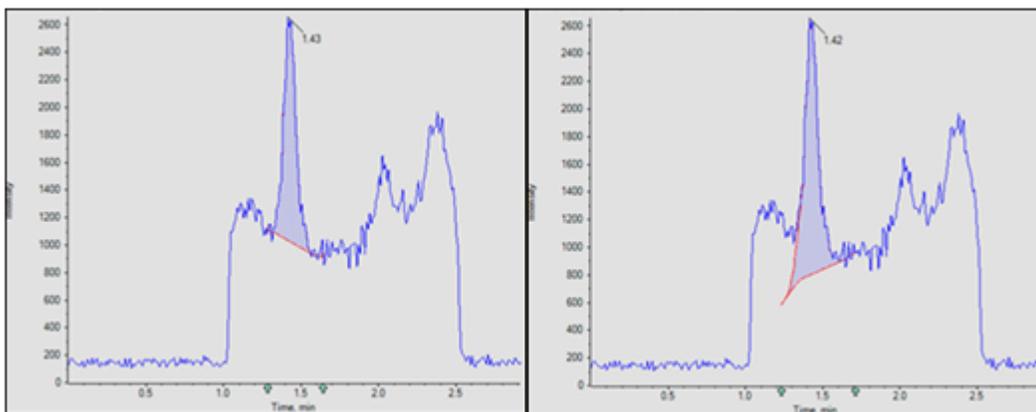


## Local versus Global Peak Baselines

The peak baseline can be local or global. If the local option is selected, then the quantitation software assesses changes to the baseline locally. The global option uses the entire chromatogram as the baseline.

For an example showing when the local baseline should be used, refer to the following figure. The left graph shows a chromatogram that was properly integrated using the local baseline. The right graph shows the same chromatogram, improperly integrated using the global baseline.

**Figure A-8 Use Global Baseline**



## Linear versus Non-linear Peak Baselines

The peak baseline can be set to linear or non-linear. The non-linear option estimates the baseline under each peak. The linear option fits a line between the points at the beginning and end of that specific group of peaks. For examples of linear and non-linear baselines for co-eluting peaks, refer to [Figure A-9](#) and [Figure A-10](#). Items 1 to 4 are convolved peaks. Item 5 shows the baseline, as derived with the different options.

A non-linear baseline is recommended for multiple peaks. For a single peak, the difference between the linear and non-linear baseline is insignificant.

Figure A-9 Example of a Linear Baseline

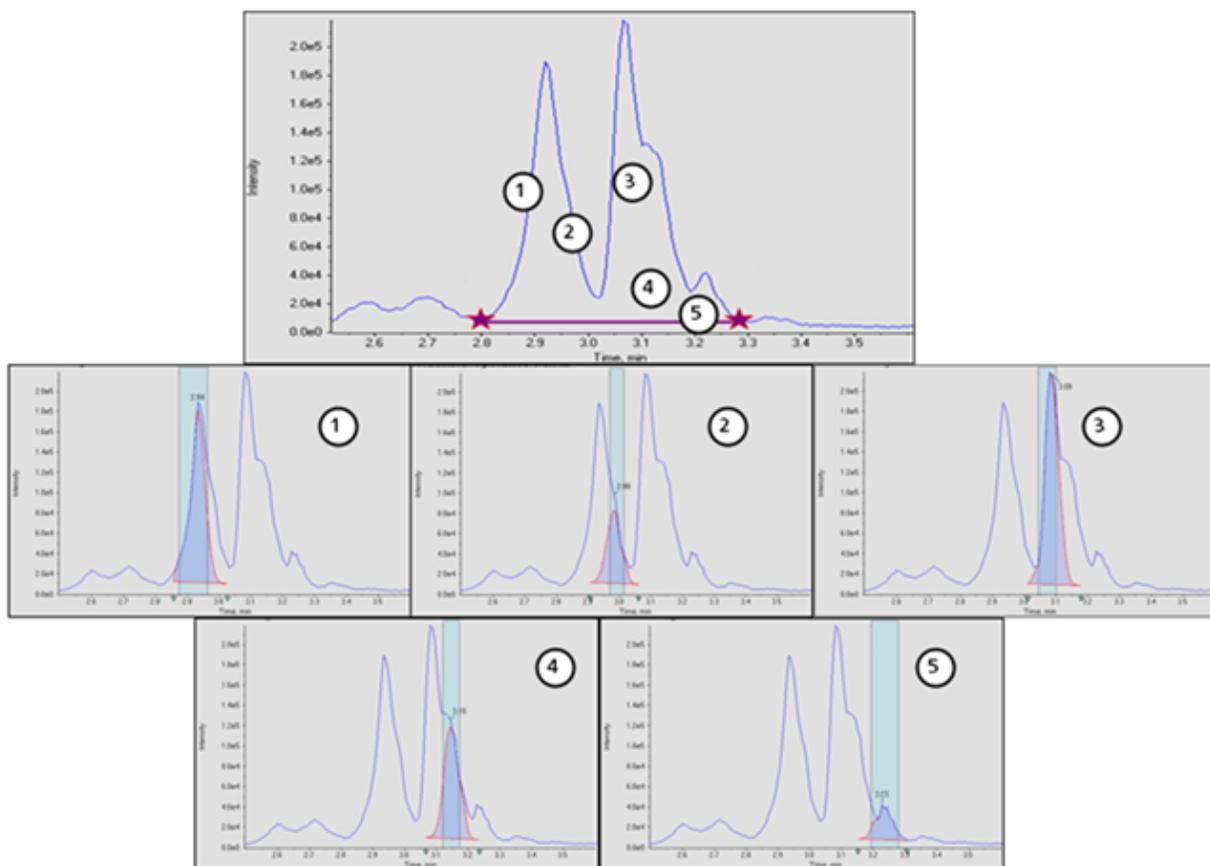
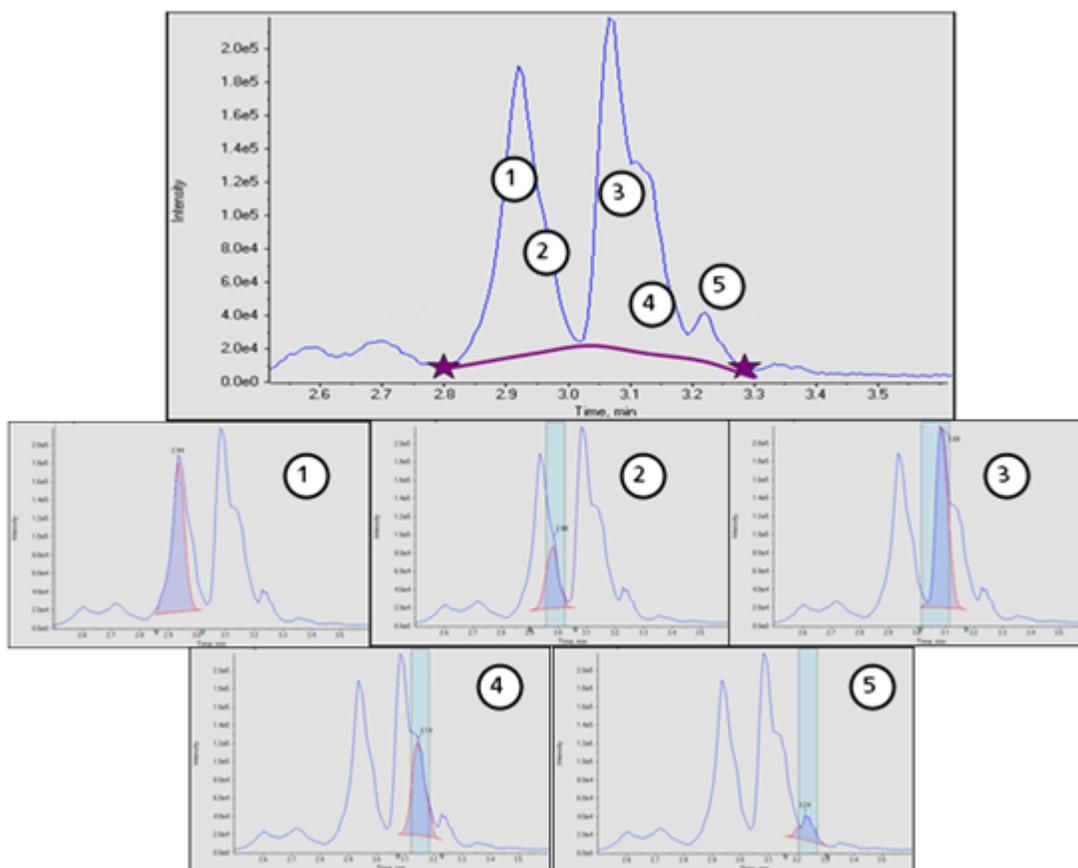


Figure A-10 Example of a Non-linear Baseline



## MQ4 Integration Algorithm Parameters

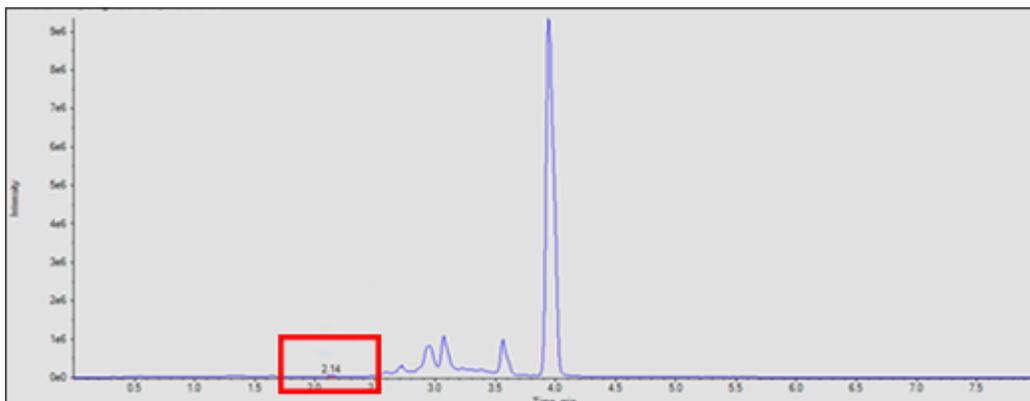
The following parameters are used to identify and report the peak of interest. For a complete list of available parameters, refer to the Help System.

### Noise Percentage

This parameter is used to estimate the noise level in the chromatograms. The specified percentage of the data points with the smallest intensity are assumed to be noise.

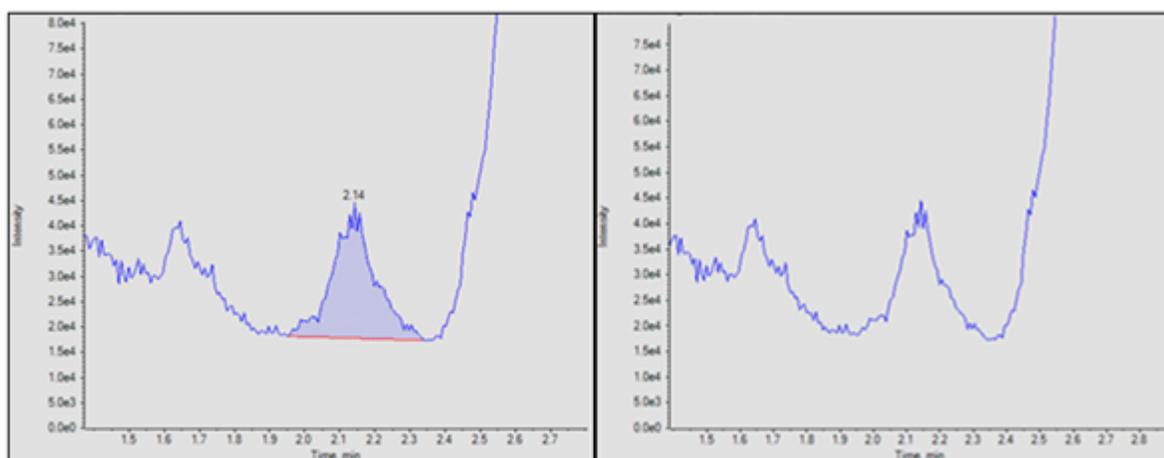
Typical values range from 20% to 60%. If small peaks in the presence of larger peaks are not being found, then the noise percentage should be lowered. The following figure shows an example of a small peak in the presence of an extremely large peak. This peak is not found when the noise percentage is set to 90% but is found when the noise percentage is set to 40%.

**Figure A-11 Peak of Interest**



In the following figure, the left graph shows the noise percentage set to 40%. The right graph is set to 90%.

**Figure A-12 Noise Levels**



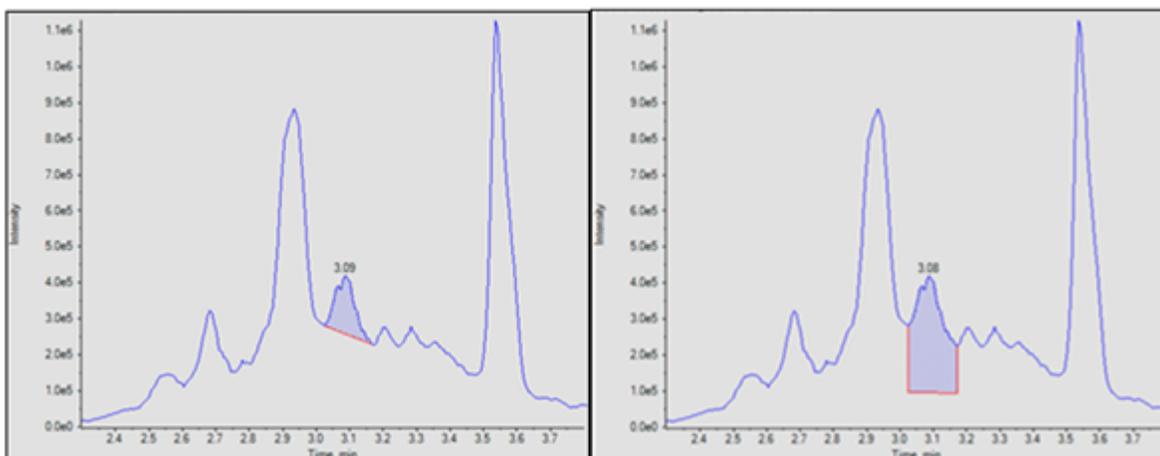
### Baseline Subtract Window

After smoothing, but before other processing, chromatograms are baseline subtracted to remove humps in the data. For each data point, the baseline is calculated using the data points on both the left and right side of the current point with minimum intensity, within the subtraction window.

The exact value of this parameter is not critical, provided that it is set at least a few times larger than the expected peak width.

In the following figure, the left graph shows the baseline subtraction window set to 0.1 minutes and the right graph shows the baseline subtraction window set to 1 minute.

Figure A-13 Baseline Subtraction Window



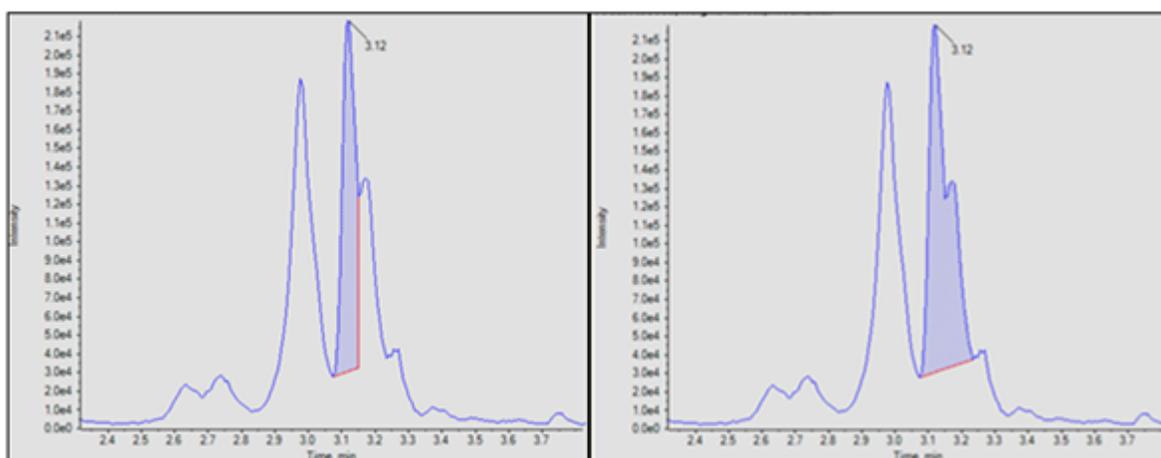
## Peak Splitting

This parameter controls whether a potentially noisy peak is found as one single peak or as two or more separate peaks. If the dip between two potential peaks is less than the specified value, then a single peak is found. Otherwise, two peaks are found.

Setting this parameter to a large value will prevent noisy peaks from being split and found as two separate peaks. However, a smaller value needs to be used if there are two closely eluting (overlapping) distinct peaks.

In the following figure, the left graph shows Peak Splitting set to two points. The right graph shows Peak Splitting set to three points.

Figure A-14 Peak Splitting



## Regression

The area or height of the analyte peaks is plotted against the known concentrations in the Calibration Curve and Metric Plot. Subsequently, a line is fitted to the points. This regression line is used to calculate the concentration of the unknown samples.

### Regression Equations

This section describes the equations used to calculate the regression curves. In the following equations,  $x$  represents the analyte concentration for standard samples and  $y$  represents the corresponding peak area or height. The exact variables used for the regression depend on whether an internal standard is being used and whether the peak area or the peak height is used as shown in the following table.

**Table A-1 Regression Variables**

Internal Standard Used?	Area Used?	$x$	$y$
Yes	Yes	$C_a / C_{is} / DF$	$A_a / A_{is}$
Yes	No	$C_a / C_{is} / DF$	$H_a / H_{is}$
No	Yes	$C_a / DF$	$A_a$
No	No	$C_a / DF$	$H_a$

where:

- $C_a$  = actual analyte concentration
- $C_{is}$  = internal standard concentration
- DF = dilution factor
- $A_a$  = analyte peak area
- $A_{is}$  = internal standard peak area
- $H_a$  = analyte peak height
- $H_{is}$  = internal standard peak height

### Weighting Types

The following table shows how the weighting factor ( $w$ ) is calculated for each of the seven weighting types.

Table A-2 Weighting Types

Weighting Type	Weight (w)
None	Always 1.0.
1 / x	If $ x  < 10^{-5}$ , then $w = 10^5$ . Otherwise, $w = 1 /  x $ .
1 / x <sup>2</sup>	If $ x  < 10^{-5}$ , then $w = 10^{10}$ . Otherwise, $w = 1 / x^2$ .
1 / y	If $ y  < 10^{-8}$ , then $w = 10^8$ . Otherwise, $w = 1 /  y $ .
1 / y <sup>2</sup>	If $ y  < 10^{-8}$ , then $w = 10^{16}$ . Otherwise, $w = 1 / y^2$ .
ln (x)	If $x < 0$ , then an error is generated. If $x < 10^{-5}$ , then $w = \ln 10^5$ . Otherwise, $w =  \ln (x) $ .
ln (y)	If $y < 0$ , then an error is generated. If $y < 10^{-8}$ , then $w = \ln 10^8$ . Otherwise, $w =  \ln (y) $ .

## Correlation Coefficient

In the regression equations,  $x$ ,  $y$ , and  $w$  are as defined previously. All sums are calculated over all standard samples, with the exception of those standard samples that are marked as not used.

The correlation coefficient is calculated as:

where:

$$D_y = \sum w \sum wy^2 - (\sum wy)^2$$

- $y_c$  = Y-value calculated using the appropriate equation for the regression type

$$D_{yc} = \sum w \sum wy_c^2 - (\sum wy_c)^2$$

## Regression Types

In the Analytics workspace, the following types of regression are available:

- Mean (Metric Plot pane only)
- Median (Metric Plot pane only)
- Linear ( $y = mx + b$ )
- Linear through Zero ( $y = mx$ )
- Mean Response Factor
- Quadratic ( $y = a^2 + bx + c$ )

- Power
- Wagner
- Hill

---

**Note:** The **Remove outliers automatically from the calibration curve** option on the Regression Options dialog in the Calibration Curve pane automatically applies the automatic outlier removal rules to the selected components of interest. Refer to the Help.

---

### Linear

The linear calibration equation is:

$$y = mx + b$$

The slope and intercept are calculated as:

$$m = (\sum w \sum wxy - \sum wx \sum wy) / D_x$$

$$b = (\sum wx^2 \sum wy - \sum wx \sum wxy) / D_x$$

where:

$$D_x = \sum w \sum wx^2 - (\sum wx)^2$$

### Linear Through Zero

The linear through zero calibration equation is:

$$y = mx$$

The slope is calculated as:

$$m = \sum wxy / \sum wx^2$$

### Mean Response Factor

The mean response factor calibration is:

$$y = mx$$

This is the same equation as for the linear through zero calibration. However, the slope is calculated differently as:

$$m = \sum w(y/x) / \sum w$$

and the standard deviation of the response factor as:

$$\sigma = \sqrt{(nD/(n-1))} / \sum w$$

where:

$$D = \sum w^* \sum wy^2 / x^2 - (\sum wy / x)^2$$

---

**Note:** Points whose x value is zero are excluded from the sums.

---

If there is some linearity and some curvature in the line of points, then use power regression instead of linear or quadratic regression to produce a line somewhere between these fits.

## Quadratic

The quadratic calibration equation is:

$$y = a_2x^2 + a_1x + a_0$$

The polynomial co-efficients are calculated as:

$$a_2 = (b_2/b_0 - b_5/b_3) / (b_1/b_0 - b_4/b_3)$$

$$a_1 = b_5/b_3 - a_2b_4/b_3$$

$$a_0 = (\sum wy - a_1\sum wx - a_2\sum wx^2) / \sum w$$

where:

$$b_0 = \sum wx / \sum w - \sum wx^2 / \sum wx$$

$$b_1 = \sum wx^2 / \sum w - \sum wx^3 / \sum wx$$

$$b_2 = \sum wx / \sum w - \sum wxy / \sum wx$$

$$b_3 = \sum wx^2 / \sum wx - \sum wx^3 / \sum wx^2$$

$$b_4 = \sum wx^3 / \sum wx - \sum wx^4 / \sum wx^2$$

$$b_5 = \sum wxy / \sum wx - \sum wx^2 y / \sum wx^2$$

## Power

The power function calibration equation is:

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$$y = ax^p$$

The equations for the linear calibration are used as described above to calculate the slope (m) and intercept (b), except that x in those equations is replaced by ln x and y is replaced by ln y. When this is done, a and p are calculated as:

$$a = e^b$$

$$p = m$$

If any of the x- or y-values are negative or zero, then an error is reported.

### Wagner

The Wagner calibration equation is:

$$\ln y = a_2 (\ln x)^2 + a_1 (\ln x) + a_0$$

The equations for the quadratic calibration are used as described above to calculate  $a_0$ ,  $a_1$ , and  $a_2$ , except that x in those equations is replaced by ln x and y is replaced by ln y.

If any of the x- or y-values are negative or zero, then an error is reported.

### Hill

The Hill calibration equation is:

$$y = (a + bx^n) / (c + x^n)$$

It is not possible to provide an analytical function for solving for a, b, c, and n. Instead, the co-efficients are determined using the iterative Levenberg-Marquardt method.

## Automatic Removal of Outliers

An optional feature allows the software to remove outliers from the calibration curve automatically. This time-saving feature is useful for applications with many compounds with different linear range and sensitivity.

When this feature is enabled, the software iteratively surveys all data points to identify a starting range, consisting of four consecutive points, that provides the best linear regression and satisfies user-specified rules for outlier removal. The algorithm calculates multiple regressions for all permutations of the starting points. It considers all valid regressions that satisfy the user-specified rules and takes all of them through the expansion sequence. For all of the valid starting ranges, the success of each expansion depends on the total number of used points, the range of the used levels, and the point with the worst absolute accuracy error in the regression before and after the expansion. The regression that spans the largest range and satisfies the rules is the "winning" regression.

**Note:** If four data points are not available, then the software will use three. The algorithm will not be applied if less than three points are available.

---

The rules for automatic removal of outliers are defined in the processing method, and include the following:

- Minimum correlation coefficient ( $r$ )

**Note:** This option uses the correlation coefficient, not the coefficient of determination ( $r^2$ ).

---

- Maximum allowed accuracy error for standard replicates at the lower level of quantitation (LLOQ)
- Maximum allowed accuracy error for standards above the LLOQ
- Maximum percentage coefficient of variation (CV) for multiple replicates of a standard at LLOQ

**Note:** If %CV is greater than the specified value, then the algorithm removes replicates in decreasing order of accuracy error until the %CV of remaining replicates is less than this value.

---

- Maximum percentage CV for multiple replicates of a standard at all levels above the LLOQ

**Note:** If %CV is greater than the specified value, then the algorithm removes replicates in decreasing order of accuracy error until the %CV of remaining replicates is less than this value.

---

- Whether the specified total number of outliers excludes outliers below the LLOQ and upper limit of quantitation (ULOQ)
- Maximum number of outliers that can be removed for a concentration level
- Total number of outliers that can be removed from a calibration curve

**Note:** This algorithm is applied to all standards, including those manually excluded.

---

**Note:** If the number of replicates that is used to produce the regression is different for each standard level, then the automatic outlier removal feature does not work perfectly and must only be used as a starting point. Review each calibration curve manually.

---

**Tip!** Make sure that the tolerance thresholds for standards accuracy in the Acceptance Criteria for the processing method match the thresholds in the Rules for Automatically Removing Outliers for Calibration Standards dialog.

---

## Results Tables

A Results Table is a compilation of the quantitative and qualitative information associated with a set of samples. It includes the calculations for concentration and accuracy determined as a result of interpolating the standard calibration curve. The library search results, formula finding results, and other qualitative analysis results are also available in the Results Table. Area, height, and other numerical characteristics can be shown. The number and type of columns in the Results Tables can be edited for simplified viewing.

## Calibration Curves

A calibration curve, also known as a standard concentration curve, is a method for determining the concentration of a substance in an unknown sample by comparing the unknown sample to a set of standard samples of known concentration. The calibration curve is a plot of how the instrument responds (the analytical signal) to changes in the concentration of the analyte (the substance to be measured). The operator prepares a series of standards across a range of concentrations near the expected concentration of the analyte in the unknown sample.

Calibration standards are used to build calibration curves. Incorrect readings or missing readings on some of the calibration samples might indicate issues with the analytical run. Follow acceptable methods found in literature and regulatory agency guidances to create a calibration curve. Examples of good practices in the preparation of calibration curves include:

- Preparing calibration standards in a blank matrix in which the analyte is to be measured.
- Generating a calibration curve for each analyte to be measured.
- Making sure of the coverage of the expected concentration range of the analyte, including typical and atypical specimens.
- Using six to eight standards to generate the curve.

This is not a comprehensive list and other guidances should be used in determining the best practice in developing a calibration curve for the laboratory.

---

**Note:** In some analytical runs, single-point calibration standards are used. Single-point calibrations are performed using a matrix blank sample and a single standard concentration. The relationship between instrument response and analyte concentration is determined by the line created by these two points. Both the acquisition and processing methods should be validated before being accepted for their intended use.

---

## Signal to Noise Ratio

When performing quantitative mass spectrometry data processing, it is important to determine whether a given peak is significant or not, where *significant* typically means *exceeding background noise*.

## Relative Noise and Signal-to-Noise Calculations

Usually the peak height is compared to background noise measured in a peak-free region where the noise is typically estimated as either one or three times the standard deviation of the data points in this range. This approach is less than ideal for the following reasons:

- It is subjective, as the noise region is selected manually.
- A background region without a peak might not exist or the region might be too narrow for an accurate estimate of the noise.
- The noise at the peak position might be quite different from that in the selected noise region.
- The factor of 'one or three' is also subjective and different authorities have different recommendations.
- The apparent noise can be altered if the data have been pre-processed. For example, smoothed, or thresholded.

The concept of Relative Noise ( $R_n$ ), makes it easy to develop a simple method to calculate the expected noise at any point in the data, for comparison with the measured signal. This is a robust, objective metric that can be used to calculate signal-to-noise (S/N) and to evaluate and compare instrument and assay performance. There are many applications of the relative noise concept, one of which is the calculation of S/N.

The basic algorithm works as follows:

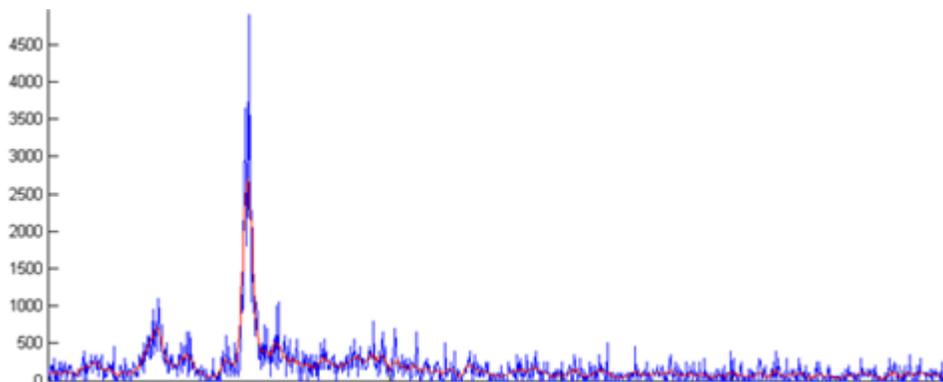
1. Devise a noise model that will allow the user to calculate the expected noise at any point in the data record, given the level of the underlying signal at that point.

The noise model can be determined from theoretical considerations or it can be modeled from real measurements for a particular system. For pulse counting detectors, the standard deviation of a signal, and therefore the expected noise, is proportional to the square root of the signal and therefore varies with the signal. In other systems there will be a constant 'white noise' component, possibly combined with an intensity-dependent component.

2. Estimate the underlying signal from the measured signal.

This task can be achieved in many ways, but the simplest is to generate a smoothed version of the data. Refer to the figure: [Figure A-15](#).

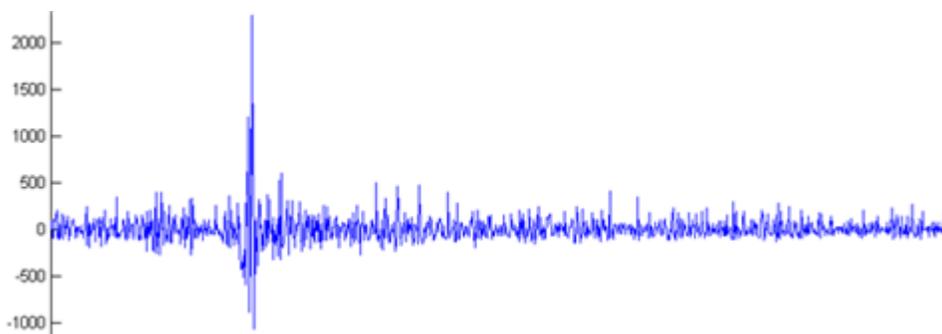
**Figure A-15 Overlay of Raw and Smoothed Data**



3. Measure the actual noise across the data using all points, both peaks and background.

This is achieved by subtracting the underlying signal estimate from the measured signal at each point in the data where the smoothed signal has been subtracted from the original. The result is known as the delta noise. The range of the delta noise is reasonably constant, except where there are large peaks, because the noise is dependent on the signal and therefore greater where the signal is larger. Refer to the figure: [Figure A-16](#).

**Figure A-16 Plot of the Delta Noise Values of Each Data Point**



4. At each data point, calculate the ratio of the measured noise to the expected noise.

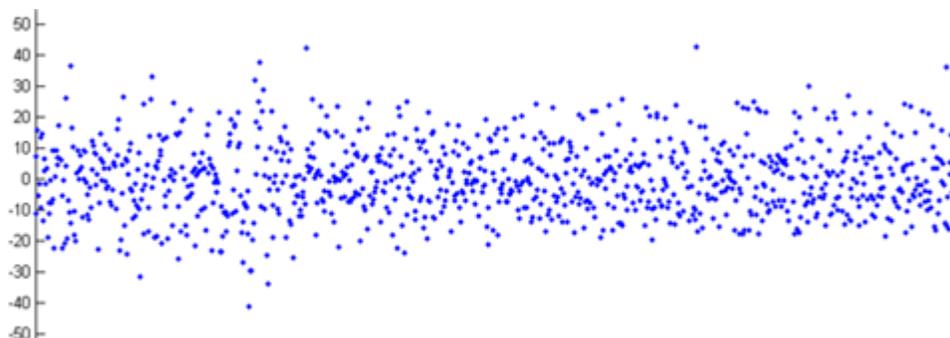
That is, at every data point, divide the noise measured in step 3 by the value that the noise model predicts, in this case the square root of the intensity. If the noise model is good, then the software generates a series of values that mostly remain bounded by some limits. Refer to the following figure. This figure also shows the plot of

$$\Delta noise / \sqrt{intensity}$$

---

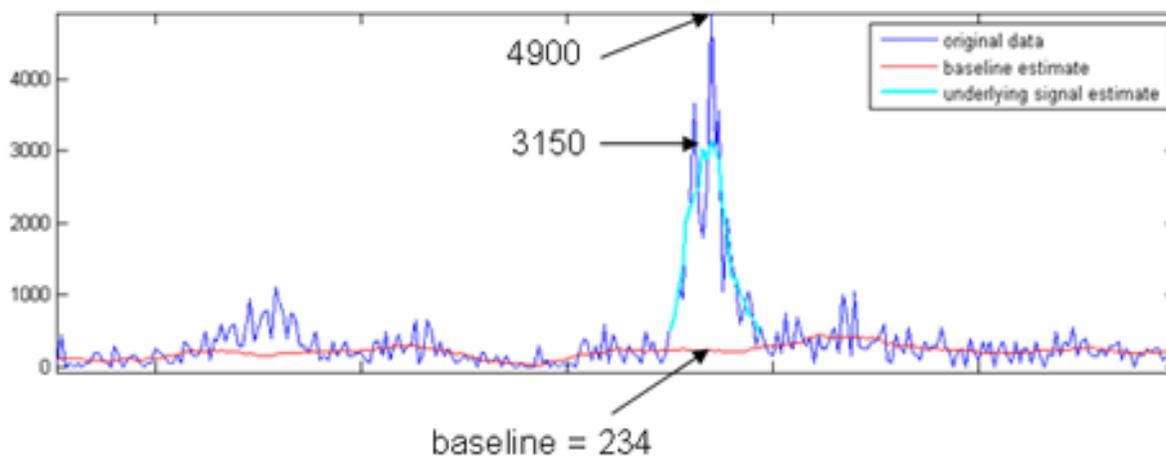
**Note:** This step reduces the large variation in the delta noise and results in a well constrained set of values.

---

**Figure A-17 Noise Model**

5. Calculate the standard deviation of the ratio values. This is the  $R_n$ , an estimate of the most likely relationship between the actual delta noise and that predicted by the model. In the preceding figure, this results in a value of 9.5.

The following figure shows an example of how relative noise can be used to calculate S/N.

**Figure A-18 Overlay of Raw Data, Underlying Signal Estimates, and Baseline Estimates**

As described previously:

$$\text{noise} = R_n \times \sqrt{(\text{baseline})}$$

In this example:

$$\text{noise} = 9.5 \times \sqrt{234} = 145$$

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If the apex of the peak is used as the signal, then the S/N is 34 (4900/145) and if the height of the smoothed signal is used, then the S/N is 22 (3150/145).

When reporting the S/N, the MQ4 integration algorithm uses the procedure described here and the peak apex as the signal. Because the AutoPeak integration algorithm is fitting a model to the peak, it uses the height of the fitted profile. This results in a smaller reported S/N. However, it is a more accurate value because it is less affected by possible noise spikes. The AutoPeak integration algorithm also has a more sophisticated approach to baseline estimation, so for these two reasons, the S/N values reported by the two algorithms are not identical, although they will usually be similar.

In summary, compared to the usual approach of estimating the noise as the standard deviation of a background region, the relative noise approach to calculating S/N has the following advantages:

- It is much less subjective because a background region need not be selected manually.
- An accurate S/N can be predicted even if no peak-free regions exist in the chromatogram.
- For the AutoPeak and Summation integration algorithms, the baseline, and therefore the noise, is estimated near the peak of interest. For the MQ4 integration algorithm, the baseline is the intensity of the data point at the user-specified noise percentage. For example, if the user-specified noise percentage is 40%, and there are 100 data points, then the MQ4 integration algorithm sorts the data points from the smallest to largest intensity, and uses the intensity of the data point with the fortieth-smallest intensity.

This can make a large difference to the reported S/N value because the background region selected for the usual approach might be much quieter than the background near the peak. As described previously, the S/N calculated using the Relative Noise approach might give smaller values than the usual approach. However, they are more accurate and useful values. Refer to [Figure A-18](#).

To make the **Signal / Noise** column visible in the Results Table, refer to [Customize the Results Table](#).

### Note on Signal-to-Noise when Using the AutoPeak Integration Algorithm

Because the AutoPeak integration algorithm calculates signal-to-noise more accurately (and therefore more accurately predicts CVs), if the 1-sigma signal-to-noise approach is used, then consider decreasing the minimum acceptable signal-to-noise value on any standard operating procedures (SOPs), based on empirical data from the laboratory.

### Signal-to-Noise Using Peak-to-Peak

If this signal-to-noise algorithm is used, then the software calculates the signal-to-noise ratio by taking the standard deviation of all of the chromatographic data points between the specified background start and background end times. The software calculates the signal-to-noise ratio for the active chromatogram, subtracts the average background signal from the selected peak, and

then divides the subtracted signal by the peak-to-peak noise level. It then differentiates the noise and peak regions based on the maximum intensities of each region. Upon completion, the active chromatogram is labeled with the signal-to-noise ratio.

## Signal-to-Noise Using Standard Deviation

When this signal-to-noise algorithm is used, the software calculates the signal-to-noise ratio of chromatographic peaks and labels them. This algorithm requires that two regions be selected on the chromatogram:

- The noise region
- The peak of interest

The software then determines which region contains the peak and which region contains the noise, based on maximum intensities in each selection. It subtracts the average background signal intensity from the peak signal intensity and then divides the subtracted signal by a user-specified factor multiplied by the standard deviation of the noise region.

## Define Noise Regions

Use this procedure to define noise regions if the standard deviation or peak-to-peak algorithm is used.

---

**Note:** Only one signal-to-noise algorithm can be used in a Results Table. To apply a different signal-to-noise algorithm to the data, change the project defaults and then create a new Results Table.

---

1. In the project default settings, select the **Standard Deviation** or **Peak-to-Peak** signal-to-noise algorithm.

---

**Tip!** To open the project default settings, click **Projects > Project default settings**.

---

2. Create a processing method.
3. On the Integration page, click **Options > Show Noise Regions**.
4. (If required) Use the mouse to adjust the noise region.

---

**Note:** The noise region must be set for each transition.

---

5. Process the data.
6. In the Peak Review pane, click **Options > Show Noise Regions**.
7. (If required) Use the mouse to adjust the noise region.

## Calculated Columns

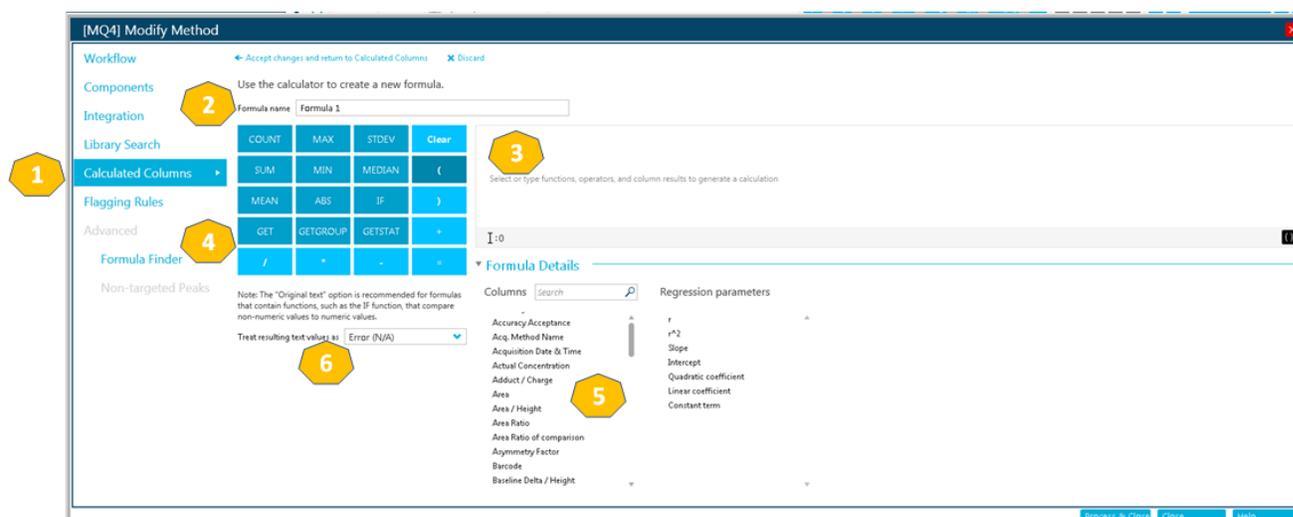
Calculated columns are formulas that result in new custom columns being added to a Results Table. After a formula is created and the data is processed (or reprocessed), the results of the formula are shown in a new custom column.

### Navigating the Calculated Column Interface

Calculated columns are created in a processing method. They can be imported and exported as `frm1` files for later use or sharing.

The following figure shows the interface for the formula editor.

**Figure A-19 Calculated Columns UI**



Item	Description
1	The <b>Calculated Columns</b> step in the workflow of the processing method. Click to open the Calculated Columns page. Then click <b>Add Formula</b> (not shown).
2	The <b>Formula name</b> field. Type a name for the formula.  <b>Note:</b> The formula name cannot contain the names of functions on the calculator, square brackets, or rounded brackets.
3	The <b>Formula</b> field.

Item	Description
4	<p>A calculator containing commonly used functions and operators. The following additional operators can be typed in the formula field:</p> <ul style="list-style-type: none"> <li>• &gt; (greater than)</li> <li>• &gt;= (greater than or equal to)</li> <li>• &lt; (less than)</li> <li>• &lt;= (less than or equal to)</li> <li>• != (not equal to)</li> </ul> <p>For more information about these operators and functions, refer to the <i>Help System</i>.</p>
5	<p>Available regression parameters and Results Table columns.</p> <hr/> <p><b>Note:</b> This list is not available in <code>qsession</code> tables.</p> <hr/>
6	<p>The <b>Treat resulting text values as</b> menu allows the user to configure how text entries are handled. This option is important in Results Table columns that may contain both numeric and text outputs.</p> <p>For example, calculated concentration columns can contain numeric values along with non-numeric values such as N/A, degenerate, and infinity.</p>

---

**Note:** When the user starts to type a formula that uses an array of samples, a sample selection item becomes available.

---

## Simple Extraction of Non-default Information

The calculated columns feature allows users to show information that is not available by default in Results Tables.

For example, to show  $R^2$  as a column in the Results Table, the user can create a formula equal to  $R^2$ .

**Figure A-20 Creating a Custom Column with Calculated Columns**

[← Accept changes and return to Calculated Columns](#) [✕ Discard](#)

Use the calculator to create a new formula.

Formula name

COUNT	MAX	STDEV	Clear
SUM	MIN	MEDIAN	(
MEAN	ABS	IF	)
GET	GETGROUP	GETSTAT	+
/	*	-	=

[r^2]

I:5

▼ Formula Details

Columns  🔍

Regression parameters

- Accuracy
- Accuracy Acceptance
- Acq. Method Name
- Acquisition Date & Time
- Actual Concentration
- r
- r^2
- Slope
- Intercept
- Quadratic coefficient

Note: The "Original text" option is recommended for formulas that contain functions, such as the IF function, that compare non-numeric values to numeric values.

Treat resulting text values as

## Simple Arithmetic

Simple formulas can be created to perform basic mathematical operations.

### Example: R<sup>2</sup>

```
[r] * [r]
```

In this example, the R<sup>2</sup> value is reproduced by using the multiplication operator (\*) to multiply the R-value by itself.

### Example: Points Per Second Collected

```
[Points Across Baseline] / ((([End Time] - [Start Time]) * 60)
```

In this example, the points across the baseline are divided by the seconds from the beginning to the end of an integrated chromatographic peak. This formula uses the division (/), multiplication (\*) and subtraction (-) operators.

## More Complex Functions

Many other functions and control structures exist. Some common ones are **MEAN()**, **MAX()**, and **MIN()**, and are shown in the calculator under the formula bar.

For a complete list of syntax details, operators, and functions, refer to the *Help System*.

### Example: MEAN([Area]) for Standards

When using a function that operates on all values, the user can select the samples to be included in the calculation.

**Figure A-21 Obtaining the Mean of the Peak Area of Standard Samples Only**

← Accept changes and return to Calculated Columns    ✕ Discard

Use the calculator to create a new formula.

Formula name:

COUNT	MAX	STDEV	Clear
SUM	MIN	MEDIAN	(
MEAN	ABS	IF	)
GET	GETGROUP	GETSTAT	+
/	*	-	=

MEAN([Area])

I:12

Formula Details

Columns

Accuracy  
Accuracy Acceptance  
Acq. Method Name  
Acquisition Date & Time  
Actual Concentration  
Adduct / Charge  
Area  
Area / Height  
Area Ratio  
Area Ratio of comparison  
Asymmetry Factor  
Barcode

Regression parameters

r  
r^2  
Slope  
Intercept  
Quadratic coefficient  
Linear coefficient  
Constant term

MEAN value will be calculated using the following sample types:

Unknowns  
 Only if the sample name contains...

Standards  
 Only if the sample name contains...

QCs  
 Only if the sample name contains...

Blanks  
 Solvent     Blank     Double blank  
 Only if the sample name contains...

Note: The "Original text" option is recommended for formulas that contain functions, such as the IF function, that compare non-numeric values to numeric values.

Treat resulting text values as:

### Example: Combining Functions

Simple arithmetic and more complex functions can be combined. For example, to calculate the mean points per second collected, use the following formula:

```
MEAN([Points Across Baseline]/(((End Time]-[Start Time])*60))
```

## IF Statements

The **IF** function performs a logical test and returns one value for a true result, and another for a false result. Nested **IF** functions can be used to test more than one condition. The **IF** function can be combined with other logical functions such as **and** and **or** to extend a logical test.

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**Note:** "&&" and "||" can be used for **and** and **or**, respectively. The **and** and **or** operators must be surrounded by spaces, but the && and || operators do not need to be.

---

The basic **IF** statement syntax is as follows:

```
if(<condition>;<value if true>;<value if false>)
```

- *<condition>* is a value or logical expression that can be evaluated as true or false.
- *<value if true>* is a value to be returned and shown in the corresponding Results Table column when *<condition>* evaluates to true.
- *<value if false>* is a value to be returned and shown in the corresponding Results Table column when *<condition>* evaluates to false.

**Note:** The **IF** function symbol can be selected from the calculator, typed, or copied from another source. It can be used in **if** or **IF** syntax.

---

The **IF** function allows other numeric functions, such as **MEAN**, **STDEV**, and so on, to be used within the formula as well, in the *<condition>*, *<value if true>*, or *<value if false>* expressions.

### **Example: <condition>**

Some examples of a *<condition>* include:

```
[Peak Area]>5000
```

```
[Component Name]='Analyte 1'
```

```
[Retention Time]> 1 and [Retention Time]<2
```

### **Example: <value if true> and <value if false>**

*<value if true>* and *<value if false>* can be either numeric or text.

```
if([Retention Time]> 1 and [Retention Time]<2; '1-2 min RT window';  
'not applicable')
```

### **Example: Mean Value for Internal Standard Area**

In this example, the mean value of the internal standard (IS) area is calculated across the desired samples and compared to a value of 1e6. The mean IS area value is shown in the corresponding Results Table column if **MEAN ([IS Area])** is greater than 1e6, that is, if the

---

<condition> is true. If **MEAN ([IS Area])** is less than 1e6, that is, if the <condition> is false, then the Results Table column contains **Review IS performance**, the <value if false>.

```
IF (MEAN([IS Area])>=1e6;'MEAN([IS Area])';'Review IS performance')
```

---

**Note:** Only **IF** functions can contain multiple calculations.

---

## Treat Resulting Text Values As

The **Treat resulting text values as** option determines how text is interpreted in a custom Results Table column that contains either text or a combination of numbers and text. For example, the **Sample Type** column contains only text, the **Precursor Mass** column contains only numeric values, and the **Calculated Concentration** column might contain both numeric values and text.

Depending on the functions used in a formula, the **Treat resulting text values as** option enables specific interpretation of the text values in the column on which the calculation is based. The available options include:

- **Zero**
- **Ignore (blank)**
- **Error (N/A)**
- **Original text**

---

**Note:** For more information about these options, refer to the *Help System*.

---

If calculations are based on the **COUNT**, **MAX**, **STDEV**, **SUM**, **MIN**, **MEDIAN**, **GET**, **GETGROUP**, **SLOPE**, **INTERCEPT**, **MAD**, or **GETSTAT** functions, then the recommended options are **Zero**, **Ignore (blank)**, or **Error (N/A)**. These options are also recommended in **IF** statements when the formula contains columns that are expected to have numeric values.

**Original text** is the recommended option in **IF** statements where components of the <condition>, <value if true>, and <value if false> expressions might be both numeric and text, especially when additional functions are used.

---

**Note:** In **IF** statements with more than one <condition>, failure to evaluate even one <condition> results in a <value if false> output in the custom results table column.

---

### Example

In this example, the columns used in the formula might contain both text and numeric values. Therefore, the **Original text** option is recommended.

```
IF ([Sample Type]='Unknown' && (GET([Calculated Concentration];  
'Analyte 1')+GET([Calculated Concentration];'Analyte 2'))<=15;
```

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---

```
'Low Range';IF([Sample Type]='Unknown'&&(GET([Calculated  
Concentration]  
; 'Analyte 1')+GET([Calculated Concentration]; 'Analyte 2'))  
<=65; 'Normal Range'; 'Over normal range'))
```

This **IF** formula contains both the **Sample Type** and **Calculated Concentration** columns. Values in the **Sample Type** column should be treated as **Original text**. For the **Calculated Concentration** column, it might be necessary to treat non-numeric values such as **<0** and **Degenerate** as **Zero**.

Because non-numeric values must be treated differently, we recommend that the user split the formula into multiple smaller formulas, for finer control of non-numeric values.

# Calibrate a System Configured with Contact Closure

## B

---

If contact closure is configured on the system, it can be used to calibrate the system in both batch and manual mode:

- Batch mode: The system can be calibrated with either the CDS or an LC method. Refer to [Calibrate the System in Batch Mode](#).
- Manual mode: The system can be calibrated with either the CDS or an LC method. Start the method by clicking **Start** or **Start with LC** in the MS Method workspace. When the status changes to **Load**, then start injection on the LC device.

---

**Note:** The MS Tune workspace does not support the contact closure feature. MS Tune does not wait for the contact closure signal.

---

## Calibrate the System in Batch Mode

Use either a CDS or an LC method to calibrate the system.

### Calibrate the System Using the CDS

If the system is communicating with an external device using contact closure, then follow these guidelines to calibrate the system using a CDS:

- Configure the Auto-Calibration properties, including the number of samples between calibrations.
- Synchronize the methods on the LC system and the mass spectrometer to allow time for calibration between samples. The following sections describe two different options for doing so.
- After submitting the batch, wait for the initial calibration to complete, and then, when the system goes into Loading state, start injection on the external device.

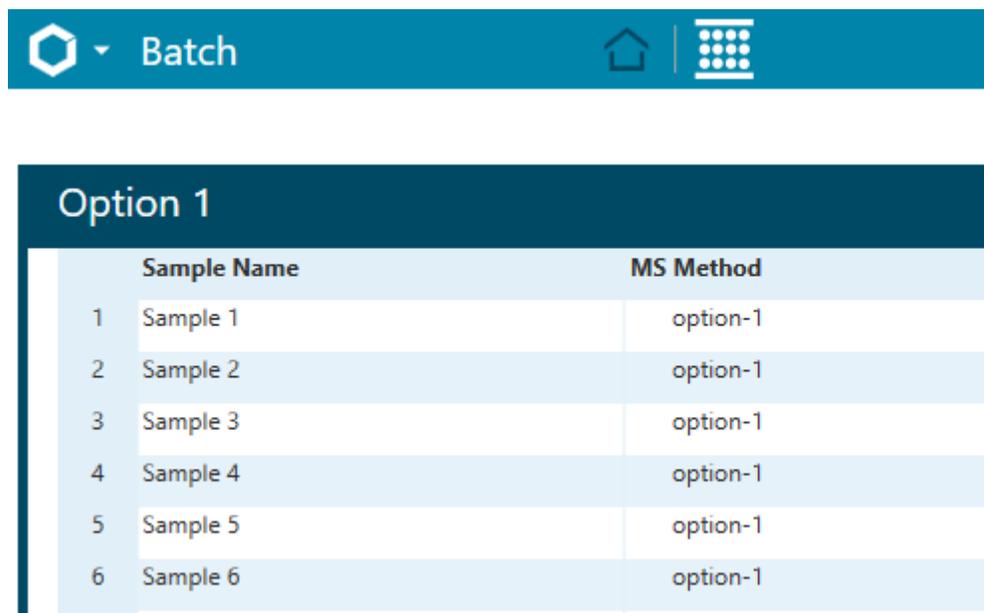
#### Option 1

To synchronize the LC system and the mass spectrometer, make sure that the LC methods are at least two minutes longer than the mass spectrometer methods.

The following examples show the batch and queue in SCIEX OS, and the corresponding schedule on the external device for a batch where calibration is performed after every third sample.

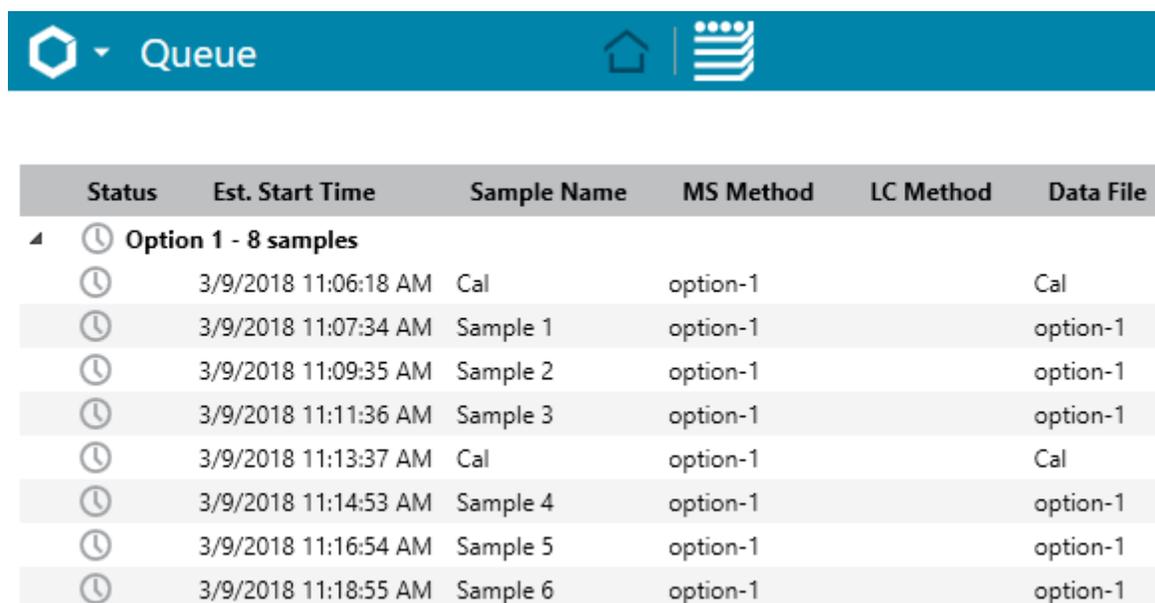
## Calibrate a System Configured with Contact Closure

Figure B-1 CDS Calibration: Example Batch



Option 1	
	MS Method
1 Sample 1	option-1
2 Sample 2	option-1
3 Sample 3	option-1
4 Sample 4	option-1
5 Sample 5	option-1
6 Sample 6	option-1

Figure B-2 CDS Calibration: Example Queue



Status	Est. Start Time	Sample Name	MS Method	LC Method	Data File
⌚	Option 1 - 8 samples				
⌚	3/9/2018 11:06:18 AM	Cal	option-1		Cal
⌚	3/9/2018 11:07:34 AM	Sample 1	option-1		option-1
⌚	3/9/2018 11:09:35 AM	Sample 2	option-1		option-1
⌚	3/9/2018 11:11:36 AM	Sample 3	option-1		option-1
⌚	3/9/2018 11:13:37 AM	Cal	option-1		Cal
⌚	3/9/2018 11:14:53 AM	Sample 4	option-1		option-1
⌚	3/9/2018 11:16:54 AM	Sample 5	option-1		option-1
⌚	3/9/2018 11:18:55 AM	Sample 6	option-1		option-1

Table B-1 Sample Sequence on the External Device

Time (mm:ss)	Injection
00:00	Sample 1

**Table B-1 Sample Sequence on the External Device (continued)**

Time (mm:ss)	Injection
12:00	Sample 2
24:00	Sample 3
36:00	Sample 4
48:00	Sample 5
60:00	Sample 6

### Option 2

This option is suitable for a workflow with a short LC method.

Follow these guidelines to synchronize the LC system and the mass spectrometer:

- For all calibrations except the first one in the batch, configure the external device to inject a blank sample whenever calibration is scheduled to occur. For example, if three samples are acquired between calibrations, then make sure that every fourth injection is a blank sample.
- Make sure that the blank sample run time on the external device is 2 minutes or greater. (CDS calibration takes 2 minutes.) Make sure that the method Duration is less than or equal to the time between injections.

The following examples show the batch and queue in SCIEX OS, and the corresponding schedule on the external device for a batch where calibration is performed after every third sample.

## Calibrate a System Configured with Contact Closure

Figure B-3 CDS Calibration: Example Batch

The screenshot shows a software interface for a 'Batch' operation. At the top, there is a blue header bar with a home icon, a grid icon, and the text 'Batch'. Below this is a navigation bar with buttons for 'Auto-Calibrate...', 'Plate Layout...', 'New', 'Open', and 'Save'. The main content area is titled 'Option 2' and contains a table with the following data:

	Sample Name	MS Method
1	Sample 1	option-2
2	Sample 2	option-2
3	Sample 3	option-2
4	Sample 4	option-2
5	Sample 5	option-2
6	Sample 6	option-2

Figure B-4 CDS Calibration: Example Queue

The screenshot shows a software interface for a 'Queue' operation. At the top, there is a blue header bar with a home icon, a list icon, and the text 'Queue'. Below this is a table with the following data:

Status	Est. Start Time	Sample Name	MS Method	LC Method	Data File
4	🕒	Option 2 - 8 samples			
	🕒	Cal	option-1		Cal
	🕒	Sample 1	option-1		option-1
	🕒	Sample 2	option-1		option-1
	🕒	Sample 3	option-1		option-1
	🕒	Cal	option-1		Cal
	🕒	Sample 4	option-1		option-1
	🕒	Sample 5	option-1		option-1
	🕒	Sample 6	option-1		option-1

Table B-2 Sample Sequence on the External Device

Time (mm:ss)	Injection
00:00	Sample 1

**Table B-2 Sample Sequence on the External Device (continued)**

Time (mm:ss)	Injection
02:00	Sample 2
04:00	Sample 3
06:00	Blank
08:00	Sample 4
10:00	Sample 5
12:00	Sample 6

### Calibrate the System Using the LC System

If the system is communicating with an external device using contact closure, then follow these guidelines to calibrate the system using the external device:

- In the properties for the mass spectrometer, configure a valve to simulate the external device.
- Create an LC method for the valve.
- Make sure that the method duration is less than or equal to the time between injections on the external device.
- Configure the Auto-Calibration properties for the batch: Select the ion reference table, and set the frequency of calibration. For **Calibrant delivery**, select the LC method for the valve, and for **MS method**, select the MS method to be used.

## Calibrate a System Configured with Contact Closure

---

**Figure B-5 LC Calibration: Automatic Calibration Editor**

Batch - Automatic Calibration Editor

Provide ion reference and calibrant delivery settings to be applied automatically, at the correct frequency during acquisition

Ion reference table: Beta Galactosidase Digests

Calibrate every: 3 samples

Calibrant delivery: valve-method

MS method: lc-calibration

Rack Type:

Rack Position:

Plate Type:

Plate Position:

Vial Position:

---

**Note:** Make sure to enter a retention time for each peptide in the reference table.

---

- Submit the batch and start the queue. Make sure that the entries in the queue match the entries in the schedule on the external device.
- Start injection on the external device.

The following examples show the batch and queue in SCIEX OS, and the corresponding schedule on the external device for a batch where calibration is performed after every third sample. The duration for the MS method is 1 minute. The duration for the calibrate method is also 1 minute.

Figure B-6 LC Calibration: Batch

The screenshot shows the 'Batch' calibration interface. At the top, there is a navigation bar with a home icon, a gear icon, a bottle icon, a grid icon, and a list icon. Below the navigation bar, there are buttons for 'Auto-Calibrate...' (checked), 'Plate Layout...', 'New', and 'Open'. The main area is titled 'LC-calibration' and contains a table with the following data:

	Sample Name	MS Method
1	Sample 1	lc-calibration
2	Sample 2	lc-calibration
3	Sample 3	lc-calibration
4	Sample 1	lc-calibration
5	Sample 2	lc-calibration
6	Sample 3	lc-calibration
7		

Figure B-7 LC Calibration: Queue

The screenshot shows the 'Queue' calibration interface. At the top, there is a navigation bar with a home icon, a gear icon, a bottle icon, a grid icon, and a list icon. Below the navigation bar, there is a 'Start' button. The main area contains a table with the following data:

Status	Est. Start Time	Sample Name	MS Method	LC Method	Data File	Project
⌚	LC-calibration - 8 samples					
⌚	12/18/2017 1:57:03 PM	Cal	lc-calibration	valve-method	Cal	
⌚	12/18/2017 1:59:04 PM	Sample 1	lc-calibration		Sample	
⌚	12/18/2017 2:00:05 PM	Sample 2	lc-calibration		Sample	
⌚	12/18/2017 2:01:06 PM	Sample 3	lc-calibration		Sample	
⌚	12/18/2017 2:02:07 PM	Cal	lc-calibration	valve-method	Cal	
⌚	12/18/2017 2:04:08 PM	Sample 1	lc-calibration		Sample	
⌚	12/18/2017 2:05:09 PM	Sample 2	lc-calibration		Sample	
⌚	12/18/2017 2:06:10 PM	Sample 3	lc-calibration		Sample	

## Calibrate a System Configured with Contact Closure

---

**Table B-3 Sample Sequence on the External Device**

Time (mm:ss)	Injection
00:00	Calibrant
01:00	Sample 1
02:00	Sample 2
03:00	Sample 3
04:00	Calibrant
05:00	Sample 1
06:00	Sample 2
07:00	Sample 3

## Calibration in Manual Mode

This section describes how to use contact closure to calibrate the system when running a method manually in the MS Method workspace.

### Calibrate the System with the CDS

1. In the MS Method workspace, open the method to be run.
2. Click **Advanced > Calibrate**.
3. In the **Ion Reference Table** field, select **X500 Positive Calibration Solution** or **X500 Negative Calibration Solution**, depending on the polarity of the method.
4. Select **Apply Calibration**.
5. Click **OK**.
6. Click **Start**.

### Calibrate the System Using the LC Method

If the system is communicating with an external device using contact closure, then follow these guidelines to calibrate the system using the external device:

- In the properties for the mass spectrometer, configure a valve to simulate the external device.
  - Create an LC method that contains a valve and has a duration less than or equal to the duration of the MS method.
1. In the MS Method workspace, open the MS method to be run.
  2. Click **Start with LC** and select the LC method.

## Calibrate a System Configured with Contact Closure

---

3. When the system status changes to **Loading**, start injection on the LC device.

# Exact Masses and Chemical Formulas

# C

## Reserpine

Table C-1 Reserpine Exact Masses (C<sub>33</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>)

Description	Mass
Molecular Ion C <sub>33</sub> H <sub>41</sub> N <sub>2</sub> O <sub>9</sub>	609.28066
Fragment C <sub>23</sub> H <sub>30</sub> NO <sub>8</sub>	448.19659
Fragment C <sub>23</sub> H <sub>29</sub> N <sub>2</sub> O <sub>4</sub>	397.21218
Fragment C <sub>22</sub> H <sub>25</sub> N <sub>2</sub> O <sub>3</sub>	365.18597
Fragment C <sub>13</sub> H <sub>18</sub> NO <sub>3</sub>	236.12812
Fragment C <sub>10</sub> H <sub>11</sub> O <sub>4</sub>	195.06519
Fragment C <sub>11</sub> H <sub>12</sub> NO	174.09134

## Peptide ALILTLVS

Table C-2 Peptide ALILTLVS Exact Mass

Name	Sequence	Mass	Charge State
Precursor Ion	ALILTLVS	829.5393	1+
b8	ALILTLVS	811.5288	1+
b7	ALILTLV	724.4967	1+
b7-18	ALILTLV	706.4862	1+
b6-18	ALILTLV	607.4178	1+
y5	LTLVS	532.3341	1+
b5	ALILT	512.3443	1+
b5-18	ALILT	494.3337	1+
b4	ALIL	411.2966	1+
b3	ALI	298.2125	1+

Table C-2 Peptide ALILTLVS Exact Mass (continued)

Name	Sequence	Mass	Charge State
Internal fragment y b	IL or LI	227.1754	1+
Internal fragment y b	LT or TL	215.139	1+
b2	AL	185.1285	1+
a2	AL	157.1335	1+
Immonium Ions	I or L	86.09643	1+

---

## Introduction

This document provides a tutorial overview of some of the tools and functionality available in the software. It does not provide a detailed description of every available operation but it does explain some of the more common workflows that the software can address.

## Organization

While some functions and operations are specific to certain applications and workflows, most are generic and are used frequently when exploring qualitative data. This section of the document provides a brief introduction to the concepts of the software and a description of some of the most common and essential operations. Subsequent sections describe approaches to specific workflows and use the sample data files supplied with the software.

The sample files are available at [sciex.com/software-support/software-downloads](http://sciex.com/software-support/software-downloads), under **SCIEX OS resources**. Copy the whole project to the D:\SCIEX OS DATA folder on the computer. The following sample files are used in the examples in this tutorial:

- Bromocrip\_IDA-DBS alone\_T=1.wiff
- Bromocrip\_IDA-DBS in plasma\_T=0.wiff
- Bromocrip\_IDA-DBS in plasma\_T=1.wiff
- DataSET61.wiff
- DataSET62.wiff
- DataSET63.wiff
- DataSET64.wiff
- DataSET65.wiff
- DataSET66.wiff
- RP\_digests.wiff
- RP\_Intact.wiff
- Bromocriptine.mol

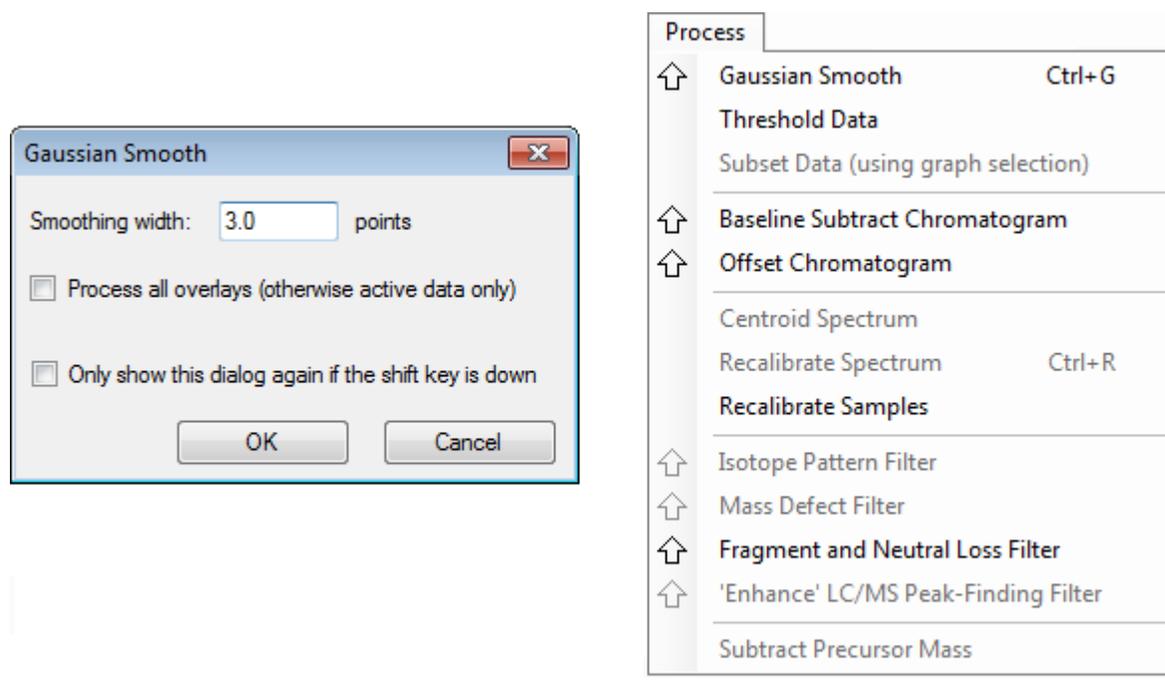
The Bromocriptine files are from negative mode IDA analyses of an incubation with rat liver microsomes. Bromocrip\_IDA-DBS alone\_T=1.wiff was obtained from the one hour time point, while the other two are for the zero and one hour time points spiked into plasma. The Bromocriptine.mol file contains the molecular structure for Bromocriptine. DataSET61 to DataSET66 are files collected from Loratadine and its impurities. The different datasets

represent different concentration levels. The RP\_Intact.wiff file is from an analysis of intact myoglobin. The RP\_digests.wiff file is from an analysis of tryptically digested myoglobin.

## Options

The software provides many options to fine-tune the way that commands behave. Some, as shown in [Figure D-1](#), provide a check box that allows the dialog to be shown only if the **Shift** key is pressed. This eliminates the need to interact with the dialog if changes to the parameters are not required. The menu for these commands contains an upwards-pointing arrow.

**Figure D-1 Options**



## Panes

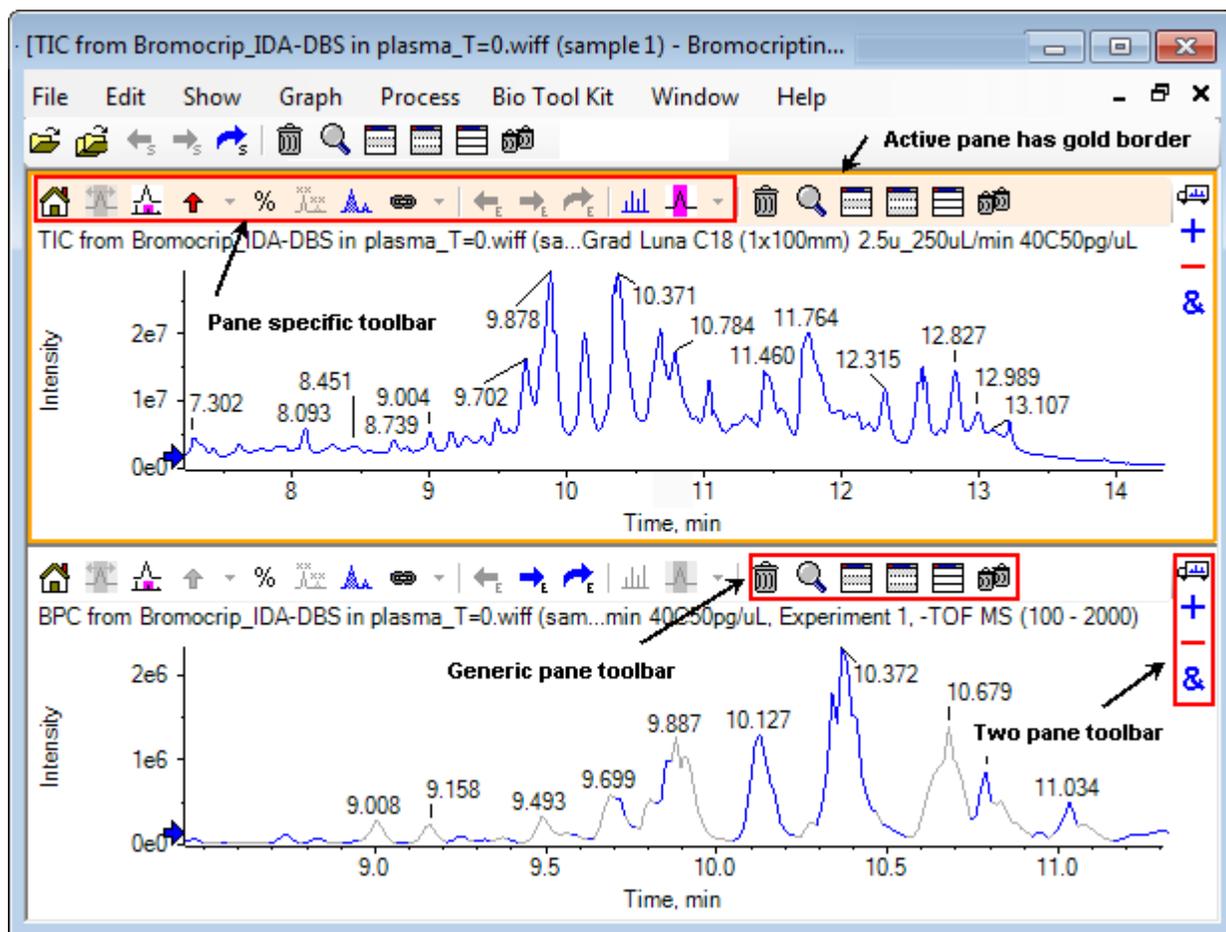
While the software uses windows to expose and receive information, the basic user interface component is a pane. A window can contain one or more panes, but only one pane can be active at a time. Panes receive commands from the menus and toolbars. Menus and toolbars provide ways to manipulate panes or the data they contain.

Panes can contain graphs such as spectra and chromatograms, heat maps, or tables, as well as more specialized views. Typical processing operations either create panes to show information, or work on the data shown inside a pane. Every pane contains generic single- and two-pane tools. Most panes have additional tools that are specific to the type of pane. The additional tools provide access to the more common commands.

## Explorer Tutorial

An example of a common window is shown in [Figure D-2](#). The window contains two panes, with the active pane, the chromatogram, identified by the colored border and toolbar.

**Figure D-2 Example of Panes Within a Window**



The common pane operations are summarized in [Generic Pane Toolbar](#) and [Two-Pane Toolbar](#). The pane specific operations are summarized in [Graphs](#).

### Generic Pane Toolbar

Click an icon to use the generic single-pane operations.

**Table D-1 Generic Pane Toolbar Icons**

Icon	Name (Tooltip)
	Deletes this pane
	Expands the active pane to fill the window

**Table D-1 Generic Pane Toolbar Icons (continued)**

Icon	Name (Tooltip)
	Hides this pane
	Hides all other panes
	Shows all currently hidden panes
	Deletes all other panes (hold the Ctrl key to delete only panes after this one)

**Note:** Similar icons are also available in the master toolbar, located just below the menu bar. Clicking one of the icons in the master toolbar has the same effect on the active pane as clicking the icon in the active pane. This toolbar can be useful if the active pane has been resized and some of the icons are not visible.

#### **Deletes this Pane**

If multiple panes are open, then use this icon to delete the corresponding pane. If only one pane is open, then the icon is not available.

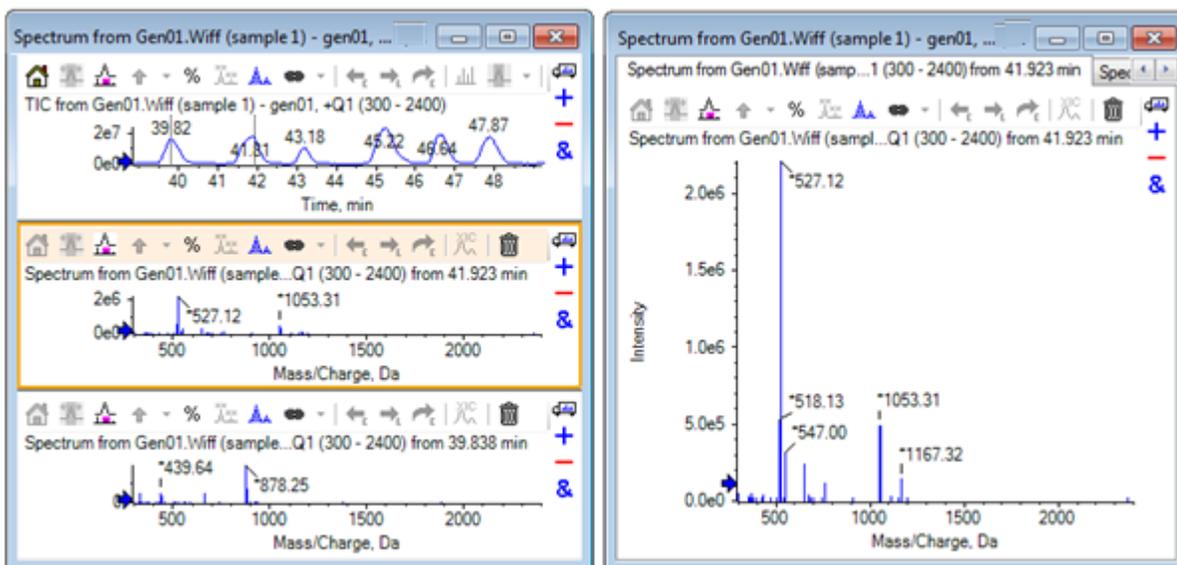
#### **Expands Active Pane to Fill Window**

Use this icon to expand the pane to fill the entire window or to return the pane to the original size. If the window contains several panes, then this icon temporarily focuses on one of them.

A separate tab is shown at the top of the window for each pane. Click the appropriate tab to switch between panes.

**Note:** If the titles of the panes are long, then all of the tabs might not be visible. Use the arrow buttons to the right of the tabs to scroll through them. Click the icon again to return to the original view, showing all of the panes.

Figure D-3 Example of Expanded Pane



### Hides this Pane

Use this icon to hide the corresponding pane so that the other panes in the window fill the available space. This icon is useful if the user wants to view a subset of the panes, but do not want to permanently delete the other panes.

### Hides all Other Panes

Use this icon to hide all of the panes except for the corresponding pane. The result is somewhat similar to clicking the **Expands active pane to fill window** icon, because in both cases only the corresponding pane remains and fills the available space. The difference is apparent when another pane is subsequently created. In the expanded pane case, that new pane becomes active and fills the available space. In the hidden panes case, the two panes (the original active pane and the new pane) are both visible.

### Shows all Currently Hidden Panes

Use this icon to show all of the panes that have been hidden.

### Deletes all Other Panes

If the Ctrl key is not pressed, then this icon deletes all of the panes in the window except the corresponding pane. This option is useful for cleaning up and reprocessing the sample. Any currently hidden panes are also deleted.

If the Ctrl key is pressed, then only the panes that are after the corresponding pane are deleted. This option is useful if there are many panes open and only a certain number of the initial ones are required. In this case, hidden panes are not deleted.

## Two-Pane Toolbar

Drag the icon to use the two-pane operations (availability depends on the pane type). The source pane is the one containing the selected icon and the target is the second pane.

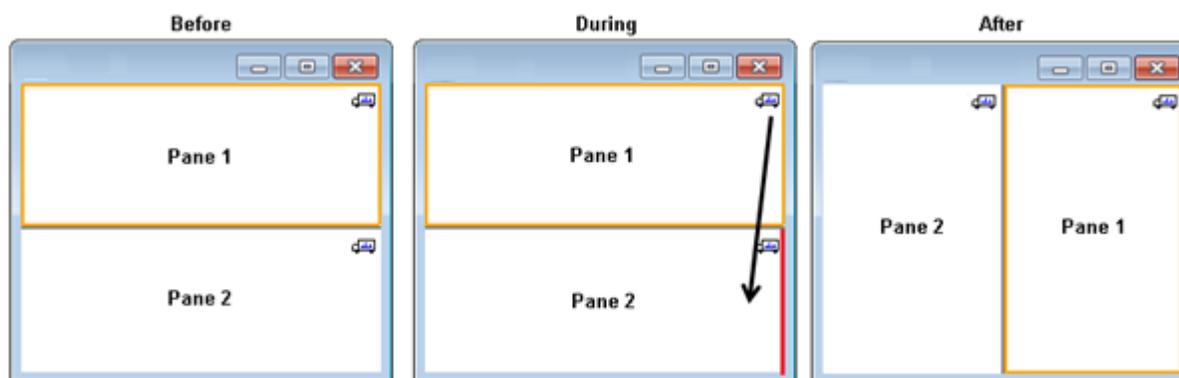
**Table D-2 Two Pane Toolbar Icons**

Icon	Name (Tooltip)
	Drag and drop to rearrange the panes.
	Drag to another graph to add the active data to the active data of the other graph. (Hold the Ctrl key to add the active data to all data sets in the other graph.)
	Drag to another graph to subtract the active data from the active data of the target graph. (Hold the Ctrl key to subtract from all data sets of the target. Hold the Shift key to keep negative values.)
	Drag to another graph to overlay the active data in the target graph. (Hold the Ctrl key to overlay all data sets, not just the active one.)

### Drag and Drop to Rearrange the Panes

This icon is shown in the top right corner of each pane and is used to change the relative positions of the panes. Click the icon in one pane and then drag it to the top, bottom, left, or right portion of a second pane. Depending on where the mouse is released, the first pane changes positions, relative to the second. As the cursor is dragged, one side of the second pane is highlighted in red to indicate where the first pane is placed. [Figure D-4](#) shows the result of dragging this icon from the top pane to the right portion of the bottom pane.

**Figure D-4 Result of Dragging the Icon from the Top Pane to the Right Portion of the Bottom Pane**



**Note:** Panes can be dragged from one window to another.

### Drag to Another Graph to Add the Active Data to the Other Graph's Active Data

Use this icon to sum two data sets together, point-by-point. The source data (from the pane that was originally clicked) is added to the target data (the pane over which the icon is released). The title of the modifying data updates to indicate that it has been modified.

---

**Note:** Only two data sets of the same type can be added together. For example, a spectrum cannot be added to a chromatogram.

---

**Note:** If the target graph contains more than one overlaid trace, then, by default, the source data is added to the active target data only. If the Ctrl key is pressed, then the source is added to all of the data sets in the target.

---

### Drag to Another Graph to Subtract the Active Data from the Target's Active Data

Use this icon to subtract the source data from the target data. This icon is most useful for background subtracting a mass spectrum.

---

**Note:** If the target graph contains more than one overlaid trace, then, by default, the source data is only subtracted from the active target data. If the Ctrl key is pressed, then the source is subtracted from all of the data sets in the target.

---

**Tip!** Normally, any data points for which the intensity in the source is larger than in the target are not kept. This means that the negative y-values are discarded. If the Shift key is pressed, then the points with negative intensity are kept.

---

### Drag to Another Graph to Overlay the Active Data in the Target Graph

Use this icon to overlay the active data in the source graph on the target graph. After the operation is completed, the target graph contains a new series with a copy of the target data.

---

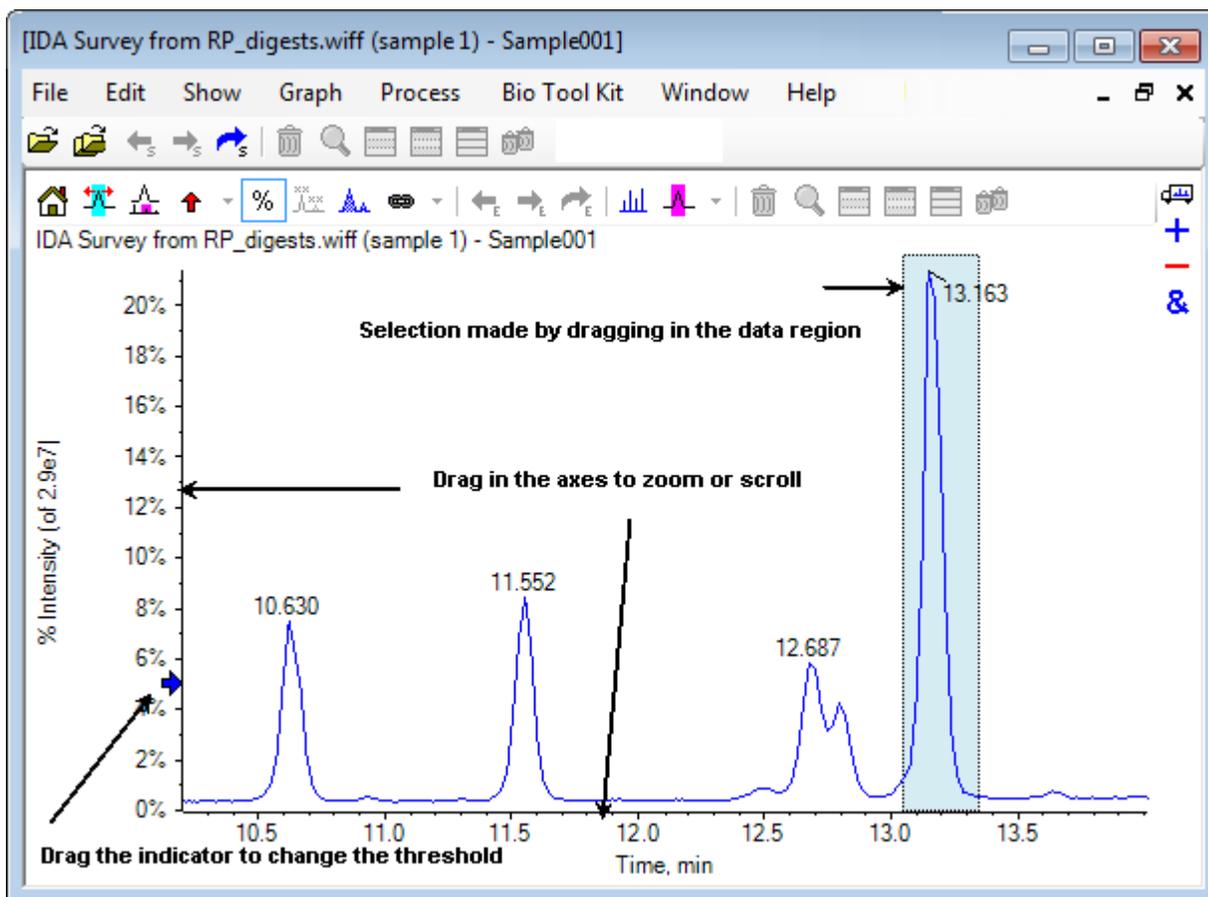
**Note:** If the source graph contains more than one overlaid trace, then, by default, only a copy of its active data is moved to the target graph. If the Ctrl key is pressed, then a copy of all of the data sets in the source graph are overlaid on the target graph.

---

## Graphs

Graphs are panes that allow data visualization and interaction. Several operations are common to all graphs, while others depend on the type of data being shown.

Figure D-5 Graphs



The generic commands are summarized as follows:

- Zooming and scrolling are performed by dragging the cursor in either the x- or y- axis region of the graph. Double-clicking resets the axis to the original range and clicking in the axis while pressing the **Shift** key reverts the graph to the previous view (undo for zoom and scroll).
- A threshold indicator can be positioned by dragging. The threshold usually determines which peaks are labeled and is sometimes used to determine which peaks are processed.
- Selections are made by dragging in the data region. Selections are used to define a part of the data to be used or processed. Select multiple regions by pressing the **Shift** key while dragging. Press the **Ctrl** key to make selections in both the x and y axes.

## Graph-Specific Toolbar

**Table D-3 Graph-Specific Toolbar Icons**

Icon	Name (Tooltip)
	Returns zoomed graph to home view
	Zooms selection to full view
	Show 'Zoom' graph (for tracking current zoom). Refer to <a href="#">Figure D-6</a> .
	Adds arrow markers for selected peaks
	Use percent y-axis.
	Label all overlaid traces
	Fill peaks
	Links graph's x-axis to others (with same units) in the window (Hold the Control key to apply to all current graphs)
	Switches data to use previous experiment
	Switches data to use next experiment
	Switches data to use a selected experiment
	Displays a spectrum for selection
	Set background subtraction range

---

**Note:** The final six icons in this toolbar, beginning with the Deletes this pane icon, are described in [Generic Pane Toolbar](#).

---

### Returns Zoomed Graph to Home View

If the plot has been zoomed, then use this icon to return to the home view, that is, the view in which both the x and y axes show their default ranges and all of the available data is visible. Double-clicking in the x-axis returns the graph to the home view. Double-clicking in the y-axis returns only that axis to its full range.

### Zooms Selection to Full View

Use this icon to zoom the plot so that the selected region fills the entire available space. Before selecting this icon, drag inside the plot to make a selection. Users can also zoom by dragging directly in the x-axis (or y-axis) of the plot.

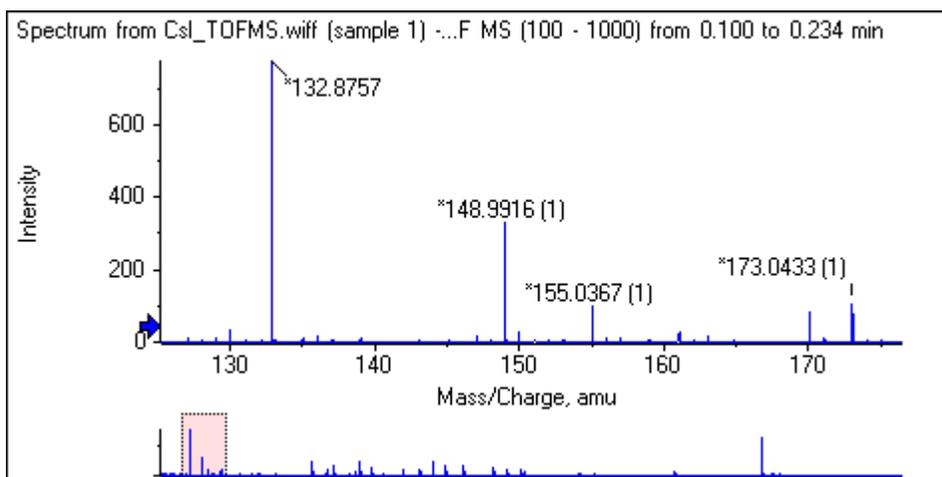
### Show 'Zoom' Graph (for Tracking Current Zoom)

Use this icon to show a small copy of the graph below the main graph as shown in [Figure D-6](#). This overview graph always shows the full available range and indicates the zoom region of the main graph using a pink selection. As the main graph is zoomed, this selection updates accordingly.

As the peak selection is dragged to a new location, the main graph scrolls, as required. Drag near the left or right edge of the selection to adjust its width. In this case, the main graph zooms, as required.

This functionality is useful for high resolution mass spectra because it is often necessary to zoom in quite far to see the required detail. The overview graph still allows the user to keep track of where the zoomed region is located with reference to the entire mass range.

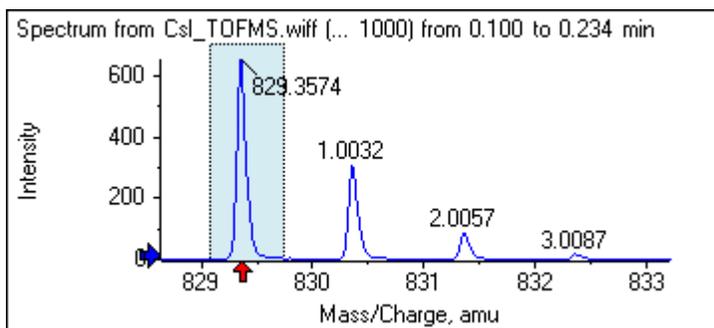
**Figure D-6 Show Overview Graph**



### Adds Arrow Markers for Selected Peaks

Use this icon to add an arrow marker to the largest peak within the currently selected region of the graph. [Figure D-7](#) shows the result of clicking this icon when the 829 peak (approximate) is selected as shown.

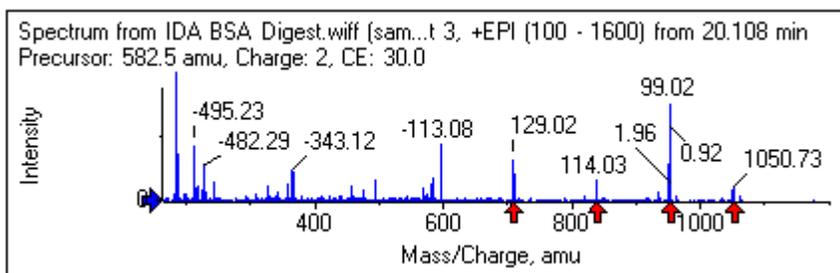
### Figure D-7 Add Single Arrow Marker



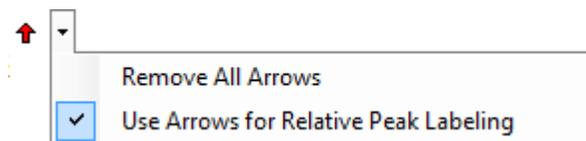
Arrows act as reference points in the data. By default, peaks that are not close to an arrow are labeled with their distance from the nearest arrow. The peak near the arrow with largest x-value is labeled with its actual x-value. Peaks near an arrow other than the last are labeled relative to the arrow with a higher x-value. In [Figure D-7](#), the peak at approximately 829 Da is labeled with its actual  $m/z$  value and the isotope peaks are labeled with their distance from this peak. Peaks to the left of the arrow (not shown) would have negative labeled values.

Arrows are most often used with spectra and provide a convenient way to look for expected mass differences such as isotopes, neutral losses in MS/MS spectra, and so on. [Figure D-8](#) shows an MS/MS spectrum of a peptide in which arrows were added at values corresponding to neutral losses of amino acid residues. For example, the peak labeled 99.02 might be a loss of valine from the peak at 1050.73 Da, the next labeled 114.03 might be an additional loss of asparagine, and so on. The peak labeled -113.08 might be a loss of leucine or isoleucine from the peak labeled 129.02 (with an actual  $m/z$  ratio near 709 Da).

### Figure D-8 Add Multiple Arrow Markers



If this relative peak labeling is not used, then clear the **Use Arrows for Relative Peak Labeling** menu item shown in [Figure D-9](#). In this case, arrows are used to mark peaks of particular interest.

**Figure D-9 Add Arrow Marker Menu**

Users can drag an arrow to a new location. If the arrow is dragged into the plot area, then this cancels the drag operation. If the user drags the arrow outside of the graph, then the arrow is deleted. Arrows can also be deleted by selecting **Remove All Arrows** from the menu shown in [Figure D-9](#).

### Use Percent Y-axis

This icon determines the y-axis scaling. When selected, overlaid traces are scaled so that the maximum value for each trace is at 100%. Using a percentage y-axis is convenient if the absolute magnitudes of the overlaid traces are very different.

### Label all Overlaid Traces

By default, when multiple traces are overlaid, only the active trace is labeled. Click this icon to label all of the traces. Click the icon again to remove all of the labels and then revert to the original view.

### Fill Peaks

Click this icon to fill the peaks for the active data, using alternating dark and light fills. This feature is useful to view the exact start and end of the peaks. Click the icon again to remove the fill and revert to the original view.

### Links Graph's X-axis to Others (With Same Units) in the Window

The axes of two or more graphs can be linked together so that when an axis in one graph is zoomed, the others automatically adjust to show the same range. This feature can be useful to compare the data in these graphs. An alternative is to overlay the data sets in the same graph. However, this is not always desired.

Click the **Links graph's x-axis to others (with same units) in the window** icon in each of the graphs to link. If the **Ctrl** key is pressed while the icon is clicked, then all of the current graphs with the same x-axis units in the same window as the active graph are linked. For example, if three spectra are visible and then **Ctrl** + the **Links graph's x-axis to others (with same units) in the window** icon is clicked in one of them, then all three of the spectra are linked to one another.

---

**Note:** In this example, if a new spectrum is subsequently generated, then it is not linked to the others. To link the new spectrum, click the associated **Links graph's x-axis to others (with same units) in the window** icon.

---

## Explorer Tutorial

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By default, only the x-axes of graphs are linked. In this case, when one graph is manually zoomed, the others automatically zoom the y-axis so that the peaks within view fill the space available.

To unlink a linked graph, click the **Links graph's x-axis to others (with same units) in the window** icon in the appropriate graph. Press the **Ctrl** key while doing so to unlink all of the graphs with the same x-axis units in the same window.

### Switches Data to Use Next Experiment

If the active data for the graph is associated with a specific experiment other than the last, then this icon replaces the data with data of the same type but for the next experiment.

For example, if the TIC for experiment 2 is active, then click this icon to switch to the TIC for experiment 3. If a spectrum from a given time is active for experiment 2, then click this icon to switch to a spectrum from the same time for experiment 3.

### Switches Data to Use Previous Experiment

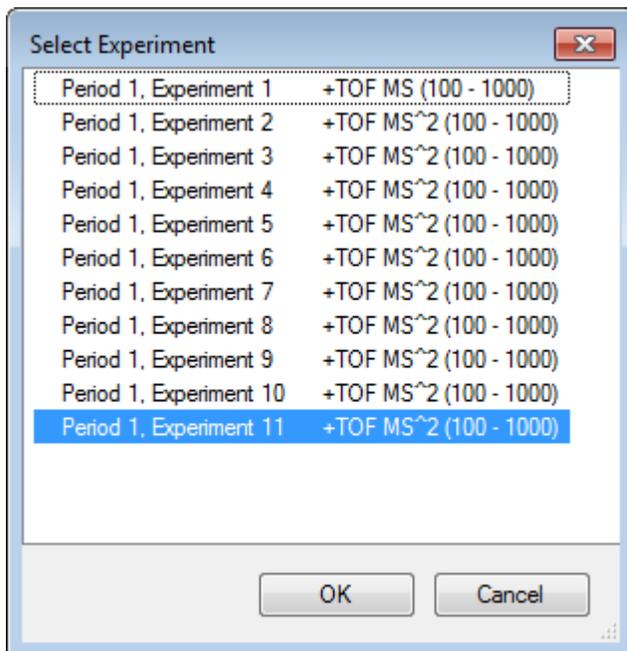
If the active data for the graph is associated with a specific experiment other than the first, then this icon replaces the data with data of the same type but for the previous experiment.

For example, if the TIC for experiment 3 is active, then click this icon to switch to the TIC for experiment 2. If a spectrum from a given time is active for experiment 3, then click this icon to switch to a spectrum from the same time for experiment 2.

### Switches Data to Use a Selected Experiment

Use this icon to select a specific experiment to use instead of scrolling through them one-by-one. Click the icon to open a dialog that lists all of the available experiments. The active sample is highlighted. Click an experiment in the list to select it and then click **OK**. Refer to [Figure D-10](#).

**Figure D-10 Select Experiment Dialog**



**Displays a Spectrum for Selection**

Use this icon to generate a mass spectrum averaged over the time range of the current selection in the graph. The same result can be achieved by double-clicking within the selection.

**Set Background Subtraction Range**

Use this icon to perform automatic background subtraction for spectra generated from the chromatogram.

**Spectrum-Specific Toolbar**

**Table D-4 Spectrum-Specific Toolbar Icons**

Icon	Name (Tooltip)
	Displays an XIC for selection

**Note:** The first eleven icons in this toolbar, beginning with the Returns zoomed graph to home view icon, are described in [Graph-Specific Toolbar](#).

**Note:** The final six icons in this toolbar, beginning with the Deletes this pane icon, are described in [Generic Pane Toolbar](#).

### Displays an XIC for Selection

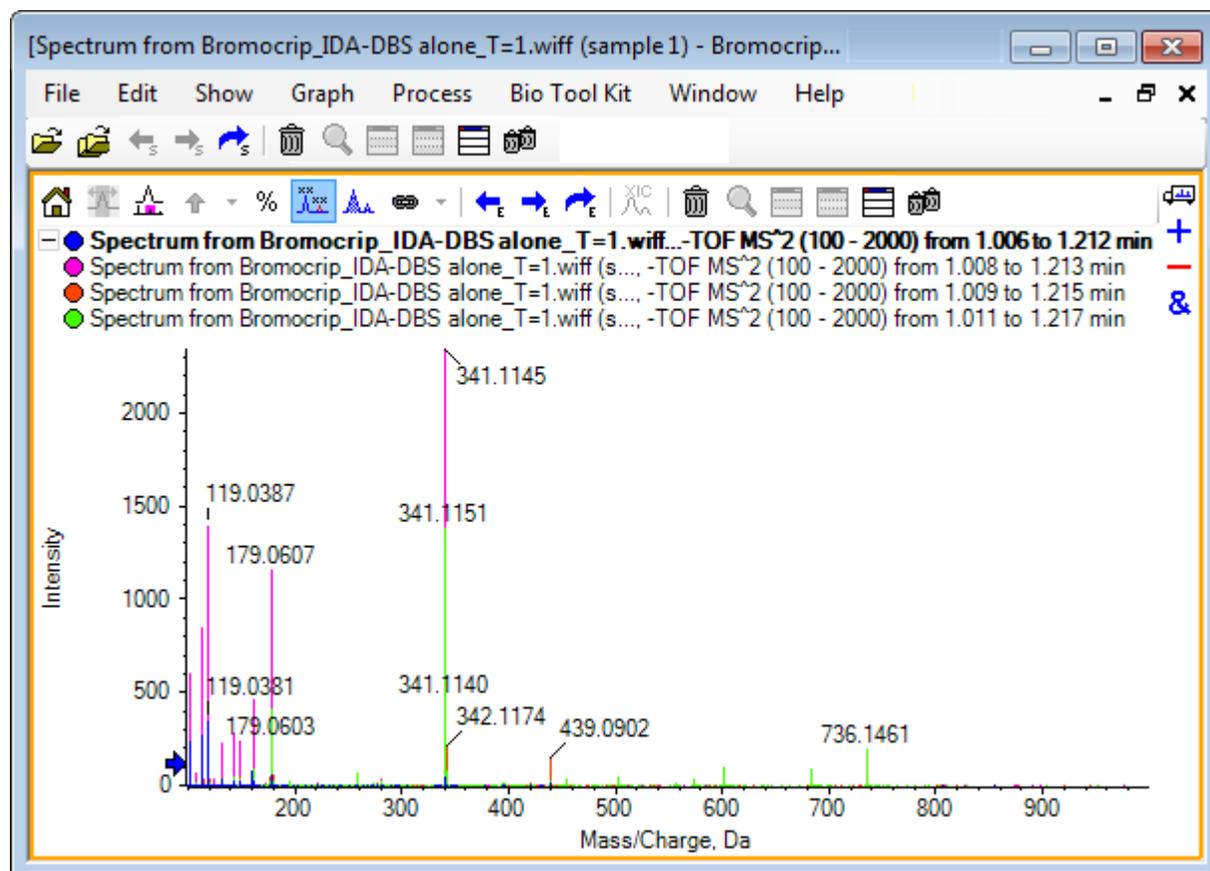
Use this icon to generate an extracted ion chromatogram (XIC) summed over the mass range of the current selection in the graph.

## Overlays

Graphs can contain different traces, called overlays, that share the same axes so that they can be easily compared. They can be generated by dragging the appropriate two-pane icon (the **Drag to another graph to overlay the active data in the target graph** icon) and are produced automatically by some pane creation commands. Refer to [Chromatograms and Spectra](#).

In [Figure D-11](#), the graph contains four spectra with the **Label all overlaid traces** icon selected. The header region of the graph shows the titles for the two spectra and colored circles indicating the trace color. The active trace is shown in bold type. This trace is the target for any processing operations, such as threshold data, smooth, and so on, and would normally be the only one labeled. Clicking the icon to the left of the title changes the icon and causes only the title of the active trace to be drawn. This feature is useful when there are many overlays. Click the icon again to reverse the process. If there are many traces and the cursor is moved over the titles, then the cursor changes to a double-headed arrow and acts like a scroll bar when dragged so that all of the titles can be accessed.

**Figure D-11 Graph Containing Four Spectra with the Label all Overlaid Traces Icon Selected**



There are several ways to switch the active trace:

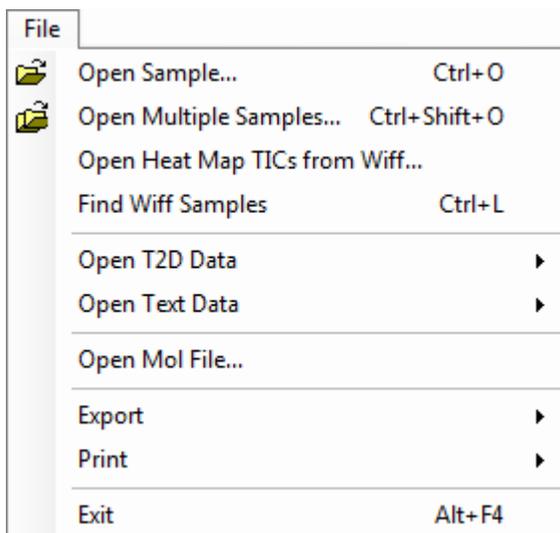
- Click the colored circle next to the title
- Click the title itself
- Click a data point in the trace (not the trace itself)

Right-clicking in a graph with overlays shows a context menu containing commands that can be used to visually edit the traces shown. The **Remove Active Trace** and **Remove All Traces Except Active** options function as expected.

## Open Files

As shown in [Figure D-12](#), the software can open different types of data files and has commands to open single or multiple samples.

**Figure D-12 File Menu**

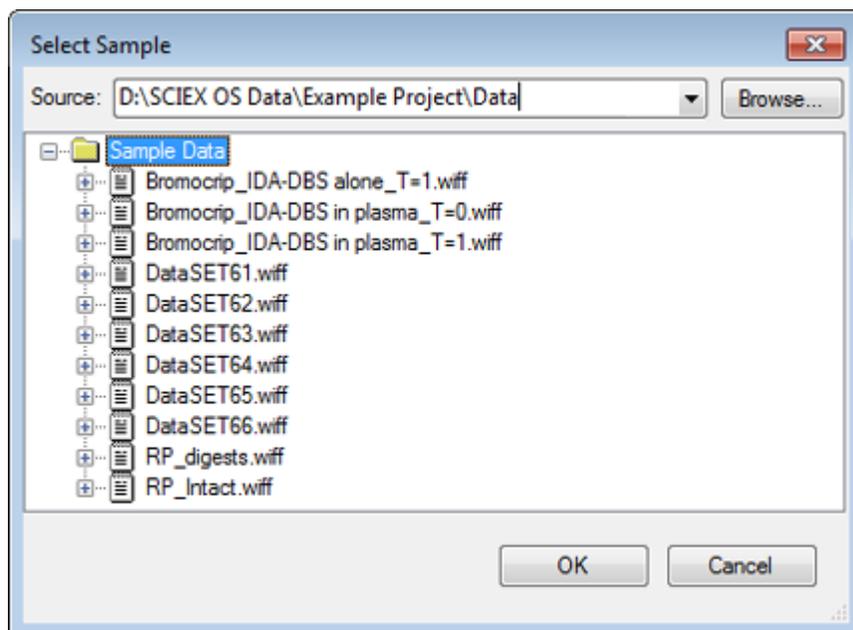


### Open Single Sample File

The **Open Sample** option opens the **Select Sample** dialog. Refer to [Figure D-13](#).

This dialog allows a single file to be selected. The resulting view depends on the command selected, with a single .scan file showing a spectrum or a Total Ion Chromatogram (TIC), and multiple scan .wiff files showing a TIC (the sum of all of the experiments if there is more than one).

Figure D-13 Select Sample Dialog



Click the icon to the left of the .wiff file to show all of the samples within the file and then select the required file name. If there is only one sample within the file, then select the file name and click **OK**.

## Open Multiple Sample Files

The **Open Multiple Samples** and **Open Heat Map TICs from Wiff** options open the **Select Samples** dialog. Refer to [Figure D-14](#).

The left panel corresponds to the **Open** dialog that allows folders to be navigated and files to be specified, and the right panel indicates the files that will be opened when **OK** is clicked. Samples can be transferred from left-to-right as follows:

- Expand the wiff file, select the sample, and then click the right-pointing arrow.
- Expand the wiff file, select the sample, and then drag it to the right panel.
- Expand the wiff file and then double-click the sample.

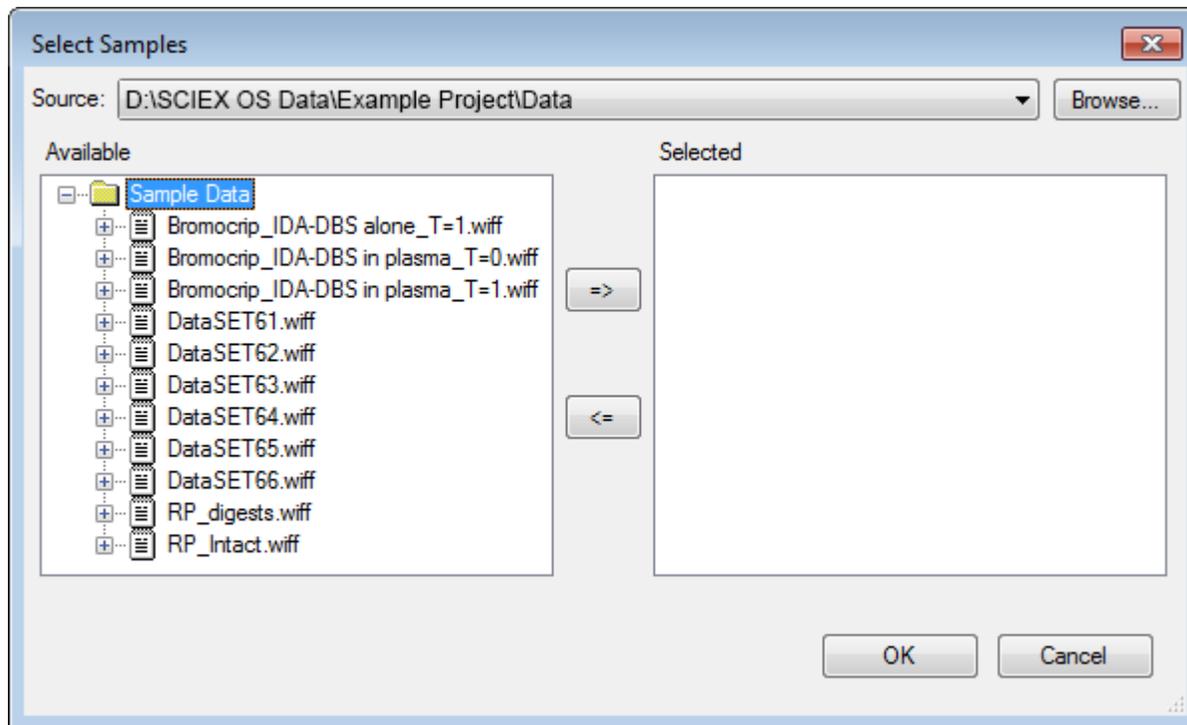
If the file contains multiple samples, then they can all be transferred by selecting the wiff file and clicking the right-pointing arrow, or by selecting the .wiff file and then dragging it to the right panel.

Samples can be transferred from right-to-left as follows:

- Expand the wiff file, select the sample, and then click the left-pointing arrow.
- Expand the wiff file, select the sample, and then drag it to the left panel.

- Double-click the sample.

**Figure D-14 Select Samples Dialog**



## Chromatograms and Spectra

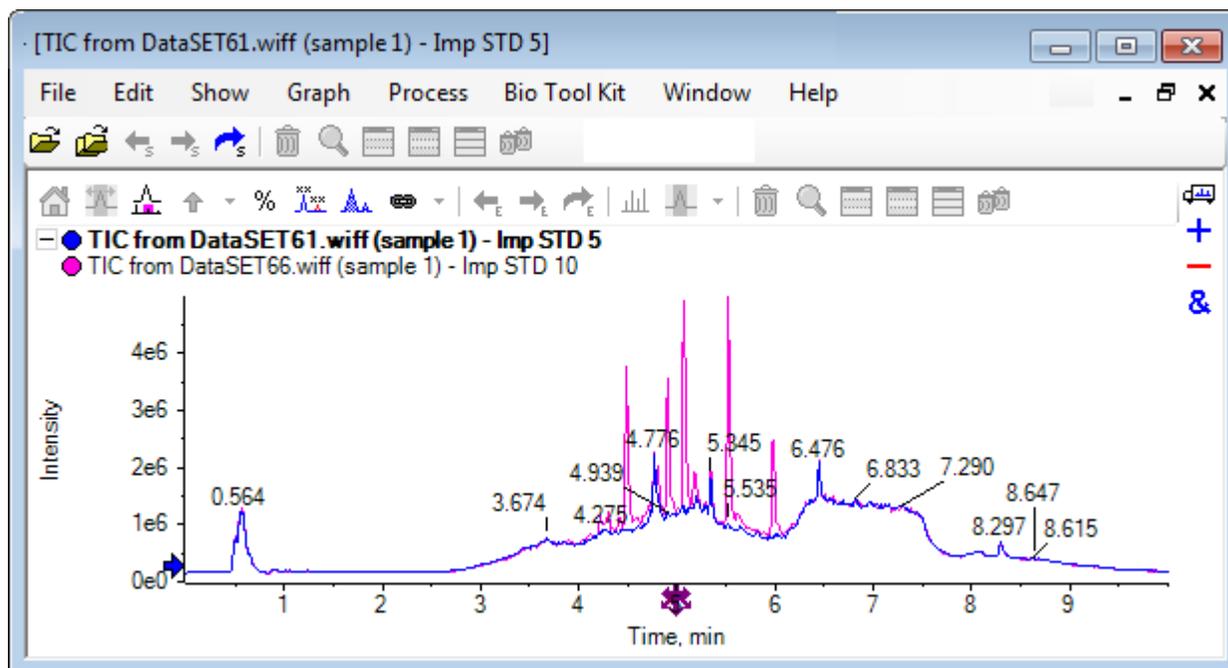
The Total Ion Chromatogram (TIC), Spectra, and Extracted Ion Chromatogram (XIC) are the most widely used data views for exploring and reviewing data. The software provides links between these data views so that users can quickly generate spectra and then XICs to determine if the peaks in the spectra are from one or more chromatographic peaks.

### Total Ion Chromatogram (TIC)

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

If the sample was acquired using looped experiments, then the TIC shown corresponds to the intensity sums of both experiments and a special arrow indicator is drawn in the x-axis to indicate this. Refer to [Figure D-15](#). If the indicator is double-clicked, then a new pane, showing overlaid individual TICs for each experiment, is shown.

Figure D-15 TIC



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

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

## Spectra

If a file only contains a single spectrum, then that spectrum is shown when the file is opened.

For data with multiple scans, derive spectra from chromatograms by making a selection in the chromatogram and either double-clicking inside it or clicking the **Displays a spectrum for selection** icon. Drag the selection rectangle in the chromatogram to update the spectrum to show the new region.

Select multiple regions by pressing the **Shift** key after completing the first selection. Double-click in any one of these selections or click the **Displays a spectrum for selection** icon to generate a new spectrum pane with the spectra overlaid.

For IDA, a prompt to overlay all of the dependent spectra or just show the first spectrum opens. In the latter case, use the left and right arrow keys to show the other spectra.

## Explorer Tutorial

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**Note:** This dialog has a Only show again if the shift key is down check box.

---

Generate background subtracted spectra in two ways:

- Generate separate spectra for the peak and background regions and then drag the subtract two-pane icon from the background spectrum to the peak spectrum.
- Define a background region by making one or two selections in the chromatogram and then clicking the **Set background subtraction range** icon. Any spectra generated when a background region is defined are automatically background subtracted. The background region is shown in the chromatogram as a pale red selection rectangle and both it and any spectrum selections can be moved to change the shown data. When a background region is defined, it can be removed by clicking the arrow next to the icon and then selecting **Clear Subtraction Range**.

---

**Note:** Arrow markers are useful in spectra because peak labels can be relative to the nearest peak marked with an arrow, which provides a quick way to determine the masses of losses or adducts. If there are multiple overlays and the Label all overlaid traces icon is selected, then each overlay is labeled relative to the arrow.

---

## Extracted Ion Chromatogram (XIC)

XICs can be generated in two ways:

- By clicking **Show > Extracted Ion Chromatogram (XIC)**.

This action opens a dialog where start and stop masses or center and width values can be typed, depending on the mode. This can be changed in the context menu, opened by right-clicking inside the dialog. The context menu also provides access to other useful commands, such as setting a default width and importing or exporting the list of masses. Users can also make the mass values persistent so that they are automatically used until removed.

- By making one or more selections in a spectrum and then double-clicking in one of them or clicking the **Displays an XIC for selection** icon.

These actions generate an XIC corresponding to each selection. By default, the program determines the largest peak in each selection range and automatically sets the XIC to correspond to the half-height low and high mass values for the peak. If the **Ctrl** key is pressed, then the entire selection width is used.

In both cases, a graph containing one overlay for each selection is shown. The selections turn into links. Dragging the links updates the XICs.

---

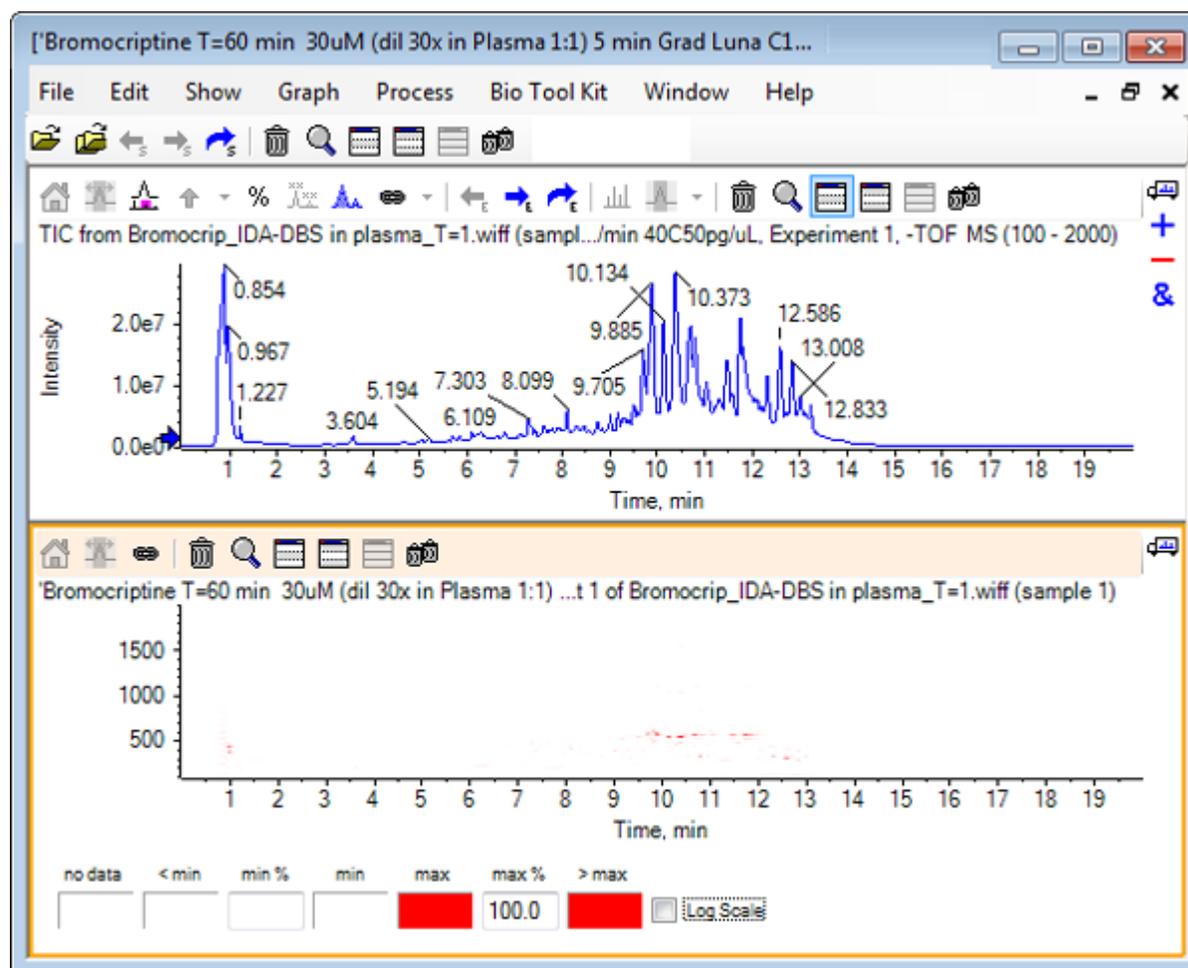
**Note:** XICs are normally calculated and shown for the entire chromatographic range, which can be slow especially if there are multiple selections and the data is from a high resolution instrument and contains many scans. A useful feature is to limit the XIC ranges to a smaller window around the retention time of the spectrum used to generate them. This can be set from the XIC tab of the dialog shown after clicking **Edit > Options > XIC** tab.

---

## Contour Plots and Heat Maps

An LC/MS contour plot (**Show > LC/MS Contour Pane**) shows all of the data from an LC/MS sample in a single pane. The example in [Figure D-16](#) shows a TIC and the corresponding contour map, which shows the data as a map of the  $m/z$  ratio versus retention time with the intensity color coded. In this case, the color controls are also shown but they can be hidden by right-clicking in the view and clearing the **Show Appearance Controls** option. Because contour plots and chromatograms have the same x-axis, they can be linked together so that zooming and scrolling affects both views similarly for comparison purposes.

**Figure D-16 TIC and Corresponding Contour Map**

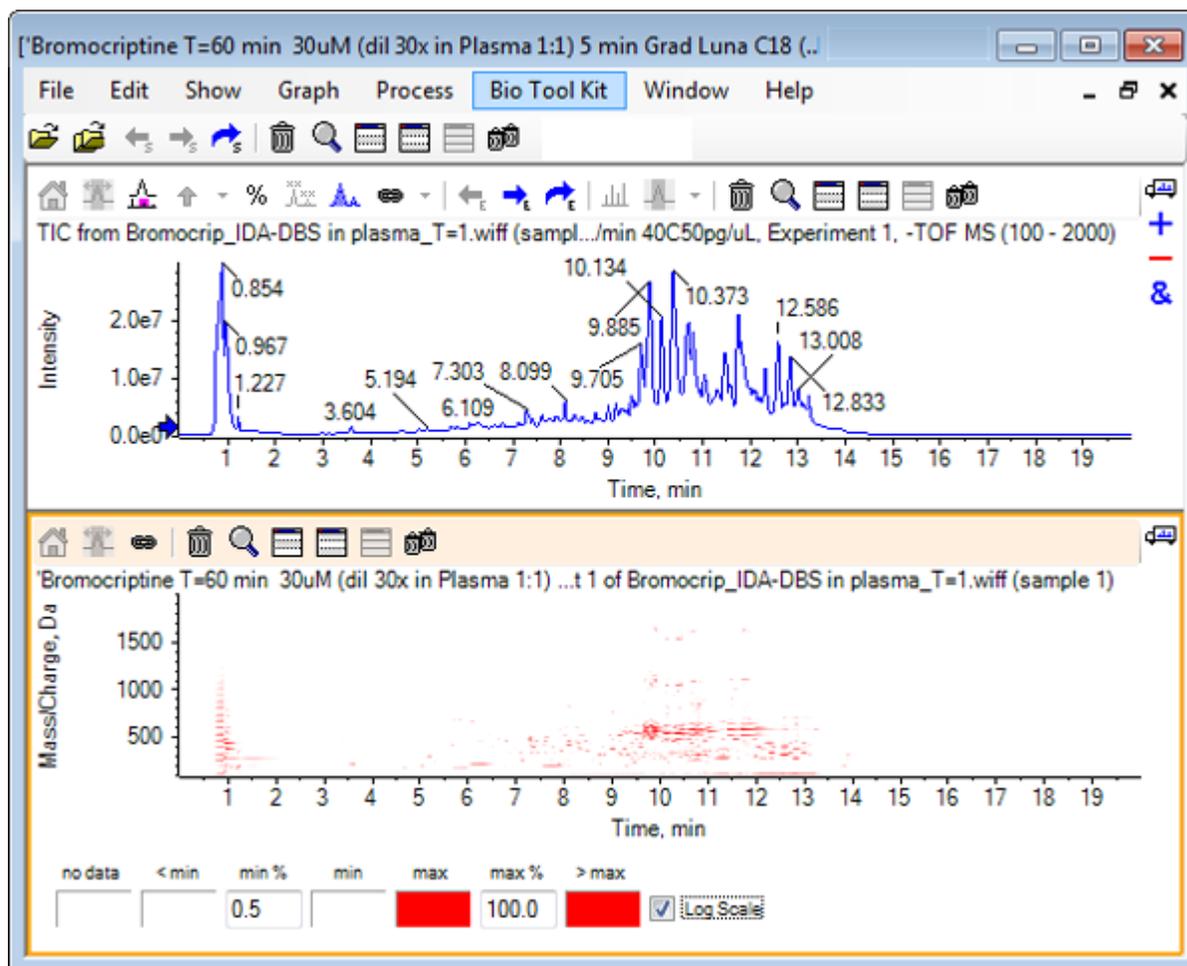


The color control uses a palette of 256 colors to show intensities in the range defined by **min %** and **max %**. Intensities below **min %** are drawn using **< min** and those above **max %** are drawn using **> max**. If the colors used for **< min** and no data are the same (as here), then any data points below **min %** disappear. This is a form of visual thresholding that can simplify the plot as

## Explorer Tutorial

shown in [Figure 1-11](#), where the **min %** value has been increased to 0.5%. For more information about color controls, refer to the *System User Guide*.

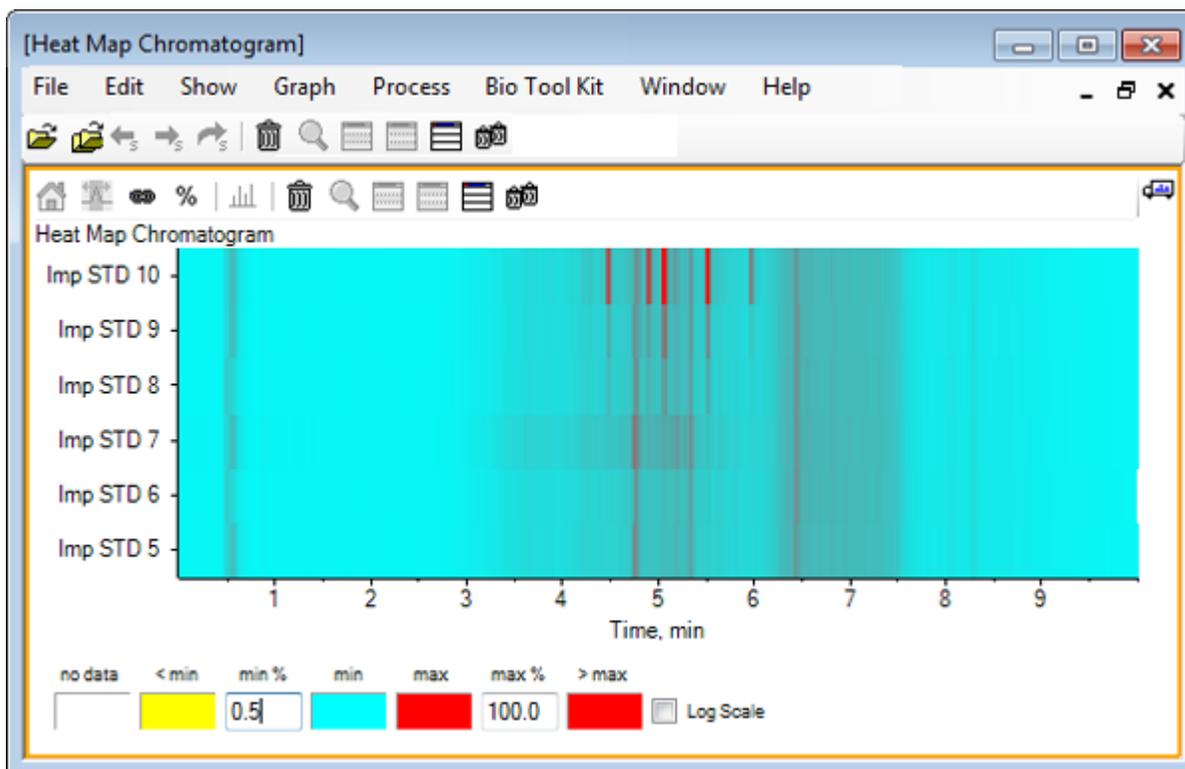
**Figure D-17 Contour Map with min% Value Increased to 0.5%**



Low intensity peaks can be emphasized by reducing **max %** so that the color palette covers a smaller intensity range, but all of the peaks greater than this value have the same color. This can also be emphasized by selecting the **Log Scale** check box. Activating the **Log Scale** requires a non-zero value of **min %** (for example, 1 or 0.1) and then maps the colors to the logarithm of the percent intensity.

The multi-sample visualization tools in the software include the ability to show the TICs, XICs, and spectra of multiple samples as series of individual heat maps, which can assist sample comparison. [Figure D-18](#) is for a series of TOF chromatograms from six analytes. Refer to [Work with Multiple Samples](#).

Figure D-18 Heat Map Chromatogram



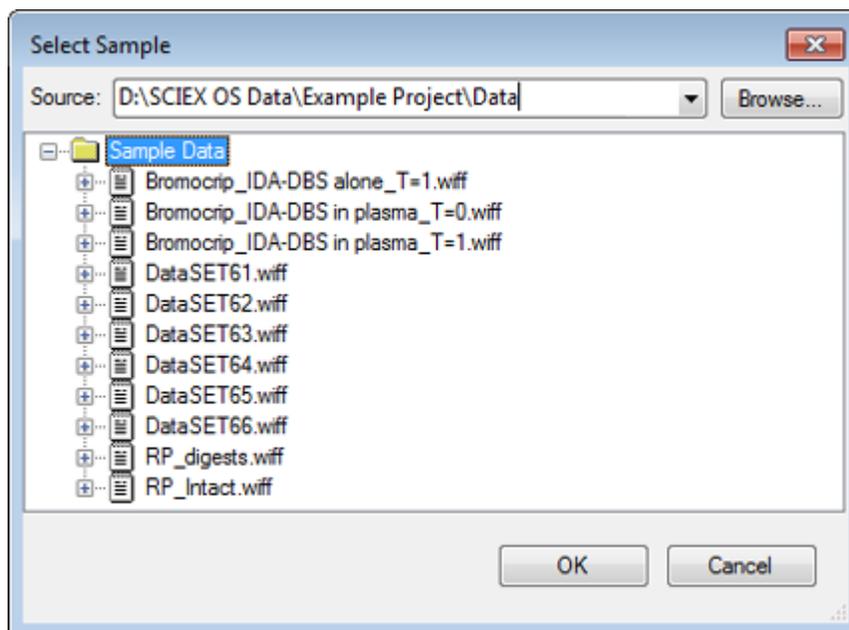
## Work with Chromatograms and Spectra

This section describes some of the most common processing options. The file used is an IDA file with a number of looped experiments, but in this example the first survey experiment is used, simulating a simple LC/MS analysis. In the following section the IDA functionality is explored.

### Open a Data File

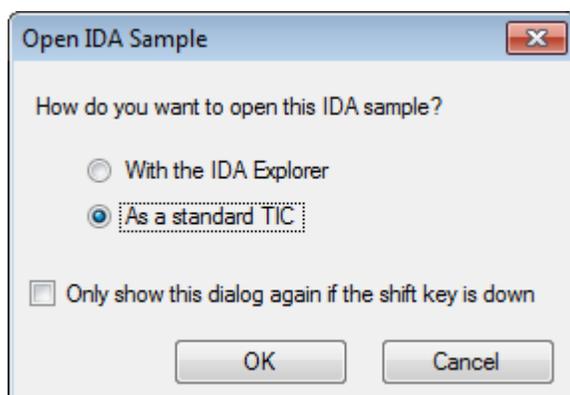
1. Click the **Open Sample** icon in the main toolbar.  
The **Select Sample** dialog opens.

**Figure D-19 Select Sample Dialog**



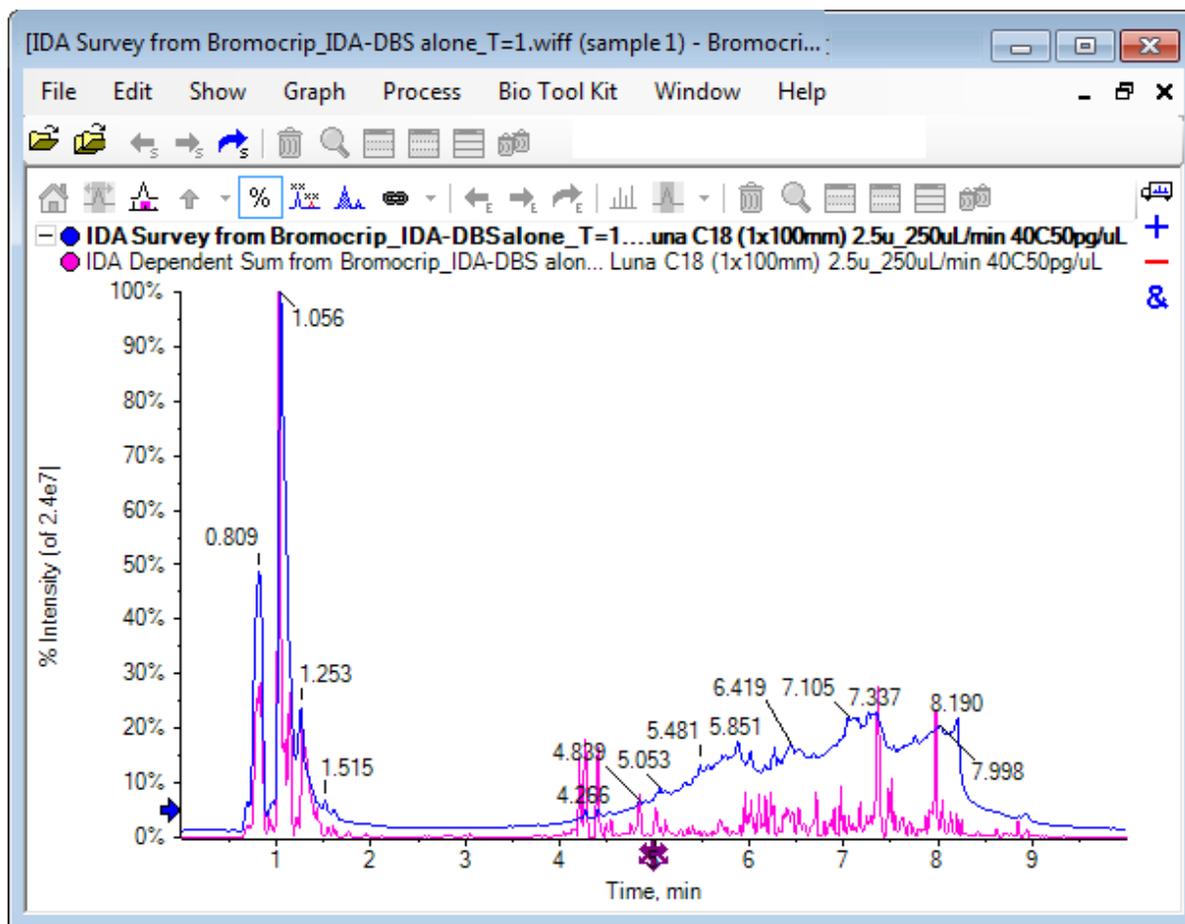
2. If the **Sample Data** folder is not already selected, then click **Browse** and navigate to the **Sample Data** folder. For information about installed data file locations, refer to [Organization](#).
3. To show all of the samples in the file, click the icon to the left of the **Bromocrip\_IDA-DBS alone\_T=1.wiff** file.  
There is only one sample in the **Bromocrip\_IDA-DBS alone\_T=1.wiff** file.
4. Select the sample name and then click **OK**.  
Because this is an IDA file, the software prompts you to specify how to open the selected sample.

**Figure D-20 Open IDA Sample**



- Click **As a standard TIC** if it is not already selected and then click **OK** to generate the TIC shown in [Figure D-21](#).

Figure D-21 TIC

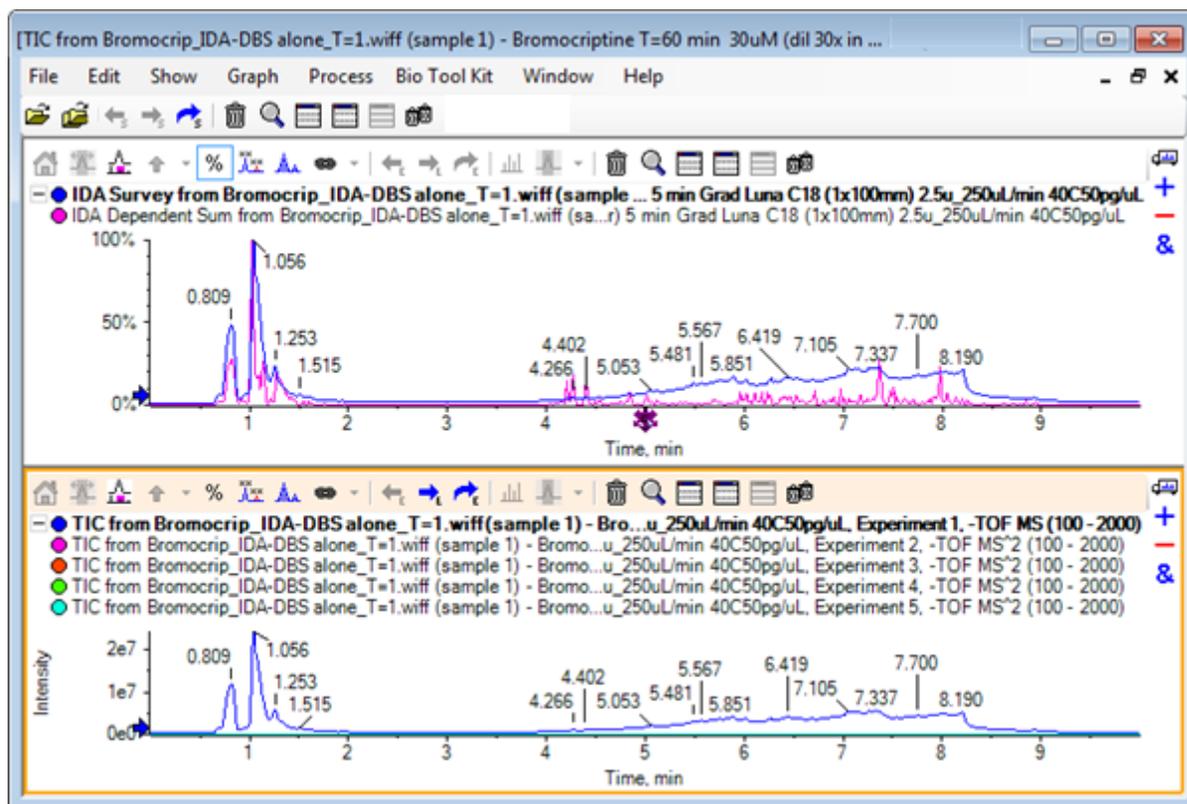


The pane has one overlay for the survey scan TIC (blue) and another for the summed dependent (product ion) scans. In this case, we want to process the survey data to show the survey TIC alone.

## Show the TIC for One Experiment

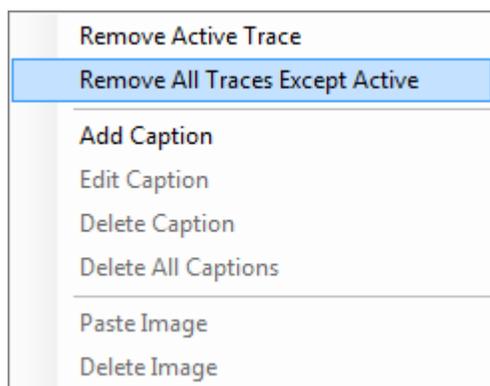
- Double-click the **Double-click to overlay individual TICs for all experiments** icon in the center of the x-axis to generate overlaid TICs for all of the experiments. The new chromatogram is the active pane. In addition, because the survey is the first experiment, it is the active trace as indicated by the bold title in the header.

Figure D-22 Overlaid TICs



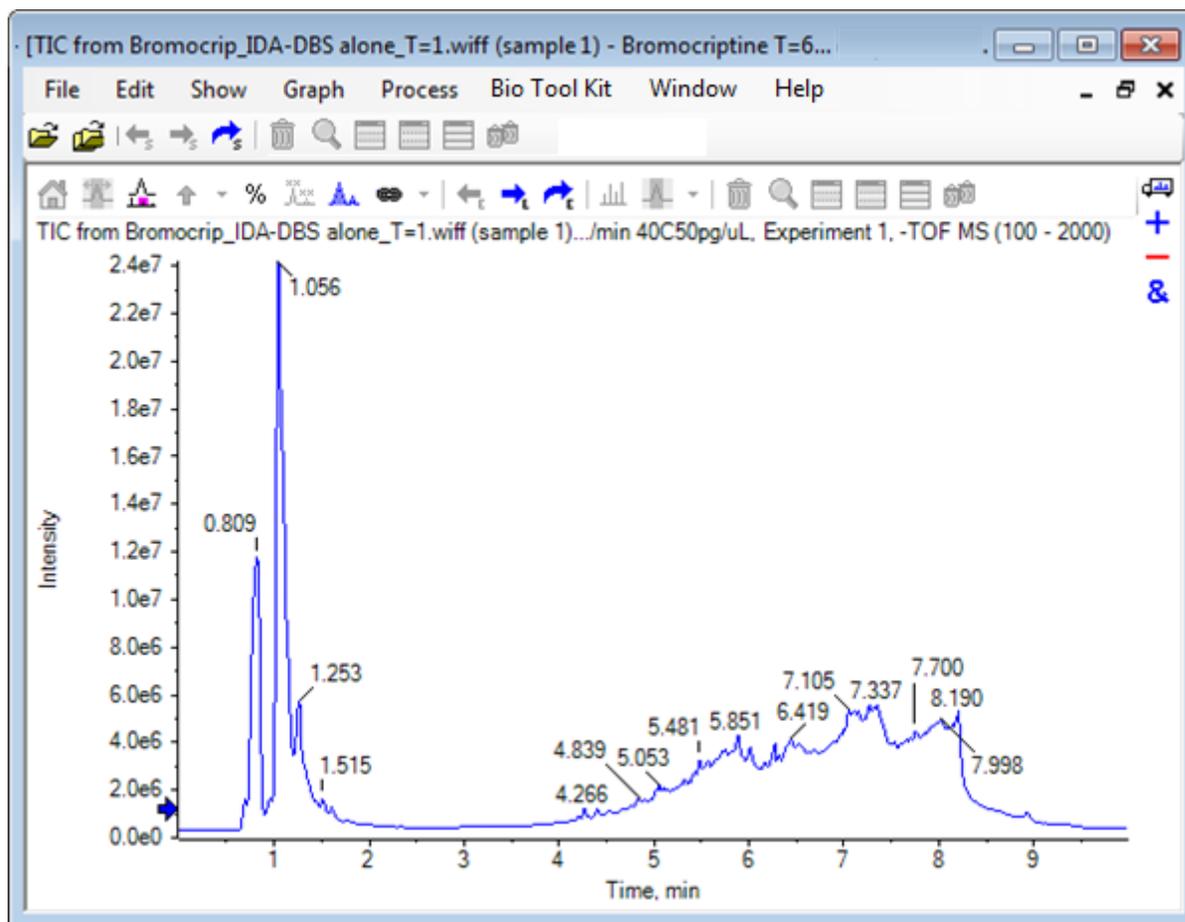
2. Right-click inside the active chromatogram pane and then click **Remove All Traces Except Active** so that only the survey TIC remains.

Figure D-23 Right-Click Menu



3. In the same pane, click the **Deletes all other panes** icon to leave just the survey TIC.

Figure D-24 Survey TIC



## Show an XIC for a Known Molecular Formula

While some apparently small peaks are obvious in the 4 min to 7 min range, it is possible that many are obscured by the background signal which is fairly intense in this data. Because this sample corresponds to a microsomal incubation of bromocriptine, use the  $m/z$  ratio of the expected molecular ion as an initial guide to peak location. The molecular formula of bromocriptine is  $C_{32}H_{40}N_5O_5Br$  and as this is negative mode data we expect to see an  $(M - H)^-$  ion.

1. Click **Show > Mass Calculators**.
2. Click the **Mass Property** tab in the **Mass Calculators** pane.
3. Type the molecular formula in the **Formula** field.
4. Type **-1** in the **Charge state** field.
5. Select **'H+' charge agent (else electron)**.

## Explorer Tutorial

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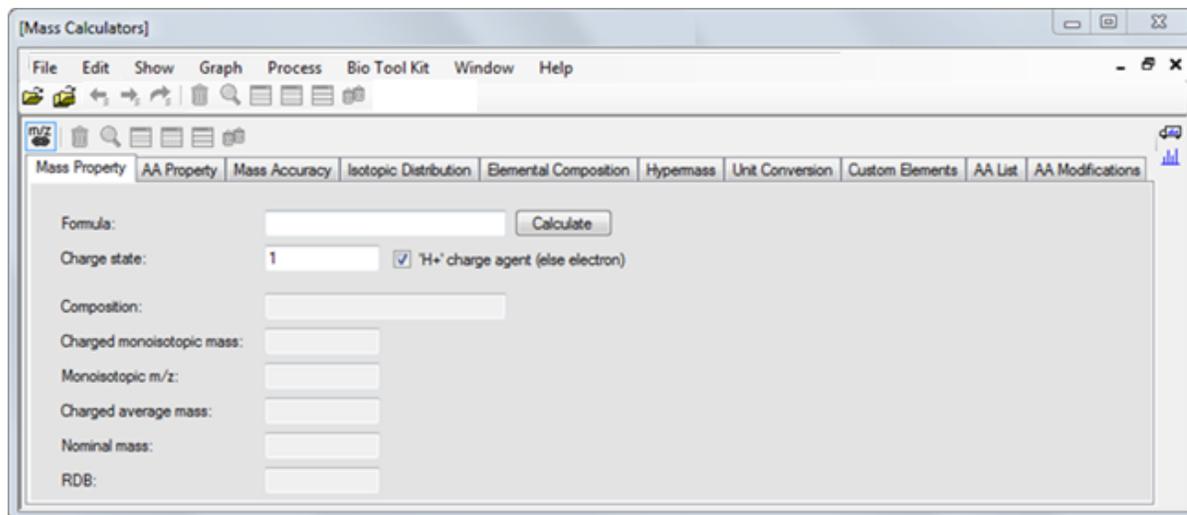
6. Click **Calculate**.

**Note:** It is also possible to manually remove one hydrogen from the molecular formula and not select the 'H+' charge agent (else electron) check box.

---

The dialog refreshes to show a number of mass values: monoisotopic, average, and so on.

**Figure D-25 Mass Calculators Pane**

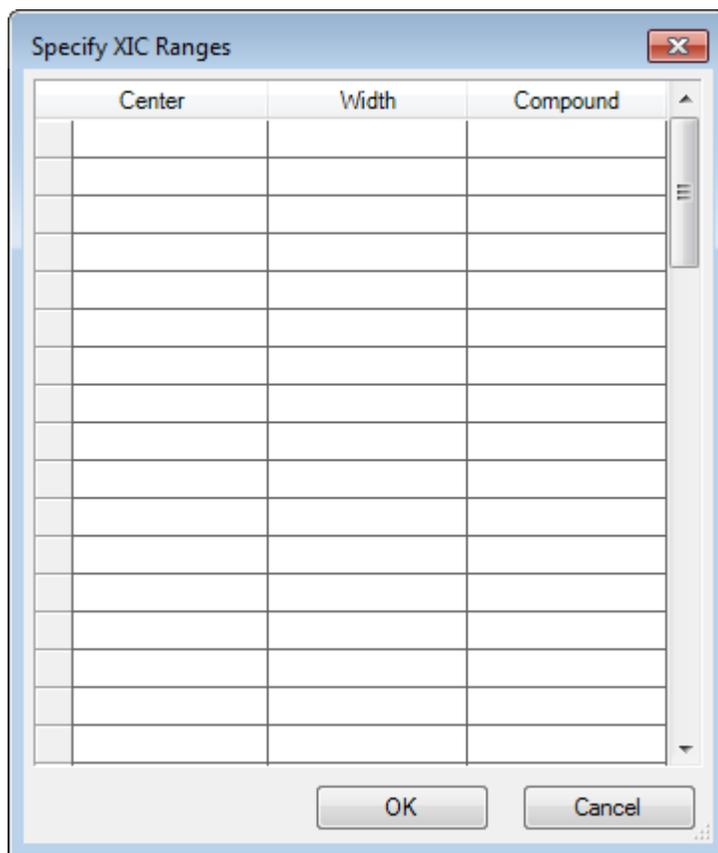


**Note:** At these mass values, the isotopes are easily resolved. Therefore, the Monoisotopic m/z value is the most appropriate value.

---

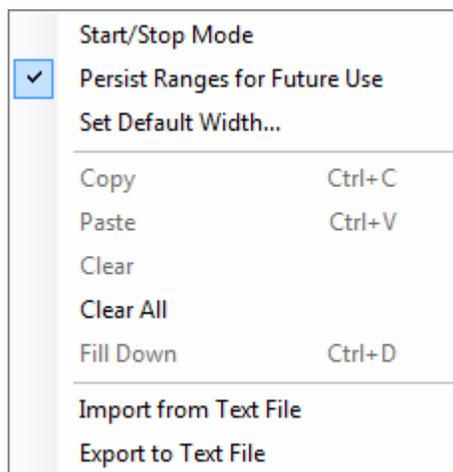
7. Select the **Monoisotopic m/z** value and then press **Ctrl+C** to copy the value to the clipboard.
8. Click the **Deletes this pane** icon to delete the **Mass Calculators** pane or click the **Hides this pane** icon to hide the pane.
9. Click **Show > Extracted Ion Chromatogram (XIC)** to open the **Specify XIC Ranges** dialog.

Figure D-26 Specify XIC Ranges Dialog



10. Right-click in the **Specify XIC Ranges** dialog to open the context menu.
11. In the context menu, do the following:
  - a. Make sure that the **Start/Stop Mode** option is not selected, so that the XIC values are entered as a center value and a width.
  - b. Click **Set Default Width**, type **0.05**, and then click **OK**.
  - c. Click **Persist Ranges for Future Use** so that the values are remembered the next time the dialog is used.

**Figure D-27 Context Menu**



- Return to the **Specify XIC Ranges** dialog.  
The dialog is now set so that only one mass must be typed for each XIC of interest and a default width is used.
- Select the first cell under **Center** and then press **Ctrl+V** to paste the mass value from step 7.
- Click **OK**.

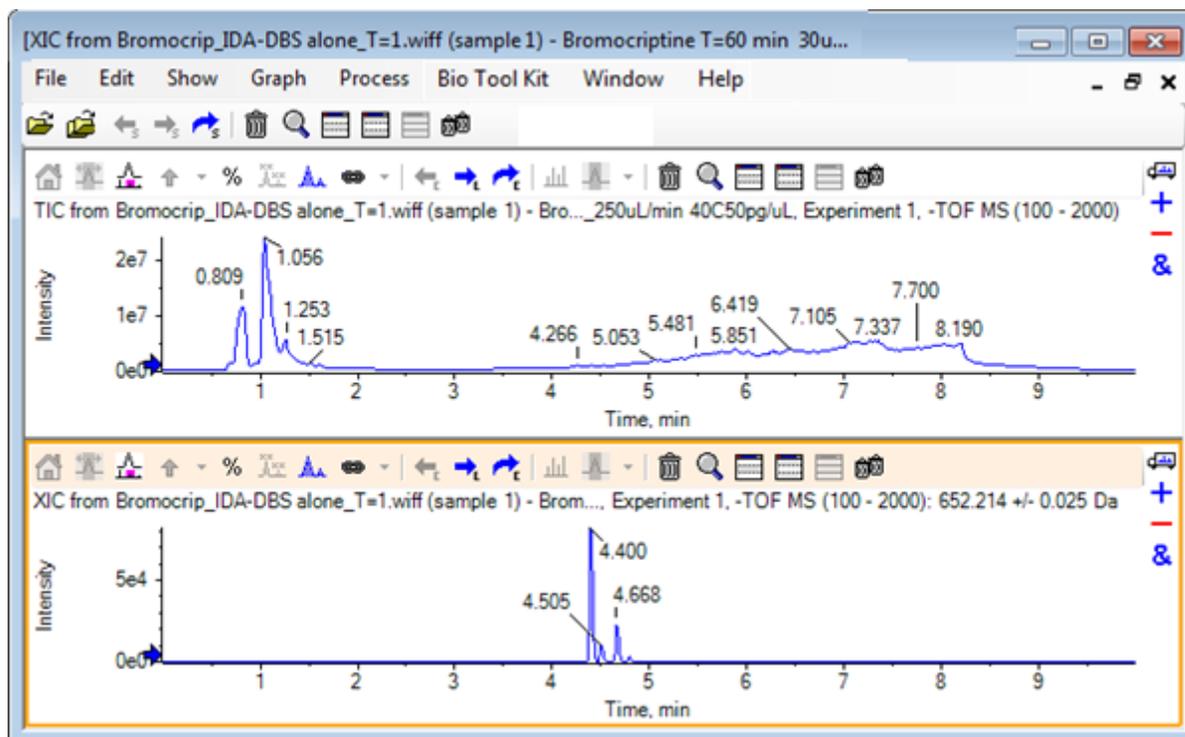
---

**Note:** Because a default width was set, it is not necessary to type an individual value.

---

The pane now contains the TIC and the XIC for the expected molecular ion of bromocriptine, which shows several peaks.

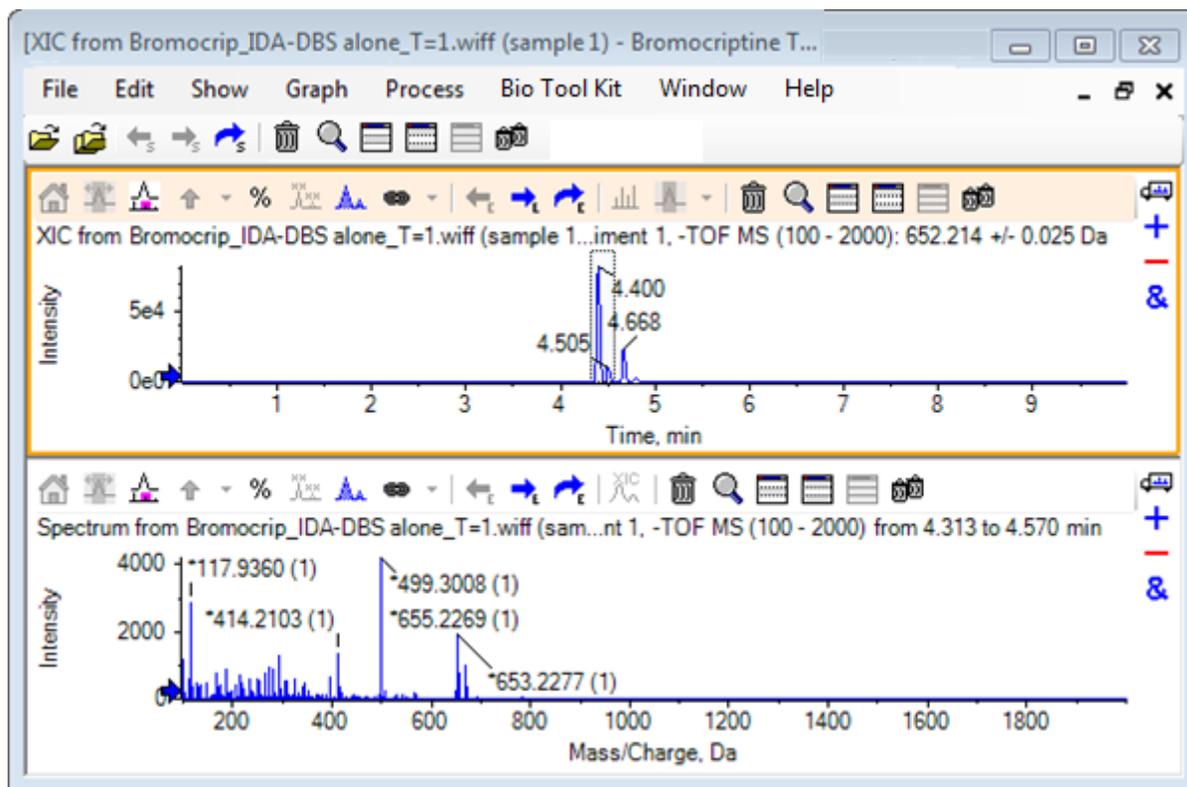
Figure D-28 TIC and XIC for the Expected Molecular Ion of Bromocriptine



## Generate and Interact with a Spectrum

1. Hide the TIC pane, make a selection around the largest peak in the XIC, and then click the **Displays a spectrum for selection** icon to generate the average spectrum for this region.

Figure D-29 Spectrum from the Largest Peak in the XIC



**Note:** In Figure D-29, the **Label** field on the **Peak Labeling & Finding** tab of the **Options** dialog (available through **Edit > Options**) is set to **Mass (Charge)**.

2. Drag the x-axis from roughly 630 Da to 700 Da to zoom the spectrum to this region.

**Note:** This might need to be done in two steps.

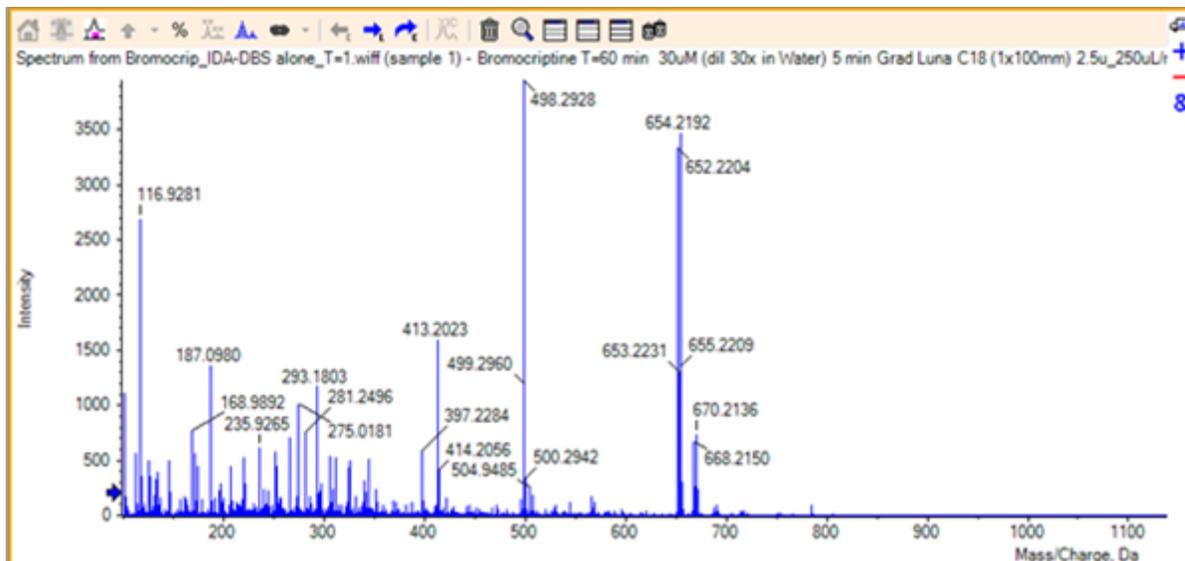
There is a peak at 652.2199, very close to the expected value of 652.2140, that also shows a bromine isotope pattern, but there is a second bromine isotope cluster starting at 668.2158. The exact  $m/z$  ratio values differ depending on the exact retention time window selected in the XIC.

**Note:** The labeling style used here shows an  $m/z$  ratio and an estimate of the charge state in brackets (based on the spacing between peaks). Peaks that appear to be monoisotopic are also marked with an asterisk. The labeling algorithm is unaware of isotopes other than  $^{13}\text{C}$  and so labels the  $^{81}\text{Br}$  isotope as singly charged but incorrectly marks it as monoisotopic.

3. Change the labeling style to the default style by clicking **Edit > Options**, navigating to the **Peak Labeling & Finding** tab, and then changing the setting to **Mass / Charge** in the **Label** field.

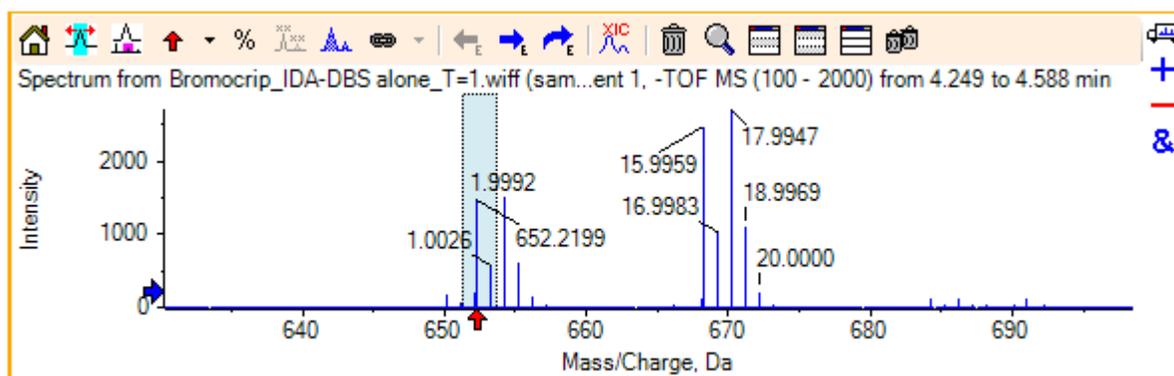
- Click **OK**.

**Figure D-30 Spectrum with Different Labeling Style**



- In the expanded spectrum, make a selection around the peak at 652.2199 and then click the **Adds arrow markers for selected peaks** icon.

**Figure D-31 Spectrum Showing ↑ on Selected Peak**



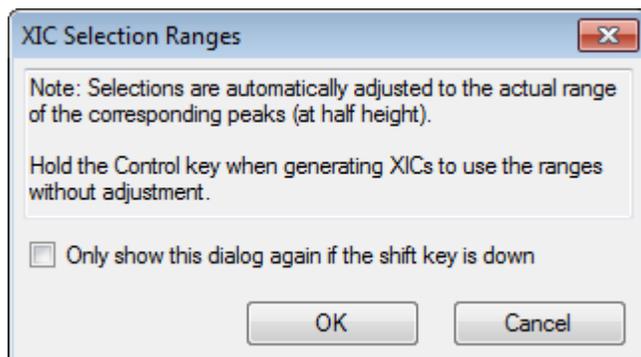
The mass labeling is now relative to the selected peak so the differences between mass peaks are shown. The label for the peak at 668.2158 now reads 15.9959, corresponding to the mass of oxygen, and suggesting that this peak is hydroxy-bromocriptine metabolite.

**Tip!** Arrows can be moved by dragging them to another peak and removed by selecting **Remove All Arrows** from the list adjacent to the arrow icon.

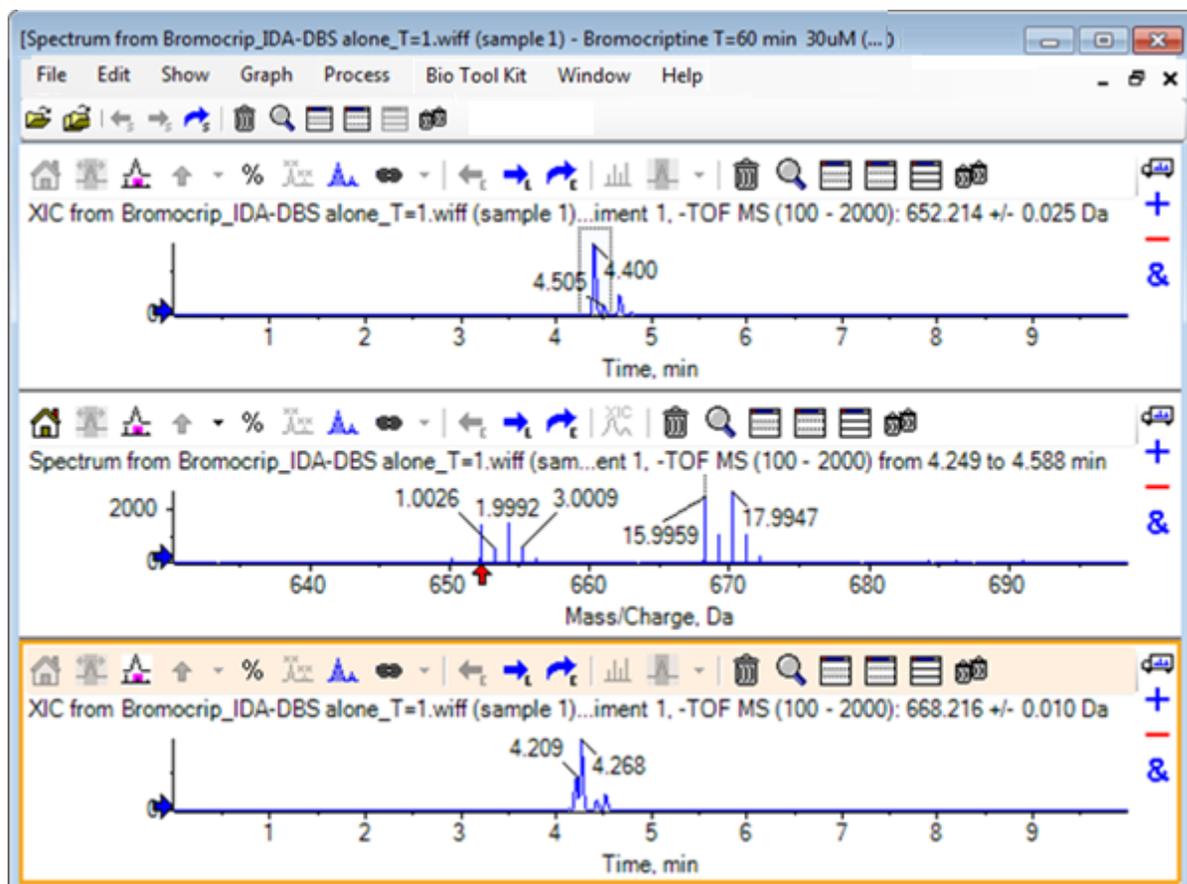
## Explorer Tutorial

6. Make a selection around the peak labeled 15.9959 and then click the **Displays an XIC for selection** icon.
7. On the **XIC Selection Ranges** dialog, click **OK**.

**Figure D-32 XIC Selection Ranges Dialog**



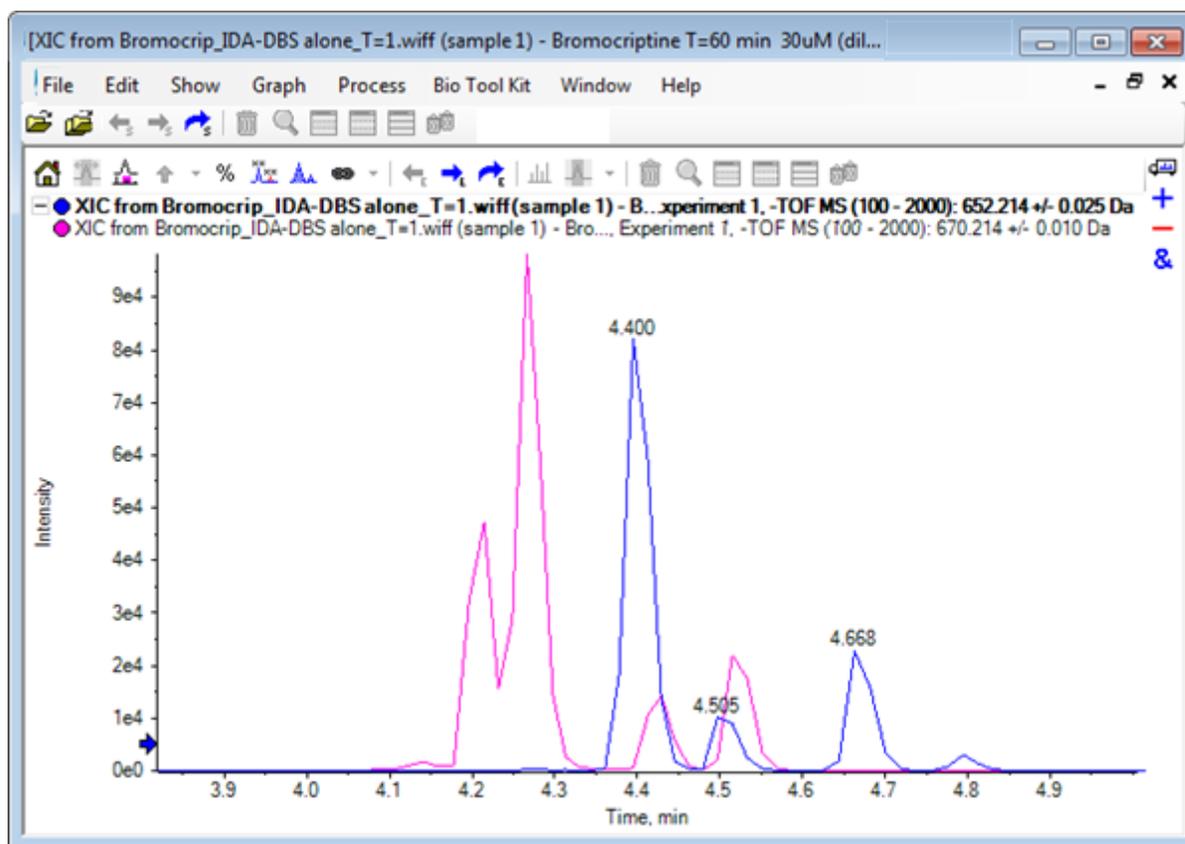
**Figure D-33 XIC**



This is a useful way to interactively generate XICs. By default, the width used for the XIC is the width of the mass peak at half height and a selection link is shown in the spectrum.

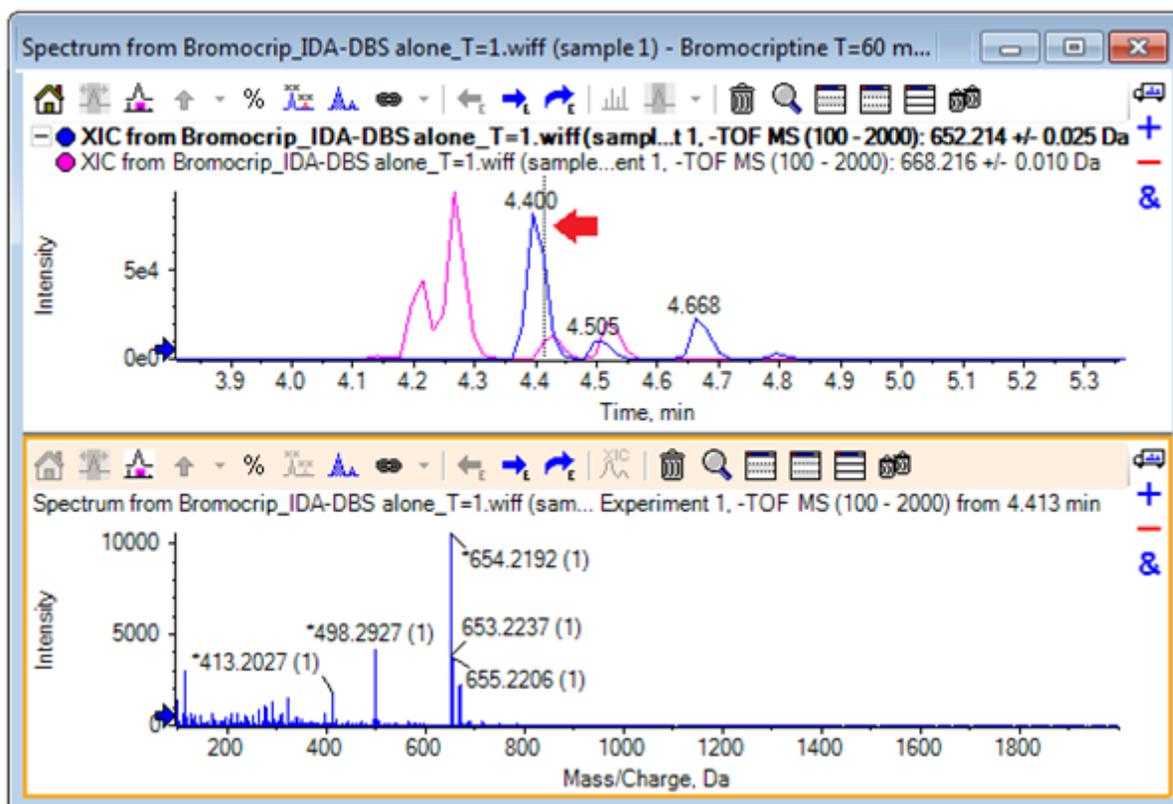
8. Drag the selection link to update the shown XIC and add more by repeating the step.
9. Click the **Drag to another graph to overlay the active data in the target graph** icon in this new chromatogram and then drag the chromatogram to the original XIC pane so that they are overlaid.

**Figure D-34 Overlaid XICs**



10. Hide or delete the second chromatogram pane and the spectrum, and then zoom the overlaid chromatograms to show the region around 4 min to 5 min. There are two peaks around 4.4 min, one from each XIC, that elute closely but not at exactly the same retention time. There are also a number of peaks in the 668.216 chromatogram, presumably indicating the presence of other hydroxy metabolites.
11. Double-click in the chromatogram pane at 4.40 min to generate the spectrum from a single scan.

Figure D-35 Spectrum from a Single Scan



A dashed line in the XIC indicates the scan shown (marked with an arrow in Figure D-35). If the line is dragged, then the spectrum updates so that the region around 4.40 min can be explored. Use the forward and backward arrow keys to move, one scan at a time. It is possible to obtain a clean spectrum for the peak for the  $m/z$  ratio of 652.214 by moving the line to a region where the signal for the 668.215 ion is zero (although even here the background is quite high), but a clean spectrum for the latter cannot be obtained this way.

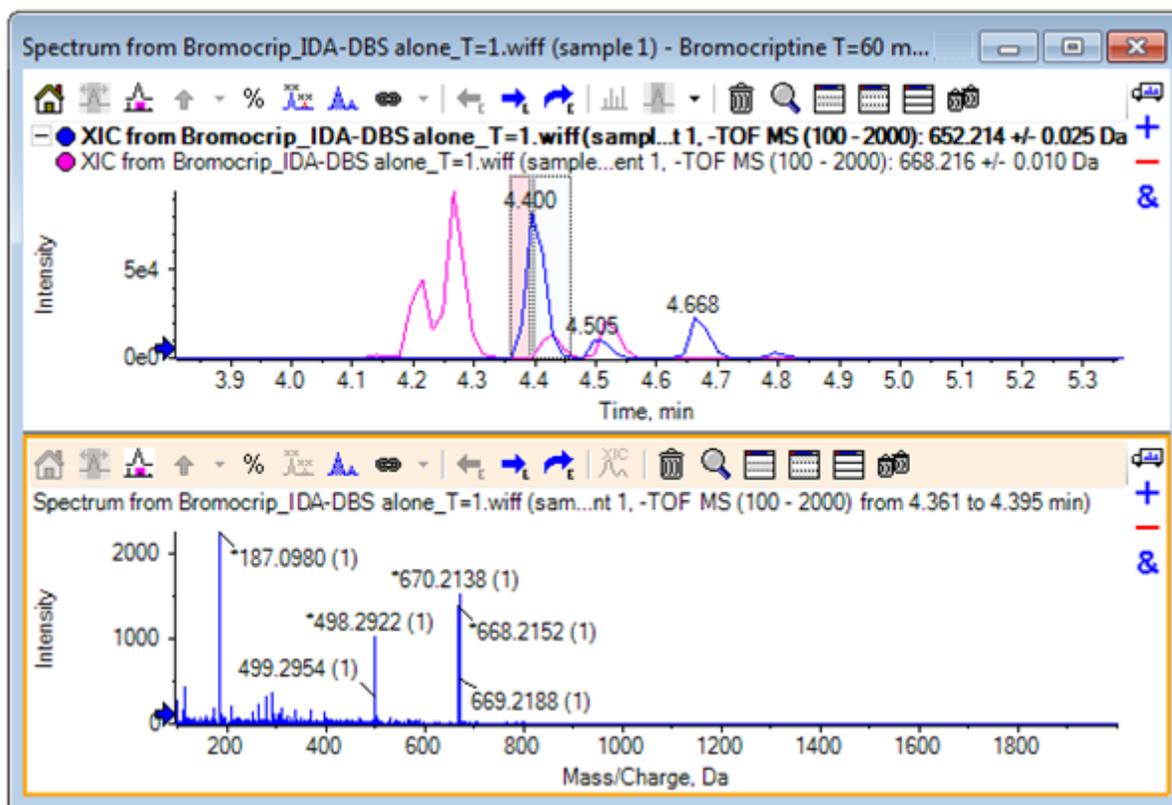
12. Delete the spectrum pane.
13. In the chromatogram pane, make a narrow selection that includes the left side of the 652 peak but avoids the 668 peak and then click the **Set background subtraction range** icon.

The selection turns pink.

When a subtraction range has been defined, it is automatically subtracted from any spectra subsequently generated. The range can be cleared by selecting **Clear Subtraction Range** from the list accessed through the small arrow to the right of the **Set Subtraction Range** icon.

14. Make another selection in the chromatogram that includes the apex of the 668 peak but as little of the 652 peak as possible and then click the **Displays a spectrum for selection** icon.

Figure D-36 Background Subtracted Spectrum for the 668 Peak



The result is a background subtracted spectrum for the 668 peak that contains little of the 652 peak. Both of the selections in the chromatogram remain linked to their respective spectra, even though the background is not visible, and can be moved to other parts of the chromatogram. Moving the spectrum selection automatically updates the shown spectrum, but if the background region is changed, then it is only applied to subsequently generated spectra.

15. Click the **Hides all other panes** icon, click in the single spectrum TIC, and then click the **Deletes all other panes** icon to only show the TIC.
16. If the TIC pane was deleted, then click **Show > Total Ion Chromatogram (TIC)**, select **Period 1, Experiment 1**, and then click **OK**.

## Use a Contour Plot

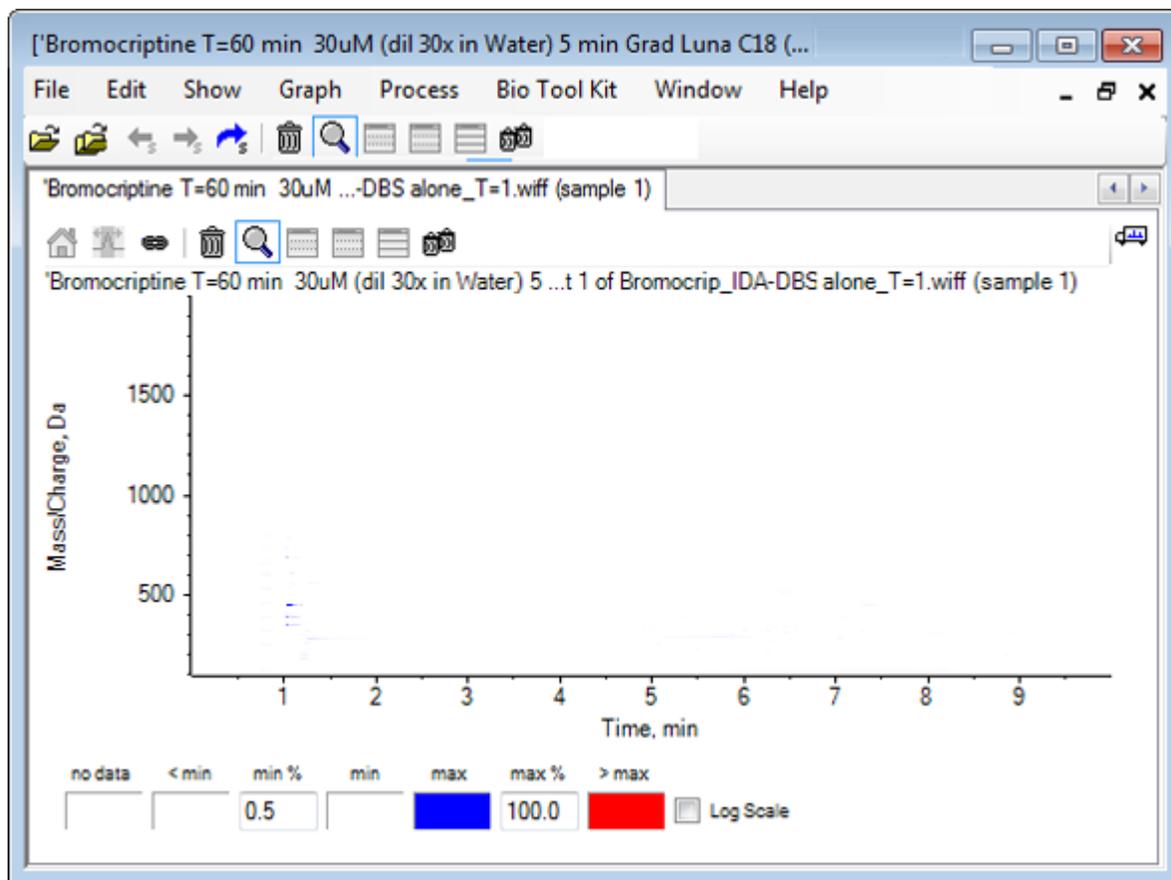
An alternative to viewing parts of a data set (chromatograms or spectra) is to use a Contour Plot to obtain a complete overview of one experiment. Contour Plots can be very informative, but it is usually necessary to adjust the viewing parameters to get the best results. In this case, the precursor compound is brominated and the Contour Plot provides a way to locate peaks with the bromine isotope pattern.

## Explorer Tutorial

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1. With the single experiment TIC active, click **Show > LC/MS Contour Pane**, and then click the **Expands active pane to fill window** icon in the toolbar of the resulting Contour Plot so that it is the only pane visible.
2. If the appearance controls (color boxes in the bottom left corner) are not visible, then right-click in the pane and click **Show Appearance Control**. Refer to [Contour Plots and Heat Maps](#) and the *Reference Guide*.

**Figure D-37 Contour Plot**

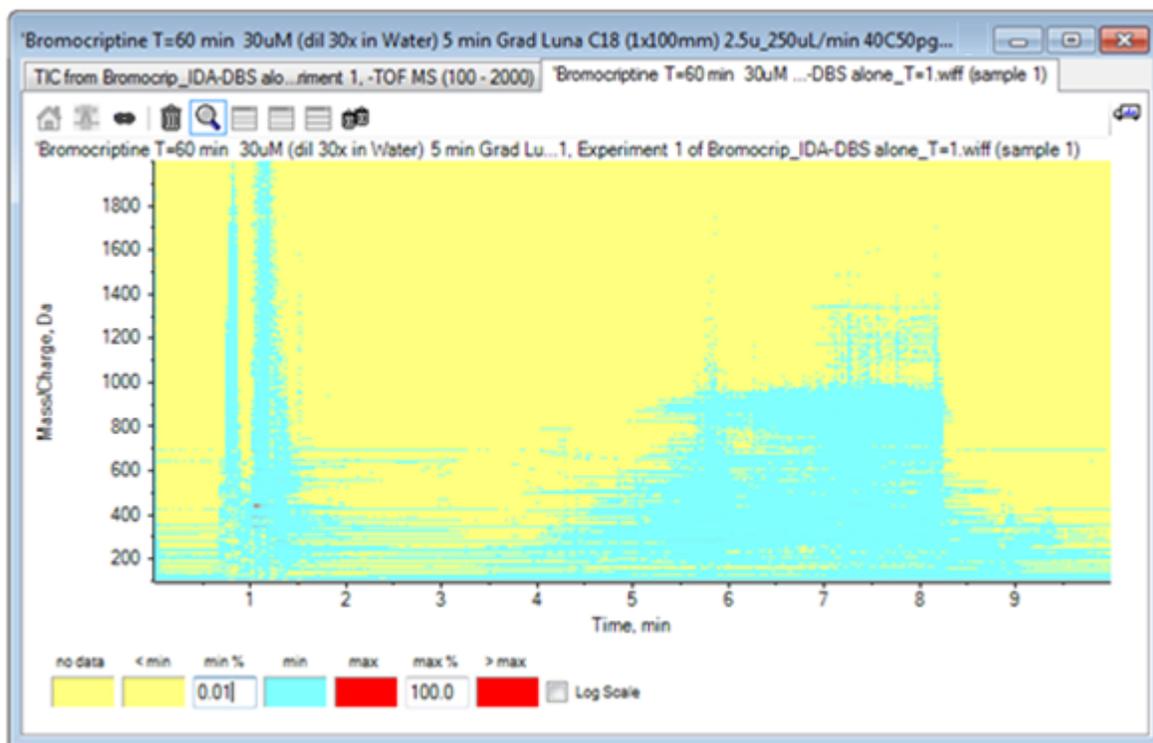


**Tip!** With the default parameters, the view is not very useful as it is dominated by low level peaks and noise that obscure the real peaks. Generate a better view by:

- Changing the minimum intensity to be shown. This changes all the data points below this level to be drawn in the same color as the points where there is no data, that is, they become invisible.
  - Changing the color mapping so that the available colors cover a narrower intensity range that enhances the visibility of small peaks.
-

3. Change the **min %** value to **0.01**. This causes all of the data points with intensities less than 0.01% of the base peak to disappear.

**Figure D-38 Contour Plot**



Much more of the structure in the data is shown. The void volume and column wash out area are clear, and there are a number of background peaks that are present at all of the retention times and are shown as horizontal lines.

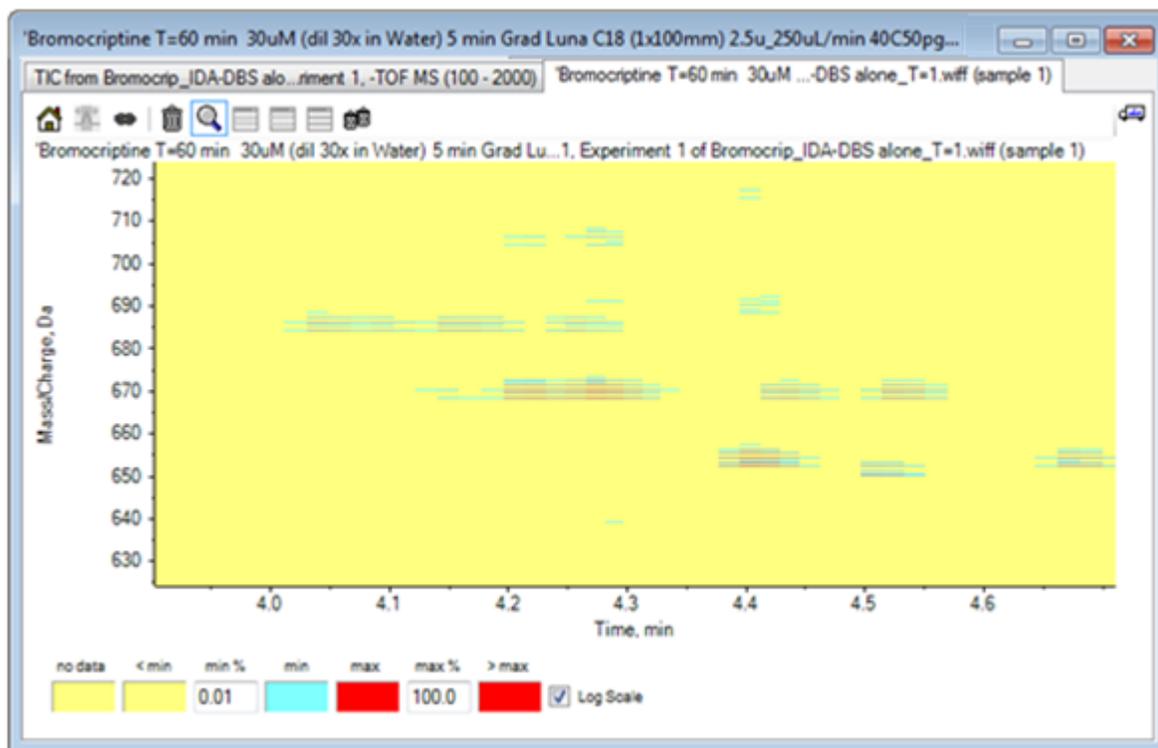
4. Select the **Log scale** check box.  
The selected colors are mapped to the logarithm of the intensity (as a percentage of the base peak intensity) which has the effect of enhancing the lower intensity peaks, for example, the cluster around 4 min to 4.5 min with masses in the 600 to 700 range.
5. Select this region and then click the **Zooms selection to full view** icon.

---

**Tip!** It is also possible to zoom the x and y axes independently in the usual way.

---

Figure D-39 Contour Plot



The view now shows that there are a number of brominated peaks in this area that can be distinguished by the sets of four parallel lines that correspond to the  $^{79}\text{Br}$  and  $^{81}\text{Br}$  isotopes and their  $^{13}\text{C}$  isotopes.

6. Experiment with the color control settings and observe the effects on the view.
7. When finished, close the window.  
This also closes the data file.

## Summary

In this section, the following tasks have been discussed:

- Browsing for and opening a data file to show a TIC.
- Changing the view so that only one experiment is used.
- Using the mass calculator to determine the mass of an ion from an elemental composition and using the mass to generate an XIC.
- Interactively generating spectra and chromatograms and using arrow markers on spectra to show the mass difference between peaks.
- Generating background subtracted spectra.

- Using a contour plot to generate an overview of a data set.

These operations are the basis of all interactive data processing, regardless of the type of data being shown.

## Work with the IDA Explorer

In an IDA experiment, MS/MS spectra (and possibly MS3) data are collected automatically when data in one or more survey spectra meet certain criteria. It is common to set the parameters to avoid collecting multiple spectra from the same LC peak by excluding the precursor mass (not allowing it to act as a trigger) for a certain period of time. Occasionally, redundant spectra can be collected. In addition, because IDA triggers as soon as a peak meets the criteria, it typically generates a spectrum early in the LC peak and this might not have the best quality.

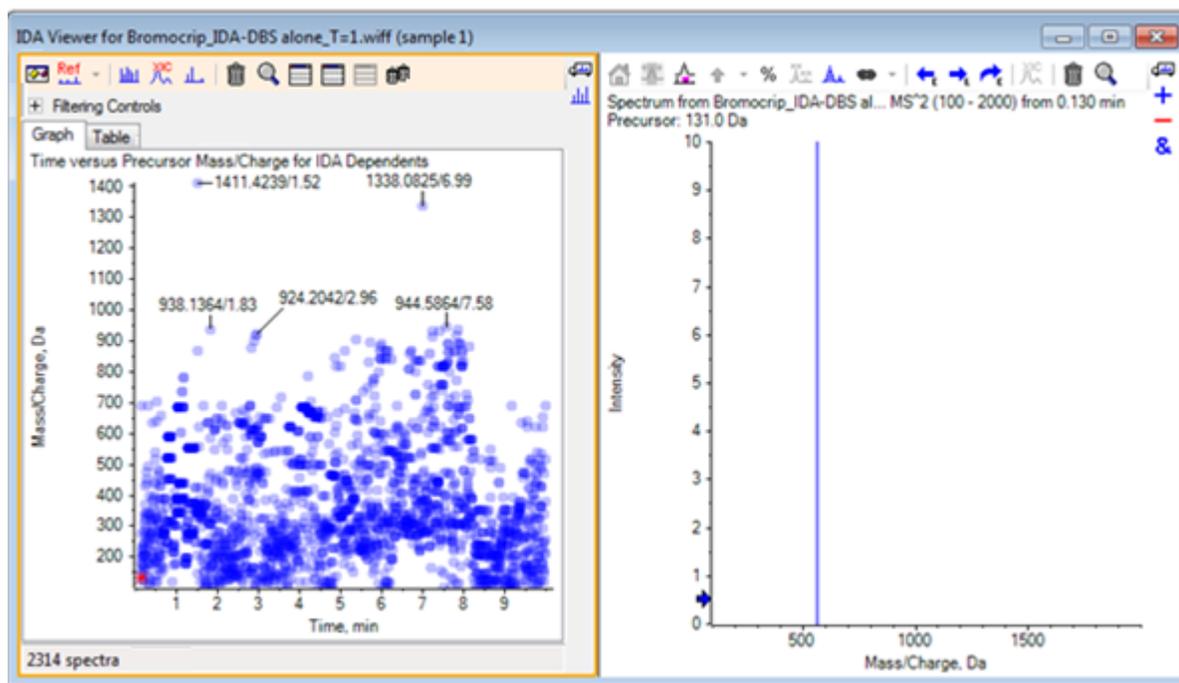
The software contains tools to show, filter, and process IDA data. Some of these are explored in this section.

Close any open windows before starting.

### Show and Merge Spectra

1. Click the **Open Sample** icon in the main toolbar.  
The **Select Sample** dialog opens.
2. If the **Sample Data** folder is not already selected, then click **Browse** and navigate to the **Sample Data** folder.
3. Select the **Bromocrip\_IDA-DBS alone\_T=1.wiff** file and then click **OK**.
4. On the **Open IDA Sample** dialog, click **With the IDA Explorer** and then click **OK**.  
The program examines all of the spectra in the data file and then generates the following graph.

Figure D-40 IDA Viewer



The left panel contains a **Graph** tab and a **Table** tab. The **Graph** tab shows a virtual contour plot where every data point represents the retention time and the  $m/z$  ratio of an ion that was selected as a precursor ion. The **Table** tab shows a tabular view of the data points on the virtual contour plot. The right panel shows the spectrum for the selected data points. Initially the first MS/MS spectrum is shown.

The contour plot uses color intensity to reflect the peak intensity. Darker colors indicate more intense peaks. Labels are drawn where possible, making sure that they do not overlap data points or each other. Zoom the Contour Plot to examine an area in more detail and show more labels.

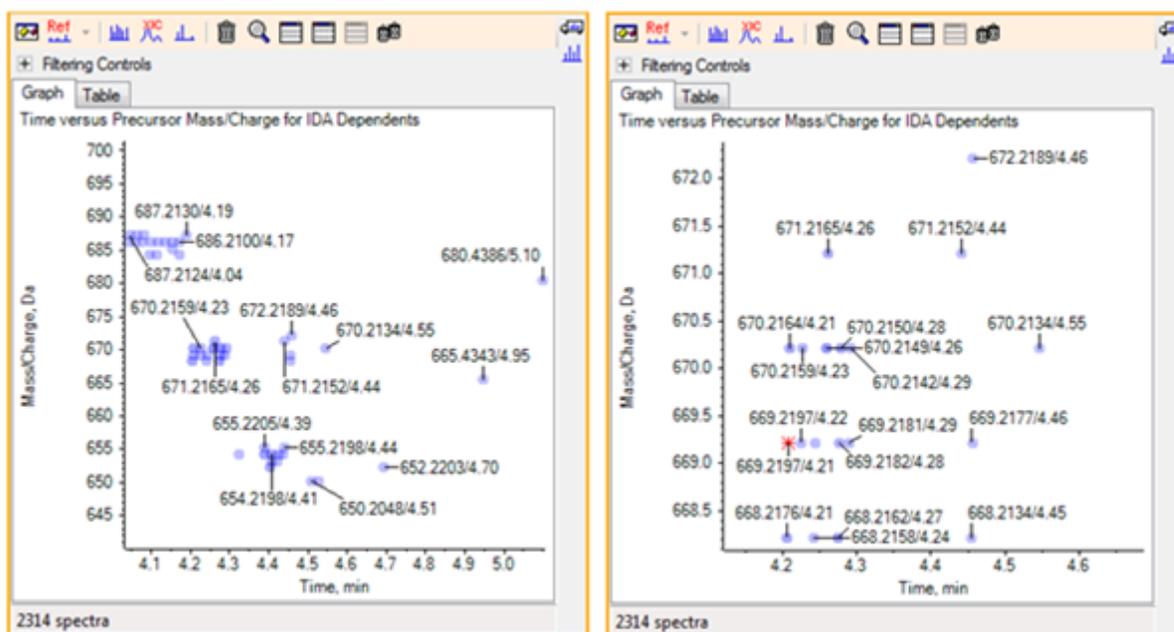
5. In the left panel, zoom the region from 4 min to 5 min and from 640 Da to 700 Da where peaks related to bromocriptine have previously been found.

The figure on the left (Figure D-41) shows just the left panel. If the current view is different, then click the **Show Options** icon and clear the **Merge spectra with similar precursor masses** check box on the **General** tab of the **Options** dialog.

A large number of MS/MS spectra have been collected in this area and although the chromatographic peaks are very narrow, several of these are probably from the same peaks. Furthermore, MS/MS spectra have been collected for each peak in the isotope cluster.

6. Zoom the graph further to focus on the cluster of peaks at an  $m/z$  ratio of 668 Da to 672 Da. Refer to the right panel in Figure D-41.

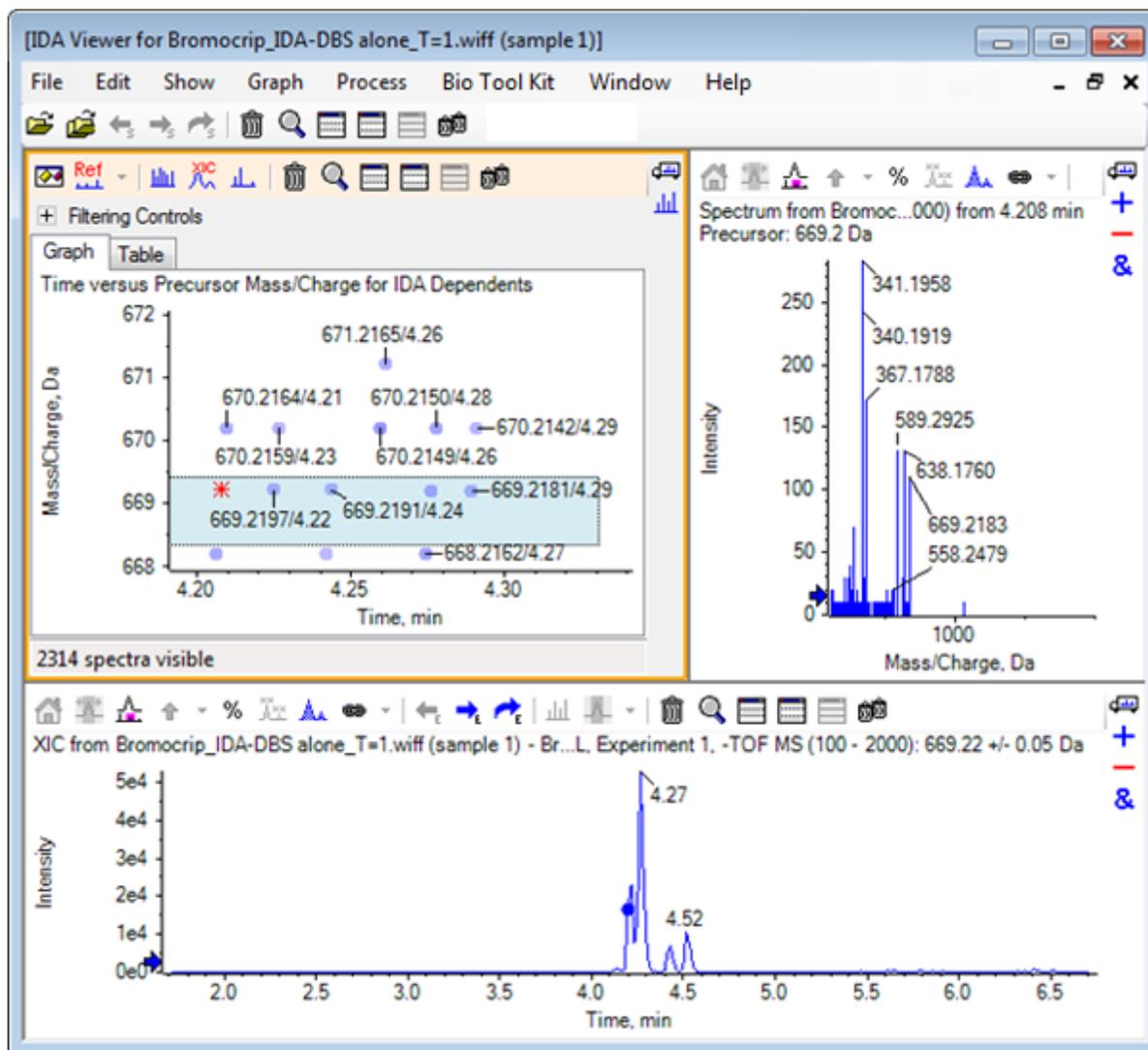
Figure D-41 IDA Viewer



7. Select the first of the 669.2197 peaks (shown as an asterisk in the right panel above) and then click the **Displays an XIC for selection** icon to show the XIC for this precursor mass in the survey scan.

Initially selecting the peak causes the corresponding MS/MS spectrum to be shown.

Figure D-42 XIC for Precursor Mass in Survey Scan



If there is an unlabeled data point in the Contour Plot, then move the cursor over it to show the  $m/z$  ratio and retention time label so that the times of the product ion scans are related to the survey chromatogram.

For the 669.2 peak, the first three scans are related to the first XIC peak at 4.21 min, which is also where a 668.2 scan was generated, the second two scans are related to the peak at 4.27 min, and the last scan is from the peak at 4.42 min (669.2177/4.46). No scan was performed for the 669.2 peak at 4.52 min, but a scan was obtained for the 670.2 peak.

**Note:** The scan times are slightly different because they are obtained sequentially, even if they are detected in the same survey scan. The smaller isotope peaks might not be detected as early as the larger ones.

8. Draw a selection rectangle around the first five 669.2 scans, right-click, and then select **Select Points in Graph Selection**.

This causes the spectrum pane to overlay all of the MS/MS spectra.

The system has acquired more scans than necessary. By reducing the number of spectra to process and merging those that are too close to be different compounds, we can obtain higher quality results. Merging uses both mass and retention time to determine these scans.

9. Click **Show Options** icon, select the **Merge spectra with similar precursor masses** check box, and then set the **Mass tolerance** to **10** ppm and the **RT gap tolerance** to **0.03** min. (the peaks in this analysis are approximately 2 sec. wide).
10. Click **OK**.

---

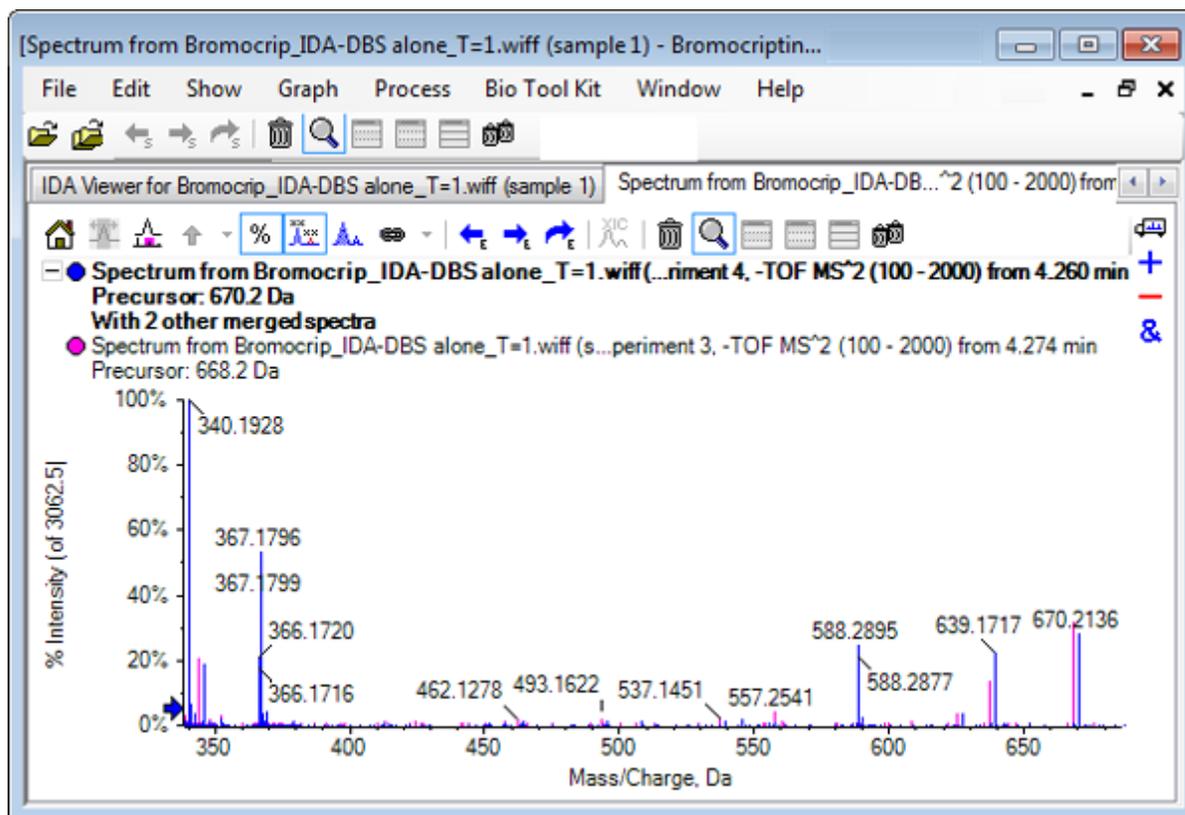
**Note:** This part of the dialog also allows users to define how XICs are to be extracted. The mass width should match the resolution or peak width of the instrument and it is useful to limit the time range used because this speeds processing.

---

Merging the data in this way results in three peaks for 669.2 at 4.21 min, 4.28 min, and 4.46 min. The status bar at the bottom of the IDA Viewer pane shows the progress as the data is being merged and then shows the total number of dependent spectra after the merging is complete.

11. Click the data point at 670.2149/4.26 and then press the **Ctrl** key and click the point at 668.2162/4.27.
12. In the MS/MS spectrum pane, click the **Expands active pane to fill window** icon, the **Use percent y-axis** icon, and the **Label all overlaid traces** icon, and then zoom the x-axis to show the region from 340 to 680.

Figure D-43 Spectrum: Region from  $m/z$  340 to 680 Zoomed



Because these two precursors correspond to the Br isotopes, the spectra should be identical except for ions that retain the Br atom, which are shown as a pair of peaks separated by two Da. In this example the fragments (668.2 trace) at 344.0441, 625.1765, and 637.1712 have retained the Br atom while those at 340.1925, 367.1796 and 588.2877 have not.

Place an arrow at the 588.2877 peak and then observe that the 668 and 670 peaks are now labeled with the mass of the Br isotopes plus 1, indicating that the 588.2877 corresponds to the loss of HBr.

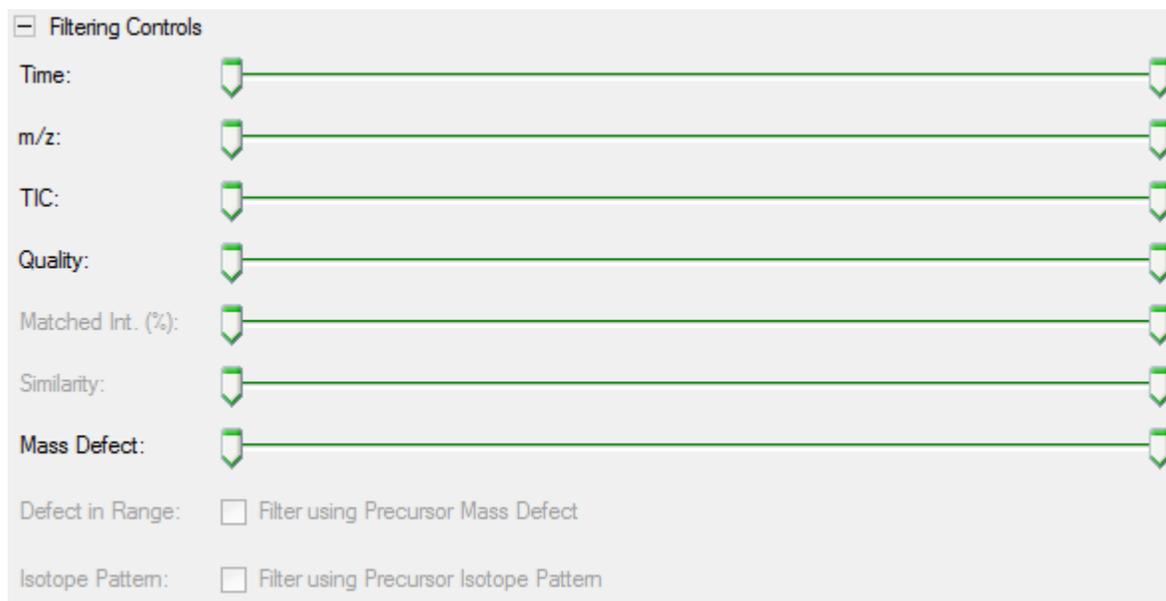
13. Remove the arrow from the spectrum, click the **Expands active pane to fill window** icon, and then zoom out the Contour Plot to see all of the data points.

## Filter IDA Data

The IDA Explorer contains a number of filters that can be used to reduce the amount of data to be visualized or processed. These are described in this section.

1. In the Contour Plot, click the **Expands active pane to fill window** icon, and then click the icon next to **Filtering Controls** just below the toolbar.

Figure D-44 Filtering IDA Data



This window shows several sliders and check boxes, each corresponding to a different filtering criterion that can be used to adjust the amount of data shown. Retention time (**Time**) and the  $m/z$  ratio (**m/z**) can be selected here or by zooming the view.

The other filters are as follows:

- **TIC**: Sets limits for the summed intensity of peaks in the MS/MS spectrum. This is usually used to remove small, noisy scans.
- **Quality**: This is the fraction of the summed intensity that is greater than the equivalent of 1 count, that is, is less likely to be due to noise, and is an estimate of spectral quality.
- **Matched Int. (%)**: Assesses the fraction of the summed intensity explained by known fragments and neutral losses when using **Fragment Matching**.
- **Similarity**: Available when a reference spectrum has been set. This feature measures the fraction of the summed intensity that corresponds to common fragments and neutral losses in the reference spectrum. Refer to [Use a Reference Spectrum](#).
- **Mass Defect**: Sets a single range for the fractional part of a mass. This feature is useful for finding metabolites because the common metabolic transformations (O, O<sub>2</sub>, and so on) do not significantly change the defect from the precursor molecule so using a range close to its defect can reveal possible metabolites.
- **Defect in Range**: In addition to the single mass defect range, the software also allows users to define several defects that apply to different mass ranges. If such ranges are defined, then this check box allows users to determine whether to apply the filter or not. The ranges are set on the **Mass Defect** tab of the **Options** dialog.

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- **Isotope pattern:** This check box allows users to apply one or more isotope pattern filters to the MS survey data. That is, a data point is shown only if the selected precursor ion has the desired pattern. These patterns are defined in the **Isotope Pattern** tab of the **Options** dialog.

Each of the simple filters has two sliders so a range can be defined. Double-click any slider and then directly type a value.

2. Experiment with the settings of the sliders and notice in particular that even low minimum settings for **TIC** (for example, 1e3) or **Quality** (1) values, have a dramatic effect. Set the lower **TIC** filter to 2e3 and all of the others to 0.

The mass defect of bromocriptine is approximately 0.22, so it is unlikely that simple metabolites have values greater than this or much lower.

3. Set the **Mass Defect** filters to 0.18 and 0.23 and notice that among the remaining peaks are those in the vicinity of 4.5 min and 650 Da and that there is only one data point from an *m/z* ratio of 652.2211 in this region (4.40 min).
4. Hide the filter controls by clicking the icon next to **Filtering Controls**.

---

**Tip!** Change which filters are visible by right-clicking in the filter area, selecting **Filters** and then selecting those that are appropriate.

---

## Use a Reference Spectrum

1. In the Contour Plot, click the data point at 652.2211/4.40 (bromocriptine itself) and then click the **Set Reference Spectrum (for Similarity Scoring)** icon.

---

**Note:** It might be necessary to zoom the graph first.

---

2. Click the arrow next to the **Set Reference Spectrum (for Similarity Scoring)** icon and then make sure that **Overlay Reference Spectrum** is selected.
3. Click the data point at 654.2185/4.39.

With a reference spectrum defined and **Overlay Reference Spectrum** selected, any spectra shown also have the reference spectrum overlaid so they can easily be compared. This is useful when working with metabolites, because it provides a quick way to determine which peaks are shifted and which are not.

We have made the MS/MS spectrum of the precursor ion for the lower mass bromine isotope the reference and we have overlaid the spectrum for the higher mass isotope, so we have a similar view to the one generated earlier for the 668.2 peak. That is, bromine-containing ions can be identified by the presence of peaks two Da apart.

4. Click the **Expands active pane to fill window** icon and then in the Contour Plot, click **Table** (just below **Filtering Controls**).

**Note:** If required, move the spectrum pane below the table (using the Drag and drop to rearrange the panes icon) so that all of the columns are visible.

---

The table shows the same information as the graphical explorer, but provides additional detail. It also responds to the filtering controls so that the two views contain the same spectra. The table is linked to the spectrum view so that selecting rows causes the spectrum to update, and the rows can be sorted by clicking the column headers.

When a reference spectrum is defined two extra columns are shown: **Delta m/z** shows the difference between the precursor mass of the reference and the spectrum corresponding to the row. **Similarity** shows the similarity of the two spectra.

5. Click **Delta m/z** to sort the table and then observe that it contains several peaks differing by approximately 15.995 (the mass of oxygen) and one at 31.990 (O<sub>2</sub>) that are likely hydroxy-bromocriptine metabolites.
6. Click a row in the table to show the associated spectra.

---

**Note:** These spectra have high similarity values as do the scans with precursor masses two Da higher, which are obtained from the <sup>81</sup>Br containing ions.

---

## Summary

In this section, the following tasks have been discussed:

- Examining an IDA file using the graphical and tabular IDA Explorer views.
- Merging related spectra after determining that this is required.
- Filtering the number of spectra shown using TIC and mass defect filters.
- Overlaying spectra so they can be compared.
- Defining a reference spectrum and use the table to find likely metabolites.

These operations are fundamental to processing IDA data.

The next section describes how to use the structure tools using the MS/MS spectrum of bromocriptine.

## Work with Structure Tools

The software contains tools to help link the masses of ions to structures (saved as .mol files) and to explore possible sites for biotransformations.

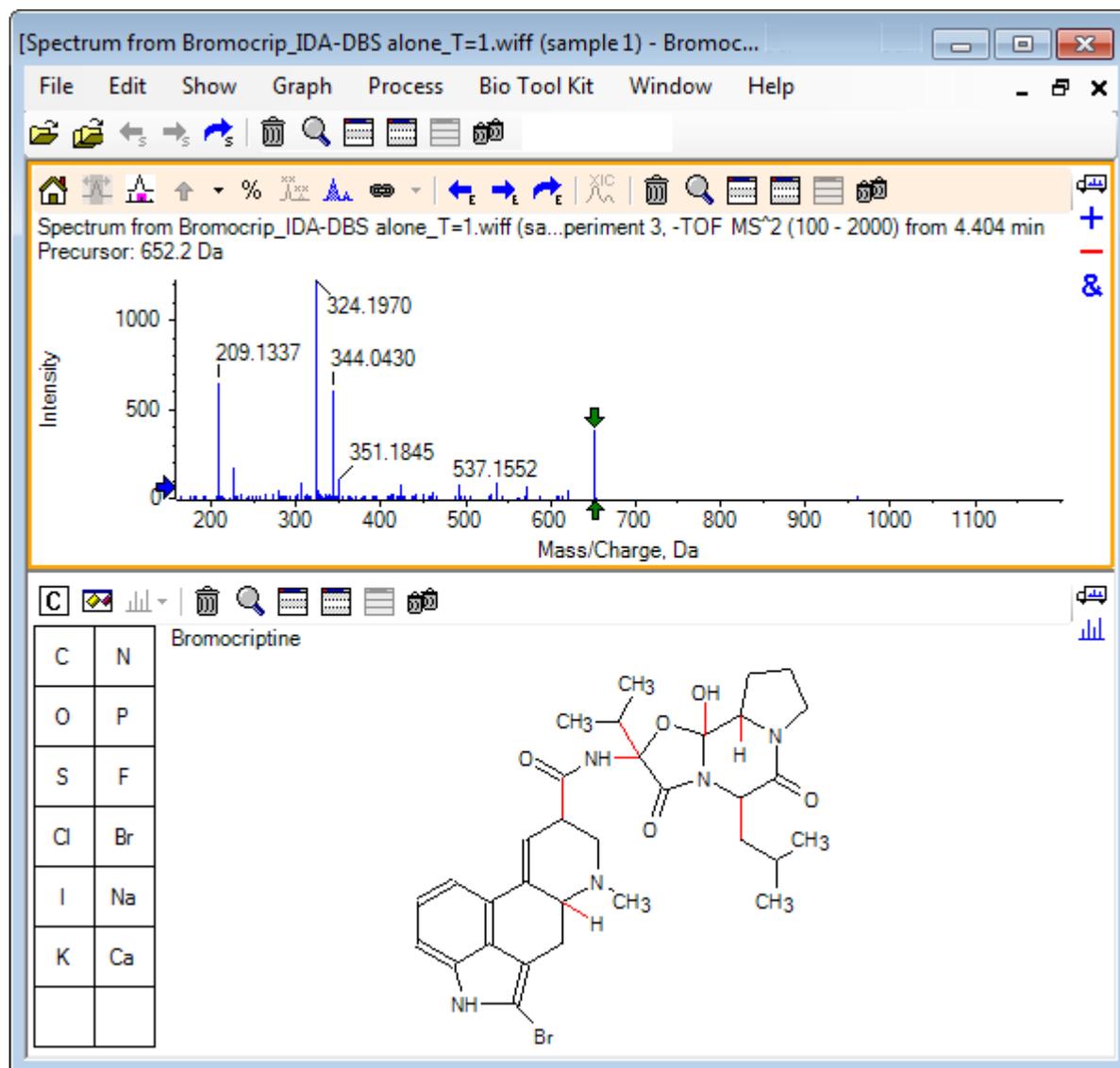
### Link a Structure to an MS/MS Spectrum

1. Locate the MS/MS spectrum of bromocriptine, 652.2211/4.40. Refer to [Work with the IDA Explorer](#).

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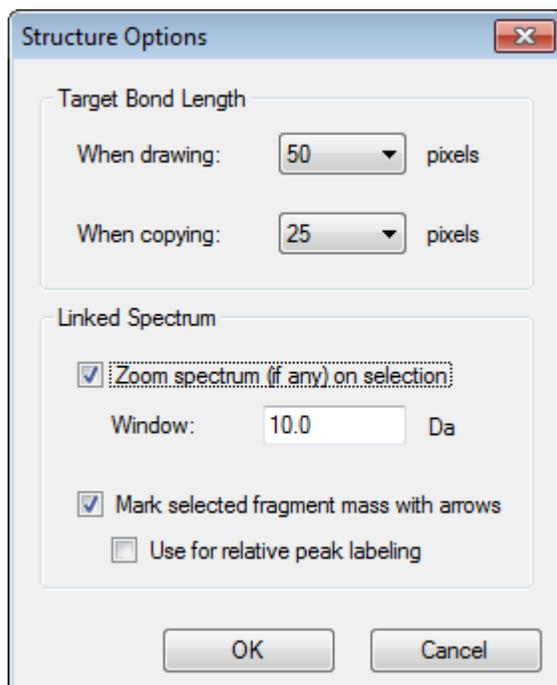
2. Click the **Hides all other panes** icon in the Contour Plot so that only the spectrum is visible.
3. Click **File > Open Mol File**.
4. In the **Select Mol File** dialog, select the **Bromocriptine.mol** file and then click **Open**. For information about installed data file locations, refer to [Organization](#).  
A new pane opens beneath the spectrum to show the structure and the tools.

**Figure D-45 Bromocriptine Structure**



5. Click the **Show options dialog** in the structure pane, make sure that the **Zoom spectrum (if any) on selection** and **Mark selected fragment mass with arrows** check boxes are selected, and then click **OK**. The other parameters can be left unchanged.

Figure D-46 Structure Options Dialog



The spectrum and structure are automatically linked because the spectrum was active when the structure pane was created. Manually link a structure to a spectrum by dragging the **Displays a spectrum for selection** icon to the appropriate spectrum.

Dragging in the structure pane causes a line (a lasso) to follow the cursor, allowing users to select all or part of the structure, which is then drawn in bold face. Because there is a linked spectrum, it zooms and scrolls to show the region around the mass of the selected sub-structure.

6. Draw a lasso around the entire molecule and the view changes to show the peak at an  $m/z$  ratio of 652.2177, which corresponds to the  $(M - H)^-$  ion.

Because the **Mark selected fragment mass with arrows** check box was selected, a red arrow is drawn above and below the peak indicating that this is the expected mass of an ion corresponding to the selected region (that is,  $(M - H)^-$  because this data is in negative mode).

---

**Note:** The title of the structure pane indicates the elemental composition and the mass of the neutral compound corresponding to the selection (that is,  $C_{32}H_{40}N_5O_5Br$  with a mass of 653.2213 Da).

---

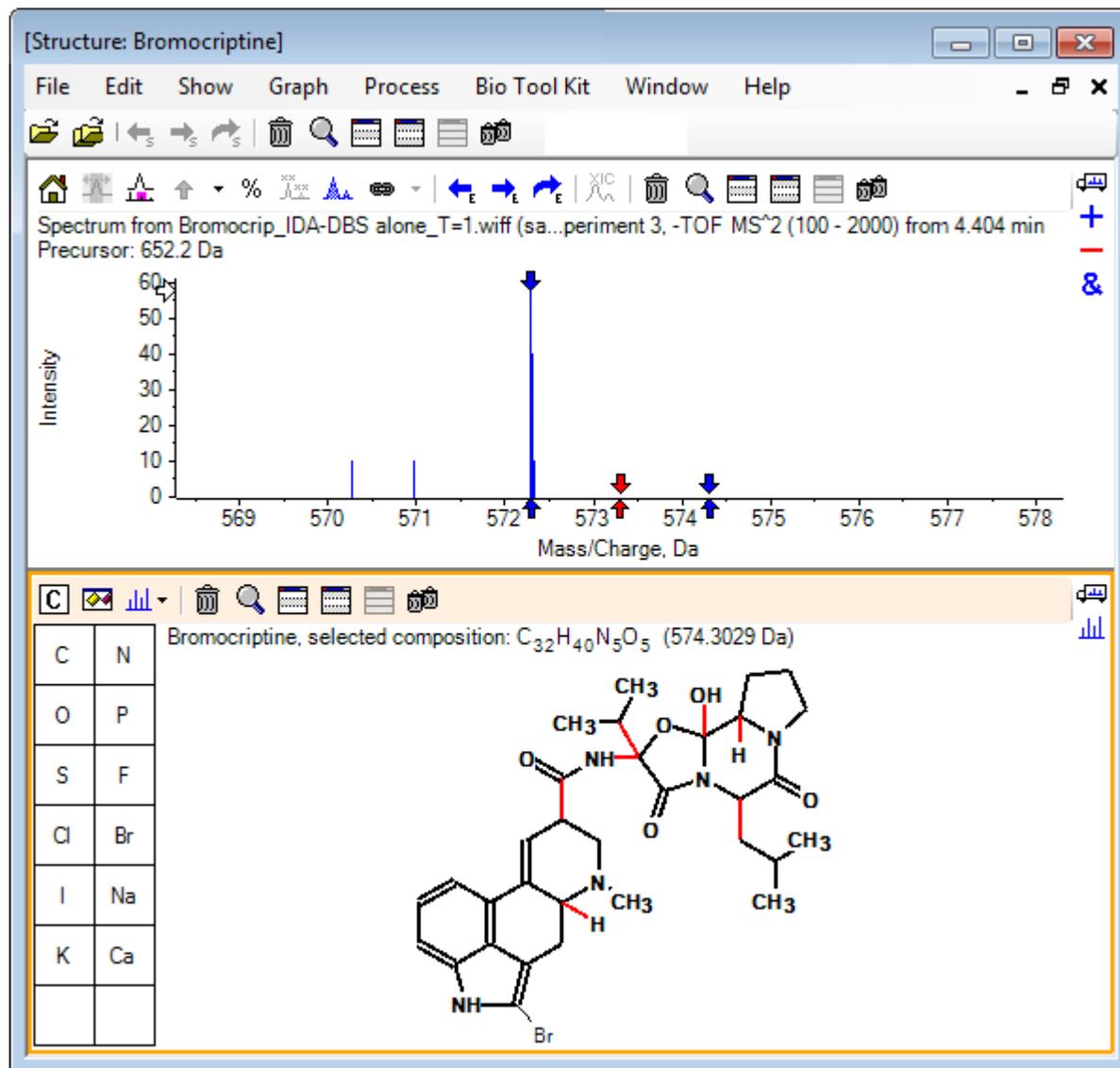
If **Mark selected fragment mass with arrows** is selected, then a green arrow is drawn on the 652.2177 peak when nothing is selected in the structure pane. This is because

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the green arrow marks the complement of the current selection and with no selection the complement is the entire molecule.

7. Select the entire molecule except the bromine atom. Refer to [Figure D-47](#).

**Figure D-47 Bromocriptine Structure**



**Note:** The bromine atom is the only one in normal font and the title in the structure pane shows the composition  $C_{32}H_{40}N_5O_5$  with a mass of 574.3029 Da. In the spectrum, the red arrow indicates the expected mass of the selection, that is, the mass of the  $(M - H)^-$  molecular ion less the mass of bromine, and there are arrows 1 Da away on either side. It is common for additional hydrogen atoms to be gained or lost during fragmentation and the software indicates this possibility by drawing a pair of blue arrows at +1 and -1 for each bond broken. In this case only one bond is broken so there are just two additional arrows.

---

The actual peak in the spectrum corresponds to one of these arrows, indicating that an extra hydrogen atom was lost, that is, HBr, so the mass of the ion corresponds to  $(M - H - HBr)^-$ .

## Work with Fragments

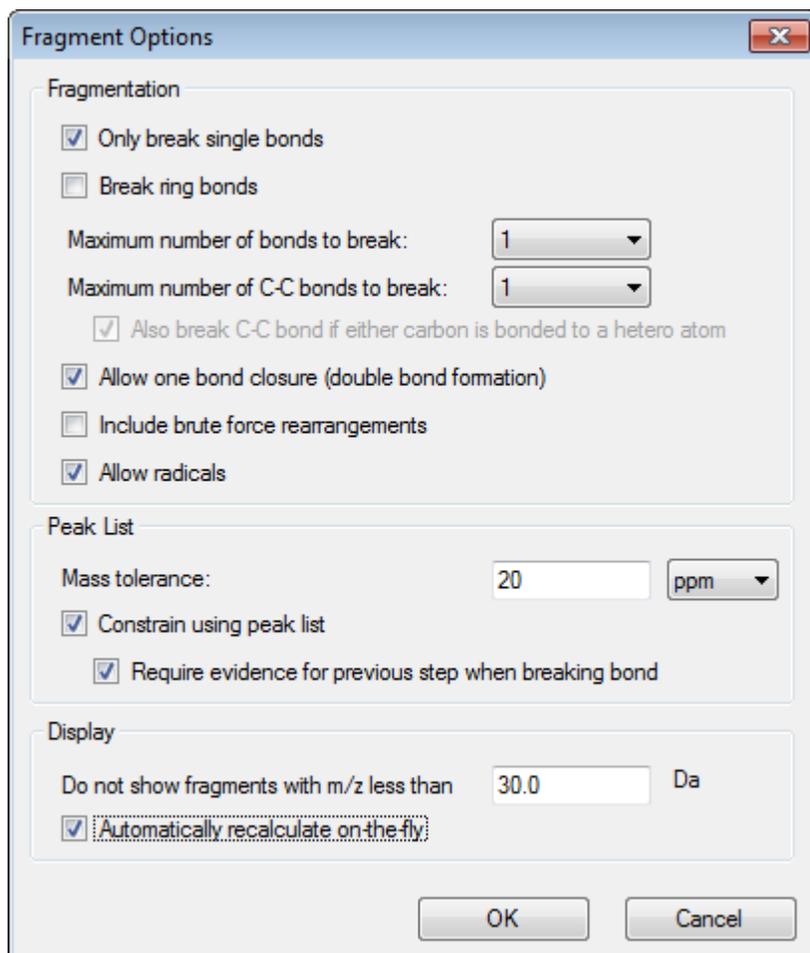
The software contains a fragment ion predictor that can generate the mass of species formed by breaking bonds and adding or removing hydrogen atoms.

**Note:** This prediction is purely arithmetic, does not use chemical logic, and tends to overestimate the fragments produced, but it is a useful tool for analyzing fragments.

---

1. With the structure pane active, click **Show > Fragments Pane**.  
A progress bar might be shown, depending on the settings in the **Fragment Options** dialog. Refer to [Figure D-48](#).
2. Click the **Show options dialog** icon, set the parameters as shown in [Figure D-48](#), and then click **OK**.

**Figure D-48 Fragment Options Dialog**



Set the options so that a small set of simple fragments are produced and then increase the number and type of the bonds broken as needed to explain observed ions. Allowing many bonds to break slows down the program and generates a large number of unlikely fragments.

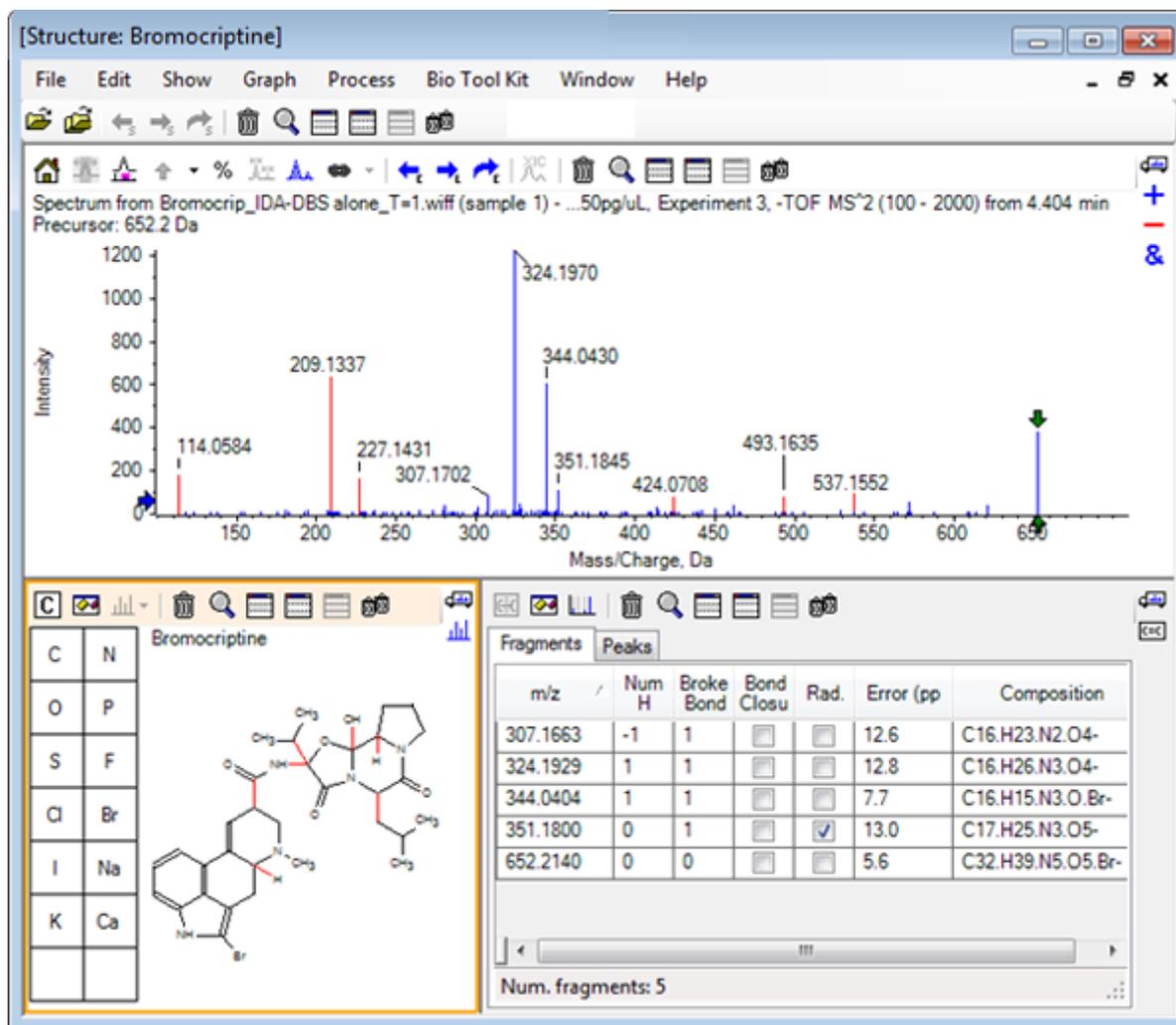
Most of the parameters in the **Fragment Options** dialog are described in the *Reference Guide*, but the following should be noted:

- If the **Automatically recalculate on-the-fly** check box is selected, then any changes to the spectrum (switching to a different one, adjusting the parameters) or selection causes the fragments to be recalculated. This is usually the desired behavior, but can impact the speed of the analysis if the options are set to produce many fragments. If this option is not used, then click the **Fragment** icon.
- **Constrain using peak list** means that the software only shows fragments that match peaks in the spectrum with the appropriate tolerance.

- **Require evidence for previous step when breaking bond** is only effective when more than one bond is broken. The program first breaks one bond and then considers breaking bonds in the resulting pieces. If this option is selected, then there must be ions corresponding to the pieces before they are broken further.

With these parameters, the view should resemble [Figure D-49](#) but might be slightly different because only peaks above the threshold setting (also labeled) are considered.

**Figure D-49 Bromocriptine Structure**



**Note:** Peaks in the spectrum are colored to indicate those assigned (blue) and those unassigned (red) matching the peaks in the Fragments tab.

The fragments pane contains two tabs:

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- **Fragments:** In this example, the list is short because not many fragments are generated under these conditions and only a few of these match peaks in the spectrum, as required, because the **Constrain using peak list** check box was selected.
- **Peaks:** Shows a table listing the peaks in the spectrum that are above the threshold, their intensities, and whether they were assigned to a fragment. For assigned peaks the mass error is also shown.

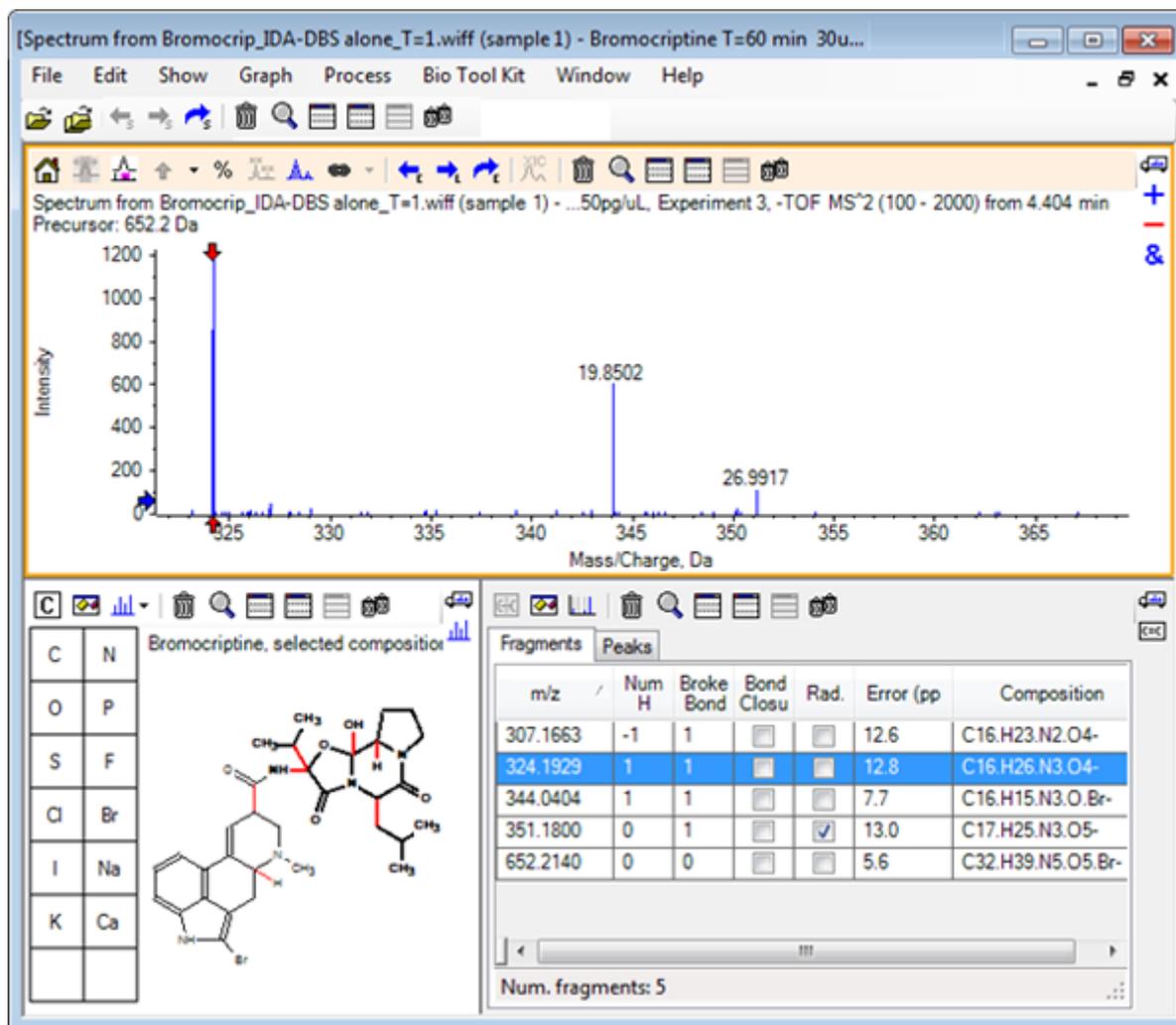
Figure D-50 Fragments Pane

Mass/Charge	Intensity (	Assign	Error (ppm)	Radica
114.0584	14.88	<input type="checkbox"/>		<input type="checkbox"/>
209.1337	52.33	<input type="checkbox"/>		<input type="checkbox"/>
227.1431	13.74	<input type="checkbox"/>		<input type="checkbox"/>
307.1702	7.20	<input checked="" type="checkbox"/>	12.6	<input type="checkbox"/>
324.1970	100.00	<input checked="" type="checkbox"/>	12.8	<input type="checkbox"/>
344.0430	49.22	<input checked="" type="checkbox"/>	7.7	<input type="checkbox"/>
351.1845	9.08	<input checked="" type="checkbox"/>	13.0	<input checked="" type="checkbox"/>
424.0708	6.62	<input type="checkbox"/>		<input type="checkbox"/>

Matches: 5 of 11 peaks, 66.0% of total intensity

3. In the **Fragments** tab, select the row for an  $m/z$  ratio of 324.1929. The peak is marked with a red arrow to show that this is the expected mass, and the corresponding substructure is drawn in bold in the structure pane.

Figure D-51 Fragmentation Dialog



**Note:** The composition and mass in the title of the structure pane now reflect the mass of the ion rather than that of the neutral.

- Examine the structures assigned for the other fragments.

They are all related to the central amide bond that separates the two cyclic parts of the molecule and seem possible.

**Note:** The assigned elemental compositions are consistent with the overlaid spectra that was generated in [Use a Reference Spectrum](#) where the presence of Br in fragments was deduced by comparing the spectra of the  $^{79}\text{Br}$ - and  $^{81}\text{Br}$ - containing molecular ions.

- Zoom the spectrum so that the whole mass range is visible.

## Explorer Tutorial

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Two of the major peaks are assigned, an  $m/z$  of 324.1970 and an  $m/z$  of 344.0430, corresponding to the two sides of the molecule, and are drawn in blue. However, a number of peaks are still unassigned.

6. Open the **Options** dialog and then change the **Maximum number of bonds to break** to **2**.

---

**Note:** Depending on the threshold setting, this option might cause some small peaks to be assigned, but not the more abundant ones ( $m/z$  ratios of 114.0584, 209.1337 and 227.1431 for example). If the spectrum is labeled relative to a red arrow, then click in the structure pane to clear any selection to show the absolute mass values.

---

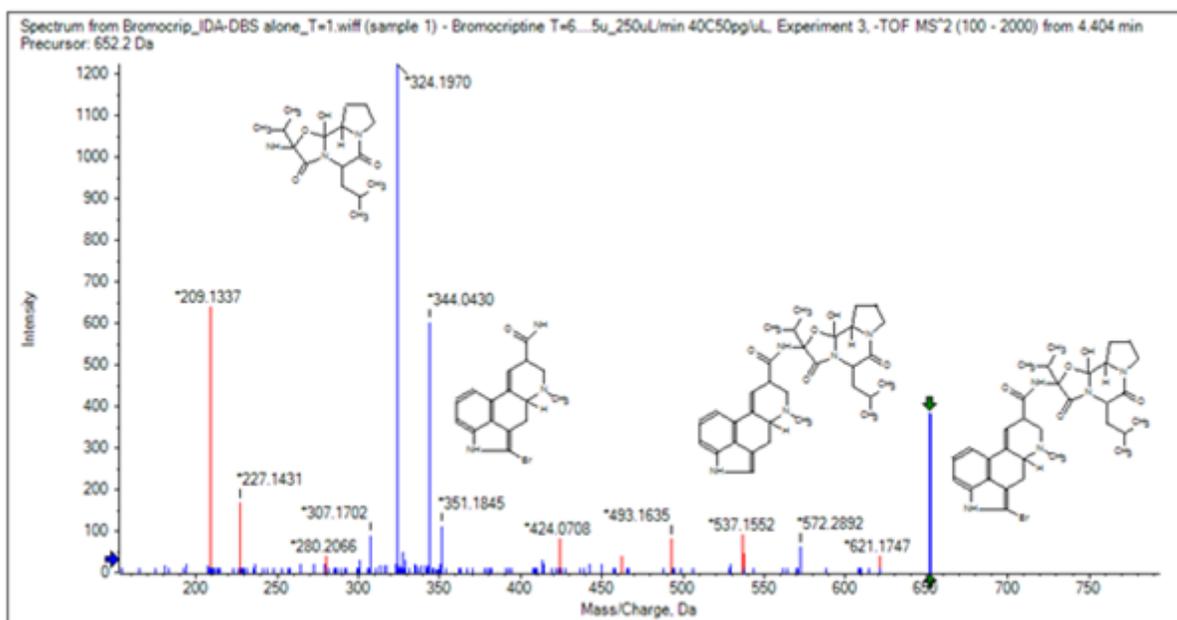
7. Select the **Break ring bonds** check box and then click **OK**.  
A number of additional ions are now matched, including those at an  $m/z$  of 209.1337 and 227.1431. Selecting the new masses in the **Fragments** pane to highlight the substructures shows that these are related to ring cleavages in the cyclic peptide part of the molecule. These ions are likely to be useful in determining metabolic transformation sites in this region.

## Add Substructures to a Spectrum

Select parts of the structure and then use them to annotate the spectrum for future reference. Depending on the size of the spectrum pane, use the **Options** dialog in the structure pane to adjust the **Target Bond Length** for copying.

1. In the **Fragment Options** dialog, clear the **Break ring bonds** check box to simplify the number of fragments.
2. In the fragments pane, select a row corresponding to one of the more abundant ions to highlight the corresponding substructure.
3. Click inside the structure pane.
4. Click **Edit > Copy**.
5. Right-click in an active spectrum pane and then click **Paste Image**.  
This causes an image of the substructure to be pasted into the spectrum pane.
6. Move the image by dragging it to the desired location. An image can be deleted completely by right-clicking it and then selecting **Delete Image**.  
Images are linked to the spectrum, that is, the mass intensity positions, so they move as users scroll and zoom.
7. Repeat step 2 to step 6 for other fragment ions to generate a final image similar to [Figure D-52](#).

Figure D-52 Spectrum with Added Substructures



8. Click **File > Print > Print Preview Window** to verify the positioning of the substructures. Because the matched ions are drawn in blue, they are easy to associate with the corresponding structures.
9. Copy the image and then paste it into a drawing program to add lines or other features.

## Work with Related MS/MS Spectra

In some applications, it is useful to be able to compare the spectrum of a modified compound, a metabolite for example, to the spectrum and structure of the precursor compound.

1. Use the IDA Explorer to show the Contour Plot again. Select the peak at 668.2176/4.21 and then hide the Contour Plot.

Because the structure and fragments panes are linked to the spectrum, they have updated to reflect the new spectrum, but the structure is still that of the precursor compound while the spectrum was obtained from a compound with an additional oxygen atom (16 Da higher in mass). In many cases there are still some matches, indicating the parts of the molecule that are unchanged, but in this case none of the significant ions match and are drawn in red.

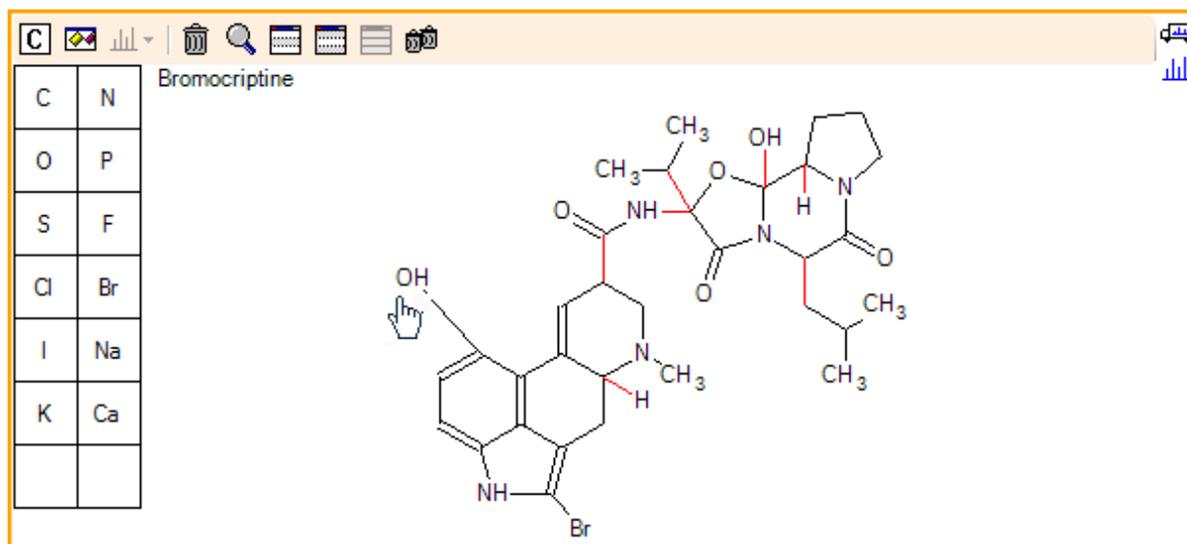
The structure pane contains some simple drawing tools that allows modifications to the structure to see if matches can be found.

2. The left side of the structure pane contains a grid with element symbols. Click **O** and then drag it towards the main structure  
When the atom is close to the structure, it is joined by a bond that follows the cursor as it is dragged close to the structure.

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3. Drag the **O** symbol so that a bond is drawn to the lower part of the structure (ergoline) and then release the mouse (for example, place the new atom on the phenyl ring). [Figure D-53](#) shows the process.

**Figure D-53 Structure Pane**

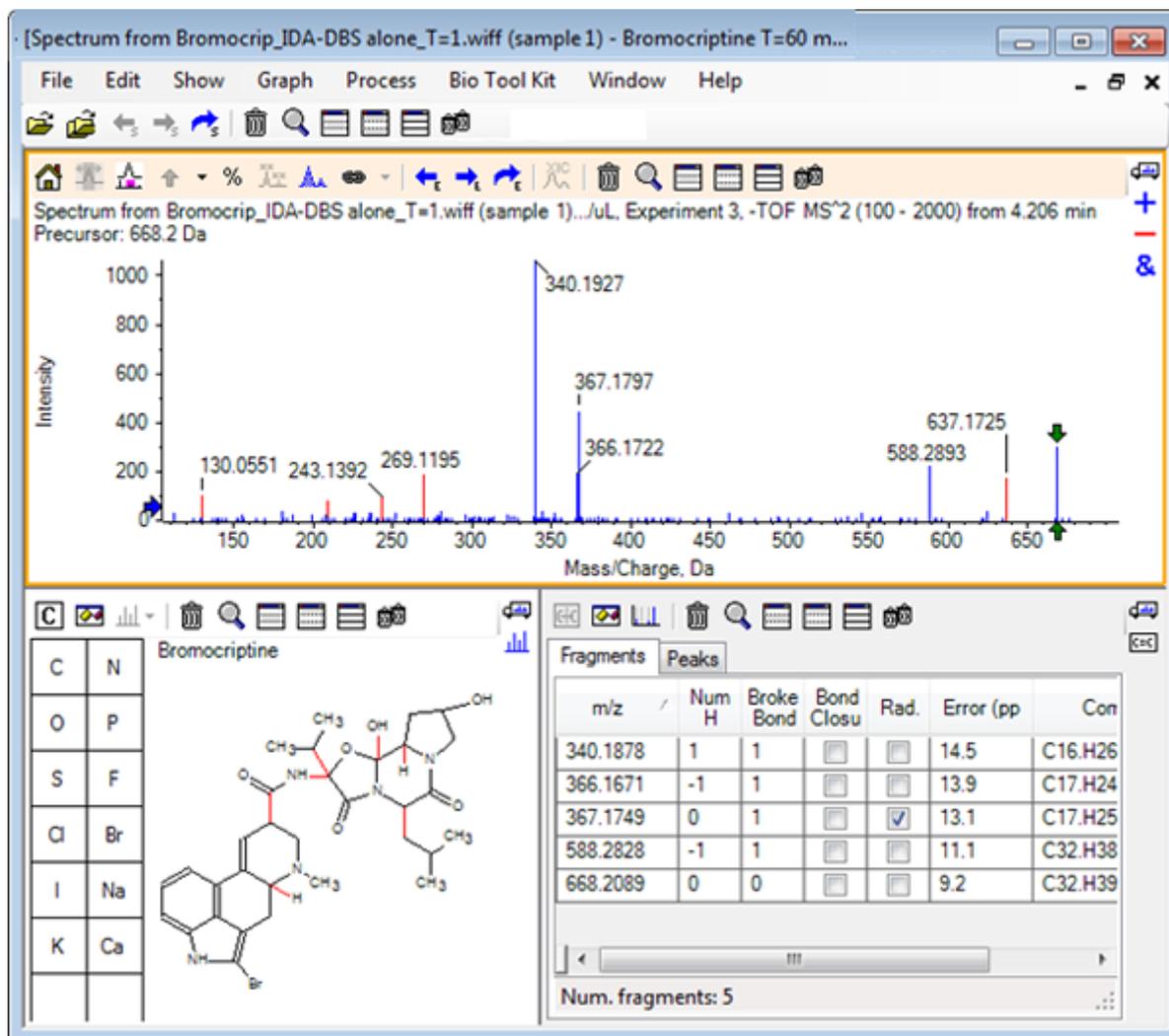


The spectrum updates again and shows two matches: the molecular ion at 668.2089 and the ion corresponding to the loss of HBr at 588.2828. This suggests that the overall elemental composition is now correct, but the fact that the major fragments do not match suggests that the atom has not been added to the right part of the molecule.

4. Click the **OH** group just added and then drag it to the pyrrolidine ring in the upper part of the structure. Make sure that only the atom being moved is drawn in bold. Otherwise, the entire highlighted substructure is moved.

As shown in [Figure D-54](#), this causes the ions at 340.1927, 366.1722, and 367.1797 to be matched and the corresponding substructures are hydroxylated forms of ions matched in the spectrum of the precursor compound.

Figure D-54 Spectrum from Bromocriptine



Many of the unmatched low mass peaks were present in the precursor spectrum, or are hydroxylated equivalents, that were matched when the algorithm was allowed to break ring bonds, but there is a high mass ion at 637.1725 that is likely due to a simple fragmentation step and yet is unmatched.

- In the **Fragments** tab, select the row for 668.2089 so that it is labeled and the other ions are labeled relative to it.  
This shows that the peak at 637.1725 corresponds to the loss of 31.0364 from the precursor molecule that could be  $\text{CH}_3\text{NH}_2$  or  $\text{CH}_3\text{O}$ . Because this ion was not observed in the spectrum of the precursor molecule it seems most likely that it is derived from hydroxylation occurring at one of the methyl groups in the cyclic peptide part of the structure.
- Click twice in the structure pane to deselect the structure and then drag the new **OH** group to one of the methyl groups at the right of the structure.

## Explorer Tutorial

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7. Open the **Fragment Options** dialog, set the **Mass tolerance** to 30 ppm, and then click **OK**. The 637 ion is now matched and selecting this row in the fragments pane shows that the ion might correspond to the loss of a methoxy moiety.
8. Open the **Fragment Options** dialog, select the **Break ring bonds** check box, and then click **OK**. The majority of the fragments can now be matched although the ion at 209 can only be matched if three bonds are allowed to break (the two needed for the precursor molecule plus the loss of the additional oxygen atom).

---

**Note:** The Fragments pane now contains multiple rows for some of the masses, such as 637.1905. Each of these rows corresponds to a different possible fragment (and even more are generated if three bonds are allowed to break). The Peaks tab in the fragments pane only shows the match deemed to be the best based on a combination of the mass accuracy, the number of bonds broken, whether the fragment is a radical, and so on. In this case, the best match corresponds to a fragment that could have been generated for the precursor compound but was not observed, so the additional options shown in the Fragments tab can be useful in suggesting potential pathways that are not obvious.

---

## Summary

In this section, the following tasks have been discussed:

- Inputting a structure as a .mol file and then linking it to a spectrum.
- Selecting parts of the structure and then determining whether there is a corresponding mass peak.
- Generating a fragments pane and then setting the parameters to observe simple fragments.
- Working with the **Fragments** and **Peaks** tabs to show matching compositions, substructures, and mass peaks.
- Modifying the **Fragment Options** to allow more complex fragmentation pathways.
- Adding substructures to a spectrum pane.
- Modifying the structure to explore the fragmentation of related molecules such as metabolites.

In general, it is good practice to start by allowing simple fragmentation processes and additional fragmentation options (additional bonds, ring bonds) as needed to explain observed ions. This is consistent with the fact that ions fragment typically fragment in a series of steps, with simpler fragments forming first, rather than in a concerted step that breaks multiple bonds. Of course a simple fragment might be unstable and immediately fragment further so that it is not observed. In addition, allowing a large number of fragmentation steps requires more processing time and takes longer to complete.

When comparing related molecules, it can be helpful to overlay the reference spectrum (precursor molecule) and the modified form, and then link the view to a structure or fragments pane, which updates as the active spectrum is swapped. However, the coloring applied

to matched and unmatched ions can be hard to distinguish if there are overlays, so it is recommended that you work with single spectra until a familiarity with the program and the views is gained.

## Work with Multiple Samples

Although it is common to work with a single sample, there are occasions when additional information can be gained by comparing or visualizing several at a time. This section illustrates some of the tools available in the software first for two samples and then for multiple samples.

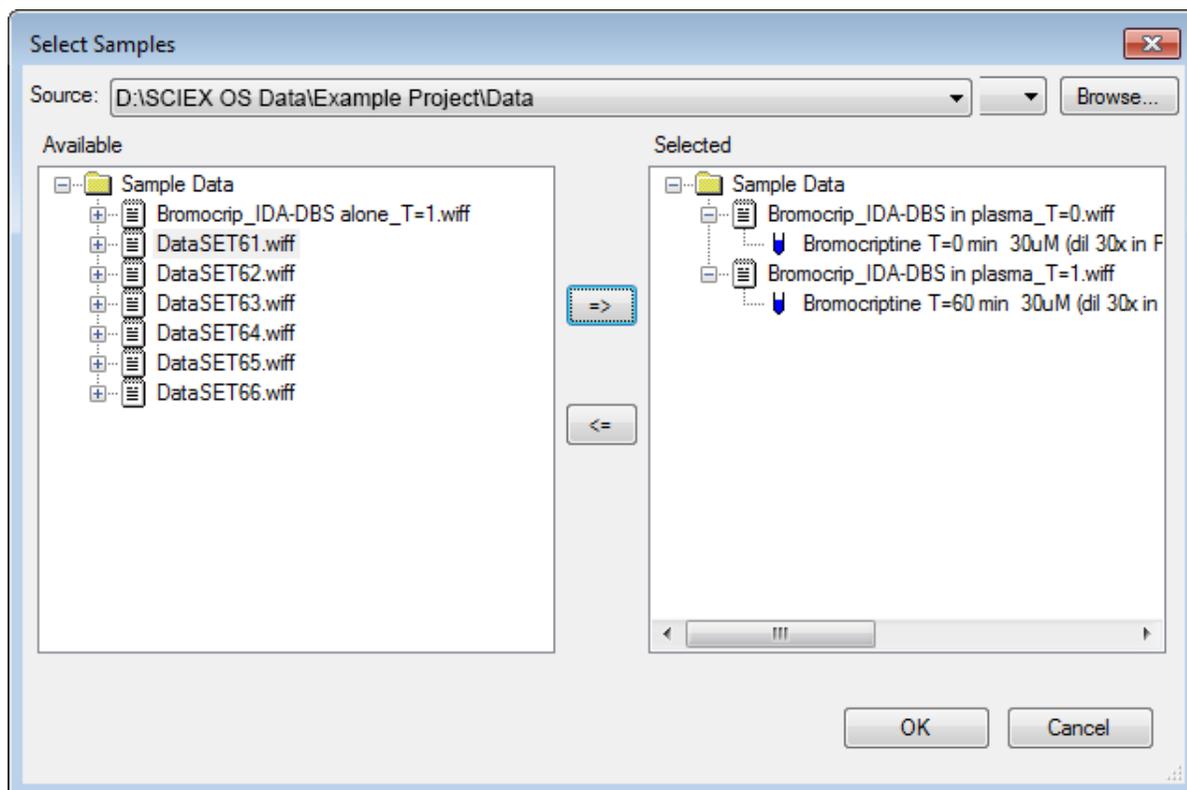
### Work with Two Samples

A common workflow is to compare two samples obtained under different conditions to determine the changes. For example, two different time points following administration of a pharmaceutical drug. The data being compared for this exercise (T = 0 hour and T = 1 hour) is from an incubation of bromocriptine with rat liver microsomes spiked into plasma.

Close all of the open windows before starting.

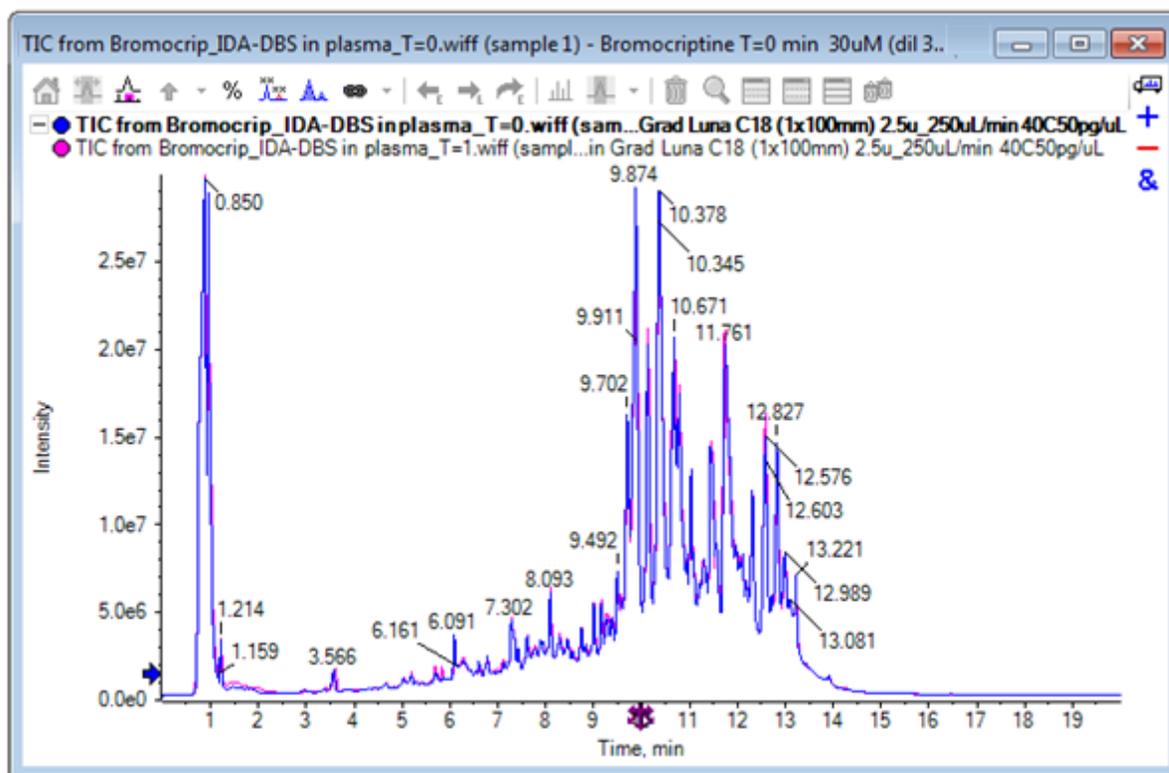
1. Click **File > Open Multiple Samples**, and then browse to the folder containing the sample data.
2. Select the **Bromocrip\_IDA-DBS in plasma\_T=0.wiff** and **Bromocrip\_IDA-DBS in plasma\_T=1.wiff** files and then drag the files to the right side of the window.
3. Click **OK**.

**Figure D-55 Select Multiple Samples**



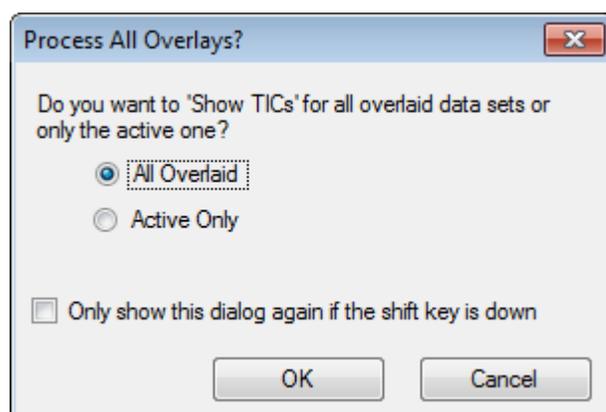
In contrast to opening a single IDA file where separate TICs are shown for the survey and dependent scans, with multiple IDA files a single TIC of all data is shown for all samples. In this case, there are two TICs as shown in [Figure D-56](#).

Figure D-56 TICs



4. Click **Show > Total Ion Chromatogram (TIC)** to open the **Select Experiment** dialog.
5. Select **Period 1, Experiment 1 - TOF MS (100 - 2000)** and then click **OK**.

Figure D-57 Process All Overlays Dialog



The **Process All Overlays** dialog, which is shown whenever overlaid traces are processed, allows users to choose whether to process all of the traces or just the active one.

## Explorer Tutorial

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Processing all of the traces is useful because subsequent operations affect all traces (samples).

6. Select **All Overlaid**.
7. Select the **Only show the dialog again if the shift key is down** check box to make this choice the default action.
8. Click **OK**.

A pane containing overlays of the survey TICs is generated. The chromatography is very reproducible and the metabolite peaks intense so some can be found by zooming and comparing the chromatograms (examine the region around 6 min.), but usually additional work is required. There are a number of ways to generate views that are more easily compared. For this example, a base peak chromatogram is used.

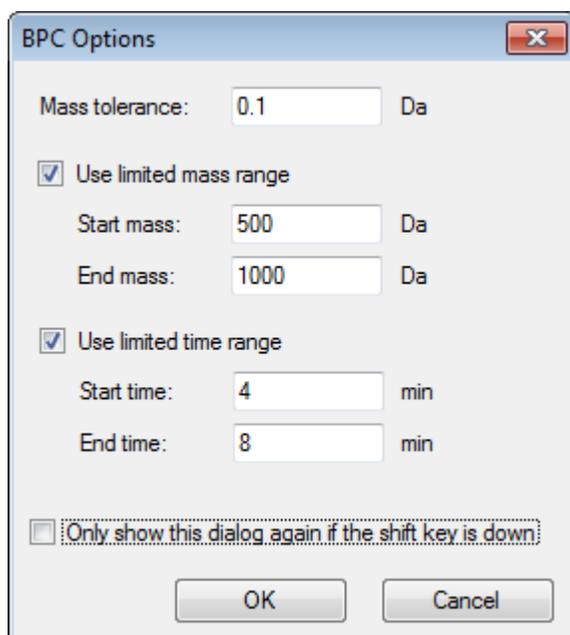
---

**Note:** If **File > Open Heat Map TICs from Wiff** is clicked, then the strip views can be generated directly without showing the overlaid chromatograms first.

---

9. Hide the original TIC pane and then click **Show > Base Peak Chromatogram (BPC)**.
10. In the **BPC Options** dialog, modify the settings, as required, to match the values in [Figure D-58](#) and then click **OK**.

**Figure D-58 BPC Options Dialog**

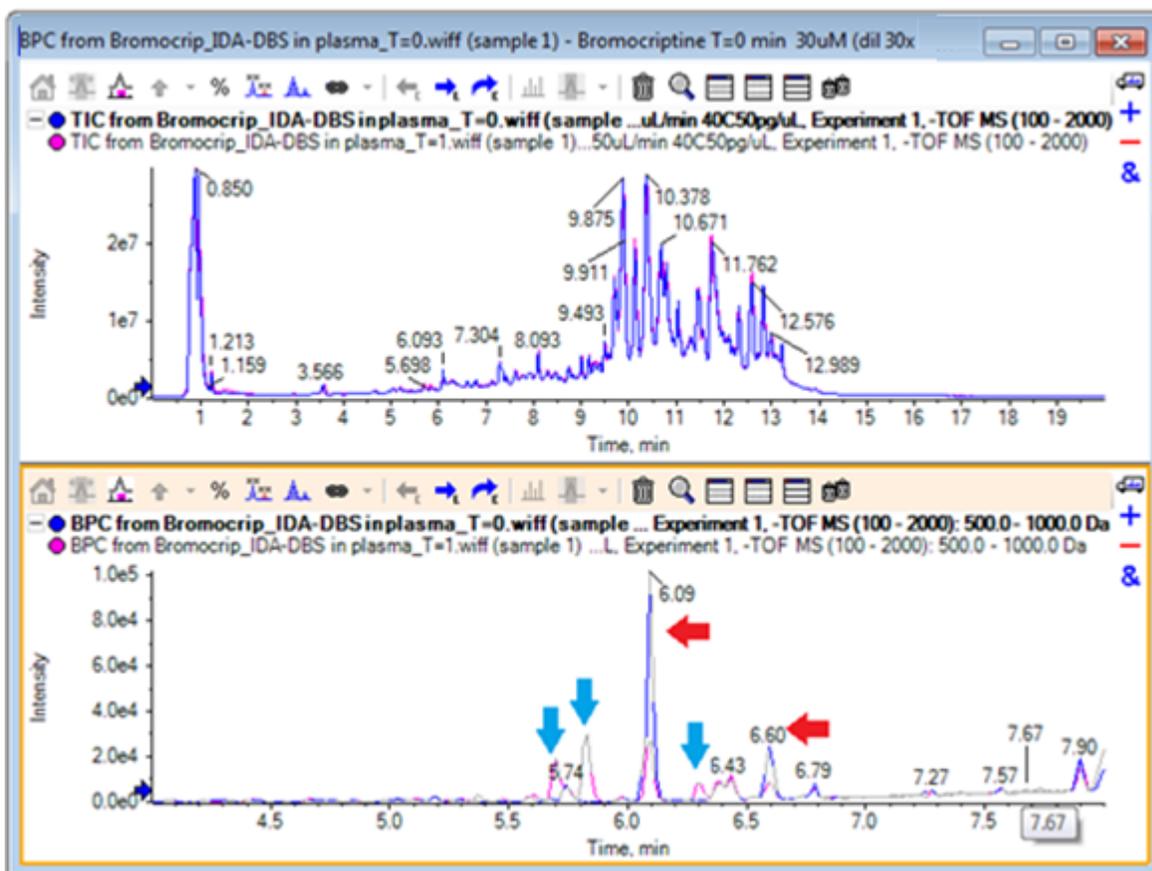


A base peak chromatogram is constructed by plotting the intensity of the largest peak in each scan as a function of retention time. To provide additional information, each trace switches between its normal color and grey when the base peak mass changes by more than the mass tolerance specified in this dialog.

Optionally, you can limit the mass range considered, which can avoid artifacts caused by noisy background peaks for example, and set the retention time range to speed processing. Because we know the mass of bromocriptine is approximately 652, simple metabolites are not below an  $m/z$  ratio of 500.

- In the **Process All Overlays** dialog, make sure that the **All Overlaid** option is selected and then click **OK**.  
A new pane shows the BPC, which is much simpler and easier to compare than the original TICs.

Figure D-59 BPC



There are two peaks (marked with red arrows) that appear to decrease in the 1 hour sample (pink) compared to the T = 0 sample (blue). These correspond to bromocriptine (6.09 min.) and an isomer. There are also three peaks (blue arrows) that are present in the T = 1 sample but not in the T = 0. These are potential metabolites.

**Note:** The BPC can be very useful, but it only reflects the behavior of the most intense ion (in the mass range chosen). Mass peaks that never become the base peak can never be shown so use other tools when looking for differences between samples.

## Explorer Tutorial

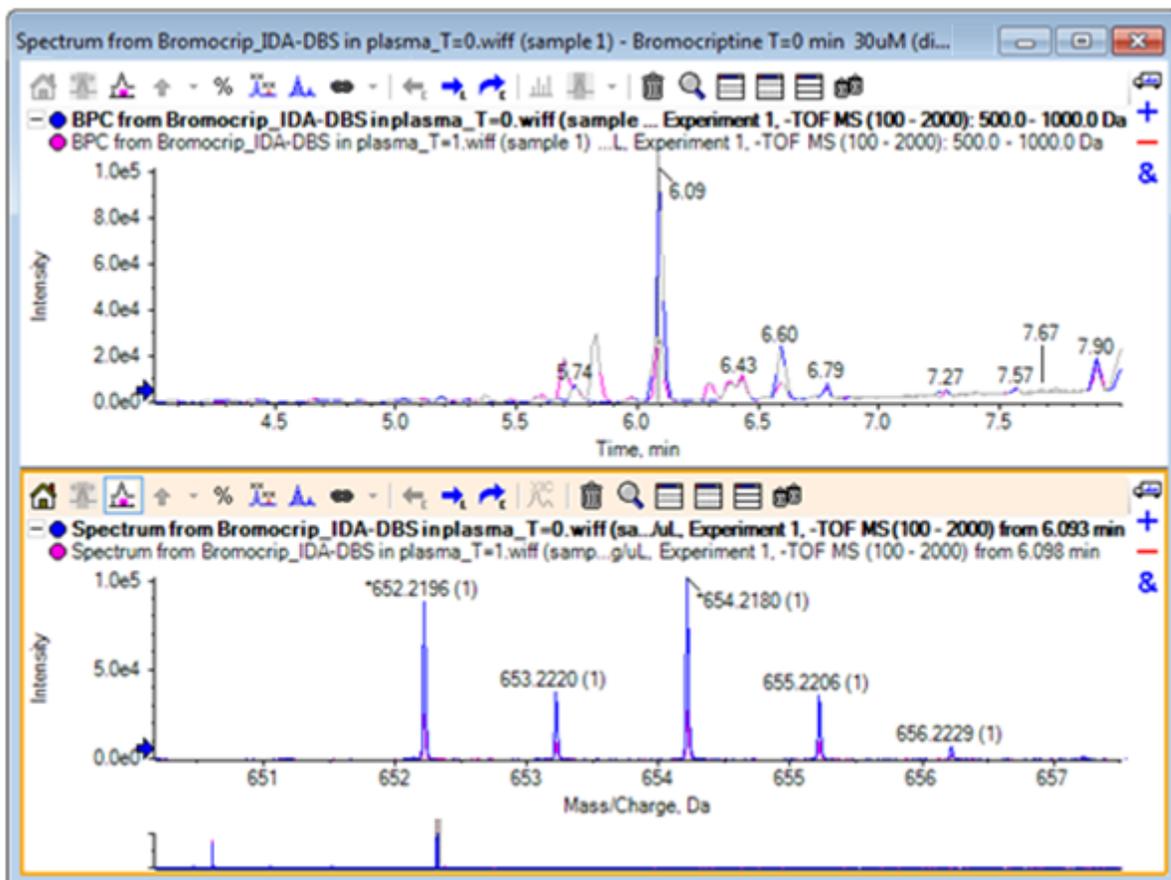
---

- Hide the TIC pane.
- Double-click in the BPC pane at 6.09 min.
- Select **All Overlaid** in the **Process All Overlays** dialog and then click **OK**. This generates two overlaid spectra.
- In the spectrum pane, click and then zoom to show the isotope cluster at around an  $m/z$  ratio of 652. Refer to [Figure D-60](#).

The spectrum pane contains overlaid spectra from the two samples so that they can be easily compared. In this example, it is clear that the intensity in the T = 1 hr sample (pink) is less than in the T = 0 sample.

The overview graph is very valuable when looking at high resolution data like this, because it provides a way to look at the details while keeping the whole spectrum visible.

**Figure D-60 Isotope Cluster at Around an  $m/z$  Ratio of 652**



- In the Chromatogram pane, move the cursor over the line that shows the spectrum time (previously double-clicked).

- When the cursor changes to a double-ended arrow, drag it to the peak at approximately 5.8 min.

The spectrum continues to show the expanded mass range, which now only has noise and small peaks. To show the large pink peaks in the main window, drag the pink rectangle in the overview graph, indicated by a black arrow below. The view renormalizes when the mouse is released.

For [Figure D-62](#), the **Label all overlaid traces** icon was selected.

**Figure D-61 BPC and Spectrum**

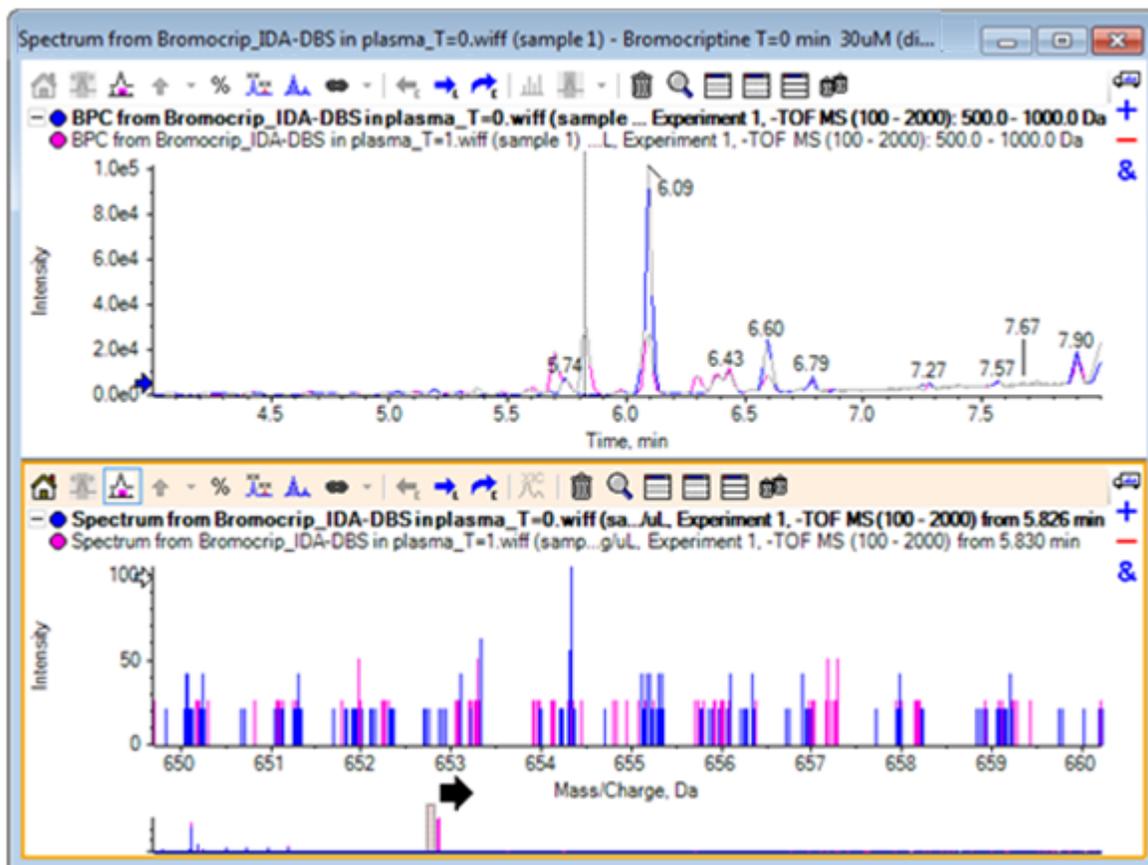
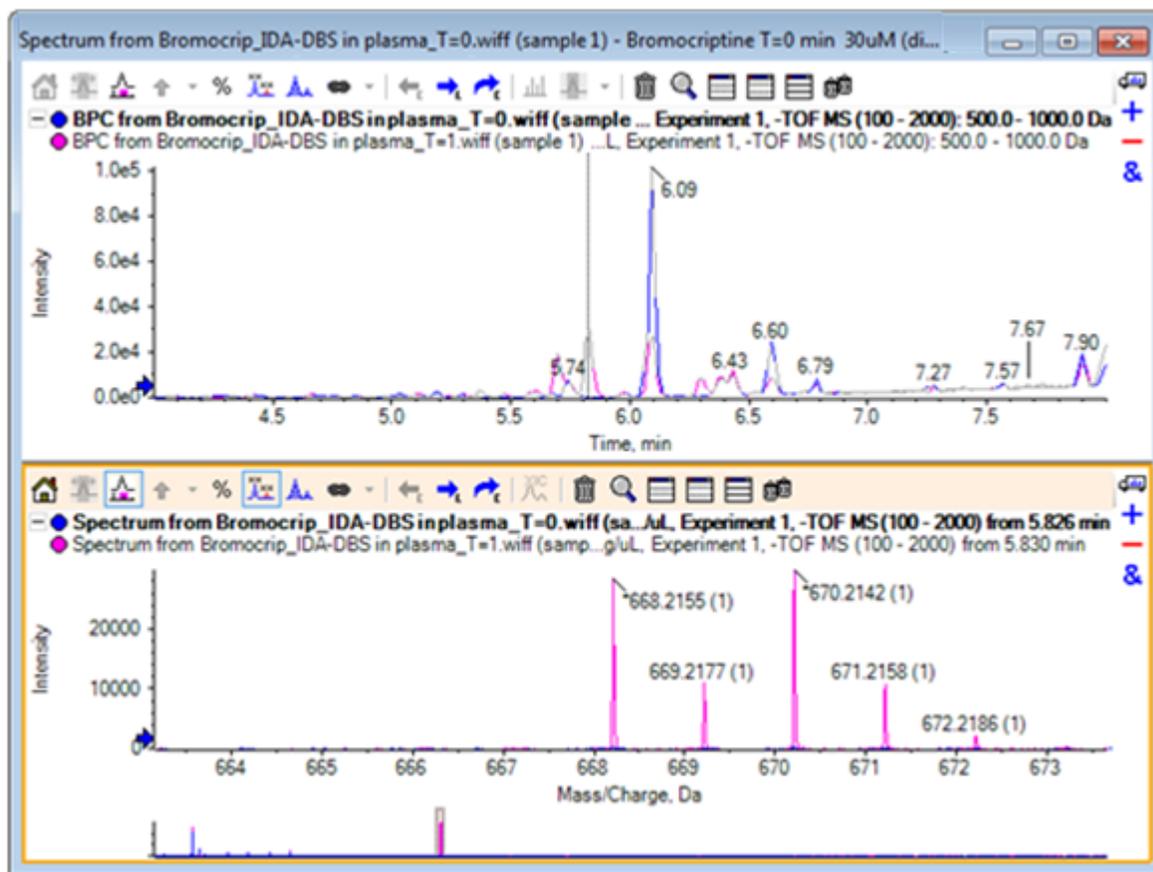


Figure D-62 BPC and Spectrum with Label All Overlaid Traces Option Applied



These peaks are absent from the T = 0 sample.

18. Close all of the windows before continuing.

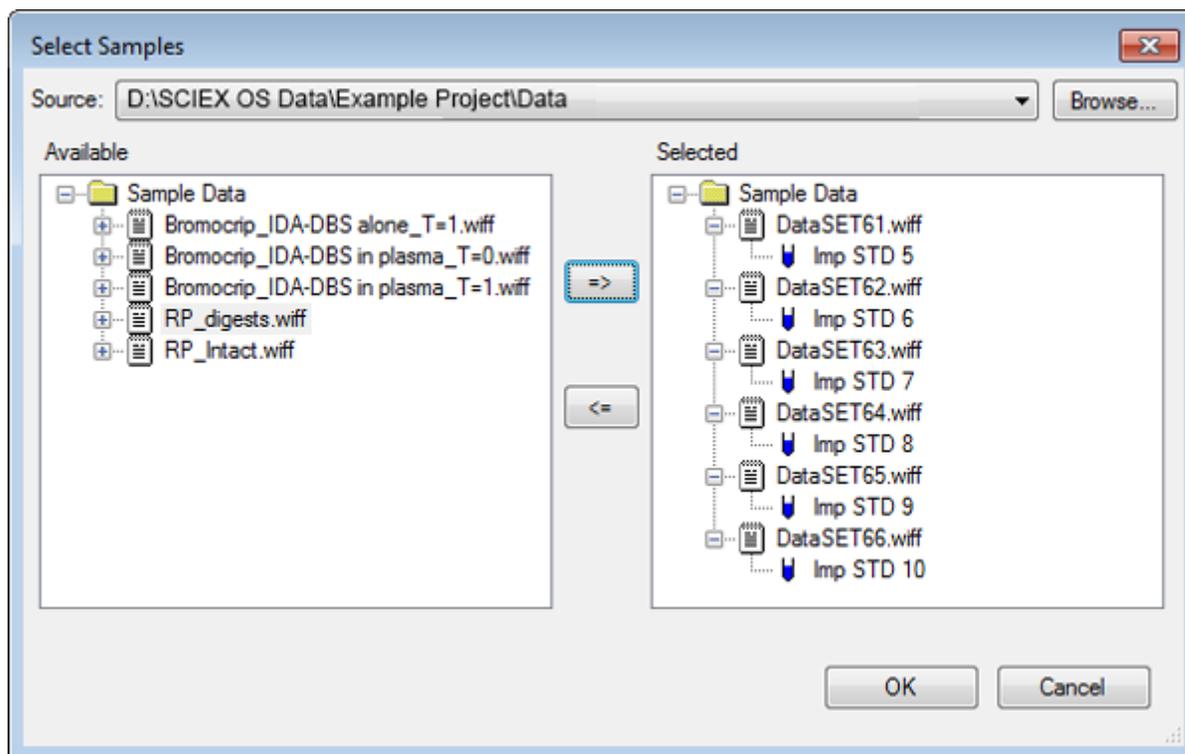
## Work with More than two Samples

With more than two samples overlaid, windows can become confusing and differences harder to associate with the correct sample. The software contains other tools to help show the data from many samples.

The data set used for the example is from an impurity profile analysis from six different data sets.

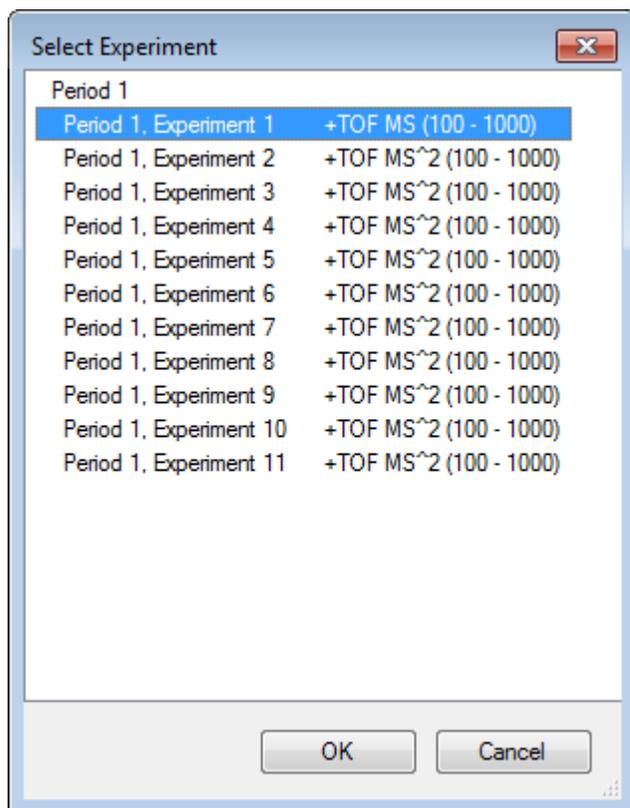
1. Click **File > Open Multiple Samples**.
2. Select the **DataSet61.wiff to DataSet66.wiff** files and then move them to the **Selected** panel.

Figure D-63 Multiple Samples Selected



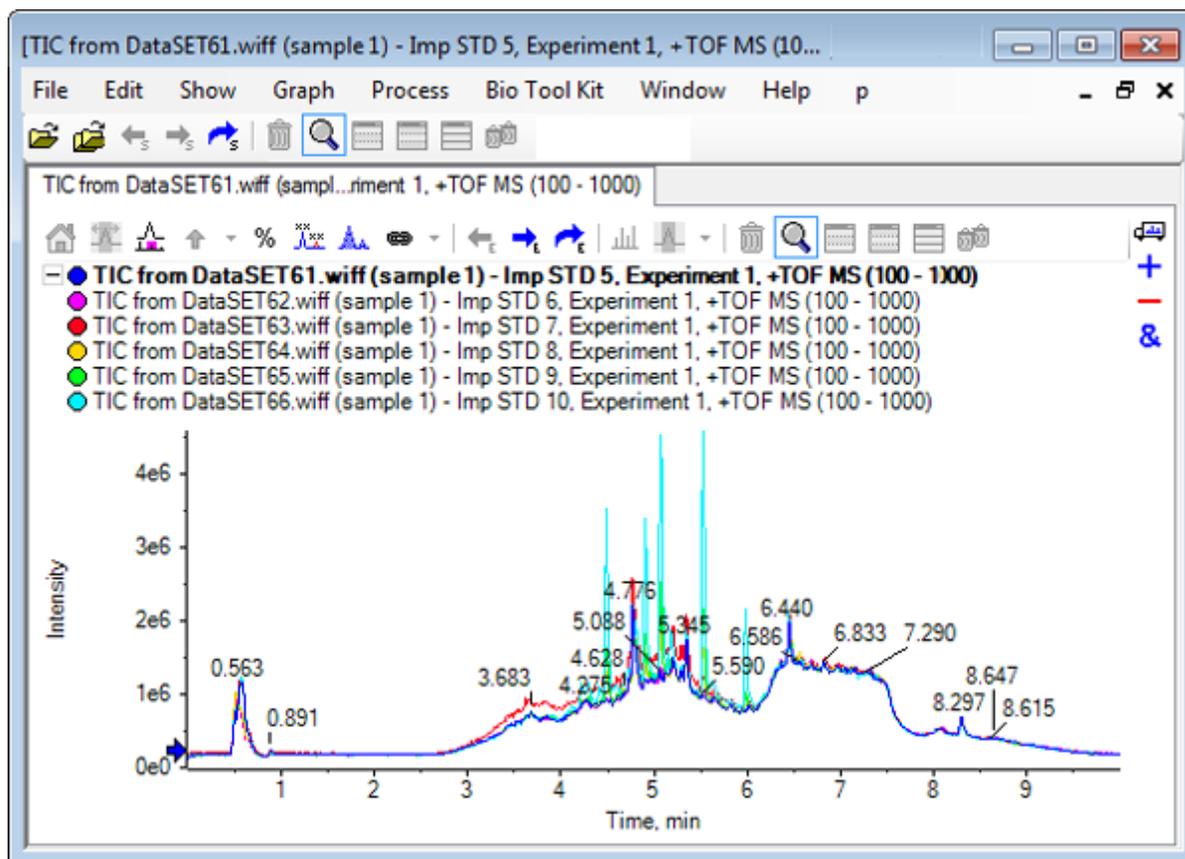
3. Click **OK**.
4. Click **Show > Total Ion Chromatogram (TIC)**.
5. Select **Period 1, Experiment 1** from the **Select Experiment** dialog.

**Figure D-64 Select Experiment Dialog**



6. Click **OK**.
7. In the **Process All Overlays** dialog, select **All Overlaid** and then click **OK**. The graph shows the overlay of a TIC chromatogram for each sample in the file.

Figure D-65 Overlaid TICs from Experiment 1 of DataSet61.wiff through DataSet66.wiff



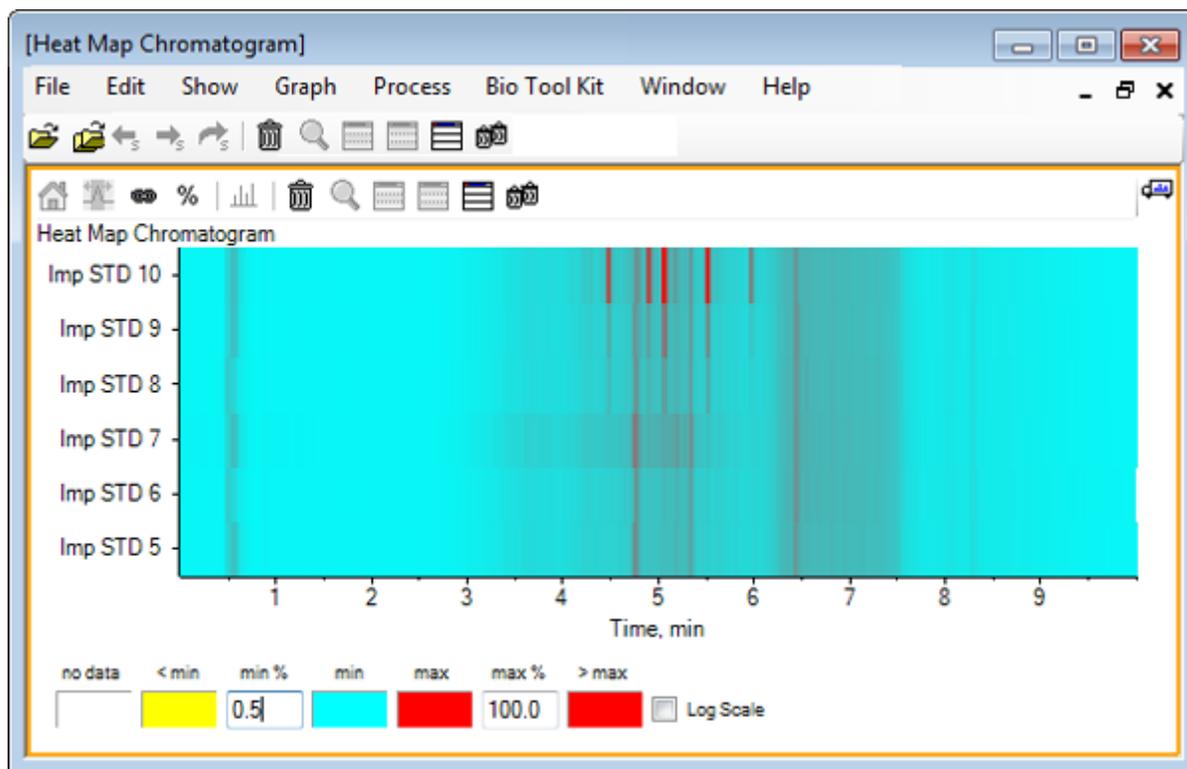
The title of the active trace is shown in a bold font. Clicking the icon to the left of this title collapses the headers to a single line which provides more room for the information.

8. Click **Show > Overlaid Traces as Heat Map** and then in the resulting pane, set the color controls so that **min%** is **0.5**, and **max%** is **100**.

**Tip!** Right-click and then select **Show Appearance Control** if the controls are not visible.

9. Click inside the chromatogram pane and then click the **Hides all other panes** icon.

Figure D-66 Heat Map Chromatogram



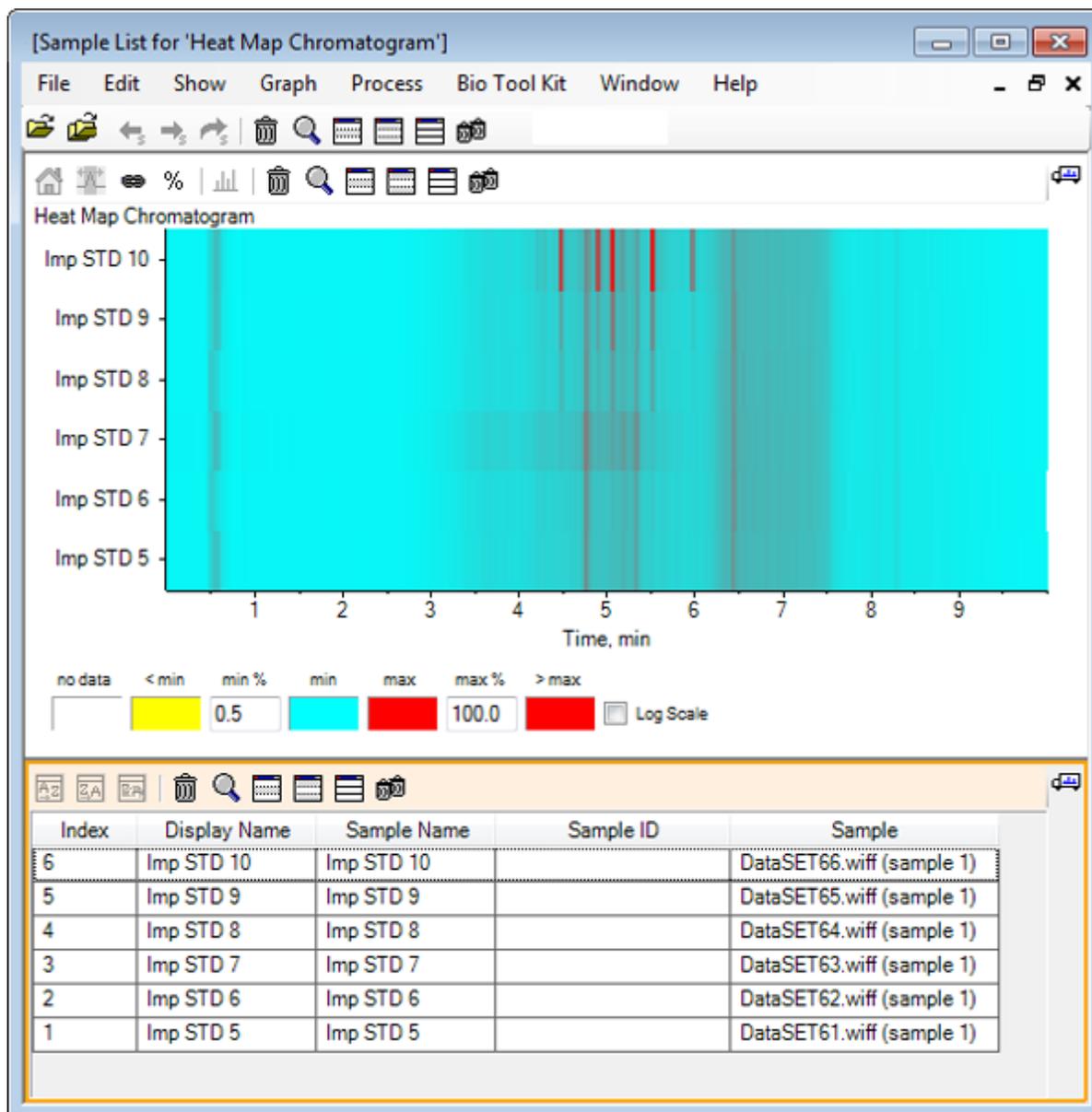
Each sample is represented by a single horizontal strip that shows its TIC, color-coded according to intensity. Using the color scheme above, yellow represents points where no data was acquired or the intensity is less than 0.5% of the largest intensity in any sample, blue represents 0.5%, and red represents the most intense signal.

The window shows six to seven peaks (between 4.5 min and 6.5 min) and that the responses vary except for the peak at 6.5 min.

The order of the peaks is the same as the order in which the samples were acquired and might not be ideal. In this example, the order is fine.

10. Right-click in the pane and then click **Show Samples Table**. Initially the samples table is shown to the right of the heat map. The **Drag and drop to rearrange the panes** icon in the top-right corner of the pane can be used to drag the pane to the bottom of the heat map to move the table below the original pane.

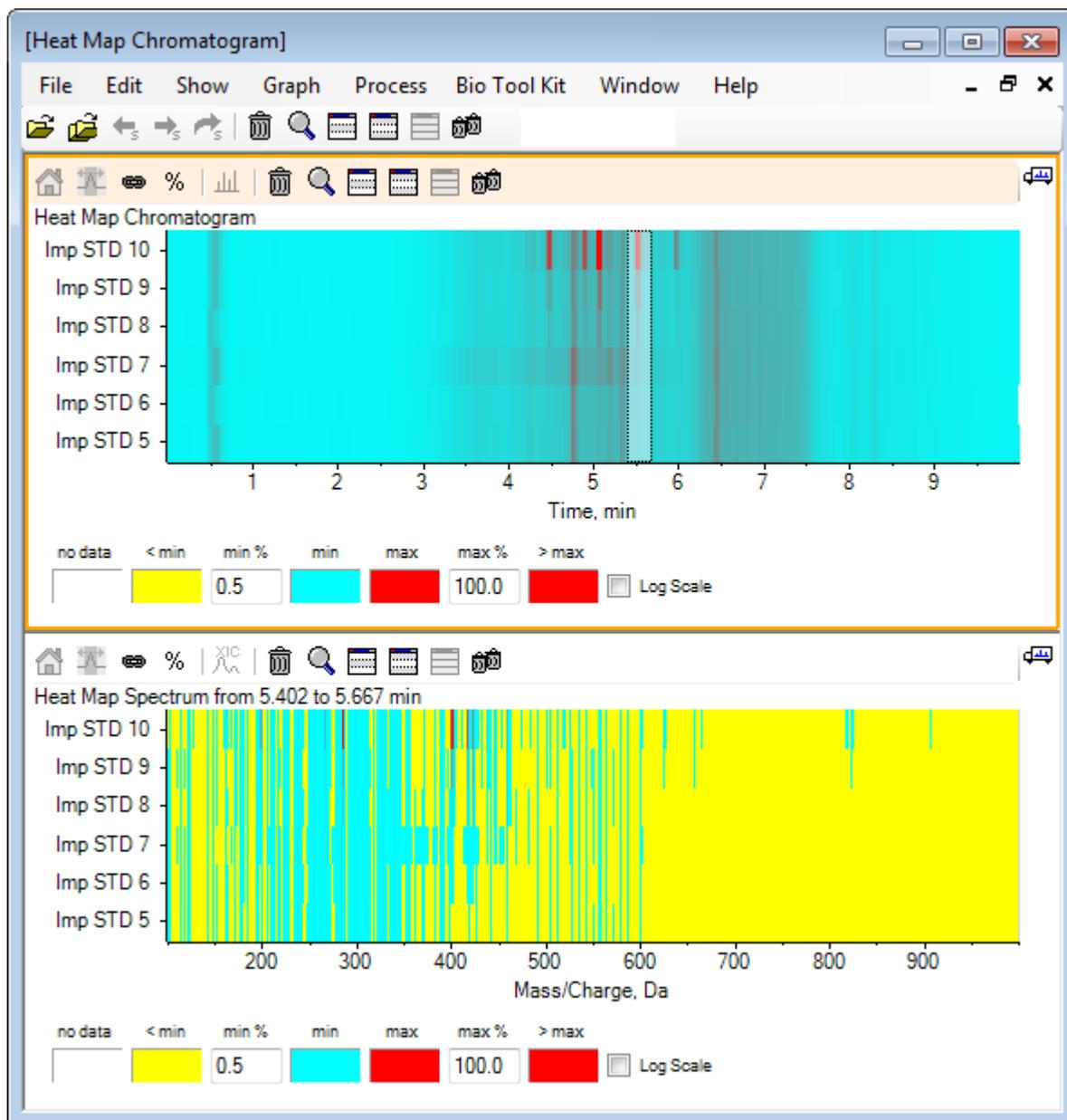
Figure D-67 Sample List for Heat Map Chromatogram



The table contains columns for the various text fields associated with each sample. The **Display Name** column is editable, the others are read-only. All of the columns can be used to sort the table and the sample view.

11. Make a selection in the Imp STD 10 around 5.5 min and then double-click inside it. A new Heat Map Spectrum pane is generated and the complete mass range is shown on the x-axis.

Figure D-68 Heat Map Spectrum

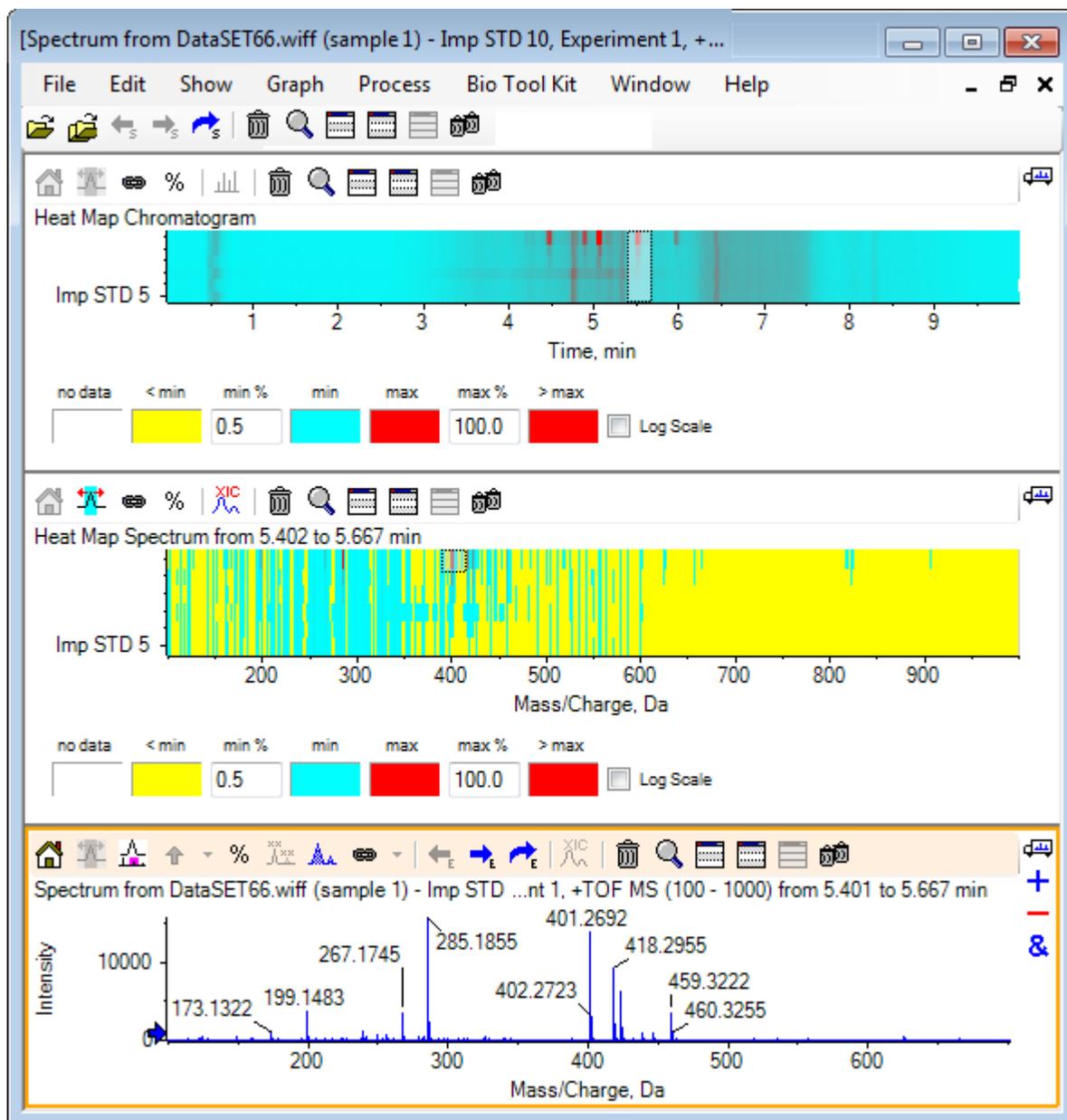


From the spectrum, it can be determined that a couple of masses (between an  $m/z$  of 400 and an  $m/z$  of 460) contribute to the higher intensity at the time region selected.

12. Select the mass around Mass/Charge Da 401 for the Imp STD 10 sample and then right-click to select **Show Spectra for Selected Samples**. This generates a spectrum for that selected sample. The spectrum at that time-point is shown. Refer to [Figure D-69](#).

- Double-click the mass around Mass/Charge Da 401 in the heat map spectrum to generate a heat map XIC.

**Figure D-69 Spectrum**



## Summary

In this section, the following tasks have been discussed:

## Explorer Tutorial

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- Working with the multiple sample tools available in the software.
- Comparing two samples with overlaid chromatograms and interactive spectra.
- Comparing multiple samples with heat map views.

## Work with the Bio Tool Kit Feature

This section illustrates some of the options available under the **Bio Tool Kit** menu item in the software.

**Note:** The Bio Tool Kit MicroApp Feature must be activated to access this functionality. Until activation is completed, the only available options are Peptide Fragments, Add Manual Reconstruct Highlights, and Remove Manual Reconstruct Highlights. Refer to Activate the Bio Tool Kit MicroApp Feature in the *Release Notes* document.

---

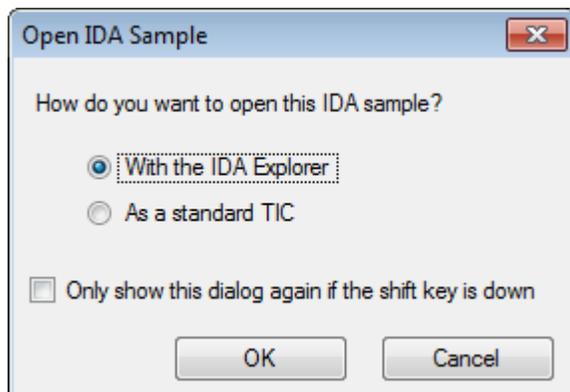
## Manual Sequence

Use this option to manually sequence MS/MS spectral data from a digested protein sample.

1. Click the **Open Sample** icon in the main toolbar.  
The **Select Sample** dialog opens.
2. If the **Sample Data** folder is not already selected, then click **Browse** and navigate to the **Sample Data** folder.
3. Select the **RP\_digests.wiff** file and then click **OK**.

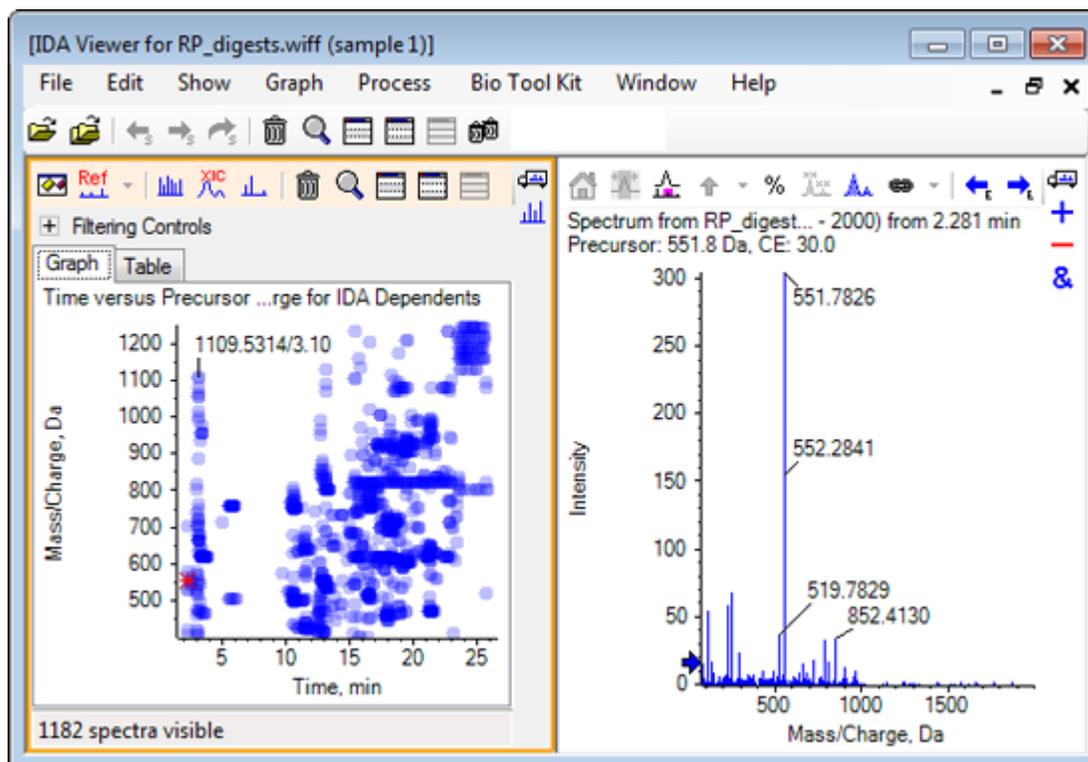
The **Open IDA Sample** dialog opens.

**Figure D-70 Open IDA Sample Dialog**



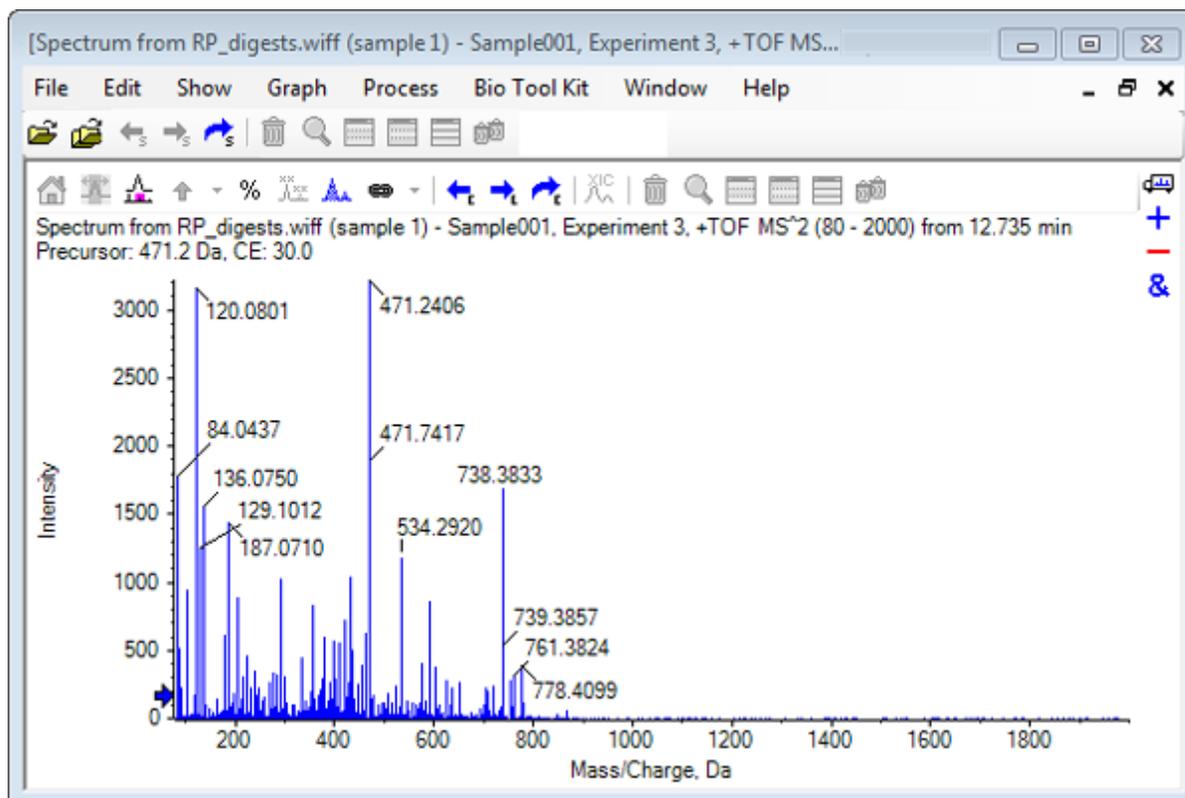
4. Make sure that the **With the IDA Explorer** option is selected and then click **OK**.

Figure D-71 Spectrum from RP\_digests.wiff



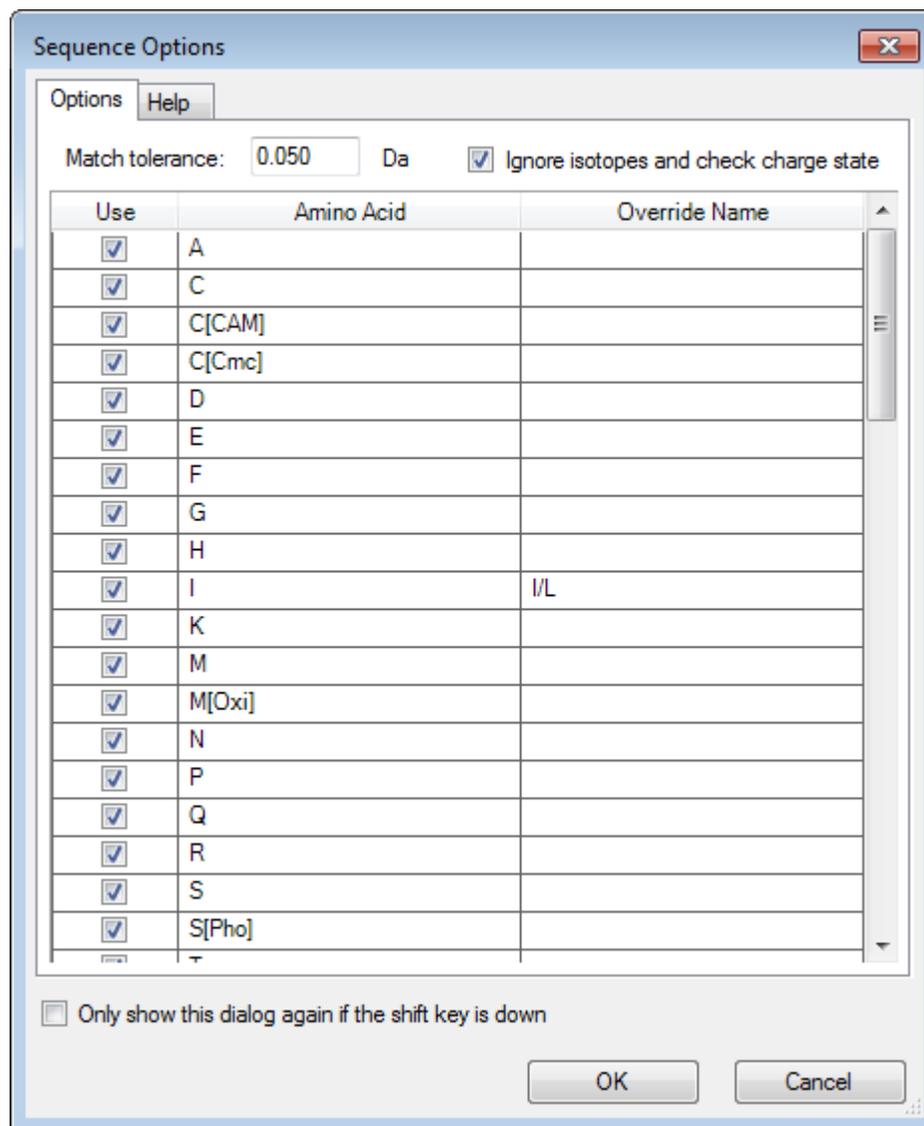
5. Click the **Table** tab.
6. Select  $m/z$  471.2398 at **Time** 12.73.
7. With the Spectrum pane active, click **Graph > Duplicate Graph**.  
A new Spectrum pane for the selected precursor (471.2) opens. The IDA Explorer pane and its associated Spectrum pane can be deleted.

Figure D-72 Spectrum for Precursor 471.2398 at Retention Time 12.73



8. Select the peak labeled **738.3833**.
9. Click **Bio Tool Kit > Manual Sequence**.  
The **Sequence Options** dialog opens.

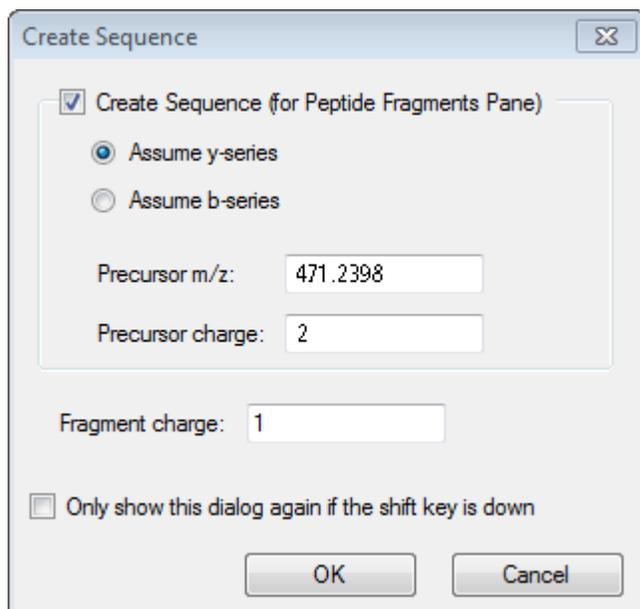
Figure D-73 Sequence Options Dialog



**Note:** When the check box for Ignore isotopes and check charge state is selected, isotopes and any peaks with an incorrect charge state are disregarded by the software when proposing the subsequent amino acid.

10. Click **OK**.  
The **Create Sequence** dialog opens.

**Figure D-74 Create Sequence Dialog**



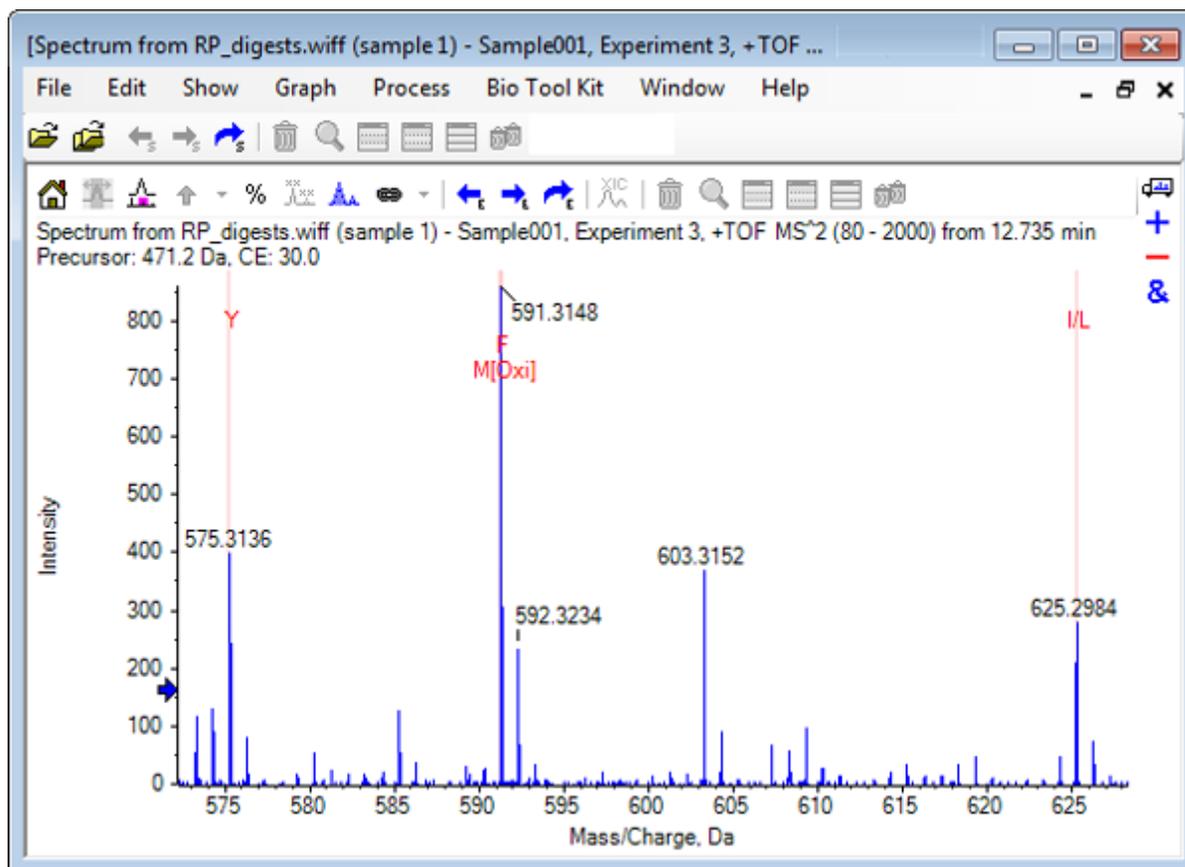
---

**Note:** This dialog enables the user to change the assumptions made for the y- and b- series ions and the charge state after the file is manually sequenced, and to see which assumption gives the best match for the data.

---

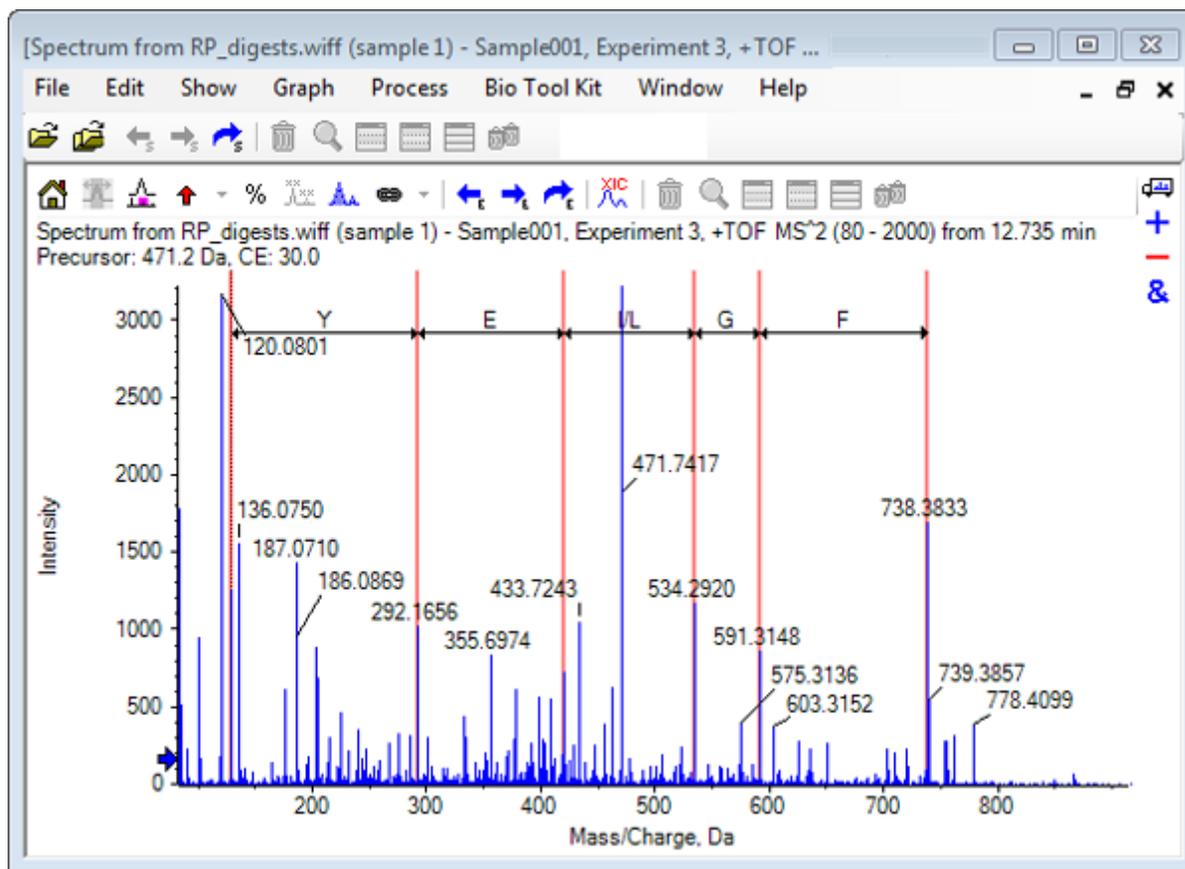
11. Make sure that the **Create Sequence (for Peptide Fragments Pane)** check box is selected.
12. Type **2** in the **Precursor charge** field.
13. Type the charge value of the selected peak to follow in the manual sequence tree in the **Fragment charge** field.
14. Click **OK**.  
The software refreshes, showing an updated Spectrum pane with red vertical lines indicating the first set of possible amino acids gained or lost on the spectral data.

Figure D-75 Manually Sequenced Spectrum — Initial Possibilities



15. Double-click the caption of the red vertical line to be sequenced further.  
The software refreshes, indicating the next set of amino acids on the spectral data.
16. Repeat step 15 until all possible amino acids have been suggested.

**Figure D-76 Manually Sequenced Spectrum**



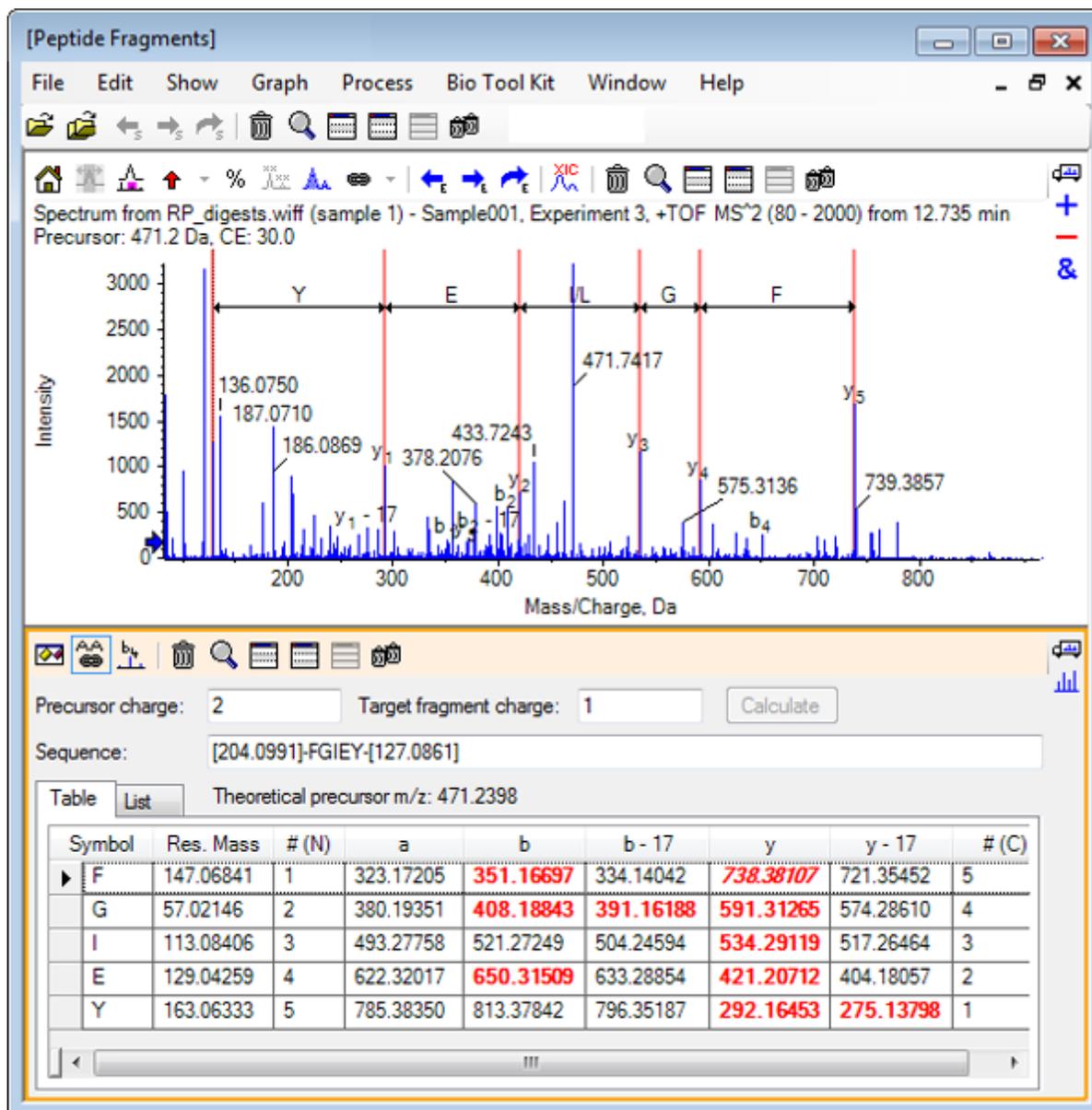
**Note:** In [Figure D-76](#), the captions were clicked in the following order: **F > G > I/L > E > Y**.

**Tip!** If the software suggests more than one possibility and you want to follow a different branch than the one suggested initially, then return the graph to the home view and repeat this procedure selecting an alternate corresponding amino acid label.

## Manual Sequencing Linked with Peptide Fragments

1. Click **Bio Tool Kit > Peptide Fragments**.  
The Peptide Fragments pane opens, linked with the manually sequenced spectrum.

Figure D-77 Peptide Fragments Pane Linked with Manually Sequenced Spectrum



**Note:** Amino acids that match the experimental data are shown in red bolded font in the columns of the Table tab. Amino acids that match the experimental data but have a different target fragment charge are shown in red italicized font in the columns of the Table tab.

2. Click the **List** tab.
3. Click **Show > Mass Calculators**.
4. Click the **AA Property** tab.

Figure D-78 Mass Calculators — AA Property Tab

The screenshot shows the [Peptide Fragments] software interface. The top pane displays a mass spectrum with peaks labeled Y, E, IL, G, and F. The x-axis is Mass/Charge, Da (0 to 800) and the y-axis is Intensity (0 to 3000). Peaks are labeled with m/z values: 84.0437, 136.0750, 433.7243, 471.7417, and y<sub>5</sub>. The bottom pane shows the AA Property tab with the following fields:

- AA sequence: [204.0991]-FGIEY-[127.0861]
- Charge state: 2
- Composition: {204.0991} C31H39N5O8 {127.0861} H2+
- Charged monoisotopic mass: 942.47962
- Monoisotopic m/z: 471.23981
- Charged average mass: 942.880

**Note:** The mass calculators are automatically linked to the manual sequenced spectrum by default. The amino acid sequence from the spectrum is shown in the **AA sequence** field.

- With the Spectrum pane active, click **Bio Tool Kit > Set Sequence Creation Parameters**. The **Create Sequence** dialog opens.

Figure D-79 Create Sequence Dialog

Create Sequence

Create Sequence (for Peptide Fragments Pane)

Assume y-series

Assume b-series

Precursor m/z: 471.2398

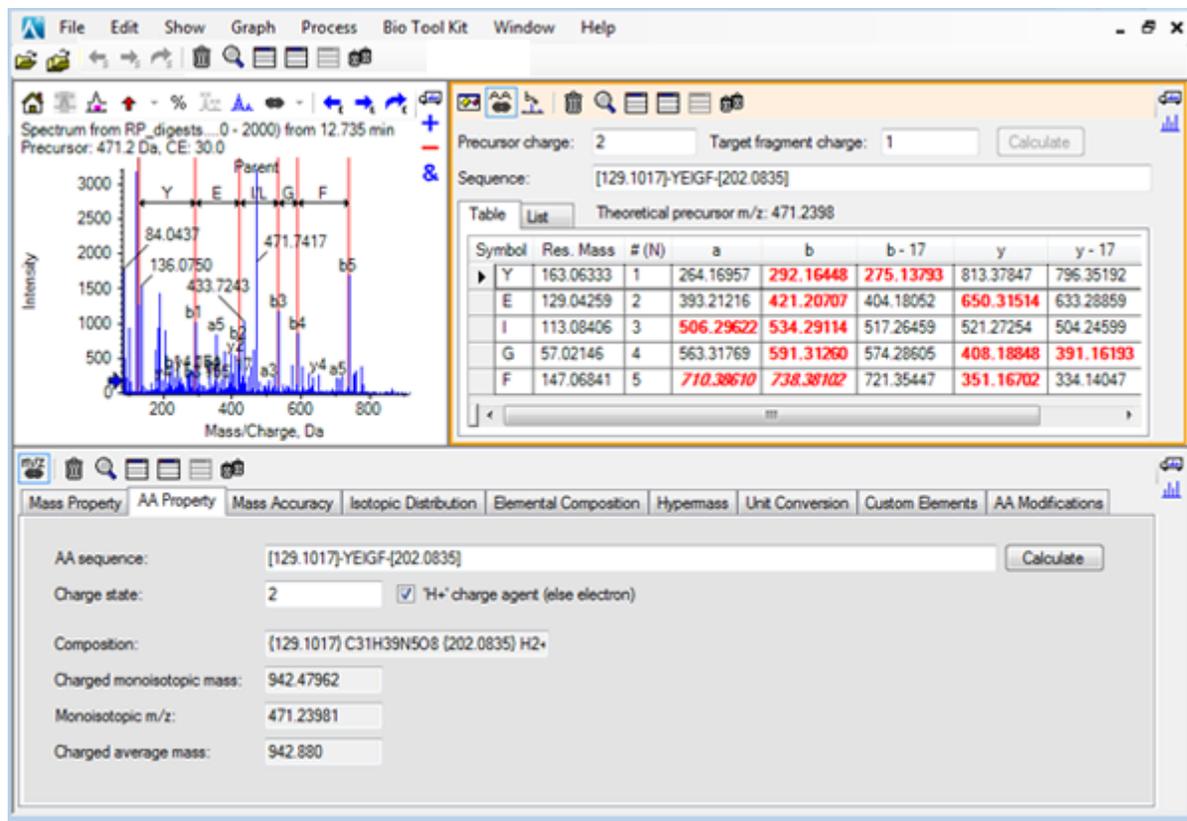
Precursor charge: 2

Fragment charge: 1

OK Cancel

6. Complete the **Create Sequence** dialog as follows:
  - Make sure that the **Create Sequence (for Peptide Fragments Pane)** check box is selected.
  - Select the **Assume b-series** option.
  - Type **471.2398** in the **Precursor m/z** field.
  - Type **2** in the **Precursor charge** field.
  - Type **1** in the **Fragment charge** field.
7. Click **OK**.  
The Peptide Fragments pane and the Mass Calculators pane refresh with the updated sequence data.
8. Click the **Table** tab in the **Peptide Fragments** pane.

**Figure D-80 Refreshed Peptide Fragments Pane Linked with Manually Sequenced Spectrum**



9. With the Spectrum pane active, click **Bio Tool Kit > Clear Manual Sequencing**. All of the manual sequence markings are removed.

## Add and Remove Manual Reconstruct Highlights

Use the **Add Manual Reconstruct Highlights** option to add markers indicating the theoretical  $m/z$  positions of a given mass to a spectrum. This feature is useful for confirming whether or not particular peaks in a spectrum correspond to the same component when spectra contains multiply-charged components. Use the **Remove Manual Reconstruct Highlights** option to remove the markers.

---

**Tip!** Drag the vertical line for the marker to a new  $m/z$  value to move the marker to a new location.

---

**Tip!** Click the vertical line for the marker or the corresponding charge state label to make the marker active. The active marker shows the  $m/z$  location.

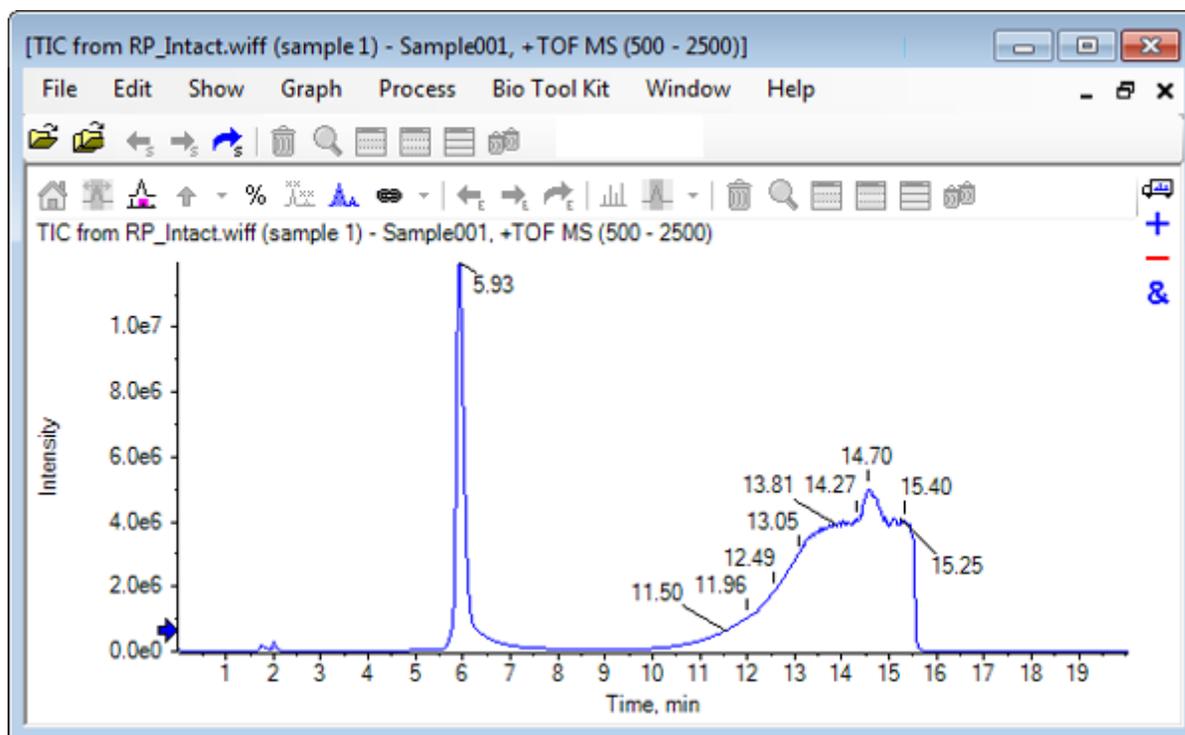
---

1. Click the **Open Sample** icon in the main toolbar.

The Select Sample dialog opens.

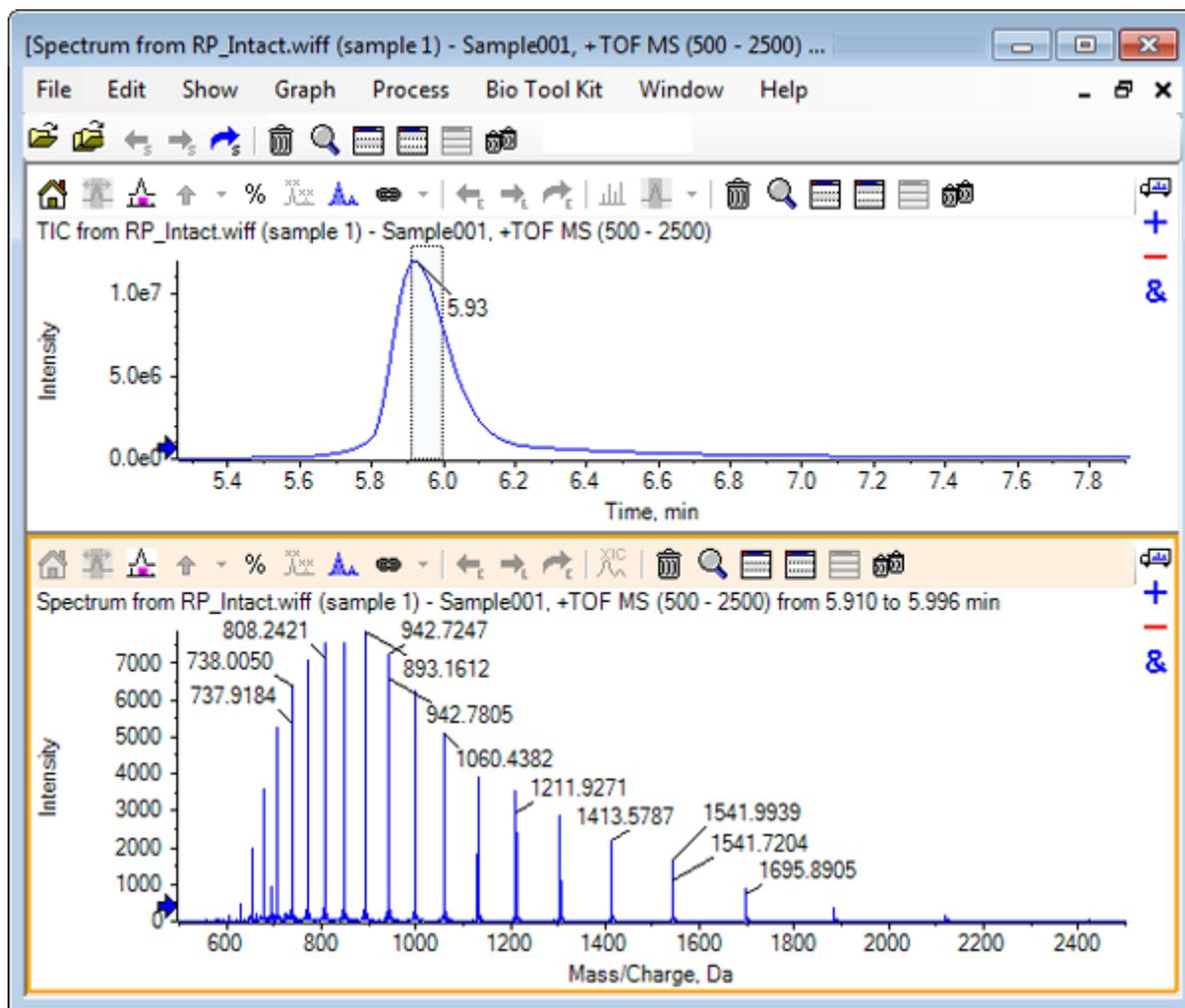
2. If the **Sample Data** folder is not already selected, then click **Browse** and navigate to the **Sample Data** folder.
3. Select the **RP\_Intact.wiff** file and then click **OK**.

**Figure D-81 TIC from RP\_Intact.wiff File**



4. Create an averaged spectrum using the top region (5.91 to 6.00 min) of the peak for myoglobin.

Figure D-82 Averaged Spectrum

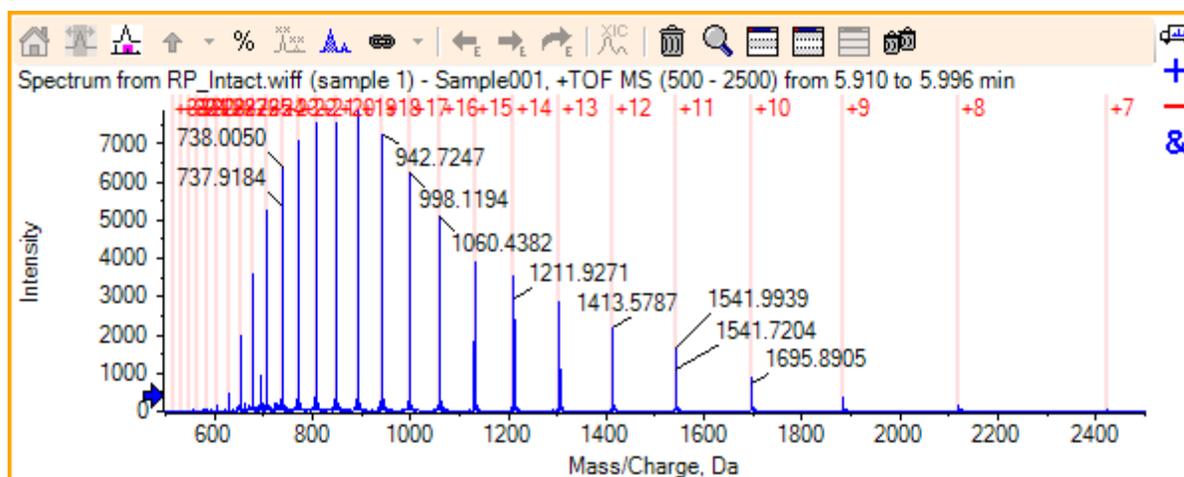


5. With the Spectrum pane active, click **Bio Tool Kit > Add Manual Reconstruct Highlights**. The **Add Manual Reconstruct Highlights to Graph** dialog opens.

Figure D-83 Add Manual Reconstruct Highlights to Graph

6. Type **16950** in the **Value** field.
7. Select **H+** as the **Charge agent** and then click **OK**.  
The graph refreshes, containing the highlights.

Figure D-84 Spectrum with Added Highlights



8. Click **Bio Tool Kit > Remove Manual Reconstruct Highlights** to remove the markers.  
The graph refreshes, with the highlights removed.

## Digest Protein

Use this option to obtain information on theoretical peptide sequences that result from a user-defined enzymatic cleavage of a specified protein.

### Toolbar

Use the icons in the toolbar to adjust the view, as required.

**Table D-5 Toolbar Icons**

Icon	Name (Tooltip)
	Find and replace in sequence
	Convert selection to uppercase
	Find sequence

---

**Note:** The final six icons in this toolbar, beginning with the Deletes this pane icon, are described in [Generic Pane Toolbar](#).

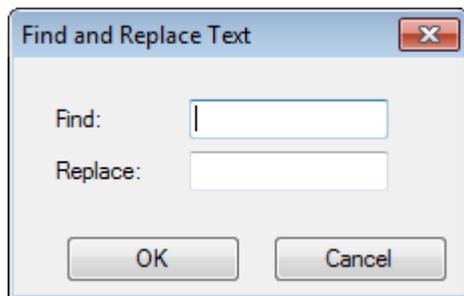
---

### Find and Replace in Sequence

Use this option to find existing text in the **Sequence** field and replace it with the new text.

1. Click the **Find and replace in sequence** icon.  
The **Find and Replace Text** dialog opens.

**Figure D-85 Find and Replace Text Dialog**



2. Type the information to be replaced in the **Find** field.
3. Type the appropriate information in the **Replace** field.
4. Click **OK**.  
The software replaces the existing text with user-specified replacement text.

### Convert Selection to Uppercase

Use this option to convert text typed in the **Sequence** field in lowercase font to uppercase font.

1. Select the appropriate text.

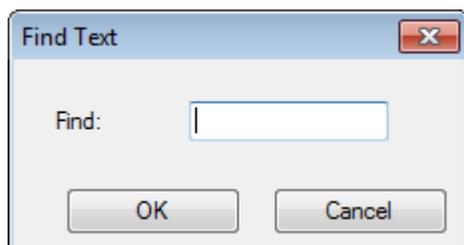
2. Click the **Convert selection to uppercase** icon.  
The software replaces the text in lowercase font with the same text in uppercase font.

### Find Sequence

Use this option to find text in the **Sequence** field.

1. Click the **Find sequence** icon.  
The **Find Text** dialog opens.

**Figure D-86 Find Text Dialog**

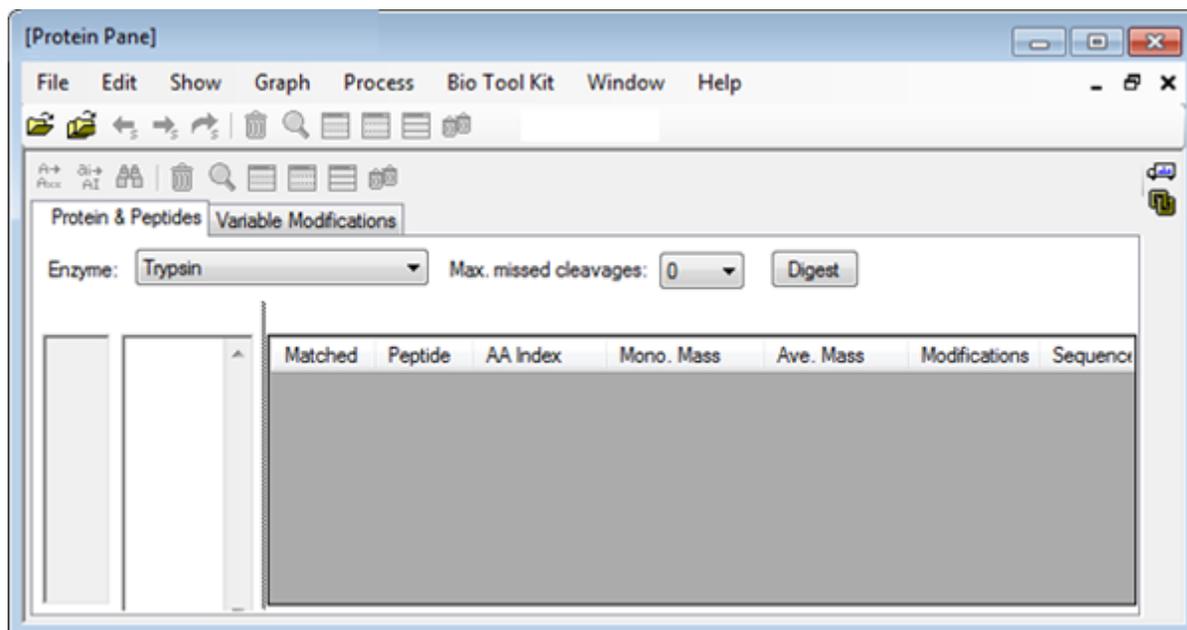


2. Type the appropriate information in the **Find** field.
3. Click **OK**.  
The software highlights the matching text.

### Theoretical Protein Digestion

1. Click **Bio Tool Kit > Digest Protein**.  
The **Protein** pane opens.

**Figure D-87 Protein Pane - Protein & Peptides Tab**



2. Type a protein or peptide sequence in the field provided.

---

**Note:** For this tutorial, GLSDGEWQQV LNVWGKVEAD IAGHGQEVLI RLFTGHPETL EKFDKFKHLK TEAEMKASED LKKHGTVVL ALGGILKKKG HHEAELKPLA QSHATKHKIP IKYLEFISDA IIVLHSHKHP GDFGADAQGA MTKALELFRN DIAAKYKELG FQG (myoglobin sequence) was used.

---

3. Select an **Enzyme**.

---

**Note:** For this tutorial, Trypsin was selected.

---

4. Select the **Max. missed cleavages**.

---

**Note:** For this tutorial, 0 was selected.

---

5. Click **Digest**.

The software populates the table with theoretical information on the digested peptides and their sequences.

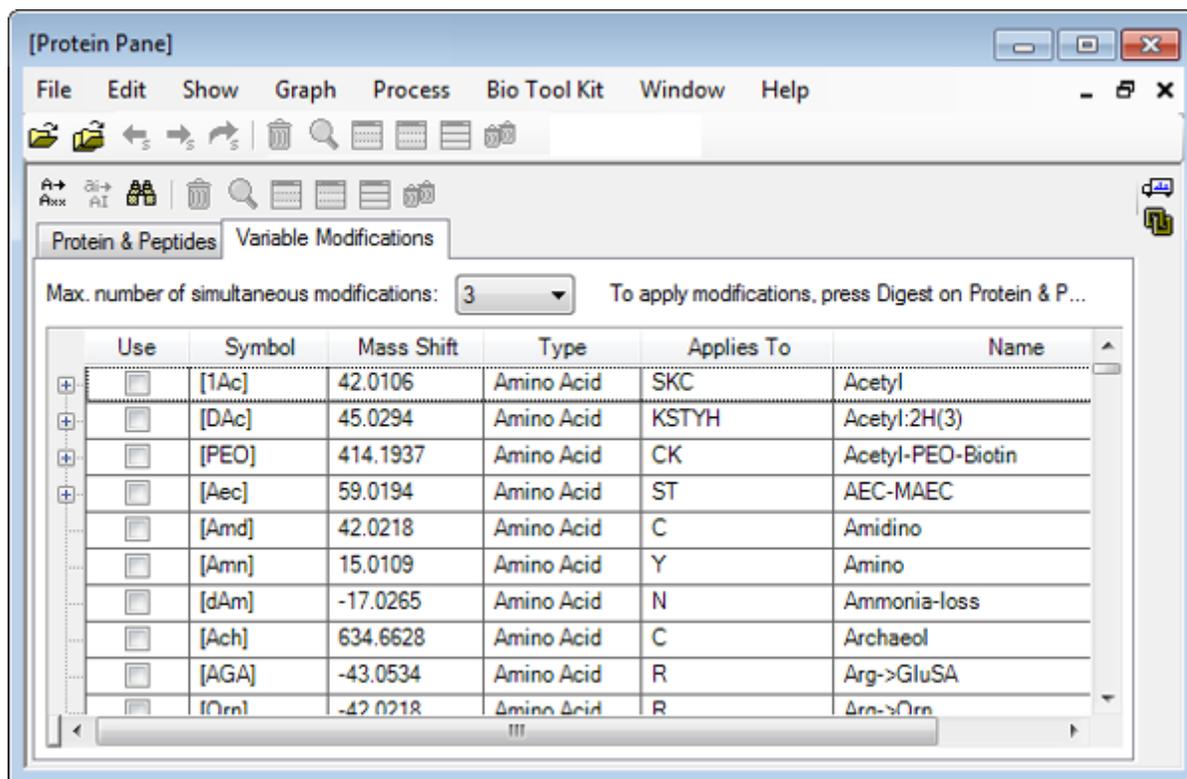
Figure D-88 Protein Pane Populated with Theoretical Information

The screenshot shows the 'Protein Pane' window with the 'Variable Modifications' tab selected. The 'Enzyme' is set to 'Trypsin' and 'Max. missed cleavages' is set to '0'. The 'AA selection' is '(None)'. On the left, a list of theoretical peptides is shown with their corresponding amino acid positions (1-151). The main table displays 21 matched peptides (T1-T21) with columns for 'Matched', 'Peptide', 'AA Index', 'Mono. Mass', 'Ave. Mass', 'Modifications', and 'Sequence'.

Matched	Peptide	AA Index	Mono. Mass	Ave. Mass	Modifications	Sequence
<input checked="" type="checkbox"/>	T1	1 - 16	1814.89515	1816.004		GLSDGEWQ...
<input checked="" type="checkbox"/>	T2	17 - 31	1605.84747	1606.799		VEADIAGHG...
<input checked="" type="checkbox"/>	T3	32 - 42	1270.65575	1271.436		LFTGHPETLEK
<input checked="" type="checkbox"/>	T4	43 - 45	408.20088	408.455		FDK
<input checked="" type="checkbox"/>	T5	46 - 47	293.17394	293.366		FK
<input checked="" type="checkbox"/>	T6	48 - 50	396.24850	396.490		HLK
<input checked="" type="checkbox"/>	T7	51 - 56	707.31599	707.801		TEAEMK
<input checked="" type="checkbox"/>	T8	57 - 62	661.32827	661.710		ASEDLK
<input checked="" type="checkbox"/>	T9	63	146.10553	146.189		K
<input checked="" type="checkbox"/>	T10	64 - 77	1377.83439	1378.679		HGTVLTAL...
<input checked="" type="checkbox"/>	T11	78	146.10553	146.189		K
<input checked="" type="checkbox"/>	T12	79	146.10553	146.189		K
<input checked="" type="checkbox"/>	T13	80 - 96	1852.95440	1854.056		GHHEAELKP...
<input checked="" type="checkbox"/>	T14	97 - 98	283.16444	283.331		HK
<input checked="" type="checkbox"/>	T15	99 - 102	469.32642	469.625		IPIK
<input checked="" type="checkbox"/>	T16	103 - 118	1884.01454	1885.194		YLEFISDAI...
<input checked="" type="checkbox"/>	T17	119 - 133	1501.66198	1502.626		HPGDFGADA...
<input checked="" type="checkbox"/>	T18	134 - 139	747.42793	747.893		ALELFR
<input checked="" type="checkbox"/>	T19	140 - 145	630.33369	630.699		NDIAAK
<input checked="" type="checkbox"/>	T20	146 - 147	309.16886	309.365		YK
<input checked="" type="checkbox"/>	T21	148 - 153	649.30714	649.701		ELGFQG

- Click the **Variable Modifications** tab.

Figure D-89 Protein Pane - Variable Modifications Tab



7. Select a **Max. number of simultaneous modifications**.

---

**Note:** For this tutorial, 3 was selected.

---

8. Select the check box in the **Use** column for the appropriate modifications.

---

**Tip!** If an icon is shown to the left of the check box, then the entire list of amino acids can be selected or just those that are applicable.

---

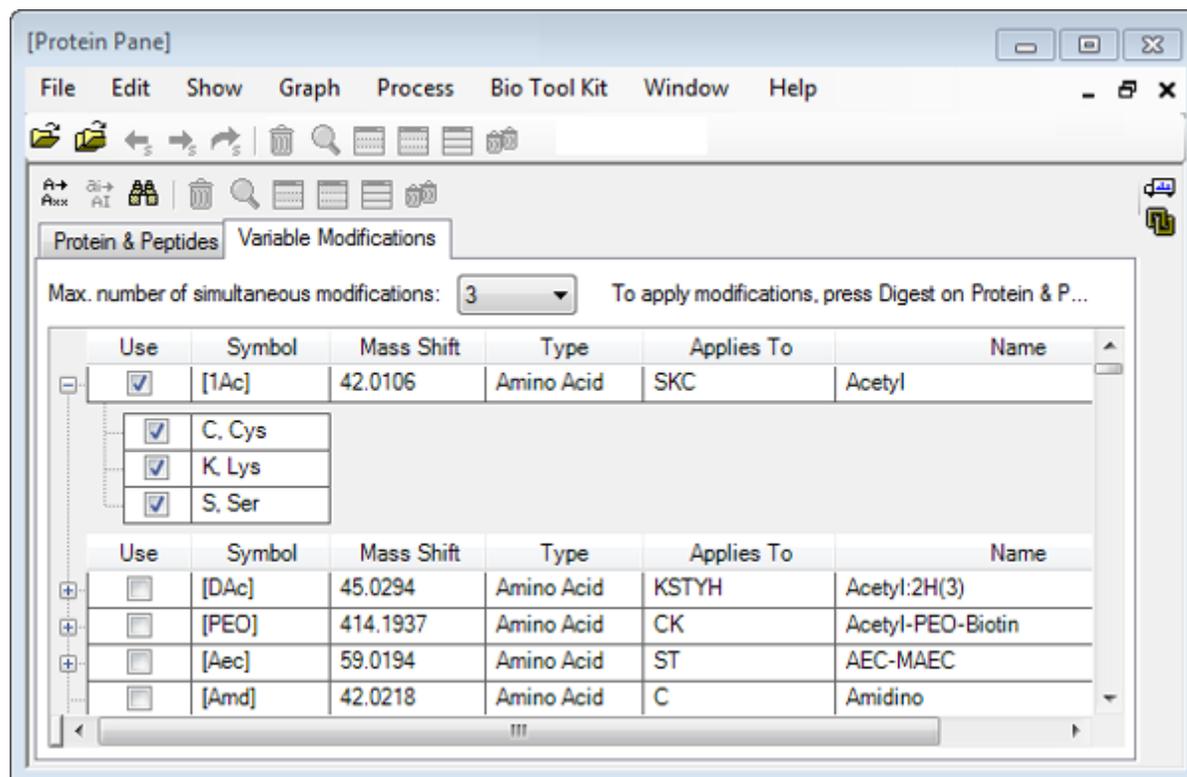


---

**Note:** For this tutorial, the check box for [1Ac] was selected.

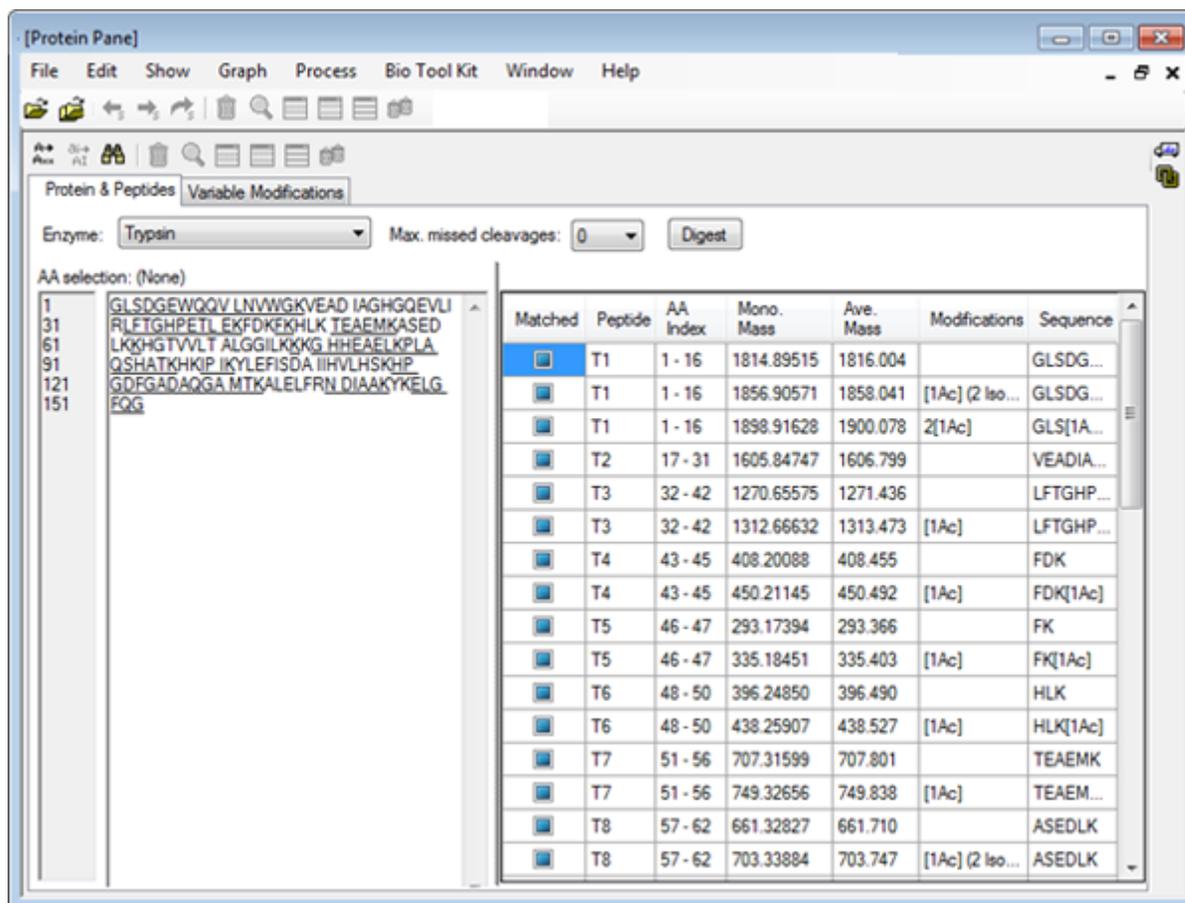
---

Figure D-90 Example of Selected Modifications



9. Click the **Protein & Peptides** tab.
10. Click **Digest**.  
The results in the table are modified to reflect the selections made by the user.

Figure D-91 Protein Pane Populated with Modified Information

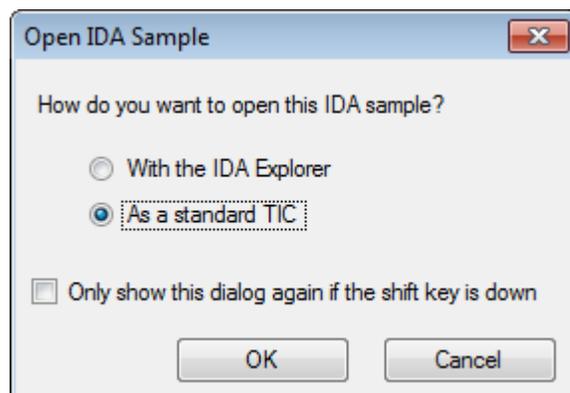


## LCMS Peptide Reconstruct

LCMS peptide reconstruction identifies spectral peaks and performs deconvolution from the identified spectral peaks. The LCMS Peptide Reconstruct tool works in two steps. First, peaks are found using the 'Enhance' peak finding algorithm. Second, the tool finds groups of peaks that form isotope series and charge series and reports the neutral mass of all of the components found.

1. Click the **Open Sample** icon in the main toolbar. The **Select Sample** dialog opens.
2. If the Sample Data folder is not already selected, then click **Browse** and navigate to the **Sample Data** folder.
3. Select the **RP\_digests.wiff** file and then click **OK**.  
The **Open IDA Sample** dialog opens.

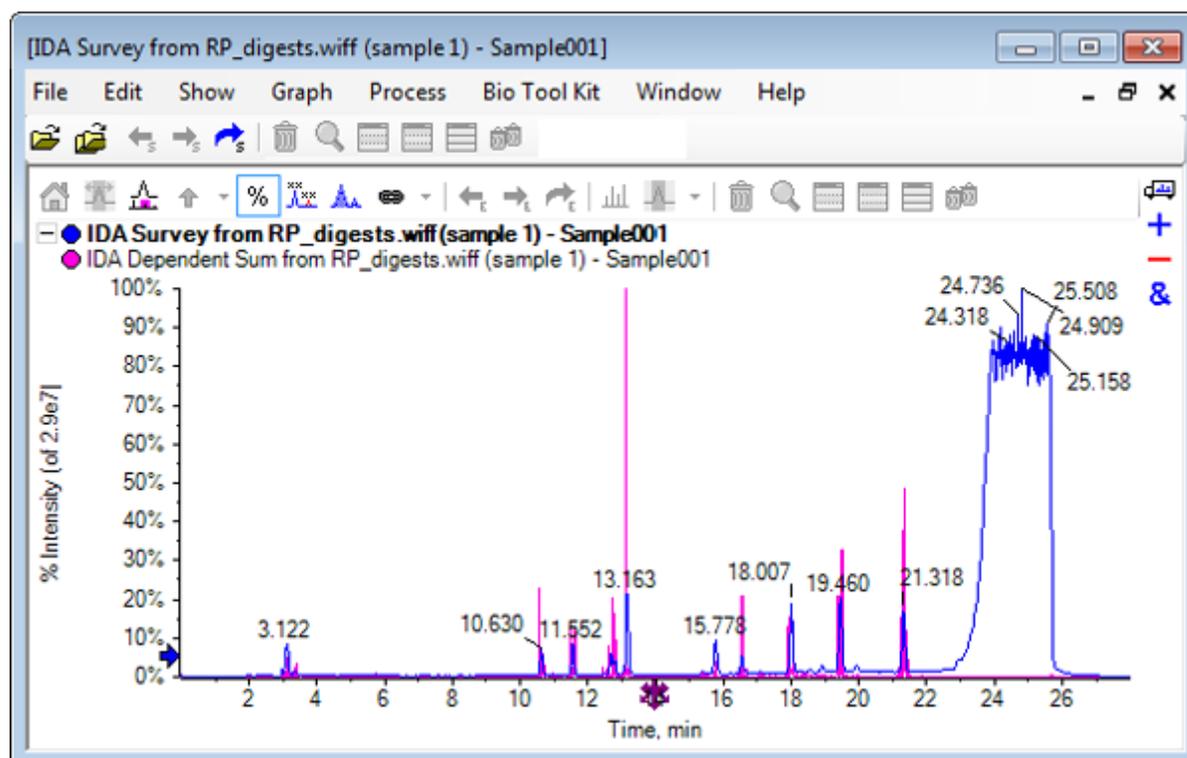
Figure D-92 Open IDA Sample Dialog



4. Make sure that the **As a standard TIC** option is selected and then click **OK**.

Make sure that the first trace, **IDA Survey from RP\_digests.wiff (sample 1) - Sample001**, is shown in bold. If required, select this trace.

Figure D-93 IDA Survey from RP\_digests.wiff



5. Click **Bio Tool Kit > LCMS Peptide Reconstruct (with peak finding)**.  
The **LCMS Peptide Reconstruct Options** dialog opens.

**Figure D-94 LCMS Peptide Reconstruct Options Dialog**

LCMS Peptide Reconstruct Options

Time Range

Minimum retention time: 0.00 min  Maximum retention time: 0.00 min

'Enhance' Peak Finding

Approximate LC peak width: sec Minimum intensity in counts: 5 counts

Perform background subtraction Chemical noise intensity multiplier: 1.5

Charge Deconvolution

Mass tolerance: 0.100 Da Maximum charge: 5

OK Cancel

6. Type the following values in the fields provided:

- **9.00** min in the **Minimum retention time** field
- Select the **Maximum retention time** check box and then type **16.00** in the field
- **6.0** sec in the **Approximate LC peak width** field

---

**Note:** The approximately peak width is used to determine offset during background subtracting.

---

- **5** counts in the **Minimum intensity in counts** field
- **1.5** in the **Chemical noise intensity multiplier** field
- **0.100** Da in the **Mass tolerance** field
- **5** in the **Maximum charge** field

---

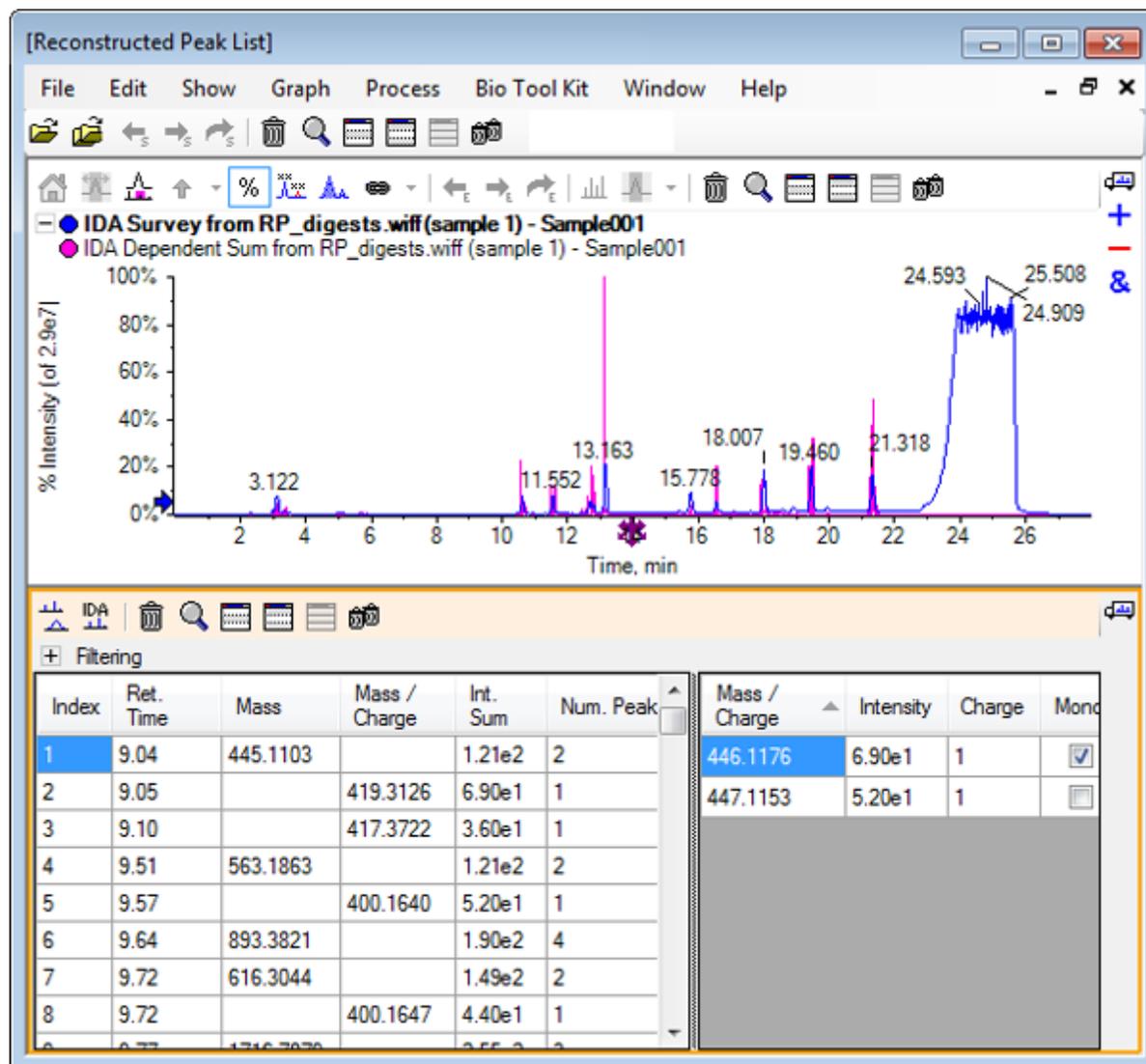
**Note:** The Mass tolerance in the Charge Deconvolution section makes sure that the reconstructed peak is matched with the theoretically digested protein and that the different *m/z* values belonging to the same peptide are grouped together.

---

7. Click **OK**.

The software shows a table of peptides, separated by retention time. The following information is provided for each peptide listed: **Index**, **Ret. Time**, **Mass**, **Mass / Charge**, **Int. Sum**, and **Num. Peaks**.

Figure D-95 Reconstructed Peak List



- Expand **Filtering** to show the available filtering options.

Available filtering options include: **Intensity threshold**, **Min. Num. Peaks**, and **Show matched peaks only**.

## Explorer Tutorial

Figure D-96 Filtering Options

Filtering

Intensity threshold:

Min. Num. Peaks:   Show matched peaks only

Index	Ret. Time	Mass	Mass / Charge	Int. Sum	Num. Peak
1	9.04	445.1103		1.21e2	2
2	9.05		419.3126	6.90e1	1
3	9.10		417.3722	3.60e1	1
4	9.51	563.1863		1.21e2	2
5	9.57		400.1640	5.20e1	1
6	9.64	893.3821		1.90e2	4

Mass / Charge	Intensity	Charge	Mono
446.1176	6.90e1	1	<input checked="" type="checkbox"/>
447.1153	5.20e1	1	<input type="checkbox"/>

9. Select one or more filters to adjust the view, as required.

**Note:** In this tutorial, the Intensity threshold was set to 2.39e4 and the Min. Num. Peaks was set to 4.

Figure D-97 Filtered Reconstructed Peak List

Filtering

Intensity threshold:

Min. Num. Peaks:   Show matched peaks only

Index	Ret. Time	Mass	Mass / Charge	Int. Sum	Num. Peaks
1	10.62	1501.6620		2.39e5	17
2	11.55	1270.6542		2.98e5	16
3	12.68	940.4651		1.93e5	9
4	13.15	1605.8489		8.62e5	18
5	15.76	563.3048		1.53e5	4
6	15.78	747.4268		1.96e5	4

Mass / Charge	Intensity	Charge	Mono
501.5605	2.98e4	3	<input checked="" type="checkbox"/>
501.8947	3.22e4	3	<input type="checkbox"/>
502.2281	1.41e4	3	<input type="checkbox"/>
502.5619	5.46e3	3	<input type="checkbox"/>
502.8962	2.39e3	3	<input type="checkbox"/>
503.2294	3.95e2	3	<input type="checkbox"/>
751.8383	3.89e4	2	<input checked="" type="checkbox"/>

## Toolbar

Use the icons in the toolbar to adjust the view, as required.

**Table D-6 Toolbar Icons**

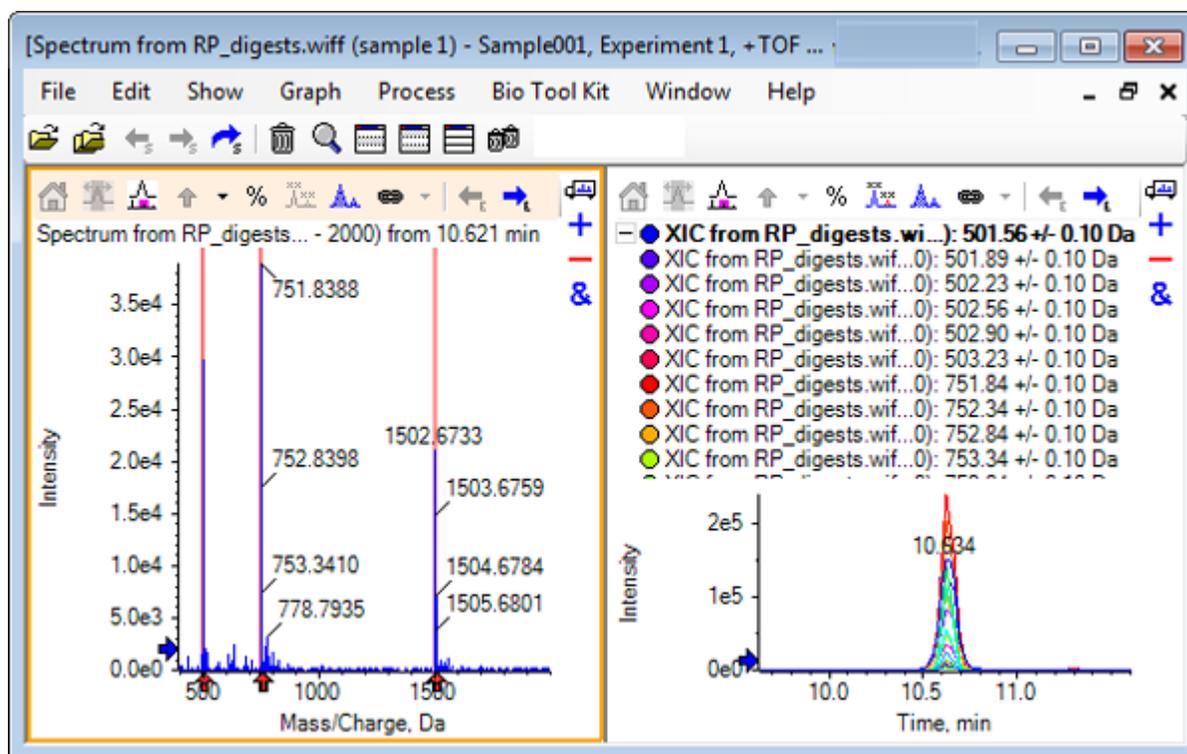
Icon	Name (Tooltip)
	Show spectrum and XIC
	Show IDA MS/MS Spectra

**Note:** The final six icons in this toolbar, beginning with the Deletes this pane icon, are described in [Generic Pane Toolbar](#).

### Show spectrum and XIC

When the **Show spectrum and XIC** icon is selected, the following spectrum and XIC panes open:

**Figure D-98 Show Spectrum and XIC Results**



For the MS spectrum generated, an arrow is shown below each peak that contributed towards the mass of the peptide. The XIC of each  $m/z$  peak that contributed towards the peptide mass is shown in as overlays in the pane on the right.

### Show IDA MS/MS Spectra

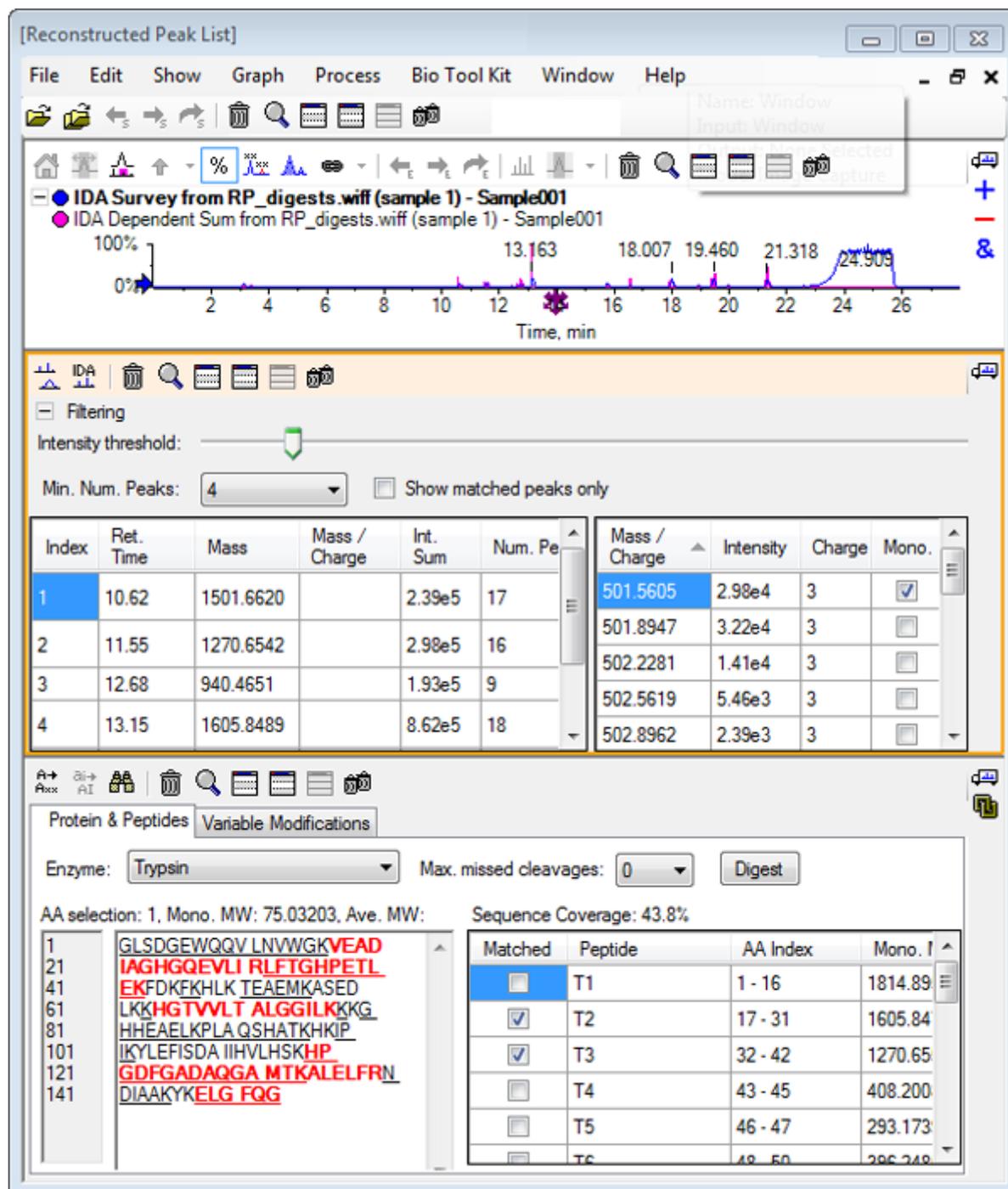
When the **Show IDA MS/MS Spectra** icon is selected, the following spectra pane opens:

### LCMS Peptide Reconstruct with Digest Protein

1. Click **Bio Tool Kit > Digest Protein**.  
The **Protein** pane opens.
2. Drag the **Drag to a protein pane to set its peak list** icon in the **Protein** pane to the **Reconstructed Peak List** pane.

The **Protein** pane refreshes, showing the peptide sequences in the Protein pane matching those in the Reconstructed Peak List. The fragments in the **Protein** pane shown in red, bolded font are those fragments with exact matches in the **Reconstructed Peak List** pane. The fragments shown in red, regular font are fragments that would have matched the fragments in the **Reconstructed Peak List** pane if they had been assigned the charge state indicated in brackets in the **Match** column of the **Reconstructed Peak List** pane. The fragments shown in black font are fragments that do not match any fragments in the **Reconstructed Peak List** pane.

Figure D-99 Theoretical Information on Protein Pane Linked to Reconstructed Peak List

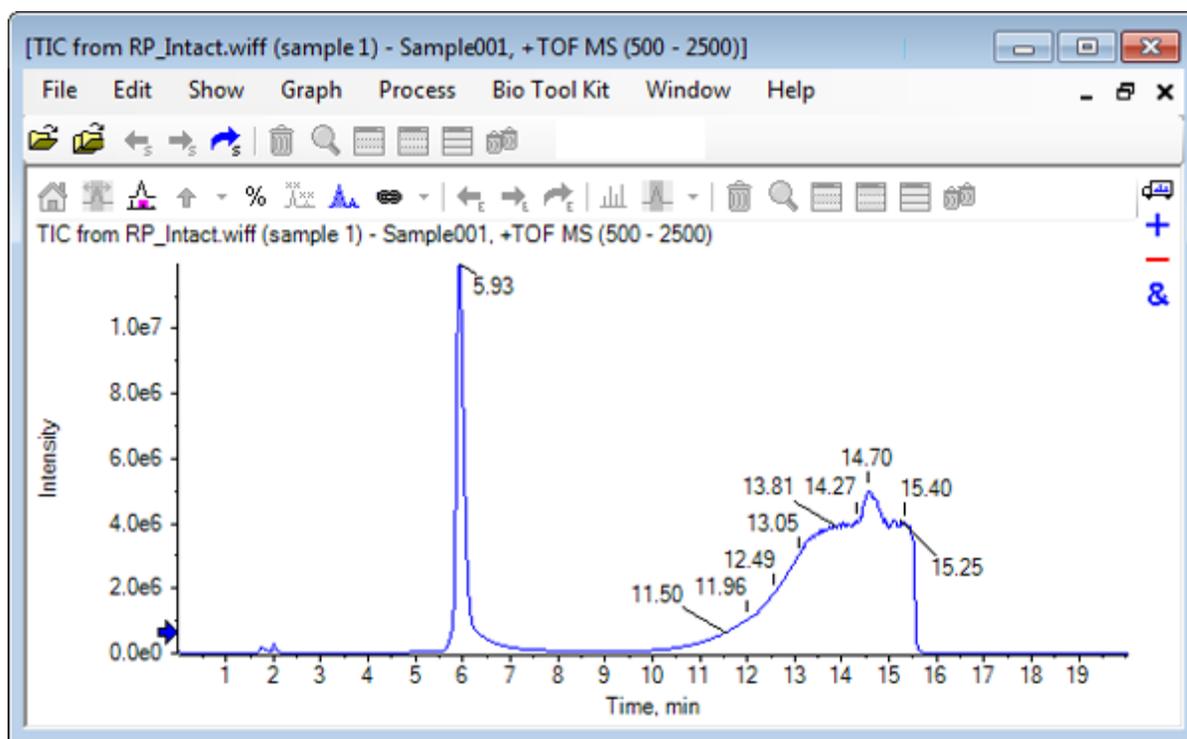


## Reconstruct Protein

Use this option to obtain the average mass (molecular weight) of an intact protein.

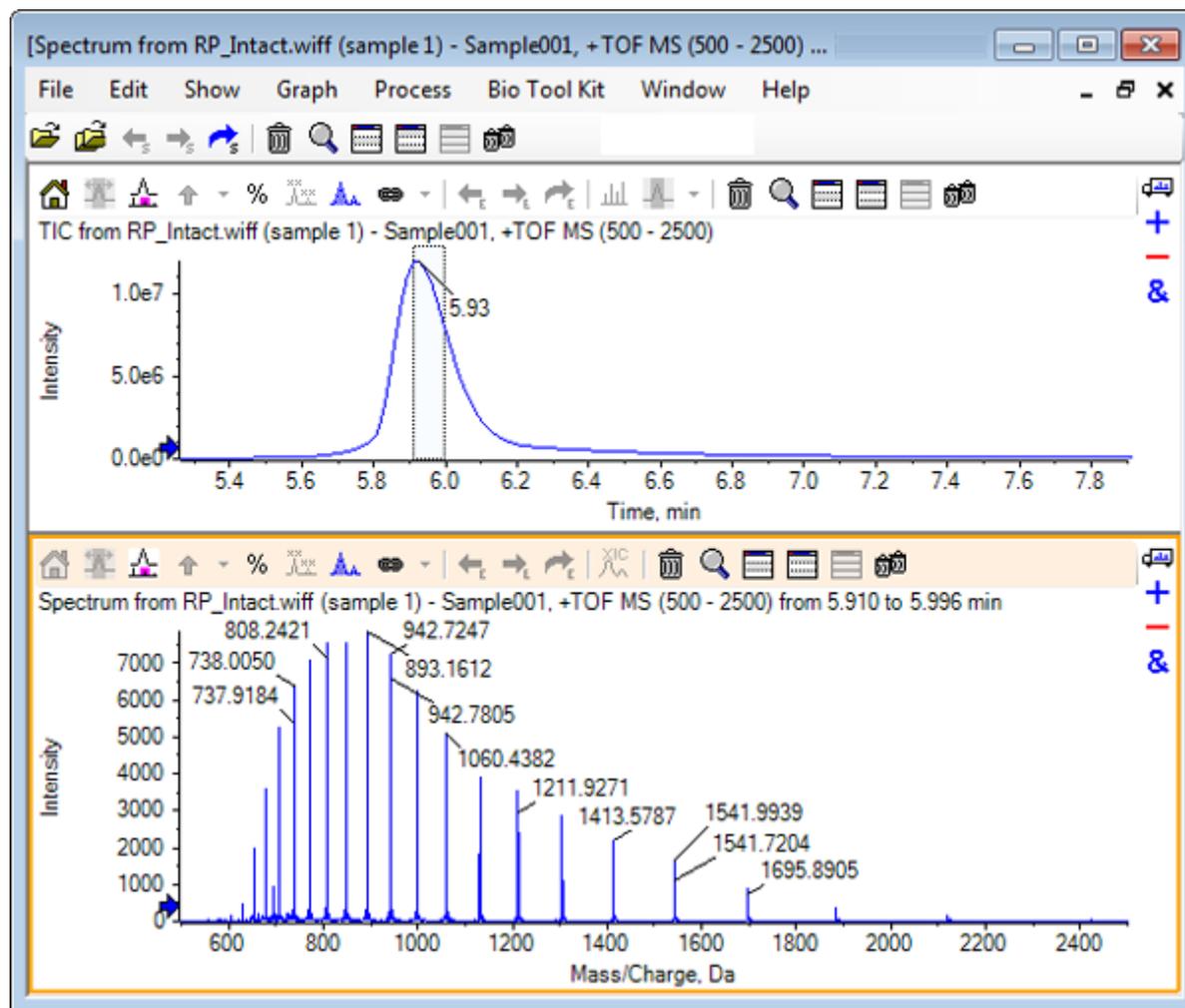
1. Click the **Open Sample** icon in the main toolbar.  
The **Select Sample** dialog opens.
2. If the **Sample Data** folder is not already selected, then click **Browse** and navigate to the **Sample Data** folder.
3. Select the **RP\_Intact.wiff** file and then click **OK**.

**Figure D-100** TIC from RP\_Intact.wiff File



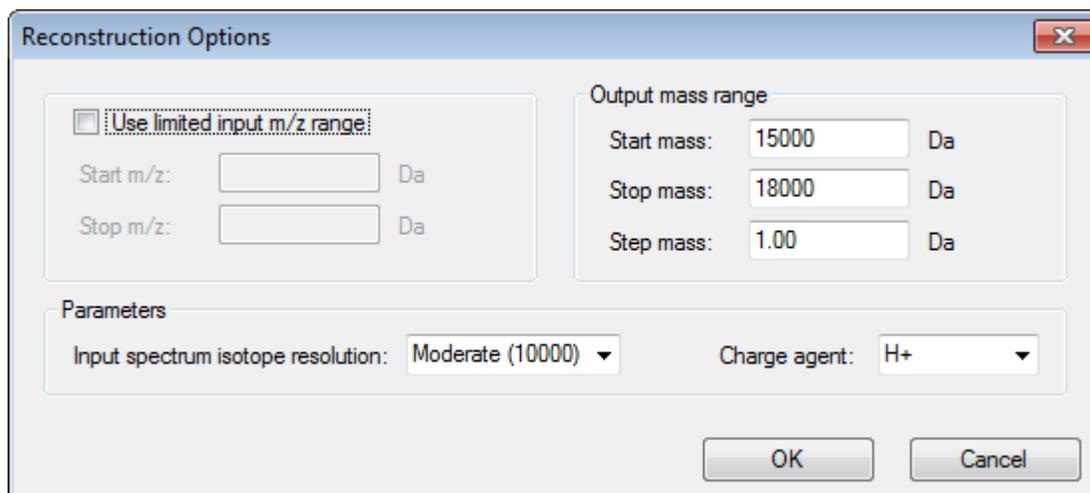
4. Create an averaged spectrum using a region of the peak at 5.93 min. Refer to [Figure D-101](#).

Figure D-101 Averaged Spectrum



5. With the spectrum pane active, click **Bio Tool Kit > Reconstruct Protein**. The **Reconstruction Options** dialog opens.

**Figure D-102 Reconstruction Options**



6. Type the appropriate values for the following options:
  - **Start mass:** 15000 Da
  - **Stop mass:** 18000 Da
  - **Step mass:** 1.0 Da
7. Select the appropriate **Input spectrum isotope resolution:** Moderate (10000).

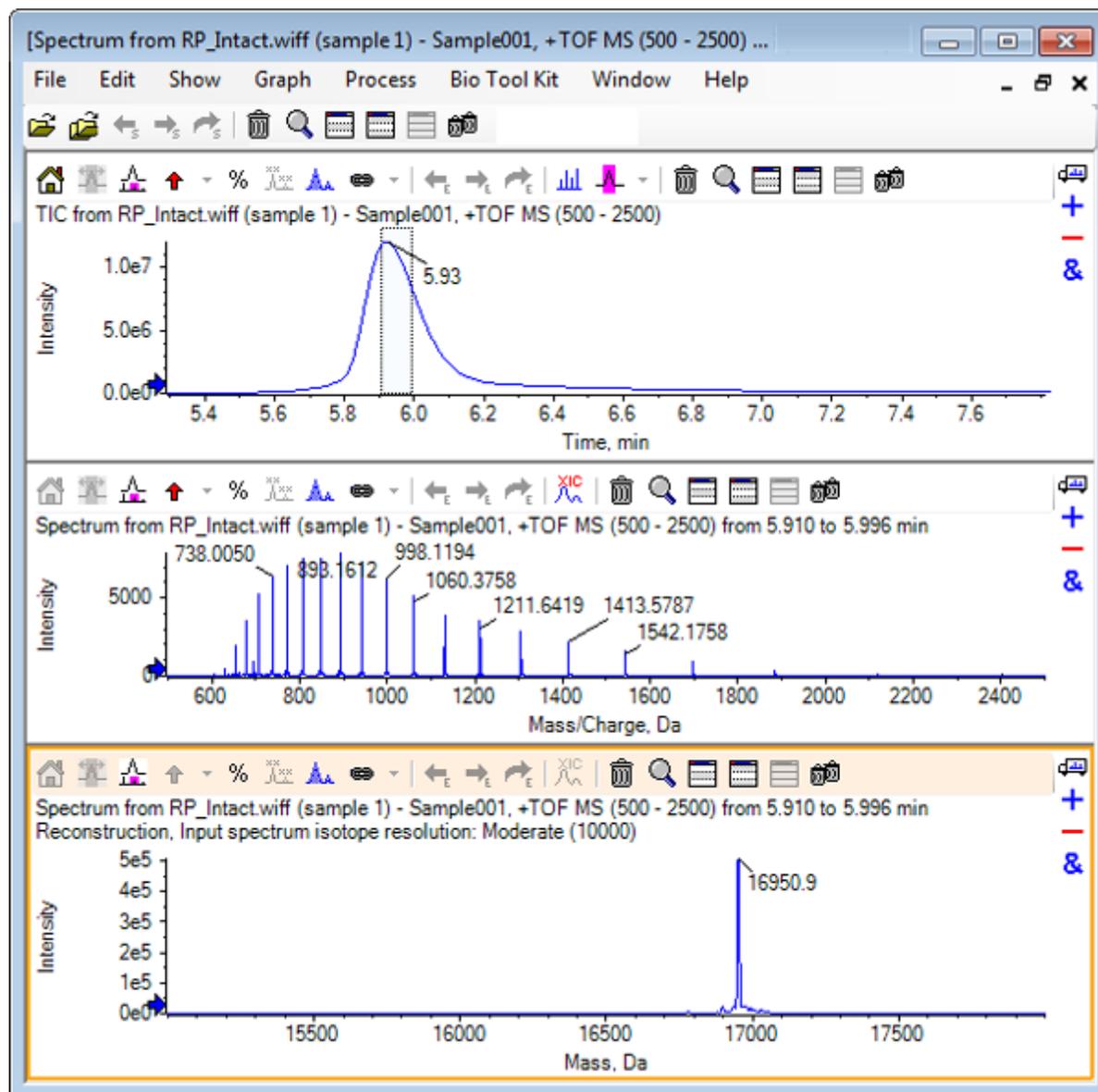
---

**Note:** For data acquired using a quadrupole system, the Peak width parameter is shown instead of the Input spectrum isotope resolution parameter.

---

8. Select the appropriate **Charge agent:** H+.
9. Click **OK**.  
The software generates a spectrum of the reconstructed protein in a separate pane with the title: **Reconstruction, Input spectrum isotope resolution [user selection]**.

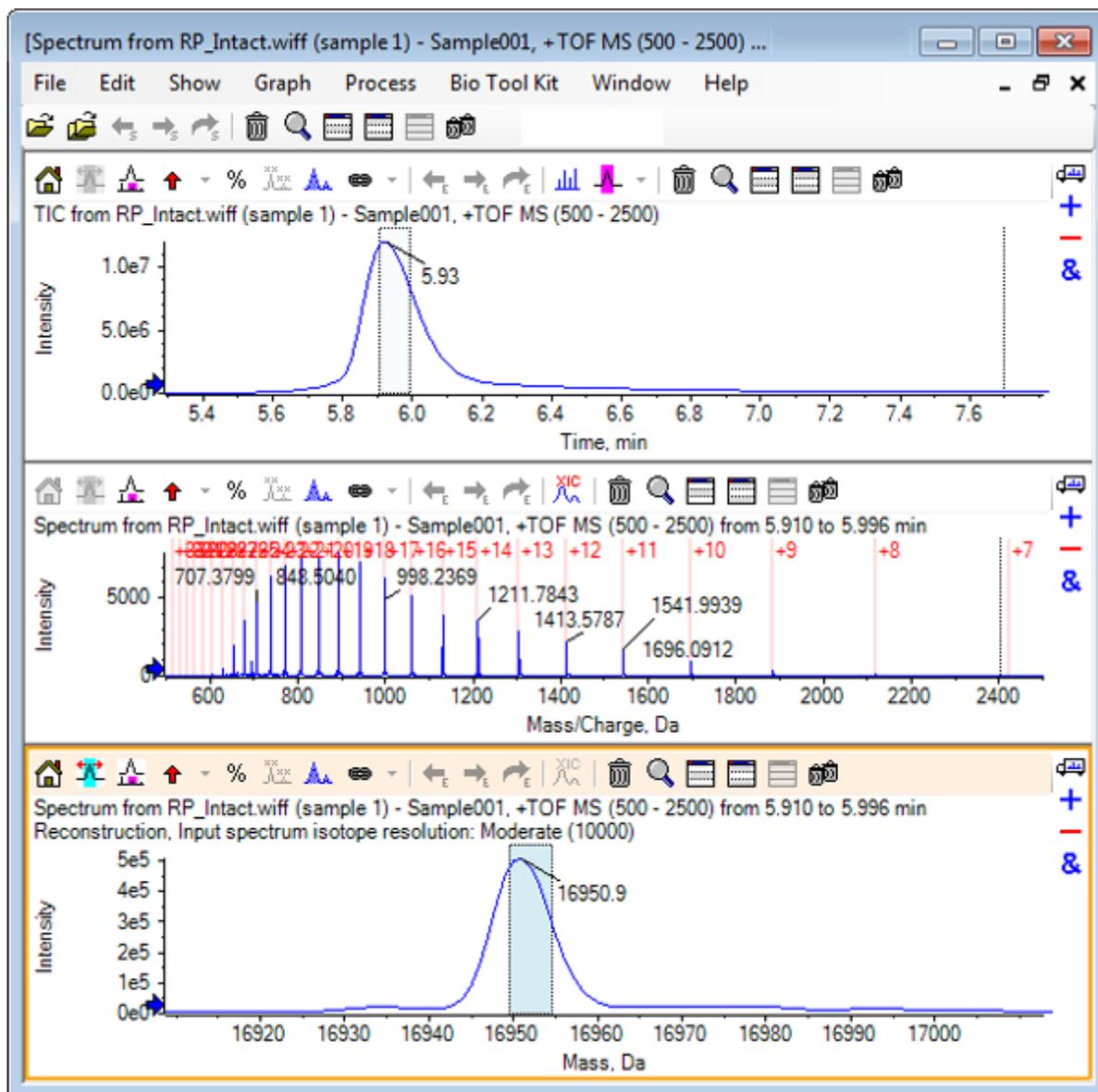
Figure D-103 Reconstruction Pane



**Note:** For data acquired using a quadrupole system, the title in the pane is: Reconstruction, Peak width [value].

10. Select the reconstructed protein peak.  
Vertical manual reconstruct highlights are added to the spectrum selected to generate the reconstructed protein.

Figure D-104 Spectrum with Manual Reconstruct Highlights



## Summary

In this section, the following tasks have been discussed:

- Manually sequencing MS/MS spectral data from a digested protein sample.
- Linking a manually sequenced spectrum with peptide fragments.
- Adding markers (Manual Reconstruct Highlights) indicating the theoretical  $m/z$  ratio positions of a given mass to a spectrum.

- Removing markers from a spectrum.
- Obtaining information on theoretical peptide sequences that result from a user-defined enzymatic cleavage of a specified protein.
- Using LCMS peptide reconstruction to identify spectral peaks and perform deconvolution from the identified spectral peaks.
- Linking theoretical information on a Protein Pane with a reconstructed peak list.
- Obtaining the average mass (molecular weight) of an intact protein.

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