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FreeStyle

User Guide

Software Version 1.4

XCALI-97962 Revision A • February 2018

ThermoFisher SCIENTIFIC

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Software version: (Thermo) Foundation 3.1 SP4 and later; FreeStyle 1.4 and later

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Contents

Preface

This guide describes how to use the Thermo FreeStyle™ application to view and analyze raw mass spectrometry data.

For contact information and information about related documentation and system requirements, see these topics.

Contents

For a list of new features, see "New Features" on page 9. To learn how to use the FreeStyle application, begin with the demonstration animations in Help.

❖ To suggest changes to documentation or to Help

Complete a brief survey about this document by clicking the button below. Thank you in advance for your help.

❖ To open the FreeStyle application

- From the Microsoft™ Windows™ taskbar, choose **Start > All Programs > Thermo FreeStyle > FreeStyle**.

–or–

- Click the **FreeStyle** icon,  , on the desktop.

Related Documentation

The FreeStyle application includes complete documentation. In addition to this guide, you can also access the application Help.

❖ **To view the product manual**

From the taskbar, choose **Start > All Programs > Thermo FreeStyle > Manual > FreeStyle User Guide.**

❖ **To open the Help system from the FreeStyle window**

Choose **File > Help**.

❖ **To view application-specific Help**

If information is available for a specific view, page, dialog box, or toolbar, click **Help** or press the F1 key.

System Requirements

Your system must meet the following system requirements.

System	Requirements
Computer	• 1.7 GHz dual core processor with 8.0 GB RAM • CD/R-ROM or DVD drive • 30 GB or greater available on drive C • Video card and monitor capable of 1280 × 1024 resolution
Software	• Adobe™ Flash™ Player (to view the demonstration animations in Help) • Adobe Reader™ 10.1 or later • Microsoft .NET Framework 4.6.2 • Microsoft Office 2013 (for exported data) • Microsoft Windows 7 SP1 (64-bit) or Windows 10 (64-bit) • Thermo Foundation™ platform 3.1 SP4 • Thermo Xcalibur™ 4.1 or 4.2 (for the NIST Library Browser)

Special Notices

Make sure you follow the special notices presented in this guide. Special notices appear in boxes; those concerning safety or possible system damage also have corresponding caution symbols.

This guide uses the following types of special notices.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Contacting Us

Contact	Email	Telephone	QR Code[a]
U.S. Technical Support	us.techsupport.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
U.S. Customer Service and Sales	us.customer-support.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
Global support	❖ **To find global contact information or customize your request** 1. Go to thermofisher.com. 2. Click **Contact Us**, select the country, and then select the type of support you need. 3. At the prompt, type the product name. 4. Use the phone number or complete the online form. ❖ **To find product support, knowledge bases, and resources** Go to thermofisher.com/us/en/home/technical-resources. ❖ **To find product information** Go to thermofisher.com/us/en/home/brands/thermo-scientific.		

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1

Introduction

Use the FreeStyle application to visualize and qualitatively analyze mass spectrometry data. A qualitative analysis focuses on identifying unknown compounds and confirming the presence of target (expected or known) compounds.

With the FreeStyle application, you can display chromatograms and spectra, detect and integrate chromatographic peaks, search mass spectral libraries, simulate mass spectra, subtract background spectra, apply scan filters, annotate plots with text and graphics, create and save layouts, and view the status of various instrument parameters during data acquisition. You can also export spectral data to the NIST™ application or mzCloud.org, calculate the elemental composition of a component from its exact mass, and perform Xtract deconvolution.

For a general understanding of mass spectrometry data and to get started with the application, see these topics.

Contents

Mass Spectra

There are many different types of mass spectrometry (MS) detectors, but the basic principles are the same in all cases: the MS ionizes the sample, separates the ions according to their mass[1], and moves the separated ions toward a detector where they are counted. The data system compiles a spectrum showing the mass distribution of the ions produced from the sample—a snapshot of ion intensities plotted against their mass-to-charge (*m/z*) ratios.

[1] In the majority of cases z=1 and the x axis becomes equivalent to mass, m.

Ionization initially produces molecular ions, but complex secondary processes can cause the molecular ions to fragment. Together with molecular ions, these fragment ions make up the mass spectrum. For individual chemical substances, a mass spectrum can be a characteristic molecular fingerprint.

Mass spectra have these common features:

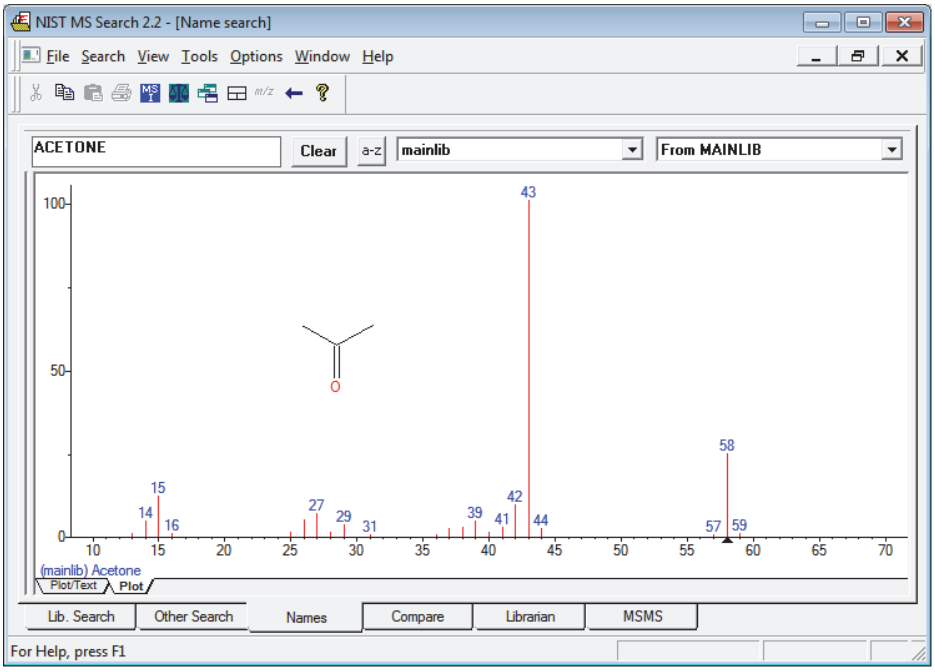
- Base Peak
- Neutral Losses
- Effect of Ionization Modes
- Adduct Formation
- Effect of Isotopes
- Isotope Patterns in High-Resolution Data

Base Peak

To plot the MS detector's response, the most abundant ion, called the base peak, is given an arbitrary abundance or intensity of 100. The application reports all other peaks as a percentage of the size of the base peak. After this normalization, the data system can compare spectra directly.

Figure 1 is an example of a NIST library spectrum showing the fragmentation of acetone C_3H_6O (molecular weight = 58 Da). Mass-to-charge labels appear above the spectrum peaks for the most abundant ions. In this example, the molecular ion (58 Da) is not the most abundant ion. The most abundant ion is the acetyl ion CH_3CO (molecular weight = 43 Da).

Figure 1. 70 eV electron ionization (EI) mass spectrum of acetone



Neutral Losses

You can use fragmentation patterns, like the pattern in Figure 1 for acetone, to determine the molecular structure of a compound. For example, the neutral loss of 15 Da from the molecular ion of acetone indicates the presence of a methyl group in the original molecule. A subsequent loss of 28 Da corresponds to the loss of CO. Table 1 lists commonly observed neutral losses, measured by the molecular weight of the compound. Assign such losses to help deduce the structure of an unknown compound. A full structural analysis generally relies on the presence of a molecular ion and the measurement of the molecular weight of the compound.

Table 1. Common neutral losses

Loss	Fragment
15	CH_3
18	H_2O
19	F
28	CO
29	C_2H_5 or CHO
35	Cl
46	NO_2
59	C_3H_7O, $COOCH_3$ or CH_2COOH
77	C_6H_5

In some cases, fragmentation is extensive, leaving little or no trace of a molecular ion. With no molecular ion, determining either the molecular weight or the structure is difficult.

Effect of Ionization Modes

The ionization mode affects the spectrum characteristics of a compound. The ionization modes for LC/MS (liquid chromatograph/mass spectrometer) instruments are different from those used with GC/MS (gas chromatograph/mass spectrometer) instruments:

- Ionization Modes for LC/MS Instruments
- Ionization Modes for GC/MS Instruments

Ionization Modes for LC/MS Instruments

LC/MS instruments use a variety of ionization techniques, collectively called atmospheric pressure ionization (API). Detectors of this type can detect positive or negative ions.

API techniques offer soft ionization, usually with little or no fragmentation. An API spectrum typically contains peaks for only the protonated or deprotonated molecule. Compounds with basic sites (such as amines) can form protonated molecules $[M+H]^+$. In the positive ion detection mode (polarity +), these ions produce a spectrum peak at the *m/z* value M+1 (where M represents the molecular weight of the neutral compound).

Compounds with acidic sites (sulphonic acids, for example) can form deprotonated molecules $[M–H]^–$. In the negative ion detection mode (polarity –), these ions produce a spectrum peak at the *m/z* value M–1.

Ionization Modes for GC/MS Instruments

GC/MS instruments offer two techniques: electron ionization (EI) and chemical ionization (CI).

EI is very commonly used because it is simple and reproducible. The fragmentation pattern is effectively determined by the energy of the impacting electrons alone (electron energy, measured in eV). Very different types of mass spectrometers that use EI can produce virtually identical spectra as long as the electron energy is the same.

This reproducibility has led to an extensive library compilation for 70 eV EI spectra. With the FreeStyle application, you can access the NIST/EPA/NIH Mass Spectral Library with over 108 000 reference EI spectra. You can use library data to select confirmatory ions for your target compounds.

Note You can purchase the NIST Mass Spectral Search application from the National Institute of Science and Technology. Thermo Fisher Scientific provides local versions of the NIST application and its libraries with the Xcalibur data system.

Chemical ionization (CI) offers a softer method of forming ions. In CI, a controlled flow of a reagent gas, commonly ammonia, methane, or isobutane, is introduced into the area where ionization occurs (the ion source). Energetic electrons that pass through the source ionize the reagent gas, as in EI. These ions can then collide with neutral molecules, causing hydrogen transfer. This process is repeated when the reagent gas ions collide with analyte molecules.

CI usually produces protonated molecules, generally at a mass one unit greater than the molecular mass of the compound. Significantly less fragmentation occurs than in comparable EI spectra. Depending on your choice of reagent gas, adduct ions can form. For example, when you use ammonia as the reagent gas, $M+NH_4$ is a typical adduct ion.

Under certain conditions, CI produces negative molecular ions formed by electron capture. The sensitivity of negative ion CI for certain classes of compounds (those containing double bonds, sulfur, phosphorus, chlorine, or bromine) can be orders of magnitude greater than positive CI or EI modes for those compounds.

For more information about the ionization modes available on your instrument, read the hardware manual and the instrument manual on how to get started.

Adduct Formation

If ionization takes place in the presence of contaminants or additives such as ammonium or sodium ions, some compounds are susceptible to adduct formation. These spectra show other ions in addition to, or instead of, the molecular ion (Figure 2).

Note The FreeStyle application can automatically add elemental composition and *m/z* annotations to the mass spectrum peaks (see "Labeling Spectrum Peaks" on page 134). To add custom annotations, such as those shown in Figure 2, you can use the application's text annotation tools.

Figure 2. Mass spectrum showing sodium and acetonitrile adducts

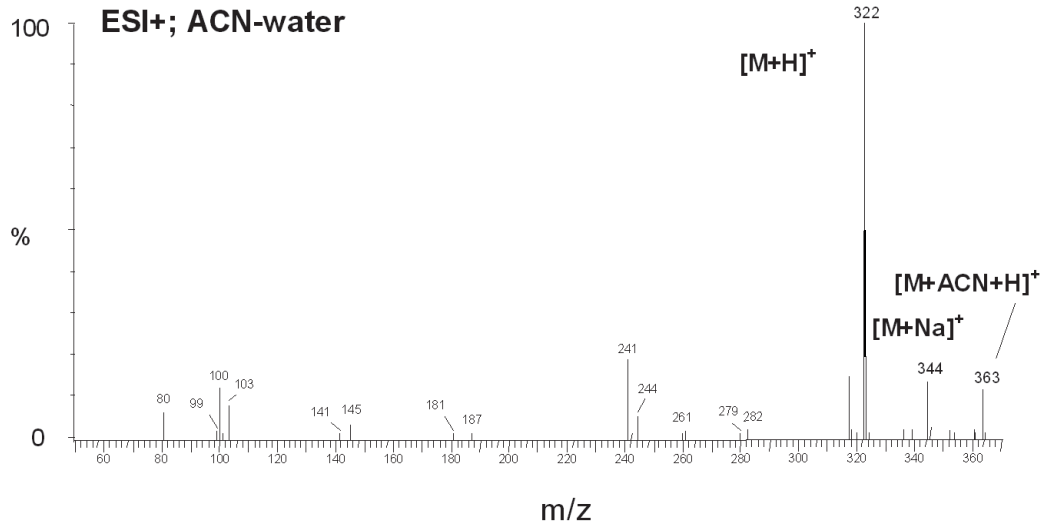


Table 2 lists common adducts for the positive and negative ESI modes.

Table 2. Common adduct ions

Cationized adducts (positive mode)		Anionized adducts (negative mode)	
$[M+NH_4]^+$	M+18	$[M+OAc]^-$	M+18
$[M+Na]^+$	M+23	$[M+Na]^-$	M+21
$[M+CH_3OH+H]^+$	M+33	$[M+Cl]^-$	M+35
$[M+K]^+$	M+39	$[M+K]^-$	M+37
$[M+CH_3CN+H]^+$	M+42	$[M+HCOO]^-$	M+59

Take care when determining molecular weights to account for possible adduct ions.

Effect of Isotopes

In some cases, the effect of less abundant isotopes might cause you to use an average molecular weight rather than one based on the most abundant isotopes. When the molecular structure of the target compound contains large numbers of certain elements, the less abundant isotopes become significant. This situation might result in a shift in the mass peaks from their expected *m/z* values.

For example, the most abundant isotope of chlorine is Cl^{35}. However, Cl^{37} occurs with a natural abundance of 24.47 percent. If a compound contains four chlorine atoms, its molecular ion is two mass units greater than that expected from a calculation based solely on Cl^{35}. Using chlorine's average atomic weight (35.453), you can correctly identify the molecular ion. Also, you observe a distribution of molecular ions across eight mass units from molecules containing between zero and four Cl^{37} atoms.

Isotope Patterns in High-Resolution Data

The mass spectra acquired with a high-resolution, accurate-mass (HRAM) mass spectrometer include isotope clusters for analytes with elements that have more than one stable isotope. The mass difference between the isotopic peaks is proportional to the mass difference of the isotopes, and the relative intensity of the isotopic peaks is proportional to the natural abundance of the isotopes.

To confirm the identity of an unknown analyte, compare the theoretical isotope pattern for its proposed chemical formula to the experimental mass spectrum. Use the Isotope Simulation Page in the Info Bar to predict the isotope pattern for any chemical formula or peptide sequence.

Analysis Modes for the Mass Spectrometer

A Thermo Scientific mass spectrometer has these analysis modes:

- Full Scan
- Selected Ion Monitoring (SIM)
- MS/MS

Full Scan

In full-scan operation, the MS detector scans repetitively over a wide mass range throughout the analysis and sends the data to the data system computer.

With the FreeStyle application, you can display the chromatograms (measured intensity versus analysis time) for full-scan MS data in these ways (plot types):

- As a total ion current (TIC) chromatogram. A TIC chromatogram represents the summed intensities of all the ions in the scanned mass range (mass spectrum) plotted against the chromatographic retention time. Each peak in the TIC represents one or more eluting compounds, which can be identified from the mass spectra recorded across the peak.
- As a mass chromatogram for a range of masses within the scan range. Mass chromatograms show the ion intensities of selected mass-to-charge ratios (*m/z*). The application extracts these mass spectra from each stored scan and plots them against the analysis time. Use this technique to increase selectivity by displaying an *m/z* value that is characteristic of the compound of interest but not present in other sample components.
- As a base peak chromatogram. Base peak chromatograms show the ion intensities of the most intense ions for each time point in the chromatogram.

Note The FreeStyle application uses the accurate mass and isotope pattern information in the full-scan MS1 data to calculate the elemental composition of unknown compounds. It then uses the accurate mass data for the fragment ions in the data-dependent MS2 scans to confirm the best matching formulas.

Selected Ion Monitoring (SIM)

In the selected ion monitoring (SIM) mode, the MS detector monitors a limited number of *m/z* values that are characteristic of a targeted compound or compounds. During an analytical run, the mass analyzer repeatedly switches between the selected *m/z* values and monitors each *m/z* value for a programmed dwell time before averaging the measured ion intensities and moving on to the next value.

SIM generates mass chromatograms only of the monitored *m/z* values, not complete mass spectra as in the full-scan mode. Without a complete mass spectrum, you cannot perform a library search to identify an unknown.

SIM is ideally suited to trace analysis and offers reduced file sizes compared to full-scan operation because SIM records only the information of interest.

MS/MS

Depending on your instrument, you might also be able to do additional stages of mass analysis called MS/MS.

In an MS/MS experiment, you select specific ions for further fragmentation while discarding all other masses. The selected ion is called a precursor (parent) ion and its fragment ions are called product ions. An ion trap mass spectrometer (a mass spectrometer with an ion trap mass analyzer) can perform additional stages of MS (called MS^n), up to MS^{10}.

The MS detector can monitor the product ions in either the full-scan mode or the SIM mode. When you set up the MS detector to monitor a specific product ion of a specific precursor (parent) ion, the scan type is called selective reaction monitoring (SRM).

You can create your own libraries of full-scan MS/MS data to use for matching.

You can display the chromatograms for full-scan MS/MS data in these plot types: TIC, mass range, base peak, or neutral fragment. With the neutral fragment plot type, you must specify the neutral fragment.

Note The query spectrum for a library search against your local mzVault database file or the online mzCloud mass spectral library must be an MS2 spectrum from a data-dependent full-scan MS/MS experiment.

Qualitative Analysis Tools

The FreeStyle application includes the following qualitative analysis tools:

- Automated library searches of local mzVault™ and NIST mass spectral databases and the online mzCloud™ database. See Chapter 6, "Searching Mass Spectrum Libraries."
- Automated peak detection algorithms for the evaluation of chromatographic data (see "Automatically Detecting and Integrating Chromatographic Peaks" on page 63).
- Elemental composition tool that determines the chemical formulas of ions by using their exact mass and isotope pattern. Includes confirmation of the best match formula by comparing the *m/z* values of the ions in the data-dependent fragmentation scans of the precursor ion to the possible set of fragment ions based on their *m/z* values. See Chapter 5, "Determining the Elemental Composition of Ions."

- Isotope simulation tool that displays a simulated mass spectrum from the following input: chemical formula, adduct species, and charge distribution. You can compare the simulated spectrum to an experimental spectrum. See Chapter 7, "Simulating Isotope Distributions."
- Xtract tool for the deisotoping and deconvolution of mass spectra. See Chapter 10, "Deconvolving and Deisotoping Spectra with the Xtract Algorithm."
- Spectrum averaging for noise reduction and mass accuracy (see "Averaging Spectra" on page 121).
- Background subtraction for the removal of spectral peaks—for example, matrix-related peaks—that are not related to the target components (see "Subtracting Background Spectra" on page 122).

New Features

The following features are new in FreeStyle version 1.4:

- Error Log View for viewing acquisition errors for the current raw data file.
- MSn Browser Page for selecting the spectra of interest from a spectrum tree.
- Manual Noise Region check box that enables the manual selection of the noise region for the ICIS and Genesis peak detection algorithms. See "Selecting the Manual Noise Region for the Genesis and ICIS Algorithms" on page 66).
- Shortcut menu (right-click) commands for the Chromatogram view:
  - Insert Chromatogram command for inserting a chromatogram trace. See "To add a chromatogram trace by using the shortcut menu command" on page 45.
  - Undo Delete Chromatogram command for displaying the last deleted chromatogram trace. See "To undo the most recent deletion of a chromatogram trace" on page 48.
  - Show Toolbox command for opening the toolbox. See "Chromatogram View Toolbox" on page 91.
- Shortcut menu (right-click) command for the Spectrum (or MultiSpectrum) view—Show Toolbox. See "Spectrum View Toolbox" on page 145.
- Spectrum Ranges dialog box for selecting the spectra of interest in the Spectrum view or the Multi Spectrum view.
- Scan Filters button in the Workspace Options toolbar for opening the Scan Filters Page in the Info Bar.
- Charge, Concentration, and Half Width parameters on the Isotope Simulation Page in the Info Bar for displaying the charge distribution for peptides and nucleotides.

- Workspace configuration setting for turning off the automatic display of the floating toolboxes. See "Selecting How You Open the Floating Toolboxes" on page 40.
- Flags and Reference Peak buttons on the Spectrum – Display Options toolbar for displaying flags, reference, and exception peaks in the Spectrum view.

2

Using the FreeStyle Window

The FreeStyle window includes multiple toolbars, views, and Info Bar pages. This chapter describes the FreeStyle window and some common tasks, such as opening raw data files, rearranging the views within a workspace, creating layout templates, zooming in on a section of a graphical view, capturing an image of a graphical view, adding images and annotations to a graphical view, and copying tabular data to a spreadsheet application.

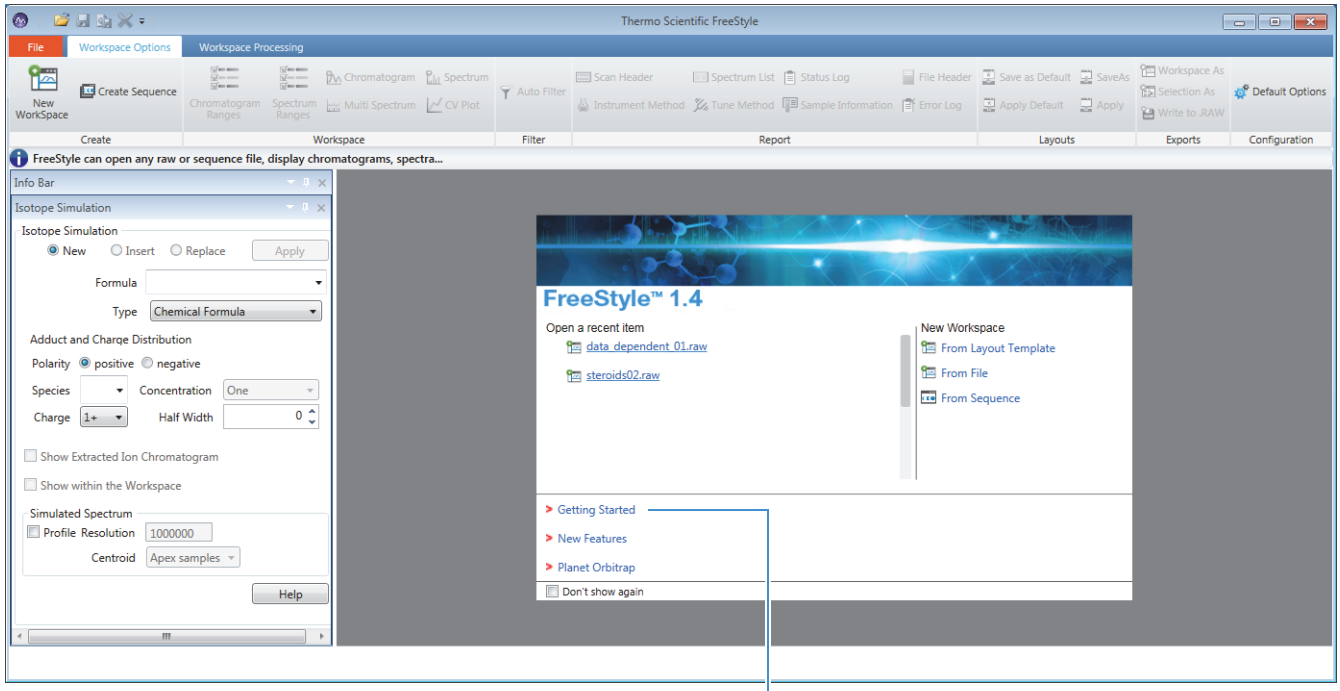
Contents

Startup Window

The FreeStyle window opens to the Isotope Simulation page in the Info Bar to the left and the getting started hyperlinks to the right (Figure 3).

You can use the Isotope Simulation page without opening a raw data file (see "Isotope Simulation Page" on page 209).

Figure 3. Startup window



File Menu

Use the File menu (see Figure 4) of the FreeStyle window to open raw data files or sequence files, create a sequence list, save the active Workspace as a template, view the list of recently opened raw data files and folders, and access the default settings.

For more information about Workspaces, see "Creating, Positioning, Previewing, and Closing Workspaces" on page 17. For more information about templates, see "Saving and Applying Layout Templates" on page 21. For more information about default settings, see Appendix A, "FreeStyle Default Settings."

Figure 4. File menu

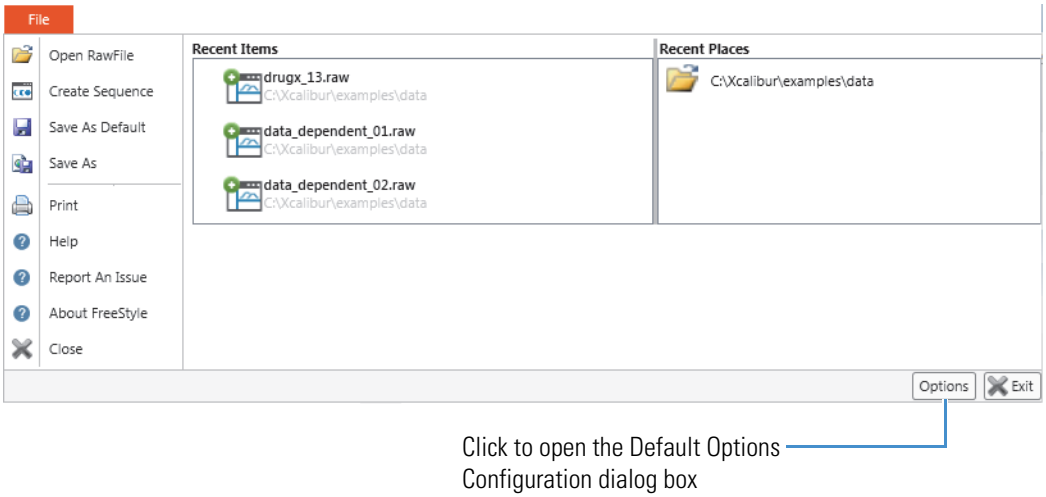


Table 3 describes the File menu commands. For information about using each command, see the related topics.

Table 3. File menu commands (Sheet 1 of 2)

Item	Description
Commands	
New Workspace	Opens a dialog box where you can choose existing raw data files (RAW) or sequence files (SLD) to open and create new Workspaces. See "Creating, Positioning, Previewing, and Closing Workspaces" on page 17.
Create Sequence	Opens a dialog box where you can choose a set of raw data files to open and create a sequence in a new Workspace. See "Working with Sequences" on page 69.
Save as Default	Automatically saves the current layout of the active Workspace to the default template file. See "Saving and Applying Layout Templates" on page 21.
Save As	Opens a dialog box where you can save the current layout to a template file with a different name. In the File Name box, type the new name and click **Save**. See "Saving and Applying Layout Templates" on page 21.
Print	Prints a report showing chromatogram and spectrum information for the currently selected Workspace. See "Copying, Exporting, and Printing Graphical Images and Tabular Data" on page 26.
Help	Opens the FreeStyle Help window.

Table 3. File menu commands (Sheet 2 of 2)

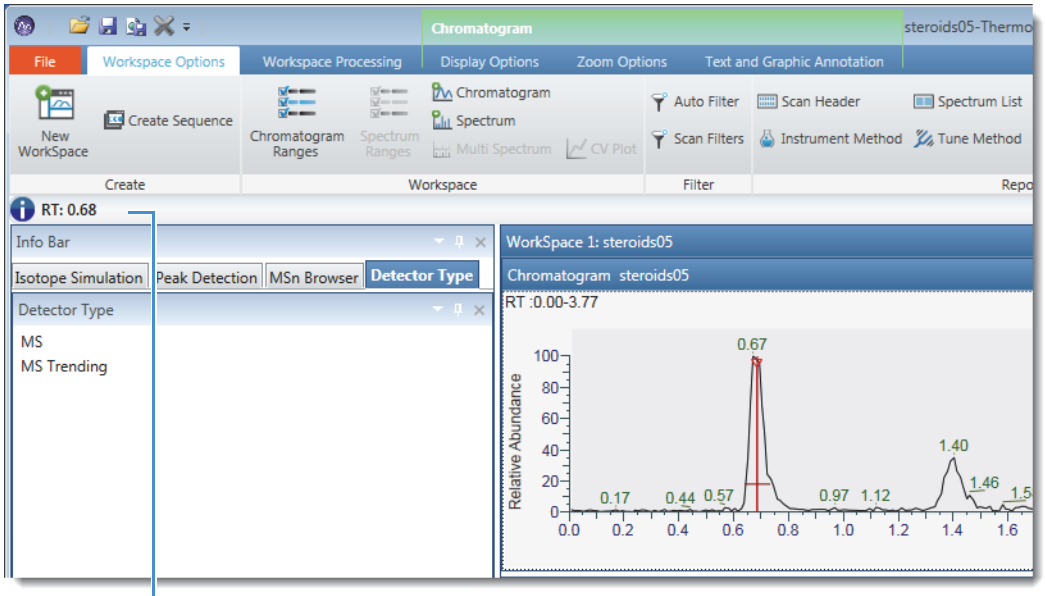
Item	Description
Report an Issue	Opens a mail dialog box in the Microsoft Outlook™ application with a default email address. You can enter a detailed description of the issue, along with the steps to reproduce it, and send this information to Thermo Fisher Scientific.
About FreeStyle	Displays the FreeStyle version information and the release and copyright dates, and shows the version information of other Thermo Scientific applications and instruments installed on your system.
Close	Closes the File menu.
Button	
Options	Displays the current configuration of the default values. See Appendix A, "FreeStyle Default Settings."
Exit	Closes the FreeStyle application.

Communicator Bar

The communicator bar, which is located immediately below the toolbar, provides information about the selected retention time in the Chromatogram view, general information about the current task, or warning messages. This symbol, ℹ, precedes general information, and this symbol , ⚠, precedes warnings.

Figure 5 shows the location of the communicator bar.

Figure 5. Communicator bar with informational message



Opening Raw Data Files or Sequence Files

In the FreeStyle application, you can open two file types—raw data files (RAW) or sequence files (SLD). A raw data file contains the data from a single acquisition run; whereas, a sequence file contains a list of associated raw data files from multiple runs. When you open either of these file types, the application creates a new Workspace view (see "Creating, Positioning, Previewing, and Closing Workspaces" on page 17).

Follow these procedures as needed:

- To open a raw data file or a sequence file
- To open a raw data file to a custom layout
- To open a file from the startup window

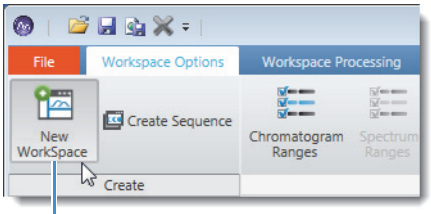
❖ To open a raw data file or a sequence file

1. Do one of the following:

   - In the menu bar, choose **File > New Workspace**.

   –or–

   - In the Create area of the Workspace Options toolbar, click **New Workspace**.



New Workspace toolbar button

2. In the Open Raw File dialog box, browse to and select a raw data file (RAW) or a sequence file (SLD), and then click **Open**.

   - For a raw data file, a new Workspace appears to the left of the Info Bar and has two stacked views (see "Factory Default Layout" on page 16). If the file includes mass spectrometry data, the Chromatogram view at the top displays the total ion current trace (TIC), and the Spectrum view at the bottom displays the first scan.
   - For a sequence file, the Sequence File Page appears in the Info Bar with a list of raw data files, and the Workspace displays data from the first raw data file in the list.

   For information about viewing a trace other than the TIC trace, see "Defining the Range of a Chromatogram Trace" on page 48.

❖ **To open a raw data file to a custom layout**

1. In the startup window, under New Workspace, click **From Layout Template**.

   Tip The From Layout Template command is only available in the startup window. To apply a custom layout to an active workspace, from the Workspace Options toolbar, choose **Apply** > ***Named Layout Template***.

2. In the Browse Templates dialog box, select a template (XML) and click **Open**.
3. In the Open Raw File dialog box, select a raw data file, and then click **Open**.

❖ **To open a file from the startup window**

Do the following:

- To open a recent file, under Open a Recent Item, click the hyperlink to the file.
- To open a raw data file or a sequence file, under New Workspace, click **From File**.
- To only open a sequence file, under New Workspace, click **From Sequence**.

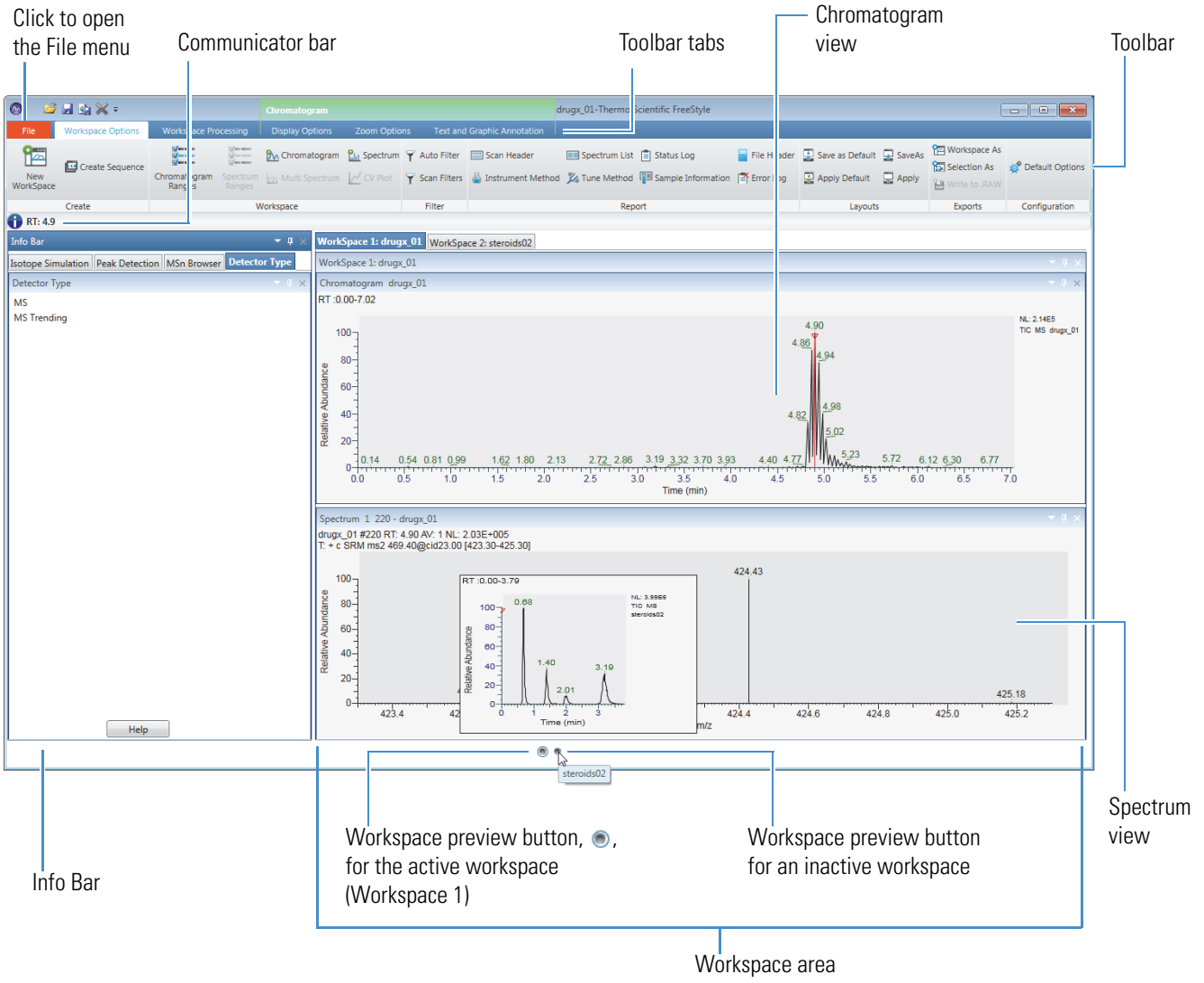
Factory Default Layout

Figure 6 shows the default FreeStyle window with two raw data files. Each raw data file appears as a separate tabbed Workspace, with only one tabbed Workspace view displayed at a time. At the bottom of the workspace area, the mouse is pointing to the preview button for the inactive Workspace—drug02.

The default layout displays the tabbed Isotope Simulation, Peak Detection, MSn Browser, and Detector Type pages in the Info Bar to the left and the stacked Chromatogram and Spectrum views in the workspace area to the right.

Note To open a new Workspace view, see the previous topic "Opening Raw Data Files or Sequence Files."

Figure 6. FreeStyle default window



Creating, Positioning, Previewing, and Closing Workspaces

The FreeStyle application creates a new Workspace view whenever you open a raw data file or a sequence of raw data files. The Workspace view displays the processed data (see Figure 6 on page 17). The Chromatogram view is the primary view. You can add multiple chromatogram plots to the Chromatogram view and select a different time point in each plot as the time base for other views such as the Spectrum view.

Follow these procedures as needed:

- To open a new Workspace
- To create multiple workspaces
- To reposition the Workspace view
- To display a Workspace when multiple Workspaces are open
- To preview the Chromatogram view for a Workspace
- To close a Workspace view

❖ To open a new Workspace

Follow the instructions in "Opening Raw Data Files or Sequence Files" on page 15.

❖ To create multiple workspaces

1. Choose **File > New Workspace**.
2. Do one of the following:
   - Select multiple raw data files and click **Open**.

     Tip Use the SHIFT key (for consecutive files) or the CTRL key (for nonconsecutive files).

   –or–

   - Drag the files into the FreeStyle window.

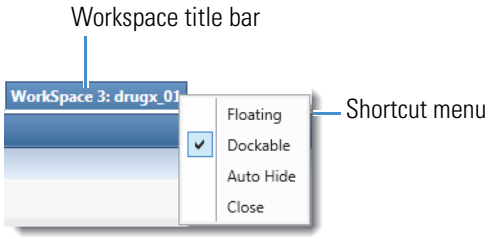
     Note Do not drag the files into the Chromatogram Ranges View because this action adds new traces to this view instead of adding new workspaces.

   A separate Workspace view appears for each raw data file. In the default factory layout, additional workspaces appear as horizontal tabs above the Chromatogram view.

❖ To reposition the Workspace view

1. Right-click the workspace title bar or click its **Window Position** icon, , to open the shortcut menu (Figure 7).

Figure 7. Workspace shortcut menu



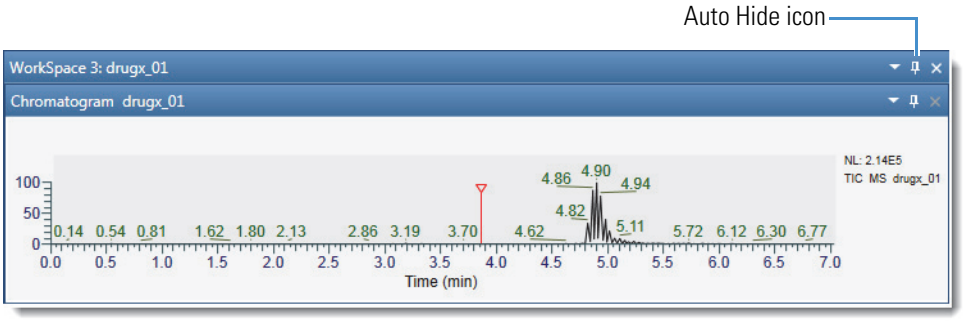
2. Do one of the following:

   - Choose **Floating** to detach the Workspace view into a floating window.
   - Choose **Dockable** to return the Workspace view back to its default position in the FreeStyle window.

   –or–

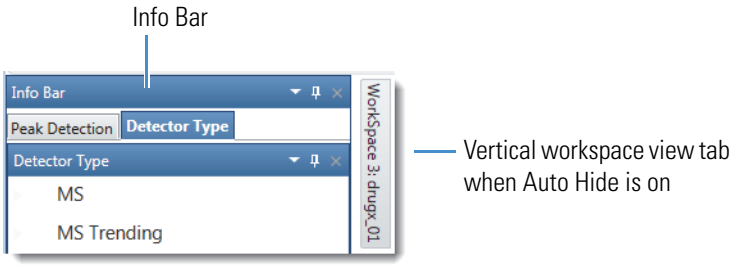
   - Choose **Auto Hide** or click the **Auto Hide** (vertical pin) icon, , in the title bar (Figure 8) to temporarily hide the Workspace view.

Figure 8. Workspace vertical Auto Hide icon



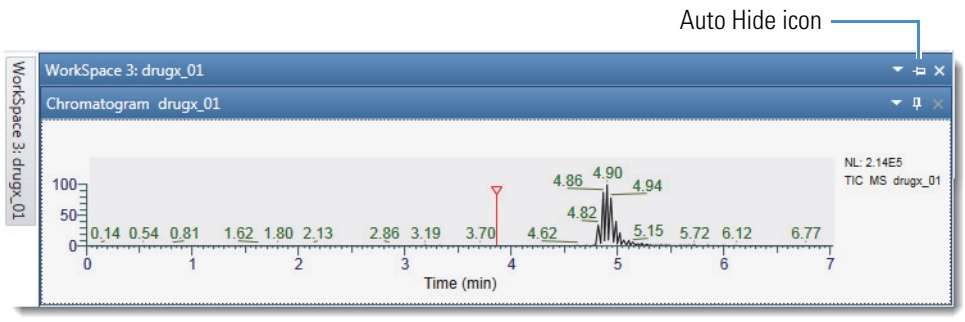
The Workspace view collapses as a vertical tab to the right of the Info Bar (Figure 9).

Figure 9. Workspace tab



Pointing to the tab displays the Workspace view again. When Auto Hide is on, the Auto Hide icon is horizontal, (Figure 10). To turn off the Auto Hide function, choose **Auto Hide** in the shortcut menu again to clear the check mark, or click the Auto Hide icon to change it back to vertical, .

Figure 10. Workspace horizontal Auto Hide icon



❖ **To display a Workspace when multiple Workspaces are open**

- Click the target workspace tab.

–or–

- Click the workspace preview button for the target Workspace.

❖ **To preview the Chromatogram view for a Workspace**

Point to its preview button for a thumbnail view (see "Factory Default Layout" on page 16).

❖ **To close a Workspace view**

- Right-click the title bar and choose **Close** (see Figure 7 on page 18).

–or–

- Click the **Close** icon,  .

Selecting the Columns to Display in a View or Dialog Box with Tabular Data

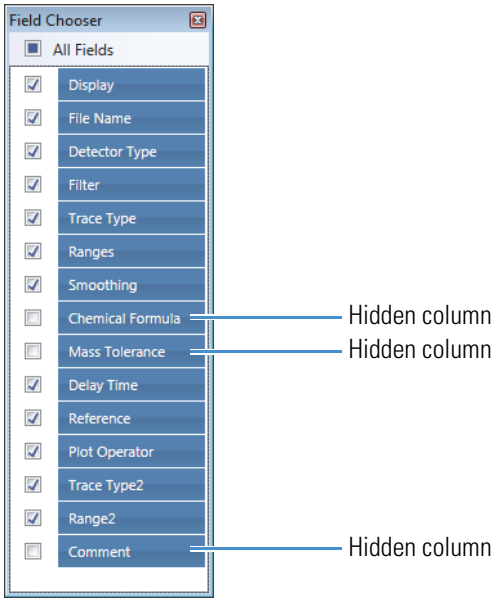
To minimize the display of infrequently used information, some of the table columns in a view or dialog box are hidden, by default. You can change which columns to display or hide in a specific table by accessing the table's Field Chooser dialog box.

❖ **To open a Field Chooser dialog box and change the column selections**

1. Click the **Field Chooser** icon,  , to the left of the table heading row.
2. Do the following:
   - To display a column, select its associated check box.
   - To hide a column, clear its associated check box.

Figure 11 shows the Field Chooser dialog box for the Chromatogram Ranges view. In this view, the following columns are hidden by default—Chemical Formula, Mass Tolerance, and Comment.

Figure 11. Field Chooser dialog box for the Chromatogram Ranges view



You can display or hide table columns in these views and dialog boxes:

- Chromatogram Ranges View
- Spectrum List View
- Status Log View
- Sample Information View
- File Header View
- Peaks List View
- Elemental Composition Results View
- Spectrum Ranges dialog box (page 115)
- Fill Down dialog box (page 85)

Saving and Applying Layout Templates

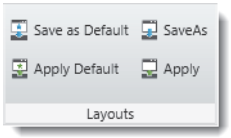
A FreeStyle template contains the settings that define the workspace layout. The template specifies what views are in a Workspace, how the views are arranged, and what labeling the views contain. The template also specifies which pages the Info Bar initially displays. For future use, you can save the template as a new XML file, or save it as the default template.

The FreeStyle application stores all templates in the following folder:

drive:\Users*your name*\AppData\Local\Thermo Scientific\FreeStyle\Templates\

❖ To save the template as the default template

1. Click the **Workspace Options** toolbar tab (see Figure 14 on page 30).
2. Click **Save As Default** in the Layouts area.



Note You can also choose **File > Save As Default**.

The application saves the layout as the default template file, DefaultTemplate.xml.

❖ To apply the default template

1. Click the **Workspace Options** toolbar tab.
2. Click **Apply Default** in the Layouts area.

❖ To name the template and save it as an XML file

1. Click the **Workspace Options** toolbar tab.
2. Click **Save As** in the Layouts area.
3. Type a name for the template and click **Save**.

IMPORTANT Do not navigate to a different folder. The application reads the template files only from the default template folder.

❖ To apply a saved template

1. Click the **Workspace Options** toolbar tab.
2. Click **Apply** in the Layouts area to open the DataBrowser Templates dialog box.
3. Select a template and click **OK**.

❖ To restore the original default factory template for a workspace

See the next topic "Restoring the Default Settings."

Restoring the Default Settings

The FreeStyle application includes a dialog box with a set of pages containing initial default settings upon installation. You can modify these settings as needed or restore them to the factory default values.

For information about these settings, see Appendix A, "FreeStyle Default Settings."

❖ **To open the Default Options Configuration dialog box**

- In the Workspace Options toolbar, click **Default Options**.

–or–

a. Choose **File** to open the File Menu.

b. In the lower right corner, click **Options**.

❖ **To restore the settings of a particular page to the factory default settings**

1. Open the Default Options Configuration dialog box.
2. In the left pane, select the appropriate page of default settings.
3. In the upper right corner, click **Revert to Factory Default Values**.

❖ **To restore all settings to the factory default settings**

1. Open the Default Options Configuration dialog box.
2. In the bottom left corner, click **Revert All to Factory Default Values**.

Zooming In and Out of a Graphical View

There are several ways to zoom in, zoom out, and reset the magnification of the *x* and *y* axes of the graphical plots. You can reset some or all of the zoom settings by using the Zoom Options Toolbar, the Chromatogram View Toolbox or Spectrum View Toolbox, or the Reset Scaling command in the view's shortcut menu. You can also change the magnification level by dragging the mouse pointer across one or both axes (see "Using the Pointer to Update the Timebase or Rescale a Graph" on page 40).

Note This topic does not provide instructions for the zoom options available in the Chromatogram and Spectrum toolboxes. You can use these zoom options to incrementally zoom in and out or to zoom in on a specific range of the *x* axis by typing the start and end times separated by a hyphen.

Follow these procedures as needed:

- To zoom in or out on the x axis
- To zoom in on a specific section of the x axis
- To zoom in or out on the y axis
- To reset the zoom level of the x and y axes

❖ **To zoom in or out on the *x* axis**

1. Click the **Zoom Options** toolbar tab.

2. To decrementally zoom in on the *x* axis, click **Zoom In X**.

   Each click zooms in on a 50% smaller section of the *x* axis—that is, it decreases the displayed range by a factor of 2. For example, in the Chromatogram view, clicking Zoom In X once decreases the displayed time range for a 10 min chromatogram from 0 to 10 min to 2.5 to 7.5 min, clicking Zoom In X twice decreases the displayed time range to 3.75 to 6.25 min, and so on.

3. To incrementally undo the zoom level, click **Zoom Out X**.

   Each click zooms out to a two-fold larger section of the *x* axis.

❖ To zoom in on a specific section of the *x* axis

Drag the pointer horizontally across the specific section of the *x* axis.

❖ To zoom in or out on the *y* axis

1. Click the **Zoom Options** toolbar tab.
2. To decrementally zoom in on the *y* axis, click **Zoom In Y**.

   Each click zooms in on a 50% smaller section of the *y* axis. For example, in a Spectrum view where the *y* axis is set to relative abundance, clicking Zoom In Y once zooms in on the 0 to 50% range, clicking Zoom In Y twice zooms in on the 0 to 25% range, and so on.

3. To incrementally undo the zoom level, click **Zoom Out Y**.

   Each click zooms out to a two-fold larger section of the *y* axis.

❖ To reset the zoom level of the *x* and *y* axes

1. To display the Zoom Options toolbar, click the **Zoom Options** tab.
2. Click **Reset**.

–or–

- Right-click the view and choose **Reset Scaling**.

Adding Text and Graphic Annotations to a Graphical View

To add custom text and graphics to the graphical views, use the Text and Graphic Annotation toolbar.

Note The toolset for the Text and Graphic Annotations toolbar are the same for the Chromatogram and Spectrum views. To add labels that the application generates from the data, use the Labels area of the Display Options toolbar.

After you select the view and open the Text and Graphic Annotation Toolbar, follow these procedures as needed:

- To place a drawing object behind the plot
- To draw a line
- To draw a box or a filled box
- To add a text label
- To remove one or more labels

❖ To place a drawing object behind the plot

Click the **Behind Graph** icon,  , before you use the pointer to draw the object.

❖ To draw a line

1. To select the line color, click the **Line** icon,  , and then select a color from the graphic.
2. Do one of the following:
   - To draw a horizontal line, click the **Horizontal Line** icon, . Then drag the pointer horizontally across the view.
   - To draw a vertical line, click the **Vertical Line** icon, . Then drag the pointer vertically across the view.
   - To draw a diagonal line, click the **Diagonal Line** icon,  . Then drag the pointer diagonally across the view.

❖ To draw a box or a filled box

1. To select the border color, click the **Line** icon,  , and then select a color from the graphic.
2. Click the **Box** icon,  , or the **Filled Box** icon,  .
3. For a Filled Box, click the **Fill** icon,  , and then select a color from the graphic.
4. Click the view where you want to place the top left corner of the box, and then drag the pointer across the view to size the box. You can continue to increase or decrease the box dimensions until you release the mouse button.

❖ To add a text label

1. Click the **Add Text** icon () to activate the text box to the right of the icon.
2. Type the text in the Text Box.

   You can enter several lines of text by pressing ENTER after each line.
3. Before you place the text label, format the text as needed:

- To select the text color, click the **Fill** icon ( ).
- To add a box around the text, click the **Boxed** icon ( ) in the Text options area.
- To set up a a text label with a callout line, click the **Callout** icon ( ) in the Text Options area.
- To change the alignment of the text, click the **Left** ( ), **Center** ( ), or **Right** ( ) icon in the Text Alignment area.

4. Click the spot in a view where you want to place the text label. For a callout, drag the callout line to the item of interest.

❖ **To remove one or more labels**

In the toolbar's Clear area, click the appropriate icon.

Copying, Exporting, and Printing Graphical Images and Tabular Data

You can copy graphical views and tabular data to the Clipboard, send the contents of a Workspace or view to a printer, or export the contents of a Workspace or view to a CSV or an EMF file.

Follow these topics as needed:

- Copying an Image of a Graphical View to the Clipboard
- Copying Tabular Data to the Clipboard
- Exporting or Printing the Contents of a View or Workspace

Copying an Image of a Graphical View to the Clipboard

You can copy an image of the following views or floating window to paste into other Microsoft™ Office applications: Chromatogram view, Spectrum view, MultiSpectrum view, and Isotope Simulation window.

❖ **To copy an image of a graphical view or floating window to the Clipboard**

Right-click the view or window and choose **Copy To Clipboard**.

Copying Tabular Data to the Clipboard

You can copy the contents of the eight report views (see "Reports" on page 36) to the Clipboard.

❖ **To copy all the contents of a report view to the Clipboard**

1. Click the view.
2. Press CTRL+C.

Exporting or Printing the Contents of a View or Workspace

To print, copy, or export the contents of a view or workspace, use the Selection As button or Workspace As button, respectively, in the Exports area of the Workspace Options toolbar.

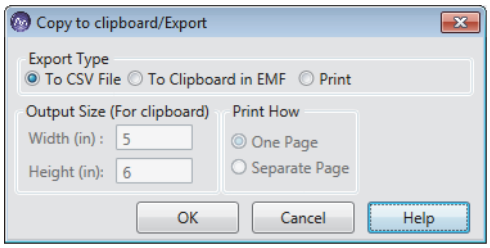
Clicking either button opens the Copy to Clipboard/Export dialog box where you select the export type, the output size for a Clipboard image, and the number of pages to print.

❖ **To export, copy, or print the contents of a view or workspace**

1. Do one of the following:
   - For the contents of a view, click the view, and then in the Workspace Options toolbar, click **Selection As**.
   - For the contents of a workspace, in the Workspace Options toolbar, click **Workspace As**.

   The Copy to Clipboard/Export dialog box opens (Figure 12).

   Figure 12. Copy to Clipboard/Export dialog box

2. In the Export Type area, select one of these options:
   - To send an enhanced metafile image to the Clipboard, select the **To Clipboard in EMF** option. Then, go to step 3.
   - To send the printing information to the selected printer, select the **Print** option. Then, go to step 4.
   - To export the data in tabular format to a CSV file, select the **To CSV File** option. Then, go to step 6.
3. For the To Clipboard in EMF option, in the Output Size (For Clipboard) area, use the Width (in.) and Height (in.) boxes to specify the size of the image.

4. For the Print option, in the Print How area, select one of the following:
   - To print the graphical views in a workspace on separate pages or all the pages in a report view, select the **Separate Page** option.
   - To print the graphical views in a workspace on one page, select the **One Page** option.
5. Click **OK**.
6. Depending on the export type, do the following:
   - For the To CSV File option, in the Export Data dialog box, select the folder location, name the file, and click **Save**.
   - For the To Clipboard in EMF option, paste the image from the Clipboard to the appropriate document.
   - For the Print option, in the Print dialog box, select the printer, the printing preferences, the page range, and the number of copies. Then, click **Print**.

Tip To create a PDF file, select the Adobe PDF printer.

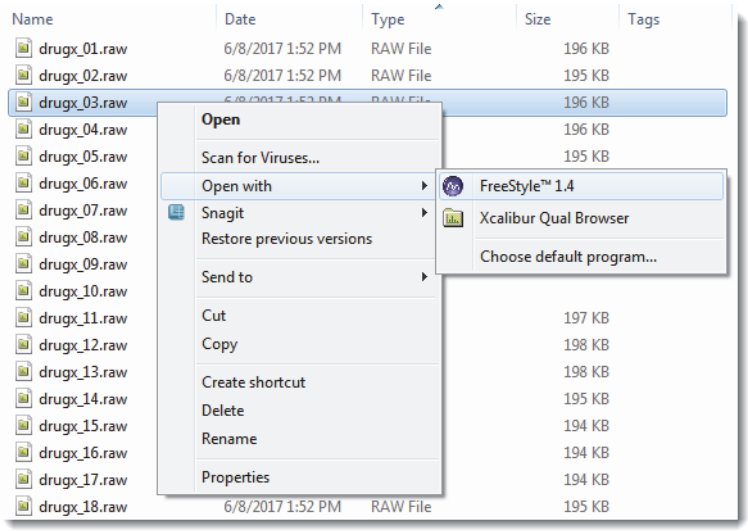
Setting FreeStyle as the Default Data Visualization Application

Currently, Thermo Fisher Scientific provides two data visualization applications: Xcalibur Qual Browser and FreeStyle.

❖ To specify FreeStyle as the default data visualization application

1. Open Windows Explorer and browse to a RAW file.
2. Right-click the file, and then choose **Open With > FreeStyle *Version*** (Figure 13).

Figure 13. Choosing FreeStyle as the default data visualization application



Toolbars

Use the FreeStyle toolbar buttons and icons to display views and to perform functions. The FreeStyle window has these toolbars:

- Workspace Options Toolbar
- Workspace Processing Toolbar
- Display Options Toolbar
- Zoom Options Toolbar
- Text and Graphic Annotation Toolbar
- Sequence Toolbar (in the Reviewing Chromatographic Data chapter)

Tip The FreeStyle window displays only the toolbars and toolbar buttons that are appropriate for the active view. For example, the Peak Detection buttons in the Workspace Processing toolbar are only available when the Chromatogram view is active, and the Library Search buttons in this toolbar are only available when a Spectrum or MultiSpectrum view is active.

The Sequence toolbar appears when you select a file on the Sequence File page.

Workspace Options Toolbar

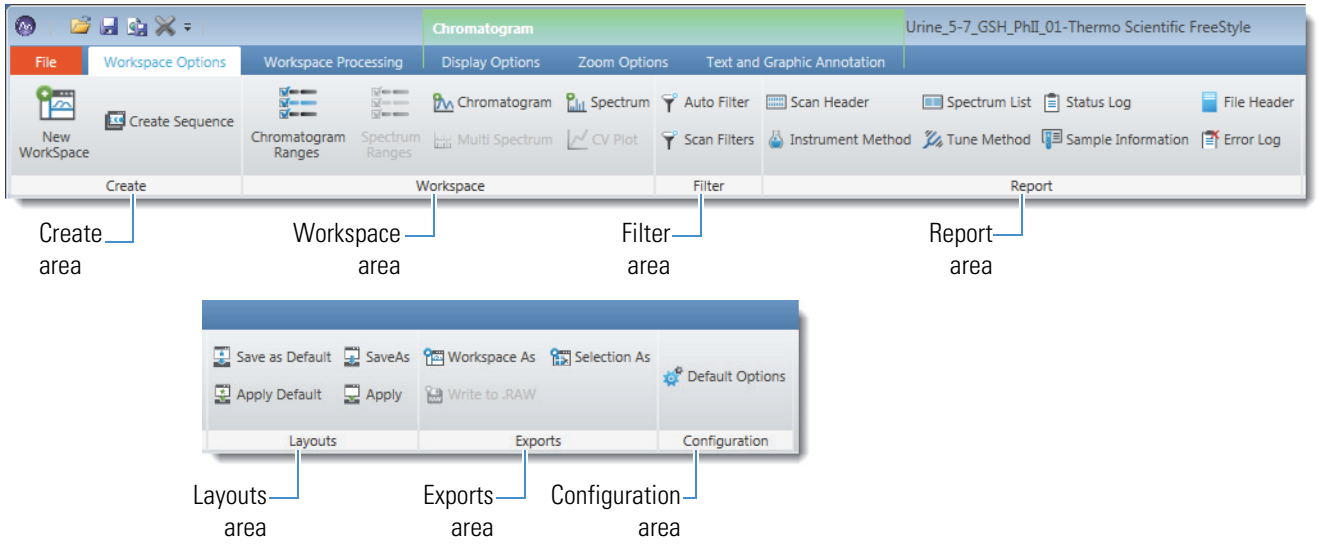
Use the buttons in the Workspace Options toolbar to create and customize workspaces.

❖ **To display the Workspace Options toolbar**

Click the **Workspace Options** toolbar tab.

Figure 14 shows the Workspace Options toolbar buttons that are available when the Chromatogram view is the active view.

Figure 14. Workspace Options toolbar for the Chromatogram view (in two parts, left and right)



Depending on the active view, these additional buttons—Spectrum Ranges, Multi Spectrum, CV Plot, and Write to .RAW—become available as follows:

- Selecting a Spectrum view or a MultiSpectrum view enables the Spectrum Ranges, Multi Spectrum, and Write to .RAW buttons.
- Opening a raw data file (RAW) with FAIMS data enables the CV Plot button.

Table 4 describes the buttons in the Workspace Options toolbar, from left to right.

Table 4. Workspace Options toolbar buttons (Sheet 1 of 3)

Button	Description
Create	
New WorkSpace	Displays the Open Raw File dialog box. You create a workspace by opening a raw data file or a sequence of raw data files. A workspace can display selected information from one or more raw data files. The workspace tab includes the file name of the raw data file when the Workspace displays information from only one raw data file.
Create Sequence	Opens the Create Sequence dialog box. Use the Create Sequence dialog box to build a sequence from raw data files.
Workspace	
Chromatogram Ranges	Opens the Chromatogram Ranges view where you can set up the chromatogram traces to display.
Spectrum Ranges	Available when a Spectrum view or a MultiSpectrum view is the active view and these views do not include a library spectrum. Displays the Spectrum Ranges dialog box, where you select which spectral traces to display in the MultiSpectrum View.

Table 4. Workspace Options toolbar buttons (Sheet 2 of 3)

Button	Description
Chromatogram	The Chromatogram view is always open. Use the Chromatogram view to work with the chromatographic peaks and to select the target time point or scan number for other views. Clicking the Chromatogram button adds another chromatogram view within the Chromatogram view. Use the Chromatogram Ranges view or the Auto Filter button to select the chromatograms of interest. Displays the Chromatogram View, where you view the chromatogram traces of the selected raw data files.
Spectrum	Displays a Spectrum View, where you view the spectrum for the chosen retention time (RT) and scan number.
Multi Spectrum	Displays the MultiSpectrum View, where you can group multiple spectra together in one view.
CV Plot	Available for raw data files with FAIMS data. Opens the CV Plot Traces dialog box, where you select the CV plots of interest.
Filter	
Auto Filter	Populates the Chromatogram view with an unfiltered plot (No Filter) and a plot for every scan filter up to the number specified on the Workspace Options page of the Default Options Configuration dialog box. Also populates the Chromatogram Ranges view with the specified number of scan filters. See "Adding Chromatogram Traces with the Auto Filter Feature" on page 46.
Scan Filters	Opens the Scan Filters page in the Info Bar (see "Using the Scan Filters Page to Display a Filtered Chromatogram" on page 54).
Report	
Scan Header	Displays the Scan Header View, where you view the scan header of the active raw data file. Click the chromatogram trace to display the scan header for the chosen retention time and scan number. See "Scan Headers and Scan Header Abbreviations" on page 266.
Spectrum List	Displays the Spectrum List View, where you view spectral peak information in a table. Click the chromatogram trace to display the spectrum list for the chosen retention time and scan number.
Status Log	Displays the Status Log View, where you view instrument readback parameters. Click the chromatogram trace to display the status log for the chosen retention time and scan number.
Sample Information	Displays the Sample Information View, where you view sample-specific information.

Table 4. Workspace Options toolbar buttons (Sheet 3 of 3)

Button	Description
Instrument Method	Displays the Instrument Method View, where you view the instrument method parameters that the instrument used to obtain the raw data file.
Tune Method	Displays the Tune Method View, where you view the tune method parameters that the instrument used to obtain the raw data file.
File Header	Displays the File Header View, where you view information from the acquisition sequence, the autosampler, and the mass spectrometer.
Error Log	Displays the Error Log View, where you view a list of error messages generated during data acquisition.
Layouts	
Save As Default	Saves the current layout as the default template file, DefaultTemplate.xml. See "Saving and Applying Layout Templates" on page 21.
Save As	Saves the current layout as an XML template file. See "Saving and Applying Layout Templates" on page 21.
Apply Default	Applies the default layout template, DefaultTemplate.xml. See "Saving and Applying Layout Templates" on page 21.
Apply	Opens the DataBrowser Templates dialog box, where you select a previously saved layout template. See "Saving and Applying Layout Templates" on page 21.
Exports	
Workspace As	Opens the Copy to Clipboard/Export dialog box, where you specify settings to print all the workspace views, export them to a CSV file, or copy them to the Clipboard (see "Exporting or Printing the Contents of a View or Workspace" on page 27).
Selection As	Opens the Copy to Clipboard/Export dialog box, where you specify settings to print the selected view, export it to a CSV file, or copy it to the Clipboard (see "Exporting or Printing the Contents of a View or Workspace" on page 27).
Write to .RAW	Opens the Export Data dialog box, where you specify settings to export a single spectrum for the active spectrum to a raw data file (see "Creating a RAW File for a Selected Spectrum" on page 124).
Configuration	
Default Options	Opens the Default Options Configuration dialog box, where you set up the default settings for the application (see "FreeStyle Default Settings" on page 249).

Workspace Processing Toolbar

The available buttons in the Workspace Processing toolbar depend on whether the Chromatogram view or Spectrum (or MultiSpectrum) view is active.

Note The following buttons are always available—Elemental Composition, Isotope Simulation, Xtract, and Library Manager.

For information about the Workspace Processing toolbar, see these topics:

- Chromatogram – Workspace Processing Toolbar
- Spectrum – Workspace Processing Toolbar

Display Options Toolbar

For information about specific Display Options toolbars, see these topics:

- Chromatogram – Display Options Toolbar
- Spectrum or Multi Spectrum – Display Options Toolbar
- Spectrum List – Display Options Toolbar

Zoom Options Toolbar

Use the buttons in the Zoom Options toolbar to adjust the display of chromatograms or spectra.

❖ To display the Zoom Options toolbar

1. Click the Chromatogram View, Spectrum View, or MultiSpectrum View to make it the active view.
2. Click the **Zoom Options** toolbar tab.

Figure 15 shows the Zoom Options toolbar, and Table 5 describes the toolbar buttons.

Figure 15. Zoom Options toolbar

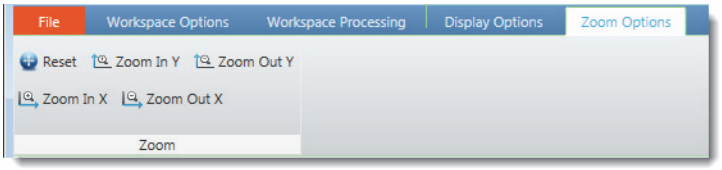


Table 5. Zoom Options toolbar buttons

Button	Description
Reset	Restores the data display to the full range of the *x* axis and *y* axis.
Zoom In Y	Zooms in on the *y* axis by a factor of two from the current baseline to show more detail. For example, you can change the *y*-axis range from 0–100 to 0–50.
Zoom Out Y	Zooms out from the *y* axis by a factor of two from the current baseline to show more data. For example, you can change the *y*-axis range from 0–25 to 0–50.
Zoom In X	Zooms in on the *x* axis by a factor of two from the center to show more detail. For example, change the *x*-axis range from 0–20 to 5–15.
	Note The first time you click Zoom In X, the application zooms in on the full time range of the active chromatogram plot. The plot's time range depends on the selected scan filter and can be shorter than the data acquisition time for the raw data file. A horizontal scrollbar automatically appears below the *x* axis of the graphical view when you zoom in.
Zoom Out X	Zooms out from the *x* axis by a factor of two from the center to show more data. For example, change the *x*-axis range from 7.5–12.5 to 5–15.

Text and Graphic Annotation Toolbar

Use the buttons and icons in the Text and Graphic Annotation toolbar to annotate chromatograms and spectra with text, lines, and boxes. You can also select color, text alignment, and various text options.

For information about working with the Text and Graphic Annotation toolbar, see "Adding Text and Graphic Annotations to a Graphical View" on page 24.

❖ To display the Text and Graphic Annotation toolbar

1. Click the Chromatogram View, Spectrum View, or MultiSpectrum View to make it the active view.
2. Click the **Text and Graphic Annotation** toolbar tab.

Figure 16 shows the Text and Graphic Annotation toolbar, and Table 6 describes the toolbar buttons and icons.

Figure 16. Text and Graphic Annotation toolbar

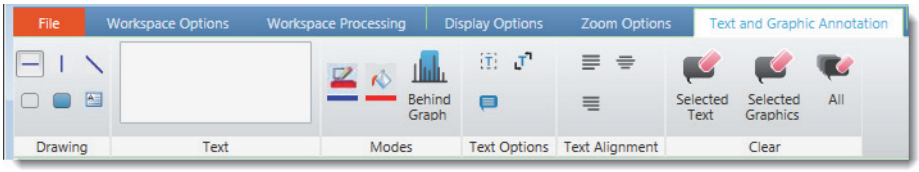


Table 6. Text and Graphic Annotation toolbar buttons and icons (Sheet 1 of 2)

Button/Icon	Description
Drawing	
Horizontal Line	Draws a horizontal line when you drag the mouse pointer horizontally.
Vertical Line	Draws a vertical line when you drag the mouse pointer vertically.
Diagonal Line	Draws a diagonal line when you drag the mouse pointer diagonally.
Box	Draws an empty box when you drag the mouse pointer diagonally.
Filled Box	Draws a box filled with the fill color when you drag the mouse pointer diagonally.
Add Text	Annotates with a text label (see "To add a text label" on page 25).
Text	
Text Box	Type the text for a label in this box (see "To add a text label" on page 25).
Modes	
Line	Opens the color box to select the line color.
Fill	Opens the color box to select the fill color and the text color.
Behind Graph	Places text, lines, and boxes behind the chromatogram traces or mass spectra.
Text Options	
Boxed	Draws a box around the text label. Click before you place the text in a workspace view.
Rotated Text	Rotates the text 90 degrees counterclockwise. Click before you place the text in a workspace view.
Callout	Adds a callout line to the text label.

Table 6. Text and Graphic Annotation toolbar buttons and icons (Sheet 2 of 2)

Button/Icon	Description
Text Alignment	
Left	Aligns the text to the left of the text container. Click before you place the text in a workspace view.
Center	Aligns the text at the center of the text container. Click before you place the text in a workspace view.
Right	Aligns the text to the right of the text container. Click before you place the text in a workspace view.
Clear	
Selected Text	Deletes text annotations when you drag the pointer over them.
Selected Graphics	Deletes graphic annotations when you drag the pointer over them.
All	Deletes all text and graphic annotations.

Views

A view is a pane in the Workspace. Use the FreeStyle views to display results and to open lists for entering parameters or selecting options. You can drag views to arrange them within the workspace of the application.

To arrange the views, update the timebase, and rescale graphs, follow these topics:

- Arranging Views
- Using the Pointer to Update the Timebase or Rescale a Graph

The FreeStyle window displays these individual views (by type in alphabetical order) inside the Workspace view. In addition, Table 7 lists related topics for detailed information and the type of data the view contains for export.

Table 7. Views (Sheet 1 of 2)

View	Topic	Copy as
Primary		
Chromatogram	"Chromatogram View" on page 78	Image
Chromatogram Ranges	"Chromatogram Ranges View" on page 80	Tabular text
Multi Spectrum	"MultiSpectrum View" on page 144	Image
Spectrum	"Spectrum View" on page 142	Image
Reports		

Table 7. Views (Sheet 2 of 2)

View	Topic	Copy as
Error Log	"Error Log View" on page 234	Tabular text
File Header	"File Header View" on page 231	Tabular text
Instrument Method	"Instrument Method View" on page 227	Tabular text
Sample Information	"Sample Information View" on page 231	Tabular text
Scan Header	"Scan Header View" on page 223	Tabular text
Spectrum List	"Spectrum List View" on page 224	Tabular text
Status Log	"Status Log View" on page 226	Tabular text
Tune Method	"Status Log View" on page 226	Tabular text
Data processing – Elemental composition		
Elemental Composition Results	"Elemental Composition Results View" on page 162	Tabular text
Data processing – Chromatographic peak detection and integration		
Peaks List	"Peaks List View" on page 87	Tabular text
Data processing – Library searches		
Chemical Structure	Chemical Structure View	Image
Compounds list	"mzVault Search Results View" on page 180	Tabular text
Chemical Structure	Chemical Structure View	Image
Compounds list	"NIST Search Results View" on page 177	Tabular text
Data processing – Xtract deconvolution		
Deconvolved Spectrum	"Deconvolved Spectrum View" on page 242	Image
Xtract Results	"Xtract Results View" on page 243	Tabular text

Arranging Views

You can move or reposition the views to change the layout of the workspace, and then save the modified layout to a template. For information about templates, see "Saving and Applying Layout Templates" on page 21.

❖ To arrange views with the mouse

1. Drag the title bar of the view that you want to move to a second view until the view arranger tool appears.

2. Do one of the tasks in the following table.

Task	Procedure
Move the first view above the second view.	Drag the title bar to the up icon,  .
Move the first view below the second view.	Drag the title bar to the down icon,  .
Move the first view to the left of the second view.	Drag the title bar to the left icon,  .
Move the first view to the right of the second view.	Drag the title bar to the right icon,  .
Make both views tabbed.	Drag the title bar to the tabs icon,  . The application displays the first view and creates a tab for the second view.

Figure 17 shows the Spectrum view being dragged to the right of the Chromatogram view, and Figure 18 shows the end result.

Figure 17. Spectrum view being dragged to the right of the Chromatogram view

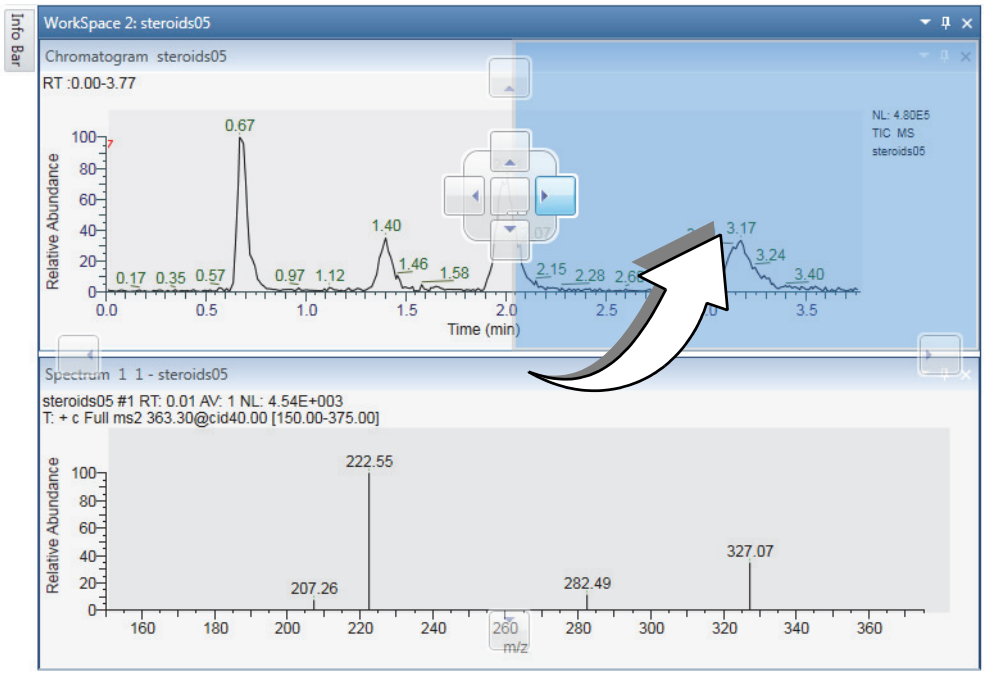
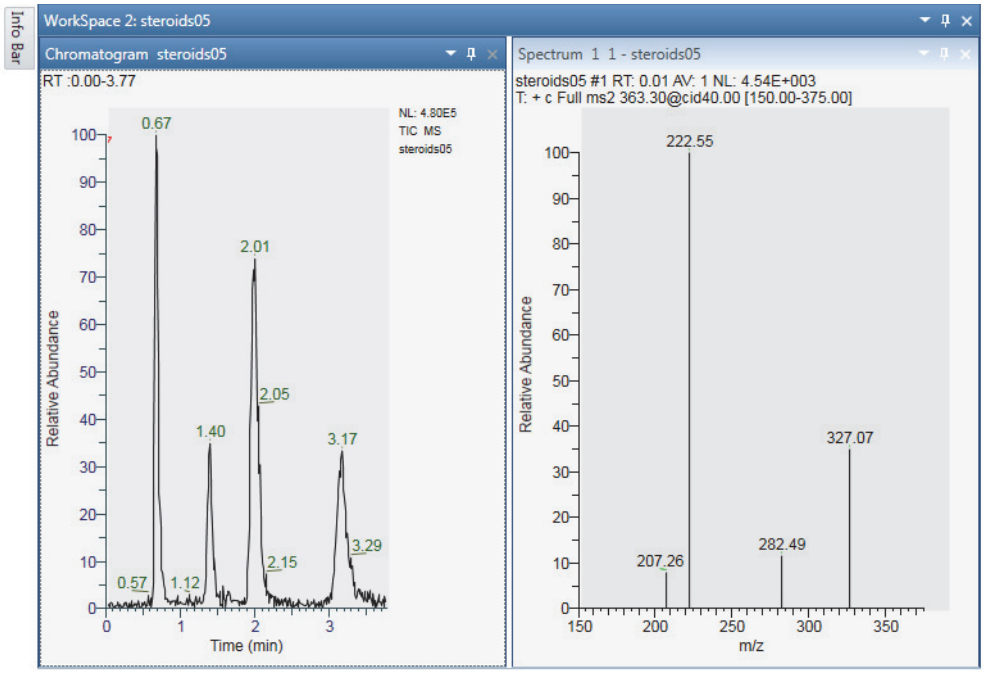


Figure 18. Result of repositioning the Spectrum view to the right of the Chromatogram view



❖ To reposition a view

Use the shortcut menu commands as you would to reposition the Workspace view. For details, see "To reposition the Workspace view" on page 18.

❖ **To close a view**

Click the **Close** icon, , in the view's title bar, or right-click the view and choose **Close**.

Note You cannot close the Chromatogram View. It is a permanent view—that is, you can apply the auto-hide option to hide it, but you cannot dismiss it.

Using the Pointer to Update the Timebase or Rescale a Graph

Within the Chromatogram View, Spectrum View, or MultiSpectrum View, use the pointer in three ways:

- To select a retention time or scan number, click the chromatogram trace at that retention time or scan number.
- To select a range, drag a line parallel to any axis.
- To select an area, drag in any diagonal direction.

Pointer actions scale the view according to the dimensions of the dragged line or area (see Table 8).

Table 8. Pointer effects

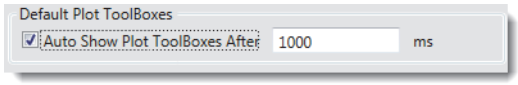
Pointer action	Effect
Drag parallel to the *x* axis	Rescales the graph to the selected *x*-axis range. The *y*-axis range might rescale depending on the selected normalization display options.
Drag parallel to the *y* axis	Rescales the graph to the selected *y*-axis range, with the same *x*-axis range.
Drag diagonally over the *x* and *y* axes	Rescales the graph to the selected *x*- and *y*-axes ranges.

In addition, when you select a retention time (RT) in the Chromatogram View, the application automatically synchronizes to display data at that selected RT for the Spectrum View, the active spectrum in the MultiSpectrum View, the Scan Header View, and the Status Log View in the Workspace.

Selecting How You Open the Floating Toolboxes

You can open a floating toolbox in the Chromatogram and Spectrum (or MultiSpectrum) views to perform various functions such as averaging scans and adding chromatographic peaks. For information about these toolboxes, see "Chromatogram View Toolbox" on page 91 and "Spectrum View Toolbox" on page 145.

How you open the floating toolboxes depends on whether the Auto Show Plot Toolboxes After check box is selected on the Workspace Options page. By default, this check box is selected, and a floating toolbox opens when you pause the pointer in the Chromatogram view or point to an *m/z* value in the Spectrum (or MultiSpectrum) view. If you clear this check box, you can only open the toolbox by choosing Show Toolbox from the view's shortcut menu.



To change the way you open the toolboxes, follow these procedures as needed.

❖ **To open the Workspace Options page**

See "To display the Default Workspace Options page" on page 258.

❖ **To make sure that pausing the pointer opens the toolbox**

1. On the Workspace Options page, select the **Auto Show Plot Toolboxes After** check box, and then type a pause time for the pointer from **100** to **2000** ms.
2. Click **Save**.

   With this setting, you can open the toolbox by pausing the pointer in the view or by right-clicking it and choosing Show Toolbox.

❖ **To open the toolbox by using the Show Toolbox command only**

1. On the Workspace Options page, clear the **Auto Show Plot Toolboxes After** check box.
2. Click **Save**.

   With this setting, you can only open the toolbox by right-clicking the view and choosing Show Toolbox.

Info Bar Pages

On the far left of the FreeStyle window, use the pages of the Info Bar to select detector, sequence, or filter options. You can also set the parameters for peak detection, isotope simulation, elemental composition, searching mass spectrum libraries, or applying the Xtract deconvolution algorithm.

The following pages appear in the Info Bar:

- Chromatogram-range pages: Only one of these two pages is available at any time.
  - Detector Type Page
  - Trace Type Page
- Scan Filters Page

- Elemental Composition Page
- Isotope Simulation Page
- Modifying an mzVault Search from the mzVault Search Page
- MSn Browser Page
- Modifying a NIST Search from the NIST Search Page
- Peak detection pages:
  - Avalon Peak Detection Page
  - Genesis Peak Detection Page
  - ICIS Peak Detection Page
  - PPD Peak Detection Page
- Sequence File Page
- Xtract Page

❖ To reposition an Info Bar page

Use the Auto Hide and Dockable shortcut menu commands as you would to reposition the Workspace view. For details, see "To reposition the Workspace view" on page 18.

Tip The Floating command to reposition a page as a floating window and the Close command to close a page are not available in the shortcut menu.

3

Reviewing Chromatographic Data

To review chromatographic data, follow these topics.

Note For information about zooming in and out of, adding images and annotations to, and copying an image of the Chromatogram view, see Chapter 2, "Using the FreeStyle Window."

Contents

Adding and Deleting Chromatogram Traces

After you open a raw data file (see "Opening Raw Data Files or Sequence Files" on page 15), you can display multiple chromatogram traces in the Chromatogram View. By default, the chromatogram traces are stacked. As you add more traces, each successive trace appears in a different color until the view contains eight traces. The color order is as follows: (1) black, (2) brick-red, (3) green, (4) blue, (5) light orange, (6) magenta, (7) blue-green, and (8) gray. Successive chromatogram traces appear in black.

The application supports the following types of chromatogram traces (Table 9). To select the TIC, BPC, Mass Range, or Neutral Fragment trace type, use the Chromatogram Ranges View.

Table 9. Supported chromatogram traces

Trace type	Description
Total ion current (TIC)	A trace of the signal of all ions combined as a function of the retention time or scan number.
Base peak (BPC)	A trace of the most intense ion signal as a function of the retention time or scan number.
Mass range	A trace of the ion signal of all ions in one or more mass ranges as a function of the retention time or scan number.
Extracted ion (EIC or XIC)	A trace of the signal of a single ion as a function of the retention time or scan number.
Neutral fragment	A trace of the ion signal of all ions that produce a specific neutral fragment as a function of the retention time or scan number.

Follow these topics as needed:

- Adding Chromatogram Traces Manually
- Adding Chromatogram Traces with the Auto Filter Feature
- Deleting Chromatogram Traces

To add an EIC trace, see "Using the Spectrum Toolbox Tools to Display an EIC Plot" on page 117.

Adding Chromatogram Traces Manually

To manually add chromatogram traces to the Chromatogram View, follow these procedures as needed:

- To add a chromatogram trace by using the Chromatogram button
- To add a chromatogram trace by using the shortcut menu command

- To add chromatogram traces by using the Chromatogram Ranges view
- To add a chromatogram trace from a raw data file in a sequence list

Note To automatically populate the Chromatogram view with traces from multiple scan filters, see the next topic "Adding Chromatogram Traces with the Auto Filter Feature."

❖ To add a chromatogram trace by using the Chromatogram button

1. Click the **Workspace Options** toolbar tab.
2. In the Workspace Options toolbar, click **Chromatogram**.

   A copy of the active chromatogram appears below the active chromatogram.

❖ To add a chromatogram trace by using the shortcut menu command

Right-click the Chromatogram view and choose **Insert Chromatogram**.

A copy of the active chromatogram appears below the active chromatogram.

❖ To add chromatogram traces by using the Chromatogram Ranges view

1. If the Chromatogram Ranges View is closed, click **Chromatogram Ranges** in the Workspace Options toolbar.
2. In the last row, select the check box in the Display column.

   The application populates the row with a copy of the currently selected row in the list, and a duplicate chromatogram trace appears in the Chromatogram view. To fill multiple rows, see "Using the Fill Down Feature" on page 85. To populate the Chromatogram Ranges view with ranges from a spreadsheet file, see "To specify the ranges of the chromatogram traces by using data in a spreadsheet file" on page 53.

❖ To add a chromatogram trace from a raw data file in a sequence list

1. Open an existing sequence file (SLD), or create a temporary sequence list by clicking **Create Sequence** in the Workspace Options toolbar and selecting a set of raw data files.
2. To display the Sequence toolbar, click the **Sequence File** tab in the Info Bar.
3. Click **New Trace**.
4. Click a raw data file in the sequence.

   The application adds the raw data file to the chromatogram ranges list and displays the new chromatogram trace in the Chromatogram View.

Adding Chromatogram Traces with the Auto Filter Feature

Use the Auto Filter button to repopulate a Chromatogram view with these possible characteristics:

- A plot showing the chromatogram without any scan filters
- Plots for each scan filter applied to the chromatogram up to the number of scan filters in the data file or the maximum number specified on the Default Workspace Options Page, whichever is fewer

Note To manually add and delete traces in the Chromatogram view, see "Adding and Deleting Chromatogram Traces" on page 44.

❖ To specify the maximum number of chromatogram traces to display

1. Open the Workspace Options page (see "Default Workspace Options Page" on page 258).
2. In the #Number of Auto Filter box, type an integer from **1** to **500**.

   The default maximum number of traces is 8.

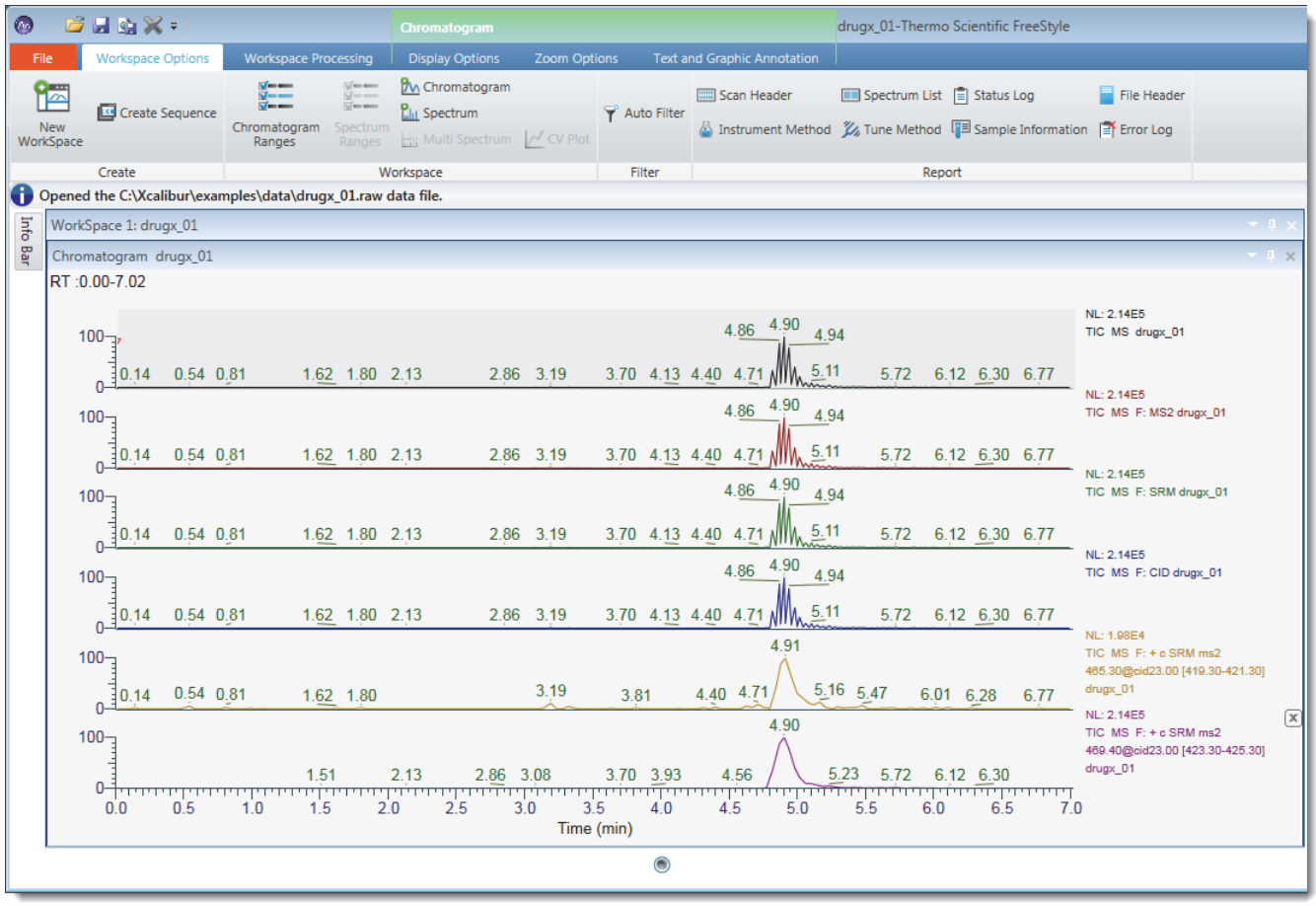
❖ To automatically add plots for all the scan filters to a Chromatogram view

In the Workspace Options Toolbar, click **Auto Filter**.

The application populates the Chromatogram view with an unfiltered plot (No Filter) and a plot for every scan filter up to the number specified on the Default Workspace Options page of the Default Options Configuration dialog box.

Figure 19 shows the result of clicking the Auto Filter button for drugx_01.raw in the *drive*:\Xcalibur\examples\data folder.

Figure 19. Chromatogram view for a data file with six scan filters and one unfiltered TIC



Deleting Chromatogram Traces

To delete chromatogram traces or undo the most recent deletion of a chromatogram trace in the Chromatogram View, follow these procedures as needed:

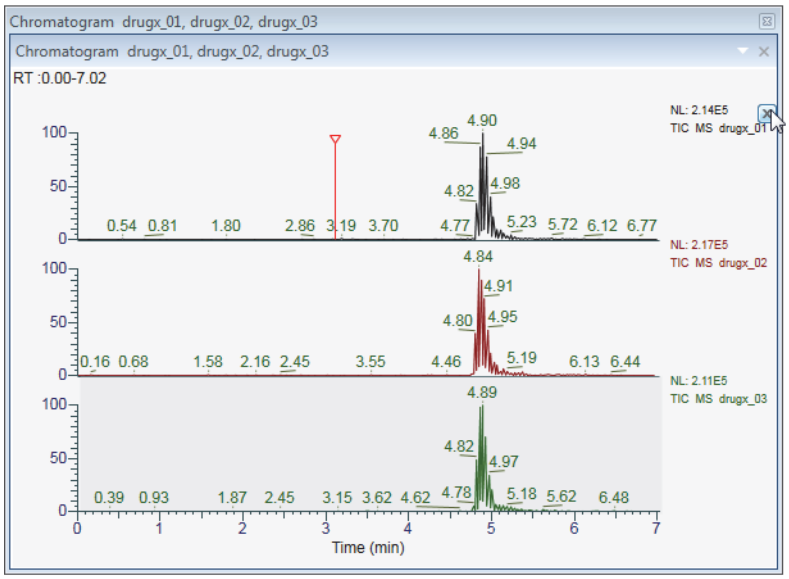
- To delete a trace by clicking the delete icon
- To delete a trace by using the Chromatogram Ranges view
- To undo the most recent deletion of a chromatogram trace

❖ To delete a trace by clicking the delete icon

1. In the Chromatogram view, point to the name of the trace that you want to delete.

   Figure 20 shows a Chromatogram view with three stacked traces. The pointer is on the delete icon, ⊠, for the first trace.

Figure 20. Chromatogram view with three stacked traces



2. Click the delete icon, , to delete the trace.

Note When the view has only one trace, you cannot delete the trace.

❖ **To delete a trace by using the Chromatogram Ranges view**

- Clear the trace's associated check box in the Display column.

–or–

a. Select the row, and then press the Delete key.

b. At the prompt, click **Yes**.

❖ **To undo the most recent deletion of a chromatogram trace**

Right-click the Chromatogram view and choose **Undo Delete Chromatogram**.

Defining the Range of a Chromatogram Trace

The following parameters define the range for a chromatogram trace—raw data file, detector or instrument status measurement, scan filter, trace type, and mass range for the Mass Range trace type. After you open a raw data file (see "To open a raw data file or a sequence file" on page 15), you use the Chromatogram Ranges View to select the parameter settings for each chromatogram plot.

You can copy the rows in the Chromatogram Ranges View to the Clipboard, and you can copy the data from a CSV file into the Chromatogram Ranges view.

To define the chromatogram ranges, follow these topics as needed:

- Defining Chromatogram Ranges Manually
- Defining Chromatogram Ranges by Using a Spreadsheet File
- Setting Up Instrument Status Traces with the MS Trending Feature

Defining Chromatogram Ranges Manually

To specify the range for each chromatogram trace manually, follow these procedures as needed:

- To specify the ranges of the chromatogram traces manually
- To specify the mass range by entering a chemical formula or peptide sequence
- To specify smoothing for a plot
- To specify reference (fixed) plots

For information about setting a delay time or using the plot operators, see "Chromatogram Ranges View" on page 80. For information about adding multiple rows with the same settings, see "Using the Fill Down Feature" on page 85.

❖ To specify the ranges of the chromatogram traces manually

1. Open a raw data file.
2. In the Workspace Options toolbar, click **Chromatogram Ranges**.

   Note Each row in the Chromatogram Ranges view defines one trace in the Chromatogram view.

3. In the Chromatogram Ranges view, do the following for each trace:
   a. If the file of interest is not listed, in the File Name column, browse to and select the file.
   b. In the Detector Type column, select one of the following from the list of available detectors:
      - MS—Mass spectrometry data
      - MS Trending—Instrument status readings for the mass spectrometer
      - UV—Data from an analog detector
      - PDA—Wavelength scan data from a photodiode array detector
      - A/D Card—Data from an analog-to-digital converter

Note The raw data files generated by an LC/MS or LC/MS/MS experiment typically contain multiple scan filters to optimize data collection. See Appendix B, "Scan Filters and Scan Headers." Use the Chromatogram Ranges view or the Filter page of the Info bar to select a scan filter.

c. For MS data, select a scan filter from the Filter list or from the Scan Filter page.

Tip To display the Scan Filter page in the Info Bar, in the Workspace Options toolbar, click **Scan Filters**.

d. In the Trace Type column, do one of the following:

- For UV data, select the channel.
- For PDA data, select one of the following to determine the signal intensity for each measured time point:
  - Total Scan—Plots the total signal.
  - Wavelength Range—Plots the total signal for the wavelength range that you specify in the Ranges box.
  - Spectrum Maximum—Plots the maximum signal.
- For A/D data, select the card.
- For MS data, select **TIC**, **Mass Range**, **Base Peak**, or **Neutral Fragment** (see "Mass Spectra" on page 1).
- For MS Trending data, select the status readback.

Note Entering a chemical formula or peptide sequence in the Chemical Formula column automatically changes the trace type to Mass Range and populates the Range column with a calculated *m/z* value.

e. To specify a mass range, do the following:

- For MS data and the Mass Range, Base Peak, or Neutral Fragment trace type, specify the mass range by doing one of the following:
  - In the Ranges box, type the mass range. Use a hyphen to define the mass range.

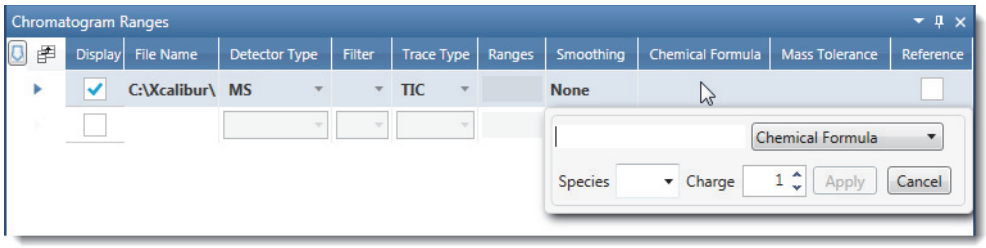
  –or–

  - See the next procedure "To specify the mass range by entering a chemical formula or peptide sequence."
- For PDA data and the Wavelength Range trace type, in the Ranges box, type the wavelength range. Use a hyphen to define the wavelength range.

f. For the Mass Range trace type, in the Mass Tolerance column, specify the mass tolerance and the units.

❖ To specify the mass range by entering a chemical formula or peptide sequence

1. In the Chemical Formula column, click the table cell to open a dialog box.



2. Select **Chemical Formula** or **Peptide**.
3. Type a chemical formula or a peptide sequence.

   Use the IUPAC nomenclature (periodic table symbols) for chemical formulas. Use the one-letter amino acid abbreviations for peptide sequences. See Appendix C, "One- and Three-Letter Abbreviations for Amino Acid Residues."
4. Select an adduct species and a charge.
5. Click **Apply**.

   The application populates the Chemical Formula column with the specified formula or peptide sequence. It also populates these columns:

   - Trace Type: Mass Range
   - Ranges: *m/z* value of specified ion
   - Mass Tolerance: 0.05 amu for ion trap data or 5 ppm for FTMS data

   The EIC trace for the specified mass range appears in the Chromatogram view.

❖ To specify smoothing for a plot

1. In the Smoothing column, select one of these smoothing algorithms: **None**, **Gaussian**, or **Moving Mean**.
2. For Gaussian and Moving Mean smoothing, select the smoothing level from a list of odd integers: **3**, **5**, **7**, **9**, and **11**.

   The smoothing level increases as the selected value increases.

❖ To specify reference (fixed) plots

> **Note** A reference plot is a fixed plot—that is, the application does not replace a reference plot when you compare raw data files in a sequence set.

Select the check box in the Reference column.

Setting Up Instrument Status Traces with the MS Trending Feature

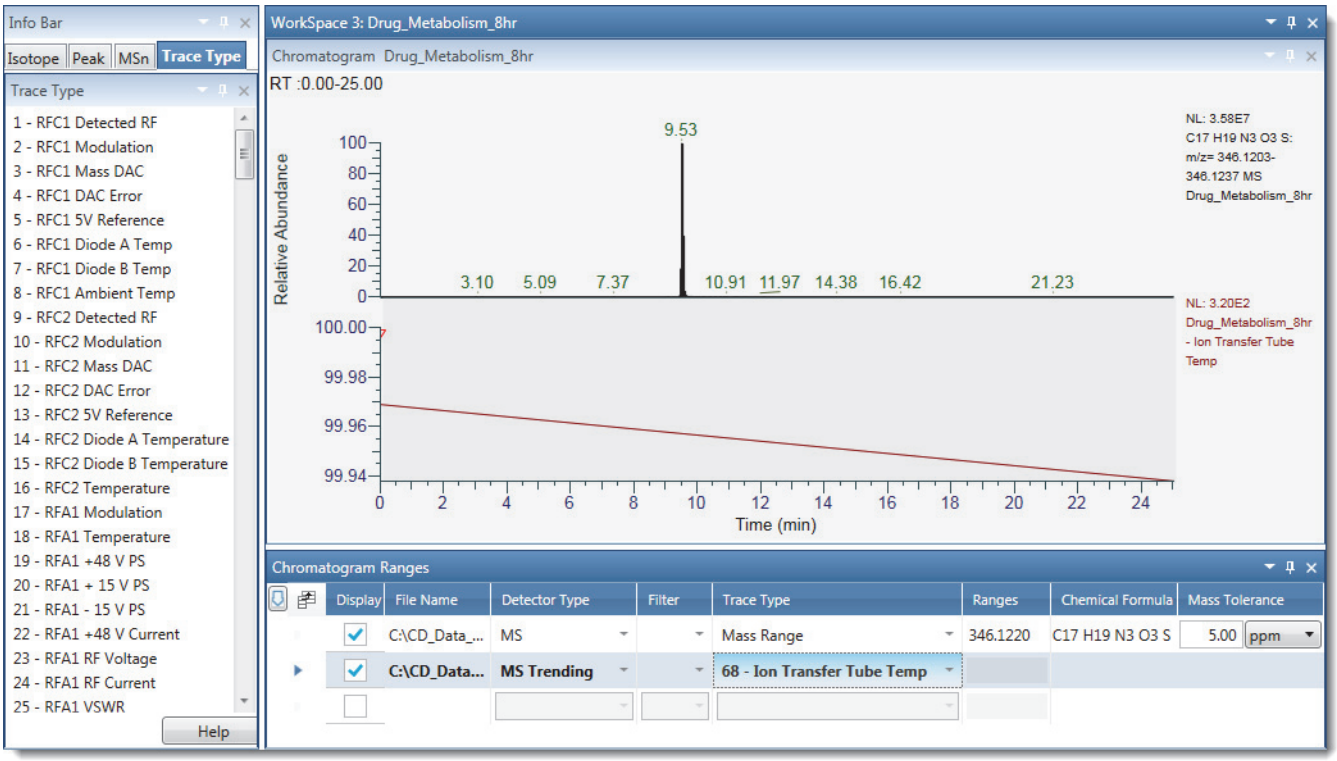
In the FreeStyle application, you can set up traces for the MS detector's status readbacks and compare these traces to possible disturbances in the chromatographic data.

❖ To add instrument status traces to the Chromatogram view

1. Open the Chromatogram Ranges View.
2. For each status readback of interest, do the following:
   a. Add a plot to the Chromatogram view (see "To add a chromatogram trace by using the Chromatogram button" on page 45 or "To add chromatogram traces by using the Chromatogram Ranges view" on page 45).
   b. In the Detector Type list of the Chromatogram Ranges view, select **MS Trending**.
   c. In the Trace Type list, select a readback parameter.

Figure 21 shows the ion transfer tube temperature readback as a function of time.

Figure 21. Status readback displayed in the Chromatogram view



Defining Chromatogram Ranges by Using a Spreadsheet File

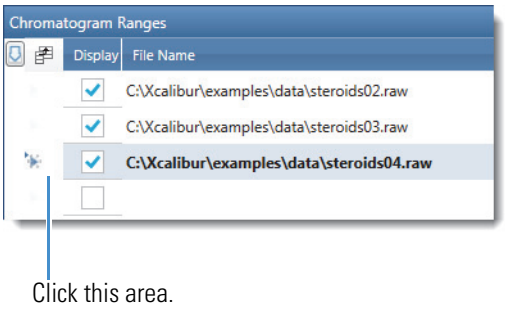
For information about editing the columns in the Chromatogram Ranges view, see "Chromatogram Ranges View" on page 80.

To add defined chromatograms by using the data in a spreadsheet file, follow this procedure.

❖ **To specify the ranges of the chromatogram traces by using data in a spreadsheet file**

1. Copy rows from the Chromatogram Ranges view to a spreadsheet file as follows:
   a. In the Workspace Options toolbar, click **Chromatogram Ranges**.
   b. Click in the area to the left of a row to select the row to copy (Figure 22).

      Figure 22. Example pointer location to copy a row

      Tip Use the CTRL key to select non-adjacent rows. Use the SHIFT key for adjacent rows, or drag the pointer across a group of rows.

   c. Press CTRL+C.
   d. Open the Excel file and select the starting cell that you want to paste into.
   e. Press CTRL+V, and then save the file.

      In the Excel file, the pasted rows appear to the right and down from the starting cell.

   Tip To copy the entire contents of the Chromatogram Ranges view to a CSV file, click **Selection As** in the Workspace Options toolbar.

2. Open the CSV file and edit its contents. Do not change the column format.
3. Paste rows from a spreadsheet file into the Chromatogram Ranges view, as follows:
   a. In the spreadsheet file, select the rows that you want to copy. Make sure that this block is highlighted and has the same number of columns as in the Chromatogram Ranges View.
   b. Click **Copy** or press CTRL+C.
   c. In the Chromatogram Ranges view, select the row where you want to paste the data.

d. Press CTRL+V.

The FreeStyle application overwrites the selected row and the rows below it with the copied rows.

Using the Scan Filters Page to Display a Filtered Chromatogram

Use the Scan Filters Page or the Filters column of the Chromatogram Ranges View to display filtered chromatograms.

❖ **To display a filtered chromatogram**

1. Open the raw data file of interest.
2. In the Workspace Options toolbar, in the Filter area, click **Scan Filters**.

   The Scan Filters page appears in the Info Bar. By default, the Time Range (min) box contains an asterisk and the Track check box is clear. The asterisk means that the time range is set to the full length of the acquisition time for the raw data file.
3. (Optional) To limit the displayed time range to a portion of the acquisition time, do one of the following:

   - In the Time Range (min) box, type the retention-time range as follows:
     Start time-End time

   –or–

   - Select the **Track** check box, and then in the Chromatogram view, drag the pointer across the retention time of interest.
4. Select a filter in the filter list.

   The filtered chromatogram appears in the Chromatogram view.

Setting Up the Display Options for a Chromatogram Trace

For the Chromatogram View, you can use the buttons in the Chromatogram – Display Options Toolbar to stack or overlay chromatogram traces, format and label the chromatogram peaks and axes, specify how the application normalizes the chromatogram traces, and change the *y*-axis scale.

Note Except for the Decimal Places button, all the buttons and icons are available on the Chromatogram – Display Options toolbar. The Decimal Places button becomes available after you add the Retention Time label to your chromatograms.

Although the Peak Area, Signal To Noise, and Height buttons are always available, the application only adds these labels to integrated peaks.

To customize the display options for chromatogram plots, see these topics:

- Formatting Chromatogram Plots
- Labeling Chromatogram Peaks or Local Maxima
- Normalizing Chromatogram Traces
- Changing the Y-Axis Scale of the Chromatogram View

Formatting Chromatogram Plots

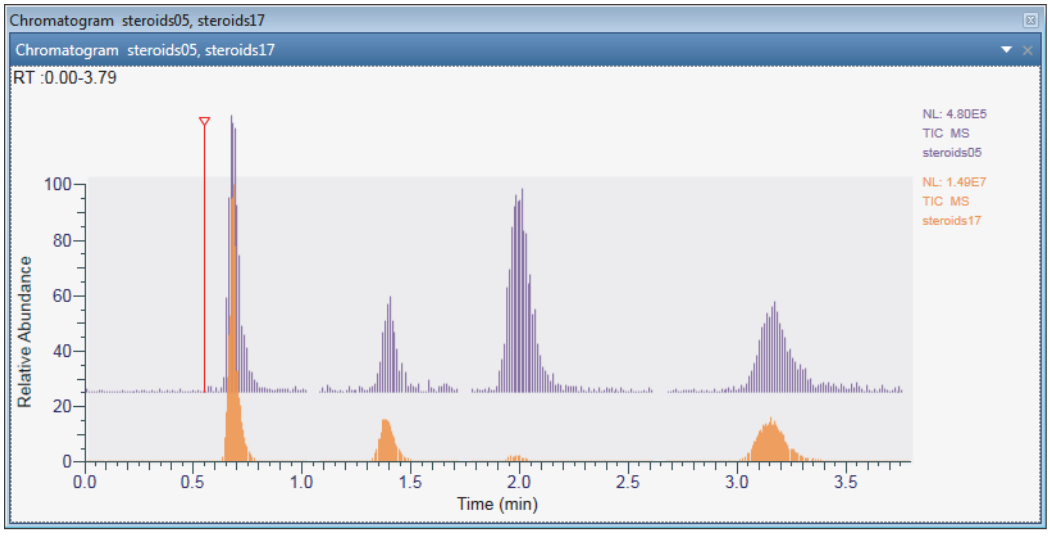
Use the Format area of the Chromatogram – Display Options Toolbar to format the chromatogram plots. You can display chromatogram traces as point-to-point graphs (line graphs) or as discrete sticks for each scan. When the Chromatogram view contains multiple plots, you can stack the plots or overlay them.

With the default factory settings, the application displays multiple traces as stacked line graphs, with the top graph plotted in black. The Stack, Overlay, Point-to-Point, and Stick format options apply to all the plots. To select different colors for overlaid plots, select the color for each active plot before you overlay them. Use the Elevation slider to change the percentage overlay of the plots. Setting the slider full left overlays the traces to the same elevation on the *y* axis. Use the Skew slider to offset the *x* axis of the plots. You cannot offset the *x* axis when the traces are 100% overlaid to the same elevation on the *y* axis.

For information about all the display options, see "Setting Up the Display Options for a Chromatogram Trace" on page 54.

Figure 23 shows two overlaid stick plots.

Figure 23. Overlaid stick plots



Labeling Chromatogram Peaks or Local Maxima

Use the Labels section of the Chromatogram – Display Options Toolbar to label the chromatogram traces. When using the default factory layout, the application labels the retention time of each localized maxima in the chromatogram plots to two decimal places. When you apply a peak detection algorithm or manually draw the chromatographic baseline, the application only labels the retention time of the detected peaks.

For information about all the display options, see "Setting Up the Display Options for a Chromatogram Trace" on page 54.

To label chromatogram traces, follow these procedures as needed:

- To add the peak area, peak height, and signal-to-noise labels to each detected peak
- To remove a label
- To change the number of decimal places for the numeric labels
- To change the labeling threshold

❖ To add the peak area, peak height, and signal-to-noise labels to each detected peak

1. Run a peak detection algorithm or manually draw the chromatographic baseline.
2. In the Chromatogram – Display Options toolbar, click the following buttons:
   - To add the peak area label, click **Peak Area**.

     Note The letters *MA* or *AA* next to the value indicate manual integration or automatic integration, respectively.

   - To add the peak height label, click **Height**.

     Note The letters *MH* or *AH* next to the value indicate manual integration or automatic integration, respectively.

   - To add the signal-to-noise label, click **S/N Signal To Noise**.

     Note The Avalon peak detection algorithm does not calculate a signal-to-noise ratio. For the Genesis and ICIS peak detection algorithms, you can choose to report the root mean square (RMS) noise values. For manual peaks, the application calculates the RMS signal-to-noise ratio.

     The letters *SN* next to the value indicate that it is a signal-to-noise ratio.

❖ To remove a label

In the Chromatogram – Display Options toolbar, click the associated button.

❖ **To change the number of decimal places for the numeric labels**

Click **Decimal Places** and select a value from **0** to **5** from the list.

❖ **To change the labeling threshold**

In the Label Threshold box, type a percentage from **0** to **100**.

For a value of 0%, the application labels all the trace maxima or detected peaks. At a value of 100%, the application labels only the highest data point on the trace. When the peak apex of a detected peak falls below this height, the application does not label the peak.

Normalizing Chromatogram Traces

Use the Normalization area of the Chromatogram – Display Options Toolbar to normalize chromatogram traces. You can apply local or global normalization or turn off normalization.

Changing the normalization type changes the *y*-axis scaling:

- Local normalization scales each trace independently to its largest peak in the displayed time range.
- Global normalization scales each trace to the largest peak across the traces in the displayed time range.
- Turning off normalization scales each trace to the largest peak across the traces in the full time range, regardless of the displayed time range.

Follow these topics as needed:

- Applying Local Normalization
- Applying Global Normalization
- Turning Off Normalization
- Changing the Y-Axis Range

For information about all the display options, see "Setting Up the Display Options for a Chromatogram Trace" on page 54.

Applying Local Normalization

When you apply local normalization (the default setting), the application normalizes the chromatogram traces so that, within the displayed time range, the most intense peak for each trace is set to 100 percent of the *y*-axis scale.

❖ **To apply local normalization**

1. Click the Chromatogram view to make it active.
2. In the Normalization area of the Chromatogram – Display Options Toolbar, click **Local**.

Figure 24 shows an example of local normalization. Each trace is independently scaled to its largest peak in the displayed time range, which is the full time range.

Figure 24. Local normalization with full time range displayed

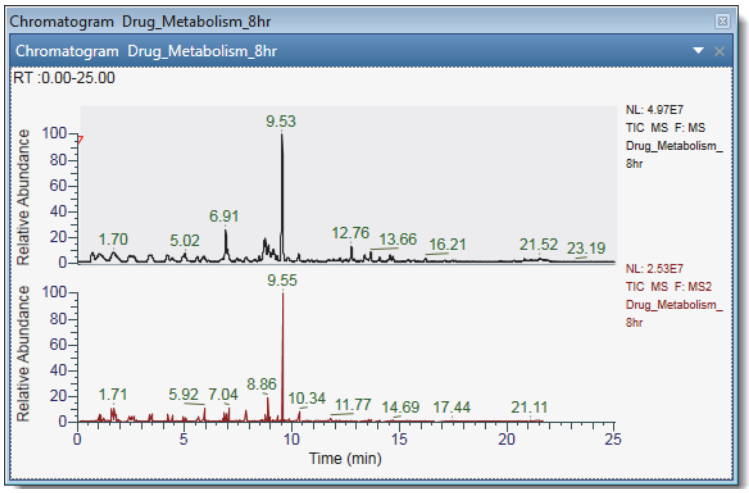
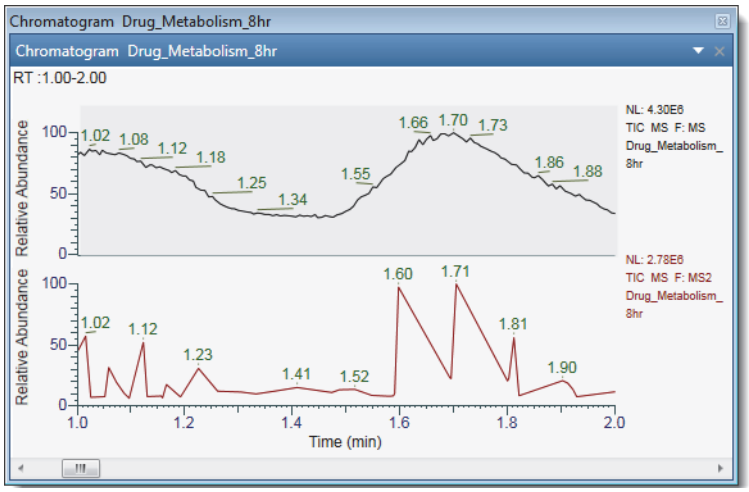


Figure 25 shows the TIC traces for the same scan filters as those shown in Figure 24, but only the 1 to 2 min time range is displayed. In this case, each trace is independently scaled to its largest peak in the displayed time range of 1 to 2 min.

Figure 25. Local normalization for the 1 to 2 min time range



Applying Global Normalization

For global normalization, the application normalizes the chromatogram traces so that the most intense peak of all traces is scaled to 100% of the *y* axis.

❖ To apply global normalization

1. Click the Chromatogram view to make it active.
2. In the Normalization area of the Chromatogram – Display Options Toolbar, click **Global**.

Figure 26 shows an example of global normalization. Both traces are scaled to the largest peak across the traces, rather than being independently scaled to the largest peak in each trace.

Figure 26. Global normalization with the full time range displayed

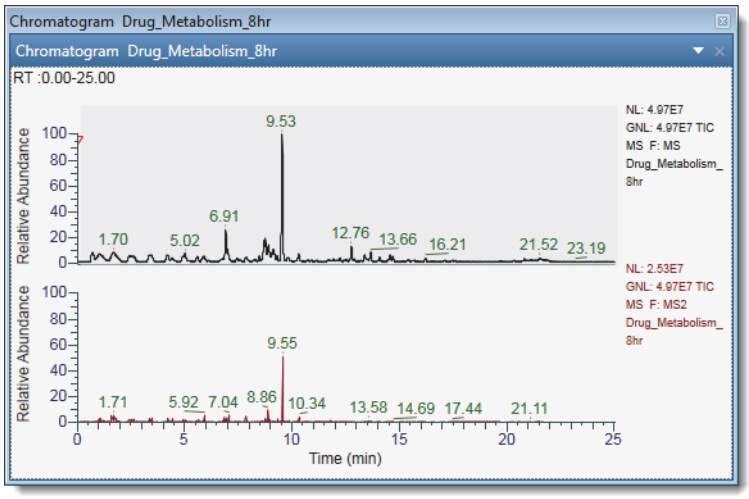
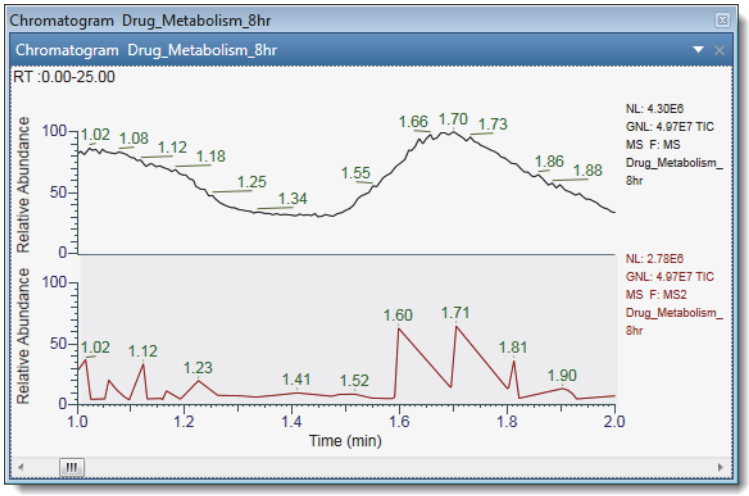


Figure 27 shows the TIC traces for the same scan filters as those shown in Figure 26, but only the 1 to 2 min time range is displayed. In this case, each trace is scaled to the largest peak across the traces in the displayed time range of 1 to 2 min.

Figure 27. Global normalization with the 1 to 2 min time range displayed



Turning Off Normalization

When you turn off normalization, the application scales all the traces by using the maximum and minimum absolute values in the full time range, regardless of the time range displayed.

❖ To turn off normalization

1. Click the Chromatogram view to make it active.
2. In the Normalization area of the Chromatogram – Display Options Toolbar, click **Off**.

   A comparison of Figure 28 and Figure 29 shows the effect of turning off normalization. Regardless of the time range displayed, the traces are scaled to the largest peak across the traces in the full time range. In addition, the Max box displays the absolute intensity of the largest peak (apex height).

Figure 28. Normalization turned off with the full time range displayed

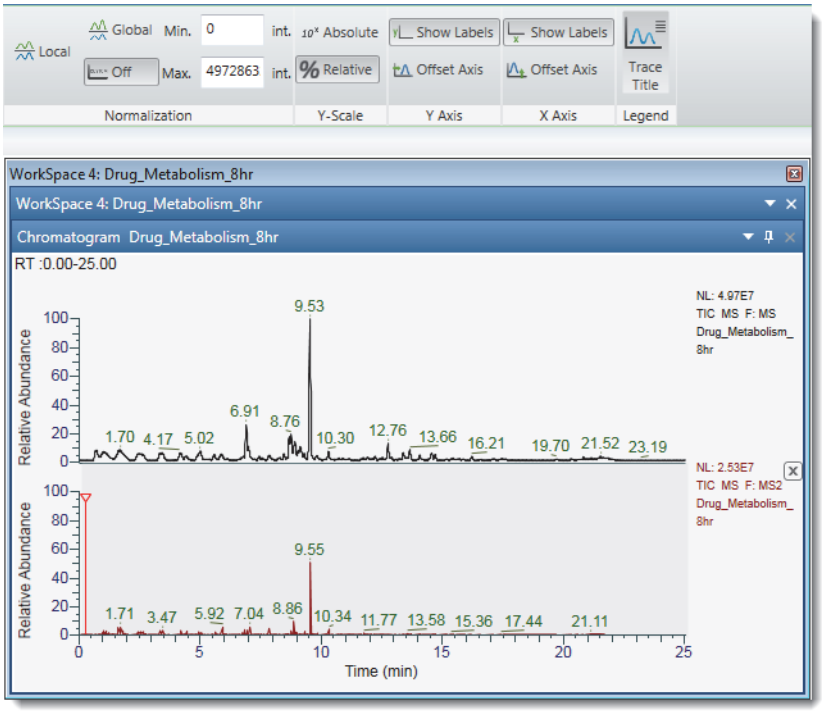
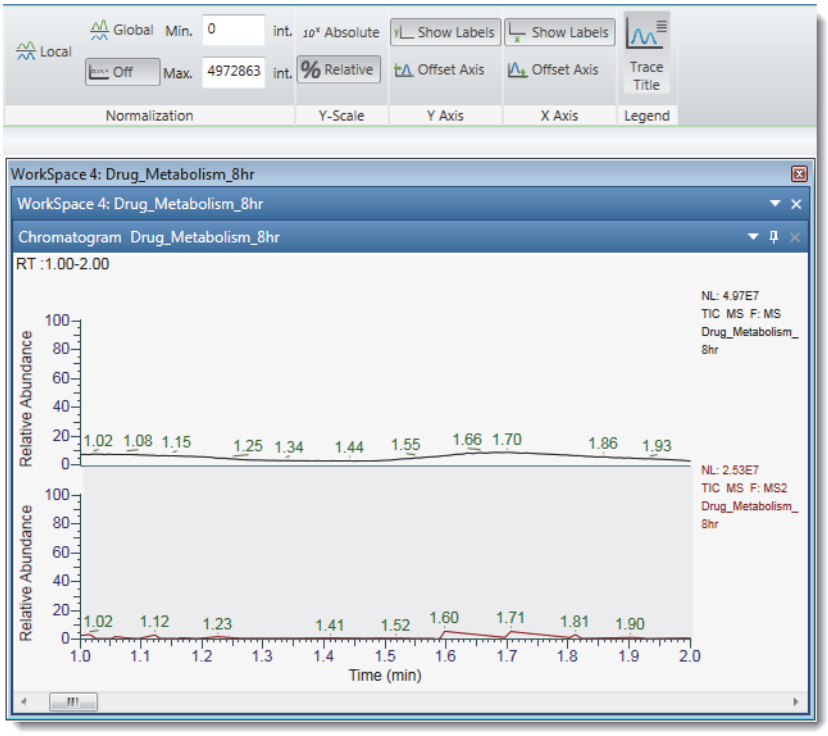


Figure 29. Normalization turned off with the 1 to 2 min time range displayed



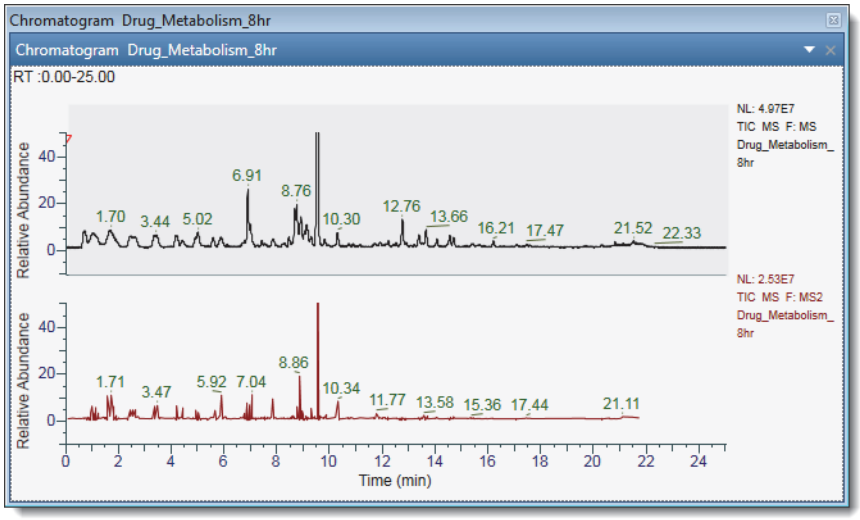
Changing the Y-Axis Range

Use the Min and Max boxes in the Normalization area of the Chromatogram – Display Options Toolbar to change the *y*-axis range from its default of full zoom (0–100%) to another zoom level.

Note Regardless of whether the *y*-axis scaling (Y Scale) is set to Absolute or % Relative, when normalization is turned on (local or global), the Min and Max boxes accept positive and negative values as percentages of the full *y*-axis scale. The Max value must be greater than the Min value.

For example, to zoom in on the –10 to 50% portion of the *y* axis, type –10 in the Min box and 50 in the Max box. Figure 30 shows the result.

Figure 30. Modified *y*-axis range of –10 to 50%



Changing the Y-Axis Scale of the Chromatogram View

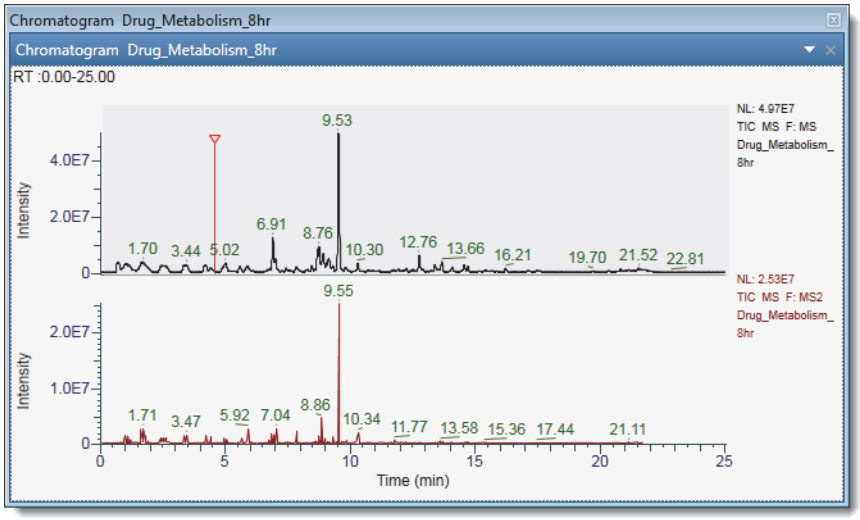
Relative abundance is the default *y*-axis scale (units) for the Chromatogram view. You can change the scale from relative abundance to absolute intensity.

❖ To change the *y*-axis units from relative abundance to absolute intensity

1. Click the Chromatogram view to make it active.
2. In the Y-Scale area of the Chromatogram – Display Options Toolbar, click **10x Absolute**.

   Figure 31 shows a Chromatogram view with the *y*-axis scale set to intensity and normalization set to local.

   Figure 31. Units for the *y* axis set to absolute intensity



Automatically Detecting and Integrating Chromatographic Peaks

Use the Peak Detection page in the Info Bar to select and apply any of the automated peak detection algorithms—Genesis, ICIS, Avalon, or PPD. When using the Genesis or ICIS algorithm, you can manually select the chromatographic time range that the application uses to determine the baseline noise level.

Follow these procedures as needed:

- To display the Peak Detection page
- To run any of the peak detection algorithms
- To run the elemental composition algorithm
- To reset the zoom level in the Chromatogram view

❖ To display the Peak Detection page

1. Open a raw data file (RAW) or a sequence file (SLD).

   The Peak Detection page appears in the Info Bar.

2. Click the **Peak Detection** tab.

❖ To run any of the peak detection algorithms

1. In the Select Algorithm list, select one of these algorithms:
   - Genesis—Use for mass spectrometry data.
   - ICIS—Use for mass spectrometry data.
   - Avalon—Use for UV or PDA data. This algorithm detects negative chromatographic peaks more accurately than the ICIS or Genesis algorithms.
   - PPD—Use to minimize the number of optimization parameters.
2. If the Chromatogram view contains multiple plots, do one of the following:
   - To process all the plots, select the **Apply to All Plots** check box.
   - To process only the active plot, clear the **Apply to All Plots** check box.
3. If necessary, modify the parameters settings for the selected algorithm.

   For information about the parameters settings for each algorithm, see these topics:
   - "Genesis Peak Detection Page" on page 97
   - "ICIS Peak Detection Page" on page 102
   - "Avalon Peak Detection Page" on page 105
   - "PPD Peak Detection Page" on page 108

For information about selecting the manual noise region for the ICIS or Genesis algorithms, see "Selecting the Manual Noise Region for the Genesis and ICIS Algorithms" on page 66.

4. (Optional) To save the modified settings as the default settings for the selected algorithm, click **Save As Defaults**.

   Note You can view the saved settings on the Peak Detection page of the Default Options Configuration dialog box. See Appendix A, "FreeStyle Default Settings."

5. Depending on the algorithm, do one of the following:

   - For the Genesis, ICIS, or PPD algorithm, click **Apply**.
   - For the Avalon algorithm, click **Auto Calc Initial Events**. If necessary customize the integration by adding more events to the table. Each time you add an event, the algorithm automatically reintegrates the plots.

   After the peak detection finishes, the application does the following:

   - Adds the detected peaks to the peak list in the Peaks List View.
   - Colors the integrated region of the detected peaks in the Chromatogram View.
   - Draws a baseline under the integration region.
   - Adds square integration markers at the beginning and end of the integration region. You can change the integration region by dragging the markers.

   Note If the instrument method used to acquire the raw data file specified the compound names of the analytes, the application displays these names above the detected peaks. The instrument control software for the Thermo Scientific TSQ Endura™ and TSQ Quantiva™ mass spectrometers provides this feature.

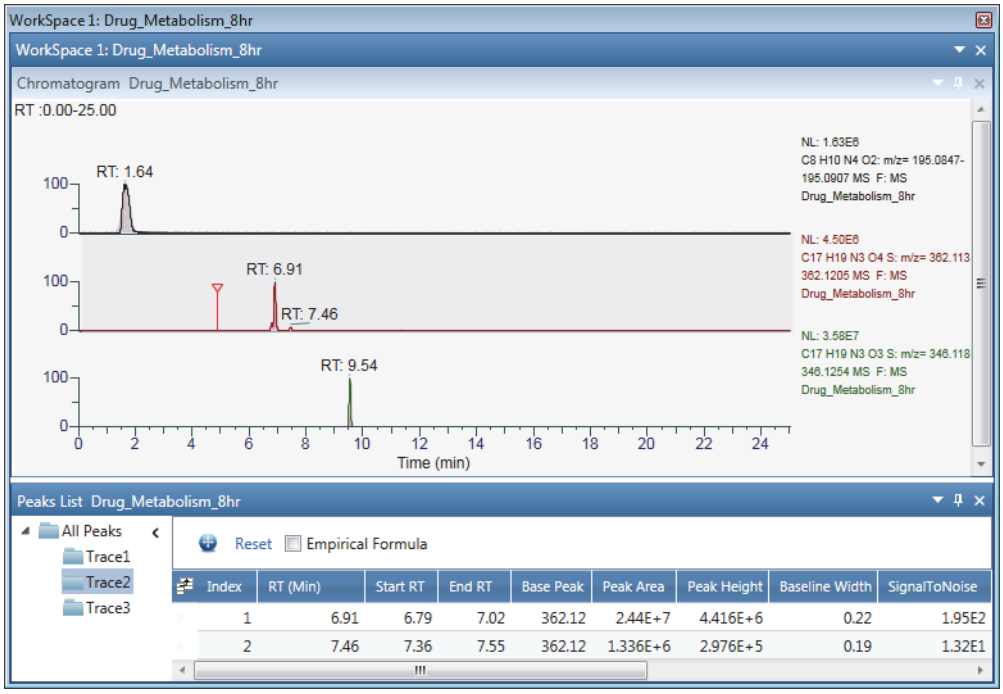
❖ To check the integration for each chromatographic peak

1. If you applied the algorithm to multiple plots, in the Peaks List view, select the plot from the trace list.

   Note The Peaks List view defaults to displaying a list of all the peaks (All Peaks) detected across the chromatogram plots.

   Figure 32 shows the second trace (Trace 2) selected from a list of three chromatogram plots.

Figure 32. Peak list for Trace 2

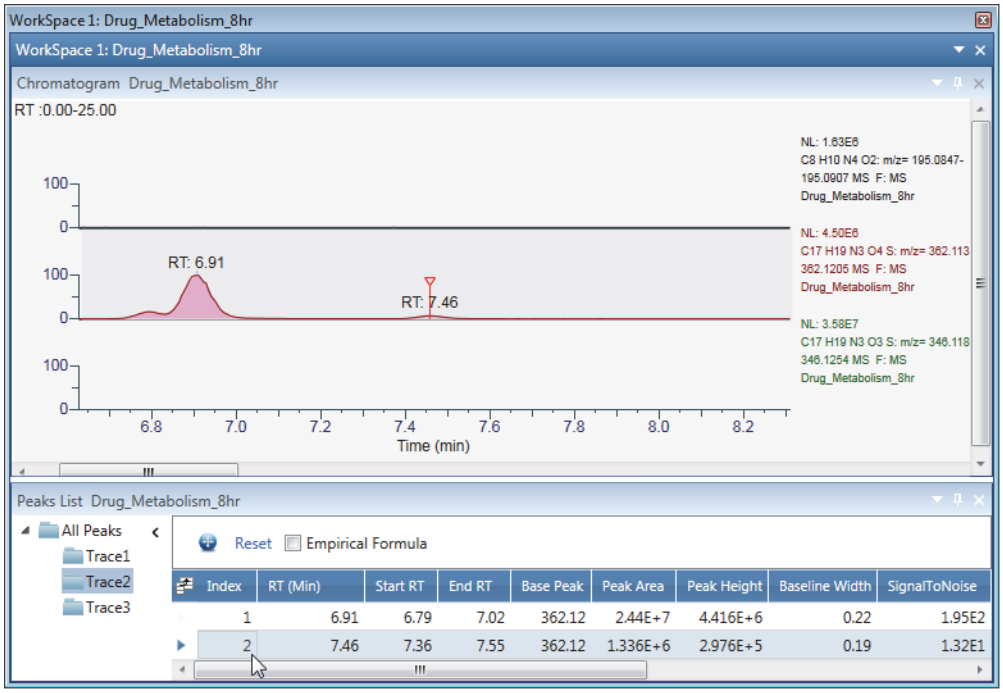


2. To check the integration of a peak, select its row in the peak list.

   In the Chromatogram view, the application zooms in on the selected peak.

   Figure 33 shows the selection of the second peak in the second chromatogram plot.

Figure 33. Zoom level set to the second peak in the second chromatogram plot



❖ **To run the elemental composition algorithm**

Select the **Empirical Formula** check box.

The Empirical Formula column appears to the left of the File Name column.

❖ **To reset the zoom level in the Chromatogram view**

In the Peaks List view, click the **Reset** icon,  .

Selecting the Manual Noise Region for the Genesis and ICIS Algorithms

You can manually select the noise region for the Genesis and ICIS peak detection algorithms.

❖ **To specify the manual noise region for the Genesis or ICIS peak detection algorithm**

1. Open the Peak Detection page (see "To display the Peak Detection page" on page 63).
2. In the Select Algorithm list, select **ICIS** or **Genesis**, and then specify the appropriate parameter settings.
3. Click the **Workspace Processing** toolbar tab.
4. In the Peak Detection area of the toolbar, click **Select Manual Noise Range**.
5. Drag the pointer horizontally across a region of the chromatographic baseline (the *x* axis).

   In the Chromatogram view, the application indicates the noise region with a red line and updates the peak integration. On the Peak Detection page, it automatically selects the Manual Noise Region check box and populates the RT Range box with the selected time range. In the Peaks List view, the application updates the detected peaks list.
6. Do the following as needed:

   - To change the noise region, in the Workspace Processing toolbar, click **Select Manual Noise Range**, and then drag the pointer horizontally across a different region of the chromatographic baseline.
   - To undo the manual noise region, in the Workspace Processing toolbar, click **Clear Manual Noise Range**, and then drag the pointer horizontally across the highlighted noise region.

   The application automatically updates the peak detection and integration.

Manually Adding and Deleting Chromatographic Peaks

You can manually add peaks to the Peaks List View without running a peak detection algorithm. You can also delete peaks from the Peaks List view.

Follow these topics as needed:

- Manually Adding Chromatographic Peaks
- Deleting Chromatographic Peaks

Manually Adding Chromatographic Peaks

❖ **To manually add peaks to the peak list**

1. Click the Chromatogram View to make it the active view.
2. Turn on the Add Peak pointer as follows:
   a. Open the Chromatogram View Toolbox.
   b. Click the **Add Peak** icon,  .

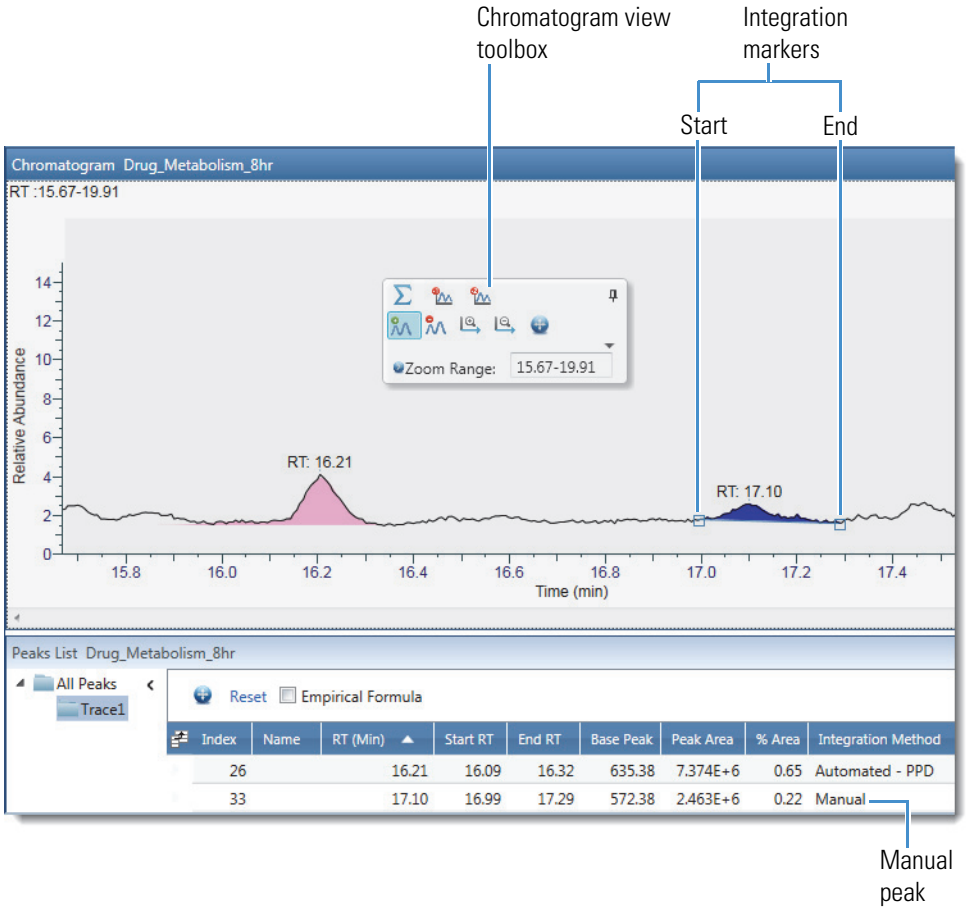
   Tip You can also turn on the Add Peak pointer by clicking Add Peak in the Chromatogram – Workspace Processing Toolbar.
3. Drag the pointer,  , along the bases of the peaks that you want to integrate and add to the peak list.

   After the peak detection finishes, the application does the following:

   - Adds the detected peaks to the peak list in the Peaks List view, and identifies their integration method as Manual.
   - Colors the integrated region of the detected peaks blue in the Spectrum View or MultiSpectrum View.
   - Draws a baseline under the integration region.
   - Adds square integration markers at the beginning and end of the integration region. You can change the integration region by dragging the markers.

   Figure 34 shows a manual peak and its integration markers.

Figure 34. Manual peak with integration markers and peaks list with a manual peak



4. To turn off the Add Peak pointer, click the **Add Peak** icon again.

Deleting Chromatographic Peaks

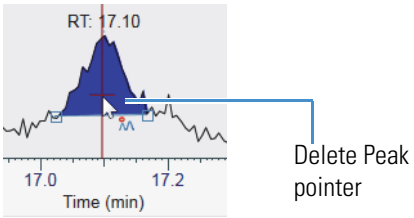
You can delete chromatographic peaks, regardless of their integration method.

❖ To delete chromatographic peaks

1. Click the Chromatogram view to make it active.
2. Turn on the Delete Peak pointer as follows:
   a. Open the Chromatogram View Toolbox.
   b. Click the **Delete Peak** icon, .

Tip You can also turn on the Delete Peak pointer by clicking Delete Peak in the Chromatogram – Workspace Processing Toolbar.

3. Click the peak that you want to delete.



4. To turn off the Delete Peak pointer, click the **Delete Peak** icon again.

Working with Sequences

A sequence is a list of raw data files where the file names link to the actual RAW files. You can open an existing sequence or create a new sequence from a set of raw data files.

To open an existing sequence, see "Opening Raw Data Files or Sequence Files" on page 15.

Follow these topics as needed:

- Creating a New Sequence
- Comparing Chromatogram Traces in a Sequence

Creating a New Sequence

You can create a sequence for any raw data file set.

❖ To create a new sequence

1. Choose **File > Create Sequence**.
2. Browse to and select a set of raw data files.
3. Click **Open**.

   The Sequence File Page opens in the Info bar. By default, the new sequence list is named NoName.sld.

4. To save the sequence, do the following:
   a. At the bottom of the Sequence File page, click **Save Sequence**.
   b. Browse to an appropriate file directory, name the file, and click **Save**.

Comparing Chromatogram Traces in a Sequence

To compare one or more fixed traces against other traces, set up the fixed traces as reference traces in the Chromatogram Ranges view.

❖ To compare chromatogram traces in a sequence

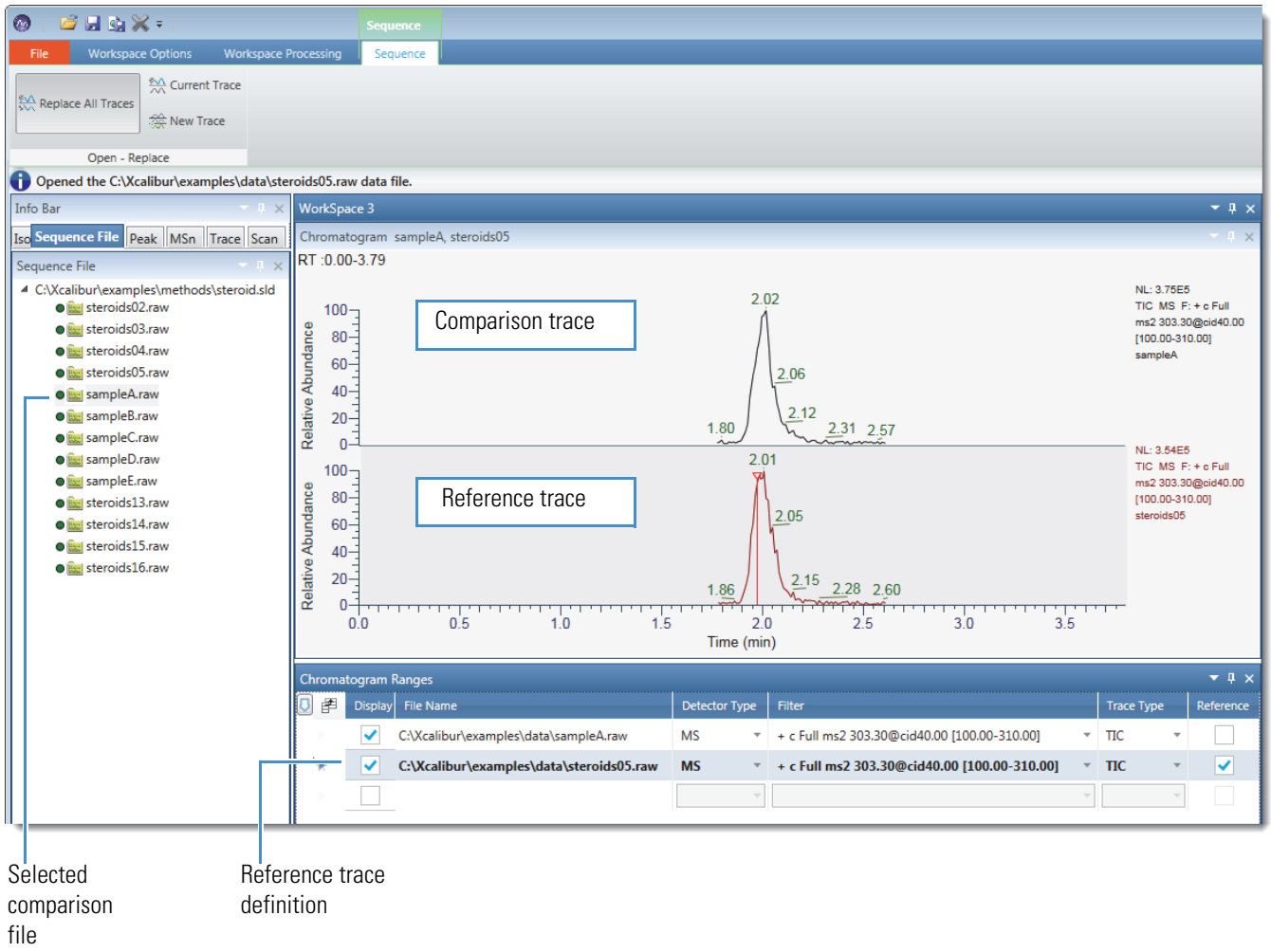
1. Open a sequence (see "Creating, Positioning, Previewing, and Closing Workspaces" on page 17) or create a sequence (see "Working with Sequences" on page 69).
2. In the sequence list, select the raw data file that you want to use as a reference file.

   The Chromatogram View displays the TIC trace for the raw data file.
3. Set up the reference trace as follows:
   a. In the Workspace Options toolbar, click **Chromatogram Ranges** to open the Chromatogram Ranges view.
   b. Define the trace that you want to use as a reference trace. For example, select the detector type, trace type, filter, and so on.
   c. Copy this definition to the last row in the Chromatogram Ranges view by selecting its corresponding check box in the Display column.

      A copy of the first chromatogram trace appears below it.
   d. Select the check box in the Reference column of the row corresponding to the reference trace.
4. Click the raw data files in the sequence to compare their chromatogram traces to the reference chromatogram trace.

   Figure 35 shows a Chromatogram view with two plots. The comparison trace for the selected file is on the top, and the reference trace is on the bottom.

Figure 35. Chromatogram view with a comparison trace and a reference trace



Selected comparison file

Reference trace definition

Chromatogram-Specific Toolbars

Use the following toolbars to format the Chromatogram view, detect chromatographic peaks, and work with sequence lists.

- Chromatogram – Display Options Toolbar
- Chromatogram – Workspace Processing Toolbar
- Sequence Toolbar

Chromatogram – Display Options Toolbar

Use the Chromatogram Display Options toolbar buttons, its Skew and Elevation sliders, and its Label Threshold box to customize the Chromatogram View.

❖ To display the Chromatogram – Display Options toolbar

1. Click the Chromatogram View to make it the active view.
2. Click the **Display Options** toolbar tab.

Figure 36 shows the Chromatogram – Display Options toolbar, and Table 10 describes the toolbar buttons.

Figure 36. Chromatogram Display Options toolbar (in two parts, left and right side)

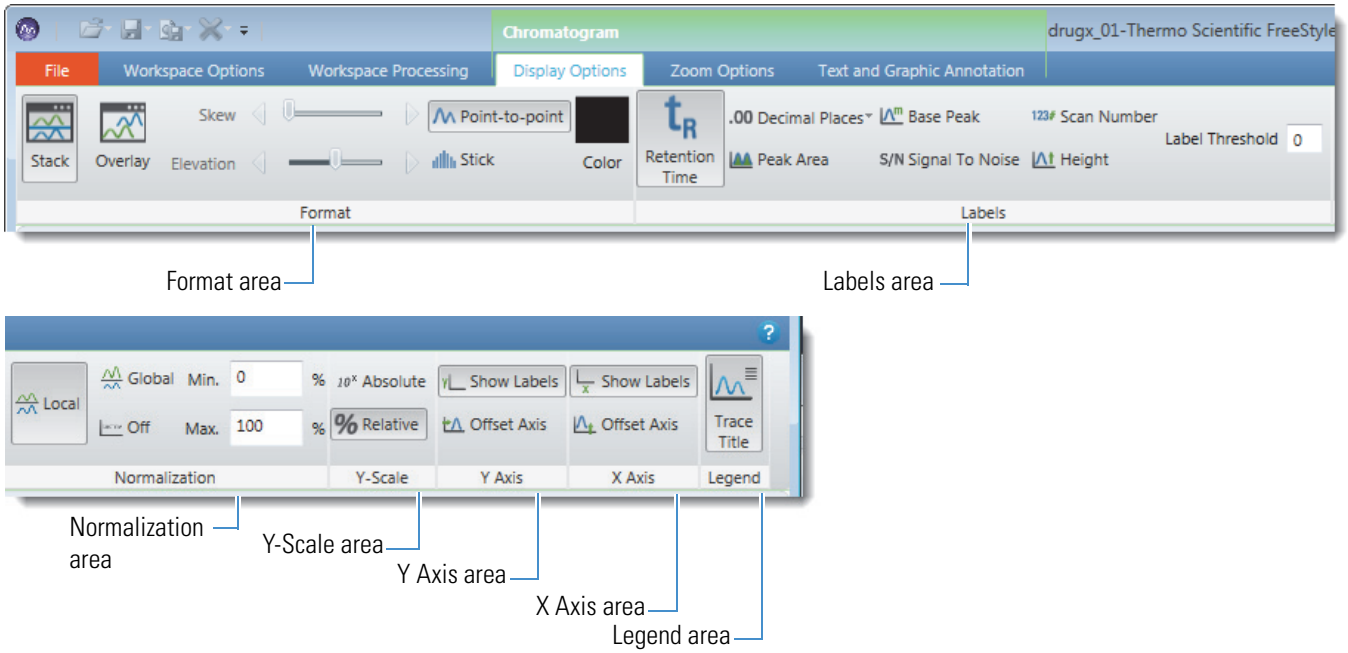


Table 10. Chromatogram – Display Options toolbar buttons (Sheet 1 of 4)

Button	Description
Format	
Use the parameters in the Format area to modify the appearance of the Chromatogram view.	
Stack	Stacks the chromatogram traces vertically.
Overlay	Overlays the chromatogram traces vertically with optional horizontal skew (time offset).
Skew	Sets the skew angle (time offset) to a value from 0–45 degrees for an overlay arrangement of chromatogram traces. To set the skew, drag the Skew slider.

Table 10. Chromatogram – Display Options toolbar buttons (Sheet 2 of 4)

Button	Description
Elevation	Sets the vertical spacing for an overlay arrangement of chromatogram traces. To set the vertical spacing, drag the Elevation slider. Move the Elevation slider to the farthest left to overlay the plots on top of each other.
Point-to-point	Connects the signal data points to form a continuous curve.
Stick	Displays the signal data points by using vertical lines.
Color	Displays the color palette, where you select the colors of the chromatogram traces.
Labels Use the buttons in the Labels area to annotate chromatogram peaks.	
Retention Time	Adds a retention time label (in minutes) above each local maxima or chromatogram peak. The order of chromatogram labels for an undetected peak, from top to bottom, is retention time, base peak, and scan number. The Chromatogram view displays the retention time on all peaks that meet the selection criteria. The letters *RT* to the left of the value indicate a retention time.
Decimal Places	Changes the number of decimal places in the retention time label. Range: 0–5
Peak Area (of integrated peaks)	Adds a peak area label above each detected chromatogram peak. The letters *MA* or *AA* next to the value indicate manual integration or automatic integration, respectively.
Base Peak	Adds a base peak mass-to-charge ratio label (in *m/z*) above each local maxima or each detected chromatogram peak. The letters *BP* next to the value indicate a base peak.

Table 10. Chromatogram – Display Options toolbar buttons (Sheet 3 of 4)

Button	Description
Signal To Noise (of integrated peaks)	Adds a signal-to-noise ratio label above each detected chromatogram peak. The Avalon peak detection algorithm does not calculate a signal-to-noise ratio. For the Genesis and ICIS peak detection algorithms, you can choose to report the root mean square (RMS) noise values. The application calculates the RMS signal-to-noise ratio for manual peaks. The letters *SN* next to the value indicate that it is a signal-to-noise ratio.
Scan Number	Adds the active scan number label above the chromatogram peak. The letter *S#* next to the value indicates the scan number.
Height (of integrated peaks)	Adds a peak height above each detected chromatogram peak. The letters *MH* or *AH* next to the value indicate manual integration or automatic integration, respectively.
Label Threshold	Sets the percentage of highest data point in the trace to 100%. The application labels only the local maxima or chromatographic peak apexes that are above the specified height threshold. Range: 0–100; default: 0
Normalization Use the parameters in the Normalization area to specify how the application normalizes the chromatogram traces. See "Normalizing Chromatogram Traces" on page 57.	
Local	Normalizes each chromatogram trace with respect to the intensity of the most intense peak in that trace.
Global	Normalizes the chromatogram traces so that the most intense peak of all chromatograms is 100 percent.
Off	Scales all chromatograms by using the maximum and minimum absolute values.
	Note If you set the normalization to Off, the *y*-axis scale to Absolute, and change the trace type, the new trace might not display. To remedy this, set the normalization to Local and then to Off.
Min.	Displays the minimum of the *y* axis. Enter a value in the box to change the minimum. The application indicates whether the value is a percentage or an intensity.

Table 10. Chromatogram – Display Options toolbar buttons (Sheet 4 of 4)

Button	Description
Max.	Displays the maximum of the *y* axis. Enter a value in the box to change the maximum value. The application indicates whether the value is a percentage or an intensity.
Y-Scale	
Use the parameters in the Y-Scale area to specify how the application scales the *y* axis. See "Normalizing Chromatogram Traces" on page 57.	
10x Absolute	Scales the chromatograms traces by using the absolute intensity values.
	Note If you set the normalization to Off, the *y* axis to Absolute, and change the trace type, the new trace might not display. To remedy this, set the normalization to Local and then to Off.
% Relative	Scales the chromatograms traces by using percentages.
Y Axis	
Use the parameters in the Y Axis area to specify how the application labels and displays the *y* axis.	
Show Labels	Shows or hides the *y*-axis label.
Offset Axis	Sets the location of the displayed plot at a specified distance from the *y* axis. The *y*-axis offset moves the *x* axis slightly to the right of the *y* axis so that you can see the plot details at low *x*-axis values.
X Axis	
Use the parameters in the X Axis area to specify how the application labels and displays the *x* axis.	
Show Labels	Shows or hides the *x*-axis label.
Offset Axis	Sets the location of the displayed plot at a specified distance from the *x* axis. The *x*-axis offset moves the *y* axis up slightly so that you can see the plot details at low *y*-axis values.
Legend	
Trace Title	Moves the title of the chromatogram trace from above the trace to beside it.

Chromatogram – Workspace Processing Toolbar

Use the buttons in the Peak Detection area of the Workspace Processing toolbar to detect peaks in chromatogram traces.

❖ To display the Chromatogram Workspace Processing toolbar

1. Click the Chromatogram View to make it the active view.
2. Click the **Workspace Processing** toolbar tab.

Figure 37 shows the Peak Detection area of the Workspace Processing toolbar. The Delete Peak button becomes available after you apply a peak detection algorithm or add a peak manually.

Figure 37. Peak Detection area of the Workspace Processing toolbar

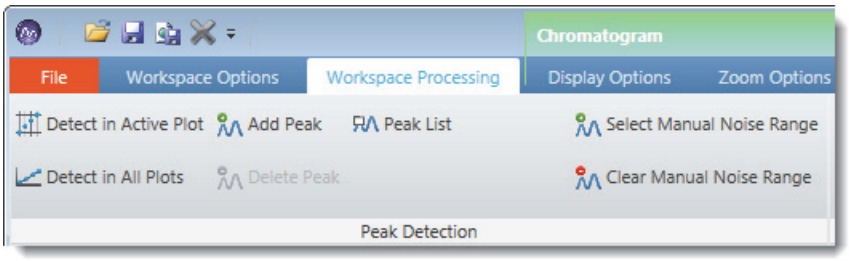


Table 11 describes the Peak Detection area buttons.

Table 11. Workspace Processing toolbar – Peak Detection area buttons (Sheet 1 of 2)

Button	Description
Detect in Active Plot	Runs the Genesis, ICIS, or Avalon peak detection algorithm on the active chromatogram plot. Click a chromatogram plot to make it active. The Chromatogram view shades the active plot area gray. See "Automatically Detecting and Integrating Chromatographic Peaks" on page 63.
Detect in All Plots	Runs the Genesis, ICIS, or Avalon peak detection algorithm on all chromatogram plots. See "Automatically Detecting and Integrating Chromatographic Peaks" on page 63.
Add Peak	Adds a peak to the peak list. See "Manually Adding Chromatographic Peaks" on page 67.
Delete Peak	Deletes a peak from the peak list. See "Deleting Chromatographic Peaks" on page 68. Available after you apply a peak detection algorithm or manually add a chromatographic peak.

Table 11. Workspace Processing toolbar – Peak Detection area buttons (Sheet 2 of 2)

Button	Description
Peak List	Displays the Peaks List View. When you run a peak detection algorithm, the Peak List view lists the peaks that the application found in the chromatogram. You can also manually add peaks to the peak list with the Add Peak button.
Select Manual Noise Range	Freezes the Chromatogram view (temporarily turns off zooming with the mouse pointer) so that you can use the mouse pointer to define a noise region (see "Selecting the Manual Noise Region for the Genesis and ICIS Algorithms" on page 66).
Clear Manual Noise Range	Freezes the Chromatogram view (temporarily turns off zooming with the mouse pointer) so that you can use the mouse pointer to clear the selection of a manual noise region (see "Selecting the Manual Noise Region for the Genesis and ICIS Algorithms" on page 66).

Sequence Toolbar

Use the Sequence toolbar buttons to determine which traces appear in the Chromatogram view as you click through the raw data files in a sequence.

❖ To display the Sequence toolbar

1. Open a sequence (see "Creating, Positioning, Previewing, and Closing Workspaces" on page 17) or create one (see "Working with Sequences" on page 69).
2. If the Sequence File page is not the active view, click the **Sequence File** tab in the Info Bar.

> **Tip** The Sequence toolbar is visible only when the Sequence File Page is the active view.

Figure 38 shows the Sequence toolbar, and Table 12 describes the toolbar buttons.

Figure 38. Sequence toolbar

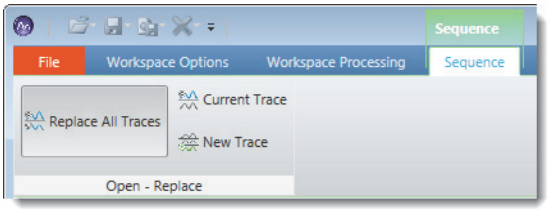


Table 12. Sequence toolbar buttons

Button	Description
Replace All Traces	Replaces all the raw data files in the chromatogram ranges list—and their chromatogram traces in the Chromatogram view—with the raw data file that you select in the sequence. Select **Replace All Traces**, and then select a raw data file in the sequence on the Sequence File Page for replacement.
	Note The application does not replace a trace that is set as a reference trace (its Reference check box is selected) with the selection from the sequence.
Current Trace	Replaces only the raw data file that you select in the chromatogram ranges list—and its chromatogram trace displayed in the Chromatogram view—with the raw data file that you select in the sequence. Select a trace row in the chromatogram ranges list, select **Current Traces**, and then select a raw data file in the sequence for replacement.
	Note The application does not replace a trace that is set as a reference trace (the box in the Reference column is selected) with the selection from the sequence.
New Trace	Adds new raw data files to the chromatogram ranges list and displays their chromatogram traces in the Chromatogram view. Select **New Trace**, and then select one or more raw data files in the sequence to add to the list.

Chromatogram-Specific Views

For information about the chromatogram-related views, see these topics:

- Chromatogram View
- Chromatogram Ranges View

Chromatogram View

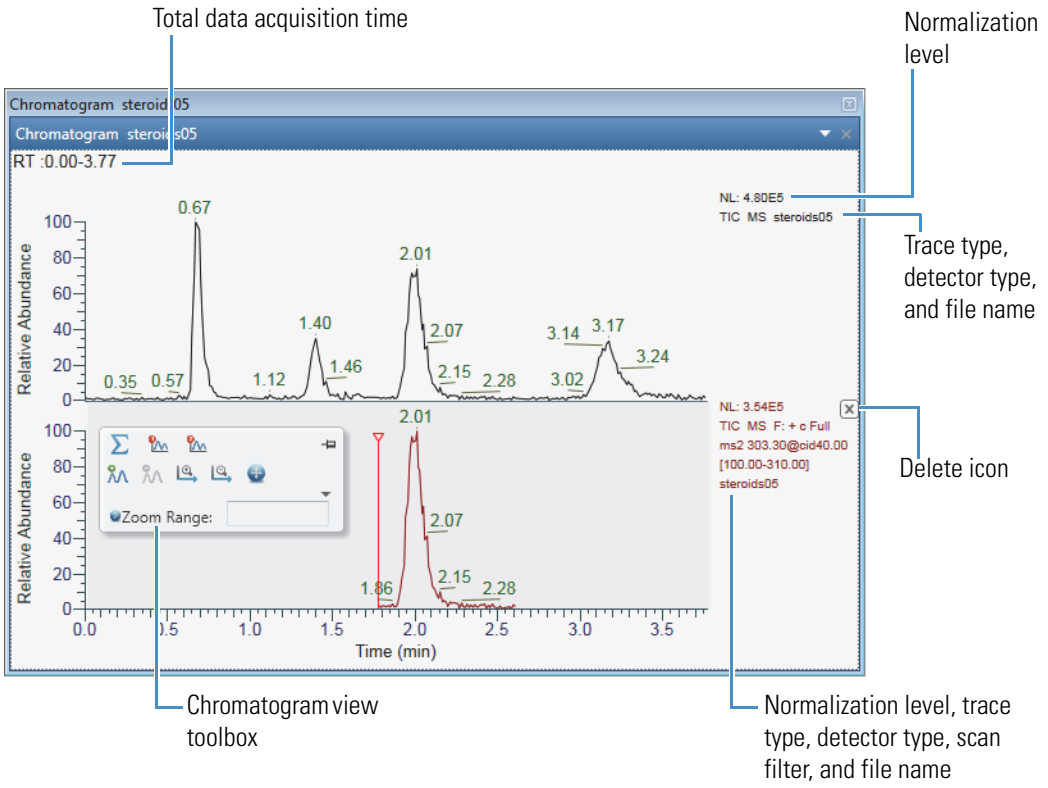
After you open a raw data file (see "Opening Raw Data Files or Sequence Files" on page 15), use the Chromatogram view to display chromatogram traces. This view provides the time base for all other views in the workspace, and is a permanent view—that is, you can hide it, but you cannot dismiss it.

To work with chromatograms, see these topics:

- "Adding and Deleting Chromatogram Traces" on page 44
- "Adding Chromatogram Traces with the Auto Filter Feature" on page 46
- "Defining the Range of a Chromatogram Trace" on page 48
- "Setting Up the Display Options for a Chromatogram Trace" on page 54

Figure 39 shows an example of a Chromatogram view with two traces. For information about the scan header that appears at the top right of each chromatogram trace, see "Scan Headers and Scan Header Abbreviations" on page 266. For information about the view's toolbox, see "Chromatogram View Toolbox" on page 91.

Figure 39. Chromatogram view with two traces and the chromatogram toolbox



Note You can set a minimum trace height value, in centimeters, in the Default Workspace Options Page. When you adjust the height of the Chromatogram view, if the height of the traces becomes smaller than the set minimum value, a scrollbar automatically appears to the right of the view.

Right-clicking the Chromatogram View displays a shortcut menu (Figure 40) with the commands described in Table 13.

Figure 40. Shortcut menu for the Chromatogram view

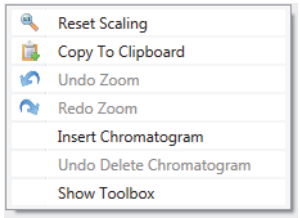


Table 13. Chromatogram view shortcut menu commands

Command	Description
Reset Scaling	Resets the scaling of all the plots in the Chromatogram view.
Copy To Clipboard	Copies an image of the Chromatogram view to the Clipboard.
Undo Zoom	Undoes the last zoom.
Redo Zoom	Reapplies the last zoom that you undid.
Insert Chromatogram	Inserts a copy of the selected chromatogram trace.
Undo Delete Chromatogram	Displays the most recently deleted chromatogram trace.
Show Toolbox	Displays the Chromatogram View Toolbox. To close the toolbox, click its pin icon.

These toolbars are available when the Chromatogram view is active:

- "Chromatogram – Workspace Processing Toolbar" on page 76
- "Chromatogram – Display Options Toolbar" on page 71
- "Zoom Options Toolbar" on page 33
- "Text and Graphic Annotation Toolbar" on page 34

Chromatogram Ranges View

Use the Chromatogram Ranges view (Figure 41) to select which chromatogram traces to display in the Chromatogram View. For instructions, see "Defining the Range of a Chromatogram Trace" on page 48.

Note For information about exporting the tabular data in the Chromatogram Ranges view to a CSV file, see "Exporting or Printing the Contents of a View or Workspace" on page 27. You can paste the contents of a CSV file to the Chromatogram Ranges view if the table columns match.

❖ To open the Chromatogram Ranges view

In the Workspace Options toolbar, click **Chromatogram Ranges**.

To specify the chromatograms of interest, see "Defining the Range of a Chromatogram Trace" on page 48.

To add, delete, and move rows in the Chromatogram Ranges view, see these topics:

- "Using the Fill Down Feature" on page 85
- "Moving a Row" on page 86
- "Deleting Rows" on page 87

Figure 41 shows an example of the Chromatogram Ranges view.

Figure 41. Chromatogram Ranges view showing the entry of a chemical formula

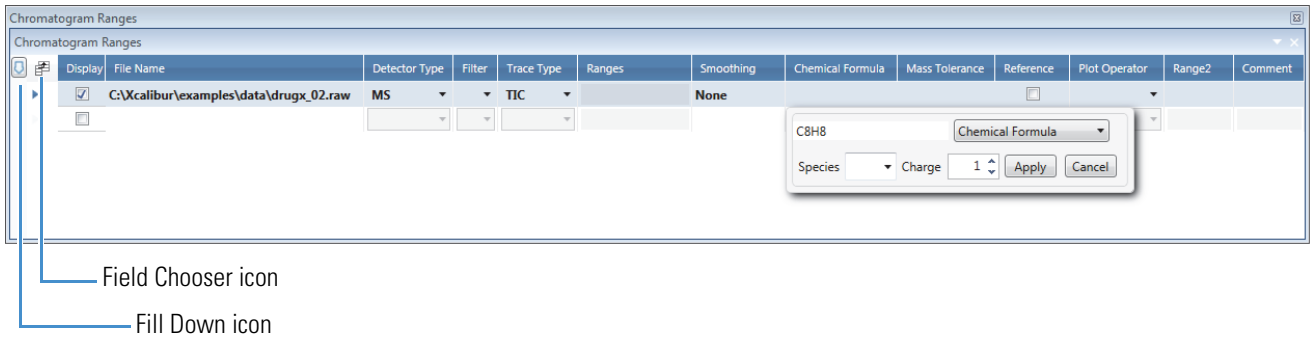


Table 14 describes the parameters and icons for the Chromatogram Ranges view. The following columns have corresponding pages in the Info Bar: Detector Type, Trace Type, and Filter.

Table 14. Chromatogram Ranges view parameters and icons (Sheet 1 of 5)

Parameter/Icon	Description
Display	Select this check box to display the chromatogram trace in the Chromatogram View. Clear this check box to remove the trace.
File Name	Displays the path and name of the raw data file. To display the Open Raw File dialog box, click … at the end of the file name.
Detector Type	Select the detector type. The application autosenses the available detector types based on the information in the raw data file. For details, see "Detector Type Page" on page 94.

Table 14. Chromatogram Ranges view parameters and icons (Sheet 2 of 5)

Parameter/Icon	Description
Filter	Select a filter to apply to the raw data file. The application autosenses the metafilters (including SRM or compound filters) in the raw data file in this order: MS, MS2, ETD, HCD, MSN, and then the individual scan filter list. For grouped filters, the Filter dropdown list displays only the first filter in the group. The Spectrum view displays the actual filter for the scan. For more information, see "Scan Filter Parameters" on page 263 and "Scan Filters Page" on page 95.
Trace Type	Select the trace type as follows: • For MS, select TIC, Base Peak, Mass Range, or Neutral Fragment. • For PDA, select Total Scan, Wavelength Range, or Maximum Spectrum. • For MS Trending, select one of the instrument status parameters, such as the API source voltage.
Ranges	Specify the *m/z* ranges of the displayed spectrum, such as 443.2, 534.6, 600–800. The application autosenses the default range from the raw data file. If the field is inactive, it is not applicable based on the trace type.
Smoothing	Select which type of smoothing, if any, to apply to the chromatogram trace. • Gaussian: Uses weighting coefficients corresponding to a Gaussian peak shape to average each data point with neighboring points to give the displayed value. • Moving mean: Uses equal weighting to average each data point with neighboring points to give the displayed value.

Table 14. Chromatogram Ranges view parameters and icons (Sheet 3 of 5)

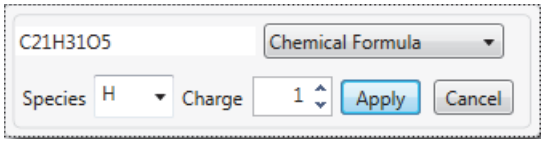
Parameter/Icon	Description
Chemical Formula	Clicking this table cell opens the following dialog box.  Select **Chemical Formula** or **Peptide**: • For a compound, select **Chemical Formula**, use the symbols in the periodic table to specify the elements in the chemical formula, select the ionization species, and type or select the charge of the adduct ion of interest. Example: For calcium copper titanate (which is made up of one calcium atom, three copper atoms, four titanium atoms, and twelve oxygen atoms), enter the chemical formula: CaCu3Ti4O12. • For a peptide, select **Peptide**, enter the peptide sequence using the one-letter symbols for the amino acid residues (see "One- and Three-Letter Abbreviations for Amino Acid Residues" on page 269), select the ionization species, and type or select the charge of the adduct ion of interest. Example: For Hexarelin (a hexapeptide), use the one-letter code—HWAWFK. When you click Apply, the Trace Type changes to Mass Range, and the mass-to-charge ratio of the specified ion appears in the Ranges column.
Mass Tolerance	Entering a chemical formula in the Chemical Formula column or selecting Mass Range in the Trace Type column enables this table cell. Specify the mass tolerance for the mass-to-charge ratio of the specified ion. Range: 0.00 to 10.00; default: 0.5 amu
Delay Time (hidden by default)	Specify a delay time (in minutes) to shift trace 1 (Trace Type column) so that it aligns with trace 2 (Trace Type2 column). A positive delay time shifts trace 1 to higher retention times by this amount. Range: –5.00 to 5.00; default: 0.00

Table 14. Chromatogram Ranges view parameters and icons (Sheet 4 of 5)

Parameter/Icon	Description
Reference	Select this check box to make the chromatogram trace a reference trace. A reference trace is always displayed in the Chromatogram view so that you can compare other traces to it.
Plot Operator	Select a plot operator, either + (add) or – (subtract). • If you select +, the Chromatogram view displays trace 1 plus trace 2. • If you select –, the Chromatogram view displays trace 1 minus trace 2. For example, from a chromatogram you can use the subtract (–) plot operator to subtract contributions from a solvent or from other noise. For MS detectors, these are the trace 1 and trace 2 combinations: • mass range ± mass range • mass range ± base peak • base peak ± mass range • base peak ± base peak • TIC – mass range • TIC – base peak For PDA detectors, these are the trace 1 and trace 2 combinations: • wavelength range ± wavelength range • wavelength range ± spectrum maximum • spectrum maximum ± wavelength range • spectrum maximum ± spectrum maximum • total scan – wavelength range • total scan – spectrum maximum
Trace Type2 (hidden by default)	Select the type of trace to add to or subtract from trace 1.
Range2	Specify the wavelength or mass range for trace 2.
Comment	Specify a comment for the row.

Table 14. Chromatogram Ranges view parameters and icons (Sheet 5 of 5)

Parameter/Icon	Description
Icon	
Fill Down	Opens the Fill Down dialog box for populating multiple rows with duplicate parameter settings. For details, see "Using the Fill Down Feature" on page 85.
Field Chooser	Displays the Field Chooser dialog box for selecting which columns appear in the Chromatogram Ranges view. For details, see "Selecting the Columns to Display in a View or Dialog Box with Tabular Data" on page 20.
Check boxes in the Display column	Select the check box to add the defined chromatogram to the Chromatogram view. Selecting the check box in the last row populates the row with the settings from the previous row. You can edit the File Name parameter to change the new entry to that raw data file, or click ... at the end of the file name to browse to and select a new raw data file.

Using the Fill Down Feature

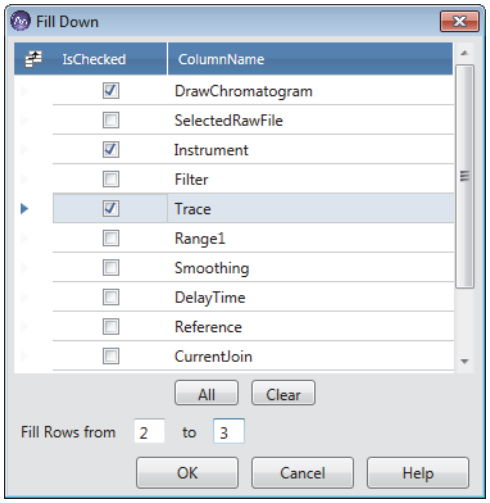
To set up several similar chromatogram traces, use the Fill Down feature in the Chromatogram Ranges View. With this feature, you can copy the selected parameter values from the currently selected row to other specified rows.

❖ To fill down data

1. In the Chromatogram Ranges View, select the trace row that you want to copy data from.
2. Click the **Fill Down** icon ( ).

   The Fill Down dialog box opens (Figure 42).

Figure 42. Fill Down dialog box



3. Do one of the following:

   - Select the check box for each parameter value that you want to fill down.
   - Click **All** to select all the check boxes.

   –or–

   - Click **Clear** to clear all the check boxes.

4. In the Fill Rows boxes, type the starting and ending row numbers, and then click **OK**.

The application copies the selected parameter values from the currently selected row and pastes them into the specified starting and ending rows. If a value is invalid, it appears in red.

Moving a Row

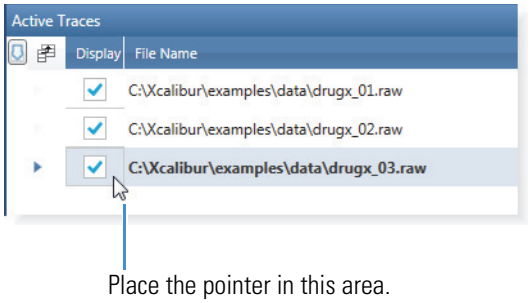
You can move a row in the Chromatogram Ranges View by dragging it to the new location.

❖ To move a row

1. Place the pointer in an area somewhere in the row that you want to move, but not inside an editable field and not in the blank area to the left of the row.

   For example, place the pointer in the area next to the Display check box (Figure 43).

Figure 43. Example pointer location to move a row



2. Drag the row up or down to move it to the new location.

   As you drag the row above or below another row, that other row temporarily turns red. After you drop the row, it appears above or below the red row.

Deleting Rows

You can delete rows in the Chromatogram Ranges View. For each row that you delete, the application automatically deletes the corresponding trace in the Chromatogram View.

Note When the view has only one row, you cannot delete the row.

❖ To delete rows in the Chromatogram Ranges view

1. Select the rows that you want to delete as follows:

   - To select a single row, click the arrow icon , ▶, that appears when you point to the left of the Display column.
   - To select adjacent rows, drag the pointer across the group of rows (in the column to the left of Display column) or use the SHIFT key.
   - To select nonadjacent rows, press the CTRL key and click to the left of the Display column for each row.

   Selected rows are highlighted in blue.

2. Press the DELETE key and click **Yes** in the confirmation dialog box to remove the selected rows.

Peaks List View

The Peaks List view lists the chromatogram peaks that the peak detection algorithm automatically finds and also lists the peaks that you manually add.

❖ To display the Peaks List view

- Apply the ICIS, Genesis, Avalon, or PPD peak detection algorithm (see "Automatically Detecting and Integrating Chromatographic Peaks" on page 63).

> **IMPORTANT** Applying the peak detection algorithm the first time automatically opens the Peaks List view. However, if you then close this view and apply the peak detection algorithm again, the Peaks List view does not automatically reopen. To manually open this view, click the **Workspace Processing** toolbar tab, and then click **Peak List**.

- Manually add a peak (see "Manually Adding and Deleting Chromatographic Peaks" on page 67).

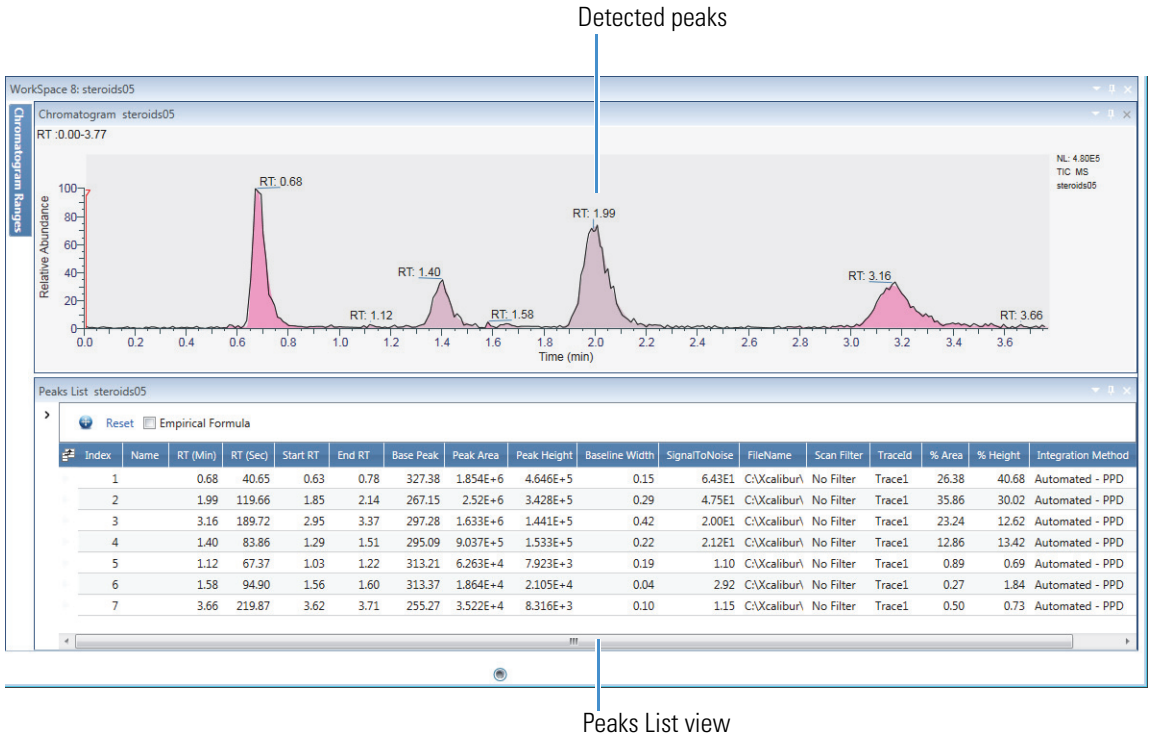
–or–

a. Click the **Workspace Processing** toolbar tab.

b. Click **Peak List**.

Figure 44 and Figure 45 show examples of the Peaks List view. In Figure 45, the Name column is populated with the compound name specified in the instrument method for the selected scan filter. The table values are read-only.

Figure 44. Peaks List view



Index	Name	RT (Min)	RT (Sec)	Start RT	End RT	Base Peak	Peak Area	Peak Height	Baseline Width	SignalToNoise	FileName	Scan Filter	TraceId	% Area	% Height	Integration Method
1		0.68	40.65	0.63	0.78	327.38	1.854E+6	4.646E+5	0.15	6.43E1	C:\Xcalibur\	No Filter	Trace1	26.38	40.68	Automated - PPD
2		1.99	119.66	1.85	2.14	267.15	2.52E+6	3.428E+5	0.29	4.75E1	C:\Xcalibur\	No Filter	Trace1	35.86	30.02	Automated - PPD
3		3.16	189.72	2.95	3.37	297.28	1.633E+6	1.441E+5	0.42	2.00E1	C:\Xcalibur\	No Filter	Trace1	23.24	12.62	Automated - PPD
4		1.40	83.86	1.29	1.51	295.09	9.037E+5	1.533E+5	0.22	2.12E1	C:\Xcalibur\	No Filter	Trace1	12.86	13.42	Automated - PPD
5		1.12	67.37	1.03	1.22	313.21	6.263E+4	7.923E+3	0.19	1.10	C:\Xcalibur\	No Filter	Trace1	0.89	0.69	Automated - PPD
6		1.58	94.90	1.56	1.60	313.37	1.864E+4	2.105E+4	0.04	2.92	C:\Xcalibur\	No Filter	Trace1	0.27	1.84	Automated - PPD
7		3.66	219.87	3.62	3.71	255.27	3.522E+4	8.316E+3	0.10	1.15	C:\Xcalibur\	No Filter	Trace1	0.50	0.73	Automated - PPD

Figure 45. Peak List view with a populated Name column

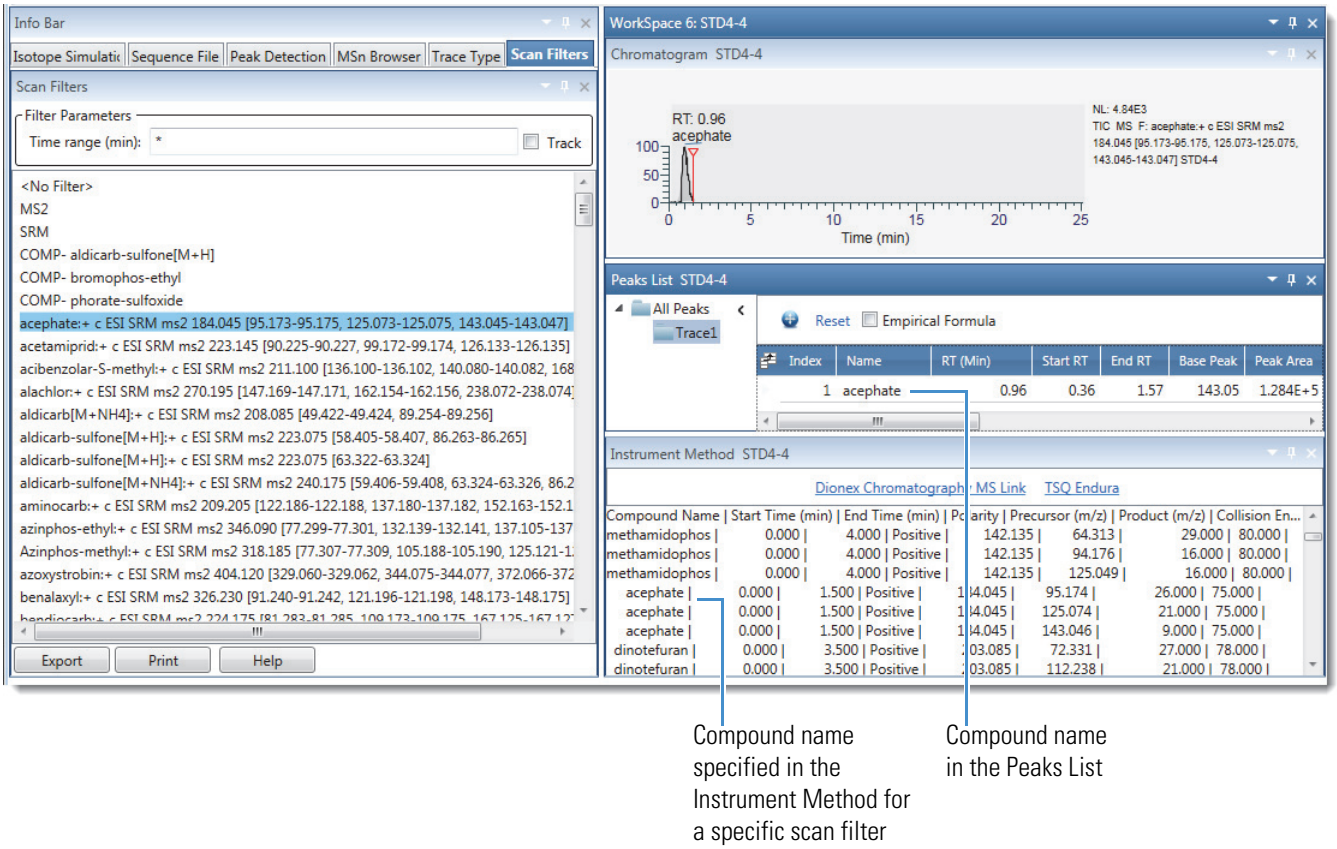


Table 15 describes the parameters and icons for the Peaks List view.

Table 15. Peaks List view parameters and icons (Sheet 1 of 3)

Parameter	Description
Reset 	Restores the data display in the Chromatogram view to the full range of the *x* axis and *y* axis after you zoom in by clicking a row in the peaks list.
Field Chooser 	Displays the Field Chooser dialog box, where you select which fields appear in the peaks list (see "Selecting the Columns to Display in a View or Dialog Box with Tabular Data" on page 20).
Elemental Composition	Select to run the elemental composition algorithm. This algorithm requires full-scan data.
Field	
Index	Unique identification number for each chromatogram peak. The index increments from lowest to highest retention time in a trace and from the top trace to the bottom trace.

Table 15. Peaks List view parameters and icons (Sheet 2 of 3)

Parameter	Description
Name	Compound name from the instrument method (Figure 45). **Note** For some Thermo Scientific mass spectrometers, for example, the triple quadrupole product line, the instrument method that you use for data acquisition includes a table where you can associate a compound name with each scan filter. When you view the scan filters on the Scan Filters page, the associated compound names appear next to the scan filter. You cannot edit the names in the FreeStyle application.
RT (Min)	Retention time (in minutes) corresponding to the apex of the peak.
RT (Sec)	Retention time (in seconds) corresponding to the apex of the peak.
Start RT	Retention time corresponding to the start of the chromatographic peak, where the detection signal increases beyond the threshold criteria.
End RT	Retention time corresponding to the end of the chromatographic peak, where the detection signal decreases below the threshold criteria.
Base Peak	Mass-to-charge ratio of the most abundant ion at the apex of the peak. The Peak List view displays 0.00 if no mass spectral data is present.
Peak Area	Area of the peak in units of counts × seconds.
Peak Height	Number of counts at the peak apex.
Baseline Width	Difference (in minutes) between the start and end retention times.
Signal To Noise	Signal-to-noise ratio of the integrated peak.
Empirical Formula	Chemical formula determined by the elemental composition algorithm. By default, this column is hidden. Selecting the Empirical Formula check box adds the column to the table and runs the elemental composition algorithm.
File Name	Displays the path and name of the raw data file. To display the Open Raw File dialog box, click the browse icon, ..., at the end of the file name.

Table 15. Peaks List view parameters and icons (Sheet 3 of 3)

Parameter	Description
Scan Filter	Select a filter to apply to the raw data file. The application autosenses the metafilters (including SRM or compound filters) in the raw data file in this order: MS, MS2, ETD, HCD, MSN, and then the individual scan filter list. For grouped filters, the Filter list displays only the first filter in the group. The Spectrum view displays the actual filter for the scan. For more information, see "Scan Filter Parameters" on page 263.
Trace ID	Number that identifies the chromatogram trace. The Trace ID increments from the top trace to the bottom trace.
% Area	Peak area as a percentage of the total peak area.
% Height	Peak height as a percentage of the total peak height.
Integration Method	Displays the integration method: ICIS, Genesis, Avalon, PPD, or manual.

Chromatogram View Toolbox

Use the Chromatogram view toolbox to create average and background subtracted spectra, add manual peaks, and zoom in or out of the Chromatogram View.

How you open the toolbox depends on your workspace setting (see "Default Workspace Options Page" on page 258). With the factory default setting, you can open the toolbox in two ways:

- Pause the pointer in the view. The toolbox opens with its pin icon in the unpinned (horizontal) position,  . To keep the toolbox open, click its pin icon.

–or–

- Right-click the view and choose Show Toolbox. The toolbox opens with its pin icon in the pinned (vertical) position,  . To close the toolbox, click its pin icon.

Figure 46 shows the floating toolbox, and Table 16 describes its icons and fields.

Figure 46. Chromatogram view toolbox

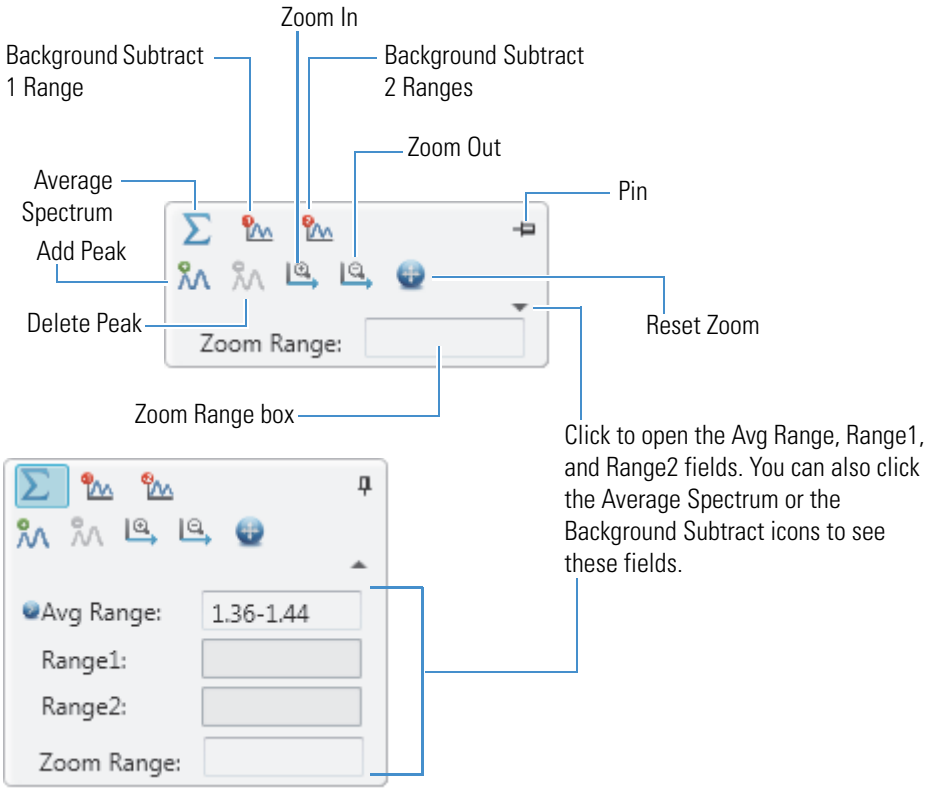


Table 16. Chromatogram view toolbox icons and text boxes (Sheet 1 of 2)

Icon/ Field	Description
Σ Average Spectrum and the Ave Range box	Averages the mass spectra over a selected retention-time range. To create an average spectrum, see "Averaging Spectra" on page 121.
Background Subtract 1 Range icon and the Range 1 box	Subtracts the mass spectrum of one retention-time range from the active mass spectrum. To create a background subtracted spectrum, see "Subtracting Background Spectra" on page 122.
Background Subtract 2 Ranges and the Range 1 and Range 2 boxes	Subtracts the mass spectra of two retention-time ranges from the active mass spectrum. To create a background subtracted spectrum, see "Subtracting Background Spectra" on page 122.

Table 16. Chromatogram view toolbox icons and text boxes (Sheet 2 of 2)

Icon/ Field	Description
Pin	When the pin is in the horizontal position, the toolbox auto-hides when you move the pointer away from the current location. To keep the toolbox visible, click the pin icon to change it to a vertical pin.
Add Peak	Adds a peak manually to the peak list in the Peaks List View (see "Manually Adding and Deleting Chromatographic Peaks" on page 67). Clicking the Add Peak icon again turns off the Add Peak pointer, and resumes the mouse pointer's zoom functionality.
Delete Peak	Deletes a manual peak from the peak list (see "Deleting Chromatographic Peaks" on page 68). Clicking the Delete Peak icon again turns off the Delete Peak pointer, and resumes the mouse pointer's zoom functionality. Available when the peaks list includes a peak.
Tip You can also use the Add Peak and Delete Peak buttons in the Chromatogram – Workspace Processing Toolbar to add and delete manual chromatographic peaks.	
Zoom In	Displays a smaller portion of the *x* axis.
Zoom Out	Displays a larger portion of the *x* axis.
Reset Zoom	Resets the zoom to display the entire chromatogram trace.
Zoom Range	Enter a retention time (RT) range within the chromatogram RT limits and then press ENTER. The view rescales to display the chromatogram within the entered time range.

Chromatogram-Specific Pages in the Info Bar

Before you open a workspace in the FreeStyle window, the Info Bar contains only the Isotope Simulation page (see "Isotope Simulation Page" on page 209). Opening a raw data file adds the Peak Detection, MSn Browser, and Detector Type pages to the Info Bar; whereas, opening a sequence file opens these pages and the Sequence Files page. The Detector Type page lists the detectors used to acquire data for the active raw data file and the MS Trending selection if the raw data file contains mass spectrometry data.

Note If you click a row in the Filter or Trace Type columns in the Chromatogram view, the application replaces the Detector Type page with the Scan Filters page or the Trace Type page, respectively.

These are the pages in the Info Bar:

- Detector Type Page
- Trace Type Page
- Scan Filters Page
- Genesis Peak Detection Page
- ICIS Peak Detection Page
- Avalon Peak Detection Page
- PPD Peak Detection Page
- Sequence File Page

Detector Type Page

The detector type determines what types of traces the Chromatogram View displays. Use the Detector Type page of the Info Bar to select the detector type. The detector types shown on this page are the same as those in the Detector Type list in the Chromatogram Ranges View.

These are the possible detector types:

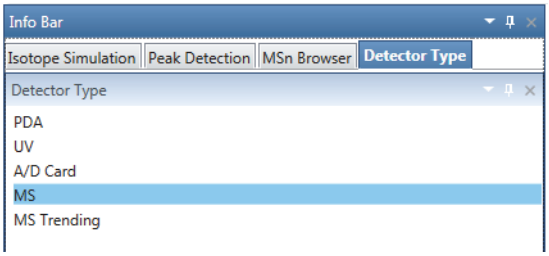
- MS—Mass spectrometry data
- A/D Card—Data from an analog-to-digital converter
- MS Trending—Instrument status readings for the mass spectrometer
- UV—Data from an analog detector
- PDA—Wavelength scan data from a photodiode array detector

❖ To display the Detector Type page

Click the **Detector Type** tab in the Info Bar.

Figure 47 shows a typical Detector Type page.

Figure 47. Detector Type page



❖ To display a plot in the Chromatogram view

On the Detector Type page, do one of the following:

- To display an unfiltered plot of the MS data, click **MS**.
- To display a total scan plot for the PDA data, click **PDA**.
- To display a plot for a UV channel, click **UV**.

- To display a plot for the data acquired with an analog-to-digital converter, click **A/D Card**.
- To display a plot for one of the MS detector's status readbacks, click **MS Trending**, click a row in the Trace Type column in the Chromatogram Ranges view, and then, on the Trace Type page, click a trace type (instrument readback).

Trace Type Page

Use the Trace Type page to select the type of trace that the Chromatogram view displays. The available trace types depend on which detector type you select on the Detector Type Page:

- For MS detectors, choose the TIC, mass range, base peak, or neutral fragment trace type.
- For PDA detectors, choose the wavelength range, total scan, or spectrum maximum trace type.
- For analog detectors, choose the analog 1, 2, 3, or 4 trace type.
- For MS Trending, choose any of the instrument status selections.

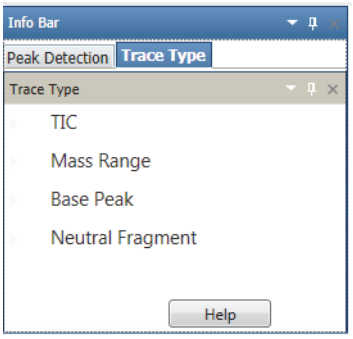
The trace types that the Trace Types page lists are the same as in the Trace Type list in the Chromatogram Ranges View.

❖ To display the Trace Type page

1. Open the Chromatogram Ranges View.
2. Click the dropdown arrow in the Trace Type column.

Figure 48 shows trace types for an MS detector type.

Figure 48. Trace Type page



Scan Filters Page

Use the Scan Filters page to select the type of scan filter, if any, that the application applies to the raw data file. The filter types on the Scan Filters page are the same as in the Filter box dropdown list in the Chromatogram Ranges View.

For instructions on using the Scan Filters page, see "Using the Scan Filters Page to Display a Filtered Chromatogram" on page 54.

Note For grouped filters (MS, MS2, HCD, and so on), the Scan Filters page displays only the first filter in the group. The scan header in the Spectrum view displays the actual filter for the scan.

❖ To display the Scan Filters page in the Info Bar

In the Workspace Options toolbar, in the Filter area, click **Scan Filters**.

Figure 49 shows a Scan Filters page.

Figure 49. Scan Filters page

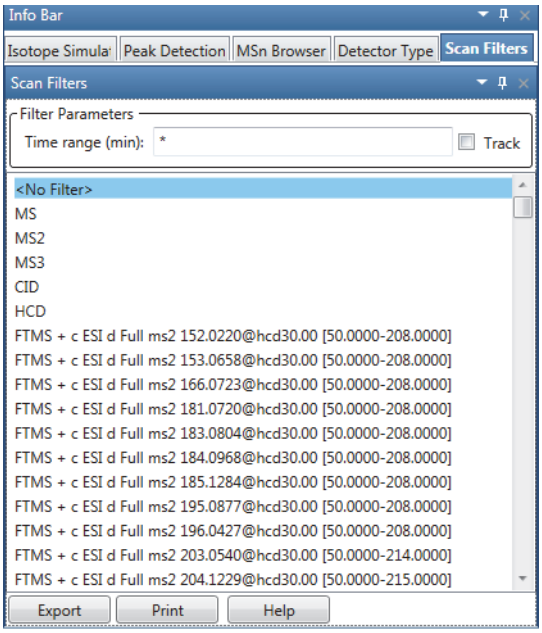


Table 17 describes the parameters on the Scan Filters page.

Table 17. Scan Filters page parameters (Sheet 1 of 2)

Parameter	Description
Filter Parameters	
Time Range (min)	Specifies the time range to display in the filter list. Filters outside the specified time range do not appear in the filter list. The specified time range must be within the retention time range for the data file. Use an asterisk to specify the full retention time range.
Track	Select to set up the time range by dragging the pointer across the Time axis in the Chromatogram view.

Table 17. Scan Filters page parameters (Sheet 2 of 2)

Parameter	Description
Filter list	
<No Filter>	Select to display an unfiltered chromatogram.
MS	Select to display a TIC chromatogram for the full-scan MS1 data.
MSn (where n = 2 or higher)	Select to display a TIC chromatogram for the MSn data.
Scan filters	Select to display a chromatogram for a specific scan filter.
Buttons	
Export	Copies the file name of the raw data file and the contents of the Scan Filters page to the Clipboard.
Print	Opens the Print dialog log box for selecting the printer and print preferences for printing the contents of the Scan Filters page.
Help	Opens the Help to the Scan Filters page topic.

Genesis Peak Detection Page

The Genesis peak detection algorithm is the original Thermo Xcalibur™ peak detection algorithm and has been provided for backward compatibility with Xcalibur 1.0 data system studies. Use the Genesis Peak Detection page of the Info Bar to specify peak identification and peak integration criteria that the Genesis algorithm applies toward chromatograms in the Chromatogram View.

For information about using the Genesis peak detection algorithm, see "Automatically Detecting and Integrating Chromatographic Peaks" on page 63.

❖ To display the Genesis Peak Detection page

1. Click the **Peak Detection** tab in the Info Bar.
2. In the Select Algorithm list, select **Genesis**.

Figure 50 shows the parameters for the Genesis peak detection algorithm.

Figure 50. Genesis Peak Detection page

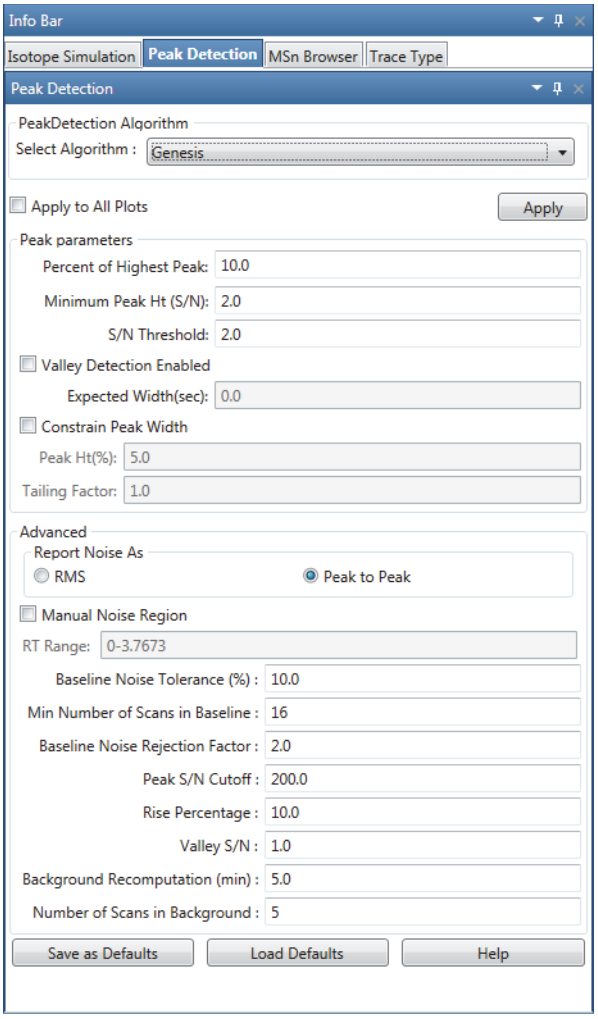


Table 18 describes the parameters for the Genesis Peak Detection page.

Table 18. Genesis peak detection parameters (Sheet 1 of 4)

Parameter	Description
Application of Settings	
Apply to All Plots	Apply the peak identification and integration settings to all displayed chromatograms. To apply the criteria to only the active chromatogram, clear this option.

Table 18. Genesis peak detection parameters (Sheet 2 of 4)

Parameter	Description
Peak Parameters	
Percent of Highest Peak	Specify a percentage threshold to limit the number of peaks to be submitted for further processing. The algorithm discards any detected peak that has an intensity less than the threshold percentage of the most intense peak. Range: 0.0–100.0; default: 10.0
Minimum Peak Ht (S/N)	Specify the peak signal-to-noise (S/N) value to equal or exceed as a criterion for peak detection. The algorithm ignores all chromatogram peaks that have S/N values less than the minimum peak height S/N value. Range: 1.0 (all peaks) to 999.0; default: 2.0
S/N Threshold	Specify the threshold for detecting peak edges. The algorithm calculates the S/N ratio by using only the baseline signal. It excludes any extraneous or minor detected peaks from the calculation. Range: 0.0–999.0; default: 2.0
Valley Detection Enabled	Select the valley detection approximation method to detect unresolved peaks. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.
Expected Width (sec)	Specify the expected peak width (in seconds). This value controls the minimum width that a peak must have if you selected the Valley Detection Enabled option. With valley detection selected, the algorithm ignores any valley points nearer than the *expected width*/2 to the top of the peak. If it finds a valley point outside the expected peak width, the algorithm terminates the peak at that point. The algorithm always terminates a peak when the signal reaches the baseline, independent of the value set for the expected peak width. Range: 0.0–999.0; default: 0.0
Constrain Peak Width	Limits the peak width of a component during peak integration. To control when peak integration is turned on and off, specify a peak height threshold and a tailing factor.

Table 18. Genesis peak detection parameters (Sheet 3 of 4)

Parameter	Description
Peak Ht (%)	Specify the peak height (as a percentage) where the algorithm tests the width of target peaks. Range: 0.0–100.0; default: 5.0
Tailing Factor	Specify a factor that controls how the algorithm integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading edge of a constrained peak. Range: 0.5–9.0; default: 1.0
Advanced	
RMS	Calculate noise as a root-mean-square (RMS) value.
Peak to Peak	Calculate noise as a peak-to-peak value.
Manual Noise Region	Specify a region of the chromatogram that the algorithm uses to determine noise. See "Selecting the Manual Noise Region for the Genesis and ICIS Algorithms" on page 66.
RT Range	Specify the RT range that the algorithm uses to determine noise. The RT range must be within the chromatogram range.
Baseline Noise Tolerance (%)	Specify a percentage value that controls how the algorithm draws the baseline in the noise data. The higher the baseline noise tolerance value, the higher it draws the baseline through the noise data. Range: 0.0–100.0; default: 10.0
Min Number of Scans in Baseline	Specify the minimum number of scans that the algorithm uses to calculate a baseline. A larger number includes more data in determining an averaged baseline. Range: 2–100; default: 16
Baseline Noise Rejection Factor	Specify the baseline noise rejection factor. This factor controls the width of the RMS noise band above and below the peak detection baseline. The algorithm applies this factor to the raw RMS noise values to raise the effective RMS noise that the algorithm uses during peak detection. It responds by assigning the left and right peak boundaries above the noise and, therefore, closer to the peak apex value. This action effectively raises the peak integration baseline above the RMS noise level. Range: 0.1–10.0; default: 2.0

Table 18. Genesis peak detection parameters (Sheet 4 of 4)

Parameter	Description
Peak S/N Cutoff	Specify the S/N level that the algorithm defines as the top of the peak edge. For example, if the S/N level at the apex is 500 and the Peak S/N Cutoff value is 200, the algorithm defines the right and left edges of the peak when the S/N reaches a value less than 200. Range: 50.0–10 000.0; default: 200.0
Rise Percentage	Specify how much above the baseline the trace can rise (as a percentage) after passing through a minimum inflection point in the trace (before or after the peak). If the trace exceeds this value, the algorithm applies valley detection peak integration criteria. It applies this test to both the left and right edges of the peak. The rise percentage criterion is useful for integrating peaks with long tails. Range: 0.1–500.0; default: 10.0
Valley S/N	Specify the S/N criterion that the algorithm uses for valley detection. Range: 1.0–100.0; default: 1.0
Background Recomputation (min)	Specify the background recomputation interval (in minutes). The algorithm periodically recalculates the representative background scan it uses for background subtraction to compensate for the possibility that the composition of the background might change over the course of a run. The background recomputation interval is the time interval between these recalculations. Range: 0.5–10.0; default: 5.0
Number of Scans in Background	Specify the number of background scans the algorithm uses to determine the background. Range: 2–100; default: 5
Button	
Apply	Starts peak detection by using the Genesis peak detection algorithm.
Save As Defaults	Saves the current settings as default settings. After you save the settings as defaults, you can restore these values at any time by clicking Load Default.
Load Defaults	Restores the current default settings.
Help	Opens the FreeStyle Help to the Genesis peak detection topic.

ICIS Peak Detection Page

The ICIS peak detection algorithm is the default algorithm that was designed for MS data. It is known for peak detection efficiency at low MS signal levels.

Use the ICIS Peak Detection page of the Info Bar to specify peak detection and integration criteria. The application applies the peak detection algorithm to the active plot or all the plots in the Chromatogram view.

For more information, see "Automatically Detecting and Integrating Chromatographic Peaks" on page 63.

❖ To display the ICIS peak detection parameters

1. Click the **Peak Detection** tab in the Info Bar.
2. In the Select Algorithm list, select **ICIS**.

Figure 51 shows the parameters for the ICIS peak detection algorithm.

Figure 51. ICIS Peak Detection page

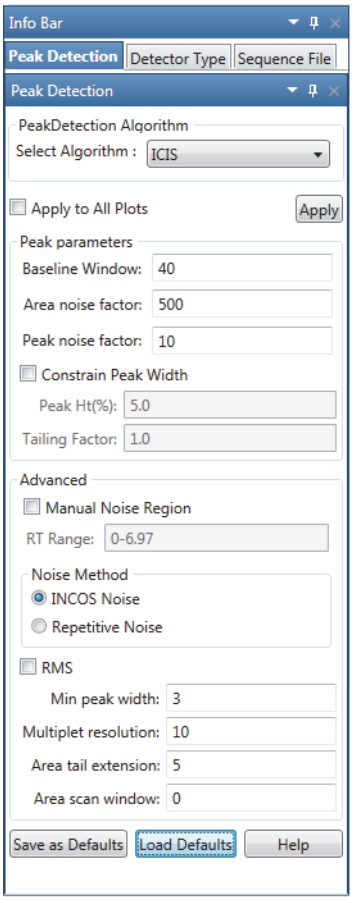


Table 19 describes the parameters for the ICIS peak detection algorithm.

Table 19. ICIS Peak Detection page parameters (Sheet 1 of 2)

Parameter	Description
Application of Settings	
Apply to All Plots	Apply the current chromatogram peak identification and integration settings to all displayed plots. To apply the criteria to only the active plot, clear this option.
Peak Parameters	
Baseline Window	Specify the number of scans, from the apex down each side of the peak, that the peak detection algorithm uses to determine the minimum baseline for the peak. A higher number of scans means a wider scan range, resulting in a lower baseline. Range: 1–500; default: 40
Area Noise Factor	Specify the noise level multiplier that the peak detection algorithm uses to determine the peak edge after the location of the possible peak. Range: 1–500; default: 500
Peak Noise Factor	Specify the noise level multiplier that the peak detection algorithm uses to determine the potential peak signal threshold. Range: 1–1000; default: 10
Constrain Peak Width	Limits the peak width of a component during the peak integration of a chromatogram. You can then set values that control when peak integration turns on and off by specifying a peak height threshold and a tailing factor.
Peak Ht (%)	Specify how much above the baseline (as a percentage) a signal must be of the total peak height before integration turns on or off. Range: 0.0–100.0; default: 5.0
Tailing Factor	Specify a factor that controls how the algorithm integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading edge of a constrained peak. Range: 0.5–9.0; default: 1.0
Advanced	
Manual Noise Region	Specify a region of the chromatogram that the peak detection algorithm uses to determine noise. You can enter the retention time (RT) in the RT Range box. See "Selecting the Manual Noise Region for the Genesis and ICIS Algorithms" on page 66.

Table 19. ICIS Peak Detection page parameters (Sheet 2 of 2)

Parameter	Description
RT Range	Specify the RT range that the algorithm uses to determine noise. The RT range must be within the chromatogram range.
INCOS Noise	Use a single pass algorithm to determine the noise level.
Repetitive Noise	Use a multiple pass algorithm to determine the noise level. In general, this algorithm is more accurate in analyzing the noise than the INCOS Noise algorithm; however, it might take longer depending on the data.
RMS	Calculate noise as root mean square (RMS). By default, the algorithm uses peak-to-peak for the noise calculation. If you determine the noise region manually, the algorithm automatically selects RMS.
Min Peak Width	Enter the minimum number of scans required in a peak. Range: 0–100; default: 3
Multiplet Resolution	Specify the minimum separation in scans between the apexes of two potential peaks. This criterion determines if two peaks are resolved. Range: 1–500; default: 10
Area Tail Extension	Specify the number of scans past the peak endpoint to use in averaging the intensity. Range: 0–100; default: 5
Area Scan Window	Specify the number of scans on each side of the peak apex to include in the area integration. Range: 0–100; default: 0 **Note** Specifying zero scans means that the algorithm includes all scans in the area integration, from the peak start to the peak end.
Button	
Apply	Starts the ICIS peak detection algorithm.
Save As Defaults	Saves the current settings as the default settings.
Load Defaults	Restores the current default settings.
Help	Opens the FreeStyle Help to the ICIS peak detection topic.

Avalon Peak Detection Page

The Avalon peak detection algorithm supports detectors other than mass spectrometers, and detects negative chromatographic peaks more accurately than the Genesis or ICIS peak detection algorithms. Use the Avalon Peak Detection page in the Info Bar to specify peak identification and integration criteria. You can apply this algorithm to the active plot or all the plots in the Chromatogram View.

❖ To display the Avalon Peak Detection page

1. Click the **Peak Detection** tab in the Info Bar.
2. Select the **Avalon** search algorithm.

Figure 52 shows the parameters for the Avalon peak detection algorithm.

Figure 52. Avalon Peak Detection Settings page

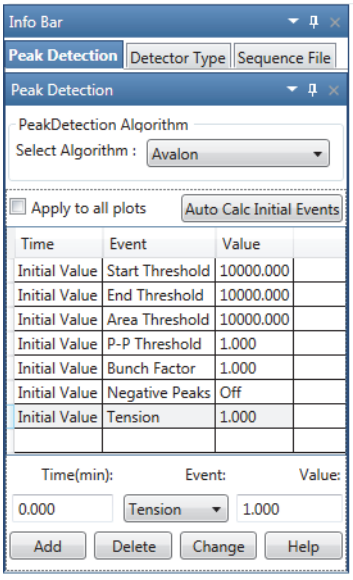


Table 20 describes the parameters for the Avalon peak detection algorithm.

Table 20. Avalon Peak Detection page parameters (Sheet 1 of 4)

Parameter	Description
Application of Settings	
Apply to All Plots	Apply the current chromatogram peak identification and integration settings to all displayed chromatogram plots. To apply the criteria to only the active plot, clear this check box.

Table 20. Avalon Peak Detection page parameters (Sheet 2 of 4)

Parameter	Description
Event List	
Event List	Specify the settings for initial events and user-defined timed events in the event list. To calculate values for initial events, click **Auto Calculate Initial Events**. To change the settings in the event list, highlight the row and then enter the revised settings in the boxes below the list. Click **Add** to add a new row of entered values to the event list. Click **Change** to update automatically both the event list and the chromatogram display. There are seven permanent integration events, identified by the Initial Value setting in the Time column. These are the default integration events that the Avalon integration algorithm requires. You can change the value of an initial entry integration event, but you cannot delete it or change its time value.
Event List Entry	
Time	Specify the value for the Time column for the highlighted entry in the event list. You cannot change the time entry for initial value events.

Table 20. Avalon Peak Detection page parameters (Sheet 3 of 4)

Parameter	Description
Event	Select the event type from these options: • Start/End Threshold: Directly related to the RMS noise in the chromatogram, this value is the start or end threshold, the fundamental control used for peak detection. • Area Threshold: Controls the area cutoff. The algorithm does not detect any peaks with a final area less than the area threshold. This control is in units of area for the data. • P-P Threshold: The peak-to-peak resolution threshold controls how much peak overlap must be present before two or more adjacent peaks create a peak cluster. Peak clusters have a baseline drop instead of valley-to-valley baselines. This option is specified as a percentage of the peak height overlap. • Negative Peaks: Allows or denies negative peaks. • Bunch Factor: The number of points that are grouped together during peak detection. It controls the bunching of chromatographic points during integration and does not affect the final area calculation of the peak. The Bunch Factor must be an integer between 1 and 6; a high bunch factor groups peaks into clusters. • Tension: Controls how closely the baseline must follow the overall shape of the chromatogram. A lower baseline tension traces the baseline to follow changes in the chromatogram more closely. A higher baseline tension follows the baseline less closely over longer time intervals. This option is specified in minutes. • Tangent Skim: For fused peaks that are significantly different in size, the tangent skim method allocates area to the various peaks. By default, the algorithm chooses the tallest peak in a cluster as the parent. You can also identify which peak in the cluster is the parent. The algorithm detects tangent skim peaks on one or both sides of the parent peak. Tangent skim automatically resets at the end of the peak cluster. The threshold and bunch factor parameters are the most important ones in controlling peak detection.
Value	Specify the value for Value column for the currently highlighted entry in the event list. The range of factors allowed for each value is specific to each event.

Table 20. Avalon Peak Detection page parameters (Sheet 4 of 4)

Parameter	Description
Button	
Auto Calc Initial Events	Searches for the best values of initial events that detect peaks in the data. This button is active if you have a raw data file open. The Avalon algorithm automatically estimates the initial values for the detection of peaks based on the data in the current raw data file, and then displays those initial values in the event list. Any timed event in the event list is unchanged. Automatic calculation of initial events determines initial values for the following events only: start threshold, end threshold, area threshold, P-P [resolution] threshold, bunch factor, negative peaks, and tension. Additionally, you can specify timed events for these events in the same event list.
Add	Add a row to the event list. When you click Add, both the event list and the chromatogram update automatically with the added specification in the currently selected chromatogram.
Delete	Removes a highlighted event from the event list. You cannot delete initial values.
Change	Updates a highlighted entry in the event list. When you click Change, peak detection with the updated specification occurs automatically for the currently selected chromatogram. For initial events, the algorithm changes only the values, and not the events.
Help	Opens the FreeStyle Help to the Avalon peak detection topic.

PPD Peak Detection Page

The PPD peak detection algorithm fits the data to model peak functions and has no integration settings.

Use the PPD Peak Detection page of the Info Bar to specify peak detection criteria. The application applies the peak detection algorithm to the active raw data file displayed in the FreeStyle window.

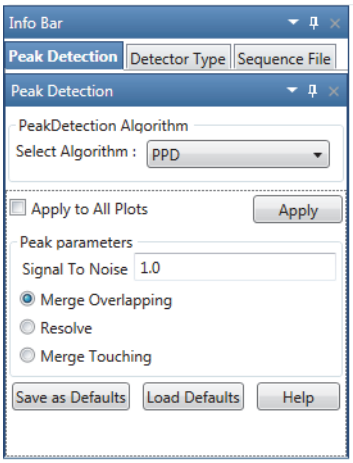
For information about using the PPD peak detection algorithm, see "Automatically Detecting and Integrating Chromatographic Peaks" on page 63.

❖ To display the PPD Peak Detection page

1. Click the **Peak Detection** tab in the Info Bar.
2. Select the **PPD** algorithm.

Figure 53 shows the settings for the PPD peak detection algorithm.

Figure 53. PPD Peak Detection page



For information about adding peak labels, see "Labeling Chromatogram Peaks or Local Maxima" on page 56.

Table 21 describes the parameters for the PPD peak detection algorithm.

Table 21. PPD Peak Detection page parameters (Sheet 1 of 2)

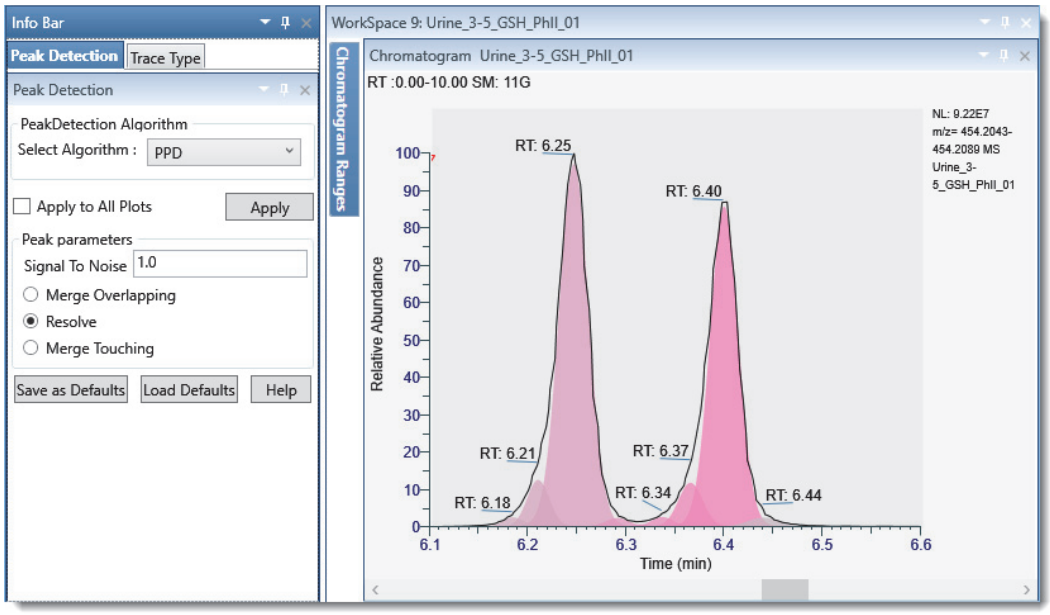
Parameter	Description
Application of Settings	
Apply to All Plots	Applies the current chromatogram peak identification and integration settings to all displayed plots. To apply the criteria to only the active plot, clear this option.
Peak Parameters	
Signal To Noise	Specifies the absolute signal-to-noise threshold. A value of 0 applies no filtering. Range: 0.00–100.00; default: 1.0
Merge Overlapping	(Default) Merges overlapping peaks into one peak. Use this option for LC/MS data to avoid reporting small shoulders as separate peaks.
Resolve	Resolves overlapping or touching peaks into two chromatographic peaks. Use this option for GC/MS data.
Merge Touching	Merges two peaks whose edges barely overlap at the 1% intensity level.
Button	
Apply	Starts the PPD peak detection algorithm.
Save As Defaults	Saves the current settings as the default settings.

Table 21. PPD Peak Detection page parameters (Sheet 2 of 2)

Parameter	Description
Load Defaults	Restores the current default settings.
Help	Opens the FreeStyle Help to the PPD peak detection topic.

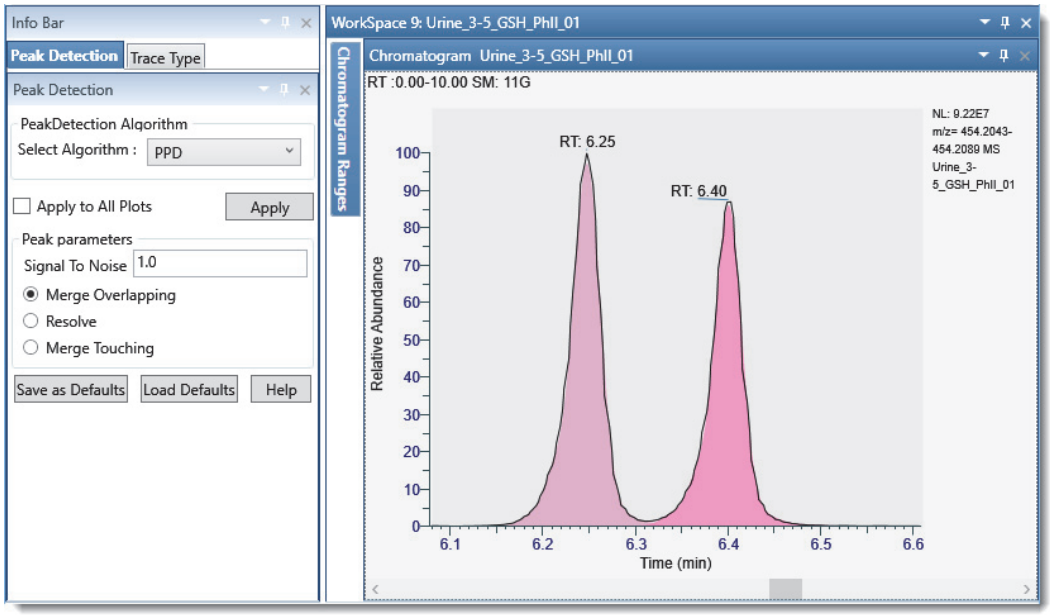
The PPD peak detection algorithm automatically resolves all the chromatographic peaks, and selecting the Resolve option displays all the detected peaks (see Figure 54).

Figure 54. PPD peak detection with the Resolve option



By default, the Merge Overlapping option is selected. For LC/MS data, use Merge Overlapping to avoid reporting small shoulders as separate peaks. Compare Figure 55 where the overlapping peaks are merged and reported as two peaks to Figure 54 where all the peaks are resolved and reported as seven separate peaks.

Figure 55. PPD peak detection with the Merge Overlapping option



Sequence File Page

The Sequence File page of the Info Bar displays a sequence of raw data files that you created or opened. Click a raw data file in the list to display its trace in the Chromatogram View.

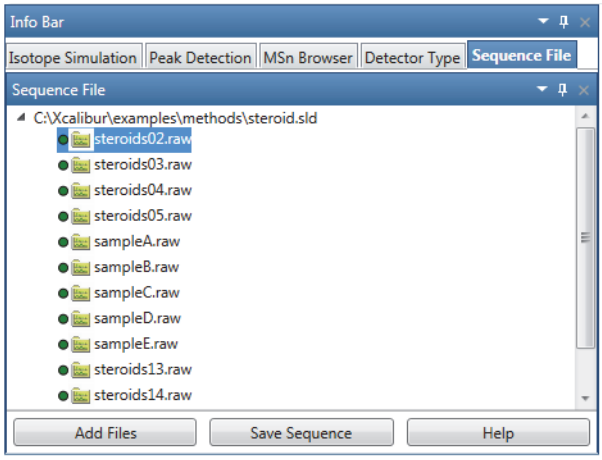
❖ To display the Sequence File page

Create or open a sequence of raw data files.

For more information, see "Creating, Positioning, Previewing, and Closing Workspaces" on page 17 or "Working with Sequences" on page 69.

Figure 56 shows a typical Sequence File page.

Figure 56. Sequence File page



4

Working with Spectra

Use the spectra-specific toolbars, views, and Info Bar pages of the FreeStyle application to review spectral data from the mass spectrometer or PDA detector.

Note For information about scan headers, zoom options, and custom annotations, see "Scan Headers and Scan Header Abbreviations" on page 266, "Zooming In and Out of a Graphical View" on page 23, and "Adding Text and Graphic Annotations to a Graphical View" on page 24, respectively.

To display spectra of interest, follow these topics.

Contents

Displaying the Scan for a Time Point in a Chromatogram

Follow this procedure to display the scan for a particular time point of a chromatogram.

❖ **To display the scan corresponding to a particular time point in a chromatogram**

1. Display the chromatogram plots of interest as described in "Defining the Range of a Chromatogram Trace" on page 48.
2. If the Spectrum view is closed, open it by clicking **Spectrum** in the Workspace Options toolbar.
3. Click the chromatogram plot at the retention time or scan number that you want to view.

   In the Chromatogram view, a red vertical marker,  , indicates the selected data point. The Spectrum view displays the spectrum for that retention time or scan number. On the computer keyboard, use the right or left arrow keys to increment or decrement the scan number.

   Note If you do not apply a scan filter, the scan number increments and decrements by one. If you do apply a scan filter, the scan number increments and decrements to the next scan number that meets the filter criteria.

Adding Spectrum Views to the Workspace

You can add multiple Spectrum views and MultiSpectrum views to the workspace.

❖ **To add a Spectrum view**

1. Open a raw data file (see "Opening Raw Data Files or Sequence Files" on page 15).

   With the factory default layout, the Chromatogram view appears at the top and the Spectrum view appears at the bottom and displays scan #1.
2. To add another Spectrum view to the workspace, do one of the following:
   - In the Workspace Options toolbar, click **Spectrum**.

     A copy of the active spectrum appears in the new Spectrum view.
   - Open the MSn Browser page and select the fragmentation spectra of interest (see "Selecting Spectra from an MSn Tree" on page 126).

     A new Spectrum view opens for each selected spectrum.

For information about displaying a different spectrum in the Spectrum view, see these topics:

- Displaying the Scan for a Time Point in a Chromatogram
- Selecting Spectra by Using the Spectrum Ranges Dialog Box

Selecting Spectra by Using the Spectrum Ranges Dialog Box

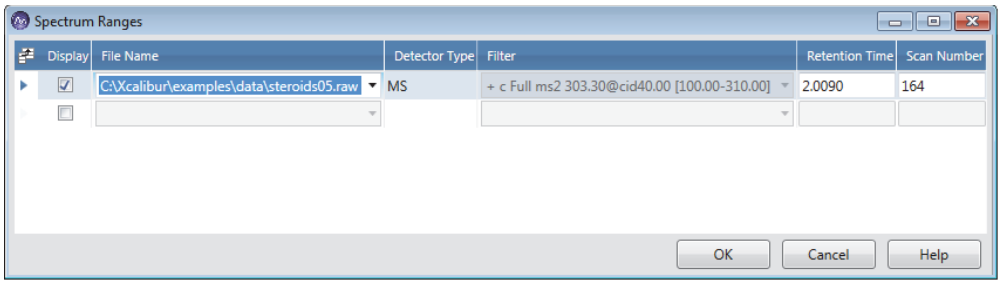
You can use the Spectrum Ranges dialog box to select the spectrum to be displayed in a Spectrum view or the spectra to be displayed in a MultiSpectrum view.

❖ To open the Spectrum Ranges dialog box

1. Click either a **Spectrum** view or a **MultiSpectrum** view to make it active.
2. In the Workspace Options toolbar, click **Spectrum Ranges**.

   The Spectrum Ranges dialog box opens with a list of the scans currently displayed in the selected view (Figure 57).

Figure 57. Spectrum Ranges dialog box



❖ To select different scans

Note In the FreeStyle 1.4 application, you cannot change the detector type or the scan filter from the Spectrum Ranges dialog box.

1. Do any of the following:
   - To add another spectrum plot, select the last check box in the Display column.
   - For a MultiSpectrum view, to remove a spectrum plot, clear its associated check box.
   - In the File Name list, select a different raw data file.

     The list only includes the raw data files that are open in the current FreeStyle session.
   - In the Retention Time box, type a different retention time, and then click elsewhere in the same row.

     The corresponding scan number appears in the Scan Number box.
   - In the Scan Number box, type a different scan number, and then click elsewhere in the same row.

     The retention time of the selected scan appears in the Retention Time box.
2. Click **OK**.

   If you add multiple plots to a Spectrum view, the Spectrum view becomes a MultiSpectrum view.

Creating a MultiSpectrum View and Changing Its Spectrum Plots

To compare spectrum plots using the same normalization level, you must use the MultiSpectrum View (see "Applying Local or Global Normalization in a MultiSpectrum View" on page 133).

To set up the spectrum plots in a MultiSpectrum view, follow these procedures as needed:

- To change a Spectrum view to a MultiSpectrum view
- To add spectrum plots to a MultiSpectrum view
- To select a different spectrum for any of the plots in a MultiSpectrum view

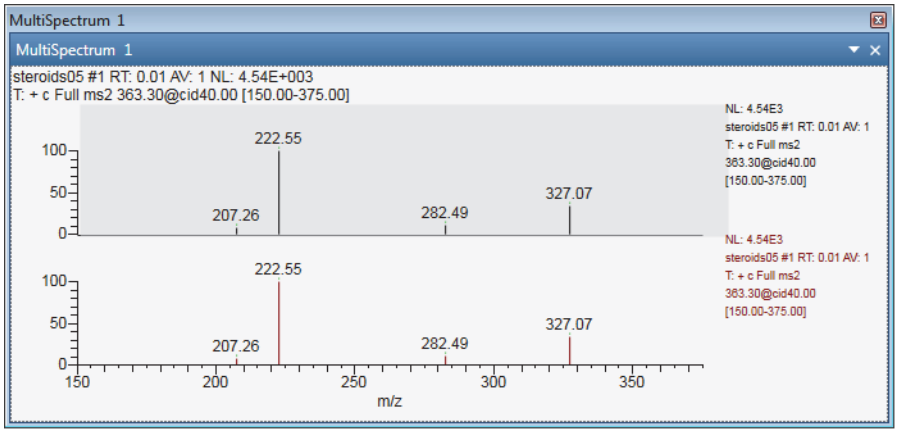
❖ To change a Spectrum view to a MultiSpectrum view

Note The Multi Spectrum button is only available when a Spectrum view or a MultiSpectrum view is active.

1. Click the **Workspace Options** toolbar tab.
2. Select a Spectrum view and click **Multi Spectrum**.

   The view's title bar changes to MultiSpectrum and a copy of the selected spectrum appears below the original spectrum (Figure 58).

Figure 58. MultiSpectrum view



❖ To add spectrum plots to a MultiSpectrum view

1. Click the MultiSpectrum view to make it active.
2. In the Workspace Options toolbar, click **Multi Spectrum**.

   A copy of the last plot appears at the bottom of the view.

❖ **To select a different spectrum for any of the plots in a MultiSpectrum view**

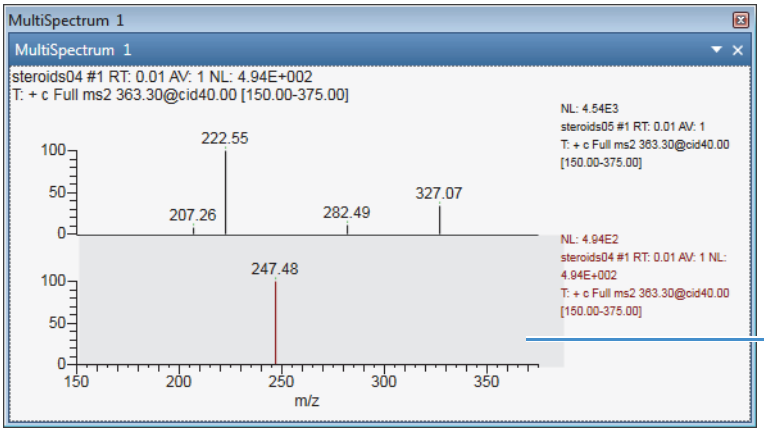
- Use the Spectrum Ranges dialog box (see "Selecting Spectra by Using the Spectrum Ranges Dialog Box" on page 115).

–or–

a. Click the plot to make it active.

An active plot has a darker background than the other plots in the view (Figure 59).

Figure 59. MultiSpectrum view with plots from different raw data files



b. In the Chromatogram view, select a point by using the pointer or the left and right keyboard keys.

Using the Spectrum Toolbox Tools to Display an EIC Plot

The Spectrum toolbox has two icons for adding EIC plots to the Chromatogram view—the EIC Mass icon and the EIC Range icon. To add a plot for multiple discrete *m/z* values, use the EIC Mass icon. To add a plot for contiguous *m/z* ranges, use the EIC Range icon.

Tip To display an EIC plot for a single mass-to-charge value, double-click the mass-to-charge label for the peak in the Spectrum view. The Spectrum view displays the EIC trace for the selected mass-to-charge ratio ± 1 Da.

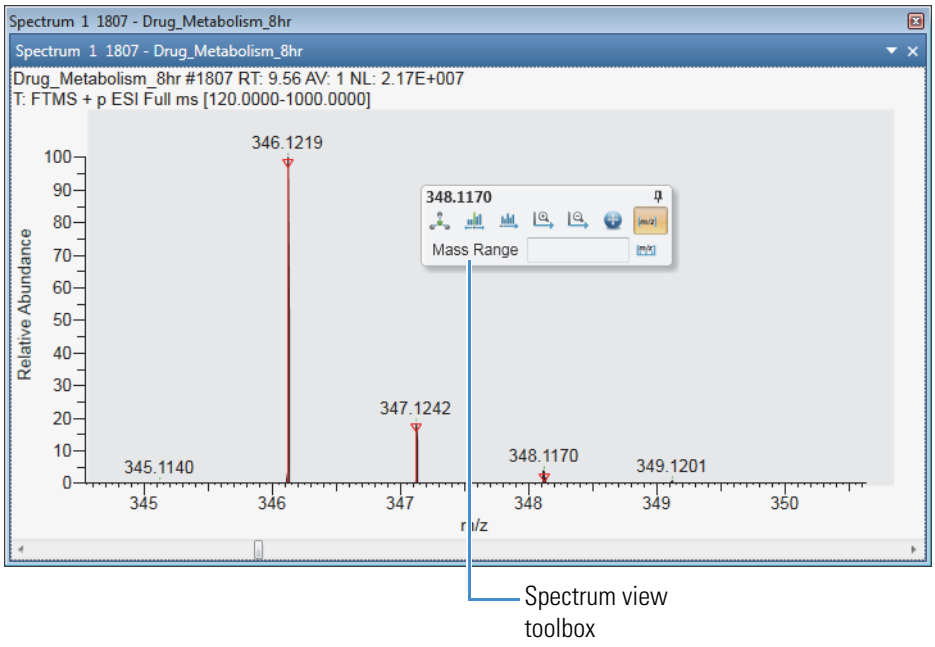
❖ **To display an EIC plot for multiple discrete *m/z* values**

1. Open the Spectrum View Toolbox.
2. Click the **EIC Mass** icon,  .

   If the toolbox was unpinned, the application pins it so that it remains visible.
3. In the Spectrum view, click the mass-to-charge label for each peak that you want to use for the EIC plot.

A red vertical marker indicates a selected peak. Figure 60 shows the selection of three mass peaks.

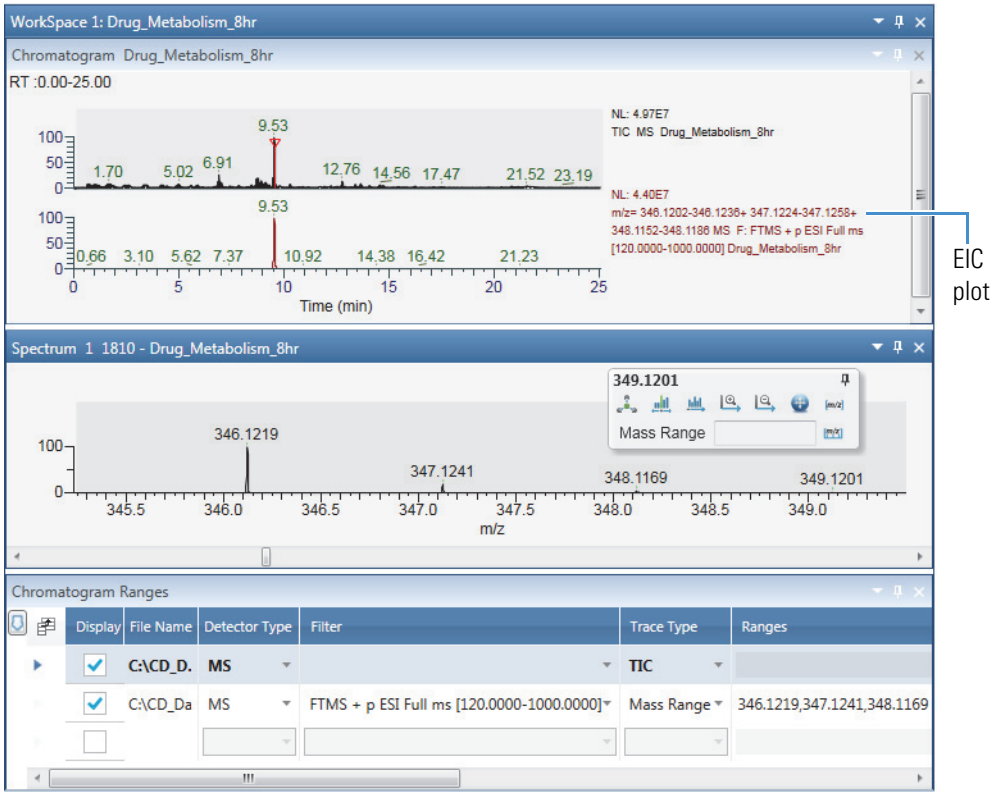
Figure 60. Spectrum view with the selection of three mass peaks



4. Click the **EIC Mass** icon again to create an EIC plot from the selected masses.

   The selected masses appear in the Ranges column in the Chromatogram Ranges View. A new EIC plot appears at the bottom of the Chromatogram view (Figure 61).

Figure 61. EIC plot created from three mass peaks



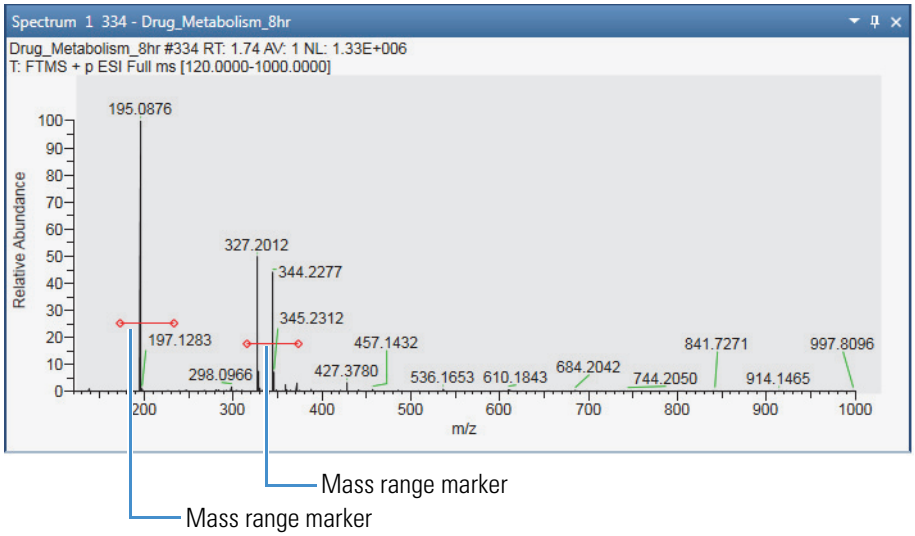
❖ To specify the mass ranges for an EIC plot

1. Open the Spectrum View Toolbox.
2. Click the **EIC Range** icon, .

   If the toolbox was unpinned, the application pins it so that it remains visible.
3. In the Spectrum view, drag the pointer horizontally through a contiguous or noncontiguous mass range to select the mass range that you want to use for the EIC plot.

   A horizontal red line with start and end markers indicates the start and end of the selected range (Figure 62).

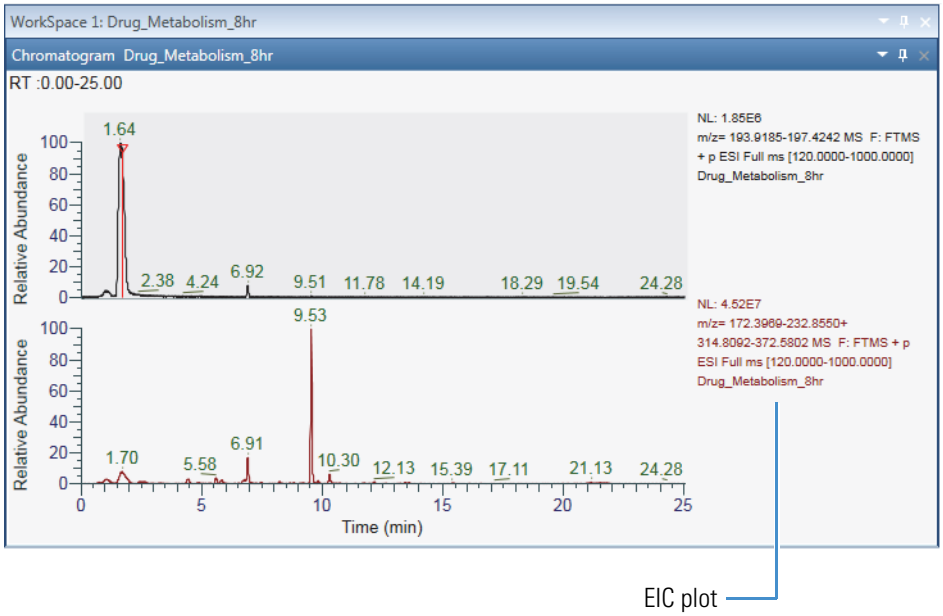
Figure 62. Two mass ranges selected in the Spectrum view



4. To create an EIC plot from the selected mass ranges, click the **EIC Range** icon again.

   The selected mass ranges appear in the Ranges column of the Chromatogram Ranges view, a new EIC plot appears at the bottom of the Chromatogram view, and the application unpins the toolbox. Figure 63 shows the EIC plot for two noncontiguous mass ranges.

Figure 63. EIC plot for two noncontiguous mass ranges



Averaging Spectra

You can increase mass accuracy and reduce noise in a spectrum by averaging the spectrum over an appropriate scan range.

To generate an average spectrum, the application uses the original intensity versus frequency data from the mass spectrometer, bins the data into frequency intervals, and then uses the instrument's mass calibration file to convert the frequency data to *m/z* values.

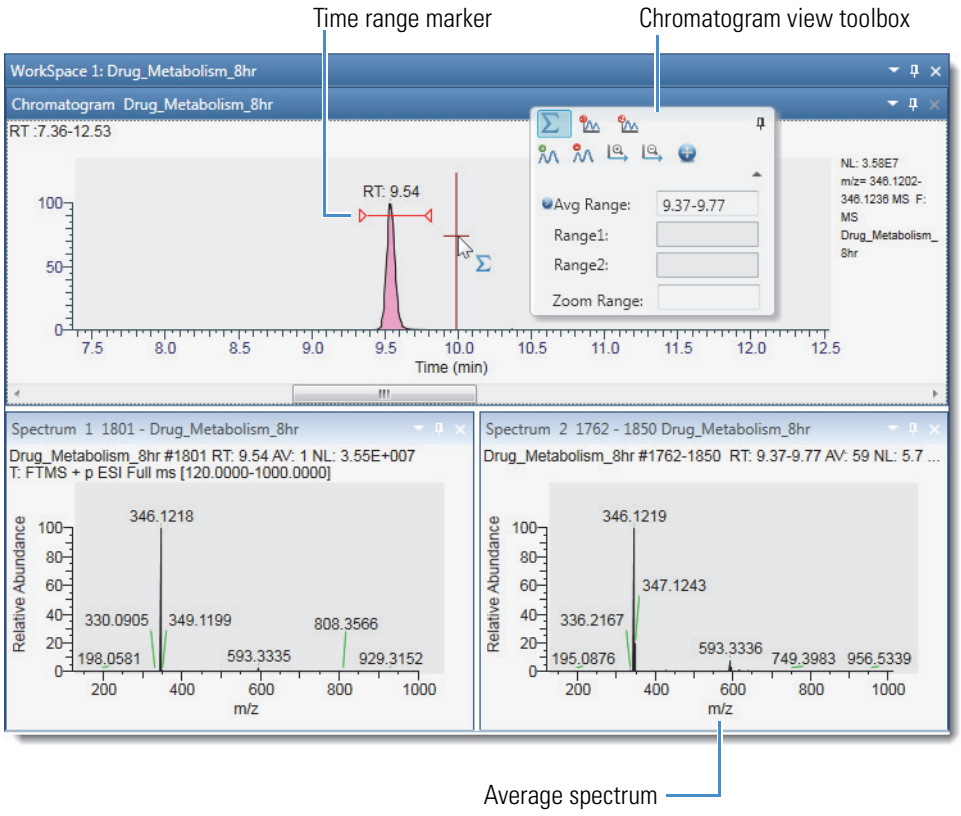
❖ To average a spectrum

1. Open the Chromatogram View Toolbox.
2. Click the **Average Spectrum** icon, Σ.
3. Drag the pointer through the time range that defines the scans to average, or enter the time range in the Avg Range box.

   A red line in the Chromatogram view marks the time range. You can undo spectrum averaging by clicking the Average Spectrum icon again.

   Figure 64 shows a Workspace view with two Spectrum views. The Spectrum view on the right shows the average spectrum for the time range defined in the toolbox.

Figure 64. Workspace with Chromatogram view and two Spectrum views



Subtracting Background Spectra

The solvent or other noise can create unwanted background spectra. You can subtract them from the spectrum for one or two ranges. The background subtraction algorithm subtracts an average of the selected scans and redraws the spectrum. The spectrum view header shows the number of subtracted scans. For example, SB: 12 indicates that the algorithm has applied background subtraction (SB) to the spectrum by using 12 scans. To the right of that is subtraction range_1 and range_2.

❖ To subtract background spectra from the spectrum

1. Open the Chromatogram View Toolbox.
2. Click the **Background Subtract 1 Range** icon or the **Background Subtract 2 Ranges** icon to background subtract over one or two ranges, respectively.
3. Drag the pointer through the time ranges that define the scans to subtract: once for one range or twice for two ranges. Or, enter the time ranges in the Range 1and Range 2 boxes.

   You can undo the background subtraction by clicking the Background Subtract 1 Range icon or the Background Subtract 2 Ranges icon again.

   Figure 65 shows the process of dragging the pointer across range 1.

Figure 65. Background subtraction process for the first time range

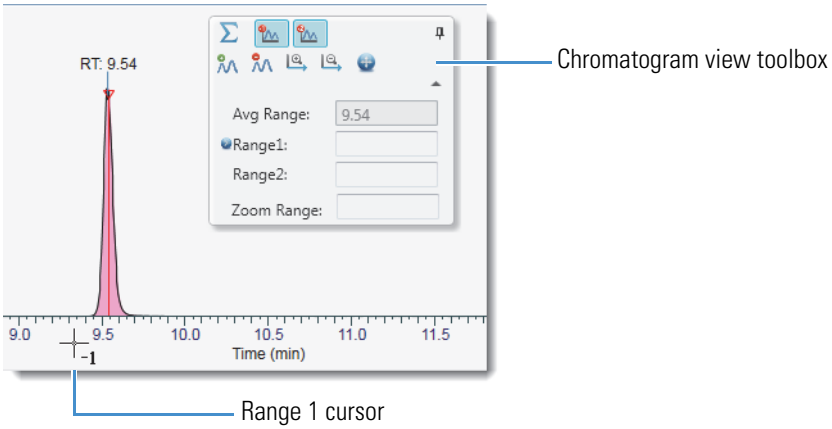


Figure 66 shows the process of dragging the pointer across range 2.

Figure 66. Background subtraction process for range 2

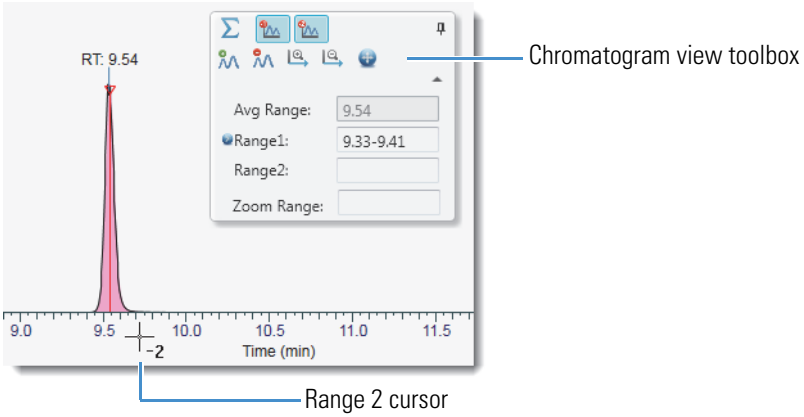
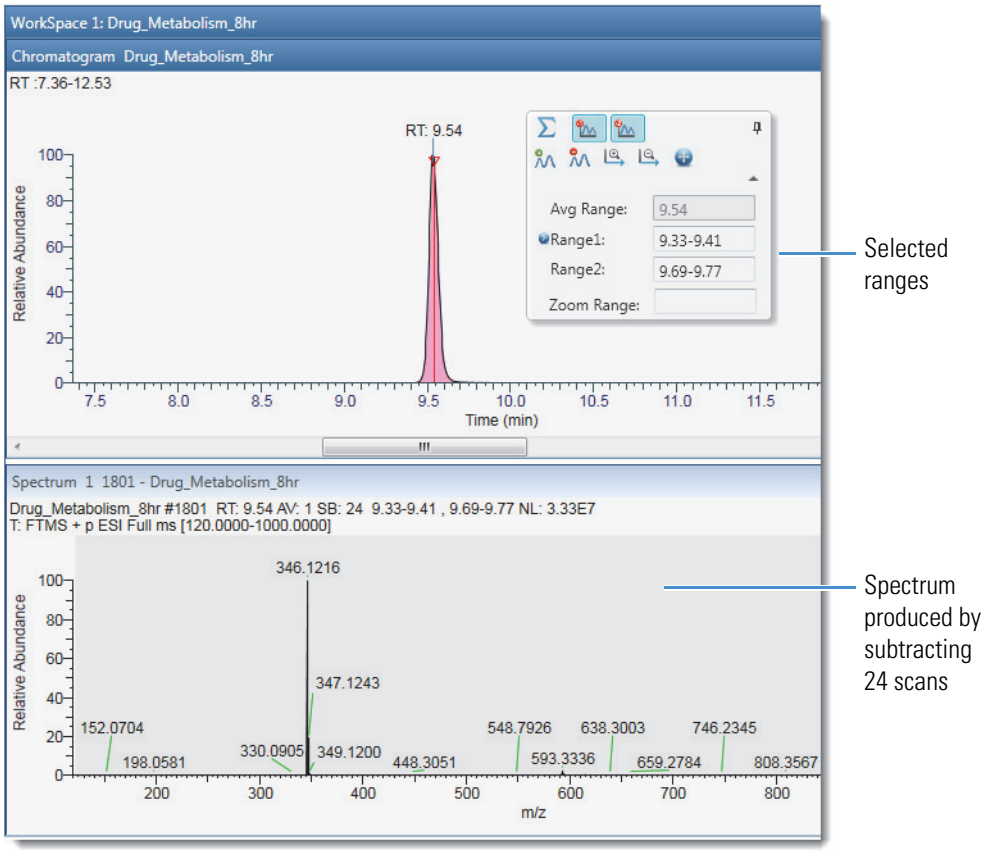


Figure 67 shows the toolbox with the selected background-subtraction ranges and the spectrum produced by subtracting 24 scans.

Figure 67. Spectrum produced by subtracting 24 scans



Creating a RAW File for a Selected Spectrum

Raw data files can contain gigabytes of data. To work with only a selected spectrum, you can export the spectrum to a new RAW file. The new RAW file will contain the selected spectrum and the sample data from the original RAW file and have a much smaller file size. The creation date of the new RAW file

❖ To export a spectrum to a new RAW file

1. Open a raw data file.
2. Open the spectrum of interest in a Spectrum or Multi Spectrum view.

   The spectrum can be a single scan, a portion of a scan, an averaged spectrum, or a composite spectrum.
3. Click the spectrum plot of interest to make it active.
4. In the Spectrum – Workspace Options toolbar, click **Write to .RAW**.

   The Export Data dialog box opens. The File name box contains the original file name appended with Scan[*scan number*].
5. Browse to an appropriate storage folder and rename the file as necessary.
6. Click **Save**.

   The new RAW file only contains the exported spectrum and the export date, which is listed in the File Header report as the acquisition date and time.

Viewing Data-Dependent Scans

The application can label the peaks that triggered data-dependent scans with triangles called precursor markers.

❖ To turn on the precursor flag label

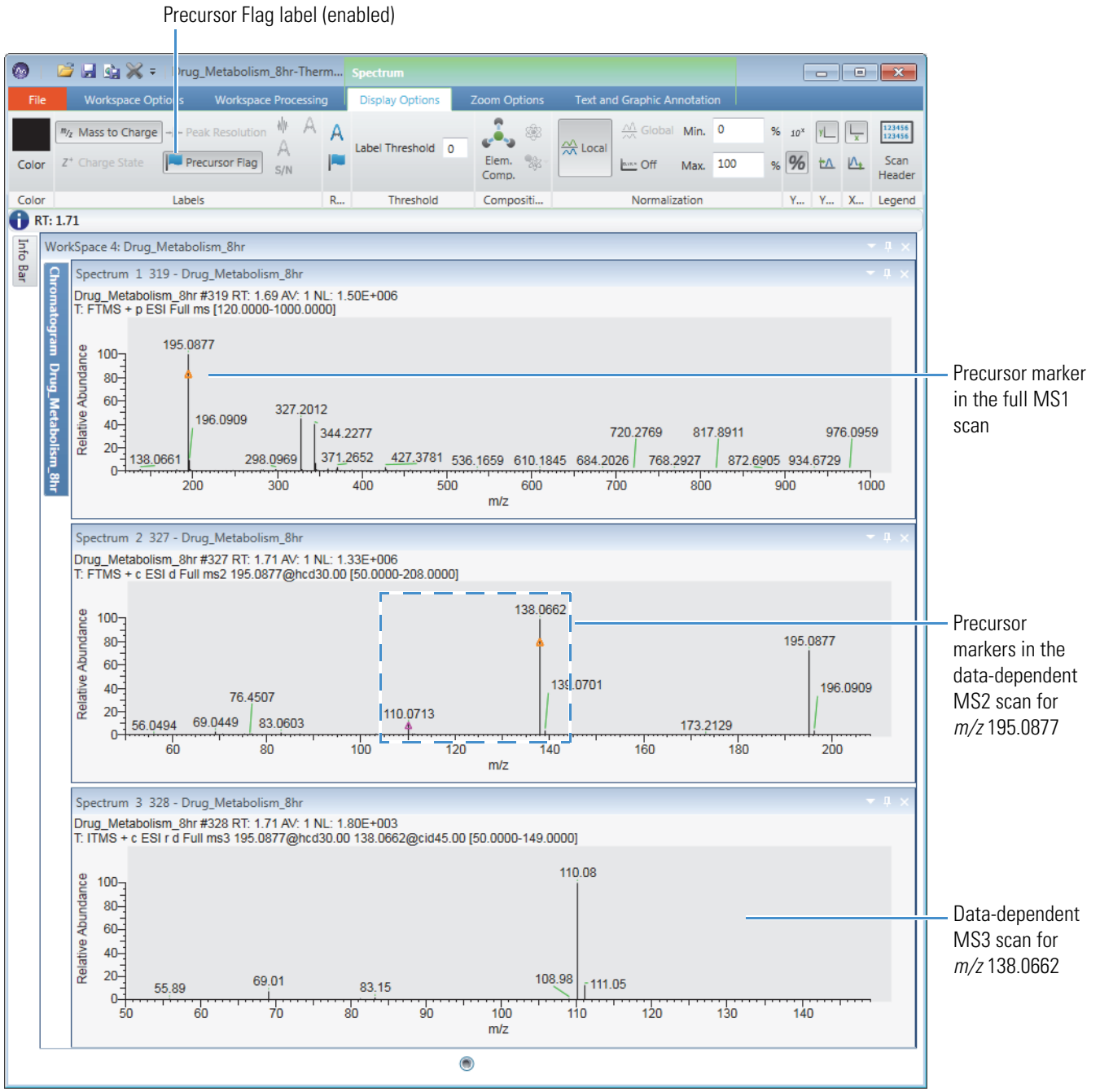
1. Click the **Spectrum** view to make it active.
2. In the Workspace Options toolbar, click the **Display Options** tab.
3. In the Labels area of the Display Options toolbar, click **Precursor Flag**.

❖ To display the fragmentation scan for a precursor ion

Double-click a peak's precursor marker to display its product ion spectrum in a separate Spectrum view.

Figure 68 shows a full MS1 scan with one precursor ion at *m/z* 195.0877. The data-dependent MS2 scan for *m/z* 195.0877 has two precursor ions. Each of these ions triggered a data-dependent MS3 scan (only the product ion scan for *m/z* 138.0662 is shown).

Figure 68. MS1 and MS2 level scans with precursor markers for the data-dependent MS2 level and MS3 level scans



Selecting Spectra from an MSn Tree

On the MSn Browser page in the Info Bar view, you can select scans of interest from an MSn spectrum tree. You can also select to display an average spectrum for all the MSn scans of a specific precursor ion or a composite spectrum that includes the mass peaks from all the fragmentation scans that originate from the same precursor ion. For information about the parameter settings and the MSn tree structure, see "MSn Browser Page" on page 146.

Follow these procedures as needed:

- To display the MSn Browser page
- To filter the MSn tree by a selected time range
- To filter the MSn tree by a selected mass range
- To modify the grouping of the scan data in the MSn tree
- To expand the entire MSn tree
- To display all the individual scan items
- To display an average spectrum
- To normalize a composite spectrum

❖ To display the MSn Browser page

1. Open a raw data file (see "Opening Raw Data Files or Sequence Files" on page 15).
2. In the Info bar, click the **MSn Browser** tab.

   In the MSn Parameters area, use the Time Range and Mass Range settings in the MSn parameters area to filter the spectra in the MSn Tree area. Use the Mass Tolerance setting to change the grouping of the MSn nodes.

   In the MSn Tree area, the branches for each MS2 precursor appear as nested groups ordered by their increasing *m/z* value. Expanding an MS2 precursor node displays the MS3 precursor nodes when available, expanding the MS3 precursor nodes displays the MS4 precursor nodes when available, and so on.

   When an MSn level includes more than one scan, the application displays the average spectrum for the level. If the experimental data includes multiple fragmentation levels, for example MS2 and MS3, the MS3 level includes a composite spectrum made up of the MS2 precursor scan and the MS3 fragmentation scans. An MS4 level node includes a composite spectrum made up of the MS2 and MS3 precursor scans and the MS4 fragmentation scans.

❖ To filter the MSn tree by a selected time range

- In the MSn Parameters area, in the Time Range (min) box, type the time range that you want to review.

–or–

a. In the MSn Parameters area, select the **Track** check box.

b. In the Chromatogram view, drag the pointer across the *x*-axis range that you want to review.

The selected time range appears in the Time Range (min) box, and the MSn Tree view displays the scans within the selected time range.

❖ To filter the MSn tree by a selected mass range

In the Mass Range box, type a beginning mass and an ending mass, separated by a hyphen (***lowest mass-highest mass***). The specified mass range must be within the mass range of the scan data.

The MSn Tree view displays only the scans where the mass of the precursor ion falls within the specified mass range.

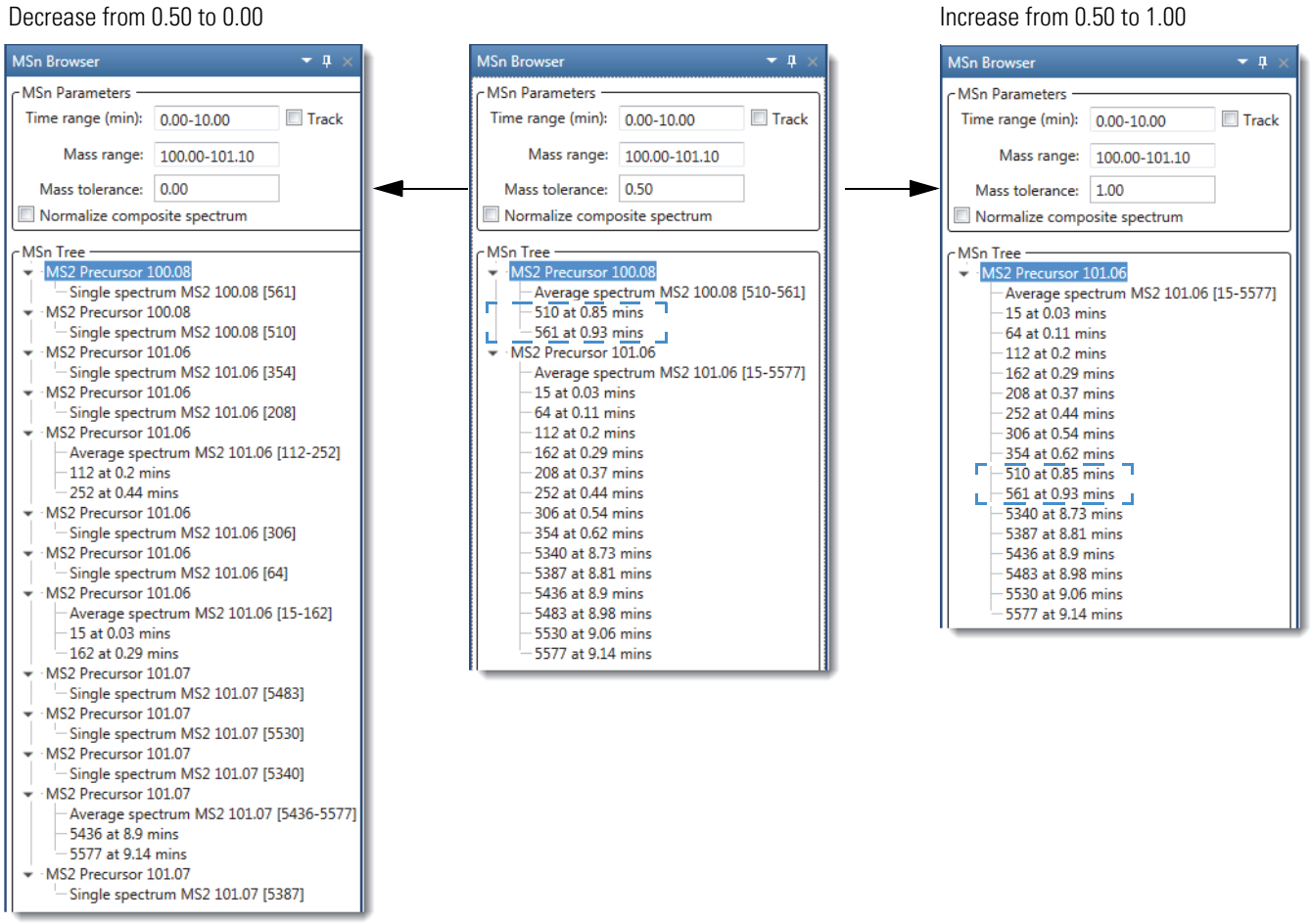
❖ To modify the grouping of the scan data in the MSn tree

In the Mass Tolerance box, type an *m/z* value from **0.00** to **10.00**.

Figure 69 shows the effect of decreasing the mass tolerance from 0.50 to 0.00 and the effect of increasing the mass tolerance from 0.50 to 1.00.

- Decreasing the mass tolerance below that of the experimental data increases the number of MS2 precursor nodes.
- Increasing the mass tolerance from 0.50 to 1.00 combines the scans under the MS2 Precursor 100.08 node with the scans under the MS2 Precursor 101.06 node, as the mass difference between these nodes is less than 1.00.

Figure 69. Effect of changing the mass tolerance on the MSn tree



❖ To expand the entire MSn tree

Right-click the MSn Tree area and choose **Expand List**.

❖ To display all the individual scan items

Right-click the MSn Tree area and choose **Include Individual Scans**.

❖ To display the spectrum for a single scan

In the MSn Tree area, double-click any of the Single Spectrum *Precursor* items or any of the *Scan Number* at *Retention Time* items.

A new Spectrum view appears in the Workspace.

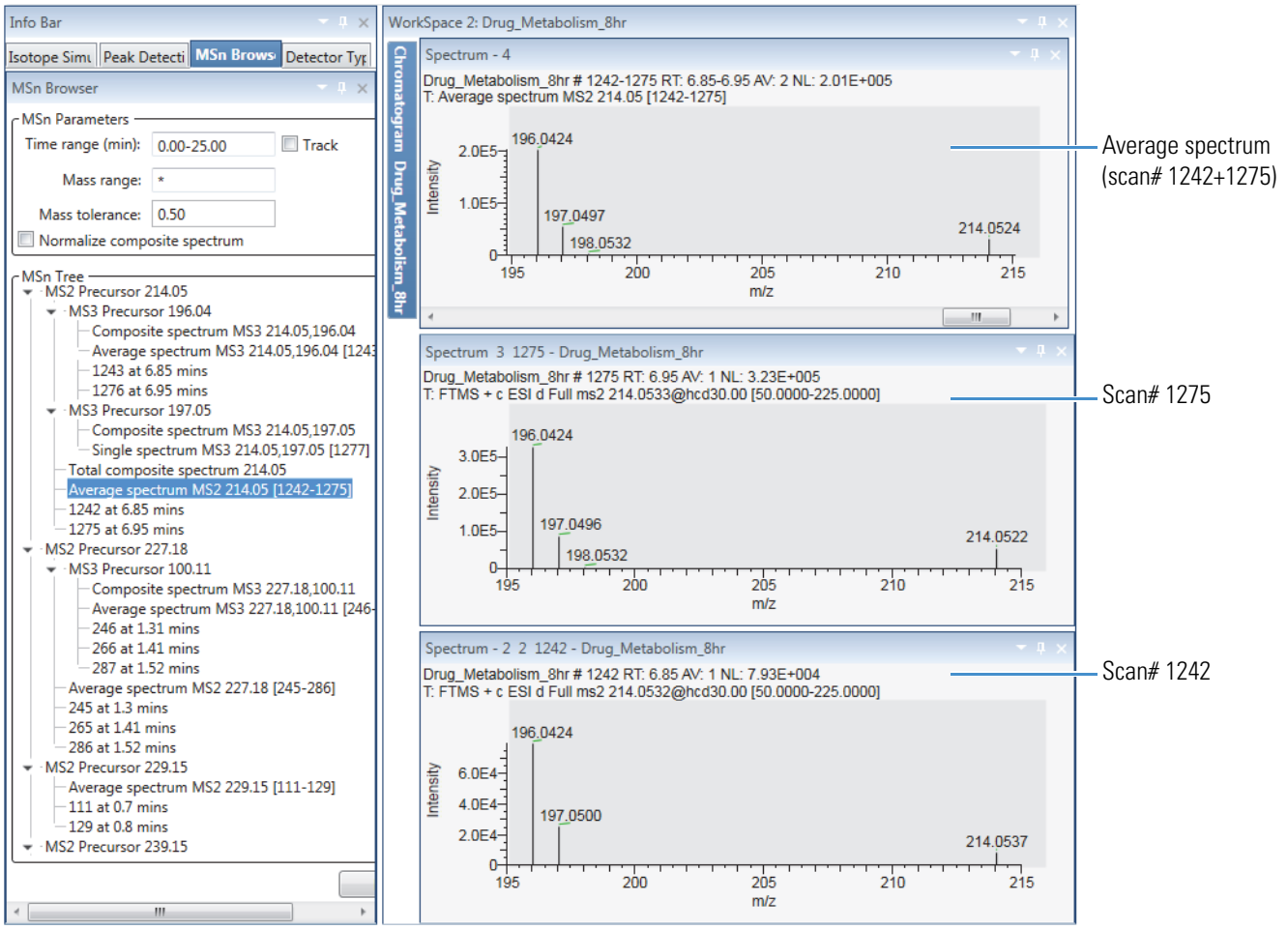
❖ To display an average spectrum

In the MSn Tree area, double-click an **Average Spectrum MSn *precursor values* (*scan range*)** item.

A new Spectrum view appears in the Workspace. The scan header includes the term Average Spectrum, the number of averaged scans, and the scan number range for the individual scans.

Figure 70 shows the individual MS2 level scans and the average MS2 level spectrum.

Figure 70. Average spectrum of two individual scans



❖ To display a composite spectrum

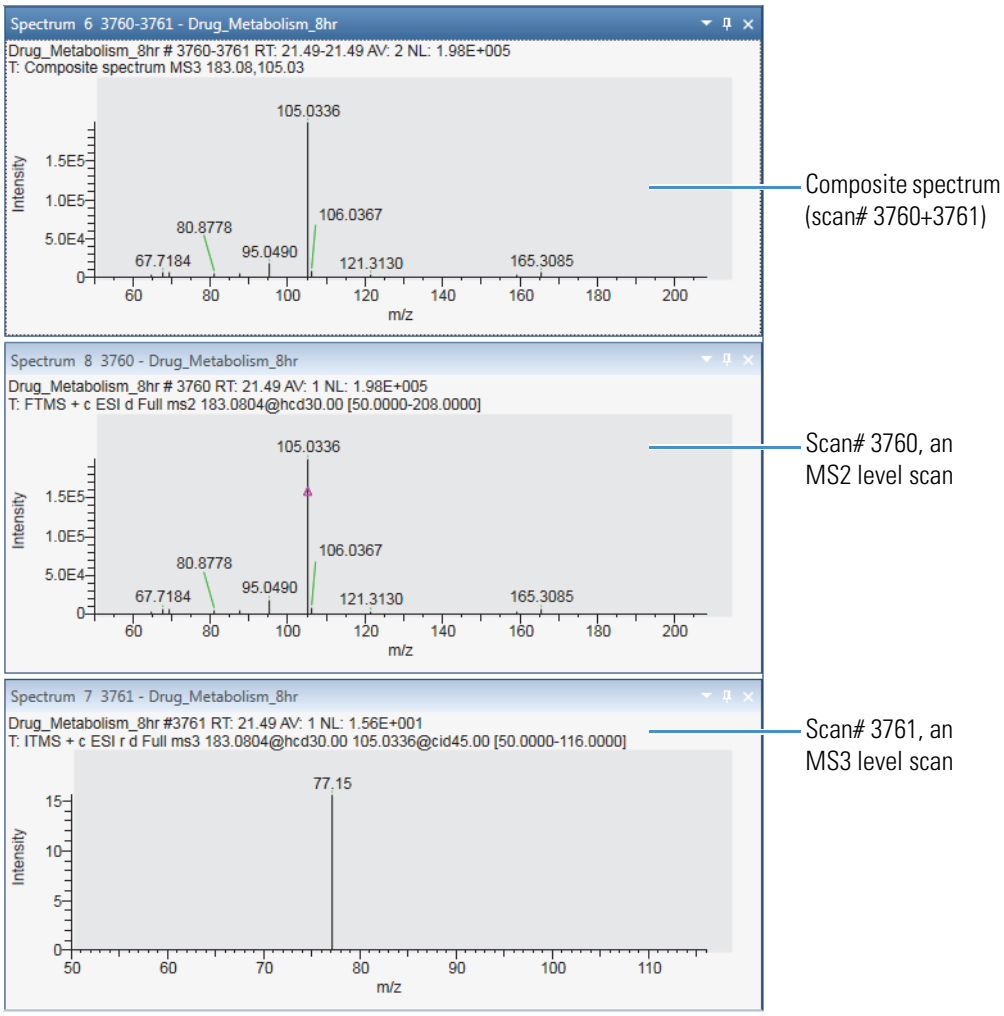
Note The MSn tree includes a composite spectrum for each branch that includes multiple fragmentation levels.

In the MSn Tree area, double-click a **Composite Spectrum MSn *precursor values*** item.

A new Spectrum view appears in the Workspace. The scan header includes the term Composite Spectrum and the number of averaged scans.

Figure 71 shows a composite spectrum and the two scans (MS2 and MS3 fragmentation levels) that the application combined to create it. Notice the intensity difference between the MS2 and MS3 scans. The spectral peak at *m/z* 77.15 is not visible in the composite spectrum.

Figure 71. Composite spectrum and individual MS2 and MS3 scans

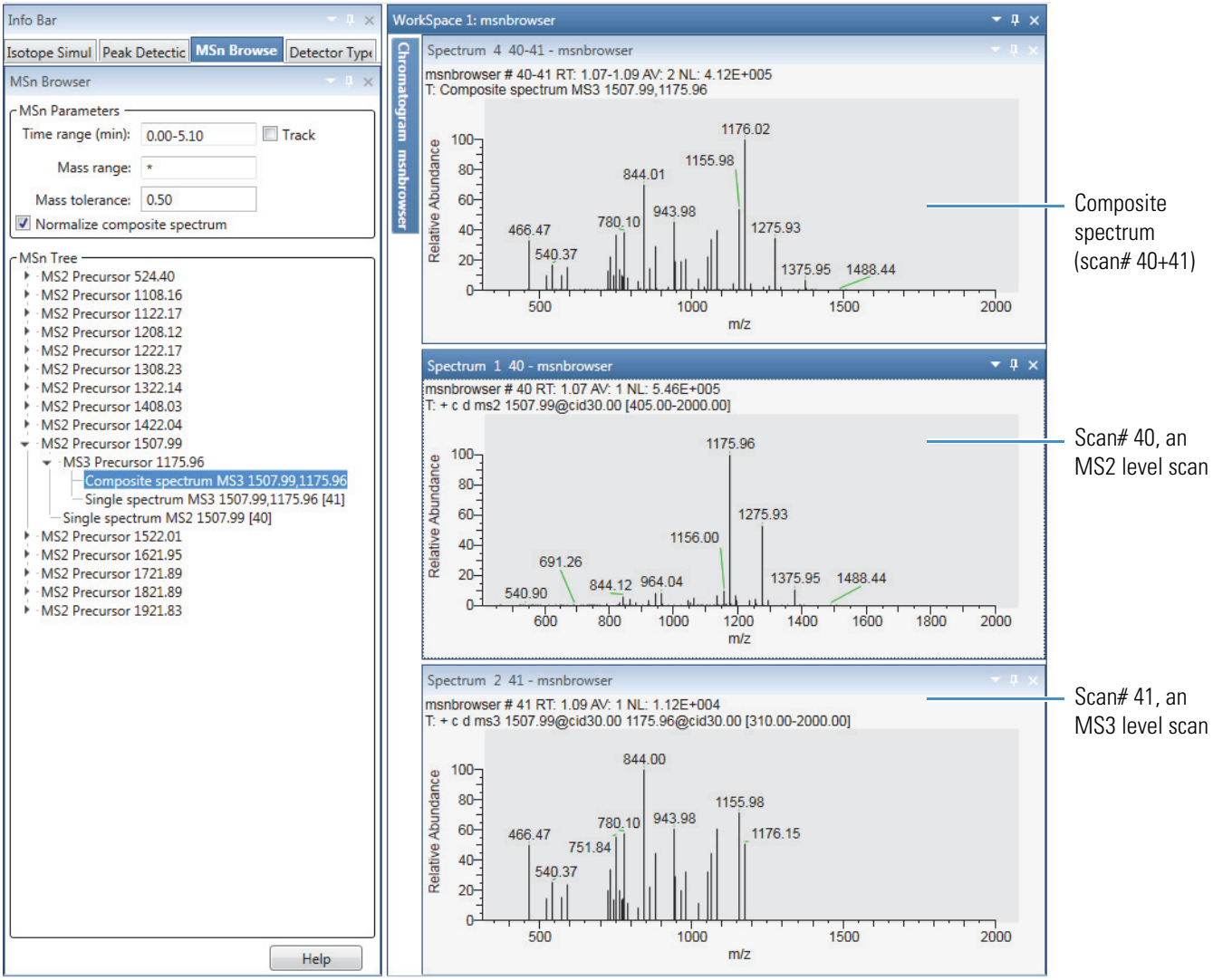


❖ To normalize a composite spectrum

Select the **Normalize Composite Spectrum** check box, and then double-click the composite spectra of interest in the MSn tree.

Figure 72 shows the normalized composite spectrum for scans 40 and 41.

Figure 72. Normalized composite spectrum versus the individual MS2 and MS3 scans



Setting Up the Display Options for a Spectrum View

Use the Spectrum or Multi Spectrum – Display Options Toolbar to customize the display of the active Spectrum or Multi Spectrum view.

For information about annotating the spectrum peaks with their theoretical elemental composition, see "Labeling Mass Spectrum Peaks with Chemical Formulas" on page 152.

To set up the display options for the Spectrum view, follow these topics as needed:

- Modifying the Scan Header for the Spectrum View
- Applying Local or Global Normalization in a MultiSpectrum View

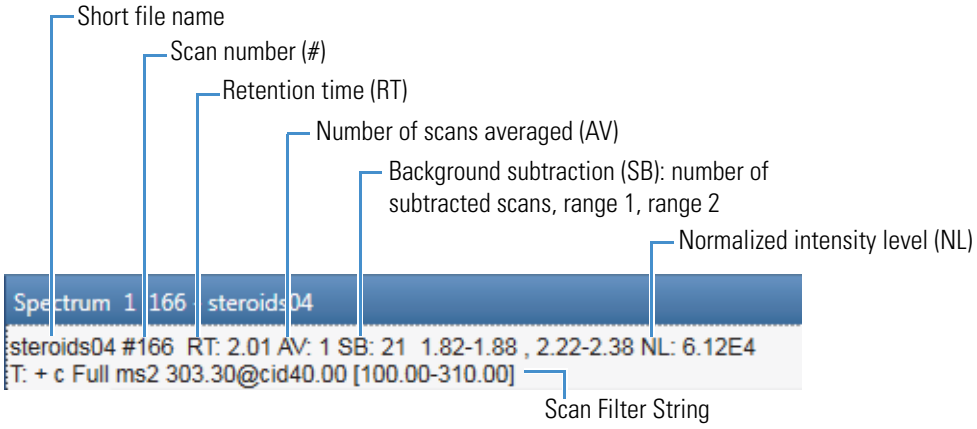
- Labeling Spectrum Peaks
- Changing the Y-Axis Scale of a Spectrum View

Modifying the Scan Header for the Spectrum View

By default, the scan header for the Spectrum view displays the following: Short File Name, Scan Number, Retention Time, Average Number of Scans, Background Subtraction Scan Numbers, Normalized Intensity, Polarity, Scan Filter String, and Mass Ranges.

Figure 73 shows a scan header for scan #166 at a retention time of 2.01 min and with background subtraction applied to two ranges.

Figure 73. Default scan header for scan #166 in steroids04.raw with background subtraction



❖ To add or delete information from the scan header for a Spectrum view

1. Open a raw data file.
2. If you are using a custom layout that does not include a Spectrum or Multi Spectrum view, in the Workspace Options toolbar, click **Spectrum** or **Multi Spectrum**.
3. Click the Spectrum (or MultiSpectrum) view of interest.

   Note The selections that you make in the Scan Header dialog box affect only the active Spectrum view (or Multi Spectrum view).

4. In the Spectrum or Multi Spectrum – Display Options Toolbar, click **Scan Header**.

   The Scan Header dialog box opens ("Scan Headers and Scan Header Abbreviations" on page 266).

5. Do the following as needed:
   - To display a field, select its corresponding check box.
   - To hide a field, clear its corresponding check box.
6. Click **OK**.

Applying Local or Global Normalization in a MultiSpectrum View

In a MultiSpectrum View, you can normalize the mass spectra to the most intense peak in all the spectra, or you can normalize the mass spectral peaks in each spectrum to the most intense peak in the spectrum.

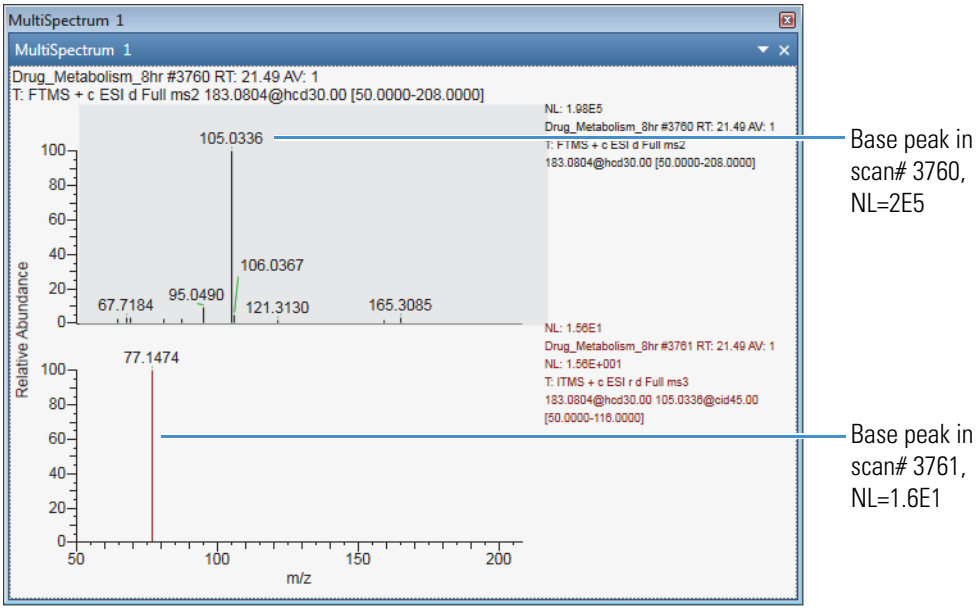
For information about displaying spectra in a MultiSpectrum view, see "Creating a MultiSpectrum View and Changing Its Spectrum Plots" on page 116.

❖ To normalize each spectrum separately

In the Normalization area of the Multi Spectrum – Display Options toolbar, click **Local**.

Figure 74 shows a set of locally normalized spectrum plots. Each spectrum is normalized to the most intense peak in the spectrum.

Figure 74. Local normalization

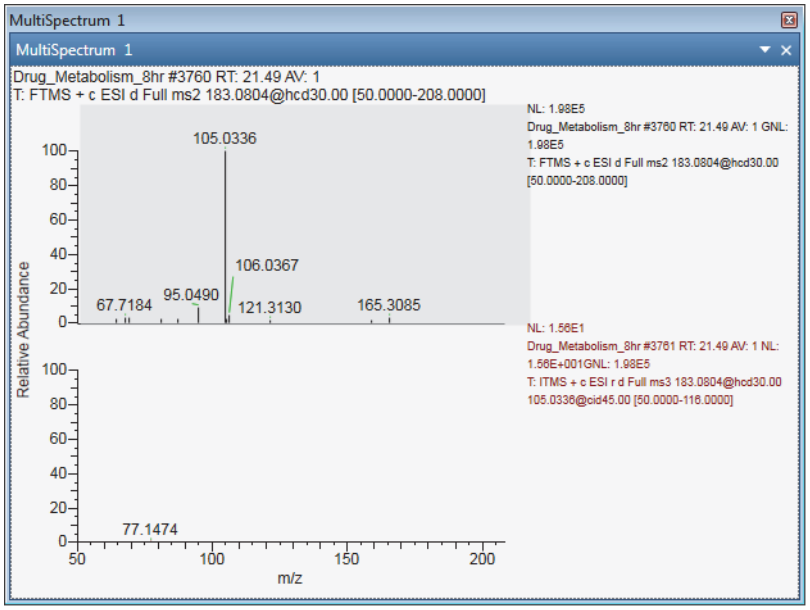


❖ To normalize the spectra against the most intense peak across the spectra

In the Normalization area of the Multi Spectrum – Display Options toolbar, click **Global**.

Figure 75 shows the effect of global normalization where both spectrum plots are normalized to the largest peak across the plots. The global normalization (GNL) is equal to 2E5, and the low intensity peak in scan# 3716 is barely visible.

Figure 75. Global normalization



Labeling Spectrum Peaks

Use the Labels area of the Spectrum or Multi Spectrum – Display Options Toolbar to add or remove labels that appear above the spectrum peaks. By default, the application adds the mass-to-charge label to each mass spectrum peak.

Note For instructions on how to use the precursor flag to display product spectra, see "Viewing Data-Dependent Scans" on page 124.

❖ To add or remove the labels for the mass spectrum peaks

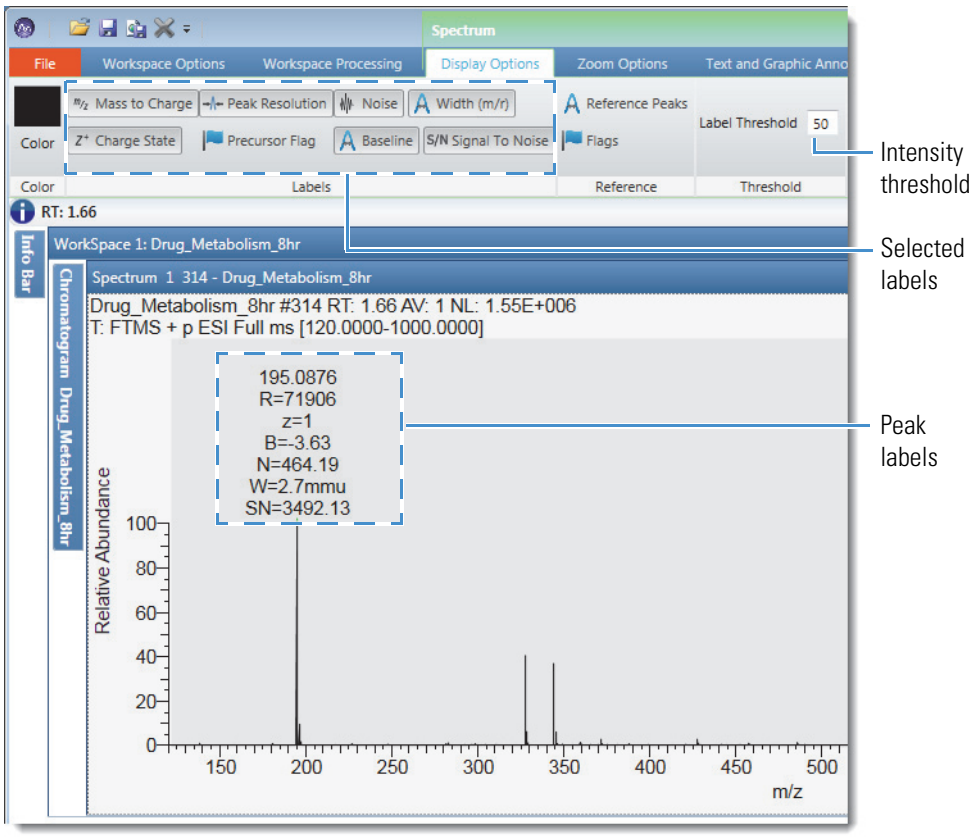
1. Open a raw data file and display a spectrum in a Spectrum view (or MultiSpectrum view).
2. Click the Spectrum (or MultiSpectrum) view of interest.
3. In the Labels area of the Display Options toolbar, click the labels that you want to display.

   Note With the default value set to 0 in the Label Threshold box, the application labels all the mass peaks.

4. (Optional) To avoid labeling low-intensity mass peaks, type a higher relative intensity value in the Label Threshold box.

   Figure 76 shows a mass spectrum with several labels and a labeling threshold of 50% relative intensity.

Figure 76. Mass spectrum with multiple labels



Changing the Y-Axis Scale of a Spectrum View

You can select the relative intensity scale or the absolute intensity scale for the *y* axis of a spectrum plot.

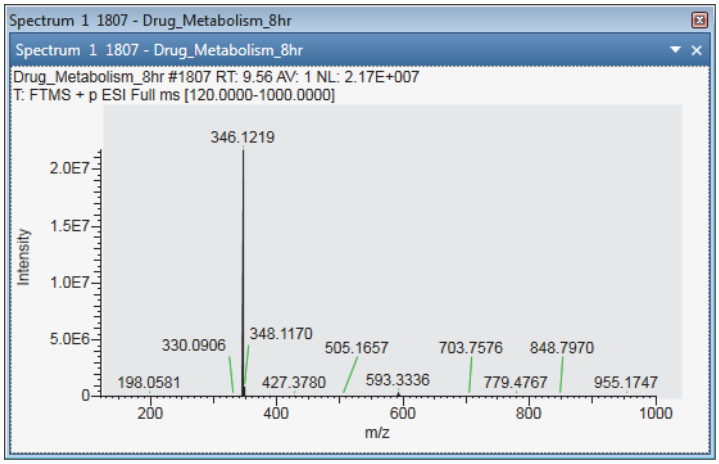
With the factory default template, a Spectrum View opens with the *y*-axis scale set to relative intensity and a *y*-axis label of Relative Abundance.

❖ To change the *y*-axis scale from relative intensity to absolute intensity

1. Click the Spectrum view to make it active.
2. Click the **Display Options** toolbar tab.
3. In the Y-Scale area of the toolbar, click **10^x Absolute**.

   The *y*-axis maximum changes from 100% to the normalization (NL) value (or slightly less than the NL value) for the scan, and the *y*-axis label changes from Relative Abundance to Intensity (Figure 77).

Figure 77. Spectrum plot of (absolute) Intensity versus *m/z* value



Spectra-Specific Toolbars

Use the spectra-specific toolbars as follows:

- Spectrum – Workspace Processing Toolbar: For determining the elemental composition of an ion; simulating isotope distributions; Xtract deconvolution; and NIST library, mzVault library, and mzCloud.org searches.
- Spectrum or Multi Spectrum – Display Options Toolbar: For customizing the Spectrum view and a MultiSpectrum view.
- Spectrum List – Display Options Toolbar: For formatting the spectrum list.

These two toolbars are specific to the Spectrum, MultiSpectrum, and Chromatogram views:

- Zoom Options Toolbar: For adjusting the display of the spectra (and chromatograms).
- Text and Graphic Annotation Toolbar: For annotating spectra (and chromatograms) with text, lines, boxes, and symbols.

Spectrum – Workspace Processing Toolbar

Use the buttons in the Elemental Analysis and Library Search areas of the Workspace Processing toolbar to analyze spectra.

❖ To display the Spectrum Workspace Processing toolbar

1. Click the Spectrum View or the MultiSpectrum View to make it the active view.
2. Click the **Workspace Processing** toolbar tab.

Figure 78 shows the Elemental Analysis and Library Search areas in the Workspace Processing toolbar, and Table 22 describes toolbar buttons.

Figure 78. Elemental Analysis and Library Search areas of the Workspace Processing toolbar

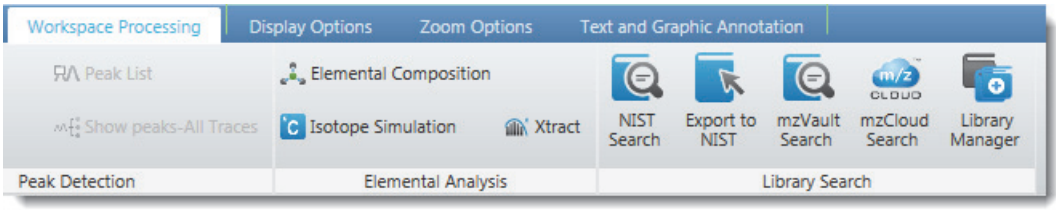


Table 22. Spectra-specific Workspace Processing buttons

Button	Description
Elemental Analysis	
Elemental Composition	Displays the Elemental Composition Page, where you calculate the best matching chemical formulas for a mass in the spectrum. See Chapter 5, "Determining the Elemental Composition of Ions."
Isotope Simulation	Displays the Isotope Simulation Page of the Info Bar, where you create a simulated isotopic distribution spectrum of a chemical formula.
Xtract	Displays the Xtract Page of the Info Bar, where you set parameters for the Xtract algorithm. See Chapter 10, "Deconvolving and Deisotoping Spectra with the Xtract Algorithm."
Library Search	
NIST Search	Opens the Library Search page in the Info Bar, where you run a NIST library search on the active spectrum and displays the results in the NIST Search Results View. You can modify the library search parameters on the Modifying a NIST Search from the NIST Search Page of the Info Bar. See "Performing a Local NIST or mzVault Library Search" on page 173.
Export to NIST	Exports a spectrum to the NIST application for a search. The NIST application opens and displays the search results (see "Exporting a Mass Spectrum to the NIST MS Search Application" on page 194).
mzVault Search	Opens the mzVault Search page in the Info Bar, runs an mzVault library search on the active spectrum, and displays the results in the mzVault Search Results View.
mzCloud	Uploads a spectrum to mzCloud.org for a search. The website opens and displays the search parameters. Select the appropriate settings and click **OK**. The website displays the results.
Library Manager	Opens the Thermo Library Manager dialog box, where you select or create NIST libraries. See "Managing Libraries" on page 195.

Spectrum or Multi Spectrum – Display Options Toolbar

Use the buttons in the Spectrum (or Multi Spectrum) Display Options toolbar to customize the Spectrum view (see "Setting Up the Display Options for a Spectrum View" on page 131). You can label the spectral peaks, determine the elemental composition for a peak, and specify how the application normalizes the spectrum.

❖ To display the Spectrum Display Options toolbar

1. Click the Spectrum View or the Multi Spectrum view to make it the active view.
2. Click the **Display Options** toolbar tab.

Figure 79 shows the Spectrum (or Multi Spectrum) Display Options toolbar.

Figure 79. Spectrum (or Multi Spectrum) Display Options toolbar (in two parts, left and right side)

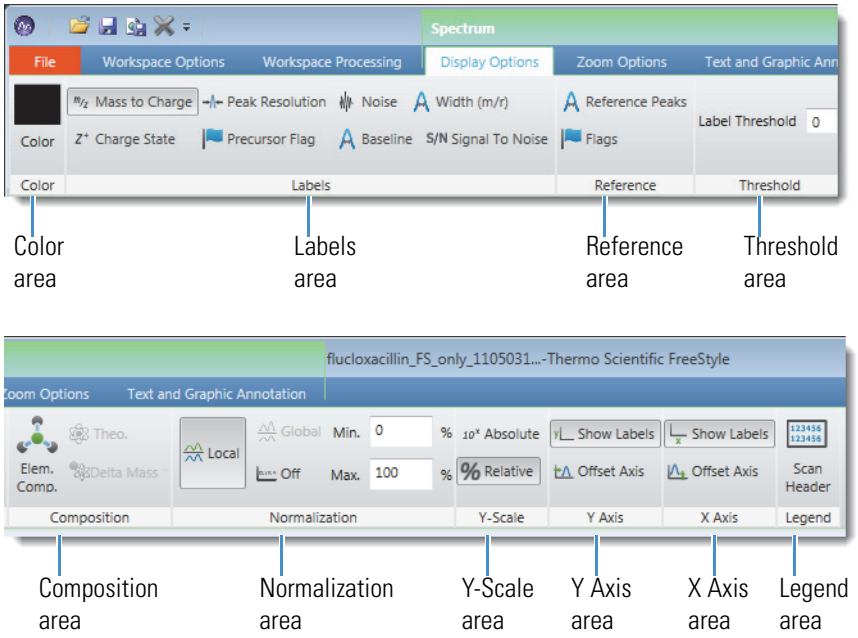


Table 23 describes the buttons in the Spectrum – Display Options toolbar.

Table 23. Spectrum Display Options toolbar buttons (Sheet 1 of 5)

Button	Description
Color	
Color	Displays the color palette, where you select the color of the spectrum.

Table 23. Spectrum Display Options toolbar buttons (Sheet 2 of 5)

Button	Description
Labels	
Use the buttons in the Labels area to annotate the mass spectrum.	
Note The following buttons are unavailable when the raw data file does not include the requested information: Z^+ Charge State, Peak Resolution, Noise, Baseline, Width, and S/N Signal To Noise.	
Mass to Charge	Adds a mass-to-charge ratio label to each peak in the spectrum.
Z^+ Charge State	Adds a charge state label to each peak in the spectrum.
Peak Resolution	Adds a peak resolution label to each peak in the spectrum.
Precursor Flag	Adds one or more pink triangles to a peak if it triggered data-dependent scans (see "Viewing Data-Dependent Scans" on page 124).
Noise	Adds a noise magnitude label to each peak in the spectrum.
Baseline	Adds a baseline label to each peak in the spectrum.
Width (m/r)	Adds a width label to each peak in the spectrum.The width equals the mass, in mmu units, divided by the peak resolution.
Signal To Noise	Adds a signal-to-noise label (intensity/noise) to each peak in the spectrum.
Reference	
Reference Peaks	Adds an R label to peaks that correspond to a reference compound used for an internal recalibration of a scan.

Table 23. Spectrum Display Options toolbar buttons (Sheet 3 of 5)

Button	Description
Flags	Displays one of the following letters or symbols above flagged peaks: • S—Saturated peaks are peaks with a signal too large to measure—that is, the signal was so high that it was outside the dynamic range of the detector, causing saturation. • R—Reference peaks are peaks from a reference compound used for an internal recalibration of a scan. • L—Lock peaks are local references used to calculate the accurate mass of nearby peaks. • E—Exception peaks are peaks from a reference compound that are not used for recalibration. These are typically small isotopes or fragments of the main references. • #—Mathematically modified peaks are peaks where the peak mass was recalculated by the instrument, usually due to a calibration process. • M—Merged peaks are peaks where the centroider combined two nearby peaks. • F—Fragmented peaks are peaks separated into multiple peaks by the centroiding activity.
Threshold	
Label Threshold	Sets the percentage of the base peak so that the application labels only the peaks above that percentage. For example, if the base peak is 100 percent and the label threshold setting is 50 percent, the application labels only the peaks in the Spectrum View or MultiSpectrum View that are at or above 50 percent. Range: 0–100; default: 0
Composition	
Use the buttons in the Composition area to determine the elemental composition of the ions associated with the peaks in the mass spectrum. See Chapter 5, "Determining the Elemental Composition of Ions."	
Elemental Composition	Determines the elemental composition of all the peaks in the spectrum.
Theoretical	Labels the peaks in the mass spectrum with the theoretical mass-to-charge ratios of the best matched ions.

Table 23. Spectrum Display Options toolbar buttons (Sheet 4 of 5)

Button	Description
Delta Mass	Labels the peaks in the mass spectrum with the differences between the experimentally determined mass-to-charge ratios and the theoretical mass-to-charge ratios. The unit options are amu (atomic mass units), mmu (millimass units), and ppm (parts-per-million).
Normalization	
Use the parameters in the Normalization area to specify how the application normalizes the mass spectrum. This function is similar to the normalization of chromatogram traces. See "Normalizing Chromatogram Traces" on page 57.	
Local	Scales the *y*-axis range as a percentage.
Global	Normalizes the spectra traces so that the most intense peak of all the spectra is 100 percent. Available for a MultiSpectrum view.
Off	Scales the spectra according to the user-specified minimum and maximum absolute intensity (counts).
Min.	Displays the minimum of the *y* axis. Enter a value in the box to change the minimum. For local normalization, the value is a percentage. For normalization set to Off, the value is an intensity.
Max.	Displays the maximum of the *y* axis. Enter a value in the box to change the maximum. For local normalization, the value is a percentage. For normalization set to Off, the value is an intensity.
Y-Scale	
Use the parameters in the Y-Scale area to specify how the application scales the *y* axis.	
Absolute	Plots the spectrum with the intensity expressed in counts.
Relative	Scales the spectrum so that the intensity of the most intense peak in the spectrum is 100 percent.
Y Axis	
Use the parameters in the Y Axis area to specify how the application labels the *y* axis.	
Show Labels	Shows or hides the *y*-axis label.
Offset Axis	Sets the location of the displayed plot a specified distance from the *y* axis. The *y*-axis offset moves the *x* axis slightly to the right of the *y* axis so that you can see plot details at low *x*-axis values.

Table 23. Spectrum Display Options toolbar buttons (Sheet 5 of 5)

Button	Description
X Axis	
Use the parameters in the X Axis area to specify how the application labels the *x* axis.	
Show Labels	Shows or hides the *x*-axis label.
Offset Axis	Sets the location for the displayed plot a specified distance from the *x* axis. The *x*-axis offset moves the *y* axis up slightly so that you can see plot details at low *y*-axis values.
Legend	
Scan Header	Opens the Scan Header dialog box, where you specify what scan header information the Spectrum View displays. For additional information, see "Scan Headers and Scan Header Abbreviations" on page 266.

Spectra-Specific Views

The FreeStyle application uses views to display results and to present lists for entering parameters or selecting options.

The FreeStyle window has these spectra-specific views:

- Spectrum View: Displays a spectrum corresponding to the retention time or scan number that you select in the Chromatogram View.
- MultiSpectrum View: Displays multiple spectra, but only the active spectrum updates corresponding to the retention time or scan number that you select in the Chromatogram view.
- Spectrum List View: Lists in tabular form the positions, intensities, and relative intensities of the peaks in the Spectrum View.

For information about the shortcut menu for the Spectrum and MultiSpectrum views, see Table 25 on page 144.

Spectrum View

The Spectrum view (Figure 80) displays a spectrum for the retention time that you either select in the Chromatogram View or specify in the Scan Ranges dialog box.

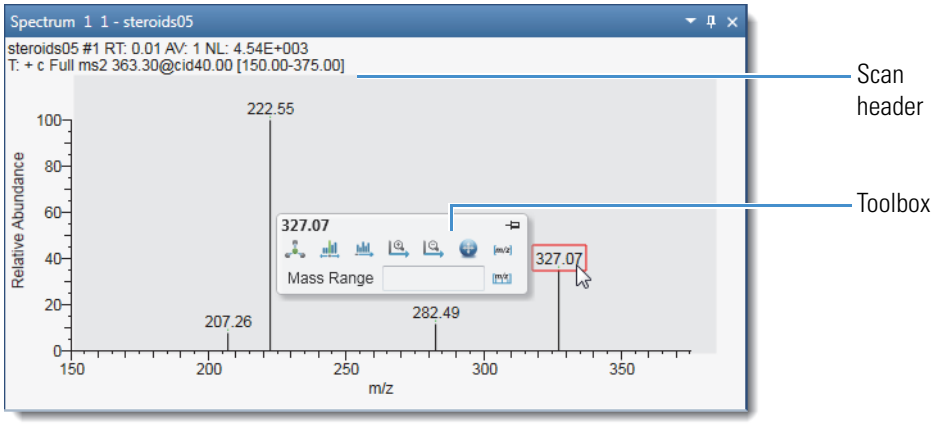
❖ To open a Spectrum view

1. Click the **Workspace Options** toolbar tab.

2. Click **Spectrum**.

Figure 80 shows an example of a Spectrum view and the Spectrum View Toolbox.

Figure 80. Spectrum view



Note You can set a minimum trace height value in centimeters on the Default Workspace Options Page. When you adjust the height of the Spectrum view, if its height becomes smaller than the set minimum value, a scrollbar automatically appears to the right of the view.

The scan header describes the experiment. Table 24 describes the default scan header information. If the scan header is not visible, resize the Spectrum view.

Table 24. Default header information symbols

Symbol	Description
#S	Scan number
RT	Retention time
AV	Averaged (followed by the number of averaged scans)
SB	Subtracted (followed by the number of subtracted scans and the scan range)
NL	Normalization level
T	Scan filter string

Right-clicking the Spectrum view displays a shortcut menu (Figure 81) with the commands described in Table 25.

Figure 81. Shortcut menu for the Spectrum and MultiSpectrum views

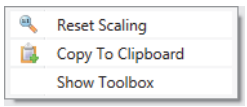


Table 25. Spectrum (or MultiSpectrum) view shortcut menu commands

Command	Description
Reset Scaling	Resets the scaling of the plot in the Spectrum view or all the plots in the MultiSpectrum view.
Copy To Clipboard	Copies an image of the Spectrum view to the Clipboard.
Show Toolbox	Displays the Spectrum View Toolbox. To close the toolbox, click its pin icon.

These toolbars are available when the Spectrum view is active:

- Spectrum – Workspace Processing Toolbar
- Spectrum or Multi Spectrum – Display Options Toolbar
- Zoom Options Toolbar
- Text and Graphic Annotation Toolbar

MultiSpectrum View

Use a MultiSpectrum view to display multiple spectrum traces when you want to compare the traces using the same normalization level or you want to simultaneously zoom in on the same mass range.

Note In the Normalization area of the Spectrum – Display Options toolbar, the Global button is unavailable when the spectra being compared are in two separate views.

Instead of zooming in on each spectrum individually in a Spectrum view, use a MultiSpectrum view to zoom in and out on all of the spectra at once. In a Multi Spectrum view, the active spectrum (the spectrum with the darker gray background) is the only one that updates when you click the chromatogram.

You can perform background subtraction, Xtract deconvolving and deisotoping, isotope simulation, or library searches on any active spectrum in this view. You can also use all the functions in the Spectrum View Toolbox.

To create and work with a MultiSpectrum view, see "Creating a MultiSpectrum View and Changing Its Spectrum Plots" on page 116.

For more information about the MultiSpectrum view and its toolbox, see "Spectrum View" on page 142 and "Spectrum View Toolbox" on page 145.

Spectrum View Toolbox

Use the Spectrum View toolbox (Figure 82) to run an elemental composition analysis, add an EIC plot to the Chromatogram view, set up relative mass labels, or zoom in or out of the Spectrum view.

How you open the floating toolbox depends on your workspace setting (see "Default Workspace Options Page" on page 258). With the factory default setting, you can open the toolbox in two ways:

- Pause the pointer on an *m/z* value in the view. The application highlights the selected value with a red rectangle, and the toolbox opens with its pin icon in the unpinned (horizontal) position, .

–or–

- Right-click an *m/z* value in the view and choose Show Toolbox. The toolbox opens with its pin icon in the pinned (vertical) position, .

Figure 82. Spectrum view toolbox

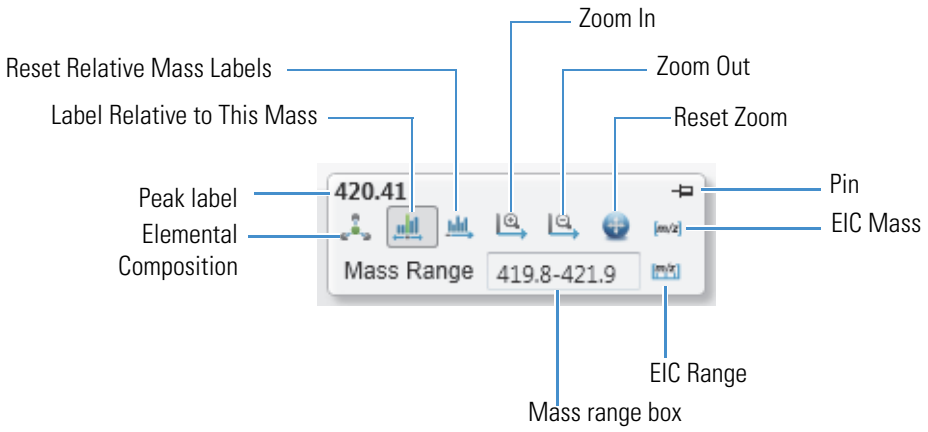


Table 26 describes the icons and the Mass Range box in the Spectrum view toolbox.

Table 26. Spectrum view toolbox icons (Sheet 1 of 2)

Icon/field	Description
Elemental Composition	Finds the best matching calculated chemical formulas for the selected peak in the spectrum. See "To start an Elemental Composition analysis from the Spectrum view toolbox" on page 156.
Label Relative to This Mass	Labels each peak in the spectrum with the difference between its mass-to-charge ratio and the mass-to-charge ratio of the selected peak (in *m/z*).
Reset Relative Mass Labels	Labels each peak in the spectrum with its mass-to-charge ratio (in *m/z*).

Table 26. Spectrum view toolbox icons (Sheet 2 of 2)

Icon/field	Description
Zoom In	Moves in on the spectrum.
Zoom Out	Moves out from the spectrum.
Reset Zoom	Resets the zoom to display the entire mass range.
EIC Mass	Uses the entered individual mass-to-charge ratio values to plot an extracted ion chromatogram (EIC). See "To display an EIC plot for multiple discrete m/z values" on page 117.
EIC Range	Uses the entered mass-to-charge ratio range to plot an extracted ion chromatogram (EIC). See "To specify the mass ranges for an EIC plot" on page 119.
Mass Range box	Enter a mass range within the spectrum *m/z* limits and then press ENTER. The view rescales to display the spectrum within the entered range. See To specify the mass ranges for an EIC plot.

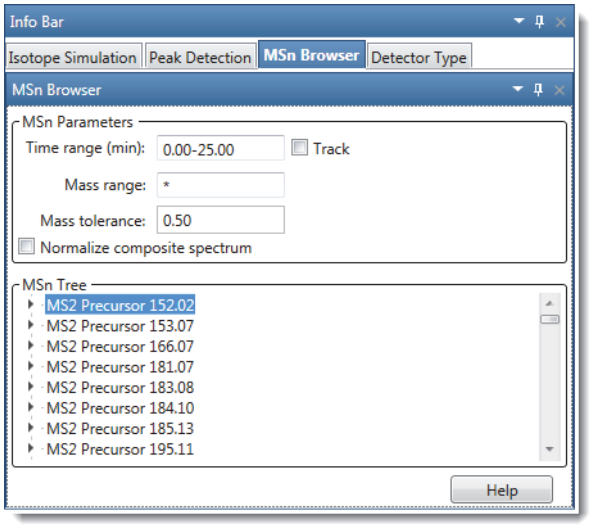
MSn Browser Page

Use the MSn Browser page in the Info Bar to select spectra of interest from an MS tree view of the spectra in a raw data file.

For information about displaying spectra, see "Selecting Spectra from an MSn Tree" on page 126.

Figure 83 shows the MSn Browser page, and Table 27 describes the parameters on the this page.

Figure 83. Info Bar – MS^n Browser page (with a collapsed MSn tree)



Note The MS^n browser information page is not available for all Thermo Scientific mass spectrometers.

Table 27. MSn Browser page parameters (Sheet 1 of 3)

Parameter	Description
MSn Parameters	
Time Range (min)	Specifies the time range of the MSn Tree (see "To filter the MSn tree by a selected time range" on page 127). Limits the spectra available for display in the MSn tree to MS2 or higher level scans acquired during the specified time range. There are two ways to change the Time Range: • Type the time range in minutes in the Time Range box. The format is *From-To*. • Select the **Track** check box, and then drag the pointer horizontally across the Chromatogram view from the minimum time to the maximum time of interest. Default: 0.00-*acquisition time* (in minutes)
Track	Activates the selection of a time range by using the mouse pointer. To reset the time range, right-click the Chromatogram view and choose **Reset Scaling**. Default: Clear
Mass Range	Specifies the mass range of the MSn Tree (see "To filter the MSn tree by a selected mass range" on page 127). The format is *From-To*. Default: * (Mass range from the instrument method)
Mass Tolerance	Specifies the mass tolerance for grouping the precursor nodes and their associated scans. Decreasing the mass tolerance to 0.00 creates an MSn Tree where most, if not all, the individual scans are associated with a separate MS2 precursor node (see "To modify the grouping of the scan data in the MSn tree" on page 127). Default: 0.05 *m/z*; Range: 0.00–10.00 *m/z*

Table 27. MSn Browser page parameters (Sheet 2 of 3)

Parameter	Description
Normalize Composite Spectrum	Normalizes the spectral peaks in a composite spectrum so that you can view low-intensity peaks from the higher-order fragmentation spectra (see "To normalize a composite spectrum" on page 130). Each MS^n spectrum is individually normalized (NL) so that its highest peak is displayed at a Relative Abundance of 100%; therefore, the relative peak heights of this display are not meaningful. For example, a composite spectrum for an MS^3 experiment displays both the MS^2 base peak and the MS^3 base peak at a Relative Abundance of 100% (unless the base peaks in the MS2 and MS3 are within the specified mass tolerance) and maintains all other relative abundances of the other ions in each spectrum. If the base peaks in the MS2 and MS3 scans are within the specified mass tolerance, the application averages these peaks, causing the Relative Abundance of the MS3 base peak to be less than 100%.
MSn Tree	
MS2 Precursor nodes	Display the precursor masses that triggered the MS2 level scans. To display the spectrum tree for each node, click the expand icon to the left of the node, or right-click the MSn Tree view and choose **Expand List**.
MSn Precursor nodes	Display the precursor masses that triggered the MSn level scans.
Average Spectrum	Double-click to display a Spectrum view with the averaged spectrum for the selected MSn level. The MSn tree includes an average spectrum when the data file includes scans for the selected scan filter.
Composite Spectrum	Double-click to display a Spectrum view with the composite spectrum for all the MSn levels down to the selected MSn level. A composite spectrum for an MSn level is a summed spectrum of all the fragmentation scans derived from the same original precursor mass down to the current level.
Single Spectrum or *Scan Number* at *Time*	Double-click to display a Spectrum view for the specified scan number.
Shortcut menu	
Include Individual Scans	Displays the individual scans (see "To display all the individual scan items" on page 128).
Normalize Composite Spectrum	Normalizes the composite spectra (see "To normalize a composite spectrum" on page 130).

Table 27. MSn Browser page parameters (Sheet 3 of 3)

Parameter	Description
Expand List	Expands all the MSn Precursor nodes (see "To expand the entire MSn tree" on page 128). Available when the MSn tree is collapsed.
Collapse List	Collapses all the MS2 Precursor nodes. Available when the MSn tree is expanded.
Export	Copies the MSn Tree to the Clipboard.
Print	Opens the Print dialog box where you can set up your print preferences and print the contents of the MSn Tree.

Determining the Elemental Composition of Ions

To determine the elemental composition of ions, follow these topics.

Contents

Note In the FreeStyle application, you can add formula labels to the peaks in a mass spectrum, or you can run an elemental composition analysis for a specific mass peak.

- Clicking Elem. Comp. in the Spectrum – Display Options toolbar adds formula labels to the peaks in a selected spectrum plot and opens the Elemental Composition page in the Info Bar.
- Clicking the Elemental Composition icon,  , in the Spectrum view toolbox runs an elemental composition analysis for the selected mass peak, and opens the Elemental Composition page in the Info Bar.
- Clicking Elemental Composition in the Workspace Processing toolbar only opens the Elemental Composition page in the Info Bar.

Labeling Mass Spectrum Peaks with Chemical Formulas

You can add formula labels to the peaks in your mass spectra.

Note When calculating the elemental compositions of all the mass peaks in a spectrum, the application uses the settings on the Default Elemental Composition Page. When you change the settings on this page, the new settings take effect after you restart the application.

Tip When determining the chemical formula for each mass peak, the application assumes that the current mass peak is an A0 peak; that is, it assumes that the peak corresponds to a monoisotopic ion, and the peaks at higher *m/z* values are part of the ion's isotope pattern. After determining the best matching formula for the current mass peak (by using the accurate mass and isotope pattern), the application analyzes the next mass peak, again assuming that the peak is an A0 peak.

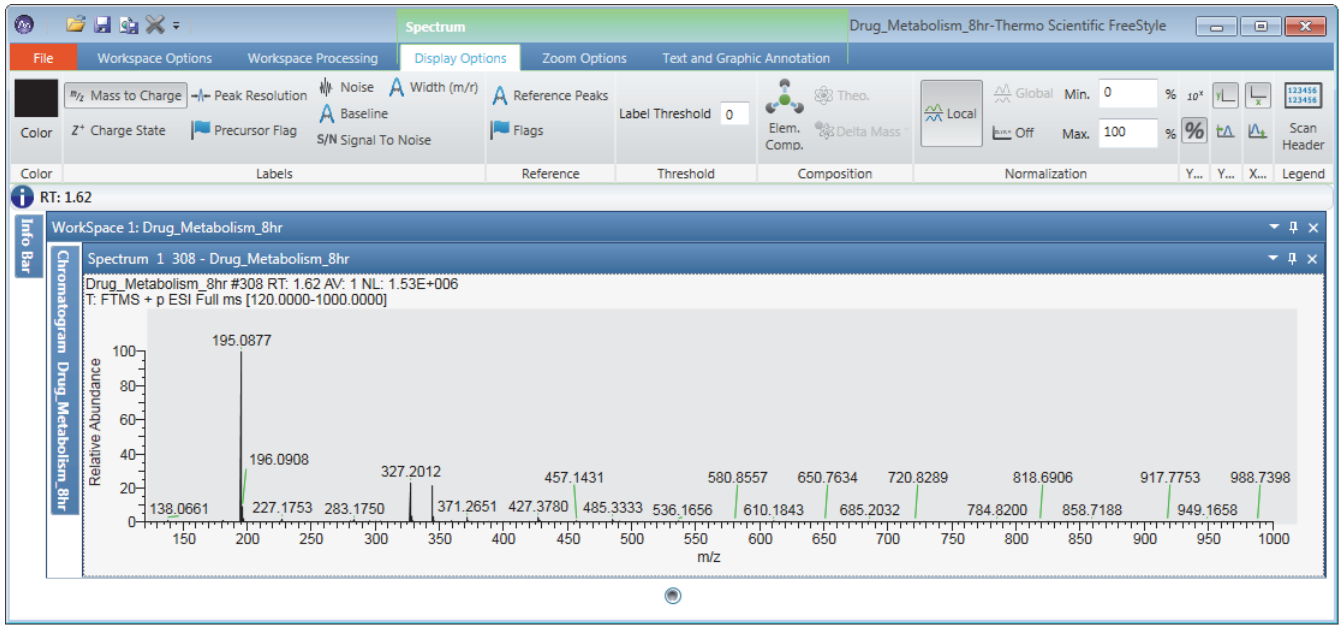
Use the Isotope Simulation Page to calculate the theoretical isotope patterns for the displayed formulas.

❖ To add formula labels to the mass peaks in a spectrum plot

1. Select a **Spectrum** view.
2. Open the Spectrum or Multi Spectrum – Display Options Toolbar.

   Figure 84 shows the Spectrum – Display Options toolbar with two unavailable buttons in the Composition area.

   Figure 84. Display Options toolbar for the Spectrum view



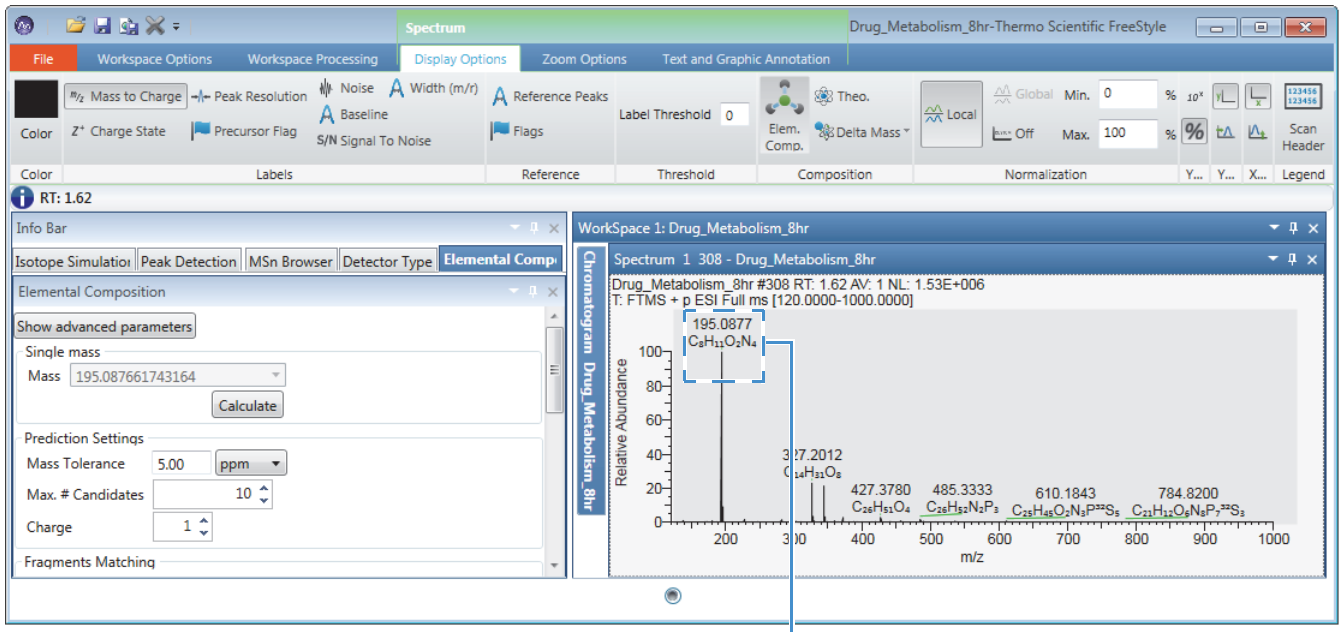
3. (Optional) To ignore low-intensity peaks, type a higher intensity threshold in the Label Threshold box.

4. In the Composition area of the toolbar, click **Elemental Composition**.

   The application labels each mass peak with a chemical formula. In addition, the Elemental Composition page appears in the Info Bar, and the Theoretical Mass (Theo.) and Delta Mass buttons become available in the Composition area of the toolbar.

   Figure 85 shows the parameters on the Elemental Composition page and the chemical formula labels in the Spectrum view. You can use the Elemental Composition page to find the best matching formulas for a specific mass peak.

   Figure 85. Mass peaks with chemical formula labels in the Spectrum view

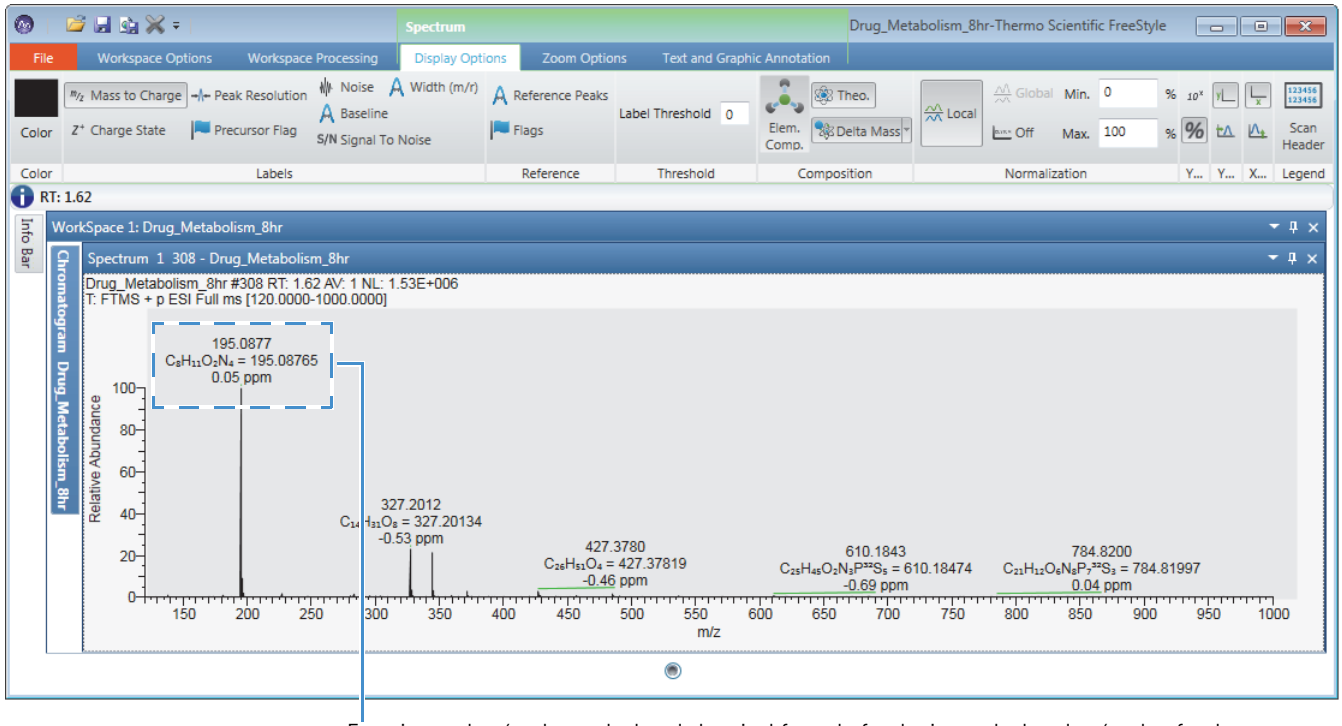


Experimental *m/z* value and calculated chemical formula for the ion

5. (Optional) To label the peaks with the theoretical mass for the displayed formula and the mass difference between the experimental *m/z* value and the theoretical mass, click **Theo.** and **Delta Mass**, respectively.

   Figure 86 shows the mass peaks labels, from top to bottom: the experimental *m/z* value, the formula and calculated *m/z* value of the ion, and the mass difference between the experimental *m/z* value and the calculated *m/z* value.

Figure 86. Mass peaks with formula, theoretical mass, and delta mass labels



Experimental *m/z* value, calculated chemical formula for the ion, calculated *m/z* value for the formula, and mass difference between the experimental *m/z* value and the calculated *m/z* value

Determining the Elemental Composition of an Ion

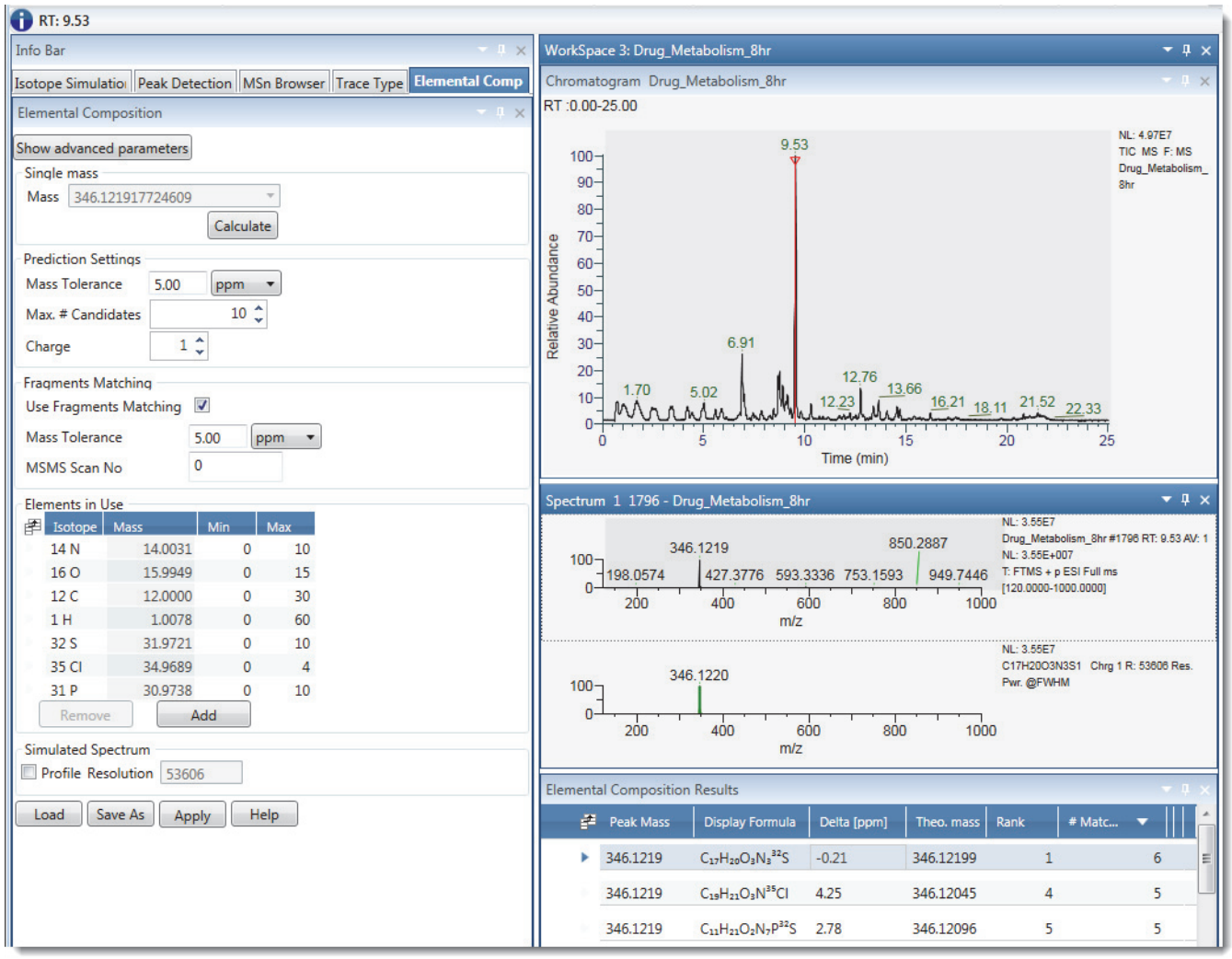
To determine the elemental composition of an ion, you select the ion of interest from a full MS scan (MS1 spectrum), and then run the elemental composition algorithm. To increase your confidence in the best matching formula, you can refine the calculation by adding the information from a fragmentation scan for the selected ion.

The elemental composition algorithm does the following:

- Determines the elemental composition of the most intense peak or the selected peak in the mass spectrum.
- Displays the Elemental Composition Results View, which lists the best matching chemical formulas.
- Displays the Elemental Composition Page of the Info Bar, where you can modify the parameters that the elemental composition algorithm uses.
- Displays the matching peaks in the theoretical spectrum in green and the non-matching peaks in red in the Spectrum View or MultiSpectrum View.

Figure 87 shows the Elemental Composition page and the Spectrum view with the theoretical isotope pattern for the calculated elemental composition.

Figure 87. Elemental Composition page and Spectrum view with a theoretical spectrum



Follow these procedures to determine the elemental composition of a precursor ion:

- To display an MS1 scan in a Spectrum view
- To start an Elemental Composition analysis from the Workspace Processing toolbar
- To start an Elemental Composition analysis from the Spectrum view toolbox
- To use the fragments matching algorithm
- To find an appropriate fragmentation scan for the fragments matching algorithm

❖ To display an MS1 scan in a Spectrum view

1. In the Workspace Options toolbar, click **Scan Filters**.
2. In the Info Bar, on the Scan Filters page, select **MS**.

3. Select a specific full-scan spectrum as follows:
   a. Open a Spectrum view or MultiSpectrum view if one is not currently open (see "Adding Spectrum Views to the Workspace" on page 114).
   b. In the Chromatogram View, click the chromatogram for the MS filter at the retention time or scan number of interest.
   c. If necessary, use the left and right arrows to find the mass spectrum that contains the peak of interest.

❖ To start an Elemental Composition analysis from the Workspace Processing toolbar

1. In the Workspace, select the **Spectrum** view or the spectrum of interest in a MultiSpectrum view.
2. Click the **Workspace Processing** toolbar tab.
3. In the Elemental Analysis area of the toolbar, click **Elemental Composition**.

   The Elemental Composition page opens in the Info Bar. The Mass box at the top of the page is populated with the *m/z* value for the selected spectrum's base peak.
4. (Optional) To select another spectral peak, double-click its *m/z* label, and then confirm the value in the Mass box.
5. (Optional) To modify the settings for the advanced parameters, do the following:
   a. Click **Show Advanced Parameters**.
   b. For information about the parameter settings, click **Help** at the bottom of the Elemental Composition page (or press the F1 key) or see "Elemental Composition Page" on page 165. Then, modify the settings as appropriate.
6. Click **Calculate**.

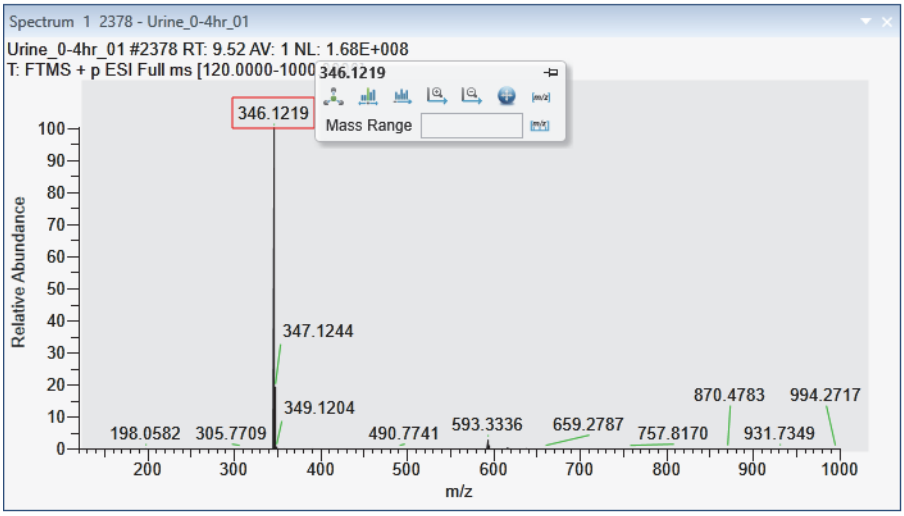
   The application runs the Elemental Composition algorithm on the selected precursor *m/z* value.

❖ To start an Elemental Composition analysis from the Spectrum view toolbox

1. In the Workspace, select the **Spectrum** view or the spectrum of interest in a MultiSpectrum view.
2. Depending on whether the toolbox appears automatically, do the following:
   a. Pause the pointer on the *m/z* value of interest.

      A red box surrounds the peak's mass-to-charge ratio label (Figure 88). If the pointer action is turned on (see "Default Workspace Options Page" on page 258), the toolbox appears within 2 s.

Figure 88. Spectrum view with a selected mass peak



b. Click the **Elemental Composition** icon, .

–or–

a. If the toolbox does not appear automatically, right-click the *m/z* value of interest and choose **Show Toolbox**.

The toolbox opens immediately.

b. (Optional) To select a different peak, point to the *m/z* label above the peak.

c. Click the **Elemental Composition** icon, , in the toolbox.

The application opens the Elemental Composition page in the Info Bar, populates the Mass box with the *m/z* value of the selected ion, runs the Elemental Composition algorithm, and displays the results in the Elemental Composition Results View.

3. (Optional) Select another peak or change the parameter settings, and click the **Elemental Composition** icon. For information about changing the elements used to determine the elemental composition, see "Adding and Removing Elements in the Elements in Use Table" on page 158.

The application automatically updates the Elemental Composition Results View.

❖ To use the fragments matching algorithm

1. In the Mass box under Single Mass, check the *m/z* value of the selected ion.
2. In the Fragments Matching area, select the **Use Fragments Matching** check box.
3. In the MSMS Scan number box, type a scan number for a fragmentation scan for the selected ion. See the next procedure "To find an appropriate fragmentation scan for the fragments matching algorithm."

4. Click **Apply**.
5. Review the results in the MSMS Coverage and MSMS Matched Peaks columns of the Elemental Composition Results view.

❖ **To find an appropriate fragmentation scan for the fragments matching algorithm**

Do one of the following:

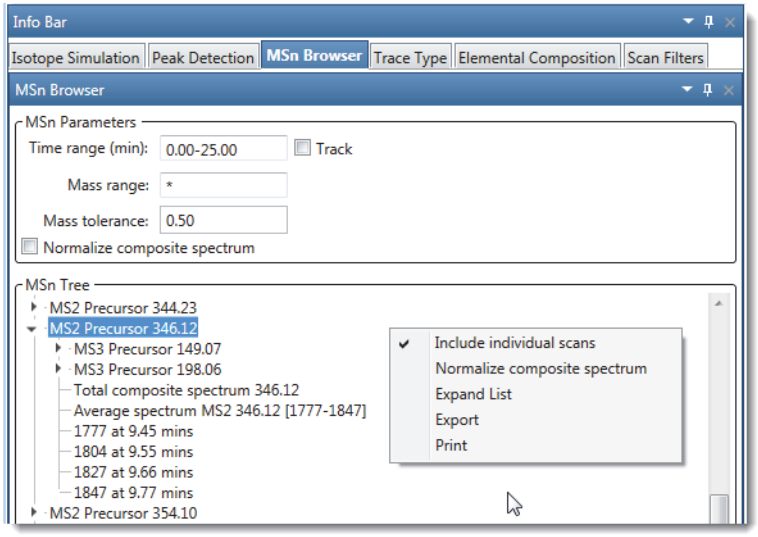
a. In the Info Bar, click the **MSn Browser** tab.

b. Expand the MS2 node for the precursor mass that you selected on the Elemental Composition page.

c. Right-click the MSn Browser page and choose **Include Individual Scans**.

Figure 89 shows the scan numbers of the individual scans for the MS2 precursor ion at *m/z* 346.12.

Figure 89. MSn Browser page showing the expanded list for *m/z* 346.12



–or–

a. Insert an MS2 filtered chromatogram for the selected ion in the Chromatogram view.

b. Select the MS2 filtered chromatogram and use the left and right arrows to display a data-dependent MS2 scan for the precursor ion in the Spectrum view.

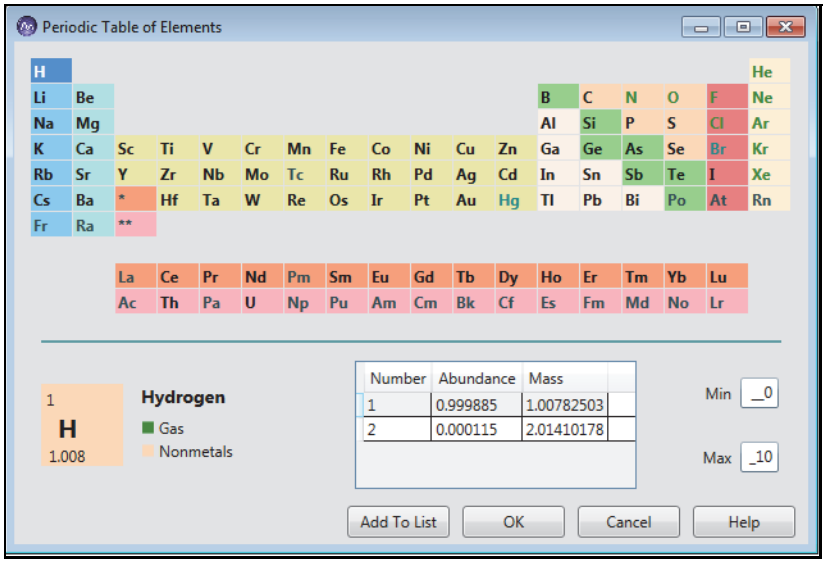
Adding and Removing Elements in the Elements in Use Table

Use the periodic table of elements to add isotopes to the Elements in Use table.

❖ **To add elements to the Elements in Use table**

1. On the Elemental Composition Page of the Info Bar, click **Add** to display the dialog box of the periodic table of elements (Figure 90).

Figure 90. Periodic table of elements



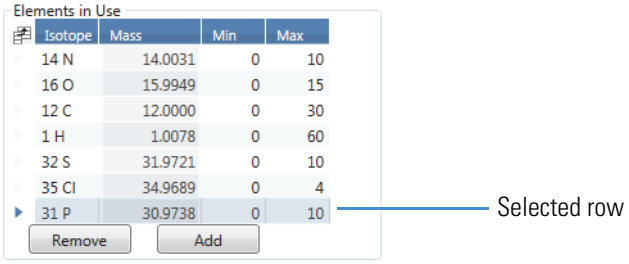
2. For each element that you want to add, select it from the table and then click **Add To List**.

   If the selected element displays several isotopes, for best results select the one with the highest abundance.

3. Click **OK** to add the selected elements to the Elements in Use table.

❖ To remove elements from the Elements in Use table

1. On the Elemental Composition Page, click to the left of the element in the table to highlight its row.



2. Click **Remove**.

Reviewing the Best Matching Formulas

After you run the Elemental Composition algorithm as described in "Determining the Elemental Composition of an Ion" on page 154, review the results in the Elemental Composition Results View. The application displays up to the maximum number of candidates that you specified on the Elemental Composition Page or the Default Elemental Composition Page and orders the best matching formulas by rank.

❖ To review the best matching formulas

1. For each row in the Elemental Composition Results view, review the formula, number of matched isotopes, and MS Coverage.
2. Compare the theoretical isotope pattern for the proposed formula, zoom in on the isotopic cluster for the mass peak of interest in the Spectrum view.

   In the theoretical isotope pattern, green sticks represent the expected mass peaks and red sticks represent the mass peaks that fall outside the mass tolerance and intensity tolerance windows at the experimental resolution.

   Figure 91 shows the elemental composition results for a mass peak at *m/z* 346.1219 and a pattern-matching intensity-threshold of 2%. Table 28 lists the four predicted isotopes (with an expected intensity above 2%) for the protonated omeprazole ion.

Figure 91. Results view for the protonated ion of omeprazole (a therapeutic drug)

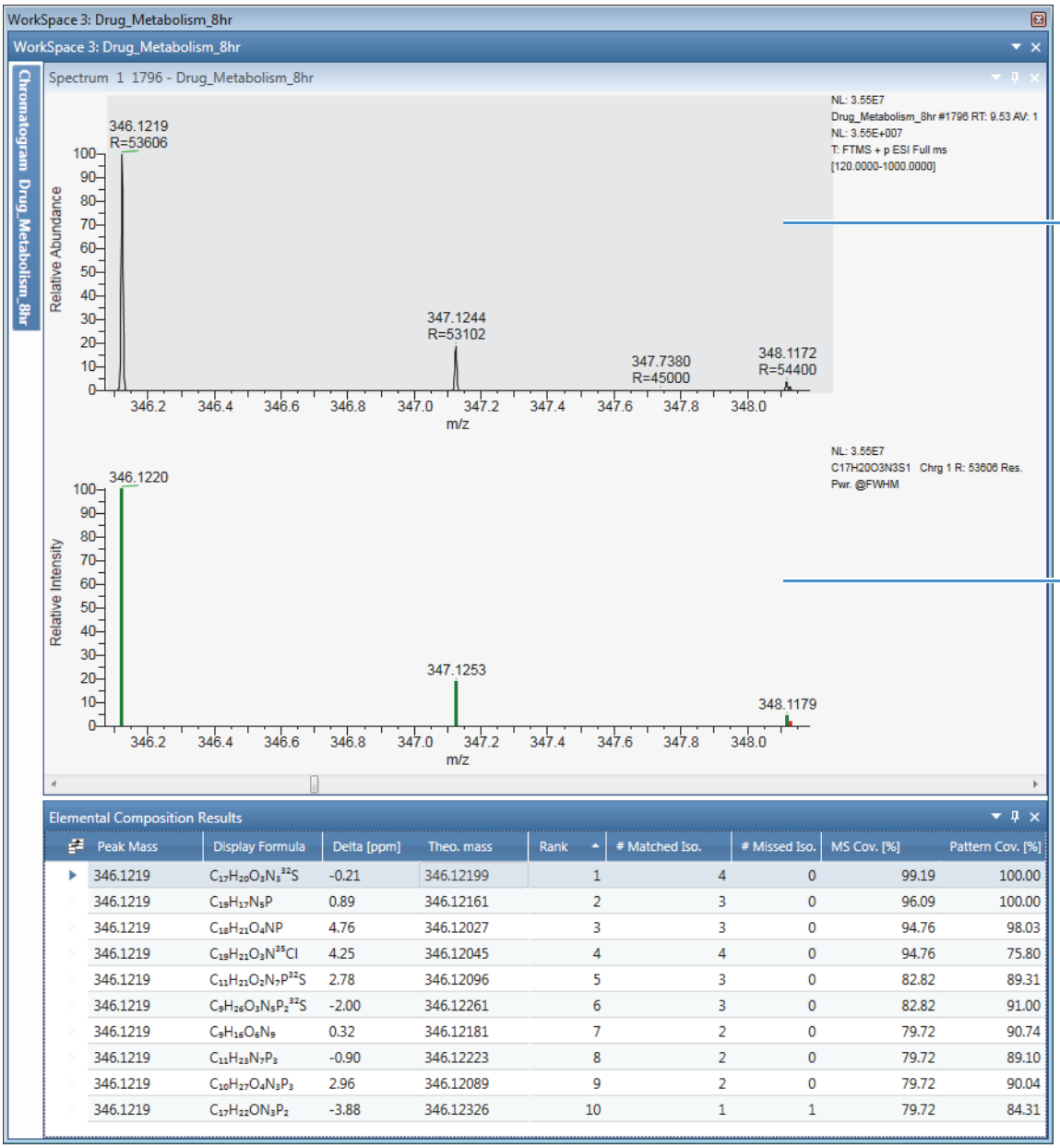


Table 28. Predicted isotopes for the protonated ion of omeprazole (Sheet 1 of 2)

Formula	Isotope type	Mass shift	Theoretical *m/z* value	Theoretical intensity [%]
$C_{17}H_{20}N_3O_3{}^{32}S$	A0	0	346.1220	100.00
$C_{16}{}^{13}CH_{20}N_3O_3{}^{32}S$	A1 (one ^{13}C)	1.00329	347.1253	19.29

Table 28. Predicted isotopes for the protonated ion of omeprazole (Sheet 2 of 2)

Formula	Isotope type	Mass shift	Theoretical *m/z* value	Theoretical intensity [%]
$C_{17}H_{20}N_3O_3{}^{34}S$	A2 (one ^{34}S)	1.99588	348.1179	4.60
$C_{15}{}^{13}C_2H_{20}N_3O_3{}^{32}S$	A2 (two ^{13}C)	2.00591	348.1278	2.24

Reviewing the MSMS Coverage Score for Matching Fragments

After you run the Elemental Composition algorithm with the additional Fragments Matching calculation (see "To use the fragments matching algorithm" on page 157), review the results in the MSMS Coverage [%] column.

❖ To compare the number fragmentation peaks to the value in the MSMS Matched Peaks column

1. In a Spectrum view, display the fragmentation scan that you entered on the Elemental Composition page, in the Fragments Matching area, in the MSMS Scan No box.
2. Compare the values for MSMS Coverage [%] and MSMS Matched Peaks to the number of mass spectrum peaks in the fragmentation scan.

Elemental Composition Results View

The Elemental Composition Results view displays the best matching calculated chemical formulas for a peak in the spectrum. You specify the criteria for the match on the Elemental Composition Page of the Info Bar and the Default Elemental Composition Page.

❖ To display the Elemental Composition Results view

Run the Elemental Composition algorithm (see "Determining the Elemental Composition of an Ion" on page 154).

Table 29 describes the columns in the Elemental Composition Results view. By default, the Combined Fit, RDB, Combined Score, MSMS Cov. [%], MSMS Shift Measure, and MSMS Matched Peaks columns are hidden. To display these columns, you must use the Field Chooser dialog box.

Table 29. Elemental Composition Results view parameters (Sheet 1 of 3)

Parameter	Description
Peak Mass	Displays the mass-to-charge ratio of the selected isotopic peak.
Display Formula	Displays the chemical formula of the match by using the elements that you specified on the Elemental Composition Page. Also displays isotope labels for elements listed in the Elements in Use table.
Combined Fit	Displays the spectral similarity score between the measured and theoretical isotope patterns. Fragments matching does not affect this value.
RDB	Displays the ring and double-bond (RDB) equivalents that the algorithm calculated for the proposed match.
Delta (ppm)	Displays the difference between the measured mass-to-charge ratio and the theoretical mass-to-charge ratio. The table lists only formulas whose mass is within the tolerance that you specified on the Elemental Composition Page, in the units (ppm, mmu, or amu) that you specified there.
Theoretical Mass	Displays the theoretical mass-to-charge ratio of the proposed match.
Rank	Displays the ranking of the proposed match by decreasing Combined Score values.
Combined Score	Displays a percentage that conveys how close the measured spectrum matches the theoretical spectrum. Fragments matching refines this value by including the relative number of matching fragments in the score. The Combined Score for a proposed formula increases if the fragmentation spectrum includes more matching fragments and decreases if the fragmentation spectrum includes fewer matching fragments relative to other formulas.
#Matched Isotopes	Displays the number of isotopes in the measured isotope pattern that match the theoretical isotope pattern for the chemical formula. A matching isotope matches the delta mass from the A0 peak (the leftmost peak in an isotope pattern) and the relative intensity of the theoretical isotope pattern within the specified tolerances.

Table 29. Elemental Composition Results view parameters (Sheet 2 of 3)

Parameter	Description
#Missed Isotopes	Displays the number of isotopes that are missing in the measured isotope pattern for the precursor ion. An isotope peak is missing if it is not found in the measured spectrum within the specified mass and intensity tolerances.
MS Coverage [%]	Displays the summed intensity of matching isotope peaks in the measured pattern relative to the summed intensity of all the peaks in the measured pattern. $\frac{\text{Summed intensity of the matching isotope peaks} \times 100}{\text{Summed intensity of all the peaks in the measured pattern}}$
	IMPORTANT Low values for all the candidates might indicate an overlapping pattern rather than a lack of good matches.
Pattern Coverage [%]	Displays the summed intensity of the matching isotope peaks in the measured MS1 spectrum relative to the summed intensity of the theoretical isotope pattern. $\frac{\text{Summed intensity of the matching isotope peaks} \times 100}{\text{Summed intensity of the theoretical isotope pattern}}$ Provides a quantitative measure of how well the measured isotope pattern matches the theoretical isotope pattern.
	Note Because the base peak (leftmost peak) is typically responsible for most of the pattern intensity, even a small decrease in the percent coverage might be important. For example, a missing peak for an isotope with two ^{13}C atoms might cause only a small decrease in the summed intensity of the measured isotope pattern.
The following columns display nonzero values when you add the fragments matching algorithm to the elemental composition analysis.	
MSMS Cov. [%]	Displays the summed intensity of the matched fragment peaks relative to the summed intensity of all the fragment peaks in the selected MSMS scan.
	Note Low values for all the candidates might indicate a contaminating compound within the isolation window for the fragmentation scan.

Table 29. Elemental Composition Results view parameters (Sheet 3 of 3)

Parameter	Description
MSMS Shift Measure	Not used for scoring. The application searches for each fragment in a range from the expected mass based on the chemical formula and the expected mass plus the delta mass between the precursor ion and its displayed formula. The MSMS Shift Measure value increases when the fragments are closer to their expected masses rather than their shifted masses.
MSMS Matched Peaks	Displays the number of matching fragments in the MSMS spectrum. Click the expand icon, +, to view the fragment list.

Elemental Composition Page

Use the Elemental Composition page of the Info Bar to calculate the best matching chemical formula for a mass-to-charge ratio in a mass spectrum. The FreeStyle application assigns formulas to components by using an isotopic pattern-matching algorithm that accounts for isotope accurate mass and intensity ratios.

The algorithm uses a single mass, usually the monoisotopic mass of a measured isotope pattern, to calculate all possible elemental compositions that lie within a tolerance window. Then, the algorithm calculates a theoretical isotope pattern for each elemental composition candidate. It calculates the fit between the theoretical and measured isotope pattern, sorting the identified candidates in decreasing order of isotopic pattern score. The isotopic pattern score value is a number between 0 percent (where the patterns are completely different) and 100 percent (where the patterns are indistinguishable by using the scoring parameters specified in the processing method).

The Fragments Matching algorithm provides confirmation of the chemical formula by comparing the fragmentation scan of the selected precursor mass to the theoretical fragmentation pattern for the chemical formula.

❖ **To display the Elemental Composition page without starting the algorithm**

In the Workspace Processing toolbar, click **Elemental Composition**.

Figure 92 shows the Elemental Composition page.

Figure 92. Elemental Composition page

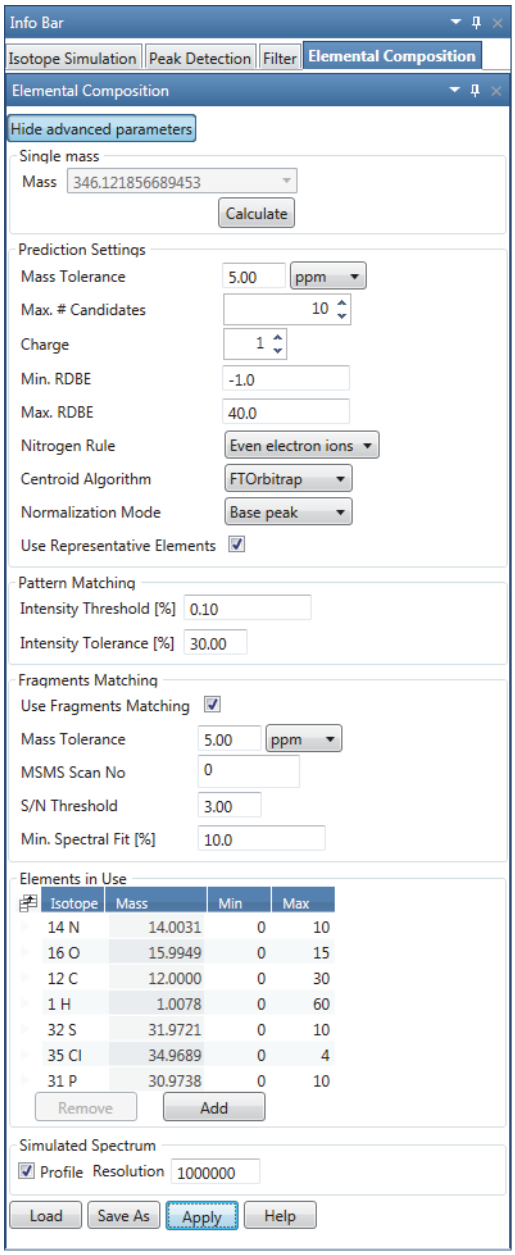


Table 30 describes the parameters for the Elemental Composition page.

Table 30. Elemental Composition page parameters (Sheet 1 of 6)

Parameter	Description
Show/Hide Advanced Parameters	Show or hide the advanced parameters. The advanced parameters are as follows: • Under Prediction Settings: Min and Max RDBE, Nitrogen Rule, Centroid Algorithm, Normalization Mode, and Use Representative Elements • Pattern Matching: Intensity Threshold and Intensity Tolerance • Under Fragments Matching: S/N Threshold and Min. Spectral Fit
Single Mass	
Mass	Specify the mass-to-charge ratio that the algorithm uses to calculate probable chemical formulas. Range: Minimum *m/z* of the measured spectrum to the maximum *m/z* of the measured spectrum
Calculate	Calculate formulas and display them in the Elemental Composition Results View.
Prediction Settings	
Mass Tolerance	Specify a mass tolerance to restrict the number of possible elemental compositions. The elemental composition algorithm returns results of the search only if the theoretical mass matches the submitted mass within the specified tolerance. Range: 0.00–100.00; default: 5.00 ppm
Units	Select the units that you want to associate with the mass tolerance. The options are amu (atomic mass units), mmu (millimass units), and ppm (parts-per-million). If you specify the error limits in ppm, the errors are mass-dependent and get larger at higher masses and smaller at lower masses. Default: ppm
Max #Candidates	Specify the maximum number of formulas to display. Range: 0–400; default: 10

Table 30. Elemental Composition page parameters (Sheet 2 of 6)

Parameter	Description
Charge	Select the charge state that you want to use to calculate the probable formulas. Range: –99 to 99; default: 1
Min. and Max. RDBE	Specify a range of values for double bonds and ring equivalents—a measure of the number of unsaturated bonds in a compound—that limits the calculated formulas to only those that make sense chemically. Limits range: –1000.0 to 1000.0 The value is calculated by the following formula: $$D = 1 + \frac{\left[\sum_{i}^{i\max} Ni\,(Vi - 2)\right]}{2}$$ where: • D is the value for the RDB equivalents • $i$max is the total number of different elements in the composition • Ni is the number of atoms of element i • Vi is the valence of atom i The calculation produces an integer such as 3, which indicates an odd-electron ion, or a number with a remainder of 0.5, which indicates an even-electron ion. Minimum value: –0.5, corresponding to a protonated, saturated compound

Table 30. Elemental Composition page parameters (Sheet 3 of 6)

Parameter	Description
Nitrogen Rule	Select to use the Nitrogen Rule in the formula calculation. Select one of the following: • Do Not Use: Do not use the Nitrogen Rule. • (Default) Even Electron Ions: Choose for even-electron ions, such as protonated species. • Odd Electron Ions: Choose for odd-electron atoms, such as radical cations.
	Note McLafferty[a] states the Nitrogen Rule as follows: "If an odd-electron ion contains no (or an even number of) nitrogen atoms, its molecular ion will be at an even mass number...[Similarly,] an odd-electron ion will be at an odd mass number if it contains an odd number of nitrogen atoms."
Centroid Algorithm	Select the centroiding algorithm for profile data. Default: FT Orbitrap Selections: FTOrbitrap, GCQ, TSQ, or MAT
Normalization Mode	Select one of the following normalization modes for the spectral peak intensities: • (Default) Base peak: The most common normalization mode. Normalizes the isotope peaks to the base peak height of 100%. Its disadvantage is the propagation of the intensity error of the base peak to all isotope intensities. • Linear: Normalizes the theoretical and measured patterns so that the sum of all isotopic pattern intensities is the same. • Quadratic: Normalizes the theoretical and measured patterns so that the error squares (intensity differences) are minimized.

Table 30. Elemental Composition page parameters (Sheet 4 of 6)

Parameter	Description
Use Representative Elements	Select the check box to use the Representative Elements table for the elemental composition calculations. Clear the check box to use the Protein Elements table. Currently the elemental composition algorithm supports two types of element tables, differing mainly in their carbon (C) abundances. • The Representative Elements table has the following C abundances: – 12C: 0.9893 – 13C: 0.0107 • The Protein Elements table has the following C abundances: – 12C: 0.989136445 – 13C: 0.010863555 Default: Selected
Pattern Matching	
Intensity Threshold [%]	Specify the isotope intensity threshold, relative to the base peak of the theoretical isotope pattern that the algorithm uses for pattern simulation. The algorithm skips isotopes below the threshold—that is, if the expected intensity of an isotopic peak is below the threshold, the algorithm does not look for the peak in the experimental spectrum, and the peak is not part of the score calculation. Range: 0.00 to 10 000.00%; default: 0.10%
Intensity Tolerance [%]	Specify the relative intensity tolerance for the isotope search. Range: 0.00 to 10 000.00%; default: 30.00%
Fragments Matching	
Use Fragments Matching	Select this check box to turn on the fragment matching algorithm, which ranks the identified candidates (chemical formulas) by the number of matching peaks in the fragmentation scan for the precursor ion. The fragment matching algorithm requires a fragmentation scan for the selected precursor ion. To perform MS/MS matching, you must enter the scan number of the fragmentation scan for the precursor ion (*m/z* value) of interest.

Table 30. Elemental Composition page parameters (Sheet 5 of 6)

Parameter	Description
Mass Tolerance	Specify the mass tolerance for the peaks in the fragmentation spectrum (expected mass versus theoretical mass).
MSMS Scan No	Specify the MS/MS scan number of the fragmentation scan for the selected precursor ion (see "To find an appropriate fragmentation scan for the fragments matching algorithm" on page 158). Default: 0 (No fragment matching); Range: 1 to the last scan number in the raw data file
S/N Threshold	Specify the signal-to-noise threshold for the peaks in the MS/MS spectrum. The fragments matching algorithm ignores peaks below this S/N threshold.
Min. Spectral Fit [%]	Restrict the candidate list to candidates that meet or exceed the minimum spectral fit value. Range: 0.00 to 100.00%; default: 10.00%
Elements in Use Define in the table which isotopes, and the number of occurrences for each isotope, to consider when the algorithm calculates possible elemental compositions for the submitted mass value. See "Adding and Removing Elements in the Elements in Use Table" on page 158.	
Field Chooser 	Displays the Field Chooser dialog box for selecting which fields appear in the Elements in Use table (see "Selecting the Columns to Display in a View or Dialog Box with Tabular Data" on page 20).
Isotope	Select the isotopes that you want the data system to consider when it calculates the possible elemental compositions for a monoisotopic ion with the given mass (A0 mass peak). To add an isotope, click **Add**. The dialog box for the periodic table of elements opens. You can also right-click in the grid and choose **Add** from the shortcut menu. To remove an isotope, click to the left of the element in the table to highlight its row, and then click **Remove** or right-click and choose **Remove** from the shortcut menu.
Min	Specify the minimum number of occurrences of an isotope in the chemical formula.
Max	Specify the maximum number of occurrences of an isotope in the chemical formula.

Table 30. Elemental Composition page parameters (Sheet 6 of 6)

Parameter	Description
Mass	Displays the exact isotopic mass for each isotope in the Elements in Use list. You cannot edit this value.
Simulated Spectrum	
Profile and Resolution	Select the **Profil**e check box and specify the resolution to plot a profile spectrum in addition to a centroid spectrum. To plot only a centroid spectrum, clear the **Profile** check box. For the profile spectrum, the baseline might not be zero due to background noise. If you know the resolution value, enter it in the Resolution box. **Tip** You can view the resolution values for each mass spectrum peak by clicking Peak Resolution in the Spectrum Display Options toolbar (see "Spectrum or Multi Spectrum – Display Options Toolbar" on page 138).
Button	
Load	Select a file (with an .limx file name extension) that contains a set of isotope limits.
Save As	Save a list of isotope limits to a file (with an .limx file name extension).
Apply	Apply the elemental composition settings to the active mass spectrum.
Help	Opens the FreeStyle Help to the elemental composition topic.

[a] McLafferty, F. W. *Interpretation of Mass Spectra*; University Science Books: Mill Valley, CA, 1980.

6

Searching Mass Spectrum Libraries

To compare a query spectrum against a set of mass spectrum libraries, follow these topics.

Contents

Performing a Local NIST or mzVault Library Search

Search your local NIST or mzVault mass spectrum libraries to identify unknown compounds.

Follow these topics in order:

1. Setting Up the Default Library Search Parameters
2. Selecting the Query Spectrum
3. Starting a Library Search

Setting Up the Default Library Search Parameters

Before you can perform an mzVault library search, you must specify the location of at least one of your local mzVault database files. The application automatically links to the NIST libraries installed during the software installation process.

Follow these procedures as needed:

- To open the Library Search page of the Default Options Configuration dialog box
- To specify the location of your local mzVault database files
- To specify the default parameter settings for a library search

❖ To open the Library Search page of the Default Options Configuration dialog box

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Library Search**.

❖ To specify the location of your local mzVault database files

1. In the Library Type area on the Library Search page, select the **mzVault** option.
2. For each database file that you want to add to the Search list, do the following:
   a. In the Search List area, browse to the location of your mzVault database files.
   b. Select an mzVault database file (DB).
   c. Click **Open**.
3. Click **Save**.

Tip After you run your first mzVault search, you can also specify the location of your mzVault database files on the mzVault Search page in the Info Bar.

❖ To specify the default parameter settings for a library search

1. In the Library Type area on the Default Library Search Page, select either the **NIST** or **mzVault** option.
2. Make the appropriate selections and entries.
3. Click **Save**.

Note If you plan to run both NIST and mzVault searches, make sure to select and save the settings for both search types.

Tip After you run a library search, selecting a different setting from the Display list on the mzVault Search page or the NIST Search page (in the Info Bar) automatically changes how the Spectrum view displays the query spectrum versus the matching library spectrum. You can also modify the search criteria from the Info Bar; however, to start the new search, you must click Apply.

Go to the next topic "Selecting the Query Spectrum."

Selecting the Query Spectrum

The query spectrum is the spectrum that you want to search against a library of mass spectra.

❖ To select the query spectrum

1. Open a raw data file that contains mass spectral data.

   IMPORTANT You can perform a library search on a mass spectrum, an average mass spectrum, or a composite mass spectrum. For an mzVault or a NIST search for a matching MS/MS spectrum, the query spectrum must be a data-dependent scan.

2. Select a mass spectrum by doing any of the following:

   - Select a data point in a chromatogram plot. If a Spectrum or Multi Spectrum view is not open, in the Workspace Options toolbar, click **Spectrum**.

     The mass spectrum for the selected time point appears in the Spectrum view or the Multi Spectrum view.

   - Click the **MSn Browser** tab, and then double-click an item in the MSn Tree.

     The selected spectrum opens in a new Spectrum view.

   –or–

   a. Click the Spectrum (or Multi Spectrum) view of interest.

   b. In the Workspace Options toolbar, click **Spectrum Ranges**.

   c. In the Spectrum Ranges dialog box, specify the spectrum of interest.

   Note You can perform a library search on a mass spectrum, an averaged mass spectrum, or a composite mass spectrum.

Go to the next topic "Starting a Library Search."

Starting a Library Search

After you set up the default options for an mzVault or NIST library search and select a query spectrum, you are ready to start the search.

❖ To start a library search

1. Set up the default search settings (see "Setting Up the Default Library Search Parameters" on page 173).
2. Select a spectrum plot (see "Selecting the Query Spectrum" on page 175).
3. Click the **Workspace Processing** tab.

4. Click **mzVault Search** or **NIST Search**.

   The selected library search page appears in the Info Bar, and the application automatically searches the specified libraries.

   When the search ends, the following items appear:

   - The library spectrum for the best hit appears in the Spectrum view as a stacked plot, a mirror plot, or an exclusion plot.

     Note From the Info Bar, you can change the display setting on the mzVault Search page or the NIST Search page without rerunning the search.

   - A list of search hits appears in the search results view.
   - The structure of the best hit appears in the chemical structure view.

Go to the next procedure "Reviewing the Results of a Local NIST or mzVault Library Search."

Reviewing the Results of a Local NIST or mzVault Library Search

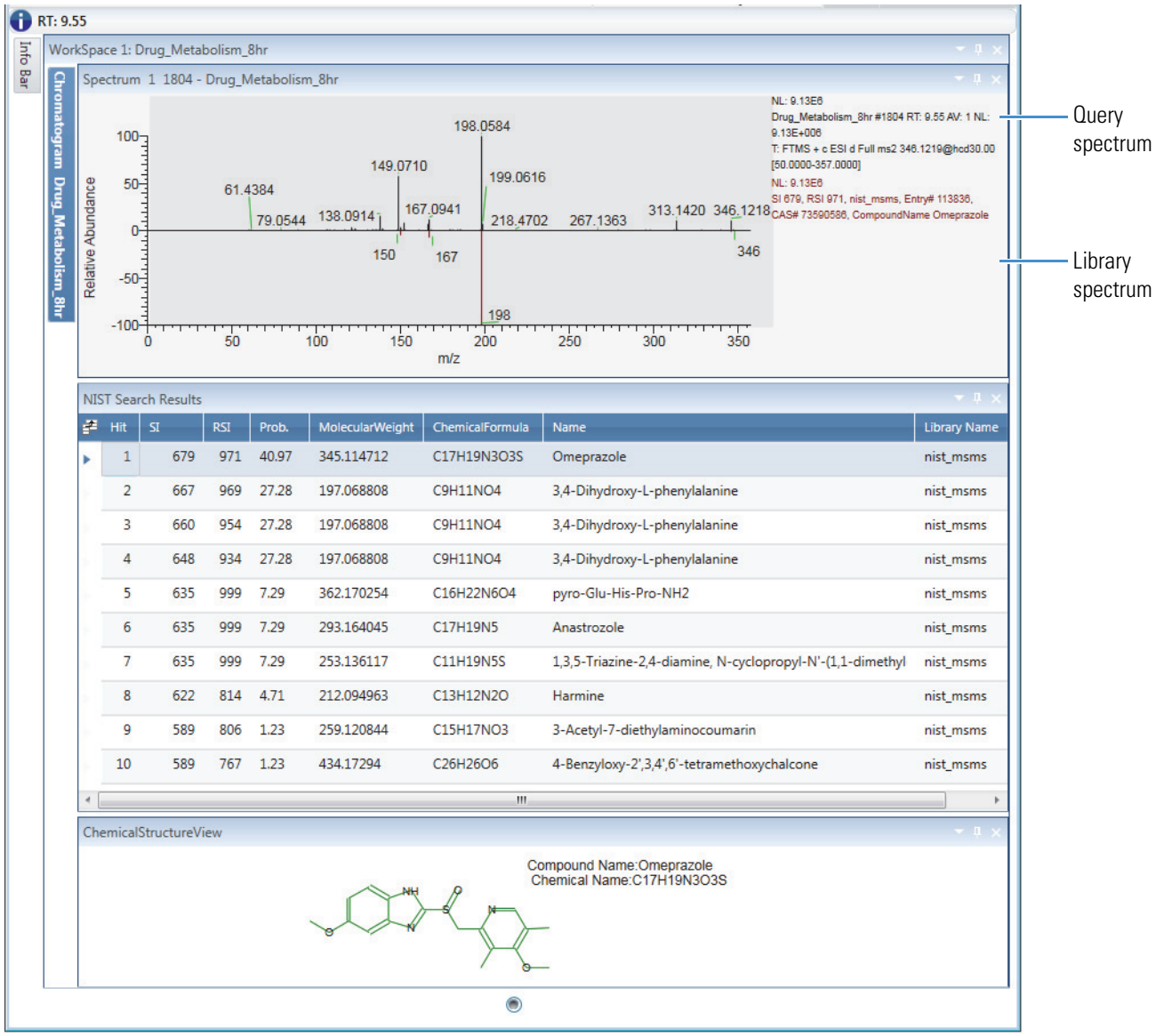
To review the results of a library search, see the appropriate topics:

- Reviewing the Results of a NIST Library Search
- NIST Search Results View
- Reviewing the Results of an mzVault Search
- mzVault Search Results View

Reviewing the Results of a NIST Library Search

Figure 93 shows the results of a NIST library search. The Spectrum view contains a mirror plot. The NIST Search Results view lists 10 hits sorted in order of probability. The Chemical Structure View displays the two-dimensional structure of the selected hit.

Figure 93. Results of a NIST library search displayed as a mirror plot



Hit	SI	RSI	Prob.	MolecularWeight	ChemicalFormula	Name	Library Name
1	679	971	40.97	345.114712	C17H19N3O3S	Omeprazole	nist_msms
2	667	969	27.28	197.068808	C9H11NO4	3,4-Dihydroxy-L-phenylalanine	nist_msms
3	660	954	27.28	197.068808	C9H11NO4	3,4-Dihydroxy-L-phenylalanine	nist_msms
4	648	934	27.28	197.068808	C9H11NO4	3,4-Dihydroxy-L-phenylalanine	nist_msms
5	635	999	7.29	362.170254	C16H22N6O4	pyro-Glu-His-Pro-NH2	nist_msms
6	635	999	7.29	293.164045	C17H19N5	Anastrozole	nist_msms
7	635	999	7.29	253.136117	C11H19N5S	1,3,5-Triazine-2,4-diamine, N-cyclopropyl-N'-(1,1-dimethyl	nist_msms
8	622	814	4.71	212.094963	C13H12N2O	Harmine	nist_msms
9	589	806	1.23	259.120844	C15H17NO3	3-Acetyl-7-diethylaminocoumarin	nist_msms
10	589	767	1.23	434.17294	C26H26O6	4-Benzyloxy-2',3,4',6'-tetramethoxychalcone	nist_msms

NIST Search Results View

The NIST Search Result view appears in the workspace after you submit a NIST library search. The library search algorithm returns a list of the best matches from the selected libraries. See "Managing Libraries" on page 195.

❖ To display the NIST Search Results view

Perform a NIST library search (see "Performing a Local NIST or mzVault Library Search" on page 173).

Table 31 describes the columns for the NIST Search Results view.

Table 31. NIST Search Result view columns

Column	Description
Hit	Relative ranking of library search matches based on decreasing SI (Search Index) values.
SI (Search Index)	Direct matching factor for the query spectrum and the library spectrum.
RSI (Reverse Search Index)	Reverse search matching factor that ignores any spectrum peaks in the query spectrum that are not in the library spectrum.
Prob	Probability factor based on the differences between adjacent hits in an SI ordered list.
Molecular Weight	Molecular weight (in daltons) of the library search match.
Chemical Formula	Chemical formula of the library search match.
Name	Name of the matched compound in the library.
Library Name	Name of the library that contains the matching compound.

Three factors describe the accuracy of the match to the submitted spectrum: SI, RSI, and Prob. With the SI and RSI matching factors, a perfect match results in a value of 1000. As a general guide, 900 or greater is an excellent match; 800–900, a good match; and 700–800, a fair match. A matching factor less than 600 is a poor match. Unknown spectra with many peaks tend to yield lower match factors than similar spectra with fewer peaks.

The probability factor is a complex parameter based on the SI matching factor and the difference between adjacent matches. If a match has an SI match factor greater than 900 and the next best match has a match factor of 300, the probability of the compound being correctly identified is high. Conversely, if several matches have very similar SI matching factors, the probability of a correct assignment is low.

The Chemical Structure View appears when you perform a NIST library search. The Chemical Structure view displays the chemical structure, the compound name, and the chemical formula of the compound that you select in the NIST Search Result view.

Reviewing the Results of an mzVault Search

For information about starting an mzVault library search, see Starting a Library Search.

Follow these procedures as needed:

- To review the matching compounds found by an mzVault library search
- To view all the matching scans for a selected compound

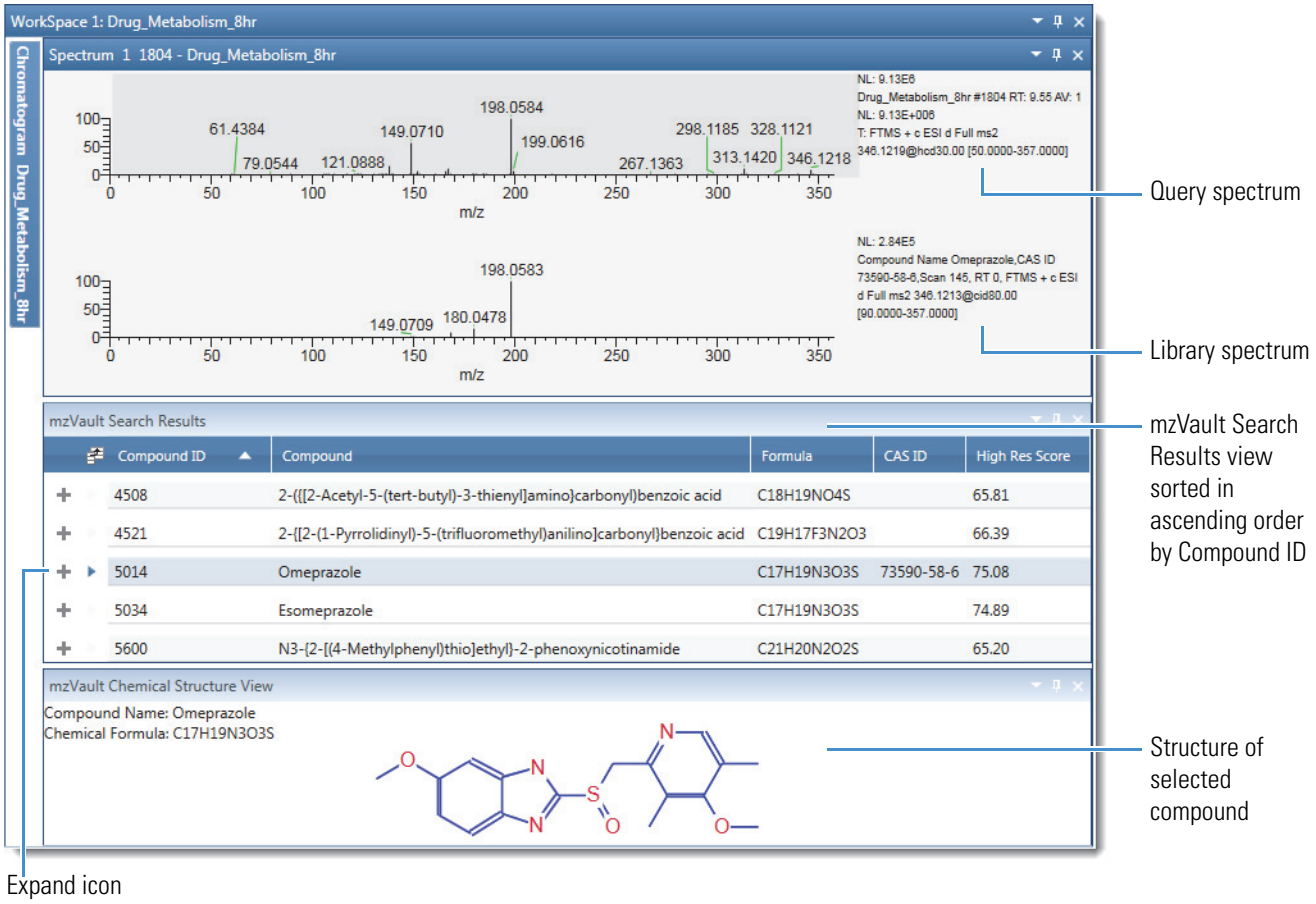
❖ To review the matching compounds found by an mzVault library search

In the mzVault Search Results view, select a compound.

The best matching spectrum for the library compound appears in the Spectrum view and its chemical structure appears in the mzVault Chemical Structure View.

Figure 94 shows the results of an mzVault library search. The query spectrum's plot is stacked above the library spectrum's plot. The mzVault Search Results view lists matching compounds in order of their compound ID numbers. The mzVault Chemical Structure view displays the two-dimensional structure of the compound with the highest score, from 0 to 100%.

Figure 94. mzVault library search results

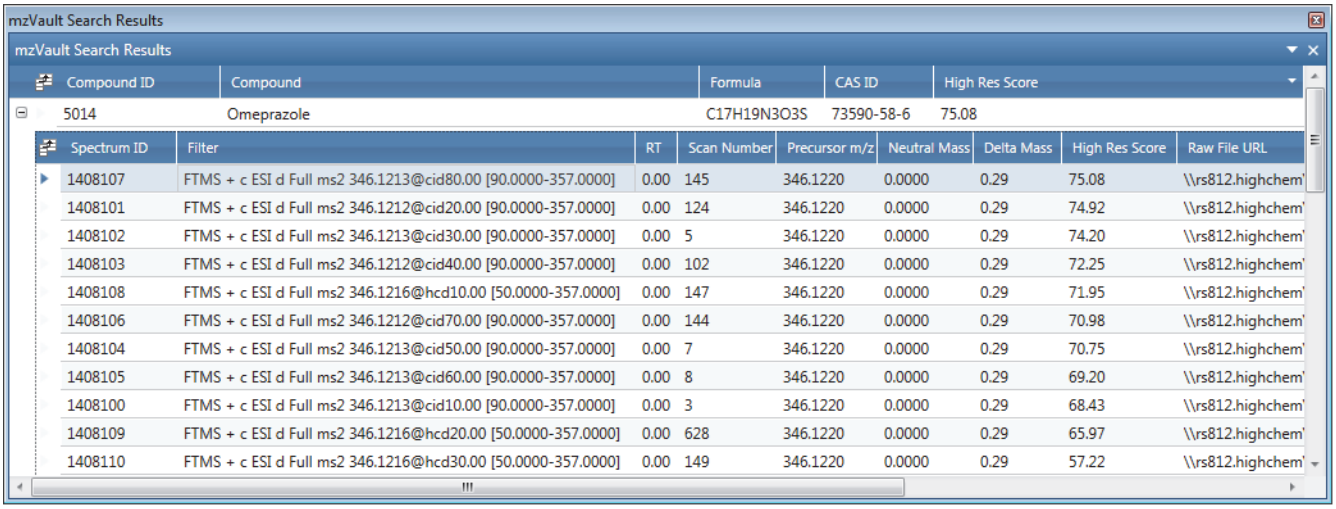


❖ To view all the matching scans for a selected compound

1. Click the expand icon, ✚, to open the complete list of matching scans.
2. To view a scan in the Spectrum view, select it in the subtable.

Figure 95 shows the subtable of matching scans in the mzVault database file. For details about the table columns, see the next topic "mzVault Search Results View." To modify and rerun the search, see "To reset the mzVault search parameters and query spectrum" on page 188.

Figure 95. Subtable of matching scans in the mzVault Search Results view



mzVault Search Results View

The mzVault Search Results view appears in the workspace after you submit an mzVault search. The mzVault search returns a list of matching compounds.

❖ To display the mzVault Search Results view

Perform an mzVault library search (see "Performing a Local NIST or mzVault Library Search" on page 173).

The mzVault Search Results view consists of the top-level compound entries and the secondary-level spectrum entries for each compound hit.

Table 32 describes the columns in the compounds table in the mzVault Search Results view.

Table 32. Compounds table in the mzVault Search Results view (Sheet 1 of 2)

Column	Description
Compound ID	Displays the assigned entry number in the mzVault library. The mzVault application assigns entry numbers in sequential order, beginning with the number 1 for the first entry.
Compound	Displays the name of the compound associated with the matching spectrum in the library.
Formula	Displays the chemical formula for the library entry.

Table 32. Compounds table in the mzVault Search Results view (Sheet 2 of 2)

Column	Description
CAS ID	Displays the unique Chemical Abstracts Service™ registry number.
High Res Score	Displays a score calculated by a proprietary algorithm that indicates how well the library spectrum and the query spectrum match.

Table 33 describes the spectrum information for each compound entry.

Table 33. Spectrum information for each compound entry

Column	Description
Spectrum ID	Displays the identification number for the library spectrum.
Filter	Displays the scan filter from the raw data file.
RT	Displays the retention time from the raw data file.
Scan Number	Displays the scan number from the raw data file.
Precursor m/z	Displays the mass-to-charge ratio of the precursor ion from the scan filter for the library spectrum.
Neutral Mass	Displays the uncharged, neutral mass of the molecule.
Delta Mass	Displays the difference in mass between the mass-to-charge ratio of the precursor ion for the library entry and the mass-to-charge ratio of the precursor ion for the query spectrum, in parts per million.
High Res Score	Displays the score calculated by a proprietary algorithm that indicates how well the library spectrum and the query spectrum match.
Raw File URL	Displays the name and path of the raw data file where the matching spectrum in the library entry came from.

Modifying the Settings for a Local NIST or mzVault Library Search

After you run a NIST or an mzVault library search, you can modify the search settings on the respective page in the Info Bar, and then restart the search.

To modify and rerun a search, see the appropriate topic:

- Modifying a NIST Search from the NIST Search Page
- Modifying an mzVault Search from the mzVault Search Page

Modifying a NIST Search from the NIST Search Page

After running a NIST search, use the NIST Search page of the Info Bar to modify the search or the display in the NIST Search Results view.

Follow these procedures as needed:

- To display the NIST Search page
- To modify the display in the NIST Search Results view
- To modify the NIST search

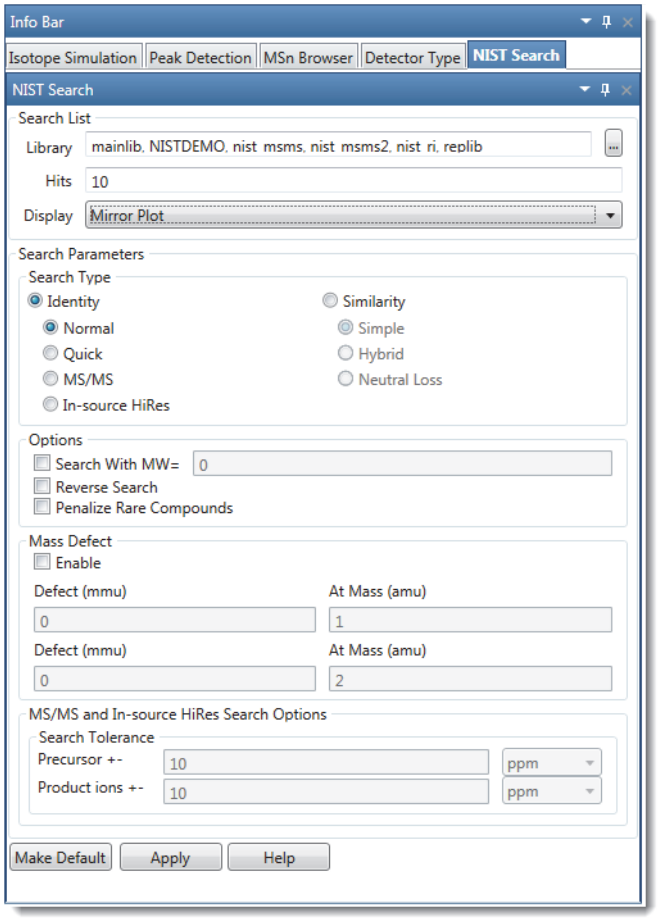
❖ To display the NIST Search page

Run a NIST library search (see "Performing a Local NIST or mzVault Library Search" on page 173).

When the search is complete, you can reset the search parameters.

Figure 96 shows the NIST Search page.

Figure 96. NIST Library Search page



❖ To modify the display in the NIST Search Results view

In the Display list on the NIST Search page, make a different selection (see Display in Table 34).

❖ To modify the NIST search

Modify the parameter settings on the NIST Search page, and then click **Apply**.

Table 34 describes the parameters for the NIST Search page.

Table 34. NIST Search page parameters (Sheet 1 of 4)

Parameter	Description
Search List	
Use the settings in this area to choose the library for library searches. Specify how many search results to display and how they are displayed.	
Library	Select the search libraries.
Hits	Select how many search results to display in the NIST Search Results View. Range: 1–100; default: 10
Display	Select how to display the library spectrum and measured spectrum, from these options: • Stacked: Displays the submitted spectrum above the currently selected library spectrum in the match list. • Exclusion: Subtracts the library spectrum from the submitted spectrum. • Mirror: Plots the submitted spectrum that points up from the *x* axis and the library spectrum that points down from the *x* axis.
Search Type	
Use the options in this area to choose the type of library search to apply. The two main search types are Identity and Similarity. They differ primarily in the weightings of the spectrum as a function of mass.	
Identity	Applies an identity-search algorithm for the library matching of spectra.
Normal	(Default) Applies a normal identity-search algorithm for library matching of spectra. Use a normal identity search for low quality or unusual spectra. The search algorithm uses a standard prescreen search filter.

Table 34. NIST Search page parameters (Sheet 2 of 4)

Parameter	Description
Quick	Applies a quick identity-search algorithm for library matching of spectra. Use this option if you are sure the spectrum or compound exists in the library. The search algorithm uses a fast prescreen search filter.
Penalize Rare Compounds	Limits the impact of rare compounds by reducing the match factor. Use this option only when you select one or more of the NIST databases (such as MAINLIB). It has no effect on spectra in user libraries or other commercial libraries. Each reference spectrum in a NIST library contains a record of other commercial databases containing information about the compound. A compound is considered rare if it is present in a limited number of these databases. If you select the Penalize Rare Compounds option, the application reduces the match factors for matched compounds that are present in few or no databases other than the NIST libraries. The maximum reduction penalty is 50 out of 1000. Selecting this option leads to a relative increase in the match factors of common compounds, placing them higher in the match list than exotic isomers with nearly identical spectra.
MS/MS	Searches for an MS/MS spectrum in a library of MS/MS spectra.
In-source HiRes	Searches for an in-source electron ionization (EI) spectrum in a library containing high-resolution in-source EI spectra—that is, a library where the EI-MS spectra are annotated with *m/z* values to several decimal places. Also searches for an MS/MS spectrum in a library of MS/MS spectra. Unlike an MS/MS search, this search does not compare precursor *m/z* values.

Table 34. NIST Search page parameters (Sheet 3 of 4)

Parameter	Description
Similarity	Applies a similarity-search algorithm for the library matching of spectra.
Simple	Applies a simple similarity-search algorithm for library matching of spectra. This option finds a large set of spectra to compare with the submitted spectrum, and is generally slower than an identity search. Use a simple similarity search in these situations: • You know that the unknown spectrum is not in the library. • The spectrum is of poor quality so that a reliable match is unlikely.
Hybrid	Applies a hybrid similarity-search algorithm for library matching of spectra. This option uses a combination of the simple and neutral-loss search strategies. The neutral-loss search requires an estimate of the unknown's molecular weight. If the unknown compound contains chemical structures that generate both characteristic ions and neutral loss patterns, the search algorithm can identify these structures from the match list produced by this search.
Neutral Loss	Applies a neutral-loss similarity-search algorithm for library matching of spectra. The neutral losses in a spectrum are the mass differences between the molecular ion and other major ions in the spectrum. Neutral-loss peaks can be very characteristic as spectral features for certain classes of compounds. In a neutral loss search, the algorithm examines the submitted spectrum and identifies the molecular ion. It submits the mass value of the molecular ion to the search algorithm along with the spectrum. The search algorithm calculates the significant neutral losses and compares them with the library data. It returns hits according to matches of the molecular ion and its neutral losses.
Options	
Search With MW=	Restricts the library search to entries with a particular molecular weight. Use the associated box to enter the molecular weight.
Reverse Search	Sorts matching library spectra by the reverse search match factor. By default, the algorithm sorts matches by the forward match factor.

Table 34. NIST Search page parameters (Sheet 4 of 4)

Parameter	Description
Mass Defect	
Use this area to set the parameters for library searches to correct for the differences between the actual masses and the nominal integer masses of the atoms in a molecule. Assign a larger mass defect (in millimass units) for more massive molecules because, in general, they are composed of more atoms than less massive molecules. More massive molecules need a larger correction factor to approximate the linear function that the application uses to calculate masses.	
Enable	Include mass defect values for library searches.
Defect (mmu)	Specify the mass defect (in millimass units). Specify a smaller value for the lower mass ranges in the first box and a larger value for the higher mass ranges in the second box.
At Mass (amu)	Specify the masses at which the application applies the specified mass defect values to calculate mass. Specify a smaller mass value in the first box and a larger mass value in the second box.
MS/MS and In-source HiRes Search Options	
Use this area to set the parameters for library searches involving the comparison of peaks in the search spectrum with library spectra whose precursor *m/z* value or product mass spectral peak might need to fall within a specified tolerance setting.	
Precursor +–	Specify the range of the *m/z* tolerance for precursor ions, in either ppm or *m/z* units. Range: 0.015 to 100 000 ppm or *m/z* 6×10^{-5} to 500; default: 10 ppm
Product Ions +–	Specify the range of mass spectral peak tolerance for product ions, in either ppm or *m/z* units. Range: 0.015 to 100 000 ppm or *m/z* 6×10^{-5} to 500; default: 10 ppm
Button	
Make Default	Saves the current settings as the default settings.
Apply	Starts the library search with the entered settings.
Help	Opens the FreeStyle Help to the NIST library search topic.

Modifying an mzVault Search from the mzVault Search Page

Use the mzVault Search page in the Info Bar to search the mzVault library for library entries that match your selected fragmentation spectrum. You can filter the search by compound name, chemical formula, or precursor *m/z* value.

Follow these procedures as needed:

- To open the mzVault Search page
- To modify the display in the mzVault Search Results view
- To reset the mzVault search parameters and query spectrum

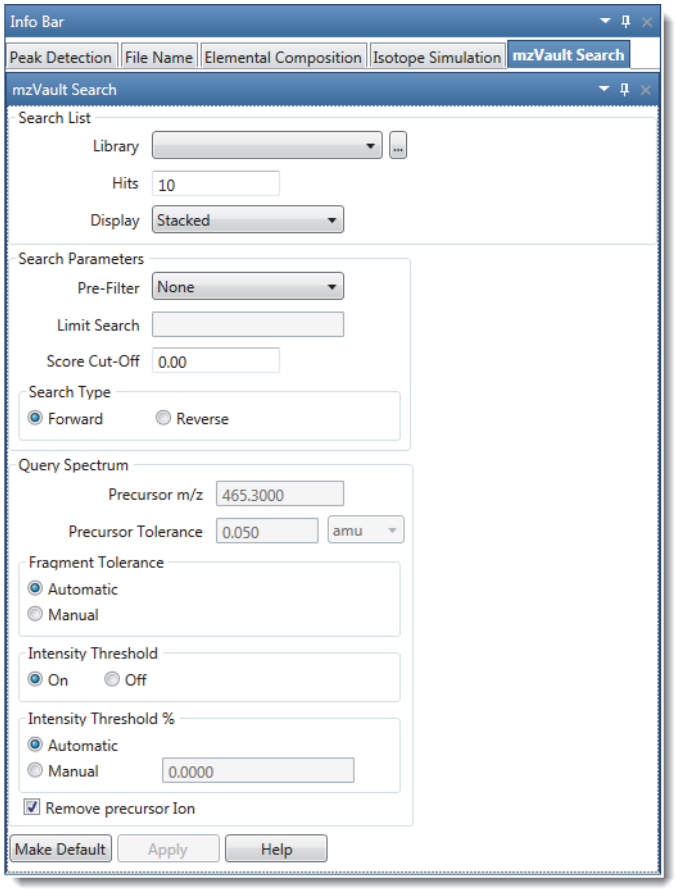
❖ To open the mzVault Search page

Run an mzVault search (see "Performing a Local NIST or mzVault Library Search" on page 173).

When the search is complete, you can reset the search parameters.

Figure 97 shows the mzVault search page.

Figure 97. *mz*Vault Search page



❖ To modify the display in the mzVault Search Results view

In the Display list on the mzVault Search page, make a different selection (see Display in Table 35).

❖ To reset the mzVault search parameters and query spectrum

1. If applicable, click the browse icon next to the Library list and select another mzVault library.
2. In the Hits box, type the maximum number of compounds that you want the search to return.
3. From the Display list, select how you want to display the matching spectra in the Spectrum view (see "Setting Up the Default Library Search Parameters" on page 173).
4. From the Pre-Filter list, select the type of filter to use:
   - (Default) **None**—Performs no filtering. Searches all the library entries for a matching spectrum. This Limit Search box is unavailable for this selection.
   - **Compound**—Filters the library entries by the name or part of the name of the compound.
   - **Formula**—Filters the library entries by the chemical formula of the library entry.
   - **Precursor m/z**—Filters the library entries by the mass-to-charge ratio of the precursor ion for the library fragmentation scans. When you select this option, the Precursor m/z and the Precursor Tolerance boxes become available.
5. For prefiltered searches, do one of the following:
   - For the Compound and Formula filters, in the Limit Search box, enter the filtering criterion. Depending on the filter selected, these criteria can be letters, numbers, or both. For example, for the Formula filter, a criterion of C12 searches for all compounds with twelve carbon atoms.
   - For the Precursor m/z filter, under Query Spectrum, select the precursor *m/z* value and its mass tolerance as follows:
     i. In the Precursor m/z box, specify the appropriate precursor *m/z* value.

        Range: Values with up to four decimal places from 0.0000 to 10 000.9000

        Default: Precursor *m/z* from the query spectrum scan filter

     ii. In the Precursor Tolerance box, specify the mass tolerance. In the units list, select the corresponding units of measure.

        Default: 5.0 ppm, 5.0 mmu, or 0.05 amu
6. (Optional) In the Score Cutoff box, specify the minimum score for a hit to be returned.

   Default: 0

7. Select the type of search to conduct, either **Forward** or **Reverse**.
8. In the Fragment Tolerance area, select the **Automatic** or **Manual** option to indicate how to specify the fragment tolerance.
9. (Optional) Select whether you want the application to filter out low intensity peaks from the query spectrum before performing the search.
10. (Optional) To have the mzVault application ignore any mass that is within 2.2 Da of the precursor ion for the purpose of calculating scores, select the **Remove Precursor** check box.

Table 35 describes the parameters for the mzVault Search page in the Info Bar.

Table 35. mzVault Search page parameters (Sheet 1 of 4)

Parameter	Description
Search List	
Use the settings in this area to choose the library for library searches. Specify how many search results to display and how they are displayed.	
Library	Select the library (database file) for the search.
Hits	Specify how many search results to display in the mzVault Search Results View. Range: 1–10 000; default: 10
Display	Select how to display the library spectrum and query spectrum: • Stacked—Displays the query spectrum above the currently selected library spectrum in the match list. • Mirror—Plots the query spectrum on the positive *y* axis from 0 to 100% intensity and the library spectrum on the negative *y* axis from 0 to –100% intensity. • Exclusion—Subtracts the library spectrum from the query spectrum.

Table 35. mzVault Search page parameters (Sheet 2 of 4)

Parameter	Description
Search Parameters	
Pre-Filter	Select one of these search limits: • None—Sets no limits on the search. • Compound—Limits the search to the specified text string. Requires a compound name or a text string in the Limit Search box. • Formula—Limits the search to the specified formula. Requires a chemical formula in the Limit Search box. • Precursor m/z—Limits the search to a mass range for the precursor ion. Requires a precursor *m/z* value in the Precursor m/z box.
Limit Search	(Available for the Compound or Formula filter) Use this box to enter a compound name or chemical formula.
Score Cut-Off	Filters out results below a percentage of the highest score. For example, if the highest score for a search result is 0.900 and the setting in this box is 60, the search does not return results with scores below 0.540 (0.9 × 60% = 0.540). Range: 0.00 to 100.00; default: 0.00
Search Type	
Forward	Conducts a forward search, which bases the search on a comparison of the peaks in the unknown spectrum against the peaks in a library spectrum. If the unknown spectrum includes a peak that is not in a given library spectrum, the score for the match is negatively affected. Use a forward search when the unknown spectrum is of high quality—that is, when it has good fragmentation and few low-intensity background peaks.

Table 35. mzVault Search page parameters (Sheet 3 of 4)

Parameter	Description
Reverse	Conducts a reverse search, which bases the search on a comparison of the peaks in a library spectrum against the peaks in the unknown spectrum. If the unknown spectrum does not contain a peak that is in the library spectrum, the score for the match is negatively affected, but the presence of additional peaks in the unknown spectrum has no effect on the score. Use a reverse search if the unknown spectrum includes peaks from several components or has a lot of background noise.
Query Spectrum	
Selecting Precursor m/z in the Pre-Filter list enables these parameters.	
Precursor m/z	Enter the mass-to-charge ratio of the precursor ion for the fragmentation scan. By default, the application uses the precursor *m/z* value in the scan filter for the query spectrum. Range: 0.0000 to 10 000.0000
Precursor Tolerance	Specifies the maximum difference in mass allowed for a precursor ion for the library spectrum relative to the precursor ion for the query spectrum, in parts per million (ppm), millimass units (mmu), or atomic mass units (amu). You can use values with one decimal place from 0.1 to 1000.0. Range: 0.001 to 1000.00; default: 0.05
Fragment Tolerance	
Automatic	(Default) Uses an internal algorithm to calculate the mass tolerance.
Manual	Selecting this option activates the mass tolerance box and the corresponding units list. Enter the mass tolerance for the fragment ions. Units: amu, mmu, or ppm Range: 0.001 to 1000.00; default: 10.000

Table 35. mzVault Search page parameters (Sheet 4 of 4)

Parameter	Description
Intensity Threshold	
On or Off	Determines whether the application filters out peaks with an intensity below the Intensity Threshold (%) setting. • (Default) On—Filters out peaks that are less than the specified value. Enables the Intensity Threshold% parameters. • Off—Does not filter out peaks on the basis of their intensity. The Intensity Threshold parameter calculates the score that indicates how well a spectrum matches a library spectrum and displays the score in the High Res Score column.
Intensity Threshold%	
Automatic	(Default) Uses an internal algorithm to calculate the threshold value.
Manual	Enables you to specify the threshold value to use for filtering spectrum peaks. The application takes the default value from the scan that you selected from the raw data file.
Additional Query Spectrum parameter	
Remove Precursor Ion	Determines whether the application removes peaks within 2.2 Da of the precursor ion. In an MS/MS experiment, the fragmentation spectrum does not normally contain a peak for the precursor ion, but sometimes it can appear and interfere with the scoring algorithm. This option corrects for the presence of a peak for the precursor ion. Default: Selected
Button	
Make Default	Saves the current settings as the default settings.
Apply	Starts the library search with the entered settings.
Help	Opens the FreeStyle Help to the mzVault search topic.

Searching the Online mzCloud Mass Spectral Database

The mzCloud mass spectral database includes thousands of fragmentation spectra.

❖ To perform an mzCloud library search for a matching fragmentation spectrum

1. In the Spectrum view, select a fragmentation spectrum.
2. In the Workspace Processing toolbar, click **mzCloud Search**.

Figure 98 shows the mzCloud™ Spectrum Search dialog box that opens in your default browser when you run an mzCloud search.

Figure 98. mzCloud search parameters in a browser

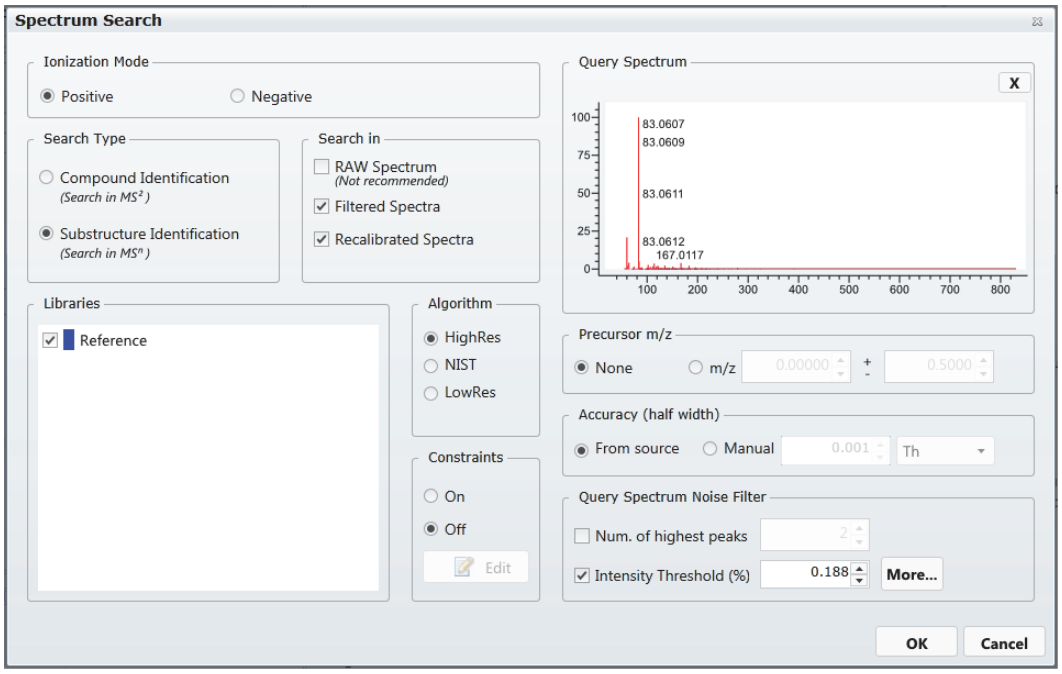
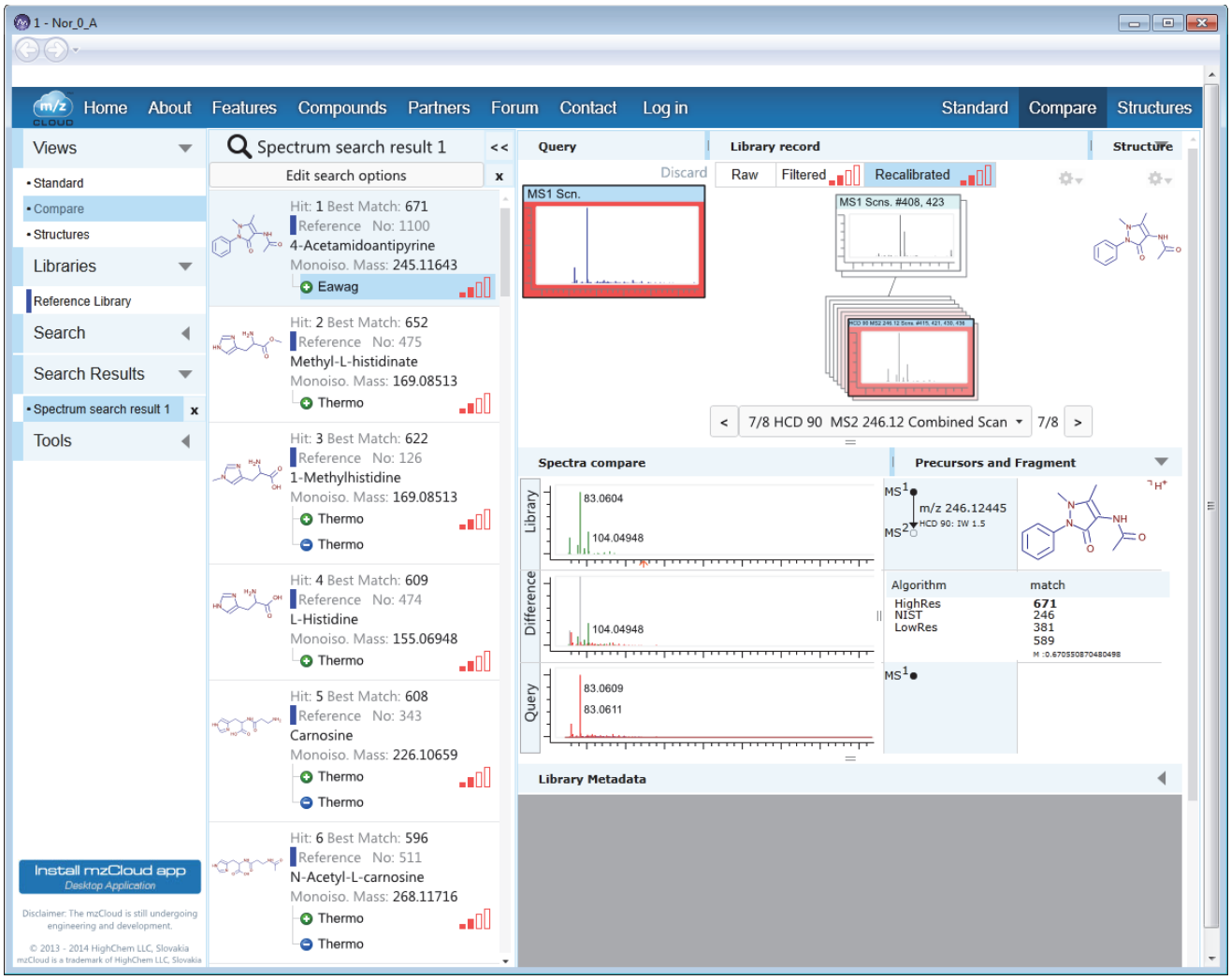


Figure 99 shows the search results for an mzCloud search.

Figure 99. mzCloud search results



Exporting a Mass Spectrum to the NIST MS Search Application

You can export a mass spectrum to the Library Search page of the NIST application.

Note Thermo Fisher Scientific provides the NIST application with the Xcalibur data system.

❖ **To export a spectrum to the NIST application**

1. Click the Spectrum view or spectrum plot of interest.
2. Click the **Workspace Processing** toolbar tab.

3. In the Library Search area of the toolbar, click **Export to NIST**.

   The Library Search page of the NIST application opens with the search spectrum displayed.

Managing Libraries

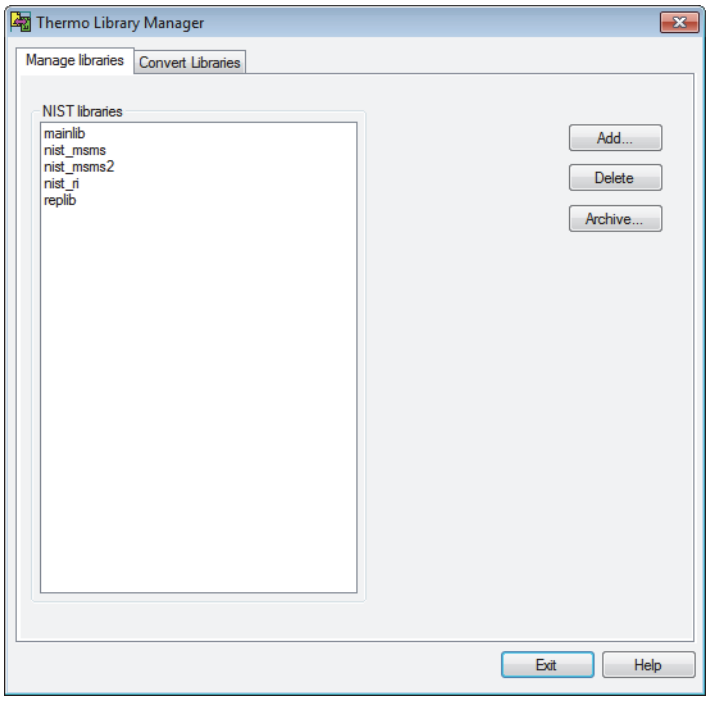
Use the Thermo Library Manager dialog box to add a library, copy a library, or delete a library.

When you add a library to the NIST libraries list, either copy the library file to the local computer or link to the library at a remote location without copying files.

❖ To add a library

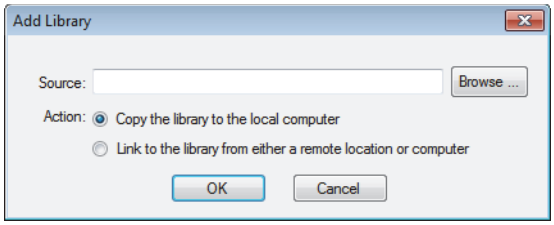
1. Click the **Workspace Processing** toolbar tab.
2. Click **Library Manager** to open the Thermo Library Manager dialog box (see Figure 100).

   Figure 100. Thermo Library Manager dialog box



3. Click **Add** to open the Add Library dialog box (see Figure 101).

Figure 101. Add Library dialog box



4. Type the path for the new library file in the Source box, or click **Browse** to locate the file.
5. Select one of two options:
   - Copy the library to the local computer.
   - Link to the library from either a remote location or computer.

   Tip Because libraries can be large, select the link option rather than the copy option to save time.

6. Click **OK**.

   The data system adds the library to the NIST Libraries list in the Thermo Library Manager dialog box.

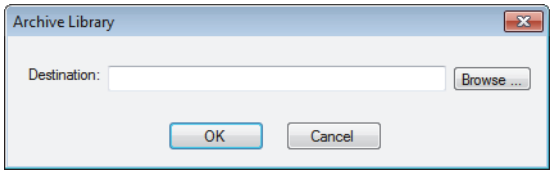
7. If you have no more tasks to complete with the Library Manager, click **Exit**.

❖ To copy a library for archiving

1. In the Thermo Library Manager dialog box (see Figure 100 on page 195), select a library in the NIST Libraries list.
2. Click **Archive**.

   The Archive Library dialog box opens (see Figure 102).

   Figure 102. Archive Library dialog box



3. In the Archive Library dialog box, type the path for the copied library file in the Destination box, or click **Browse** to find the location.
4. To copy the selected library to the remote location, click **OK**.
5. If you have no more tasks to complete with the Library Manager, click **Exit**.

You can copy a selected library to another directory on the computer or network by using the Archive feature.

❖ To delete a selected library in the NIST Libraries list

1. In the Thermo Library Manager dialog box (see Figure 100 on page 195), select a library to delete from the NIST Libraries list.
2. Click **Delete**.

   The system prompts you to confirm the deletion.
3. Click **Yes**.
4. If you have no more tasks to complete with the Library Manager, click **Exit**.

IMPORTANT After you delete a library, you cannot bring it back. Make sure that you want to delete a library permanently before you proceed, especially a shared library on the network.

Spectrum Workspace Processing Toolbar – Library Search Buttons

Use the buttons in the Library Search area of the Workspace Processing toolbar to perform library searches.

❖ To display the Spectrum Workspace Processing toolbar

1. Click the Spectrum View or the MultiSpectrum View to make it the active view.
2. Click the **Workspace Processing** toolbar tab.

Figure 103 shows the Library Search area in the Workspace Processing toolbar, and Table 36 describes the toolbar buttons.

Figure 103. Library Search areas of the Workspace Processing toolbar

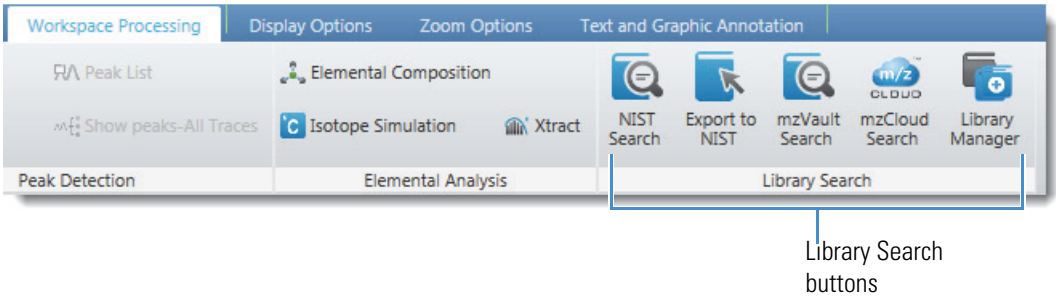


Table 36. Spectra-specific Workspace Processing buttons

Button	Description
Library Search	
NIST Search	Opens the Library Search page in the Info Bar, where you run a NIST library search on the active spectrum and displays the results in the NIST Search Results View. You can modify the library search parameters on the Modifying a NIST Search from the NIST Search Page of the Info Bar. See "Performing a Local NIST or mzVault Library Search" on page 173.
Export to NIST	Exports a spectrum to the NIST application for a search. The NIST application opens and displays the search results.
mzVault Search	Opens the mzVault Search page in the Info Bar, runs an mzVault library search on the active spectrum, and displays the results in the mzVault Search Results View.
mzCloud	Uploads a spectrum to mzCloud.org for a search. The website opens and displays the search parameters (see Figure 98). Select the appropriate settings and click **OK**. The website displays the results (see Figure 99 on page 194).
Library Manager	Opens the Thermo Library Manager dialog box, where you select or create libraries of spectra. See "Managing Libraries" on page 195.

7

Simulating Isotope Distributions

The FreeStyle application includes a utility that predicts and displays the isotopic distribution for a specified chemical formula or peptide sequence. The simulated spectrum is based on the elemental composition of the compound or peptide and the charge state distribution of its adduct ions.

To display simulated isotope distribution plots for chemical formulas and peptide sequences, see these topics.

Contents

Simulating the Isotope Distribution for a Chemical Formula or Peptide

A simulated isotopic distribution is based on a chemical formula or peptide sequence. For details about the parameter settings, see "Isotope Simulation Page" on page 209.

When you open the FreeStyle application, the Isotope Simulation page opens in the Info Bar. Without opening a raw data file, you can use the Isotope Simulation page to display the isotope pattern at a specified resolution for compounds and peptides in a separate window. After opening a raw data file (see "To open a raw data file or a sequence file" on page 15), you can also use the Isotope Simulation page to insert a simulated isotope pattern or profile spectrum in a Spectrum view and an EIC trace in the Chromatogram view of a Workspace.

❖ To display isotope simulations

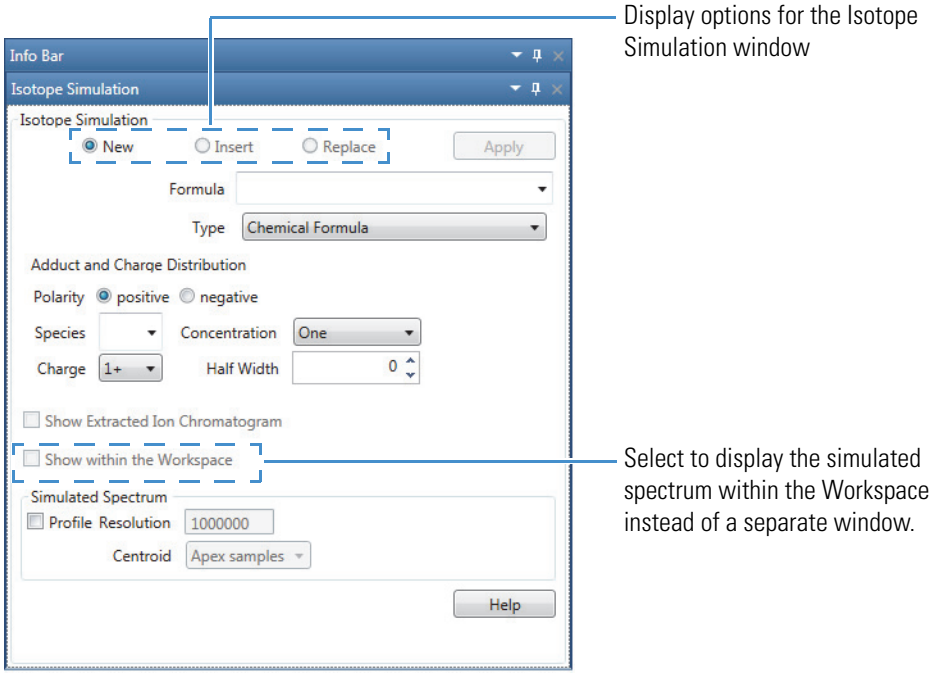
1. If the Isotope Simulation page is not visible, click the **Isotope Simulation** tab.
2. For the first simulation, do one of the following:
   - To display the simulation in the Isotope Simulation window, keep the **New** option.

   –or–

- To display the simulation in the Spectrum view of a Workspace, open a raw data file, and then on the Isotope Simulation page, select the **Show within the Workspace** check box.

Figure 104 shows the default settings on the Isotope Simulation page.

Figure 104. Isotope Simulation page without an open workspace



3. Enter the formula for a compound or the sequence of a peptide. See "To specify the formula type and the composition."

4. Under Adduct and Charge Distribution, specify the adduct species and charge distribution. See "To specify the adduct ion species and charge distribution."

5. To display an EIC trace for the specified ion in the Chromatogram view of a Workspace, open a raw data file if you have not already done so, and then select the **Show Extracted Ion Chromatogram** check box. See "To display an EIC trace in the Chromatogram view of a Workspace."

6. In the Simulated Spectrum area, specify whether to display an isotope pattern at infinite resolution or a simulated profile spectrum. See "To display the theoretical isotope pattern at infinite resolution" on page 206, or see "To display simulated profile spectra at multiple resolution settings."

   If you leave the Show within the Workspace check box clear, the Insert and Replace options become available after you run the first simulation and the Isotope Simulation window opens.

7. Click **Apply**.

For more information about setting up isotope simulations, follow these procedures:

- To display additional isotope simulations in Isotope Simulation windows
- To add an isotope simulation to a Spectrum view of a Workspace
- To specify the formula type and the composition
- To specify the adduct ion species and charge distribution
- To display an EIC trace in the Chromatogram view of a Workspace
- To display the theoretical isotope pattern at infinite resolution
- To display simulated profile spectra at multiple resolution settings
- To select the centroiding algorithm for a simulated profile spectrum
- To view formulas and a simulated profile spectrum in the Isotope Simulation window

❖ To display additional isotope simulations in Isotope Simulation windows

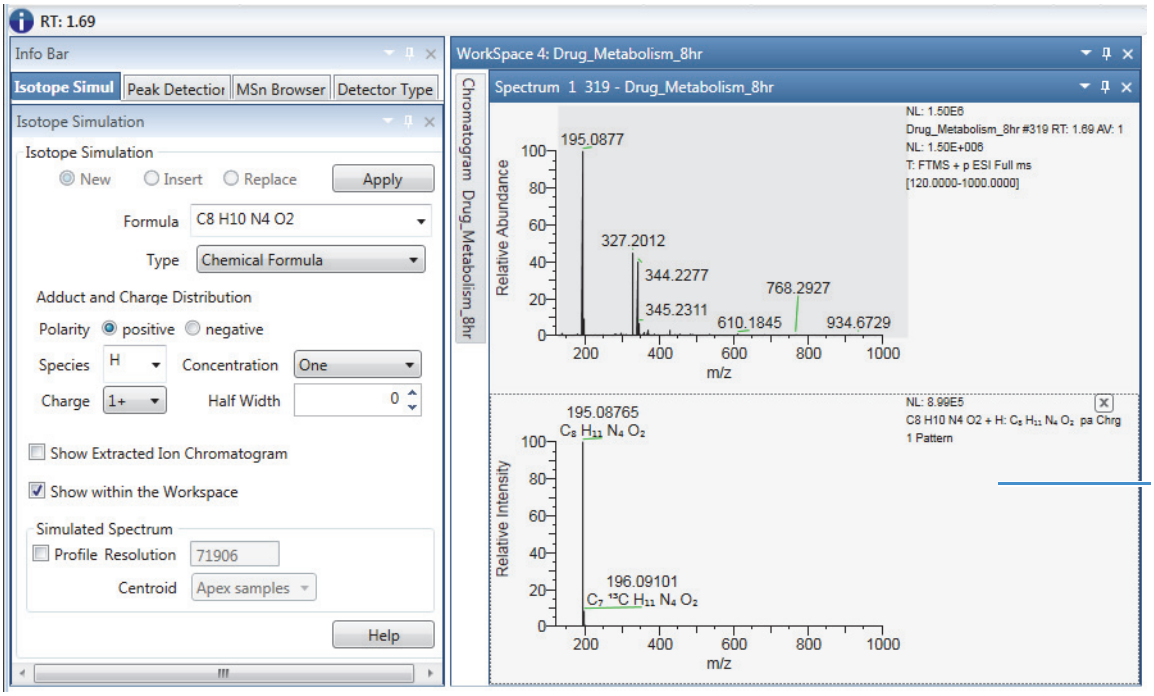
1. Follow the instructions in "To display isotope simulations," and make sure that the Show within the Workspace check box is clear.
2. Do one of the following:
   - To display additional simulations in separate Isotope Simulation windows, select **New**.
   - To insert additional simulations into one Isotope Simulation window, select **Insert**.
   - To replace the current simulation with another simulation, select **Replace**.
3. Modify the compound or peptide information, and then click **Apply**.

❖ To add an isotope simulation to a Spectrum view of a Workspace

1. Open a raw data file.
2. Select a spectrum of interest.
3. Enter the ion information and specify the simulated spectrum type as described in "To display isotope simulations."
4. Select the **Show within the Workspace** check box.
5. Click **Apply**.

   Figure 105 shows the simulated isotope pattern displayed within a Workspace.

Figure 105. Isotope simulation displayed within a Workspace

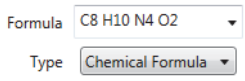


❖ To specify the formula type and the composition

Note For details, see "Formula" on page 210.

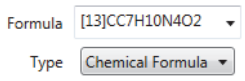
In the Type list, select one of the following, and then in the Formula box, enter the composition or the peptide sequence:

- For a compound, select **Chemical Formula** and enter the chemical formula using the chemical symbols in the periodic table.

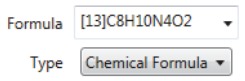


Elemental composition of caffeine

To specify a specific isotope, use square brackets before the element name to enclose the isotope mass number, for example, **[13]C**.

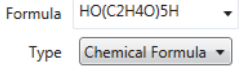


Elemental composition of caffeine where one carbon-12 atom has been replaced with carbon-13



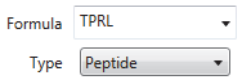
Elemental composition of caffeine where all the carbon-12 atoms have been replaced with carbon-13

To specify repeating moieties, such as those found in polymers, use parentheses.



Polyethylene glycol

- For a peptide, select **Peptide** and enter the peptide sequence using uppercase one-letter symbols for the amino acid residues (see "One- and Three-Letter Abbreviations for Amino Acid Residues" on page 269).



Kentsin, a tetrapeptide with the following amino acid sequence: Threonine-Proline-Arginine-Lysine

Note If you enter an incorrect formula, the application outlines the Formula box in red. Examples of incorrect entries include the following:

- Peptide—Lowercase letters or three-letter abbreviations
- Chemical formula—Chemical symbols that are not in the periodic table

❖ To specify the adduct ion species and charge distribution

Note For details, see "Adduct and Charge Distribution" on page 210.

Under Adduct and Charge Distribution, do the following:

a. Select the polarity of the ion.

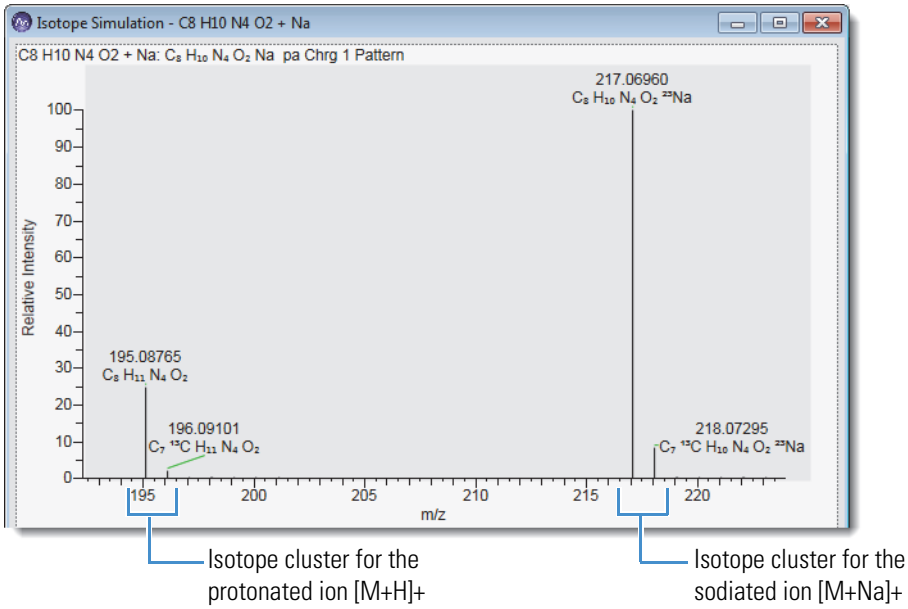
b. Select the adduct species, the charge of the most abundant ion, the concentration of the adduct species, and the half width of the charge distribution.

   For example, to simulate a spectrum for both the protonated and sodiated adduct ions of a compound in a high-concentration sodium solution, select the following:

   - Polarity: positive
   - Species: Na
   - Concentration: High
   - Half-Width: 0

Figure 106 shows the isotope clusters for the protonated and sodiated adduct ions of caffeine.

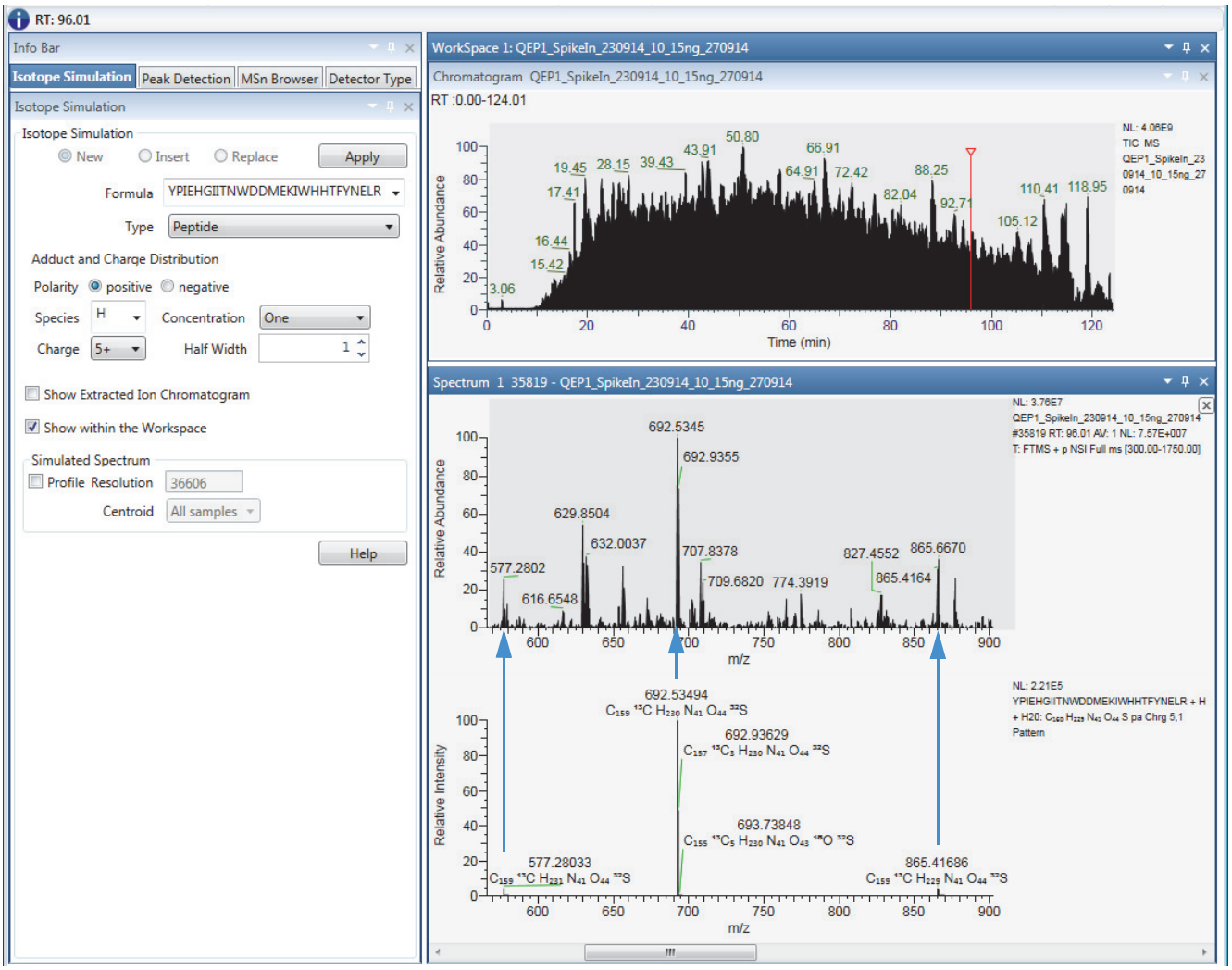
Figure 106. Isotope clusters for the protonated and sodiated adduct ions of caffeine



Note Most peptides have multiple charge sites, which means that their spectra will show multiple charge states.

Figure 107 shows an isotope simulation for a peptide with a charge distribution pattern of 5,1—that is, a charge of +5 and a half width of 1. Notice that the monoisotopic peak is not the most intense peak. In compounds with large numbers of carbon atoms, the probability that one of the atoms will be a carbon-13 atom increases. When the number of carbon atom exceeds 91, the probability that the ion includes one carbon-13 atom exceeds 100% and the M+1 peak for the (A1) ion with one carbon-13 atom becomes the most intense peak.

Figure 107. Example isotope simulation for a peptide



❖ To display an EIC trace in the Chromatogram view of a Workspace

1. To set up the ion information, follow the instructions in "To display isotope simulations."
2. Open a raw data file.
3. Select the **Show Extracted Ion Chromatogram** check box.
4. Click **Apply**.

   In the Chromatogram view of the Workspace, the EIC trace for the specified ion appears below the current trace.

   Figure 108 shows an EIC trace in the Chromatogram view.

Figure 108. Chromatogram view with an EIC trace

❖ To display the theoretical isotope pattern at infinite resolution

In the Simulated Spectrum area, make sure that the **Profile** check box is clear.

❖ To display simulated profile spectra at multiple resolution settings

Note You can set up a simulated profile spectrum to determine whether your instrument is capable of resolving the theoretical isotope pattern for a specific ion, or you can set up several simulations to determine the required resolution.

1. Select one of the display options for the Isotope Simulation window (see "To display additional isotope simulations in Isotope Simulation windows" on page 201).
2. Specify the ion and charge distribution as described in "To display isotope simulations."
3. Select the **Profile** check box, and then select the centroiding algorithm.

If you are working with raw data, the application automatically populates the Resolution box with the experimental resolution in the current raw data file.

If you have not opened a workspace, the application populates the Resolution box with the default value of 1 000 000. You can type a value from 1 to 2 000 000.

4. Click **Apply**.

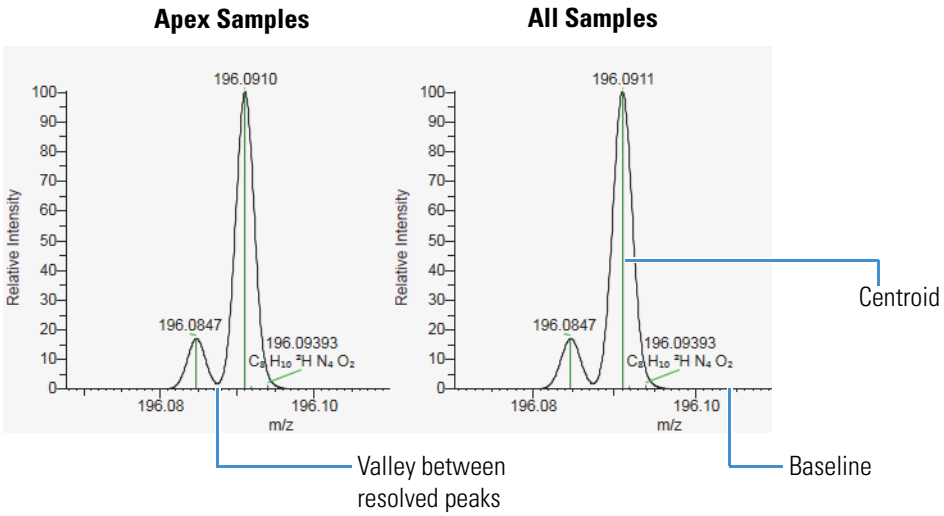
❖ To select the centroiding algorithm for a simulated profile spectrum

1. In the Simulated Spectrum area, select the **Profile** check box.
2. From the Centroid list, select one of the following to calculate the centroid for each profile peak:
   - To calculate the centroids by using the sampling points nearest the peak apex, select **Apex Samples**.
   - To calculate the centroids by using all the sampling points in the profile peak (down to the baseline or valley between resolved peaks), select **All Samples**.

Figure 109 shows a portion of a simulated profile spectrum. The two graphs show the difference between the two centroiding methods, where the left graph displays the results of the Apex Samples algorithm and the right graph displays the results of the All Samples algorithm.

Note When you display a simulated profile spectrum in the Spectrum view of a Workspace, the application displays the centroids for the Gaussian-shaped peaks as vertical green lines.

Figure 109. A1 peaks in a simulated profile spectrum (displayed within the Spectrum view of a Workspace)

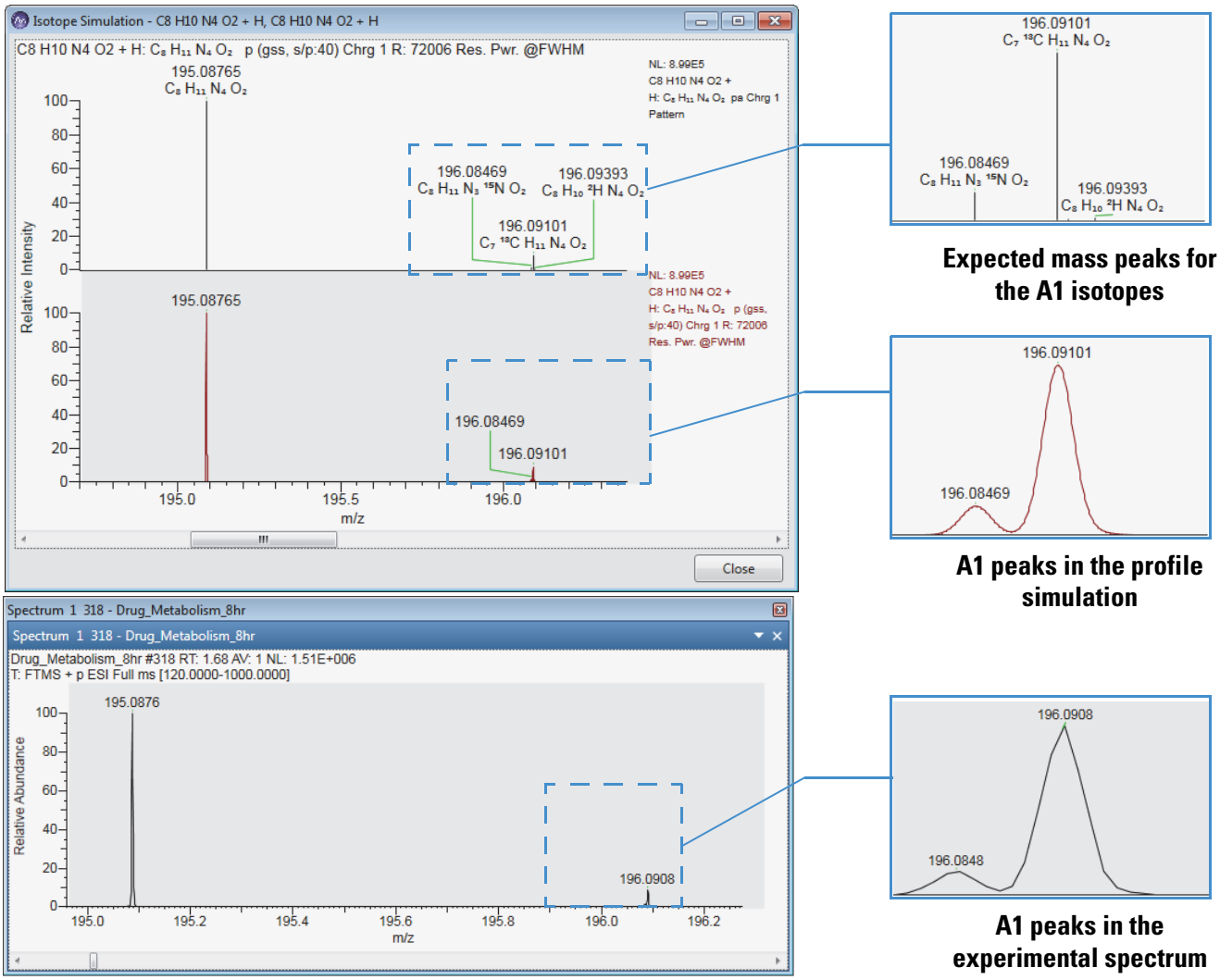


❖ To view formulas and a simulated profile spectrum in the Isotope Simulation window

1. Set up the initial simulation, make sure that the Show Within the Workspace and Profile check boxes are clear, and click **Apply**.
2. Select the **Insert** option.
3. Set up a second simulation for the same formula and charge distribution.
4. Select the **Profile** check box, and then select the centroiding algorithm.
5. Click **Apply**.

Figure 110 shows the isotope simulation and the mass spectrum for a protonated caffeine ion. At the experimental resolution, the isotope simulation shows that the A1 peaks at *m/z* 196.1 will not be completely resolved. The experimental spectrum shows a similar profile at *m/z* 196.1.

Figure 110. Simulation for caffeine versus the experimental spectrum for a caffeine sample



Isotope Simulation Page

Use the Isotope Simulation page of the Info Bar to create a simulated isotopic distribution plot. The Isotope Simulation page is always available. Without opening a workspace, you can only display a simulated spectrum in the stand-alone Isotope Simulation window. After you open a workspace, you can display an EIC trace in the Chromatogram view and add an isotope simulation plot to the Spectrum view.

For information about running the isotope simulation utility and reviewing the resulting spectrum, see "Simulating the Isotope Distribution for a Chemical Formula or Peptide" on page 199.

❖ To display the Isotope Simulation page if it is not active

Do one of the following:

- In the Workspace Processing toolbar, click **Isotope Simulation**.

–or–

- In the Info Bar, click the **Isotope Simulation** tab.

Table 37 describes the parameters for the Isotope Simulation page.

Table 37. Isotope Simulation page parameters (Sheet 1 of 5)

Parameter	Description
To select a display option for the Isotope Simulation window, see "To display additional isotope simulations in Isotope Simulation windows" on page 201.	
• New	Opens a separate Isotope Simulation window each time you click Apply.
• Insert	Adds repeated simulations to the same Isotope Simulation window. Available when an Isotope Simulation window is open.
• Replace	Replaces the current simulated spectrum with the latest simulation. Available when an Isotope Simulation window is open.
Type	Specify that the formula is either a Chemical Formula or a Peptide.

Table 37. Isotope Simulation page parameters (Sheet 2 of 5)

Parameter	Description
Formula	Enter the formula or peptide sequence for the compound or peptide that you want to create a simulated spectrum for (see "To specify the formula type and the composition" on page 202). After you enter a formula or peptide sequence, it appears in a selection list for this box.
• Chemical Formula	For a chemical formula, do the following: • Use the IUPAC symbol for each element—that is, match the notation, including the capitalization, in the periodic table of elements. • To enter a specific isotope, use square brackets before the element name to enclose the isotope mass number, for example, [13]C. • Use parentheses to specify repeating moieties such as those found in polymers, for example, HO(C2H4O)5H.
• Peptide	For a peptide sequence, do the following: • Use the single capital letter abbreviations for amino acids. • You can specify mixtures of substances by using additional symbols "+" (addition) and "×" (multiplication). Both symbols are required to specify a mixture. A valid mixture has the format substance × quantity + substance × quantity, for example, A × 2 + C × 5. See "One- and Three-Letter Abbreviations for Amino Acid Residues" on page 269. The maximum molecular weight for the formula is less than 600 000 amu.
Adduct and Charge Distribution See "To specify the adduct ion species and charge distribution" on page 203.	
Polarity	Select the Positive or Negative option. Default: Positive
Species	Select, if necessary, the adduct for the simulated spectrum. • For positive polarity, you can select H, K, Na, NH4, or no adduct. Or, you can type the chemical symbol or formula for a positive adduct such as Ca for calcium. • For negative polarity, you can select H, Cl, OH, HCOO, or no adduct. Or, you can type the chemical symbol or formula for a negative adduct.

Table 37. Isotope Simulation page parameters (Sheet 3 of 5)

Parameter	Description
Charge	Specify the charge of the adduct ion or peptide. • (Positive polarity) Range: 1–25; default: 1 • (Negative polarity) Range: –1 to –25; default: –1
Half Width	Simulate the number of additional charges on each side of the most abundant ion. Select a value from 0 to 99. For example: If you simulate charge 5 and a half width of 2, then the data system draws charges 5 ± 2, giving 7, 6, 5, 4, 3 (with the largest mass peak (normalized to 100% relative intensity) at charge 5). For most small molecules, simulate charge 1 with a half width of 0. To simulate an intensity distribution, the peaks at the edge of the distribution are shown at 5% of the height of the most abundant peak.

Table 37. Isotope Simulation page parameters (Sheet 4 of 5)

Parameter	Description
Concentration	There are four possibilities for how the adduct is added to the ion: One, Low, High, and 100%.
• One adduct	**For positive charge states** The application creates the specified charge state by adding the specified species for the first charge and H+ ions for subsequent charges. For example, a charge distribution of Charge 2+ (most abundant) and Half Width = 1 (2,1) and a Na+ adduct shows these ions and the simulated spectrum includes three isotope clusters: – Charge +1) *Chemical_Formula*Na^{+} – Charge +2) *Chemical_Formula*HNa^{+2} – Charge +3) *Chemical_Formula*$H2Na^{+3}$ **For negative charge states** When you select H as the species, each charge state shows a loss of H+ to attain the specified charge—that is, a loss of one hydrogen ion for a charge of negative one, a loss of two hydrogen ions for a charge of negative two, and so on. If the molecule does not contain hydrogen, nothing is removed. For the neutral compound C8H8: – Charge –1) C8H7 – Charge -2) C8H6 – Charge -3) C8H5 For negative species, such as Cl–, the –1 charge state shows the addition of the negative species to attain the specified charge. Higher charge states show the addition of one Cl– and the loss of one or more H+ to attain the specified charge. For the neutral compound C8H8: – Charge –1) C8H8Cl – Charge -2) C8H7Cl – Charge -3) C8H6Cl
• Low	The ion with no adduct is included at 100% intensity, one adduct at 25% intensity, two adducts at 11% intensity, and so on (to the limit of the charge distribution).
• High	The ion with N adducts are included at 100% intensity, N–1 adduct at 25% intensity, N–2 adducts at 11% intensity, and so on, where N is the absolute value of the maximum charge simulated (to the limit of the charge distribution).

Table 37. Isotope Simulation page parameters (Sheet 5 of 5)

Parameter	Description
• 100%	The ion of charge N contains M adducts (where M is the absolute value of N).
Show Extracted Ion Chromatogram	Select to display an EIC trace for the monoisotopic ion in the Chromatogram view (see "To display an EIC trace in the Chromatogram view of a Workspace" on page 205).
Show within the Workspace	Select to display the isotope simulation in the Spectrum view of a Workspace (see "To add an isotope simulation to a Spectrum view of a Workspace" on page 201).
Simulated Spectrum	
Profile and Resolution	When the Profile check box is clear, the application generates a theoretical isotope pattern with infinite resolution—that is, it displays the peaks as one-dimensional sticks rather than as Gaussian peaks similar to the intensity versus frequency data that the instrument generates. When the Profile check box is selected, for each peak in the simulated pattern, the application uses a Gaussian peak-shape model that bases the peak width on the specified resolution. The application then sums the simulated Gaussian peaks to create a simulated profile spectrum for the specified resolution. The application displays the centroid for each profile peak as a green vertical line. When you open a raw data file in the workspace, the Resolution box displays the resolution value for the most intense peak (full width at half maximum [FWHM]) in the selected scan. You can overwrite this value. **Tip** To view the resolution values for each mass spectrum peak, click **Peak Resolution** in the Spectrum or Multi Spectrum – Display Options Toolbar.
Centroid	• (Default) Apex Samples—Selects the sampling points nearest the peak apex to calculate the centroid of each simulated profile peak. • All Samples—Uses all the sampling points across the profile peak, down to the baseline or valley between resolved peaks. See "To select the centroiding algorithm for a simulated profile spectrum" on page 207.
Button	
Apply	Calculates the isotope distribution and displays the simulated spectrum by using the parameter settings.
Help	Opens the FreeStyle Help to the isotope simulation topic.

8

Working with CV Plots for FAIMS Data

The Thermo Scientific high-field asymmetric waveform ion mobility spectrometry (FAIMS) interface increases sensitivity by reducing chemical noise and matrix interferences. Use the CV Plot view to determine the optimal compensation voltages for your FAIMS-MS experiments.

Follow these topics.

Contents

CV Traces Dialog Box

The CV Traces dialog box includes check boxes for the selected traces in the Chromatogram Ranges view.

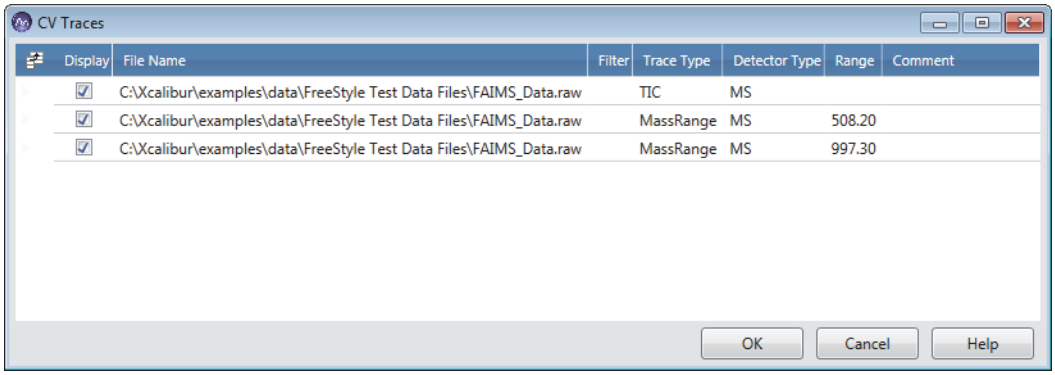
❖ **To select the traces for display in the CV Plot view**

1. Open an Xcalibur RAW file with data acquired from a mass spectrometer with the FAIMS interface.
2. In the Chromatogram Ranges view, set up the traces of interest.
3. In the Workspace Options toolbar, click **CV Plot**.

   The CV Traces dialog box opens (Figure 111).
4. Select the check boxes for the traces of interest.
5. Click **OK**.

   The CV Plot view opens. For information about working with the CV Plot, see the next topic CV Plot View.

Figure 111. CV Traces dialog box



CV Plot View

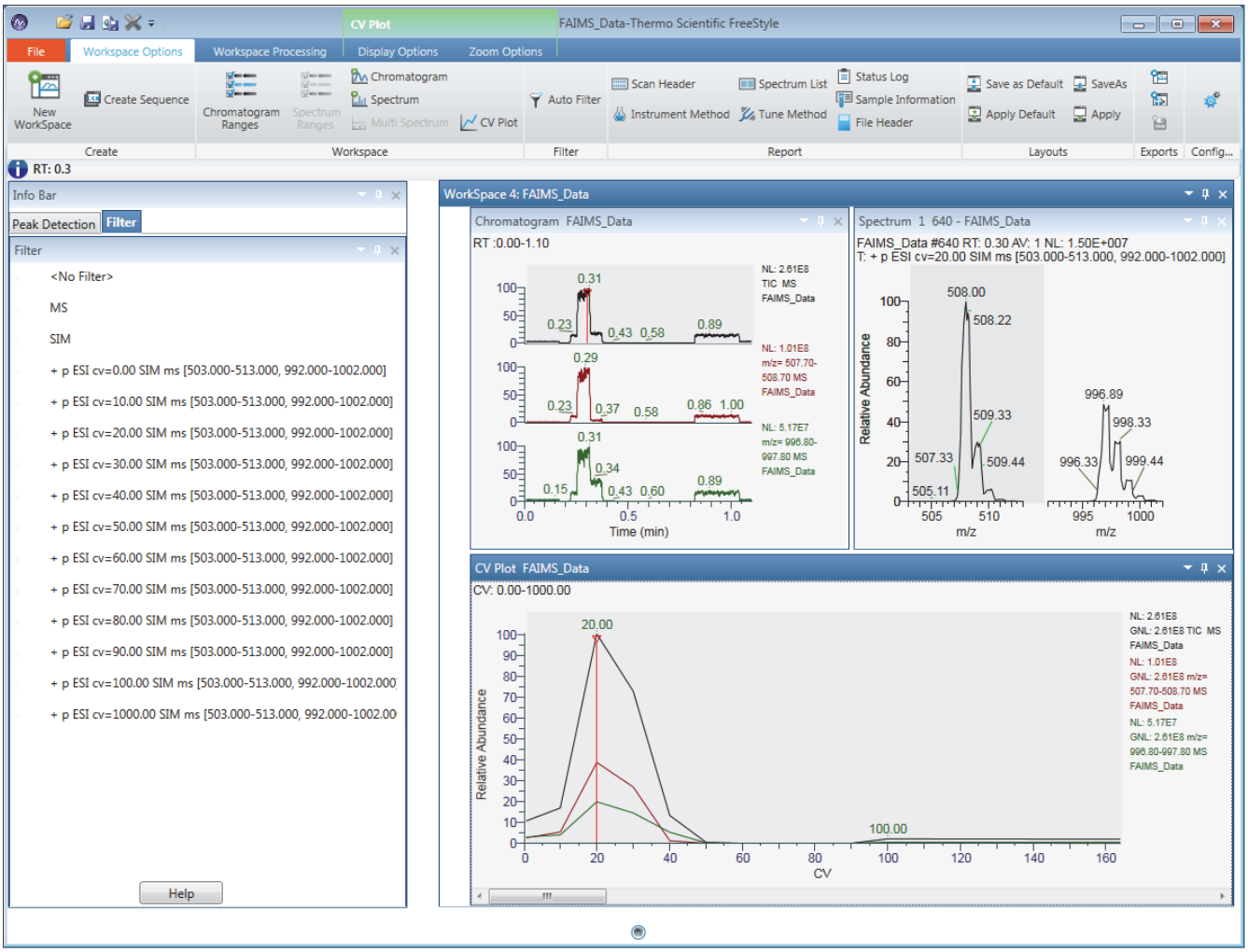
Use the CV Plot view to determine the optimum compensation voltages for your LC/MS/FAIMS experiments.

To open the CV Plot view, see "CV Traces Dialog Box" on page 215. You can reopen the CV Traces dialog box to add or remove traces from this view.

Figure 112 shows a CV Plot view with three overlaid traces: a TIC MS trace in black, a mass range *m/z* 507.2 ± 0.5 trace in red, and a mass range *m/z* 997.3 ± 0.5 trace in green.

The Filter page on the left shows scans acquired at 12 stepped compensation voltages from 0.00 to 1000 and two mass ranges. The CV Plot view, which plots the relative abundance versus the compensation voltage, shows a maximum response at a compensation voltage of 20 for both mass ranges.

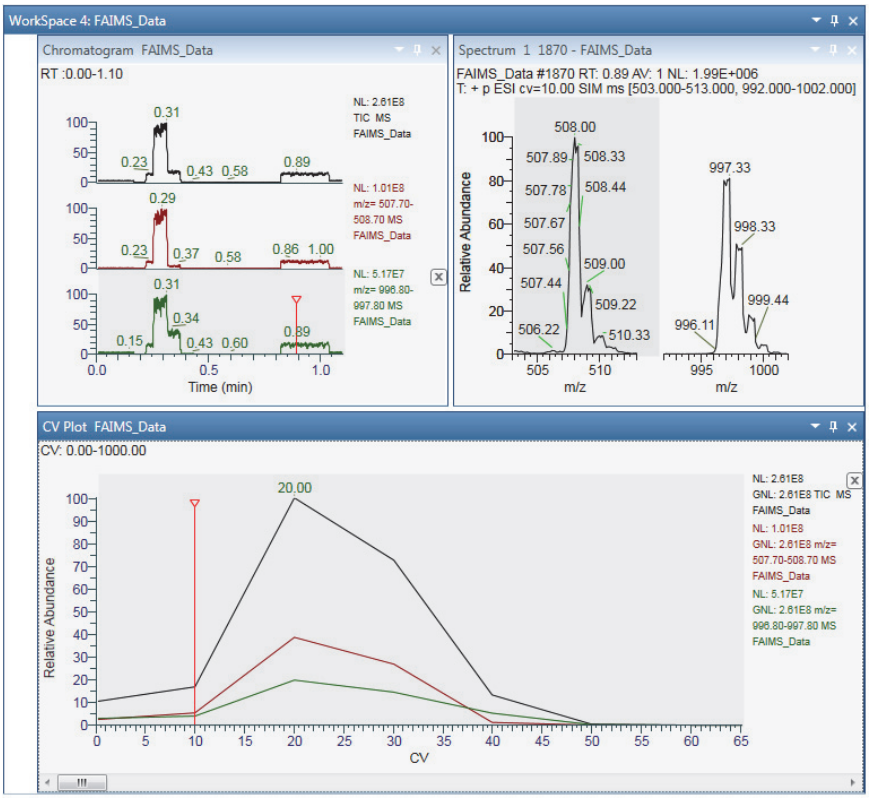
Figure 112. Workspace showing an active CV Plot view



The CV Plot view, Chromatogram view, and Spectrum view are interactive—that is, as you move the red vertical bar across the CV voltage range on the *x* axis, the traces update in the Chromatogram and Spectrum views. Use this interactive behavior to determine the optimum compensation voltage for your analytes—that is, monitor the relative abundance of the ions in the Spectrum view as you move across the CV range in the CV Plot view.

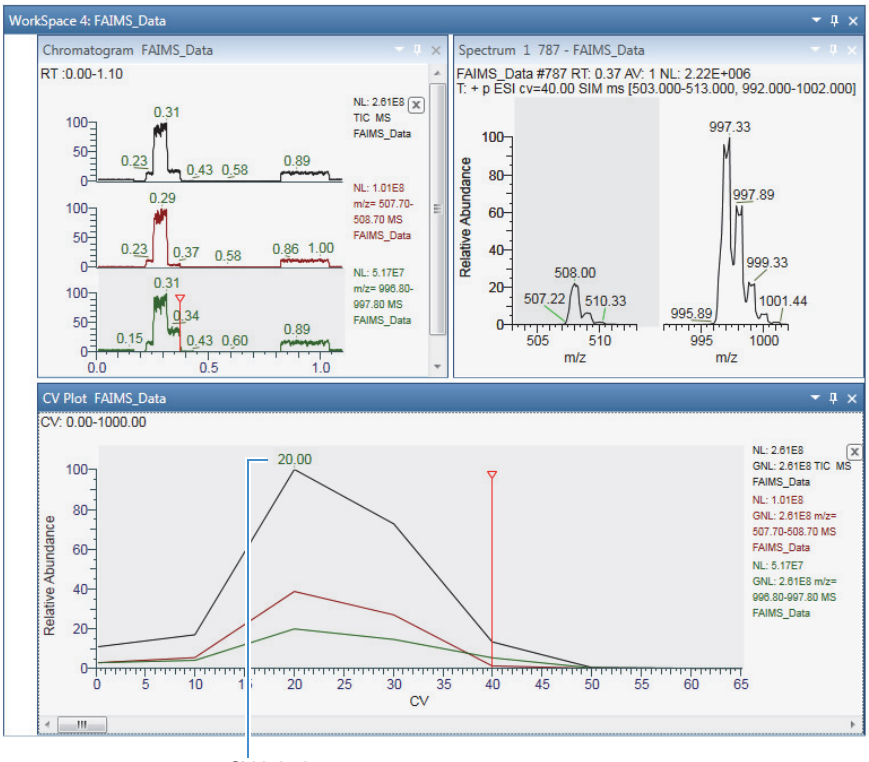
At a CV of 10, the ion at *m/z* 508.00 is relatively more abundant than the ion at *m/z* 997.33 (Figure 113).

Figure 113. Compensation voltage 10



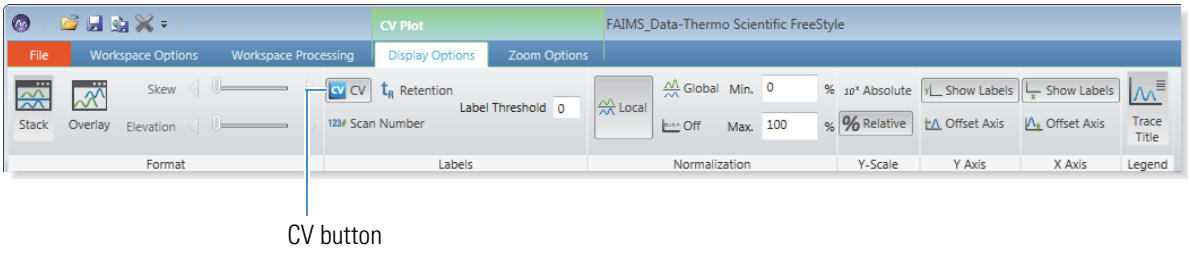
At a CV of 40, the ion at *m/z* 997.33 is relatively more abundant than the ion at *m/z* 508.00 (Figure 114).

Figure 114. Compensation voltage 40



The display options for the CV Plot view are similar to those for the Chromatogram view (see "Chromatogram – Display Options Toolbar" on page 71). Use the CV button in the Display Options toolbar to add the compensation voltage label to the *y*-axis maxima in the CV Plot view (Figure 115).

Figure 115. Display Options toolbar for the CV Plot view



9

Viewing Experiment and Instrument Information

In addition to containing the acquired data, the raw data file contains information about the scan header, instrument status, instrument method, tune method, and sample. Use the FreeStyle application to extract this information from the raw data files. Viewing the experiment information for a particular acquisition can help you interpret the results of that acquisition. Viewing the mass spectrometer readback (MS Trending detector type) information for the acquisition can help you troubleshoot anomalies in the data.

To review the experiment and instrument information in your raw data files, follow these topics.

Contents

Workspace Options Toolbar – Report Area

Use the buttons in the Report area of the Workspace Options toolbar to view information that is contained in the raw data file.

❖ **To display the Workspace Options toolbar**

Click the **Workspace Options** toolbar tab.

Figure 116 shows the Report area, and Table 38 describes the toolbar buttons, which display information-specific views.

Figure 116. Report area of the Workspace Options toolbar

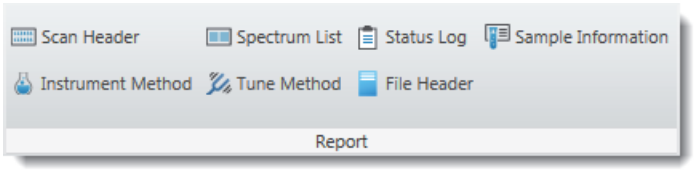


Table 38. Workspace Options toolbar – Report area buttons

Button	Description
Scan Header	Displays the Scan Header View, showing the scan header of the active raw data file. Click the chromatogram trace to display the scan header for the chosen retention time and scan number.
Spectrum List	Displays the Spectrum List View, showing spectral peak information in a table. Click the chromatogram trace to display the spectrum list for the chosen retention time and scan number.
Status Log	Displays the Status Log View, showing mass spectrometer readbacks. Click the chromatogram trace to display the status log for the chosen retention time and scan number.
Instrument Method	Displays the Instrument Method View, showing the instrument method parameters that the instrument used to obtain the raw data file.
Tune Method	Displays the Tune Method View, showing the tune parameters that the instrument used to obtain the raw data file.
Sample Information	Displays the Sample Information View, showing sample-specific information.
File Header	Displays the File Header View.

Spectrum List – Display Options Toolbar

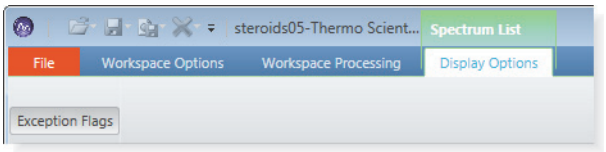
Use the Exception Flags button in the Spectrum List Display Options toolbar to add or remove the Flags column.

❖ To display the Spectrum List Display Options toolbar

1. Click the Spectrum List View to make it the active view.
2. Click the **Display Options** toolbar tab.

Figure 117 shows the Spectrum List Display Options toolbar.

Figure 117. Spectrum List Display Options toolbar



The Exception Flags button is the only button in the Spectrum List Display Options toolbar. It displays or hides the Flags column in the Spectrum List View.

Scan Header View

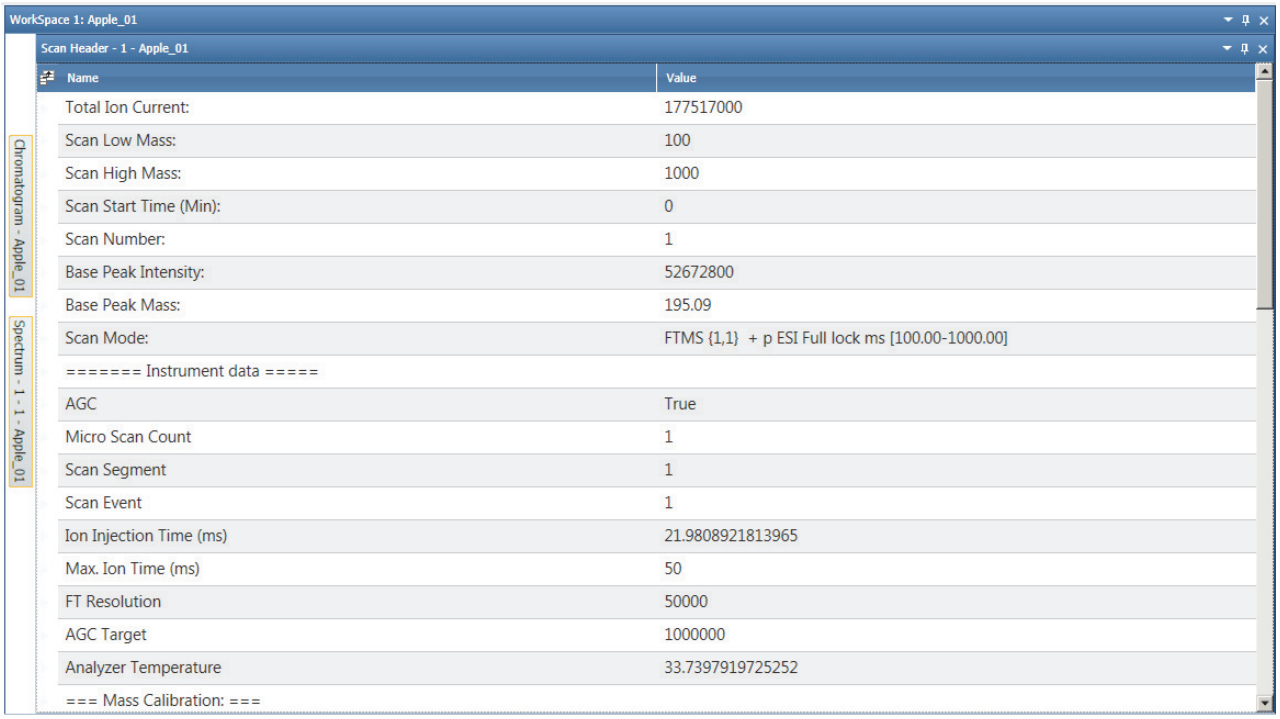
Use the Scan Header view to display scan parameters and instrument data for the retention time and scan number that you select in the Chromatogram View.

❖ To open the Scan Header view

1. Click the **Workspace Options** toolbar tab.
2. Click **Scan Header**.

Figure 118 shows an example of a Scan Header view.

Figure 118. Scan Header view



Name	Value
Total Ion Current:	177517000
Scan Low Mass:	100
Scan High Mass:	1000
Scan Start Time (Min):	0
Scan Number:	1
Base Peak Intensity:	52672800
Base Peak Mass:	195.09
Scan Mode:	FTMS {1,1} + p ESI Full lock ms [100.00-1000.00]
======= Instrument data =====	
AGC	True
Micro Scan Count	1
Scan Segment	1
Scan Event	1
Ion Injection Time (ms)	21.9808921813965
Max. Ion Time (ms)	50
FT Resolution	50000
AGC Target	1000000
Analyzer Temperature	33.7397919725252
=== Mass Calibration: ===	

The Scan Header view can include the following information:

- Scan parameters
- Instrument data
- Mass calibration parameters
- Ion optics readbacks
- Diagnostic data

Spectrum List View

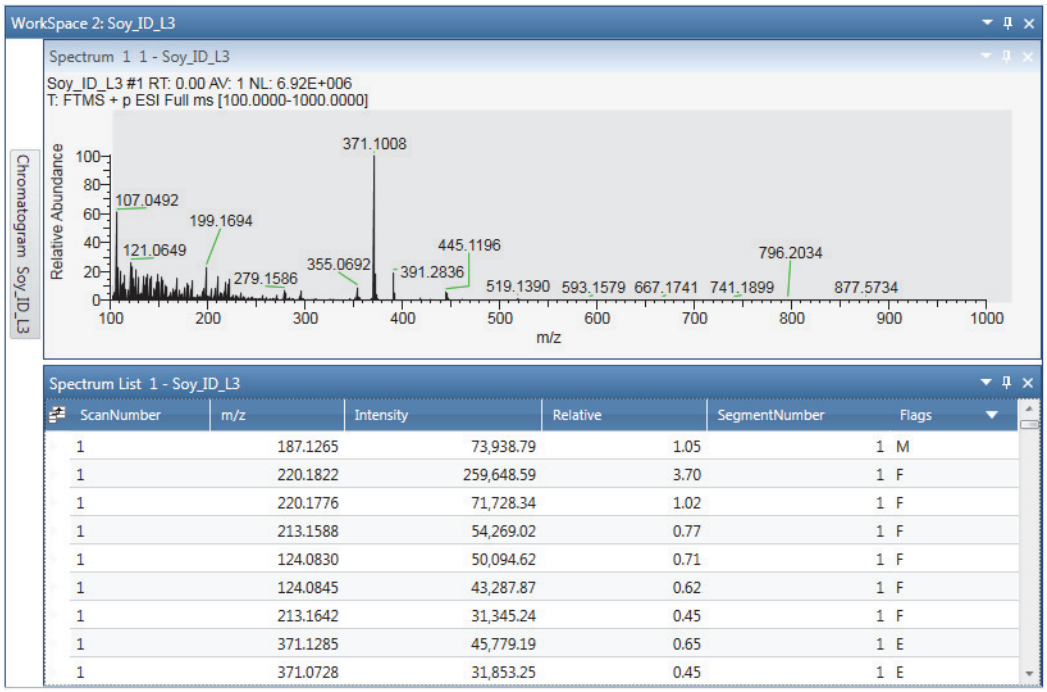
The Spectrum List view lists in tabular form the peak positions, intensities, and relative intensities of the peaks in the Spectrum view for the retention time that you select in the Chromatogram View.

❖ To open the Spectrum List view

1. Click the **Workspace Options** toolbar tab.
2. Click **Spectrum List**.

Figure 119 shows an example of a Spectrum List view and the associated mass spectrum.

Figure 119. Spectrum List view (bottom) associated with the mass spectrum (top)



Some raw data files contain flags that provide supplemental information about the peak data. The possible flags are as follows:

- R: Reference peaks are peaks from a reference compound used for an internal recalibration of a scan.
- E: Exception peaks are also peaks from a reference compound, but they are not used for recalibration. These are typically small isotopes or fragments of the main references.
- M: Merged peaks are peaks where the centroider combined two nearby peaks.
- F: Fragmented peaks are peaks separated into multiple peaks by the centroiding activity.

If the Spectrum List view does not show the Flags column (Figure 119), you can click Exception Flags in the Display Options toolbar for the Spectrum List view to see this column and the letters representing the flags.

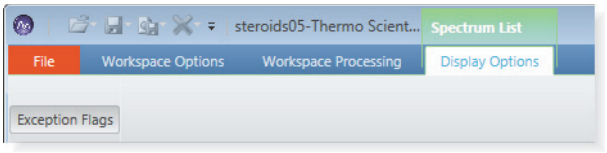
❖ To display flags in the spectrum list

Do one of the following:

a. Click the **Spectrum List** view to make it the active view.

b. Click the **Display Options** toolbar tab.

c. Click **Exception Flags**.



The Flags column appears as the last column. To hide the Flags column, click **Exception Flags** again.

–or–

a. Click the **Field Chooser** icon, , to the left of the first column heading.

b. In the Field Chooser dialog box, select the **Flags** check box.

Status Log View

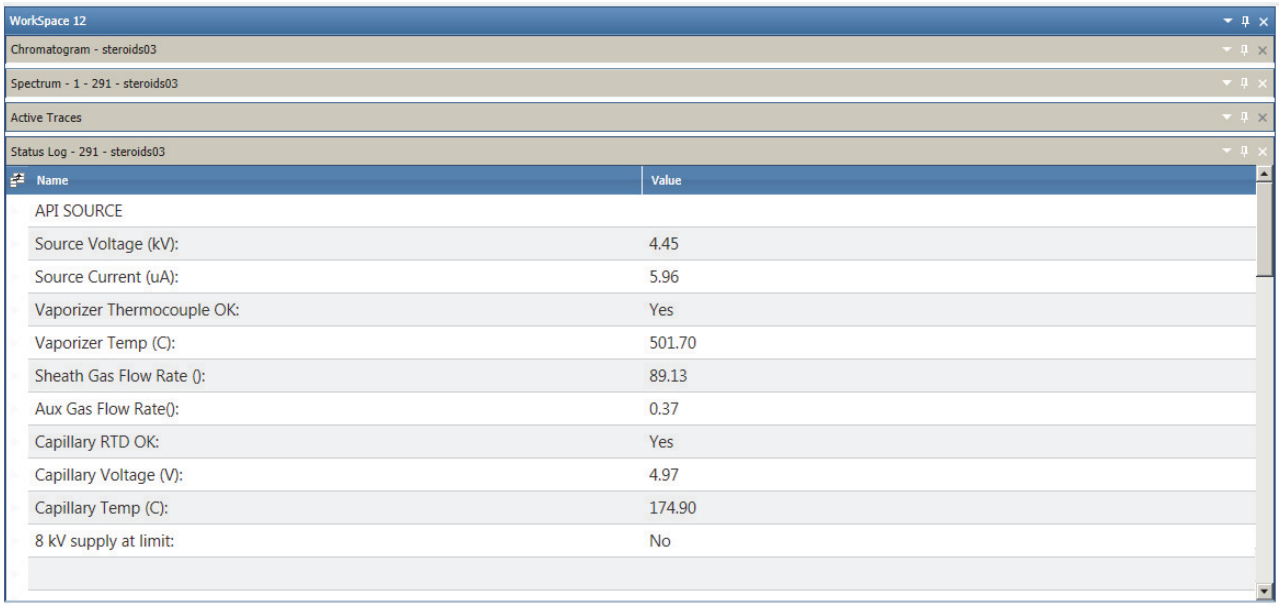
The Status Log view displays the mass spectrometer component readbacks that the system recorded at the retention time and scan number that you selected in the Chromatogram View.

❖ To open the Status Log view

1. Click the **Workspace Options** toolbar tab.
2. Click **Status Log**.

Figure 120 shows an example of the Status Log view.

Figure 120. Status Log view



For example, the Status Log view provides status information on the following mass spectrometer subsystems:

- API source
- Vacuum system
- Turbomolecular pump
- Ion optics
- Main rf voltage
- Ion detection system
- Power supplies
- Instrument status
- Autosampler
- LC pump
- Analog inputs
- Syringe pump
- Digital inputs
- UV detector

You can display individual instrument status readbacks, as a function of the retention time, in the Chromatogram view (see "Setting Up Instrument Status Traces with the MS Trending Feature" on page 52). The readback display can aid in troubleshooting anomalies in the data.

Instrument Method View

The Instrument Method view displays the instrument method. An instrument method is a set of experiment parameters that define operating settings for the autosampler, liquid chromatograph (LC), mass spectrometer, divert valve, syringe pump, and so on. Instrument methods are saved as file type .meth.

For an LC/MS system, the Instrument Method view displays the instrument method settings for the autosampler, LC pump, and mass spectrometer on separate pages. This information does not change from scan to scan.

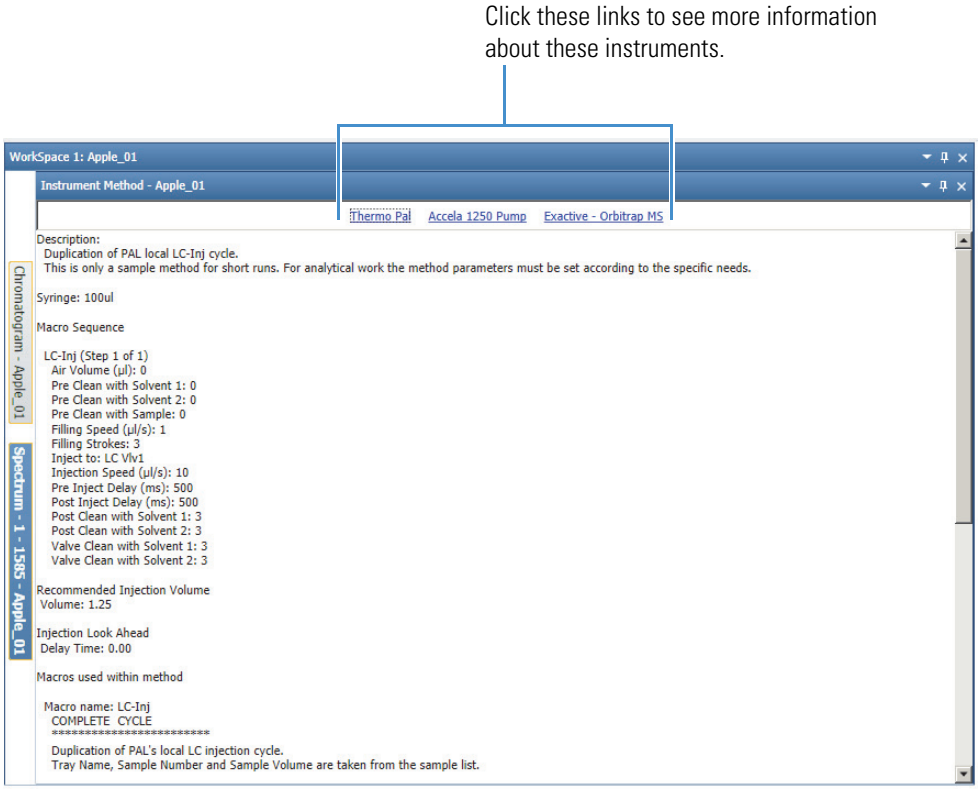
❖ To open the Instrument Method view

1. Click the **Workspace Options** toolbar tab.
2. Click **Instrument Method**.

For example, the Autosampler page lists the injection-cycle information.

Figure 121 shows an example of the Autosampler page of an Instrument Method view.

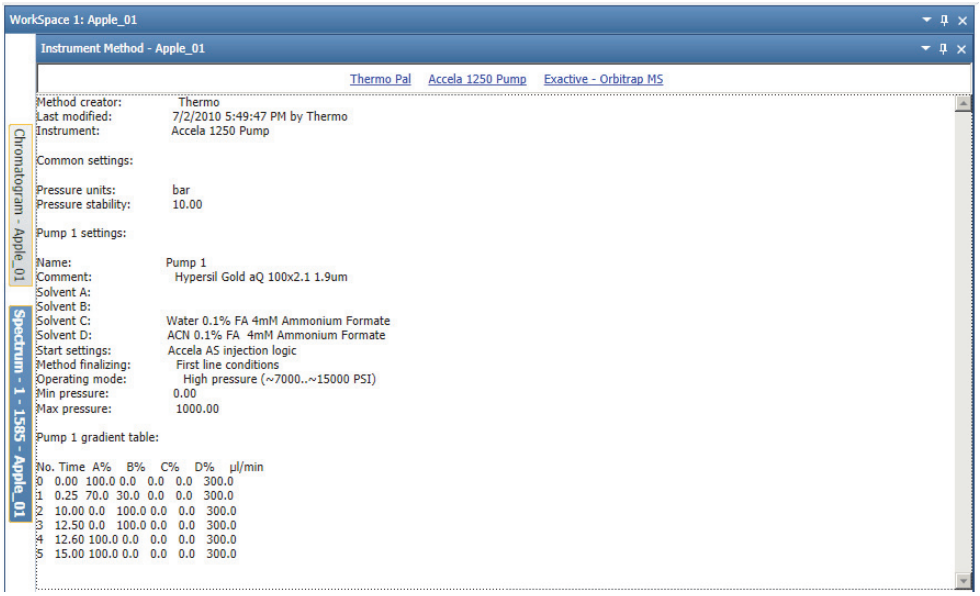
Figure 121. Autosampler page of the Instrument Method view



The LC page can include the solvent composition and the gradient information for the acquisition.

Figure 122 shows an example of the LC page of an Instrument Method view.

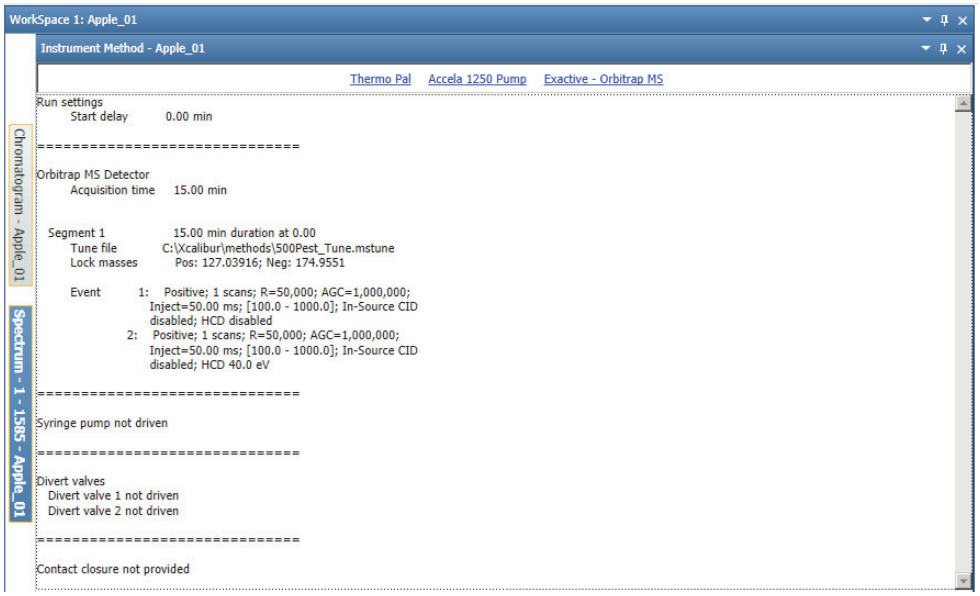
Figure 122. LC page of the Instrument Method view



The Mass Spectrometer page might list the tune file, the lock masses, the scan event descriptions, the activation types, the syringe pump settings, and the divert valve settings.

Figure 123 shows an example of the Mass Spectrometer page of the Instrument Method view.

Figure 123. Mass Spectrometer page of the Instrument Method view



Tune Method View

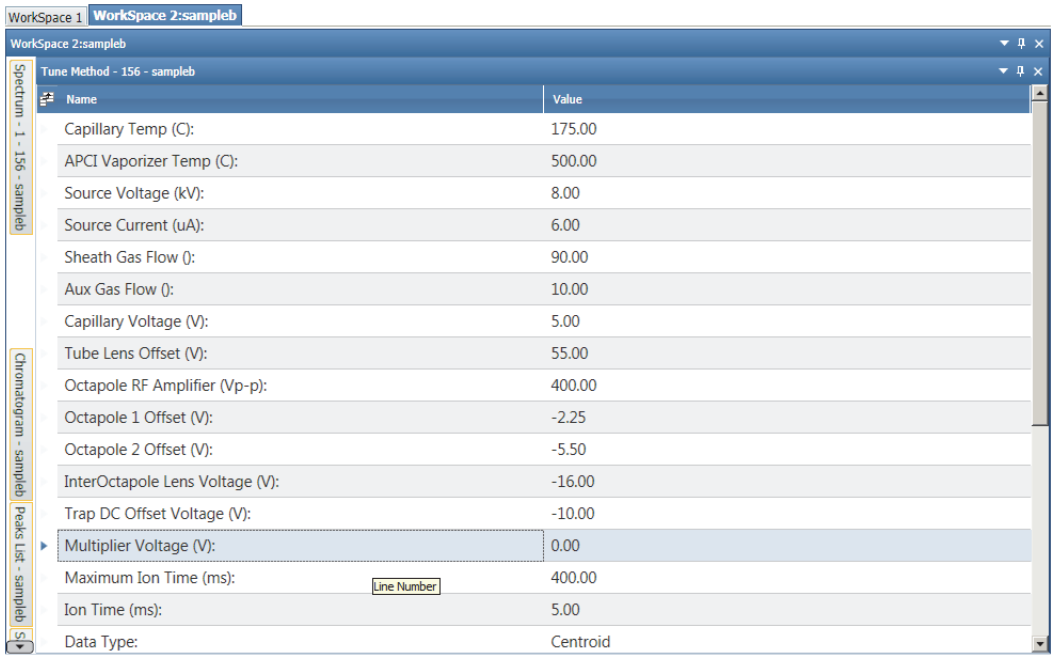
The Tune Method view displays the settings in the tune method. A tune method is a defined set of mass spectrometer tune parameters for the ion source, ion optics, and mass analyzer.

❖ To open the Tune Method view

1. Click the **Workspace Options** toolbar tab.
2. Click **Tune Method**.

Figure 124 shows an example of a Tune Method view.

Figure 124. Tune Method view



Sample Information View

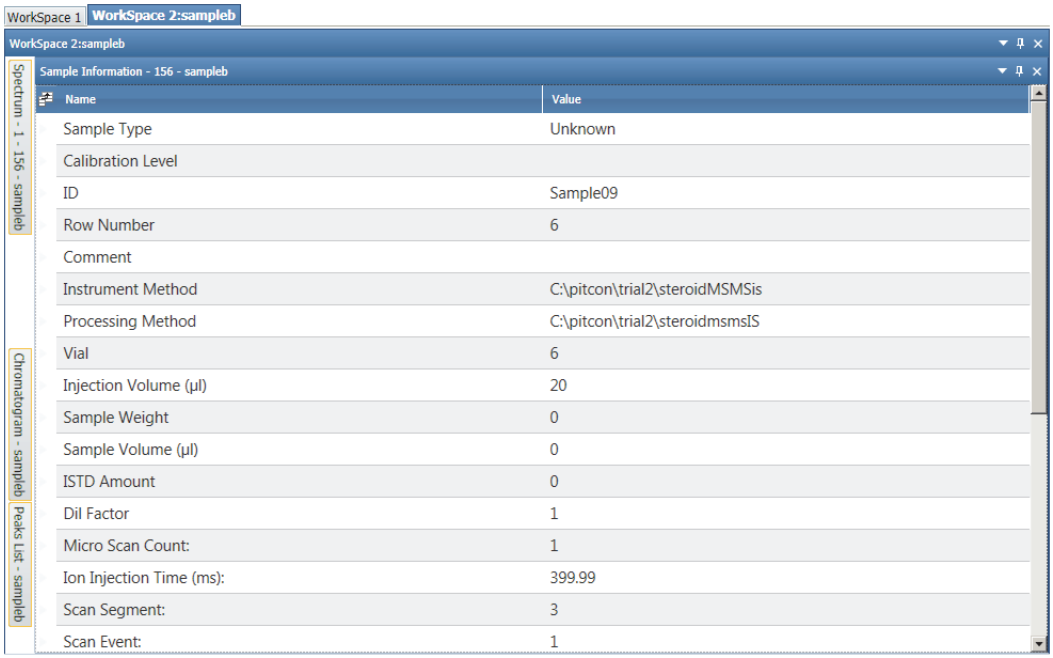
Use the Sample Information view to view sample-specific autosampler, LC pump, and mass spectrometer information in a raw data file. This information does not change from scan to scan.

❖ To open the Sample Information view

1. Click the **Workspace Options** toolbar tab.
2. Click **Sample Information**.

Figure 125 shows an example of a Sample Information view.

Figure 125. Sample Information view



File Header View

Use the File Header view to view acquisition information about the current raw data file. The File Header view displays information from the acquisition sequence, the autosampler, and the mass spectrometer.

❖ To open the File Header view

1. Open a raw data file.
2. In the Workspace Options toolbar, click **File Header**.

Figure 126 shows the File Header view.

Figure 126. File Header view

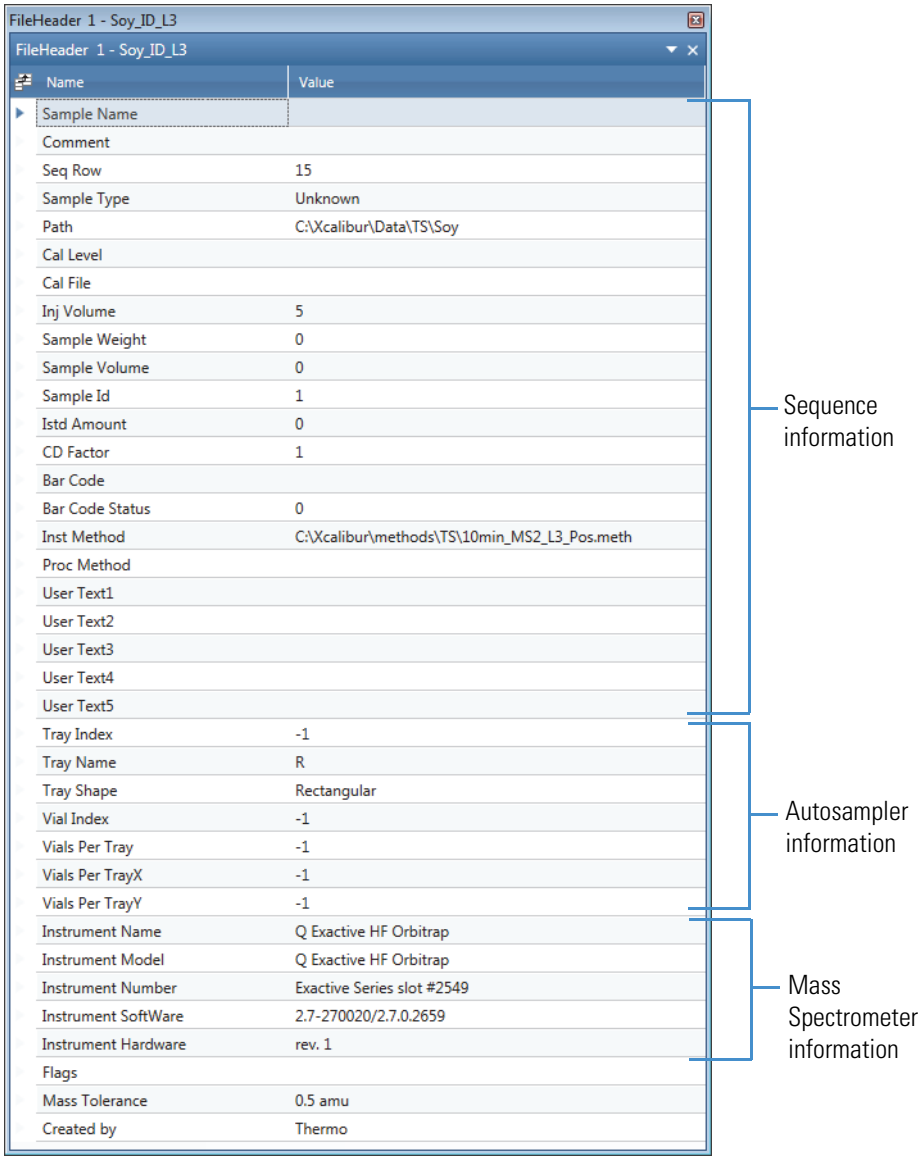


Table 39 describes the information in the File Header view.

Table 39. File Header view (Sheet 1 of 3)

Read-only parameter	Description
The following values are from the sequence row that corresponds to the raw data file.	
Sample Name	Displays the text in the Sample Name column.
Comment	Displays the text in the Comment column.
Seq Row	Displays the number of the sequence row.
Sample Type	Displays the sample type selection in the Sample Type column.

Table 39. File Header view (Sheet 2 of 3)

Read-only parameter	Description
Path	Displays the directory and file name in the Path column, which is the file's original storage location and file name.
Cal Level	Displays the calibration level in the Level column for calibration standards.
Injection Volume	Displays the injection volume, in microliters, in the Inj. Vol. column.
Sample Weight	Displays the numeric value in the Sample Wt column. The application does not use this value for any internal calculations.
Sample Volume	Displays the numeric value in the Sample Vol column. The application does not use this value for any internal calculations.
Sample ID	Displays the sample identification text in the Sample ID column.
ISTD Amount	Displays the numeric value in the ISTD Corr Amt column.
CD Factor	Displays the numeric value in the Dil Factor column. The application does not use this value for any internal calculations.
Bar Code	Displays the bar code information acquired by the autosampler's bar code reader during an injection sequence.
Bar Code Status	Indicates whether the autosampler read the bar code for an injection vial.
Inst Method	Displays the instrument method in the Inst Meth column.
Proc Method	Displays the processing method in the Proc Meth column.
User Text 1 to User Text 5	Displays the text in the respective sequence table columns. An Xcalibur sequence table can include up to five additional text columns for user-specific information. The default headings for these columns are (1) Study, (2) Client, (3) Laboratory, (4) Company, and (5) Phone. You can customize the column headings in the Xcalibur data system's User Labels dialog box.
The following values are from the autosampler. Not all autosamplers supply this information to the data system.	
Tray Index	Displays text that identifies the autosampler tray.
Tray Name	Displays text that identifies the type of autosampler tray.
Tray Shape	Displays the shape of the autosampler tray (for example, rectangular).
Vial Index	Displays a numeric value.
Vials Per Tray	Displays a numeric value.
Vials Per Tray X	Displays the number of vials across the autosampler tray.

Table 39. File Header view (Sheet 3 of 3)

Read-only parameter	Description
Vials Per Tray Y	Displays the number of vials that the autosampler is deep.
Instrument Name	Displays the name of the mass spectrometer series (for example, Orbitrap Fusion).
Instrument Model	Displays the model name of the mass spectrometer (for example, Orbitrap Fusion).
Instrument Number	Displays the serial number of the mass spectrometer.
Instrument Software Version	Displays the version number of the mass spectrometer's instrument control software that is installed on the data system computer.
Instrument Hardware Version	Displays the version number of the hardware components that are installed on the mass spectrometer.
Flags	Displays additional information about the scan data.
Mass Tolerance	Displays the default mass tolerance setting on the Mass Options page of the Xcalibur Configuration dialog box. The FreeStyle application does not use this setting.
	Tip To open the Xcalibur Configuration dialog box, from the Xcalibur RoadMap view, choose **Tools > Configuration**.
Created by	Displays the user name.

Error Log View

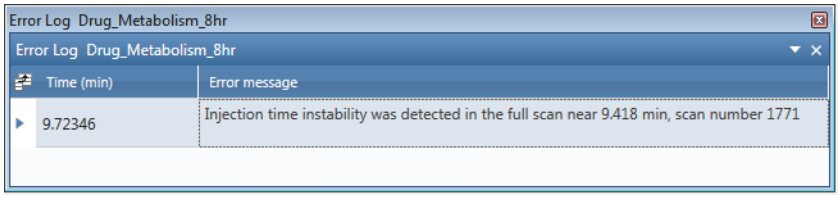
Use the error log to view errors recorded during data acquisition.

❖ To open the Error Log view

1. Open a raw data file.
2. Click the **Workspace Options** toolbar tab.
3. In the Reports area, click **Error Log**.

Figure 127 shows the Error Log view.

Figure 127. Error Log view



10

Deconvolving and Deisotoping Spectra with the Xtract Algorithm

The Xtract algorithm of the FreeStyle application uses a fitting scheme to deconvolve and deisotope isotopically resolved mass spectra of peptides, proteins, and nucleotides.

To deisotope and deconvolve the spectra for peptides, proteins, and nucleotides, follow these topics.

Contents

Understanding the Xtract Algorithm

The Xtract algorithm first examines a cluster of isotopically resolved peaks and uses the peak spacing of a cluster to determine an initial estimate of the mass of the relevant component. For peptides and proteins, it fits an averagine[1] distribution to the observed peak profile in that cluster to determine the monoisotopic mass that best reproduces that profile. (The monoisotopic mass is the mass of an ion for a given molecular formula, which is calculated by using the exact mass of the most abundant isotope of each element, for example, C = 12.000000, H = 1.007825, and O = 15.994915.)

For nucleotides, the algorithm fits a distribution that is typical for nucleotides. Finally, the Xtract algorithm combines the results for all observed charge states for each mass component to produce a single mass value for that component. The resulting spectrum shows only the monoisotopic masses and their ion populations for the components that the algorithm identified.

[1] Senko, M.W.; Beu, S.C.; McLafferty, F.W. Determination of monoisotopic masses and ion populations for large biomolecules from resolved isotopic distributions. *J. Am. Soc. Mass Spectrometry.* **1995**, Vol. 6, 226–233.

When used properly, the Xtract algorithm reduces spectral noise and provides a high-intensity mass spectrum of the monoisotopic peaks. You can use the Xtract Results table, which contains the monoisotopic mass list of the deconvolved mass-spectral peaks or the extracted deconvolved spectrum, as the input to various search engines.

Figure 128 shows an isotopically unresolved mass spectrum. The mass-spectral peaks represent different charge states.

Figure 128. Isotopically unresolved mass spectrum of myoglobin

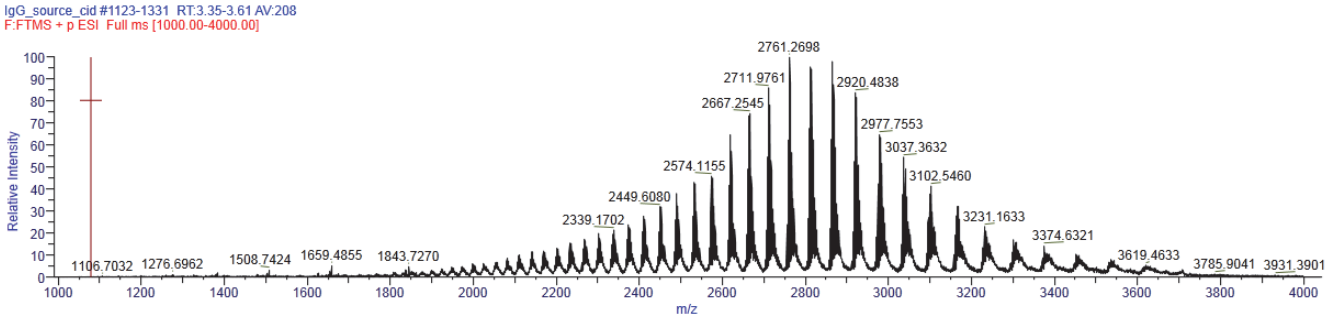
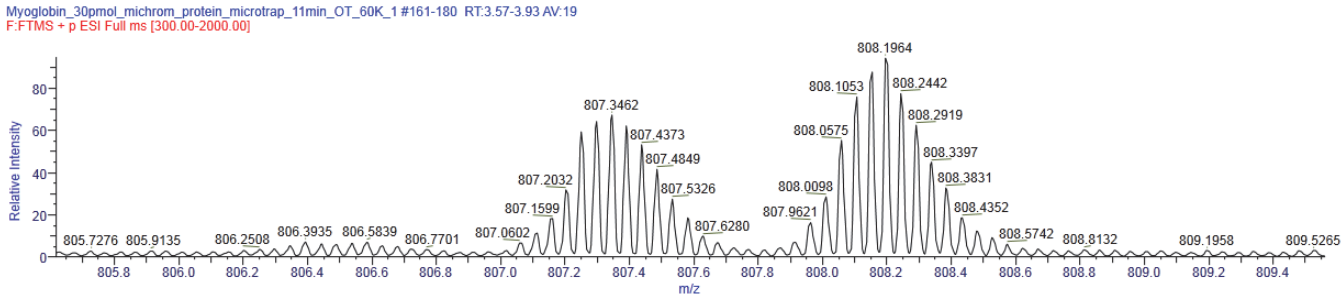


Figure 129 shows an isotopically resolved mass spectrum for one charge state.

Figure 129. Isotopically resolved mass spectrum of myoglobin for one charge state showing two components



Applying the Xtract Algorithm to an Isotopically Resolved Spectrum

❖ **To apply the Xtract algorithm to an isotopically resolved spectrum**

1. Review the default settings for the Xtract algorithm on the Xtract Options page of the Default Options Configuration dialog box as follows:
   a. In the Workspace Options toolbar, click **Default Options**.
   b. In the left pane, select **Xtract Options**.
   c. If needed, modify the settings and click **Save**.
2. Open a raw data file.
3. Select a mass spectrum.
4. Click the **Workspace Processing** tab.

5. Click **Xtract**.

   The Xtract page opens in the Info Bar. The m/z Range boxes are populated with the m/z range of the selected spectrum.

6. If necessary, modify the *m/z* range by entering a range within the selected spectrum.

7. Review and modify the settings in the Deconvolution Parameters area as appropriate.

8. Click **Apply**.

   The application runs the Xtract algorithm.

Xtract Page

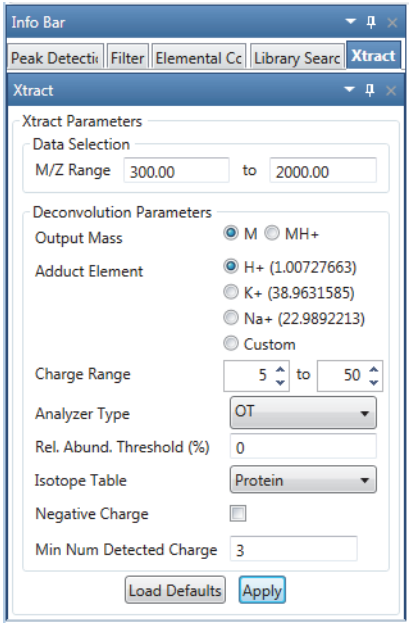
Use the Xtract page in the Info Bar to specify the parameter settings for the Xtract algorithm.

❖ To display the Xtract page

1. Click the **Workspace Processing** toolbar tab.
2. Click **Xtract**.

Figure 130 shows the default settings on the Xtract page. The *m/z* range depends on the selected spectrum.

Figure 130. Default settings on the Xtract page



Note The default parameters on the Xtract page are for protein and peptide deconvolution in the positive polarity mode.

Table 40 describes the parameters for the Xtract page.

Table 40. Xtract page parameters (Sheet 1 of 4)

Parameter	Description
Data Selection	
m/z Range	Specifies the portion of the input spectrum that the Xtract algorithm processes. The options are as follows: • Min: Specifies the lowest end of the input spectrum • Max: Specifies the highest end of the input spectrum For example, if the total mass range of the spectrum is mass 100–2000, a setting of 300–500 for the *m/z* Range parameter means that the Xtract algorithm processes only peaks with masses between *m/z* 300 and 500. Range: minimum *m/z* of the input spectrum to the maximum *m/z* of the input spectrum Default: minimum *m/z* of the input spectrum to the maximum *m/z* of the output spectrum
Deconvolution	
Output Mass	Determines whether the Xtract algorithm returns a single peak at either the monoisotopic mass or the monoisotopic MH^+ mass for each of the detected components. The options are as follows: • M: Specifies that the results file contains a single peak for the monoisotopic mass for each of the detected components. This option generates masses without adducts. • MH^+: Specifies that the results file contains a monoisotopic MH^+ mass for each of the detected components. This option generates masses with adducts. Default: M

Table 40. Xtract page parameters (Sheet 2 of 4)

Parameter	Description
Adduct Element	Specifies the adduct ions used during ESI processing. Adduct ions bring the charge to the molecule, and this charge converts it to an ion. The options are as follows: • H^+ (1.00727663): Specifies that the adduct is hydrogen. • K^+ (38.9631585): Specifies that the adduct is potassium. • Na^+ (22.9892213): Specifies that the adduct is sodium. • Custom: Specifies that the adduct is a charge carrier other than hydrogen, potassium, or sodium. When you select this option, a box opens so that you can type the mass of the custom charge carrier. Default: H^+
Charge Range	Specifies the lowest and highest charge state to be deconvolved. The options are as follows: • Low: Specifies the lowest charge state. • High: Specifies the highest charge state. For example, if you set this parameter range to 1–5, the Xtract algorithm considers only charge states 1 through 5 for deconvolution. It ignores charge states 6 and higher. Default range: 5–50
Analyzer Type	Specifies the type of mass analyzer that was used to obtain the mass spectral data. Different types of mass analyzers have different mass accuracies. The options are as follows: • FT: Specifies a Fourier transform - ion cyclotron resonance (FT-ICR) mass analyzer • OT: Specifies an Orbitrap™ mass analyzer • Sector: Specifies a magnetic-sector mass analyzer • Quad: Specifies a quadrupole mass analyzer Default: OT

Table 40. Xtract page parameters (Sheet 3 of 4)

Parameter	Description
Rel. Abund. Threshold (%)	Specifies a threshold (as a percentage) below which the Xtract algorithm filters out data for data reporting. This option sets a relative threshold as a percentage of the most abundant component in the spectrum. The most abundant peak in the deconvolved spectrum has a relative abundance of 100 percent, and all other peaks are calculated relative to that one. In the Xtract Results table of the Xtract Results View, the application shows only those components that are greater than or equal to this relative abundance threshold in the deconvolved spectrum. For example, if the highest peak has an absolute abundance of 1000, the relative abundance is 1 percent, and no peaks below an absolute abundance of 10 appear in the deconvolved spectrum. Zero (0) displays all results, and 100 displays only the most abundant component. Range: 0–100; default: 0
Isotope Table	Specifies the type of isotope table to use. Isotope tables simulate the distribution of isotopic peaks, in *m/z,* for different choices of the monoisotopic mass. The Xtract algorithm chooses the monoisotopic mass with the best fit between the theoretical and the observed isotope distribution. To generate an isotope table, the Xtract algorithm uses a chemical formula to describe the type of molecule. You can choose one of the following formulas: • Protein: Uses an averagine formula typical for peptides and proteins to generate the isotope table • Nucleotide: Uses an elemental formula typical for nucleotides to generate the isotope table Default: Protein

Table 40. Xtract page parameters (Sheet 4 of 4)

Parameter	Description
Negative Charge	Indicates whether the data was acquired in positive charge mode or negative charge mode during the ESI process. You might want to use this option when you process compounds that contain nucleotides like those found in DNA and RNA. When you acquire these compounds in negative mode, the resulting mass spectra are often clearer. Deprotonation of nucleotides, which are acidic, occurs when the compound is dissolved in a basic solution and negative voltage is applied to produce negatively charged ions. The options are as follows: • Selected: The data was acquired in negative charge mode. • Cleared: The data was acquired in positive charge mode. Default: Cleared **IMPORTANT** Do not select Negative Charge if your data was acquired in positive mode. The results will not be usable.
Min Num Detected Charge	Specifies the minimum number of charge states required to produce a component. No components with less than this minimum number appear in the deconvolved spectrum. Valid values: an integer greater than or equal to 1 Default: 3
Button	
Load Defaults	Resets all the parameters on the Xtract page to the default settings.
Apply	Runs the Xtract algorithm on the selected spectrum.

Deconvolved Spectrum View

The Xtract algorithm transforms the source mass spectrum into a deconvolved mass spectrum and displays it in the Deconvolved Spectrum view, labeled with mass units rather than with the mass-to-charge ratio on the *x* axis. The spectrum that the application displays in the Deconvolved Spectrum view is a mass spectrum that consists of the monoisotopic peaks of the components that the Xtract algorithm detected. The range of the mass spectrum is from the mass of the component with the lowest monoisotopic mass to the mass of the component with the highest monoisotopic mass. Information about each component can be found in the Xtract Results View.

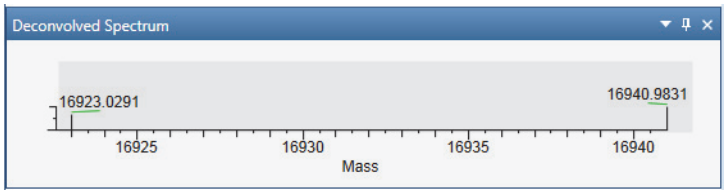
❖ To run the Xtract algorithm and display the Deconvolved Spectrum view

1. Select a mass spectrum for the Xtract algorithm to deconvolve.
2. Click the **Workspace Processing** toolbar tab.
3. Click **Xtract**.

   The Xtract page opens in the Info Bar.
4. Set the deconvolution parameters on the Xtract Page.
5. Click **Apply**.

Figure 131 shows the deconvolved spectrum for the myoglobin example.

Figure 131. Deconvolved Spectrum view showing the two monoisotopic peaks of myoglobin (located at the endpoints)



The spectrum in the Spectrum View or MultiSpectrum View shows the multiple masses (see Figure 134 on page 247) used to create the deconvolved peak displayed in the Deconvolved Spectrum view.

Xtract Results View

The Xtract Results view displays the experimentally determined results for each monoisotopic mass and charge state.

Xtract deconvolves isotopically resolved mass spectra—that is, spectra in which it is possible to distinguish separate peaks for different isotopic compositions of the same component. When the Xtract algorithm finishes processing, it displays the deconvolved spectrum in the Deconvolved Spectrum View as a set of peaks in mass and relative intensity.

It also displays the component list in the Xtract Results view as a list of masses, intensities, charge state information, and quality scores (Figure 132). The values in the columns represent the outputs of the deconvolution. Each peak in the list is composed of isotopic clusters. Each isotopic cluster in the original spectrum provides evidence for the peak in the deconvolved spectrum.

❖ To run the Xtract algorithm and display the Xtract Results view

1. Display the mass spectrum for the Xtract algorithm to deconvolve in the Spectrum or Multi Spectrum view.
2. Click the **Workspace Processing** toolbar tab.
3. Click **Xtract**.
4. Set the parameters on the Xtract Page.
5. Click **Apply**.

Figure 132 shows the Xtract Results view for the two-component myoglobin example.

Figure 132. Xtract Results view in two parts (left and right) showing the 16 923 Da and 16 941 Da components

Xtract Results

	No.	Monoisotopic Mass	Sum Intensity	Number of Charge States	Average Charge
+	1	16923.0291	572,776.58	9	20.96
+	2	16940.9831	850,259.11	12	20.56

Delta Mass	Relative Abundance	Fractional Abundance	RT Range
-17.95	67.3649	40.2503	3.654
0.00	100.0000	59.7497	3.654

Table 41 describes the parameters for the Xtract Results view.

Table 41. Xtract Results view parameters (Sheet 1 of 2)

Parameter	Description
No.	An integer, starting at one, that labels the components in the order of increasing monoisotopic mass.
Monoisotopic Mass	The weighted average of the monoisotopic masses of each charge state: $Monoisotopic\ Mass = \frac{\sum_{i}(Monoisotopic\ Mass\ of\ This\ Charge \times Charge\ Normalized\ Intensity)}{Sum\ Intensity}$ where *i* is the sequential order of the charges in the Charge column of the expanded Xtract table.
Sum Intensity	The sum of the intensities of all the charge states and the isotopes of a component.
Number of Charge States	The number of charge states that the Xtract algorithm used to calculate the monoisotopic mass.
Average Charge	The average of the charges weighted by intensity.
Delta Mass	The difference in mass (in daltons) of a component from the mass of the most intense component.
Relative Abundance	Displays the components that are above the relative abundance threshold set by the Relative Abundance Threshold parameter on the Xtract page. The application assigns the largest peak in a deconvolved spectrum a relative abundance of 100 percent. An abundance number in the Relative Abundance column represents the intensity in the same row of the Sum Intensity column divided by the greatest intensity in the Sum Intensity column multiplied by 100. For example, if the largest peak in a deconvolved spectrum has an intensity of 1000, the application assigns it a relative abundance of 100 percent. If the next most abundant peak has an intensity of 500, the application assigns it an abundance of 50 percent: $\frac{500}{1000} \times 100\% = 50\%$

Table 41. Xtract Results view parameters (Sheet 2 of 2)

Parameter	Description
Fractional Abundance	The fractional abundance of a component, which is the abundance for that peak relative to the total abundance of all peaks in the spectrum, expressed as a percentage. The sum of all fractional abundances of all peaks in a deconvolved spectrum is 100 percent.
RT Range	The retention time (in minutes), or the retention time range (if the retention time was averaged), that produced the mass spectrum that the Xtract algorithm deconvolved.

Click ✚ to expand the Xtract Results view for a component. The expanded list shows the experimentally-determined parameters for the individual charge states of a component.

In the expanded list (see Figure 133), six new columns appear: Charge State, Calculated Monoisotopic m/z, Monoisotopic Mass for This Charge, Mostabund m/z, Charge Normalized Intensity, and Fit %. These values represent the isotopic clusters with different charge states from the source spectrum that were used to produce the peak in the deconvolved spectrum.

Figure 133 shows the Xtract Results view of Figure 132 on page 243 with the 16 923 Da component expanded to show the individual charge states.

Figure 133. Xtract Results view with the 16 923 Da component expanded to show results for the nine individual charge states

Charge State	Calculated Monoisotopic m/z	Monoisotopic Mass for This Charge	Mostabund m/z	Charge Normalized Intensity	Fit %
18	941.1756	16923.0428	941.7327	83,056.26	89.10
19	891.6931	16923.0477	892.2206	105,669.90	89.20
20	847.1588	16923.0393	847.6607	90,557.22	87.60
21	806.8658	16923.0641	807.3449	73,625.01	91.80
22	770.2359	16923.0486	770.6924	64,146.69	84.10
23	736.7912	16923.0498	737.2283	64,615.90	92.20
24	706.1335	16923.0670	706.5534	47,677.56	86.80
25	677.9285	16923.0196	678.3286	27,615.76	86.40
26	651.8930	16923.0233	652.2789	15,812.27	86.10

Table 42 describes the parameters for the expanded Xtract Results view.

Table 42. Expanded Xtract Results view parameters (Sheet 1 of 2)

Parameter	Description
Charge State	The charge states of the individual isotopic clusters that constitute the total number shown in the Number of Charge States column.
Calculated Monoisotopic *m/z*	The mass-to-charge ratio of the monoisotopic mass that the Xtract algorithm calculated from the isotopic peak envelope for a specific charge state.

Table 42. Expanded Xtract Results view parameters (Sheet 2 of 2)

Parameter	Description
Monoisotopic Mass For This Charge	The mass (in daltons) that is equal to the calculated monoisotopic *m/z* multiplied by the charge state, *z*.
Mostabund *m/z*	The mass-to-charge ratio of the most abundant isotope, or the height of the tallest peak in the isotopic distribution.
Charge Normalized Intensity	The sum of the intensities of the isotopic peaks for a particular charge state.
Fit %	The quality of the match between a measured isotope pattern and an averagine distribution of the same mass. This column displays a value between 0 and 100 percent. • 0 percent indicates a poor fit between the measured pattern and the averagine pattern. • 100 percent indicates that the observed peaks in the measured isotope profile are absolutely identical to those in a theoretical averagine distribution and that any missing peaks fall below a restrictive threshold.

When you select any charge state row in the expanded Xtract Results view, the application automatically highlights in blue the following items:

- The blue spectral line in the Spectrum View or MultiSpectrum View corresponding to the most abundant *m/z* for the selected row, as shown in Figure 134 and Figure 135.

  For example, in Figure 135, a blue spectral line marks the value of 770.6924 corresponding to the Mostabund m/z value of the selected row.

- The deconvolved peak in the Deconvolved Spectrum View.

You might have to right-click and choose Reset to Scale to see the blue spectral lines.

Figure 134 shows the mass spectrum from Figure 128 on page 236 with nine blue bars that indicate the positions of the theoretical most abundant *m/z* for each of the nine charge states that the Xtract algorithm used to deconvolve the spectrum.

Figure 134. Mass spectrum of myoglobin showing the nine charge states (blue bars) that the Xtract algorithm used to calculate the 16923 Da monoisotopic mass

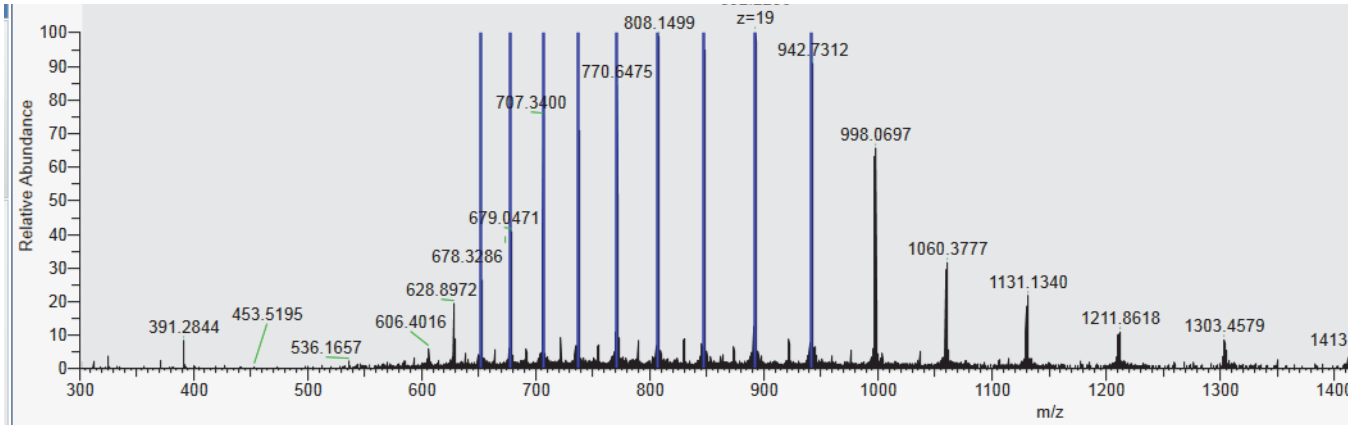
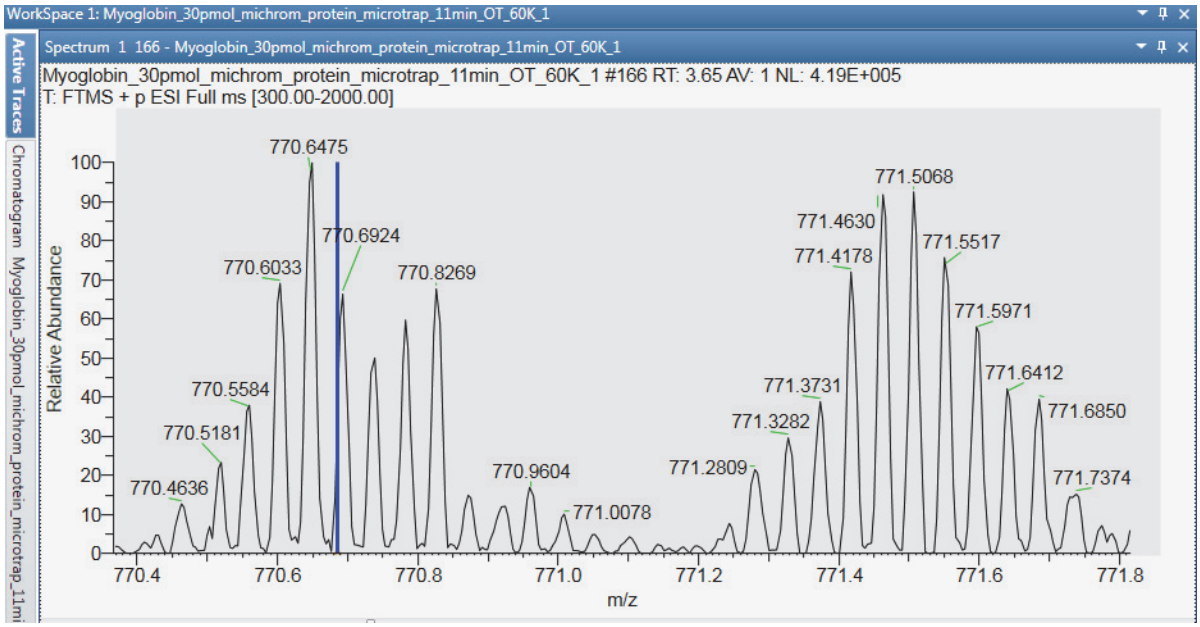


Figure 135 shows the theoretical most abundant *m/z* for the *z*=22 charge state of the 16923 Da component.

Figure 135. Mass spectrum at the *z* = 22 charge state showing the location (blue bar) of the theoretical most abundant *m/z* for the 16 923 Da monoisotopic mass



A

FreeStyle Default Settings

Use the pages of the Default Options Configuration dialog box to specify the initial default settings that the FreeStyle application uses when you start the application.

Follow these topics.

Contents

Modifying, Saving, and Resetting the Default Configuration Options

Use the pages of the Default Options Configuration dialog box to modify some of the parameter settings for the FreeStyle application.

Follow these procedures as needed:

- To open the Default Options Configuration dialog box
- To display a specific page of the dialog box
- To open the Help topic for a specific page
- To save your new parameter settings

- To cancel all your changes to the parameter settings
- To return all the configuration parameters to their factory default settings
- To apply the factory default layout to the active Workspace view

❖ **To open the Default Options Configuration dialog box**

- Open the File Menu and click **Options** at the bottom right.

–or–

- In the Workspace Options Toolbar, click **Default Options**.

The dialog box opens to the Default Mass Precision page.

❖ **To display a specific page of the dialog box**

In the left pane of the dialog box, click the page link.

❖ **To open the Help topic for a specific page**

Click **Help** at the bottom right of the page.

❖ **To save your new parameter settings**

Click **Save**.

The Default Options dialog box closes, and the application saves all the modified parameter settings.

❖ **To cancel all your changes to the parameter settings**

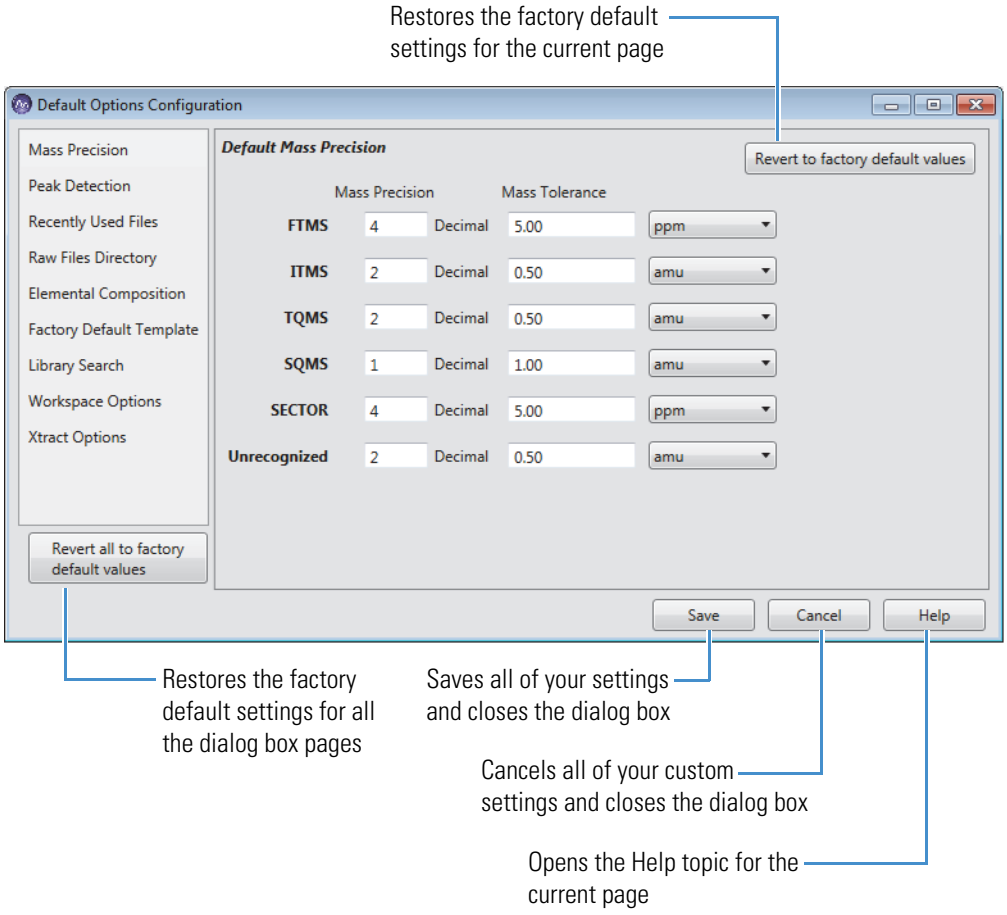
Click **Cancel**.

The Default Options Configuration dialog box closes without changing the parameter settings.

❖ **To return all the configuration parameters to their factory default settings**

At the bottom left, click **Revert All to Factory Default Values**.

Figure 136. Location of the Revert All to Factory Default Values button



❖ **To apply the factory default layout to the active Workspace view**

1. In the left pane of the dialog box, click **Factory Default Layout**.
2. At the top right of the Factory Default Template Page, click **Revert to Factory Default Values**.

Default Mass Precision Page

Use the Default Mass Precision page to specify how many decimal places the application displays, by default, and also the default mass tolerance.

❖ **To display the Default Mass Precision page**

1. Open the Default Options Configuration dialog box (see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249).
2. In the left pane, click **Mass Precision**.

Figure 136 shows the Default Mass Precision page with its original default values, and Table 43 lists the default Mass Precision page parameters.

Table 43. Default Mass Precision page parameters (Sheet 1 of 2)

Column	Description
Mass Precision	Specify the default number of decimal places in the mass-to-charge ratios that the application displays in the Spectrum View and the MultiSpectrum View. Default: • FTMS: 4 • ITMS: 2 • TQMS: 2 • SQMS: 1 • Sector: 4 • Unrecognized: 2 Range: 0–5
Mass Tolerance	Specify the default mass tolerance. The mass tolerance defines how close a measured mass must be to the mass in the mass list to be considered the same mass. Default: • FTMS: 5.00 ppm • ITMS: 0.50 amu • TQMS: 0.50 amu • SQMS: 1.00 amu • Sector: 5.00 ppm • Unrecognized: 0.50 amu Range: 0.00–1000.00 ppm or 0.00–10.00 amu
Default Mass Tolerance Units	Specify the default mass tolerance units as follows: • ppm: In parts per million. Select this option for FTMS and sector mass analyzers because the peak width is proportional to the mass-to-charge ratio. When you select ppm, the size of the window around the reference mass is proportional to the mass-to-charge ratio of the reference mass. • amu: In units of the mass-to-charge ratio. When you select amu, the size of the window around the reference mass remains the same regardless of the mass-to-charge ratio of the reference mass. • mmu: In units of one one-thousandth of an amu.

Table 43. Default Mass Precision page parameters (Sheet 2 of 2)

Column	Description
Button	
Revert to Factory Default Values	Returns the parameter settings on this page to their factory default values.
Help	Opens the Help topic for this page.

Default Peak Detection Page

Use the Default Peak Detection page to select the default peak detection algorithm and its default parameters. For a description of these parameters, see these topics: "Avalon Peak Detection Page" on page 105, "Genesis Peak Detection Page" on page 97, "ICIS Peak Detection Page" on page 102, or "PPD Peak Detection Page" on page 108.

❖ To display the Default Peak Detection page

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Peak Detection**.

To save your custom settings, see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249.

Default Recently Used Files Page

Use the Default Recently Used Files page to set the number of recently used files that the application displays in the Recent Items area of the File menu. By default, this number is set to 10. You can change the setting to a value from 0 to 10.

❖ To display the Default Recently Used Files page

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Recently Used Files**.

   The Default Number of Recently Used Files page appears (Figure 137).

Figure 137. Default Number of Recently Used Files page

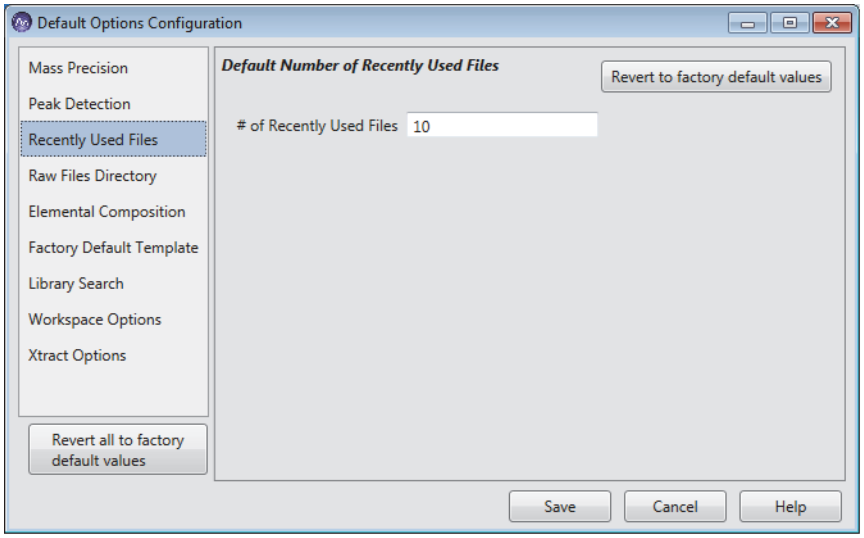


Table 44 describes the parameter and buttons on the Default Number of Recently Used Files page. To save a custom setting, see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249.

Table 44. Default Number of Recently Used Files page parameter and buttons

Parameter	Description
# of Recently Used Files	Specifies the maximum number of files that the application displays in the Recent Items area of the File menu. Range: 0 to 10; default: 10
Button	
Revert to Factory Default Values	Returns the parameter settings on this page to their factory default values.
Help	Opens the Help to the topic for this page.

Default Raw Data Files Directory Page

Use the Default Raw Data Files Directory page to specify the default location where the FreeStyle application looks for raw data files.

❖ **To display the Default Raw Data Files Directory page**

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Raw Files Directory** (Figure 138).

Figure 138. Default Raw Files Directory page

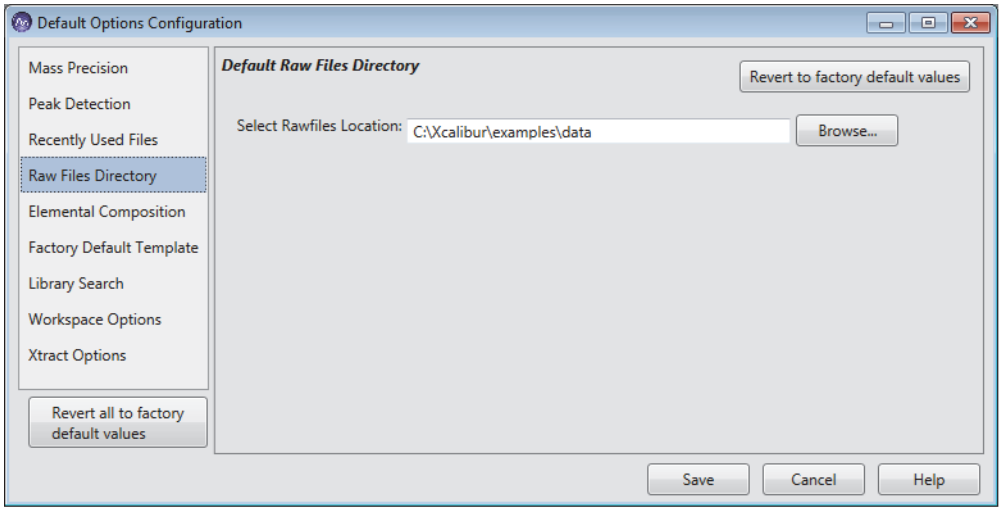


Table 45 describes the parameter and buttons on the Default Raw Files Directory page. To save a custom setting, see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249.

Table 45. Default Raw Files Directory page parameter and buttons

Parameter	Description
Select RawFiles Location	Default: C:\Xcalibur\examples\data
Button	
Revert to Factory Default Values	Returns the parameter settings on this page to their factory default values.
Help	Opens the Help to the topic for this page.

Default Elemental Composition Page

Use the Default Elemental Composition page to specify the default elemental composition search parameters. For information about the basic parameter settings and performing an elemental composition analysis, see "Elemental Composition Page" on page 165. To save your custom settings, see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249.

❖ To display the Default Elemental Composition page

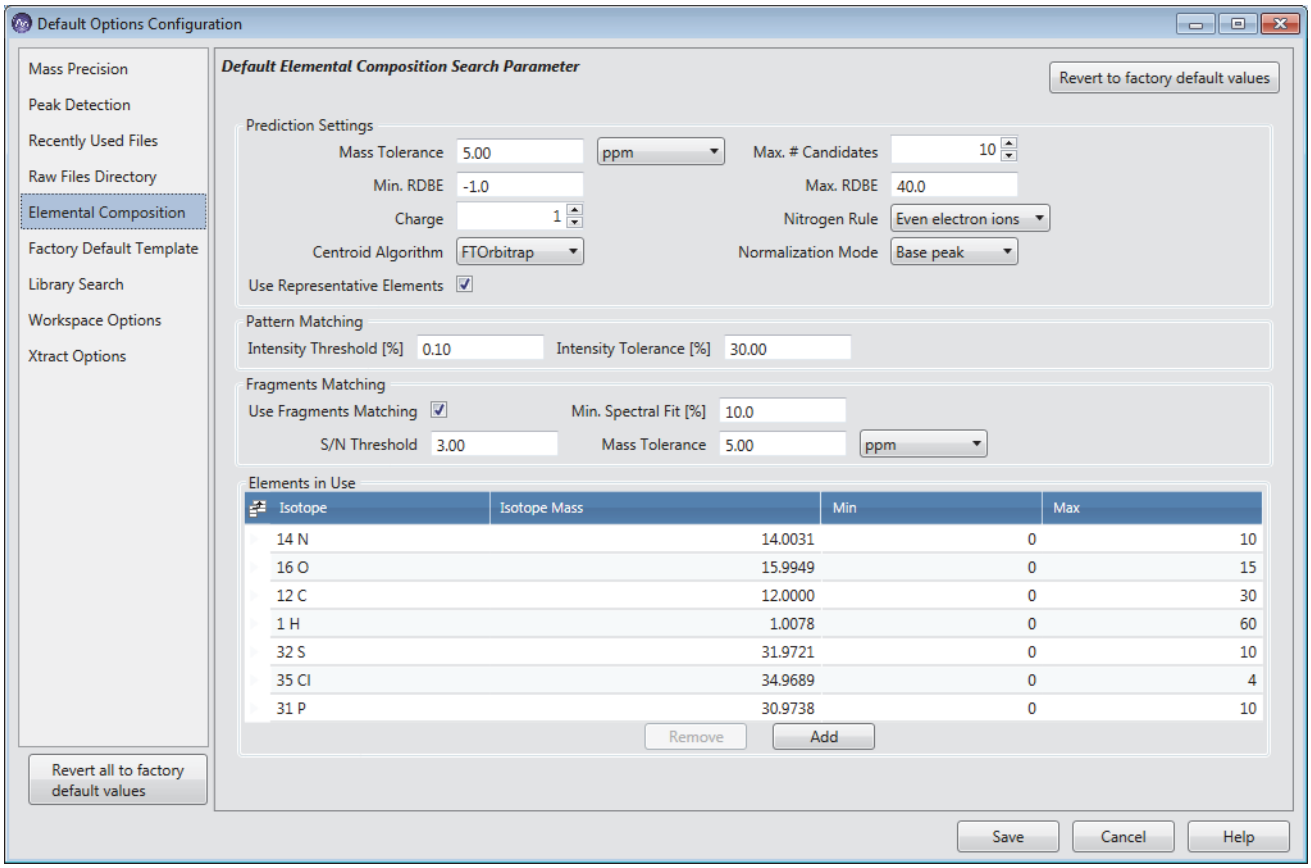
1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Elemental Composition**.

Figure 139 shows the Default Elemental Composition Search Parameter page. For information about the parameter settings, see "Elemental Composition Page" on page 165.

Figure 139. Default Elemental Composition Search Parameter page



Factory Default Template Page

Use the Factory Default Template page to apply the factory default layout to the active Workspace view (see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249). The settings on this page are read only.

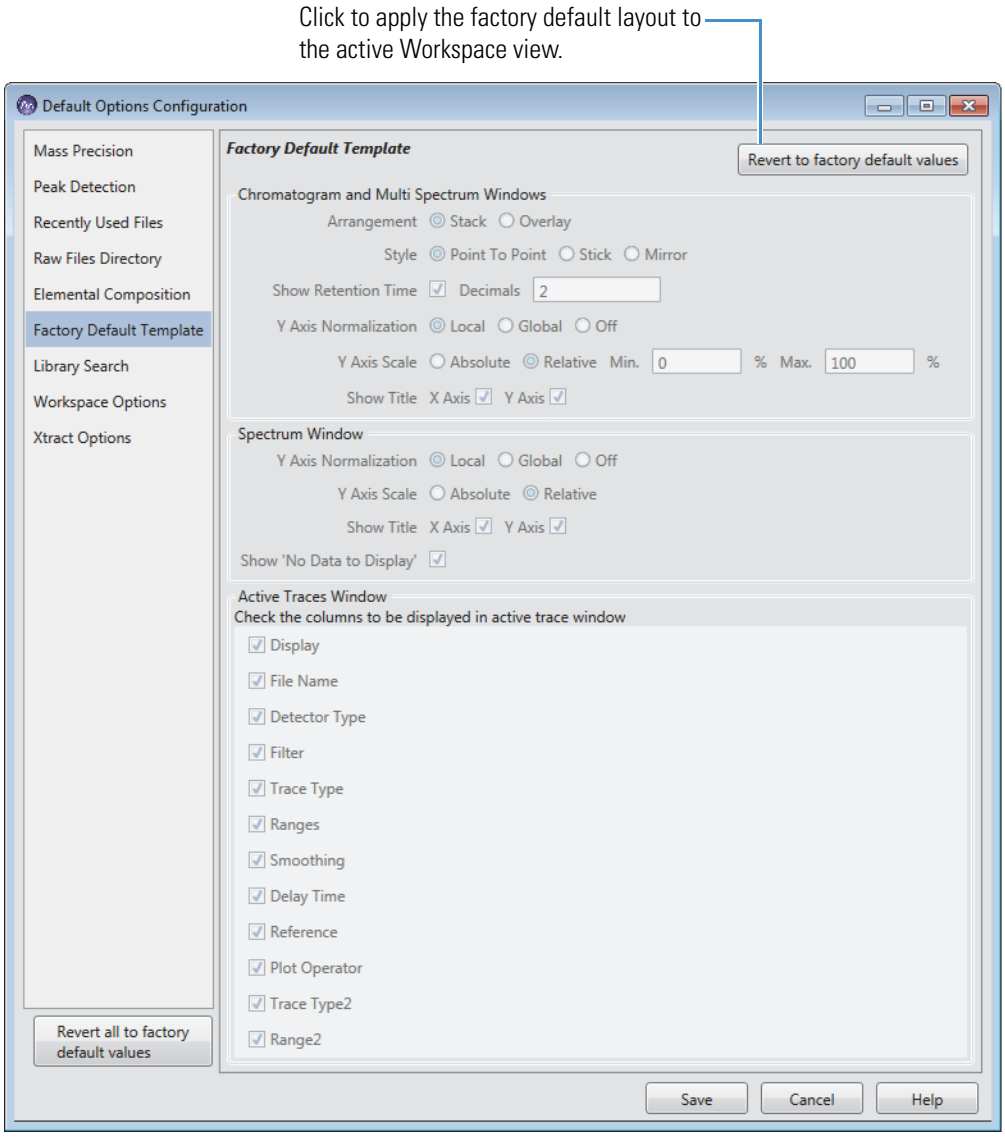
❖ To display the Factory Default Template page

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Factory Default Template** (Figure 140).

Figure 140. Factory Default Template page



Default Library Search Page

Use the Default Library Search page to specify the default library search parameters for the NIST libraries and the *mz*Vault libraries. For a description of these parameters, see "Modifying a NIST Search from the NIST Search Page" on page 182 and "Modifying an mzVault Search from the mzVault Search Page" on page 187. To save your custom settings, see "Modifying, Saving, and Resetting the Default Configuration Options" on page 249.

❖ To display the Default Library Search page

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Library Search**.

❖ To display the NIST library parameters

Select the **NIST** option in the Library Type area.

❖ To display the mzVault library parameters

1. Select the **mzVault** option in the Library Type area.
2. Make sure that the Library list in the Search List area includes the mzVault databases that you want to search (see "To specify the location of your local mzVault database files" on page 174).

Default Workspace Options Page

Use the Default Workspace Options page to specify the following:

- The minimum trace height (in centimeters) for the Chromatogram View, Spectrum View, or MultiSpectrum View
- The maximum number of scan filters to display in the Chromatogram view when applying the auto filter command (see "Adding Chromatogram Traces with the Auto Filter Feature" on page 46)

  Note When you adjust the height of these views, if the height becomes smaller than the set minimum value, a scrollbar automatically appears to the right of the views.

- How to open the Chromatogram view and Spectrum view toolboxes

❖ To display the Default Workspace Options page

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Workspace Options**.

Table 46 describes the parameters on the Workspace Options page of the Default Options Configuration dialog box.

Table 46. Workspace Options page parameters

Parameter	Description
Chromatogram and Multi Spectrum Windows	
Minimum Trace Height (cm)	Specifies the minimum height, in centimeters, for the traces in the Chromatogram and Multi Spectrum views. Range: 0.5 to 15.0; default: 2.65
Default Auto Filter	
# Number of Auto Filter	Specifies the maximum number of filtered chromatograms that the application displays in the Chromatogram Ranges and Chromatogram views. Range: 1 to 500; default: 8
Default Plot Toolboxes	
Auto Show Plot Toolboxes After	• Select to turn on the automatic display of the view's toolbox with these mouse pointer actions: – Point to the Chromatogram view for the specified time, in milliseconds. – Point to an *m/z* value in the Spectrum view for the specified time, in milliseconds. • Clear to turn off the automatic display of the view's toolbox. For more information, see "Selecting How You Open the Floating Toolboxes" on page 40. **Tip** By default, there are two ways to open the toolbox: • Right-click the view and choose **Show Toolbox**. –or– • Use the mouse pointer. Clearing this check box turns off the mouse pointer action.
Time box (ms)	Available when the Auto Show Plot Toolboxes check box is selected. Range: 100 to 2000 ms; default: 1000 ms

Default Xtract Options Page

Use the Default Xtract Options page to specify the default Xtract parameters. For more information, see Chapter 10, "Deconvolving and Deisotoping Spectra with the Xtract Algorithm."

❖ To display the Default Xtract Options page

1. In the Workspace Options toolbar, click **Default Options**.

   The Default Options Configuration dialog box opens.

2. In the left pane, click **Xtract Options**.

Figure 141 shows the Default Xtract Options page with its original default values.

Figure 141. Default Xtract Options page

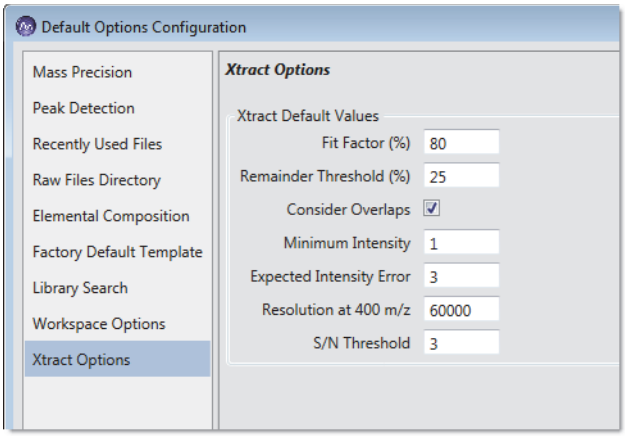


Table 47 lists the default Xtract parameters.

Table 47. Default parameters for the Xtract algorithm (Sheet 1 of 3)

Parameter	Description
Fit Factor (%)	Measures the quality of the match between a measured isotope pattern and an averagine distribution of the same mass, as a percentage. • 0% requires a low fit only. • 100% means that the measured isotope profile is identical to the theoretical averagine isotope distribution. Range: 0–100; default: 80

Table 47. Default parameters for the Xtract algorithm (Sheet 2 of 3)

Parameter	Description
Remainder Threshold (%)	Specifies the height of the smaller overlapping isotopic cluster (as a percentage) with respect to the height of the most abundant isotopic cluster when the Xtract algorithm attempts to resolve overlapping isotopic clusters. For example, if one isotopic cluster in a spectrum has an abundance of 100 and you set the Remainder Threshold parameter to 30 percent, the Xtract algorithm ignores any overlapping clusters with an abundance less than 30. Range: 0–100; default: 25
Consider Overlaps	Determines whether the Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. • Selected: The Xtract algorithm is more tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. Because this option can lead to increased false positives, select it only in cases where you expect overlapping isotopic clusters in a data set. • Cleared: The Xtract algorithm is less tolerant of errors when the spectrum intensity is significantly higher than expected for the theoretical isotopic cluster. Default: Selected
Minimum Intensity	Specifies a minimum intensity threshold to filter out possible background noise, even when you set the S/N Threshold parameter to zero. Range: 0–9999; default: 1
Expected Intensity Error	Specifies the permissible percentage of error allowed in calculating the ratio of the most abundant isotope to the next isotope that is higher in mass in the isotope series. Range: 0–9999; default: 3

Table 47. Default parameters for the Xtract algorithm (Sheet 3 of 3)

Parameter	Description
Resolution at 400 m/z	Defines the resolution of the source spectrum at *m/z* 400. This parameter is not needed if the Xtract algorithm deconvolves FTMS, Orbitrap, or Exactive data because the data contains the instrument information in the spectrum. You must set this parameter for all other spectrum types and for exported spectrum files (in -qb.raw data file format), which lack instrument information. Range: 6000–240 000; default: 60 000
S/N Threshold	Specifies a signal-to-noise (S/N) threshold, *x*, above which the Xtract algorithm considers a measured peak to be a real (accepted) peak. The Xtract algorithm ignores peaks below this threshold. Any spectral peak must be *x* times the intensity of the calculated noise for that spectrum before the Xtract algorithm considers it. Range: 0–9999; default: 3

B

Scan Filters and Scan Headers

The data system creates scan filters from scan-event settings and stores them in the raw data file. You can select or create scan filters to apply processing to a subset of the scans in the raw data file. Scan headers provide important information about the scan.

Contents

Scan Filter Parameters

Table 48 lists the scan filter parameters. Use only the fields that apply to your mass spectrometer. You can define additional scan filters by adhering to the following scan filter format.

Note Not all features are applicable for every mass spectrometer.

Table 48. Scan filter format (Sheet 1 of 4)

Feature	Option	Interpretation
Polarity	+, –	Positive or negative.
Data type	p, c	Profile or centroid.
Dependent scans	d, !d	Include dependent scans or exclude dependent scans.
TurboScans	t, !t	Include TurboScan scans or exclude TurboScan scans.
Source CID	cid, !cid	Include Source CID scans or exclude Source CID scans. (Source CID scans are scans for ions that are produced by collision-induced dissociation in the ion source.)
Scan type	FULL, Z, SIM, SRM, CRM, Q1MS, Q3MS	Full scan, ZoomScan, selected ion monitoring (SIM), selected reaction monitoring (SRM), consecutive reaction monitoring (CRM), Q1 quadrupole analysis, or Q3 quadrupole analysis.

Table 48. Scan filter format (Sheet 2 of 4)

Feature	Option	Interpretation
Scan mode	ms, ms2, ms3, ... MS10	MS^n where n = 1 to 10 Each order can be followed by the appropriate number of parents. The parents can also be omitted. Example: "ms3 345.3, 253.2" indicates an MS^3 scan with parents with *m/z* 345.3 and 253.2.
	pr	Parent (followed by the product mass)
	cng	Constant neutral gain (followed by the mass of the neutral)
	cnl	Constant neutral loss (followed by the mass of the neutral)
Mass Analyzer	ITMS, TQMS, SQMS, TOFMS, FTMS, Sector	Ion trap, triple-quadrupole, single-quadrupole, time-of-flight, Fourier transform, or magnetic sector mass spectrometry.
Photo Ionization	pi, !pi	Include photo ionization scans or exclude photo ionization scans.
Compensation Voltage	cv, !cv	Include compensation voltage scans or exclude compensation voltage scans.
Detector Valid	det, !det	Include detector valid scans or exclude detector valid scans.
Enhanced	E, !E	Include enhanced scans or exclude enhanced scans.
Wideband	w, !w	Include wideband scans or exclude wideband scans.
Supplemental Activation	sa, !sa	Include supplemental activation scans or exclude supplemental activation scans.
Multistate Activation	msa, !msa	Include multistate activation scans or exclude multistate activation scans.
Product masses or mass range of scan	[m1a–m1b, m2a–m2b, m3a–m3b, ...]	Scans with a specific mass range or mass ranges, such as SIM, SRM, and CRM. Example: [50.00–1500.00] for a scan from *m/z* 50.00 to 1500.00 If a scan is exactly 1 Da wide, it appears as a single value (the center mass). This is typical for SIM, SRM, and CRM. Filters for precursor ions in dependent scans are matched with a tolerance of *m/z* 1.0 so that minor differences in precursor mass measurements from scan to scan do not give different filters.
Segment/scan event number pairs	{segment, scan number}	Example: "{3, 4} + c ms" indicates segment 3, scan event 4 for a positive centroid MS scan The curly brackets { } are required.

Table 48. Scan filter format (Sheet 3 of 4)

Feature	Option	Interpretation
Ionization mode	APCI, ESI, EI, CI, NSI, FAB, TSP, FD, MALDI, GD	Atmospheric pressure chemical ionization (APCI), electrospray (ESI), electron ionization (EI), chemical ionization (CI), nanoelectrospray ionization (NSI), fast atom bombardment (FAB), thermospray ionization (TSP), field desorption (FD), matrix-assisted laser desorption ionization (MALDI), or glow discharge (GD). Example: "+ c ESI ms" indicates a positive centroid electrospray MS scan.
Corona on/off	corona, !corona	Corona on or corona off. Example: "+ APCI !corona ms" indicates a positive centroid APCI scan with the corona off.
Detector value	"det=## .##"	Detector value is ## .## with no spaces. Example: "+ ESI det= –800.0" indicates a positive electrospray scan at –800.0 detector units (usually volts).
MS/MS and MS^n CID energies	mass@energy	Mass is the precursor mass and energy is the CID relative energy (no units). Example: "– c ms2 196.1@25.0" indicates a negative centroid MS/MS scan of *m/z* 196.1 at 25.0 units of CID energy.
Quadrupole identification	Q1MS, Q3MS	Q1 quadrupole or Q3 quadrupole. Example: "+ c ESI Q3MS" indicates a positive centroid electrospray MS scan using Q3 quadrupole.
Accurate mass	AM, !AM, AMI, AME	Include accurate mass scans, exclude accurate mass scans, include accurate mass internal, or include accurate mass external.
Ultra	u, !u	Include ultra scans or exclude ultra scans.
Sector	BSCAN, !BSCAN	Include magnetic sector scans or exclude magnetic sector scans.
	ESCAN, !ESCAN	Include electric sector scans or exclude electric sector scans.
LOCK	lock, !lock	Include lock scans or exclude lock scans.
Multiplex	msx, !msx	Include multiplexing scans or exclude multiplexing scans.
Electron capture dissociation	ecd, !ecd	Include electron capture dissociation or exclude electron capture dissociation.
Multi-photon dissociation	mpd, !mpd	Include photo dissociation or exclude photo dissociation.

Table 48. Scan filter format (Sheet 4 of 4)

Feature	Option	Interpretation
Pulsed dissociation	pqd, !pqd	Include pulsed dissociation scans or exclude pulsed dissociation scans.
Electron transfer dissociation	etd, !etd	Include electron transfer dissociation scans or exclude electron transfer dissociation scans.
NPTR	nptr, !nptr	Include NPTR, Exclude NPTR
Higher-energy CID	hcd, !hcd	Include higher energy CID scans or exclude higher energy CID scans.
Source SID	sid, !sid	Include source SID scans or exclude Source SID scans. (Source SID scans are surface-induced scans.)
Isolation width	iw ##, ##	Isolation width value at ##, ##

Scan Headers and Scan Header Abbreviations

Use the Scan Header dialog box to select what parameters appear in the scan header of the Spectrum View or MultiSpectrum View.

❖ **To display the Scan Header dialog box**

1. Click the Spectrum View or MultiSpectrum View to make it the active view.
2. Click the **Display Options** toolbar tab.
3. Click **Scan Header**.

Figure 142 shows the default scan header selections.

Figure 142. Default scan header selections

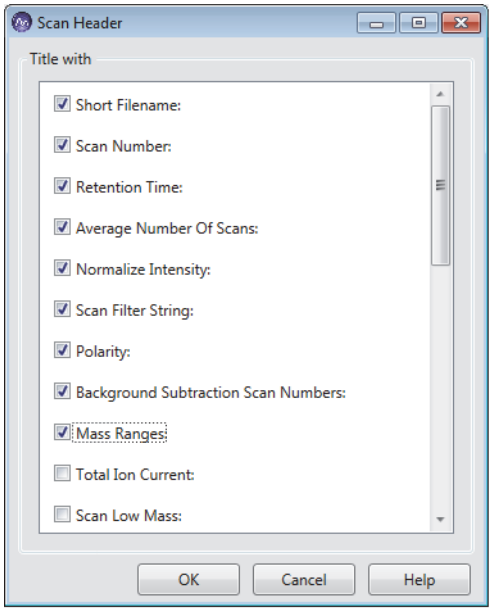


Table 49 lists the parameters that you can display in the scan header. The parameters displayed in the Scan Header dialog box depend on the data acquisition settings for the mass spectrometer used to acquire the raw data file.

Table 49. Scan header parameters and their abbreviations (Sheet 1 of 2)

Parameter	Abbreviation before the value
Short filename	NA
Scan Number	#
Retention time	RT
Average Number of Scans	AV
Normalize Intensity	NL
Scan Filter String	T:
Polarity	NA (P: when the Scan Filter String is not selected)
Background Subtraction Scan Numbers	SB
Mass Ranges	[...] (MR: [...] when the Scan Filter String is not selected)
Total Ion Current	TIC:
Scan Low Mass	SLM:

Table 49. Scan header parameters and their abbreviations (Sheet 2 of 2)

Parameter	Abbreviation before the value
Scan High Mass	SHM:
Scan Start Time (Min)	SST (Min):
Base Peak Intensity	BPI:
Base Peak Mass	BPM:
Multiple Injection	Multiple Injection::
Multi Inject Info	Multi Inject Info::
AGC (automatic gain control—On, Off, or predicted)	AGC::
Micro Scan Count	MSC:
Ion Injection Time (ms)	IIT
Scan segment	SS:
Scan Event	SE:
Master Index	Master Index::
Charge State	CS:
Reagent Ion AGC	Full parameter name
Reagent Ion Injection Time (msec)	Full parameter name
Elapsed Scan Time (sec)	EST
API Source CID Energy	Full parameter name
Average Scan By Instrument	ABSI
Charge State	CS
Monoisotopic Ion M/Z	Full parameter name
MS^n Isolation Width (n = 2 to 10) (m/z)	Full parameter name
FT analyzer settings	Full parameter names
FT analyzer message	Text message
FT resolution	Full parameter names
Conversion parameters	Full parameter names

One- and Three-Letter Abbreviations for Amino Acid Residues

Table 50 lists common one- and three-letter abbreviations for amino acid residues that you enter in the peptide/protein Formula box on the "Isotope Simulation Page" on page 209.

Table 50. Common one- and three-letter abbreviations for amino acid residues (Sheet 1 of 2)

One letter	Name	Formula	Three letter
A	Alanine	C3H5NO	Ala
C	Cysteine	C3H5NOS	Cys
D	Aspartate	C4H5NO3	Asp
E	Glutamate	C5H7NO3	Glu
F	Phenylalanine	C9H9NO	Phe
G	Glycine	C2H3NO	Gly
H	Histidine	C6H7N3O	His
I	Isoleucine	C6H11NO	Ile
K	Lysine	C6H12N2O	Lys
L	Leucine	C6H11NO	Leu
M	Methionine	C5H9NOS	Met
N	Asparagine	C4H6N2O2	Asn
O	Ornithine	C5H11N2O	Orn
P	Proline	C5H7NO	Pro
Q	Glutamine	C5H8N2O2	Gln
R	Arginine	C6H12N4O	Arg
S	Serine	C3H5NO2	Ser

Table 50. Common one- and three-letter abbreviations for amino acid residues (Sheet 2 of 2)

One letter	Name	Formula	Three letter
T	Threonine	C4H7NO2	Thr
V	Valine	C5H9NO	Val
W	Tryptophan	C11H10N2O	Trp
Y	Tyrosine	C9H9NO2	Tyr

Table 51 lists less common three-letter abbreviations for amino acid residues to enter in the peptide/protein formula for the simulated spectrum.

Table 51. Less common three-letter abbreviations for amino acid residues (Sheet 1 of 3)

Three letter	Name	Formula
Abu	2-Aminobutyric acid (2-aminobutanoic acid)	C4H7NO
Aec	Aminoethylcysteine	C5H10N2OS
Aib	Aminoisobutyric acid	C4H7NO
Aln	--	C13H11NO
Aly	Alveolysin	C12H22N2O6
Amc	--	C6H10N2O2S
Bcy	--	C10H11NOS
Bgl	--	C12H13NO3
Bly	--	C16H26N4O3S
Bse	--	C10H11NO2
Bth	--	C11H13NO2
Cmc	Carboxymethylcysteine	C5H7NO3S
Cml	--	C8H14N2OS
Cph	Chlorophenylalanine	C9H8NOCl
Cya	Cysteic acid	C3H5NO4S
Dha	Dehydroalanine	C3H3NO
Dhb	Dehydro-2-aminobutyric acid	C4H5NO
Dpr	D-proline	C5H5NO
Dty	Diiodotyrosine	C9H7NO2I2
Fcy	--	C18N29NOS
Fph	--	C9H8NOF
Ftr	--	C12H10N2O2

Table 51. Less common three-letter abbreviations for amino acid residues (Sheet 2 of 3)

Three letter	Name	Formula
Gaa	--	C4H7NO
Gcg	--	C5H5NO4
Gla	Carboxyglutamic acid	C6H7NO5
Glp	--	C5H5NO2
Hse	Homoserine	C4H7NO2
Hsl	Homoserine lactone	C4H5NO
Hya	Beta-hydroxyaspartate	C4H5NO4
Hyg	Hydroxyglycine	C5H7NO4
Hyl	Hydroxylysine	C6H12N2O2
Hyp	Hydroxyproline	C5H7NO2
Ils	Isolysine	C9H18N2O
Ity	Iodotyrosine	C9H8NO2I
Iva	Isovaline	C5H9NO
Mar	--	C7H14N4O
Mas	--	C5H7NO3
Mbt	--	C17H17NO2
Mes	--	C5H9NO3S
Mga	--	C6H10N2O2
Mgl	--	C6H9NO3
Mhi	--	C7H9N3O
Mls	--	C7H14NO
Mme	--	C6H11NOS
Mph	--	C10H11NO
Mso	Methioninesulfoxide	C5H9NO2S
Mty	C10H11NO2	
Nle	Norleucine	C6H11NO
Nls	Norlysine	C12H15N3O2
Pal	--	C8H8N2O
Pcy	--	C19H35NO2S
Pec	--	C10H12N2OS
Pip	2-Piperidinecarboxylic acid	C6H9NO

Table 51. Less common three-letter abbreviations for amino acid residues (Sheet 3 of 3)

Three letter	Name	Formula
Psr	Phosphoserine	C3H6NO5P
Pth	Phosphothreonine	C4H8NO5P
Pty	Phosphotyrosine	C9H10NO5P
Pyr	Pyroglutamic acid	C5H5NO2
Sar	Sarcosine	C3H5NO
Sas	--	C8H8NO5
Tml	E-amino trimethyl-lysine	C9H19N
Tys	Tyrosinesulfonic acid Tyr (SO3H)	C9H9NO5S

Common Isotopes

Table 52 lists the exact mass and natural abundance for some common isotopes.

Table 52. Common elements with multiple stable isotopes

Element	Isotope symbol	Mass (Da)[a]	%Abundance[b]
Bromine	^{79}Br	78.918336	50.69
	^{81}Br	80.916290	49.31
Carbon	^{12}C	12.000000	98.93
	^{13}C	13.003354	1.07
Calcium	^{40}Ca	39.962591	96.95
	^{42}Ca	41.958622	0.65
	^{44}Ca	43.955485	2.086
Chlorine	^{35}Cl	34.968853	75.77
	^{37}Cl	36.965903	24.23
Potassium	^{39}K	38.963708	93.20
	^{41}K	40.961825	6.73
Nitrogen	^{14}N	14.003074	99.63
	^{15}N	15.00011	0.37
Oxygen	^{16}O	15.994915	99.76
	^{18}O	17.999159	0.20
Sulfur	^{32}S	31.972071	95.02
	^{33}S	32.971459	0.75
	^{34}S	33.967867	4.21

[a] Mass values are for reference only.

[b] Isotopes with a natural abundance below 0.20% are not listed. Percent abundance values are for reference only.

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