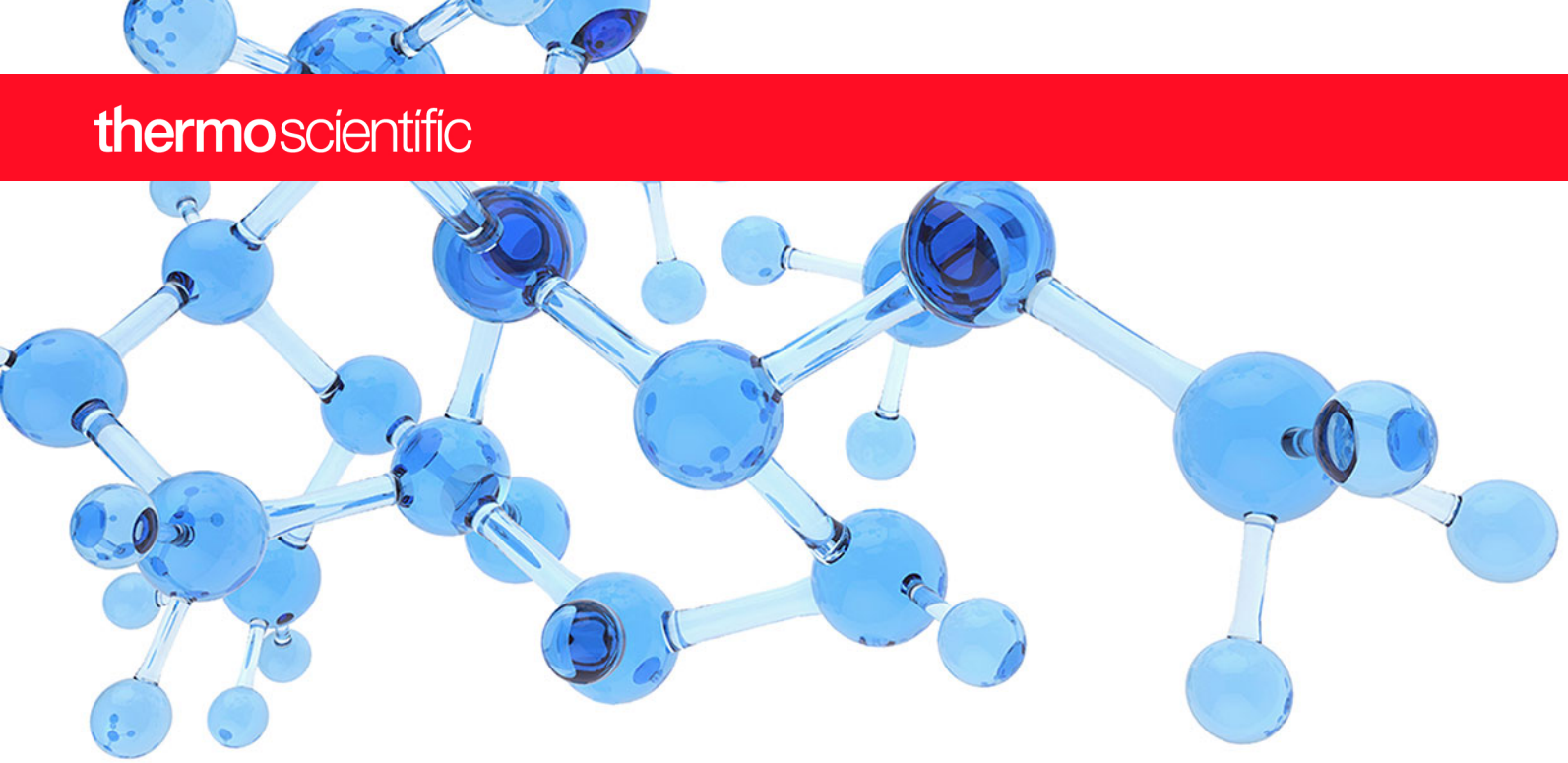
thermoscientific



Thermo

Mass Frontier

User Guide

Software Version 8.1

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ThermoFisher SCIENTIFIC

Release history: April 2023

Software version: Mass Frontier 8.1; Server Manager 8.1

For Research Use Only. Not for use in diagnostic procedures.

Contents

Preface

This guide describes how to use the Thermo Mass Frontier™ application to manage, evaluate, and interpret mass spectra.

Contents

To familiarize yourself with the Mass Frontier application, follow the tutorials that are available from the application Help menu.

Related documentation

The Mass Frontier 8.1™ application includes these manuals and tutorials as PDF files:

- *Mass Frontier 8.1 User Guide*
- *Mass Frontier 8.1 Installation Guide*
- *Mass Frontier 8.1 tutorial to Automatically Annotate Spectral Trees Using a Generated List of Fragment Structures*
- *Mass Frontier 8.1 tutorial to Curate Spectral Trees for User Libraries*
- *Mass Frontier 8.1 tutorial to Save Components to Spectral Libraries*
- *Mass Frontier 8.1 tutorial to Detect Structurally-Related Compounds with the FISh Algorithm*
- *Mass Frontier 8.1 tutorial to Identify Unknowns by Library Search and mzLogic (HRAM)*
- *Mass Frontier 8.1 tutorial to Identify Unknowns by Library Search and mzLogic (Nominal Mass)*

- *Mass Frontier 8.1 tutorial to Create a New Metabolika Pathway*

❖ To view the Mass Frontier manuals and tutorials

From the Start menu, click **Help** to view the available manuals and tutorials in PDF format.

–or–

In the upper right corner of the application window, click the Help icon,  . Then, select a PDF from dropdown list.

❖ To view user documentation from the Thermo Scientific website

1. Go to thermofisher.com.
2. In the Search box, type the name of the product, for example, Mass Frontier.
3. On the left, click **Documents & Support**.
4. From the results list, click the title to open the document in your web browser, save it, or print it.

   To return to the document list, click the browser **Back** button.

System requirements

The following table lists the minimum and recommended system configuration requirements for using the Mass Frontier 8.1 and Server Manager 8.1 applications.

Table 1 lists the hardware and software requirements for the processing computer.

Table 1. Hardware and software requirements for the processing computer

System	Minimum requirements	Recommended requirements
Hardware	• Intel compatible processor with a minimum speed of 1 GHz. • 8GB RAM • 30GB of disk space • Monitor resolution display of 1024 x 768pixels. • DVD-ROM drive (This is optional and not required if you can download from Flexera).	• Intel compatible processor with a minimum speed of 2.5 GHz. • 32GB RAM • 100GB of solid-state drive disk space • Dual monitor resolution display of 1920 x 1080pixels. DVD-ROM drive (This is optional and not required if you can download from Flexera).
Software	• Microsoft Windows™ 10 (English) 64bit. • Microsoft™ .NET Framework 4.7.2 • Adobe™ Acrobat Reader DC • Google Chrome version 81.0 or higher.	• Microsoft Windows™ 10 LTSC 64bit. • Microsoft™ .NET Framework 4.7.2 • Adobe™ Acrobat Reader DC • Google Chrome version 81.0 or higher.
System settings	• To run processing workflows with online mass spectral database searches, the computer must have an unblocked access to the mass spectral data via the internet . • The time and date settings in computer must be synchronized with the internet time. • Set the Region and Language setting to English (United States).	

Note Ensure that you have a Windows account with administrator rights to do the following:

- Install the Mass Frontier and Server Manager applications. For information on how to install Mass Frontier software, refer to the *Mass Frontier 8.1 Installation Guide*.
- Create, copy, and delete spectral libraries.

License activation or deactivation

Use the Thermo Scientific product licensing wizard to activate or deactivate the license for the Mass Frontier 8.1 application. To activate the license, you must have an activation code from Thermo Fisher Scientific.

Note For software download and licensing questions, contact support at ThermoMSLicensing@thermo.com.

To activate the Mass Frontier 8.1 software, do the following:

1. After the installation is completed, start the application from the desktop icon or Start menu.
2. Choose the appropriate licensing option:
   - For the demo version of the Mass Frontier 8.1 application, use the 60-day trial license that comes preinstalled.

   –or–

   - For purchasers of the Mass Frontier 8.1 application, set a permanent license using the activation code provided through your Flexera™ account. You cannot use a license key from an earlier version.

     You will receive an e-mail from Thermo MS licensing with subject line "**Mass Frontier 8.1 software is now ready for download!**"This e-mail the information for activation code.
3. Choose **Mass Frontier Start menu > About > License** and click **Activate License** to display the Product Licensing wizard and follow the on-screen instructions.

   For more information about trial and permanent licenses, click **Help** on the License Activation page of the wizard.

❖ Locate or request the activation code

If you have not received the activation code for Mass Frontier 8.1 software, follow this procedure:

1. Check your Junk e-mail folder.
2. If the e-mail is not in your junk email folder, log in to your account at the following URL: https://thermo.flexnetoperations.com
   - In the left navigation pane, under **Software & Services,** click **Order History.**
   - In the list of ordered products, click the order number to see the activation code.
3. If you cannot find your account, send an e-mail message to Licensing at ThermoMSLicensing@thermofisher.com with the following information in the body of the email.
   - Software application: Mass Frontier 8.1 software.
   - End user name
   - End user e-mail
   - Sale order or purchase order number

After you activate a permanent license, the Deactivate button appears. When you need to transfer the permanent license from one computer to another computer, click the Deactivate button.

IMPORTANT Depending on the features that you purchased in the Mass Frontier 8.1 software, your permanent license covers one of three options:

- Option 1: Mass Frontier 8.1 Base + Curator
- Option 2: Mass Frontier 8.1 Base
- Option 3: Curator Only

The license keys control the different features in the software and determine which ones are active. For example, for option 2, all Mass Frontier 8.1 base modules are active when you apply the license key, but the Curator module is inactive. You can purchase option 3 to activate the Curator module at a later date.

Special notices

Make sure you understand the special notices, symbols, and caution labels in this guide. Most of the special notices and cautions appear in boxes. Some symbols are also marked on the instrument itself and can appear in color or in black and white. For complete definitions, see the following table.

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.

Contact us

Contact	Email	Telephone	QR Code
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	
E.U. Technical Support	eu.techsupport.cms@thermofisher.com		

Contact	Email	Telephone	QR Code
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, if displayed, type the catalog number or product name. 4. Select the display location to view information for the best team to help you with that product. 5. 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.		

Note To provide feedback for this document, go to surveymonkey.com/s/PQM6P62 or send an email message to Technical Publications (techpubs-lcms@thermofisher.com).

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Introduction

The Mass Frontier application is a software package for the management, evaluation, and interpretation of mass spectra. To manage the large amount of mass spectral and chromatographic data, the application provides various tools and features. The application can interpret mass spectra even when it does not find a matching spectrum in your user library, as it includes the HighChem Fragmentation Library™ and an interface to the online New mzCloud™ mass spectral database.

Contents

File format changes in Mass Frontier 8.1

This topic lists the file and data format compatibility between Mass Frontier 7.0 and Mass Frontier 8.0 and Mass Frontier 8.1.

The parameter files that do not contain source data, such as FISh of Fragmentation parameter files, are not compatible between Mass Frontier 7.0, Mass Frontier 8.0, and Mass Frontier 8.1.

Table 2. File format changes (Sheet 1 of 3)

File type	File format	File suffix	Read	Write	Description
Structure	Compressed structure	.mcs	MF 7.0	MF 7.0	Chemical structure in native HighChem format
			MF 8.0	MF 8.0	
			MF 8.1	–	
	Template structure	.tml	MF 7.0	MF 7.0	Chemical template structure in native HighChem format
			MF 8.0	MF 8.0	
			MF 8.1	–	
	MDL mol file	.mol	MF 7.0	MF 7.0	Chemical structure in MDL mol format (V2000)
			MF 8.0	MF 8.0	
			MF 8.1	–	
Collection of structures	MDL sdf file	.sdf	MF 7.0	MF 7.0	Collection of chemical structure in MDL sdf format (V2000)
			MF 8.0	MF 8.0	
			MF 8.1	MF 8.1	
Mass spectra	MSP file	.msp	MF 7.0	MF 7.0	Collection of spectra in NIST MSP format
			–	–	
			MF 8.1	–	
	JDX File	.jdx	MF 7.0	–	Collection of spectra in JCAMP-DX format
			–	–	
			–	–	

Table 2. File format changes (Sheet 2 of 3)

File type	File format	File suffix	Read	Write	Description
Chromatogram	RAW file	.raw	MF 7.0	MF 7.0	Thermo Fisher Scientific chromatogram
			MF 8.0	MF 8.0	
			MF 8.1	MF 8.1	
	mzML file	.mzml	–	–	mzML chromatogram
			MF 8.0	–	
			MF 8.1	–	
	JDX file	.jdx	MF 7.0	–	Collection of spectra in JCAMP-DX format
			–	–	
			–	–	
	GCM file	.ms	MF 7.0	–	Thermo GCM chromatogram format
			–	–	
			–	–	
	ChemStation file	.ms, .D	MF 7.0	–	HP ChemStation chromatogram format
			–	–	
			–	–	
	HighChem file	.hcc	MF 7.0	MF 7.0	HighChem chromatogram format
			–	–	
			–	–	
	HighChem file	.hccx	–	–	HighChem chromatogram format
			MF 8.0	MF 8.0	
			MF 8.1	MF 8.1	
Database items	Backup file	.dbbak	MF 7.0	MF 7.0	HighChem backup of Data Manager records
			MF 8.0	–	
			MF 8.1	–	
	Backup file	.dbbakx	–	–	HighChem backup of Data Manager records
			–	–	
			MF 8.1	MF 8.1	

Table 2. File format changes (Sheet 3 of 3)

File type	File format	File suffix	Read	Write	Description
SledgeHammer	Reactions file	.mcr	MF 7.0	MF 7.0	HighChem compressed reactions format
			MF 8.0	–	
			MF 8.1	–	
	Reactions file	.mechanisms	–	–	–
			MF 8.0	MF 8.0	HighChem compressed reactions format
			MF 8.1	MF 8.1	
	Reaction restrictions	.rrs	MF 7.0	MF 7.0	Parameters of Fragments & Mechanisms generation
			–	–	
			–	–	
	Reaction restrictions	.fragres	–	–	Parameters of Fragments & Mechanisms generation
			MF 8.0	MF 8.0	
			–	–	
	Reaction restrictions	.hammer	–	–	Parameters of Fragments & Mechanisms generation
			–	–	
			MF 8.1	MF 8.1	

[1]MF 8.0 spectral libraries must be migrated to MF 8.1 format in Mass Frontier Server Manager 8.1 before they can be used in Mass Frontier 8.1. See "Migrate spectral libraries" on page 390.

[2]The Mass Frontier 8.1 does not support the migration of the Mass Frontier 7.0 libraries. However you can connect and view the Mass Frontier 7.0 libraries if you have the Mass Frontier 7.0 software installed on your computer or network.

[3]If you wish migrate Mass Frontier 7.0 libraries to Mass Frontier 8.1, install the trial version of Mass Frontier 8.0 and then convert the libraries from Mass Frontier 7.0 to Mass Frontier 8.0. and then migrate this libraries to Mass Frontier 8.1 libraries using Mass Frontier Server Manager 8.1.

Supported file and data formats

These topics list the file and data formats that the application can process, their usage in the program, and a short description.

- Supported chromatogram formats
- Supported MS spectrum formats
- Background subtraction supported chromatogram formats
- Parameter file formats

- Supported file formats in Curator
- Supported file formats in Data Manager
- Supported file formats in Metabolika
- Supported file formats in Structure Editor
- Supported file formats in Structure Grid
- Supported file formats in the Reaction Restrictions dialog box
- Supported file formats in SledgeHammer
- Supported file formats in Server Manager

Supported chromatogram formats

Table 3. Supported chromatogram formats

Data format	Read only		Read and write
	Thermo Fisher Scientific (.raw)	mzML (.mzml)	Thermo Fisher Scientific (.hccx)
GC/MS	Limited[1]	Limited[1]	Limited[1]
HRAM	Y	Y	Y
Nominal Mass	Y	Y	Y
MS1	Y	Y	Y
MSn	Y	Y	Y
Profile	Limited[2]	Limited[3]	N
Centroid	Y	Y	Y
Stepped energy spectra	Y	N	Y
All ion fragmentation	Y	Y	Y

[1] The component detection and spectral search features are optimized for LC/MS data only. Data annotation, filtering, fragmentation, and other data processing features support both GC and LC data.

[2] The application automatically centroids profile data during the import process.

[3] Data processing includes filtering, component detection, and spectral search support of centroid spectra.

Supported MS spectrum formats

The Chromatogram Processor module can export the MS spectra to a NIST MSP file or a JCAMP-DX file.

Tip To export the scan data, do the following:

1. Select a scan from the list of scans in the chromatogram data view of a Chromatogram Processor window.
2. In the Chromatogram Processor toolbar, click **Save** and select **Scans** from the dropdown list.
3. In the Save As dialog box, name the file, select its file type, and click **Save**.

Table 4. Supported MS spectrum file types

Data format	File suffix
NIST MSP spectrum files	.msp
JCAMP-DX spectrum files	.jdx

Background subtraction supported chromatogram formats

The application can recognize the following data formats for the blank sample in the Subtract Blank Filter view.

Table 5. Background subtraction

Data format	Thermo (.raw)	Generic (.mzml)	Mass Frontier 8.0 (.hccx)
GC/MS	Limited[1]	Limited[2]	Limited[1]
LC/MS	Y	Limited[2]	Y
HRAM	Y	Limited[2]	Y
MS1	Y	Limited[2]	Y
MSn	Limited[3]	Limited[2,3]	Y
Profile	N	N	N
Centroid	Y	Y	Y

[1] Background subtraction is optimized mainly for LC/MS data.

[2] Accuracy based on analyzer and Mass Tolerance settings is applied to all the peaks in an mzML file. You can also use the Chromatogram Processor's local Force Accuracy feature or the global setting for mass tolerance to specify the mass accuracy for each detected mass analyzer.

[3] The application only uses MS1 scans for background subtraction. It skips the MSn scans during processing.

Parameter file formats

Parameter files define the parameters for one or more actions applied by the Chromatogram Processor module.

Table 6. Parameter file formats

View	File type	File suffix	Description
Command processor	Chromatogram Actions	.chpro_act	Sequence of commands for processing chromatograms with their corresponding parameter settings
Force Tolerance	Force Tolerance Parameters File	.chpro_tolerance	Forces user-defined accuracy and resolution to all the scans in Chromatogram Processor
Convert to Matrix	To Matrix Parameters File	.chpro_matrix	Converts centroid peaks to matrix form
Threshold Filter	Threshold Parameters File	.chpro_threshold	Filters peaks based on intensity
Peak Elimination Filter	Peak Elimination Filter Parameters File	.chpro_pef	Filters peaks based on *m/z* value
Contaminants Elimination Filter	Exclude Ions Parameters File	.chpro_exclude	Removes contaminant peaks
Subtract Blank Filter	Subtract Blank Parameters File	.chpro_subtract	Subtract blank chromatogram from sample
Baseline and Noise Elimination	Baseline Parameters File	.chpro_baseline	Baseline reduction
Smoothing Filter	Smoothing Parameters File	.chpro_smooth	Smoothing
Joint Components Detection (JCD)	JCD Parameters File	.chpro_jcd	Detects component by JCD method
Total Extraction Components Detection (TECD)	Total Extraction Parameters File	.chpro_tcd	Detects component by TECD method
Direct Infusion Components Detection (DICD)	Direct Infusion Parameters File	.chpro_direct	Detects component from direct infusion chromatogram
FISh Detection	FISh Parameters File	.fish2.par	Fragment Ion Search
Extracted Ion Chromatogram (XIC)	XIC Parameters File	.xic_par	Shows XIC traces
Neutral Loss Chromatogram (NLC)	NLC Parameters File	.nlc_par	Shows Neutral Ion Chromatograms

Supported file formats in Curator

Table 7. Supported file formats in the Curator Module (Sheet 1 of 2)

Data type	File type	File suffix	Read	Write	Description
Spectral Tree Records	Mass Frontier Compound Data Container	.mfcdc	Y	Y	Processed spectral tree with corresponding spectra, precursor, and peak annotations
	Curator File	.curator	Y	Y	Processed spectral tree with corresponding spectra, precursor and peak annotations and parameters settings of the completed curation actions
	Fusion Complex Component	.ccomx	Y	N	Raw spectral tree from Fusion Robot
	Mass Frontier 7.0 Database Backup	.dbbak	Y	N	Raw spectral tree with precursor structure
Structure	HighChem Structure	.mcs	Y	N	Compound's structure in HighChem native format
	HighChem Template Structure	.tml	Y	N	Compound's structure in HighChem native format
	MDL Structure	.mol	Y	Y	Compound's structure in MDL mol file format (V2000)
Structures	MDL collection of structures	.sdf	Y	Y	Collection of structures in MDL format (V2000) assigned as annotation to the spectral tree.

Table 7. Supported file formats in the Curator Module (Sheet 2 of 2)

Data type	File type	File suffix	Read	Write	Description
Parameter Files	Mass Frontier Curator Actions	.curator_act	Y	Y	Sequence of commands for processing of spectral tree with corresponding parameters.
	Clear Raw Tree Parameters File	.curator_crt	Y	Y	Parameters for action step 1: Raw Spectra Exclusion
	Select Significant Spectra Parameters File	.curator_sss	Y	Y	Parameters for action step 3: Select Significant Spectra
	Remove Resonance Peaks Parameters File	.curator_rrp	Y	Y	Parameters for action step 4: Remove Resonance Peaks
	Select Relevant Peaks Parameters File	.curator_srp	Y	Y	Parameters for action step 5: Select Relevant Peaks
	Merge Replicate Spectra Parameters File	.curator_mrs	Y	Y	Parameters for action step 6: Merge Replicate Spectra
	Assign Fragments Parameters File	.curator_afr	Y	Y	Parameters for action steps 8 and 11: Assign Fragments
	Recalibrate Parameters File	.curator_rec	Y	Y	Parameters for action step 10: Recalibrate
	Assign Molecular Formulas Parameters File	.curator_afm	Y	Y	Parameters for action step 12: Assign Molecular Formulas

Supported file formats in Data Manager

Table 8. Supported file formats in the Data Manager module

Data type	File type	File suffix	Read	Write	Description
Spectral Tree Records	Mass Frontier Compound Data Container	.mfcdc	Y	Y	Processed spectral tree with corresponding spectra, precursor, and peak annotations
	Fusion Complex Component	.ccomp	Y	N	Raw spectral tree with corresponding spectra, precursor, and peak annotations
	Mass Frontier 7.0 Database Record export file	.dbbak	Y	N	Raw spectral tree with precursor structure
	Mass Frontier 8.1 Database Record export file	.dbbakx	Y	Y	Raw spectral tree with precursor structure
Structures	MDL collection of structures	.sdf	Y	Y	Collection of structures in MDL format (V2000) assigned as annotations to the spectral tree

Supported file formats in Metabolika

Table 9. Supported file types in the Metabolika Module

Data type	File type	File suffix	Read	Write	Description
Metabolika pathway	Metabolika Drawing File	.metabolikadrw	Y	Y	Single Metabolika file with full information including colors, fonts, and so on
	Metabolika Data File	.metabolika	Y	Y	Single Metabolika file without information about graphics as colors, fonts, and so on
Structure	HighChem Structure	.mcs	Y	Y	Single structure in HighChem native format
	HighChem Template Structure	.tml	Y	N	Single structure in HighChem template format
	MDL Structure	.mol	Y	Y	Single structure in MDL mol file format (V2000)

Supported file formats in Structure Editor

Table 10. Supported file formats in the Structure Editor dialog box

Data type	File type	File suffix	Read	Write	Description
Structure	HighChem Structure	.mcs	Y	N	Single structure in HighChem native format
	HighChem Template Structure	.tml	Y	N	Single structure in HighChem template format
	MDL Structure	.mol	Y	Y	Single structure in MDL mol file format (V2000)

Supported file formats in Structure Grid

Table 11. Supported file types in the Structure Grid

Data type	File type	File suffix	Read	Write	Description
Structure	HighChem Structure	.mcs	Y	N	One structure in HighChem native format
	HighChem Template Structure	.tml	Y	N	One structure in HighChem template format
	MDL Structure	.mol	Y	Y	One structure in MDL mol file format (V2000)
Structures	MDL collection of structures	.sdf	Y	Y	Collection of structures in MDL format (V2000)

Supported file formats in the Reaction Restrictions dialog box

Table 12. Supported file types for the Reaction Restrictions dialog box

File type	File suffix	Read	Write	Description
Reaction Restrictions Parameters File	.hammer	Y	Y	Parameters of fragmentation

Supported file formats in SledgeHammer

Table 13. Supported file types for the Fragments & Mechanisms module

Data type	File type	File suffix	Read	Write	Description
SledgeHammer	All Fragments & Mechanisms	.mechanisms	Y	Y	All Fragments & Mechanisms in Mass Frontier 8.0 format
	All Fragments & Mechanisms	.mcr	Y	N	All Fragments & Mechanisms in Mass Frontier 7.0 format
Structure	HighChem Structure	.mcs	N	Y	Single structure in HighChem native format
	MDL Structure	.mol	N	Y	Single structure in MDL mol file format (V2000)
Fragments	MDL collection of structures	.sdf	N	Y	Collection of generated fragments in MDL format (V2000)

Supported file formats in Server Manager

Table 14. Supported file types for the Server Manager

User library	File suffix	Read	Write	Description
User Library	-	Y	Y	User library file format. Not backward compatible with Mass Frontier 8.0 and earlier.
User Library Backup	.backup	Y	Y	User library file backup format. Not backward compatible with Mass Frontier 8.0 and earlier.

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Get Started

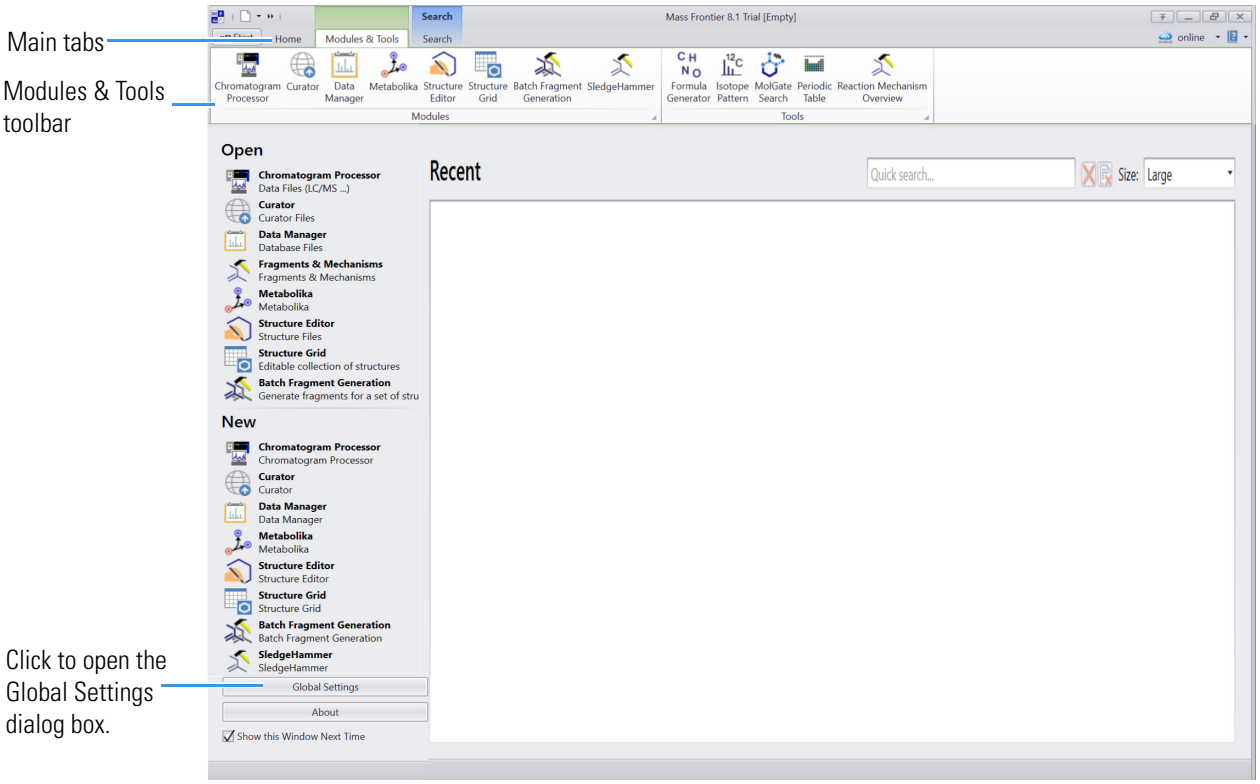
This following topics provide a general overview of the startup window, the Start menu, the modules and tools, the library search tools that you can use independently of the Chromatogram Processor module, and the window layout options.

Contents

Startup window

The first time you open the Mass Frontier application, it opens to the startup window with an active Modules & Tools toolbar. Because you have not opened any modules, the Recent area is empty (Figure 1).

Figure 1. Startup window



Start menu

Click the **Start** tab to open the Start menu.

Figure 2. Start menu with the New command selected

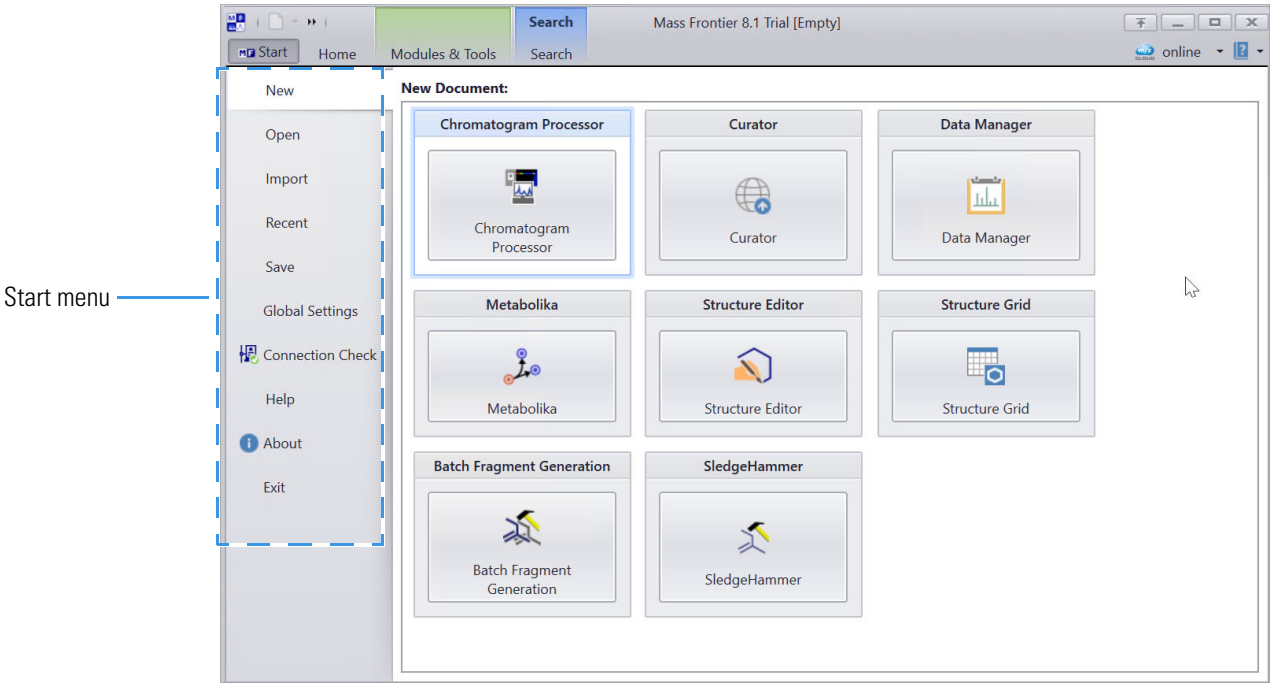


Table 15. Start menu (Sheet 1 of 2)

Command	Description
New	Displays the start buttons for the eight modules in grid format. Clicking the button for a module opens the module or a dialog box where you select the data type for the module. For the Chromatogram Processor module, the application directs you to open a chromatogram file (RAW, HCCX, or MZML). The Chromatogram Processor module opens after you open the data file.
Open	Displays a button for each of the eight modules on the right side of the application window.
Import	Opens the Import view. This view includes an Open Mass Frontier File button when a Data Manager window is open. Clicking Open Mass Frontier File opens the Import File dialog box for selecting a known data manager format: DBBAK, MFCDC, or CCOMP.
Recent	Opens the Recent view. This view displays information about each of the recently used module windows. You can click any of the window buttons to continue working in the window.
Save	Displays buttons for any opened window where you might want to save your work.

Table 15. Start menu (Sheet 2 of 2)

Command	Description
Global Settings	Opens the Global Settings dialog box.
Connection Check	Opens the Connection Check dialog box for checking your processing computer's connection to the online mzCloud mass spectral library. See "Check the connection to the mzCloud mass spectral database" on page 18.
Help	Opens links to the application's user guide, tutorials, release notes, and so on. These documents are provided as PDFs.
About	Opens the About Mass Frontier dialog box.
Exit	Closes the application.

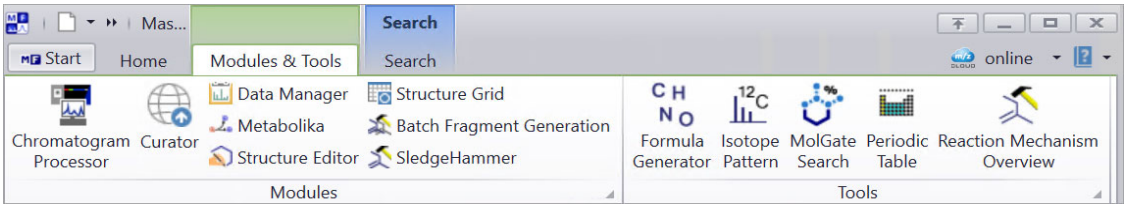
Modules and tools

To explore the modules and tools provided in the Mass Frontier application, see these topics:

- Modules
- Tools

You can access any of the modules or tools from the Modules & Tools toolbar.

Figure 3. Modules & Tools toolbar



Modules

The eight modules are the core functional blocks for processing a module-specific data type; for example, a chemical structure or a chromatogram. There are two types of modules: modules for creating new data types, such as drawing a new chemical structure, and modules that use input data, such as a chromatogram file.

You can use each module independently or as part of a workflow with other modules. For example, you can open a raw data file and detect and identify components in the Chromatogram Processor module, send identified components to the Curator module where you can curate their spectral trees (recalibrate the spectral peaks and remove noise), save the components to a custom user library, and then open a Data Manager window where you can add the saved components to the custom library.

Each module processes a specific data type and provides access to related functionalities in other modules. The Home toolbar displays different buttons and icons for each module—that is, when you open multiple modules as tabbed pages in the application window, the Home toolbar tracks with the active page. If you float a module's window, the Home toolbar becomes part of the window.

Tip For information about commonly performed tasks and workflows, such as detecting components, searching spectral libraries, creating your own custom libraries, creating Metabolika pathways, and so on, refer to the tutorials.

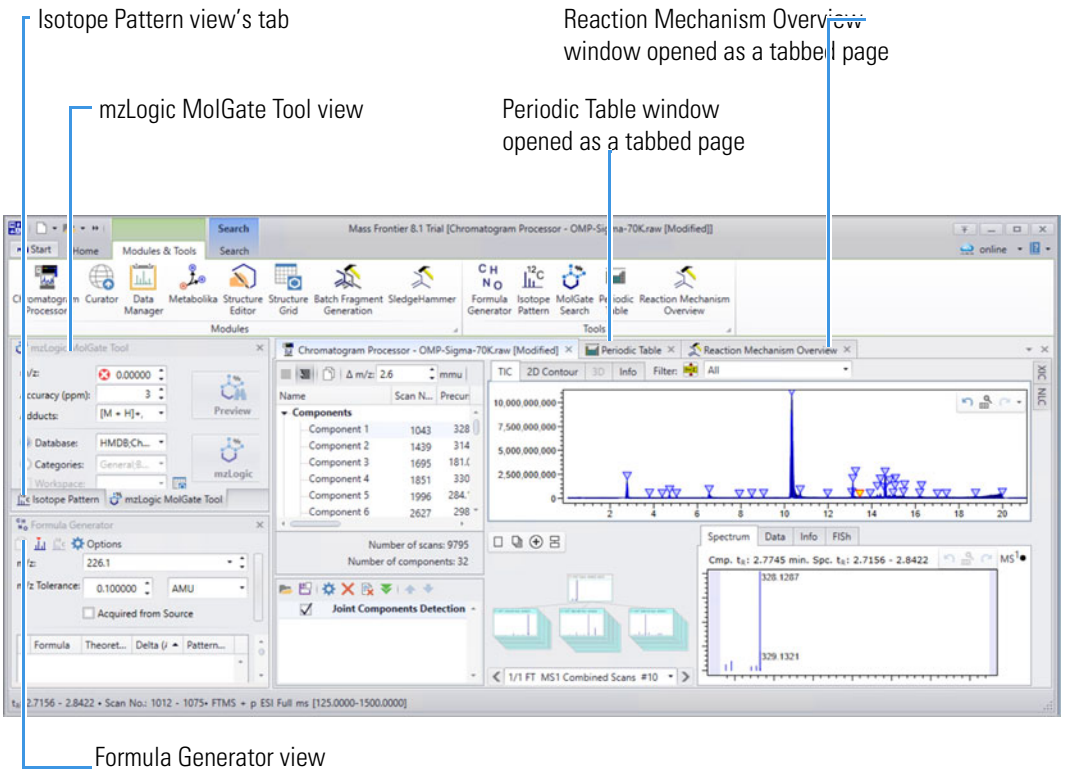
Tools

The tools that you can open by clicking the respective buttons in the Tools group of the Modules & Tools toolbar provide additional analytical or interpretation functionality. By default, the Formula Generator, Isotope Pattern, and mzLogic Search views open to the left of the tabbed module pages, and the Periodic Table and Reaction Mechanism windows open as tabbed pages. Unlike the modules, you can open only one instance of a tool.

Table 16. Mass Frontier Tools

Toolbar button	Description
Formula Generator	Opens the Formula Generator view for generating possible formulas for a specific *m/z* value. See "Formula Generator tool" on page 317.
Isotope Pattern	Opens the Isotope Pattern tool for displaying the simulated isotope pattern of an adduct ion from a user-specified formula or structure, adduct, charge, resolution, and intensity threshold. See "Isotope Pattern tool" on page 324.
MolGate Search	Opens the mzLogic MolGate Tool view for running a MolGate search for structures that match a user-specified *m/z* value and adduct species. You can send the structures to a structure grid from this view. See "MolGate Search tool" on page 328.
Periodic Table	Opens the Periodic Table window for viewing the isotopes of the elements. See "Periodic Table tool" on page 330.
Reaction Mechanism Overview	Opens the Reaction and Mechanism Overview window.

Figure 4. Application window with all five tools opened



Library searches

In the Mass Frontier application, you can run library searches from the Search tab or from within any module that includes an MS spectrum view.

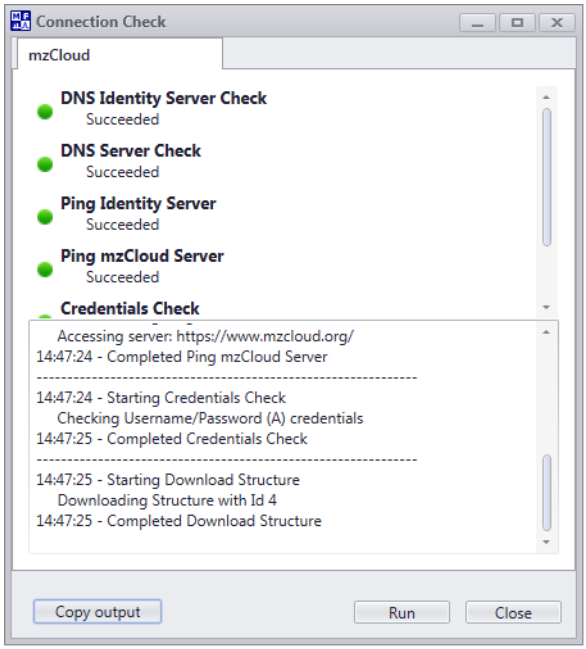
Check the connection to the mzCloud mass spectral database

Before you run a library search against the online mzCloud mass spectral database, check your computer's connection to the mzCloud server.

❖ To check the connection

1. Do one of the following:
   - In the Start menu, click **Connection Check**.
   - In the upper right corner of the application window, click online and choose **Connection Check**.
2. In the Connection Check dialog box, click **Run**.

Figure 5. Connection Check dialog box showing a successful connection



3. If the connection check fails, check the following:
   - The computer's physical or wireless connection to the Internet.
   - The computer's date and time setting. The computer must be synchronized with Internet time.

Modify the layout of the application window

You can dock and float any document (tabbed page), and some of the views.

- To dock a floating view or pane, drag the title bar to the location you want.
- To add a view or pane as a new tab in an existing tab group, drag and drop into the tab group.
- To float a view or pane, drag it away from the docked location.

Application tutorials

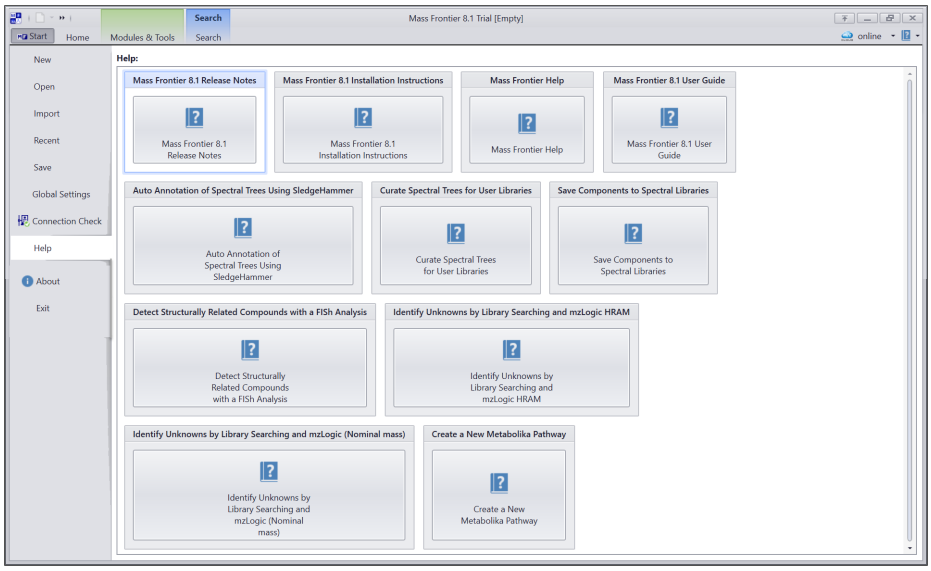
If you are unfamiliar with the Mass Frontier application, Thermo Fisher Scientific recommends that you follow the tutorials, which guide you through the basic workflows by using the demo data provided with the application.

The demo data is located in the following folder:

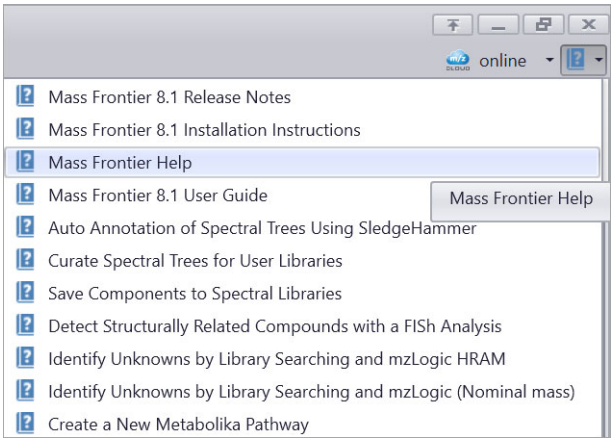
drive:\Users\Public\Public Documents\Highchem\Mass Frontier 8.1\Demo Data

You can access the user documentation for the Mass Frontier application in two ways:

- From the Start menu, choose **Help**. Then, click the link to the document of interest.



- In the upper right corner of the application window, click the **Help** icon,  . Then, select a PDF or Help file from the dropdown list.



3

Chromatogram Processor module

Use the Chromatogram Processor module to open data files from infusion or LC/MS experiments and to perform component detection, spectral deconvolution, spectral averaging, background subtraction, FISh (fragment ion search) detection, library searches, and MolGate and mzLogic analyses. This module can process Xcalibur RAW files, chromatogram files (HCCX files created with the Mass Frontier application), or mzML files from third-party mass spectrometers.

Contents

- Joint Component Detection (JCD) algorithm
- Total Ion Extraction Component Detection (TECD) algorithm
- Direct Infusion Components Detection (DICD) algorithm
- FISh analysis
- Component library searches
- mzLogic searches
- Extracted Ion Chromatogram (XIC) filter
- Neutral Loss Chromatogram (NLC) filter
- Generate reports

Open a Chromatogram Processor window

❖ **To open a new Chromatogram Processor window**

1. Do one of the following:

   - From the application Start menu, choose **Open**, and then click **Chromatogram Processor** on the right. Or, from the application Start menu, choose **New**, and then click **Chromatogram Processor** on the right.

   –or–

   - In the Modules & Tools toolbar, click **Chromatogram Processor**.

   The Open Chromatogram dialog box appears.

2. In the Open Chromatogram dialog box, select any of these file types:

   - HCCX—With the exception of the profile data, this file type includes all the data from the original raw data file and the results of any applied processing actions from the Chromatogram Processor module.
   - MZML—This file type includes the spectral data acquired with a third-party data system.
   - RAW—This file type is the proprietary file type that a Thermo Fisher Scientific data system generates during data acquisition.

   Note Large data files can take a significant time to load. The status bar at the bottom of the application window provides information about the loading progress, from reading the scan data to building the scan tree.

A new instance of the Chromatogram Processor module opens as a tabbed document with four views:

- The chromatogram data view at the upper left lists the scan data by scan level and number.

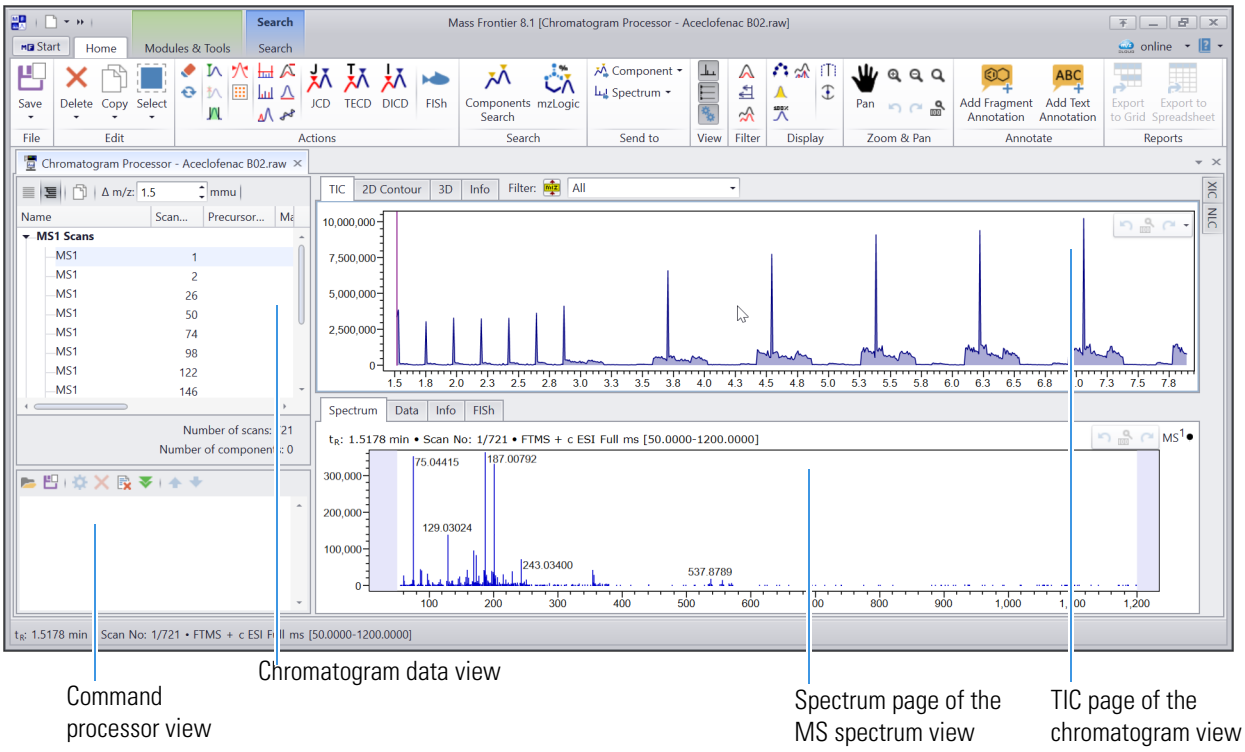
  Note Applying a component detection algorithm to the chromatogram adds a list of detected components to this view.

- The chromatogram view at the upper right displays the total ion current (TIC) chromatogram. The *y*-axis scale is set to absolute intensity.

  Tip To change the scale from absolute counts to relative intensity (versus the base mass spectrum peak), right-click the view and choose **Show Absolute Intensities**.

- The MS spectrum view at the lower right displays the first scan in the raw data file.
- The command processor view at the lower left is empty until you apply actions to the chromatogram.

Figure 6. TIC chromatogram and scan number 1 for the selected raw data file



Show or hide the Chromatogram Processor views

To show or hide the views in a Chromatogram Processor window, click the following icons in the View area of the Chromatogram Processor toolbar:

- For the MS spectrum view, click the **Show MS Spectrum** icon, .
- For the chromatogram data view, click the **Show Chromatogram Data** icon, .
- For the command processor view, click the **Show Command Processor** icon, .

You cannot hide the chromatogram view.

Zoom and scroll controls for the chromatogram and spectrum plots

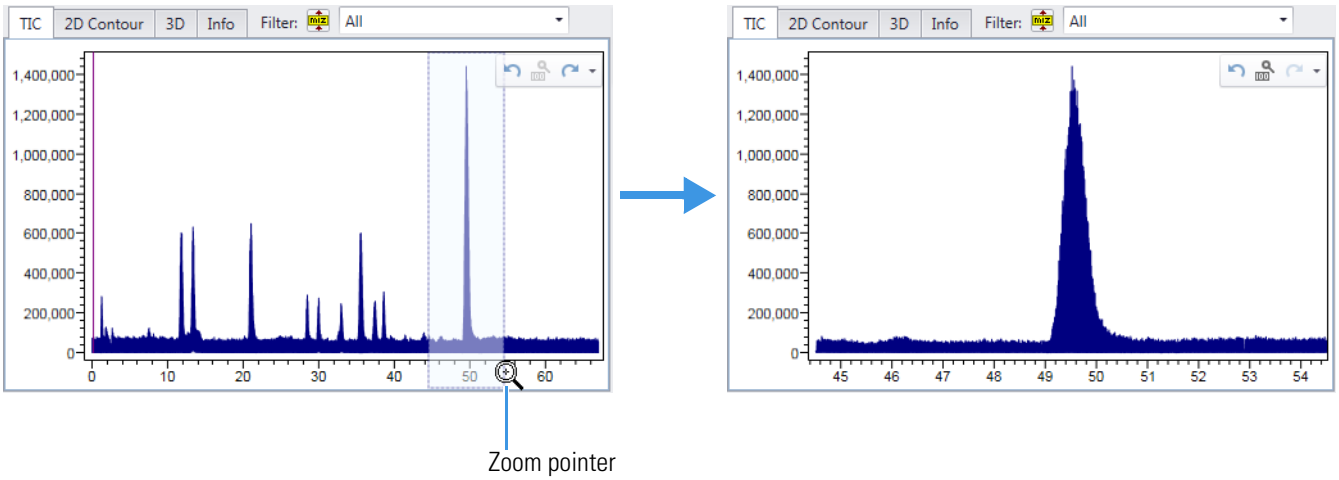
In a Chromatogram Processor window, you can zoom in or out of and scroll through the chromatogram and spectrum plots in several ways:

- Use the mouse pointer to zoom in on the x axis
- Use the ALT key to scroll the x axis
- Use the CTRL key to zoom in or out of the plot
- Use the Zoom toolbar

Use the mouse pointer to zoom in on the x axis

To zoom in on a specific retention time range in a chromatogram, drag the mouse pointer across the time range (*x* axis) and release. To zoom in on a specific *m/z* range in a spectrum, drag the mouse pointer across the *m/z* range (*x* axis) and release.

Figure 7. Zooming in on a specific retention time range



Use the ALT key to scroll the x axis

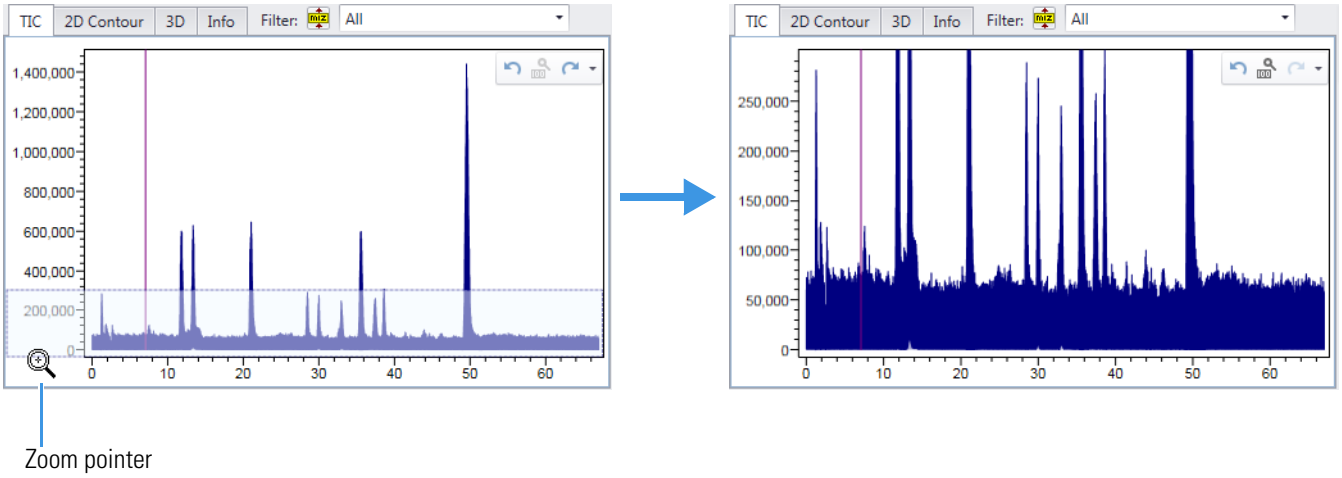
❖ **To scroll left or right through the x axis of a plot**

1. Zoom in on an *x*-axis range of the plot.
2. While pressing the ALT key, use the mouse wheel to scroll left or right through a time range or an *m/z* range.

Use the mouse pointer to zoom in on the y axis

To zoom in on a specific abundance range, drag the mouse pointer across the *y* axis.

Figure 8. Zooming in on a specific abundance range



Use the SHIFT key to scroll the y axis

1. Zoom in on a *y*-axis range of the plot.
2. While pressing the SHIFT key, use the mouse wheel to scroll Up or Down the *y* axis.

Use the CTRL key to zoom in or out of the plot

❖ **To zoom in on a rectangular section (*x* and *y* range) of a plot**

1. Point to the region of the plot that you want to zoom on. For example, point to a specific peak in a chromatogram plot.
2. While pressing the CRTL key, use the mouse wheel to zoom in or out of the plot.

Use the Zoom toolbar

Spectrum plots and chromatogram plots have a zoom toolbar in the upper right corner.

Table 17. Zoom controls

Tooltip	Icon	Description
Undo		Reverts the last zooming action.
Reset Zoom		Resets Zoom to full size.
Redo		Repeats the last zooming action.

Window states

The tab or title bar for a Chromatogram Processor module lists the file name of the opened chromatogram file and the module's current state.

A Chromatogram Processor module can be in one of five processing states:

- Locked—The module is loading or processing data.
- Read Only—You have initiated an action that is still in progress.
- Modified & Read Only—The module has completed an action and is waiting for you to accept the processed results.
- Modified—The module has completed an action and you have accepted the processed results.

When you open a new instance of the Chromatogram Processor module, it goes through the Locked and Read Only states as it reads and loads the selected file:

After the file loads and before you perform an action, the tab or title bar does not include a state.

Use the Chromatogram Processor with other modules

In the Chromatogram Processor module, you can work with other modules as follows:

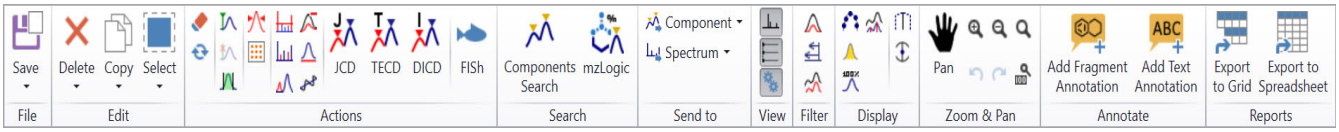
- Select a single spectrum for a Spectrum Search or an mzLogic analysis.
- Send a single component to a New Curator window.
- Send selected components to an existing or New Data Manager window.
- Load the *m/z* value of any structure or adduct from any open Structure Grid window to display XICs in the chromatogram view.
- Copy and paste a spectrum to a spectral tree in a Data Manager window.

Chromatogram Processor toolbar

The Chromatogram Processor toolbar contains the following toolbar groups:

- File toolbar group
- Edit toolbar group
- Actions toolbar group
- Search group
- Send To group
- View group
- Filter group
- Display group
- Zoom & Pan group
- Annotate group
- Reports group

Figure 9. Chromatogram Processor toolbar



File toolbar group

Table 18 describes the commands in the File toolbar group of the Chromatogram Processor toolbar.

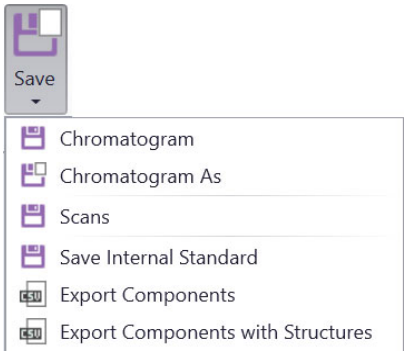


Table 18. File toolbar group

Command	Description
Save > Chromatogram	Saves the current chromatogram with the processing metadata into the HCCX file format to the current location by default or another location that you select.
Save > Chromatogram As	Saves the current chromatogram with the processing metadata into the HCCX file format to any location and prompts you when saving the file will overwrite an existing file.
Save > Scans	Exports individual chromatogram scans to the NIST (.msp) or JCAMP-DX (.jdx) file format for third-party software compatibility.
Save > Export Components	Exports the Component and Scan List into plain-text format (.txt, .csv, or .dat).
Save > Export Components with Structures	Exports the Component and Scan lists to plain-text format (.txt, .csv, or .dat) files with the accepted structure in InChI format.
	Tip To open the file in the Excel spreadsheet application, select .txt as the file type. The Excel application uses the column separator defined in the Windows settings under **Region and Language > Formats > Additional Settings**.

Edit toolbar group

Table 19 describes the commands in the Edit group of the Chromatogram Processor toolbar.

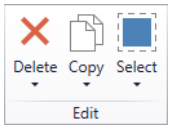


Table 19. Chromatogram Processor – Edit commands (Sheet 1 of 2)

Command	Description
Delete > Selection	Deletes the selection.
Delete > All Annotations	Deletes text and structure annotations.
Delete > All Text Annotation	Deletes text annotations.
Delete > All Structure Annotations	Deletes structure annotations.
Copy > Selection	Copies selection to the Clipboard.
Copy > Chromatogram	Copies the chromatographic data to the Clipboard as a table with two columns: total ion current (TIC) and retention time (T_R) values.

Table 19. Chromatogram Processor – Edit commands (Sheet 2 of 2)

Command	Description
Copy > Tree	Copies an image of the spectral tree to the Clipboard.
Copy > Spectrum	Copies the selected single spectrum to the Clipboard as a list of *m/z* values with their abundance, resolution, and flags.
Copy > Profiles	Copies the scan number, TIC, and retention time for each scan to the Clipboard as a list.
Copy > Spectrum to Legacy mzCloud	Copies the selected single spectrum in a format that the Legacy mzCloud web search interface supports.
Copy > Spectrum to New mzCloud	Copies the selected single spectrum in a format that the New mzCloud web search interface supports.
Select > All Scans and Components	Selects all the scans and components in the Components list.
Select > All Scans	Selects all the scans in the Components list.
Select > All Components	Selects all the components in the Components list.
Select > Scans	Selects the scans using click and drag along the *x* axis.
Select > Components	Selects the components using click and drag along the *x* axis.

Actions toolbar group

Table 20 describes the icons in the Actions group of the Chromatogram Processor toolbar.

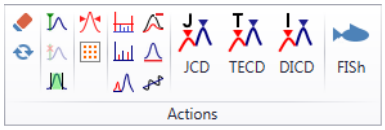


Table 20. Chromatogram Processor – Actions group (Sheet 1 of 3)

Tooltip	Icon	Description
Clear chromatogram		Removes all the components, XIC, background marks, and additional calculated properties from the chromatogram. The application saves the annotations and does not revert the scans to the initial state. To restore the modified scans, click the **Reload Original Chromatogram File** icon.
Reload original chromatogram file		Discards all changes to the chromatogram and reverts to the initial state.

Table 20. Chromatogram Processor – Actions group (Sheet 2 of 3)

Tooltip	Icon	Description
Add a scan to the background subtraction filter		Defines one or two scans used for manual background subtraction. These scans are used to eliminate the background signal from an active scan or from the average of spectral scans. The green vertical background scans are indicated by green vertical lines.
Remove the subtraction filter		Clears the background subtraction filter.
Average scans in the chromatogram		Averages scans from a selected time interval and displays the average spectrum in the MS spectrum view.
Force mass accuracy		Overrides the per-spectra accuracy calculated from the input file with a custom value.
Convert chromatogram to matrix form		Aligns ions across the chromatogram to compensate for minor *m/z* oscillations. This algorithm is an internal part of several chromatogram processing utilities, such as Baseline Correction and Noise Elimination, Smoothing, and the JCD Component Detection algorithm.
Apply threshold filter to all scans in the chromatogram		Calculates the chromatogram noise level and removes signal (spectral peaks) below the threshold. In contrast to the baseline filter, which processes the data in a chromatographic time domain, you can independently analyze and modify individual scans and remove individual spectral peaks. See "Threshold filter" on page 85.
Peak elimination filter		Removes signals (spectral peaks) that do not belong to the elemental composition or its subset. See "Peak Elimination filter" on page 79.
Remove contaminants from chromatogram		Opens the Contaminants Elimination Filter view for removing characteristic signals from common LC contaminants. See "Contaminants Elimination Filter" on page 76
Subtract blank chromatogram		Aligns the blank experiment chromatogram file and subtracts it from the active chromatogram. See "Background subtraction" on page 63.

Table 20. Chromatogram Processor – Actions group (Sheet 3 of 3)

Tooltip	Icon	Description
Baseline filter		Reduces baseline noise by analyzing the ion profiles (ion chromatograms) of all the ions appearing in the scans over the entire region of a chromatogram (processes data in a chromatographic time domain). In contrast to the threshold filter, where the individual scans and their spectral peaks are independently analyzed and modified, the baseline filter analyzes and modifies spectral peaks in a specified retention time range. See "Baseline correction and noise elimination filter" on page 69.
Apply smoothing filter to all the scans in the chromatogram		Performs smoothing for every ion (not the total ion chromatogram) found in the data file with the neighbors in a time series (time domain).
Apply Joint Components Detection		Opens the JCD view for specifying the component detection settings. See "Joint Component Detection (JCD) algorithm" on page 92
Total Ion Extraction Component Detection		Opens the TECD view for specifying the component detection settings. See "Total Ion Extraction Component Detection (TECD) algorithm" on page 101
Direct Infusion Component Detection		Opens the DICD view for specifying the component detection settings for infusion data. See "Direct Infusion Components Detection (DICD) algorithm" on page 103
FISh analysis		Opens the FISh view for specifying the FISh model and component detection settings. See "FISh analysis" on page 105

Search group

Table 21. Chromatogram Processor – Search toolbar group

Button	Description
Components Search	Opens the Component Search view for searching the selected spectral libraries for detected components. See "Component library searches" on page 119
mzLogic	Opens the mzLogic view. Combines an *m/z* structure search in MolGate with an mzCloud similarity search for the putative identification of detected components. See "mzLogic searches" on page 135

Send To group

The Send To toolbar group contains two dropdown menus: Component and Spectrum.

Table 22. Chromatogram Processor – Send To toolbar group (Sheet 1 of 2)

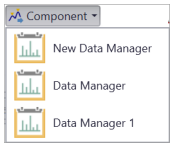
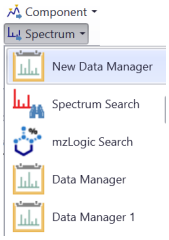
Menu command	Description
Component > New Curator	Sends one or more selected components to a Curator window.
Component > New Data Manager	Sends one or more selected components to a New Data Manager window or to any Data Manager window that is already open in Mass Frontier software.  For instance, the above figure displays options to send the components to a New Data Manager, or to the Data Manager or Data Manager1 windows which are open.

Table 22. Chromatogram Processor – Send To toolbar group (Sheet 2 of 2)

Menu command	Description
Spectrum > New Data Manager	Sends a query spectrum to the New Data Manager window or to any Data Manager window that is already open in Mass Frontier software.  For instance, the above figure displays options to send the spectrum to a New Data Manager, or to the Data Manager or Data Manager1 windows which are open.
Spectrum > Spectrum Search	Sends a query spectrum to the Spectrum Search dialog box where you can search for a matching or similar spectrum in the New mzCloud libraries and your user libraries. See "Spectrum searches" on page 348.
Spectrum > mzLogic Search	Opens the (MS^2 or MS^n) query spectrum in the mzLogic MolGate Tool view.

View group

Table 23. Chromatogram Processor – View toolbar group

Tooltip	Icon	Description
Show MS Spectrum		Displays or hides the MS spectrum view.
Show Chromatogram Data		Displays or hides the chromatogram data view.
Show Command Processor		Displays or hides the command processor view.

Related Topics

- Show or hide the Chromatogram Processor views
- Open a Chromatogram Processor window

Filter group

Table 24. Chromatogram Processor – Filter toolbar group

Tooltip	Icon	Description
Show Extracted Ion Chromatograms (XIC)		Opens and pins the Extracted Ion Chromatogram pane where you can select the *m/z* ranges of the chromatograms that you want to show or hide in the chromatogram view.
Show Neutral Loss Chromatograms		Shows or hides the individual neutral loss profiles in the chromatogram.
Show or Hide Base Peaks of the Chromatogram		Shows or hides the ion profile of the base peak in the chromatogram. A base peak is the most intense peak in a scan.

Display group

Table 25. Chromatogram Processor – Display toolbar group (Sheet 1 of 2)

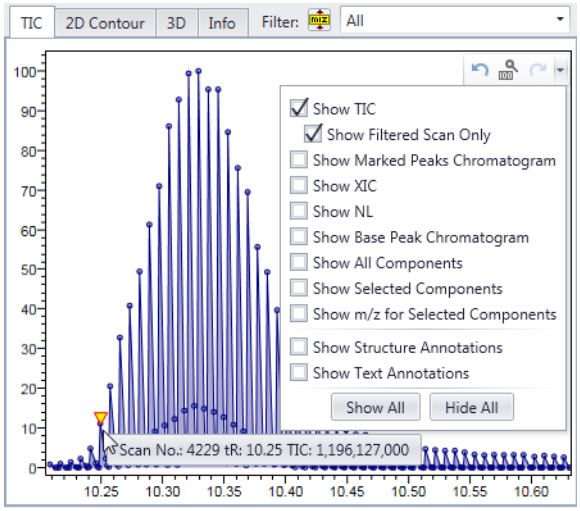
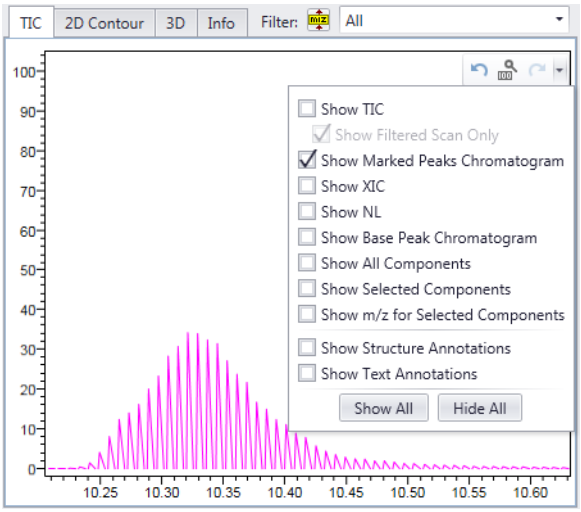
Tooltip	Icon	Description
Show scan points as dots in a chromatogram		Shows or hides dots that mark the individual scans that make up a chromatogram in the chromatogram view. 
Fill area under curves in a chromatogram		Shows or hides color filling of the chromatogram peaks.
Absolute/Relative Intensities		Switches between relative intensity and absolute intensity on the *y* axis of the chromatogram and spectrum views.

Table 25. Chromatogram Processor – Display toolbar group (Sheet 2 of 2)

Tooltip	Icon	Description
Marked Peaks Chromatogram		Shows or hides the ion profile of the marked peaks (chromatographic peaks) that match the FISh fragments in the chromatogram. A FISh chromatogram is a TIC chromatogram calculated from the *m/z* peaks for the specified FISh model. By default, the application displays calculated TIC chromatograms in pink. 
Show Accuracy		Shows or hides the accuracy of individual scans in the chromatogram view and MS spectrum view.
Show Resolution		Shows or hides the experimental resolution in the MS spectrum view.

Zoom & Pan group

Table 26. Chromatogram Processor – Zoom & Pan toolbar group (Sheet 1 of 2)

Tooltip or label	Icon	Description
Pan		Moves zoomed-in window.
Zoom In		Zooms In.
Zoom Out		Zooms Out.

Table 26. Chromatogram Processor – Zoom & Pan toolbar group (Sheet 2 of 2)

Tooltip or label	Icon	Description
Zoom Area		Selects the zoom area.
Zoom Undo		Reverts the last zooming action.
Zoom Redo		Repeats the last zooming action.
Zoom Reset		Resets zoom to full size.

Annotate group

Table 27. Chromatogram Processor – Annotate toolbar group

Button	Description
Add Fragment Annotation	Assigns a custom structure annotation to the chromatogram scans.
Add Text Annotation	Assigns a custom text annotation to the chromatogram scans.

Reports group

Table 28. Chromatogram Processor – Reports toolbar group

Button	Description
Export to Grid	Exports the components and accepted search results to a spreadsheet with collapsible scan groups for each component. See "Generate reports" on page 146.
Export to Spreadsheet	Exports the components and accepted search results to a spreadsheet with one row per scan. See "Generate reports" on page 146.

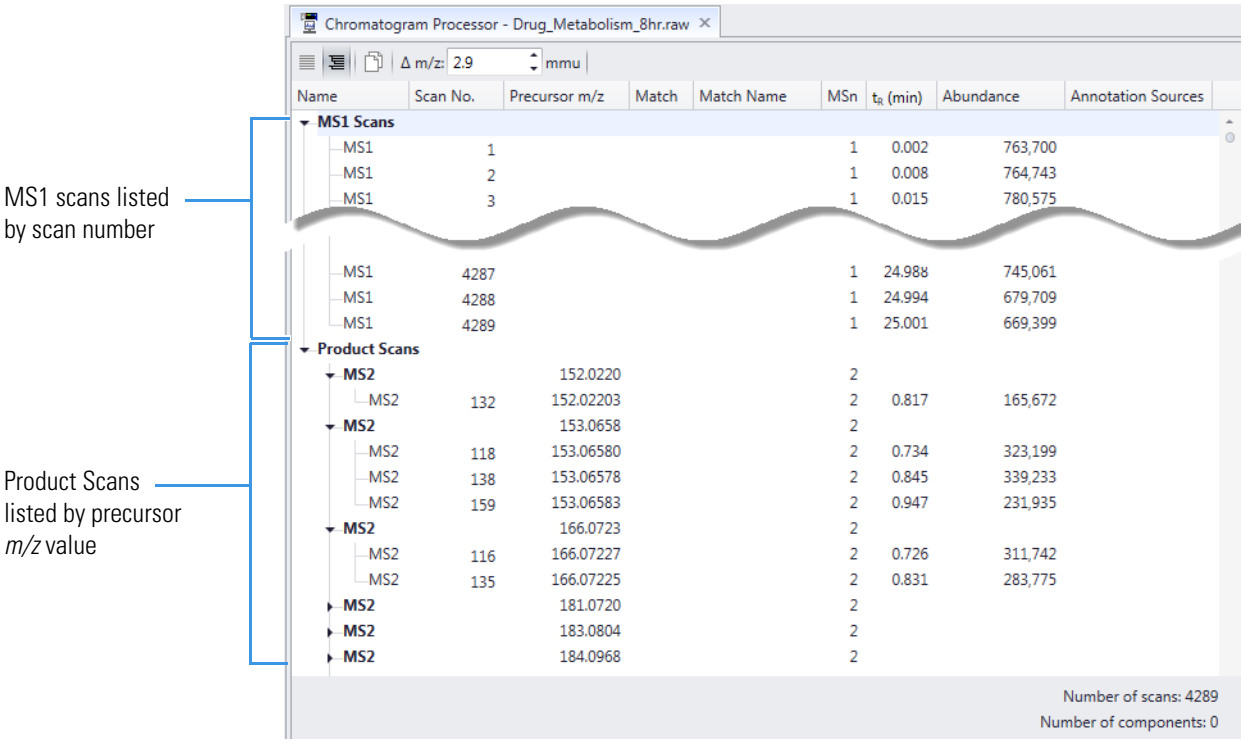
Chromatogram data view

The chromatogram data view in the upper left of a Chromatogram Processor window displays a list of the scans from the selected file. By default, the scans are separated into two lists: MS1 scans and Product Scans. After you apply any of the component detection algorithms (JCD, TECD, DICD, or FISh) this view also displays a list of components.

For details, see these topics:

- MS1 and product scans lists
- Components list
- Chromatogram data view toolbar
- Chromatogram data view column options
- Sort the data in the chromatogram data view by a single column
- Filter the chromatogram data view columns

Figure 10. Chromatogram data view with the scans displayed in the tree structure layout



MS1 and product scans lists

The scan lists are interactive with the chromatogram and MS spectrum views. Clicking a scan in the chromatogram data view automatically updates the spectrum displayed in the MS spectrum view. Clicking a retention time point in the chromatogram view displays the scan acquired at that time point on the Spectrum page of the MS spectrum view.

Note The scan lists do not support putative structures assigned from a library search.

For information about changing the layout of the scan lists, see "Chromatogram data view toolbar" on page 42.

Table 29. Columns for the MS1 and Product Scans lists

Column header	Description
Scan No.	The scan number from the original data file.
Precursor m/z	*m/z* value of the precursor ion from the parent scan within its isolation width.
Match	Displays putative annotations with their corresponding match factors from a Component Search in a regular font. Displays manually accepted annotations in a bold font.
Match Name	Displays the name of the compound that match with the library search entry.
MSn	MSn stage of the scan.
t_R (min)	Retention time of the scan.
Abundance	Total ion current of the scan.
Annotation Sources	Displays the name of the mass spectral library where the library search found a matching library entry.

Components list

Applying any of the component detection algorithms—JCD, TECD, DICD—to the chromatogram adds a list of components to the chromatogram data view.

A component is a collection of scans that the component detection algorithm groups together by *m/z* value, retention time, and peak shape in the form of a spectral tree. When you run a component detection analysis, the Components list appears above the scans lists in the chromatogram data view.

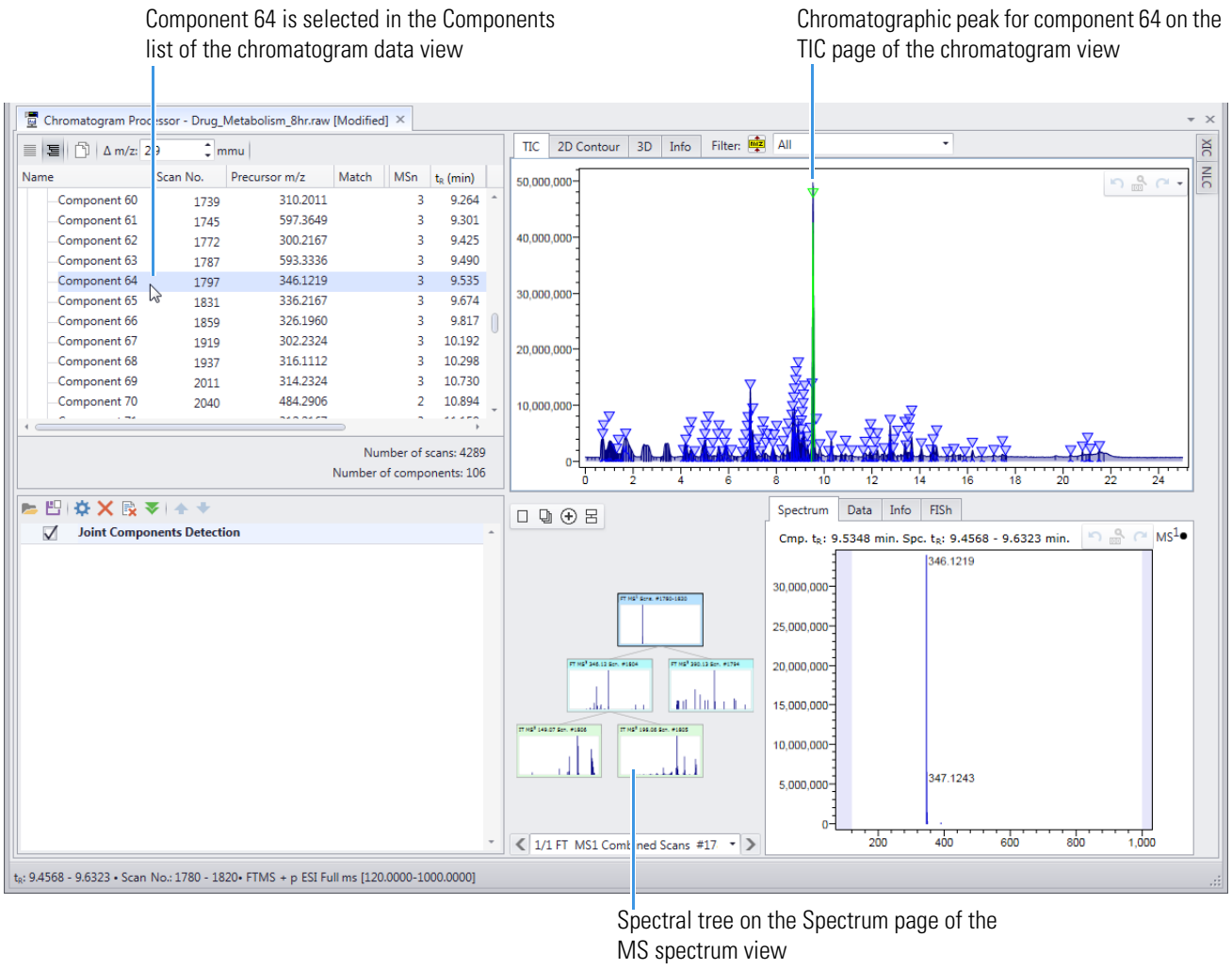
The Components list is interactive with the chromatogram and spectrum views. When you select a component in the Components list, the applications does the following:

- Displays the chromatographic peak for the component in green (default color) on the TIC page of the chromatogram view.

- Displays the compiled spectral tree for the selected component with the corresponding MSn scans in the MS Spectrum view.

Note By default, the color of a chromatogram for a selected component is green. You can select a different color in the 2D Chromatogram view of the Global Settings dialog box.

Figure 11. Chromatogram Processor window with the selection of component 64 in the chromatogram data view



The chromatogram data view displays the following component information.

Table 30. Chromatogram data view column descriptions for components

Column Header	Description
Name	Identifies the relative retention time of the component in the form of 'Component X' where X is the component's relative position on the *x* axis of the chromatogram plot.
Scan Number	Displays the scan number of the most intense MS1 scan for the component.
Precursor m/z	A component's precursor *m/z* value depends on the component detection algorithm. For more information, see these topics: • Joint Component Detection (JCD) algorithm • Total Ion Extraction Component Detection (TECD) algorithm • Direct Infusion Components Detection (DICD) algorithm
Match	A match score appears after a component search or an mzLogic search.
Match Name	Displays the name of the compound that match with the library search entry.
MSn	Displays the highest MSn scan stage for the component.
t_R(min)	Displays the retention time corresponding to the interpolated maxima of the MS1 profile for the component.
Abundance	Displays the total ion current abundance corresponding to the interpolated maxima of the component's MS1 profile.
Annotation Source	Displays the name of the mass spectral library where the library search found a matching library entry.

Chromatogram data view toolbar

There are two possible layouts for the scan lists. Use the chromatogram data view toolbar to switch between the simple list layout or the tree structure layout, to copy selected rows to the Clipboard, or to change the tolerance for grouping the product scans by their precursor ions.

Figure 12. Chromatogram data view toolbar

Table 31. Chromatogram data view toolbar descriptions

Tooltip	Icon	Description
Show Scans as a Simple List		Combines all the scans into one list: Scans. The simple list layout orders the scans by scan number.
Show Scans as a Tree Structure		Separates the scans into two lists: MS1 Scans and Product Scans. The tree view displays the precursor path for each MSn scan and groups the corresponding values together. The Components, MS1 Scans, and Product Scans lists appear as collapsible groups with corresponding chromatographic data in the tree view layout.
Copy selected items to the clipboard		Copies the selected scans to the clipboard as a plain text (.txt).
Tolerance Used to Merge Precursors		Specifies the *m/z* tolerance window used to group precursor ions. Only affects the grouping of the product scans. Default: 16 mmu

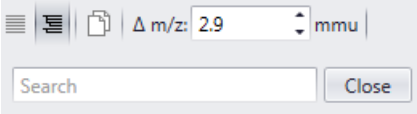
Chromatogram data view column options

To view the filter and sort commands for the chromatogram data view, right-click the column headers.

Table 32. Filter and sort commands for the chromatogram data view (Sheet 1 of 2)

Shortcut menu command	Description
Sort Ascending	Sorts the selected column list in ascending order.
Sort Descending	Sorts the selected column list in descending order.
Clear Sorting	Clears sorting and reverts to the default sorting order.
Show Column Chooser	Adds or removes specific columns from the chromatogram data view.
Best Fit	Adjusts the column width to best fit the data.
Best Fit (all columns)	Adjusts the width of all columns to best fit the data
Clear Filter	Clears the current filter.

Table 32. Filter and sort commands for the chromatogram data view (Sheet 2 of 2)

Shortcut menu command	Description
Filter Editor	Opens Filter Editor for advanced filter construction.
Show Search Panel	Shows or hides the Search box below the toolbar. 

Tip To change the order of the components by a corresponding column value, click the column headers.

Sort the data in the chromatogram data view by a single column

❖ **To sort the data in the chromatogram data view by one column**

Do one of the following:

- Right-click the column heading that you want to sort by and choose **Sort Ascending** or S**ort Descending**.
- Click the column heading until the down arrow or up arrow appears to sort the column in descending order or ascending order.

Sort the data in the chromatogram data view by two columns

❖ **To sort the data by two columns**

1. Sort the data by the primary column.
2. Press the SHIFT key and sort the data by the secondary column.

Filter the chromatogram data view columns

You can hide some of the data in the chromatogram data view by setting up the filter criteria in the Filter Editor dialog box.

❖ **To filter the data by a specific value or range**

1. Right-click the column heading of interest and choose **Filter Editor**.

   For example, to filter the data by retention time (criterion), right-click the t_R (min) column heading.

   The Filter Editor dialog box opens.

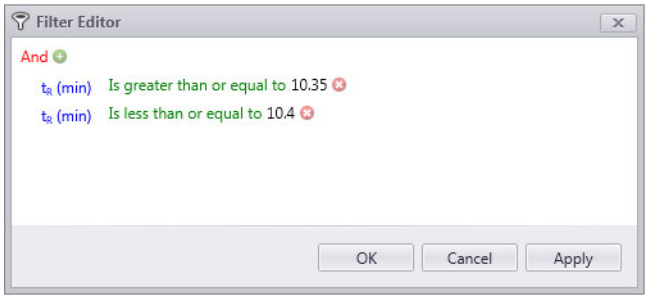
Note By default, the Boolean logic operator (in red) is set to And. Clicking this logic operator displays a dropdown list that includes And, Or, Not And, and Not Or, as well as other functions.

Figure 13. Filter Editor dialog box with the default criterion for the retention time column

2. To specify the retention time range to display, do the following:
   a. Click the operator (Begins with in green) to display the dropdown operator list and choose Is Equal To or Greater Than.
   b. To the right of the operator, click **<enter a value>** and type the first time point of the retention time range.
   c. Click the **Add** icon, .

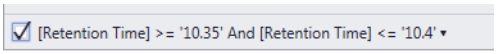
      A duplicate of the previous filter appears.
   d. Click the operator (green text) to display the dropdown operators list and select an operator.
   e. Click **<enter a value>** to the right of the operator and type the last time point of the retention time range.

Figure 14. Retention time range filter (10.35 to 10.4 min)

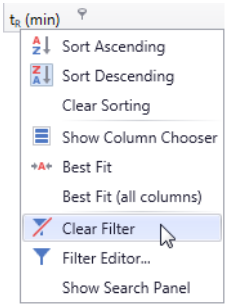


3. Click **Apply**.
4. Check the result in the chromatogram data view.
5. Click **OK**.

6. (Optional) To temporarily turn off the filter, clear the check box at the bottom left of the chromatogram data view.



Or, to clear the filter, right-click the column heading that has a filter icon, and choose **Clear Filter**.



Delete components from the chromatogram data view

❖ **To delete components in the chromatogram data view**

Select the components in the chromatogram data view, and then click **Delete > Selection** in the Edit group of the Chromatogram Processor toolbar.

Command processor view

The command processor view provides a batch-like reprocessing workflow of the chromatogram or infusion data. As you apply processing actions to the chromatogram, the actions appear in the command processor view in the order that you apply them. You can adjust the parameter settings of the actions, and then reprocess the raw data. You can save the applied actions with their parameter settings to a Chromatogram Actions file (.chpro_act), which you can use in a later Mass Frontier session or a different Chromatogram Processor window within the current session.

Tip You copy the commands in one Chromatogram Processor window to another opened Chromatogram Processor window by dragging the commands from one window to the other window. To apply the commands to the chromatogram (synchronize the actions), click the **Apply Checked Actions to the Chromatogram** icon, .

Figure 15. Command processor view

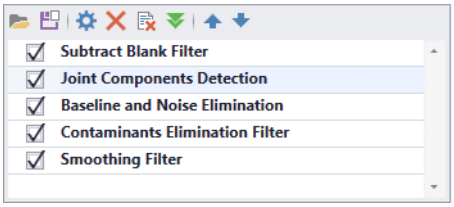


Table 33. Command processor view toolbar icons

Tooltip	Icon	Description
Load actions from a file		Loads the actions from a .chpro_act file.
Save actions from a file		Saves the actions to a .chpro_act file.
Change the parameters of a selected action		Opens the window with adjustable parameters for any of the actions
Remove selected action		Removes selected action.
Remove all actions		Removes all actions.
Apply checked actions to the chromatogram		Reprocesses the chromatogram using the actions.
Move action up		Moves action up in the command list order.
Move action down		Moves action down in the command list order.

Chromatogram view

Use the chromatogram view in the upper right of the Chromatogram Processor window to inspect the LC/MS chromatograms.

These topics describe the chromatogram view's pages and the customization options for the 2D Contour and 3D plots:

- Chromatogram view – TIC page
- Add fragment or text annotations to a chromatogram
- TIC page display options
- Chromatogram view – 2D contour page
- Customize the color mapping of a 2D or 3D plot
- Chromatogram view – 3D page
- Chromatogram view – Info page

Figure 16. Chromatogram view – TIC page

Chromatogram view – TIC page

The TIC page of the chromatogram view displays the total ion profile of the scans that you selected from the Filter list. Additionally, it displays various information depending on the type of applied processing method, such as the following:

- Uses blue triangles (▽) to indicate components identified by applying a component detection algorithms.
- Uses red triangles (▽) to indicate components identified by running a components search. The saturation of the red color indicates the value of the respective match score.

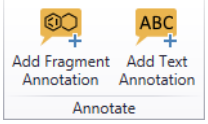
Right-click the TIC page to access its shortcut menu.

Table 34. Chromatogram view – TIC page shortcut menu

Command	Description
Reset Zoom	Resets zoom to original size.
Show Grid	Shows or hides the grid in the chromatogram plot.
Show Absolute/Relative Intensities	Switches between absolute or relative intensity on the *y* axis of the chromatogram plot.

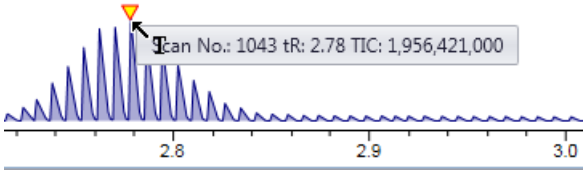
Add fragment or text annotations to a chromatogram

In the chromatogram view of a Chromatogram Processor window, you can add text or structure annotations to the displayed chromatograms.



❖ To add annotations to a chromatogram

1. Display a chromatogram on the TIC page of the chromatogram view.
2. Make sure that the Chromatogram Processor's status is not Read Only or Locked.
3. In the Chromatogram Processor toolbar, do any of the following:
   - To add a text annotation to a scan (time point) in the chromatogram, do the following:
     a. Click **Add Text Annotation**.
     b. Click the scan (time point) of interest with the text pointer (↖T).



     c. Type the text in the text entry dialog box and click **OK**.
   - To add a structure annotation to a scan in the chromatogram, do the following:
     a. Click **Add Fragment Annotation**.
     b. Click the scan of interest with the structure pointer (↖⬡).

        The Structure Editor dialog box opens.
     c. Open a structure file or draw the structure, and then click **OK**.

TIC page display options

To open a list of display options for the TIC page, click the down arrow at the right end of the toolbar.

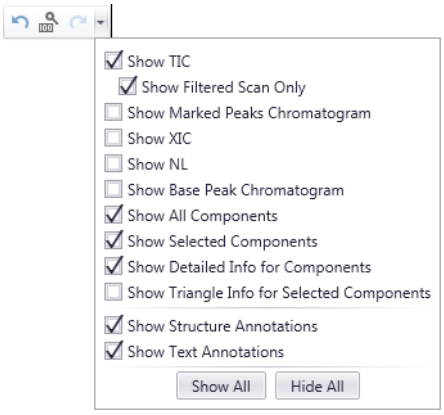


Table 35. Display menu for the TIC page of a Chromatogram Processor window

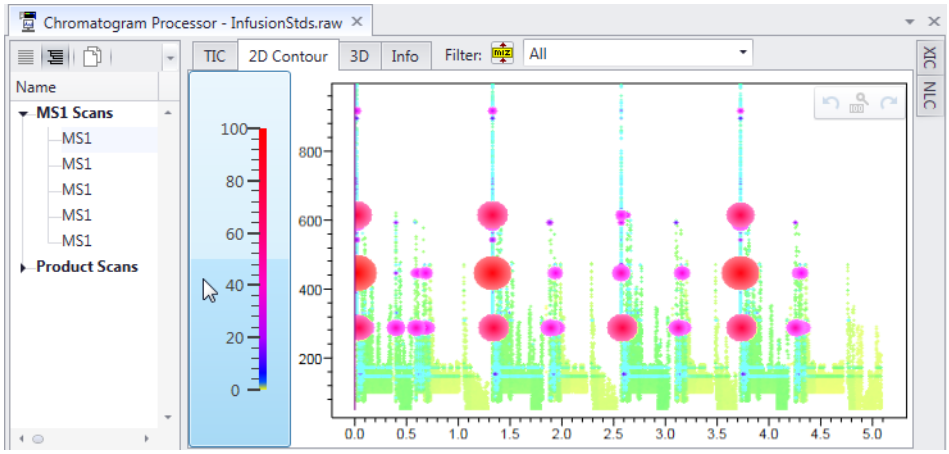
Command	Description
Show TIC	Displays the total ion current profile.
Show Filtered Scan Only	Displays the total ion current of only the selected scan filter.
Show Calculated TIC	Displays the calculated total ion current profile.
Show XIC	Displays the extracted ion chromatogram profiles for the selected *m/z* values. See "Extracted Ion Chromatogram (XIC) filter" on page 138.
Show NL	Displays the neutral loss profiles for the selected *m/z* values. See "Neutral Loss Chromatogram (NLC) filter" on page 143.
Show Base Peak Chromatogram	Displays the base peak profile across the chromatogram.
Show All Components	Displays the chromatogram for all the detected components.
Show Selected Components	Displays the chromatograms for the components that you selected in the Components list in the chromatogram data view.
Show Structure Annotations	Displays the structure annotations that you added. See "Add fragment or text annotations to a chromatogram" on page 48.
Show Text Annotations	Displays text annotations that you added.

Tip You can customize the color of the different profiles, such as components, the base peak chromatogram, and so on in the Layout/2D Chromatogram section of the Global Settings dialog box.

Chromatogram view – 2D contour page

The 2D Contour page in the chromatogram view shows the LC/MS data as a two-dimensional plot of *m/z* value versus retention time where the third dimension, intensity, is color-coded.

Figure 17. 2D Contour page of the chromatogram view



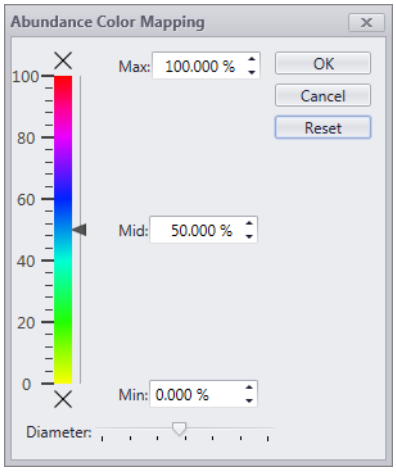
Customize the color mapping of a 2D or 3D plot

❖ To customize the color mapping of the plot

1. Click the color legend at the left.

   The Abundance Color Mapping dialog box opens.

   Figure 18. Abundance Color Mapping dialog box with the default settings

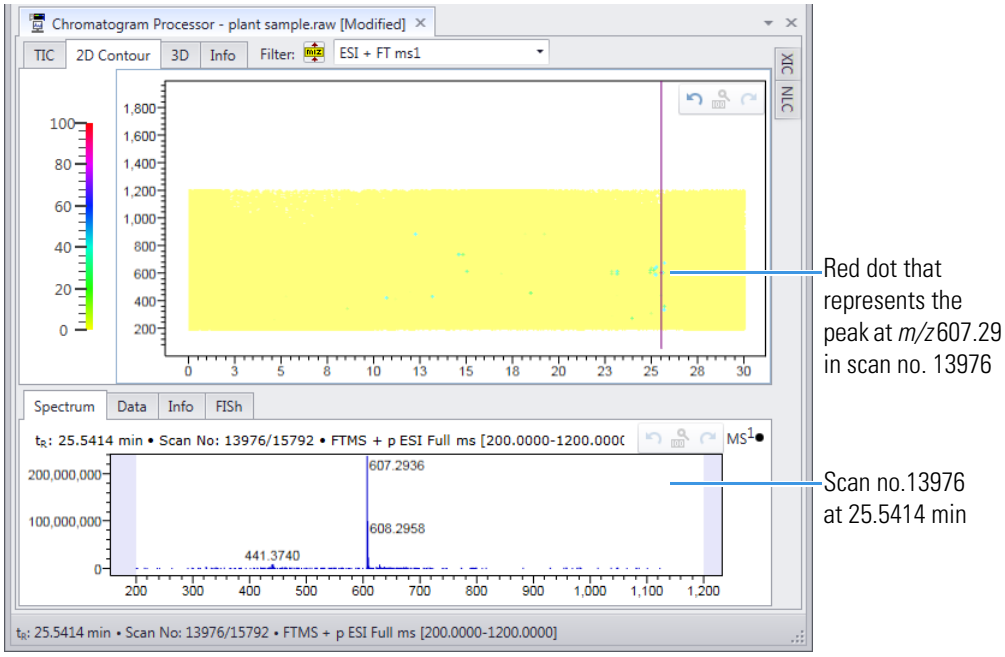


2. Use the sliders to change the appearance of the plotted intensities.

   For example, moving the Diameter slider to the right enlarges the dots that represent the spectral peaks in proportion to the relative intensity of the peaks.

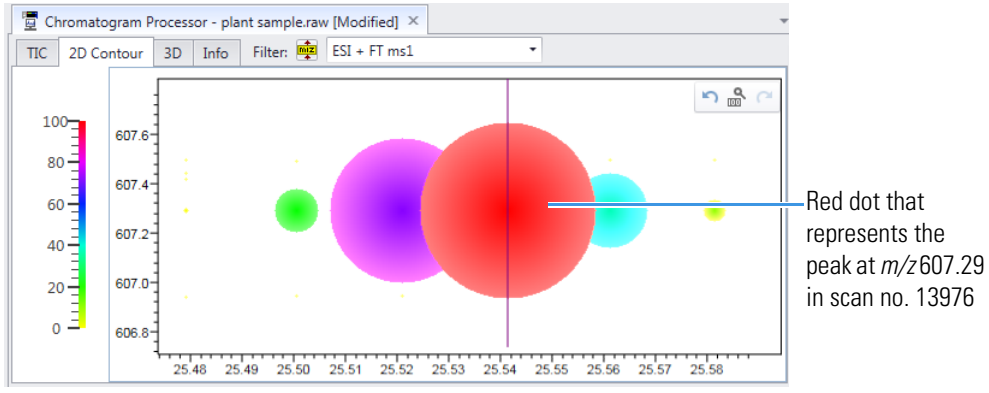
   For example, Figure 19 shows a filtered (MS1 scans) 2D contour plot with a Diameter setting of 50%. The red dot that represents the most intense peak at *m/z* 607.2936 min in scan 13976 at 25.541 min is barely visible.

Figure 19. Diameter slider set to 50%



To make the peak more visible, you can zoom in on it.

Figure 20. Zoomed in on the *m/z* 607 and 25.5 min region of the contour plot



Or, you can change the Diameter setting to enlarge the dots (see Figure 21).

Figure 21. Diameter slider set to 100%

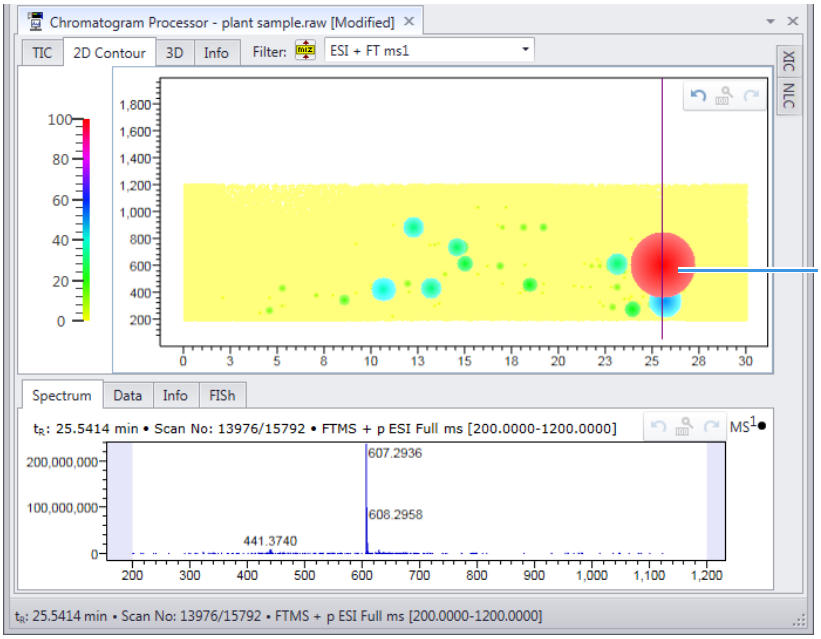
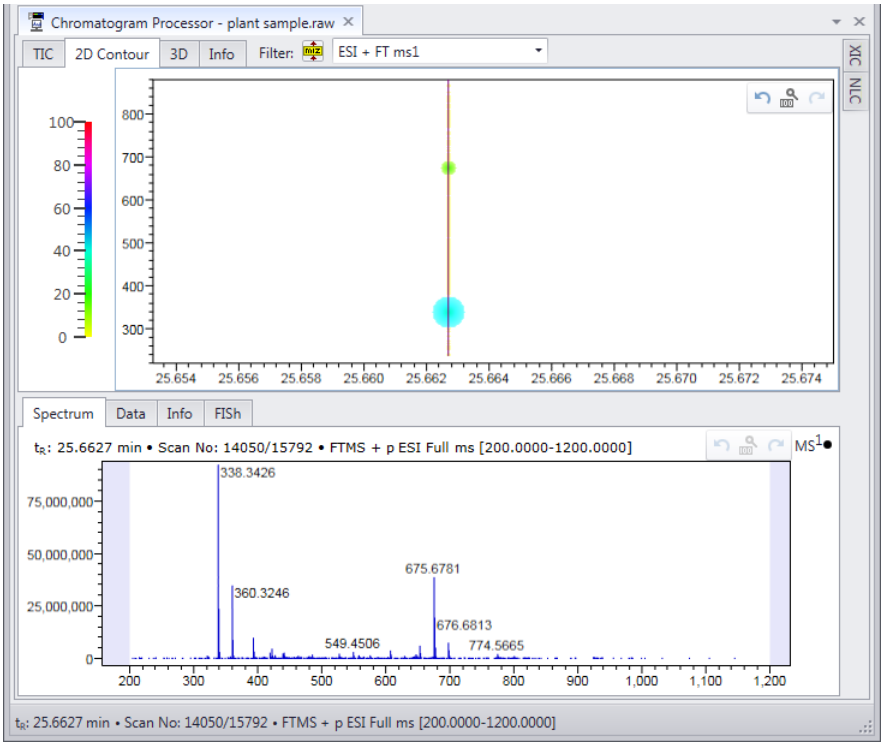


Figure 22 shows the relative sizes and colors of the dots that represent the peaks in scan 14050, where the dot at *m/z* 338 is much larger than the dot at *m/z* 676.

Figure 22. Zoomed in on scan no. 14050 with the Diameter slider set to 100%

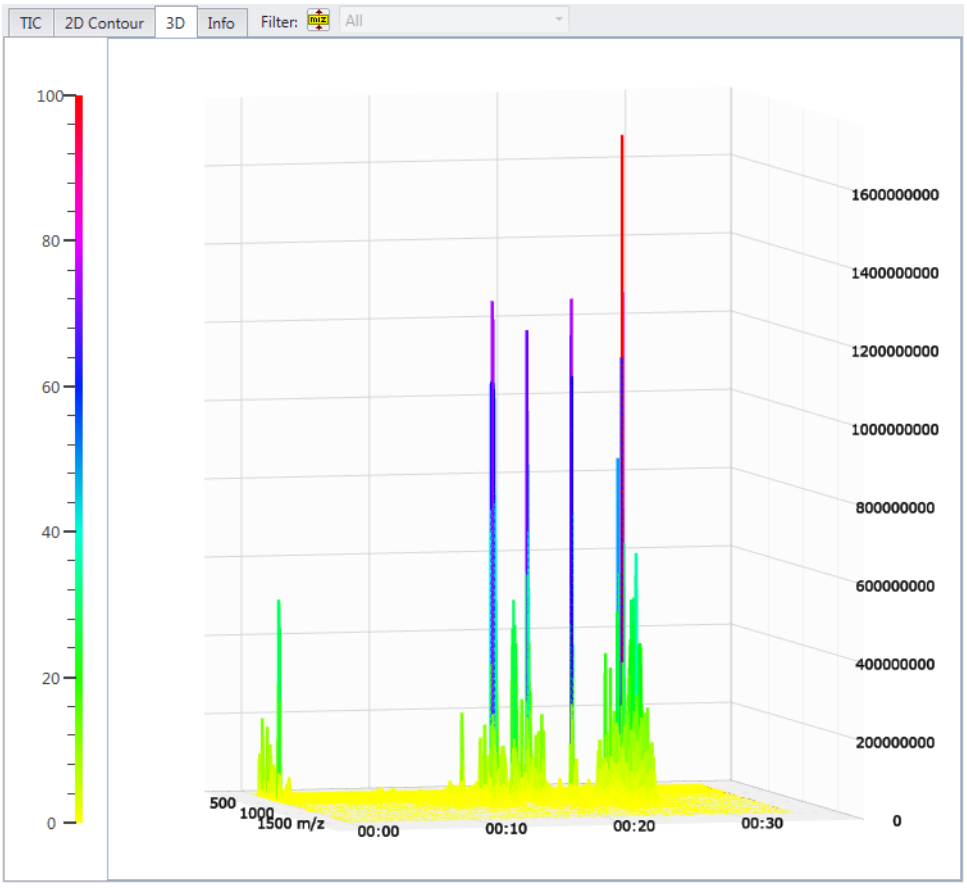


Chromatogram view – 3D page

The 3D page of the chromatogram view shows the LC/MS data in a three-dimensional plot where retention time is plotted along the *x* axis, *m/z* value is plotted along the *y* axis, and intensity is plotted along the *z* axis and is color-coded (Figure 23). You can freely rotate and navigate the 3D plot along any axis.

For information about customizing the plot, see "Customize the color mapping of a 2D or 3D plot" on page 51.

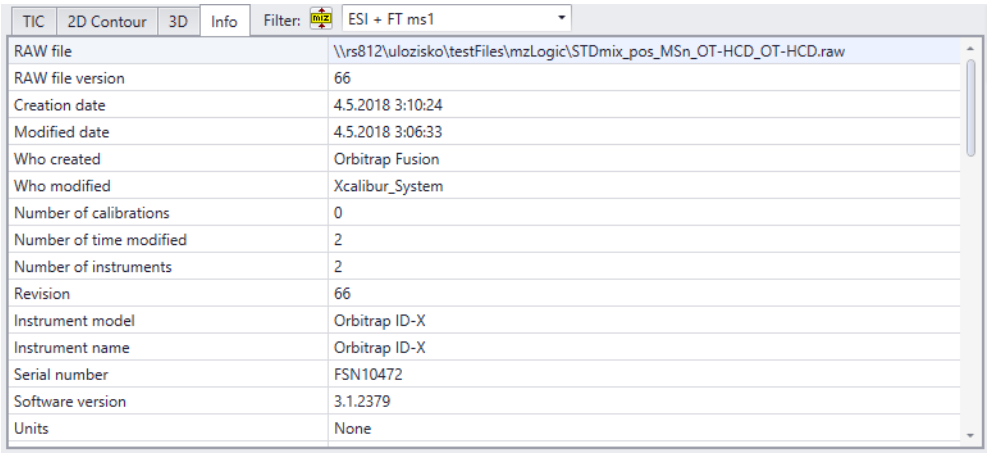
Figure 23. 3D page of the chromatogram view



Chromatogram view – Info page

The Info page of the chromatogram view shows additional information that is saved in the data file (Figure 24). Each LC/MS file format contains a different list of items that describe the sample, instrument, experimental conditions, and so on.

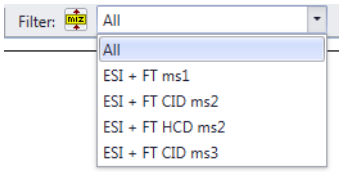
Figure 24. Info page of the chromatogram view



Chromatogram view – scan filter list

In the chromatogram view of a Chromatogram Processor window, use the dropdown Filter list to select the scan type for the chromatogram that you want to display.

Figure 25. Filter list in the chromatogram view



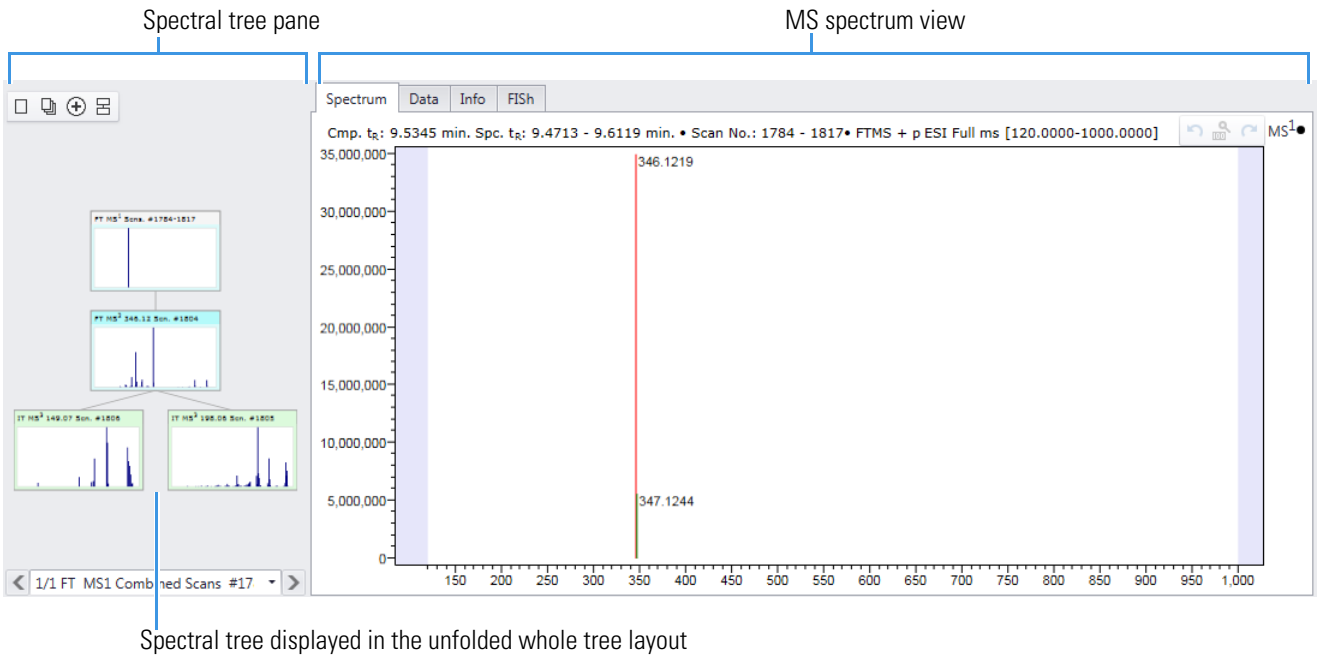
MS spectrum view

In the MS spectrum view, you can inspect the spectral tree arrangement of the selected component and detailed information about the corresponding scans on the various pages (Figure 26).

For details, see these topics:

- MS spectrum view – spectral tree pane
- MS spectrum view – Spectrum page
- MS Spectrum view – Data page
- MS spectrum view – Info page
- MS spectrum view – FISh page

Figure 26. MS spectrum view after applying a component detection algorithm



MS spectrum view – spectral tree pane

The spectral tree pane is an interactive tool for visualizing the hierarchical scan arrangement of MSn data. Click the Fold Whole Tree icon, ▢, or the Unfold Whole Tree icon, ⧉, to switch between the schematic view and the full tree view. The schematic view displays only the MSn precursor path and *m/z* value for each node. The full tree view displays scans (node items) separately and color-codes them by their ion activation method and mass analyzer.

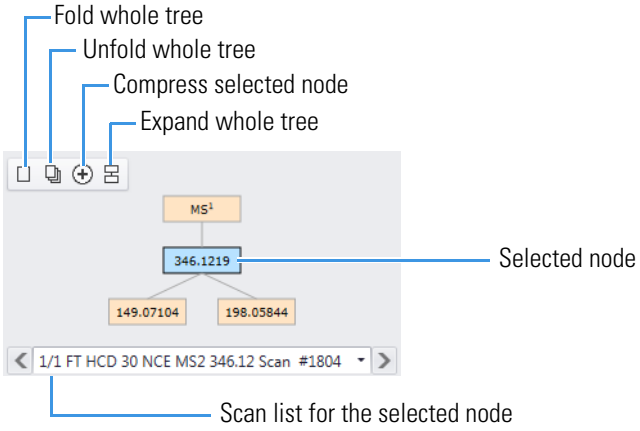
Use the scan box at the bottom of the spectral tree pane to browse the respective scans for the selected spectral tree node.

IMPORTANT When a spectral tree contains more than 50 nodes, it opens in the folded layout. When a node in a spectral tree contains more than 200 scans (node items), this particular node is folded.

You cannot unfold spectral trees with more than 50 nodes or nodes with more than 200 node items.

To view a specific scan, click the spectral tree node, and then use the scan box at the bottom of the spectral tree pane to select the scan.

Figure 27. Schematic (folded tree) layout of a spectral tree



Note The spectral tree pane appears after you apply component detection (JCD, TECD, DICD).

MS spectrum view – Spectrum page

The Spectrum page displays the scan spectrum. Right-click the Spectrum page to display a shortcut menu with commands for modifying the plot, running library searches, and so on.

Table 36. Shortcut menu commands on the Spectrum page of the MS Spectrum view (Sheet 1 of 2)

Command	Description
Reset Zoom	Resets zoom to full size.
Show Grid	Displays or hides the grid lines in the spectrum plot.
Show Absolute Intensities	Switches between displaying absolute or relative intensity on the *y* axis of the spectrum plot.
Show XIC m/z	Adds the *m/z* value of the selected peak to the XIC list to show the profile across the chromatogram. Available only after you select a spectrum peak. To add an *m/z* value to the list in the Extracted Ion Chromatogram pane, right-click a specific peak in the spectrum, and then choose **Show XIC m/z**.
Show FISh Explanation m/z	Displays or hides the FISh fragment explanations. Available only after you apply a FISh analysis. See "FISh analysis" on page 105)
Copy MS Spectrum	Copies MS Spectrum to the Clipboard.
Copy MS Spectrum to Legacy mzCloud	Copies a selected single spectrum in a format supported by the Legacy mzCloud web search interface.

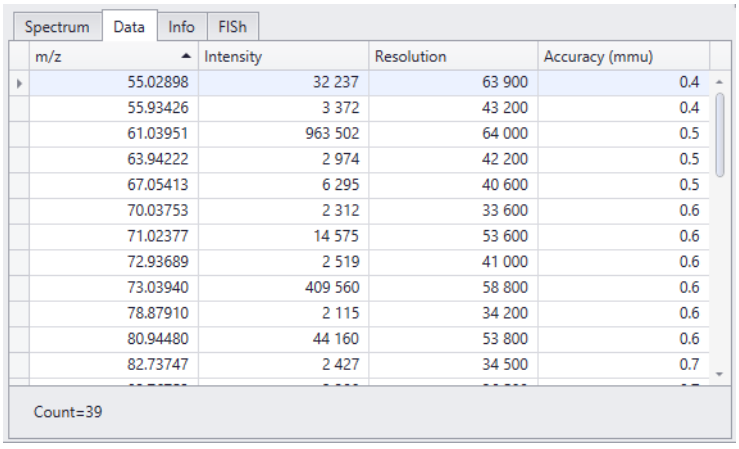
Table 36. Shortcut menu commands on the Spectrum page of the MS Spectrum view (Sheet 2 of 2)

Command	Description
Copy MS Spectrum to New mzCloud	Copies a selected single spectrum in a format supported by the New mzCloud web search interface.
Data Manager	Send the selected spectrum to the New Data Manager window. **Note** If there are any open Data Manager windows, then the name of window will be displayed in shortcut menu. You can choose and directly send the selected spectrum to open Data Manager window.
Spectrum Search	Opens the Spectrum Search dialog box with the current spectrum.
mzLogic Search	Opens the mzLogic MolGate Tool window with the current spectrum.
Peak Search	Opens the Peak Search dialog box. If you right-click a specific spectral peak, its corresponding *m/z* value and accuracy automatically appear in the Peak Search dialog box.

MS Spectrum view – Data page

The Data page lists the spectrum peaks in a table format, with one row per peak.

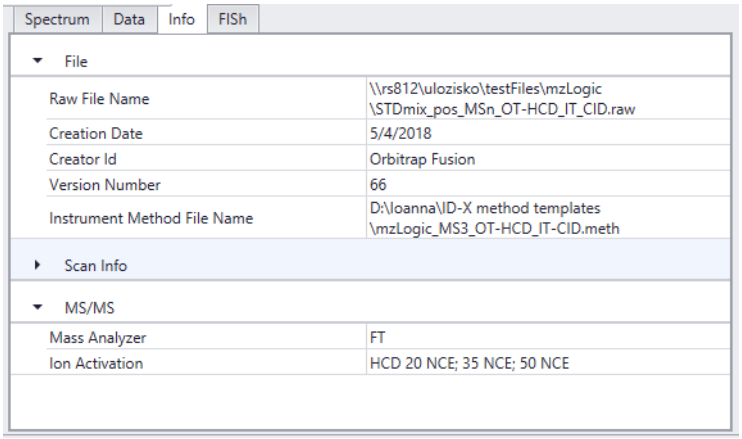
Figure 28. Data page of the MS spectrum view



MS spectrum view – Info page

The Info page displays additional scan metadata that the data system recorded during data acquisition.

Figure 29. Info page of the Spectrum view in Chromatogram Processor



MS spectrum view – FISh page

The FISh page of the Spectrum view shows a list of peaks (*m/z* values) in the scan that are explained by the FISh model fragments.

Note The FISh model used to process the OMP-Sigma-70k.raw example file was generated by using the Omeprazole FISh fragments.sdf structure file and one modification from the predefined modifications list—oxidation (N,S). The JCD parameter settings were loaded from the OMP-Sigma-70K.chpro_jcd example file.

For more information about the FISh algorithm, refer to the *Detecting Structurally-Related Compounds with a FISh Analysis* tutorial.

Figure 31 shows the explanation for a spectral peak (*m/z* 167.09329) from a neutral loss ion in scan #3184 in the OMP-Sigma-70k.raw raw data file. The m/z column displays the peak's *m/z* value. The Explanations column displays the neutral loss mass, the matching structure from the parent ion in red, and the structure of the neutral loss in blue.

The protonated ion of omeprazole is the parent ion.

Figure 30. Protonated ion of omeprazole

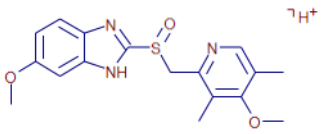


Figure 31. FISh page of the spectrum view in a Chromatogram Processor window with an explanation for a fragment (blue structure) from a neutral loss

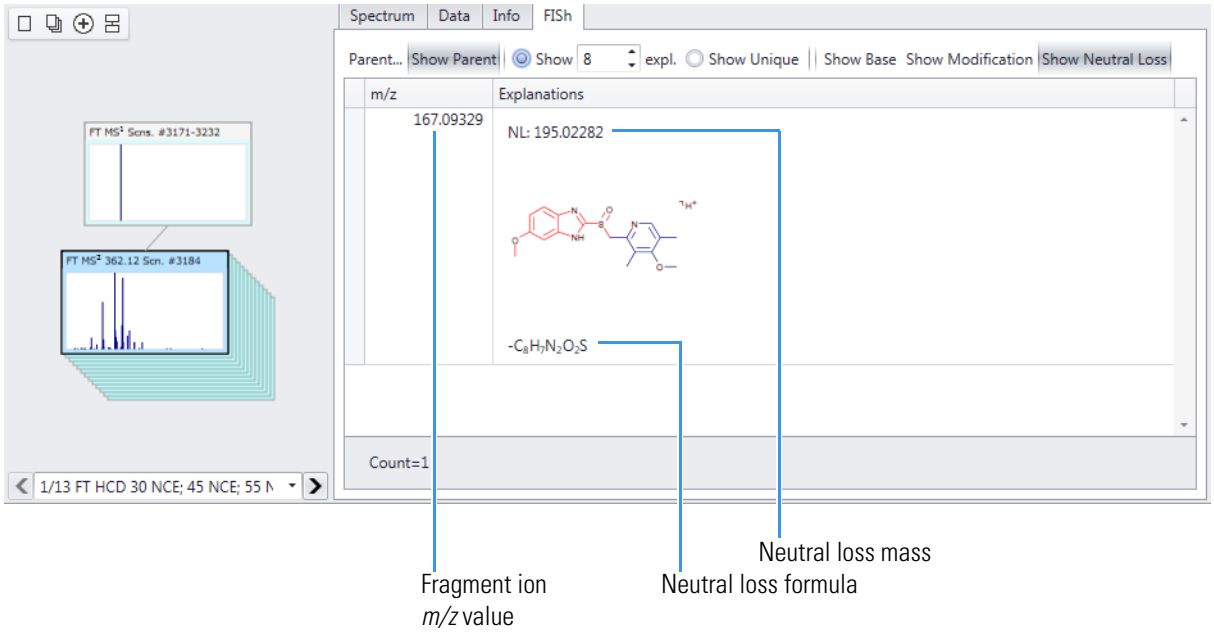


Figure 31 shows the explanations for the spectral peaks from a combination of any of the FISh model fragments and the specified oxidation modification. The m/z column displays the peak's *m/z* value. The Explanations column displays the ion's *m/z* value, the matching structure from the parent ion, the structure of the modification, and the formula of the FISh model fragment and its modification.

Figure 32. FISh page for peaks from the specified modification of the specified fragments

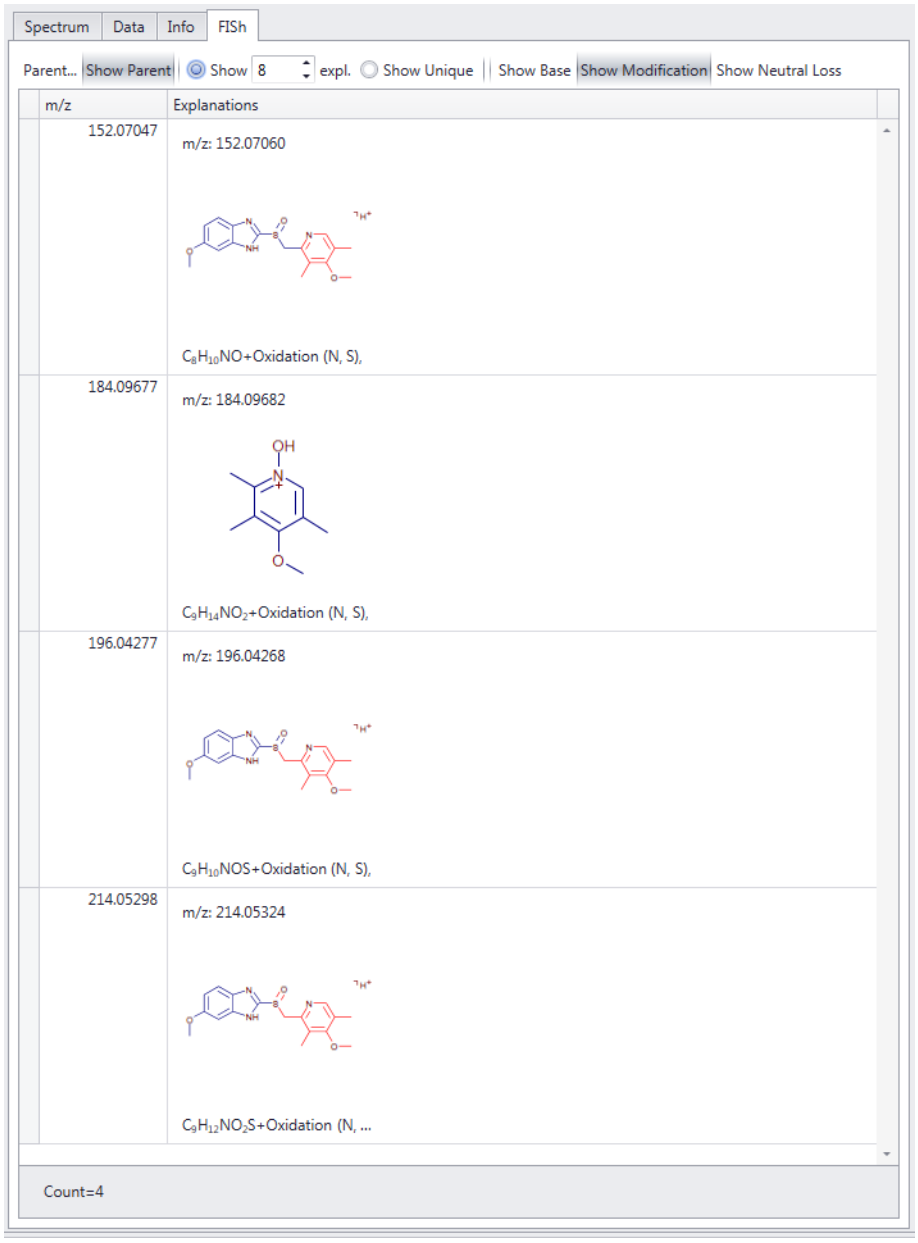


Figure 33 shows scan# 3184 with color-coded peaks from the FISh analysis.

Table 37. Description for color coded peaks from FISh analysis in scan# 3184

Color code for peak	Description
Lime-green peak	Represent a neutral loss fragment.
Light-blue peaks	Indicate that the fragments are generated by the specified modification
Red peaks	Represent a match for the specified FISh fragments.

Figure 33. Spectrum page showing the spectral peaks for the neutral loss fragment, four fragments from the specified modification, and eight explained fragments

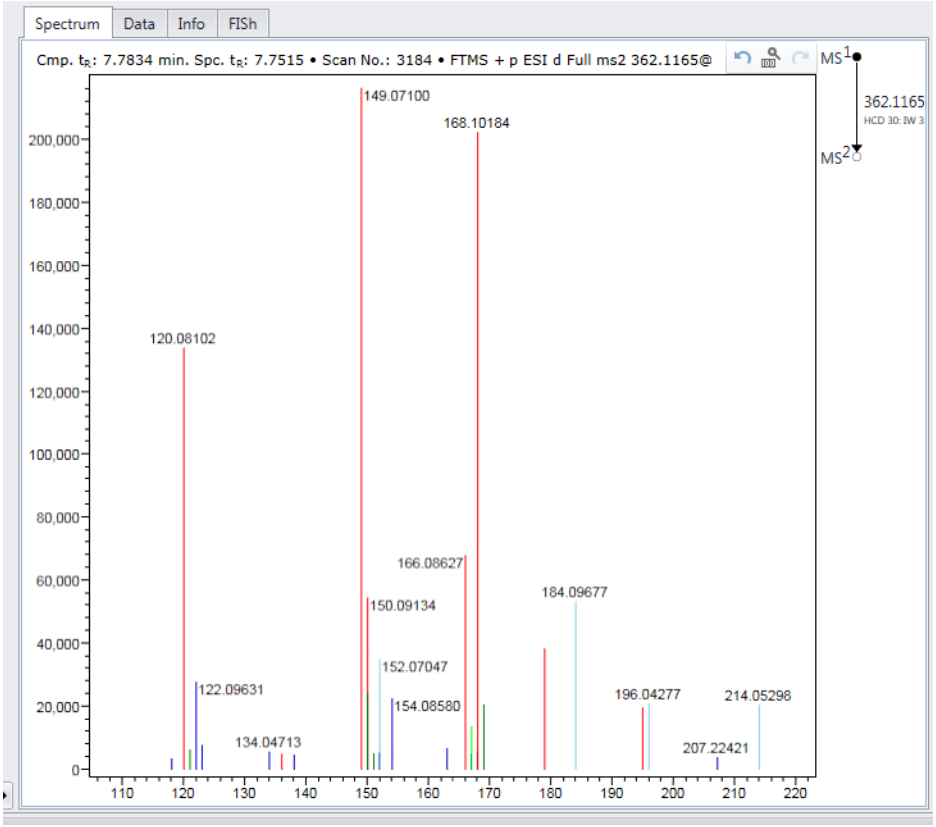


Table 38. FISh page option descriptions (Sheet 1 of 2)

Option	Description
Parent	Opens the Structure Editor with the source structure of the selected FISh explanation.
Show Parent	Switches the view between the actual FISh explanation and its source structure (parent). When you display the parent structure, its common substructure with the fragment appears in red.
Show	Limits the number of displayed isobaric explanation structures for any *m/z* value.
Show Unique	Displays only the first fragments in each *m/z* group.
Show Base	Displays the explanation for a spectral peak defined directly by any fragments or *m/z* values in the FISh model. The explanation includes the fragment's exact *m/z* value, its structure, and its formula.

Table 38. FISh page option descriptions (Sheet 2 of 2)

Option	Description
Show Modification	When the FISh model includes modifications, the Explanation column displays the fragment's exact *m/z* value, the fragment's structure and formula, and a description of the modification.
Show Neutral Loss	Displays the explanation defined by the neutral loss. To calculate the neutral loss, the application uses the input fragment with the highest *m/z* value as the molecular ion. It then calculates the neutral loss between all other fragments and this molecular ion. These values are compared with the neutral losses between the precursor peak and all the peaks in all the product spectra in the chromatogram. The Explanation column on the FISh page displays the *m/z* value of the neutral loss, the fragment structure that has an identical neutral loss in the input set of fragments, and the formula corresponding to the neutral loss. This option is meaningful only when you select the Neutral Mass Loss check box on the Options page of the FISh Detection view, and the set of fragments for the FISh model includes the molecular ion on the Model page.

Background subtraction

The background subtraction algorithm subtracts the ion profiles in the supplied background file from the ion profiles in the active chromatogram, removing the chemical noise peaks. The subtraction algorithm uses only the MS1 scans.

Supported blank MS data formats: LC/MS, tandem MSn (only MS1 is used), centroid, HRAM, nominal mass

Supported file formats:

- Thermo Fisher Scientific (.raw), generic (.mzml), HighChem Chromatogram file (.hccx)
- Background Subtraction Parameter file (.chpro_subtract)

For details, see these topics:

- Subtract background noise from a chromatogram
- Subtract Blank Filter view parameters

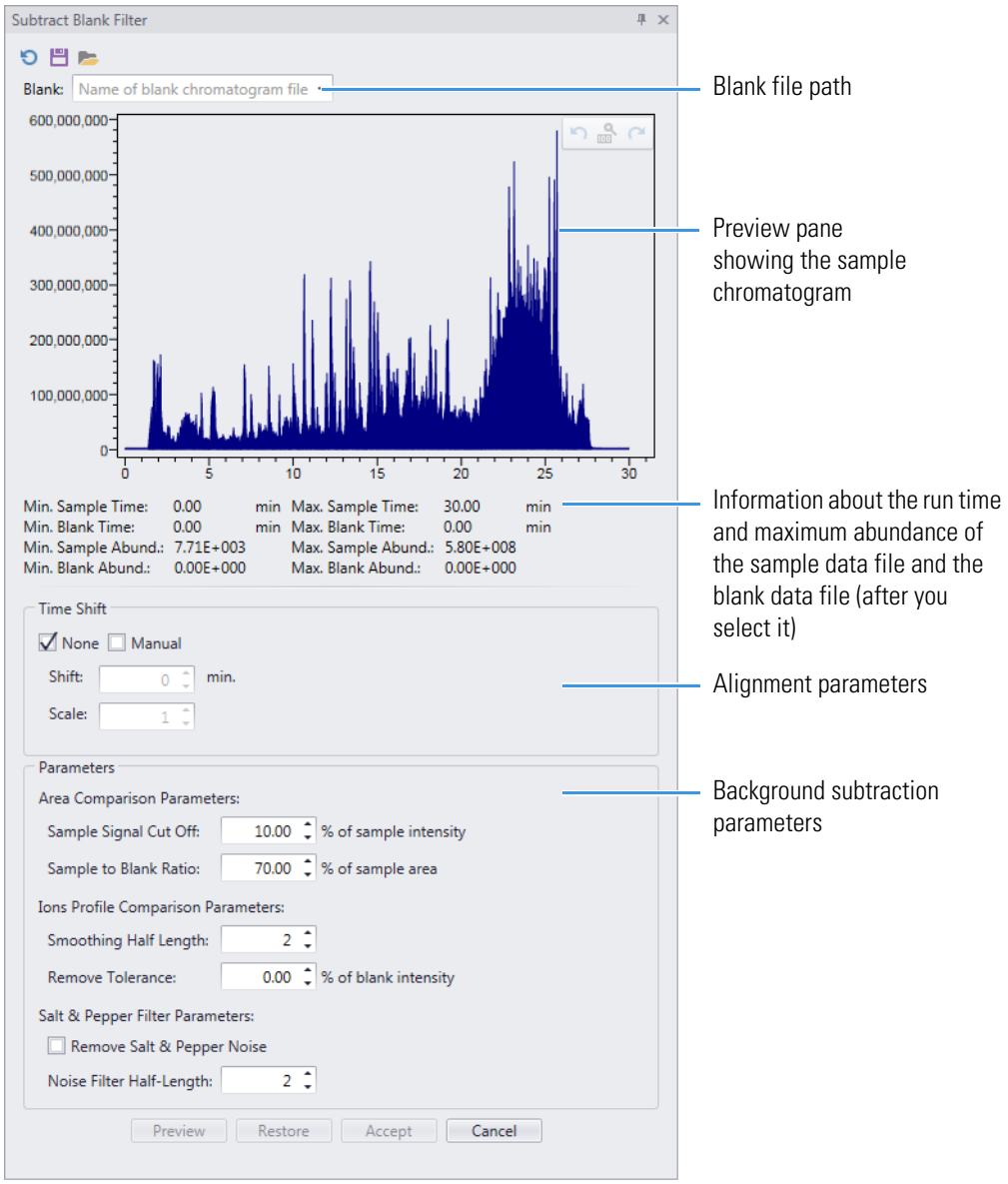
Subtract background noise from a chromatogram

❖ To subtract the background noise from a chromatogram

1. In a Chromatogram Processor window, open a data file.
2. In the chromatogram view, open the TIC page or the 2D Contour page.
3. From the Actions toolbar group, click the **Subtract Blank Experiment from All the Scans in the Chromatogram** icon, .

   The Subtract Blank Filter view opens (Figure 34).

Figure 34. Subtract Blank Filter view with the default parameter settings

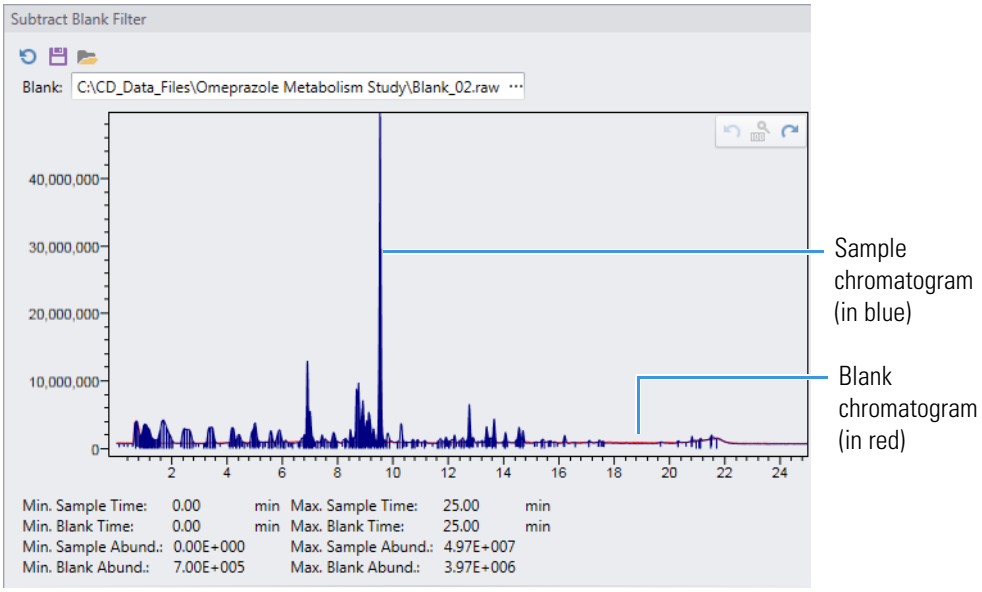


4. Select a blank sample from the same experiment type and sequence run if available (HCCX, MZML, or RAW).

> **Note** Use the Time Shift parameters to adjust the relative retention times of the two chromatograms if you are comparing samples from different sequence runs or if the retention times of the analytes drifted during the sequence run.

Figure 35 shows a sample chromatogram and a blank chromatogram in the preview pane. The two chromatograms have the same run time.

Figure 35. Preview pane with a sample chromatogram and a blank chromatogram



5. (Optional) In the Time Shift area, adjust the relative *x*-axis position of the blank chromatogram as follows:

   a. Select the **Manual** check box.

   b. In the Shift box, enter a value from **–2.50** to **2.50**.

      A negative value shifts the blank chromatogram to the left. A positive value shifts the blank chromatogram to the right.

Figure 36. No time shift

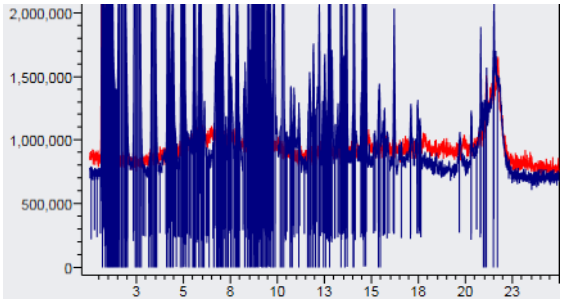
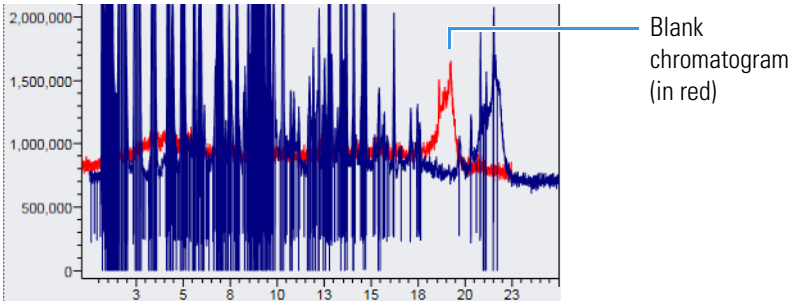


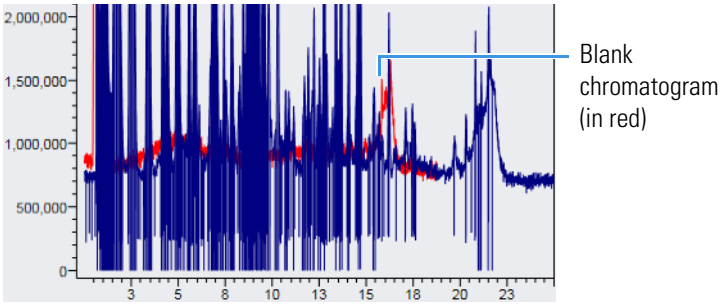
Figure 37. –2.5 minute shift



c. In the Scale box, enter a value from **0.75** to **1.25**.

A value <1 compresses the run time and a value >1 lengthens the run time of the blank chromatogram.

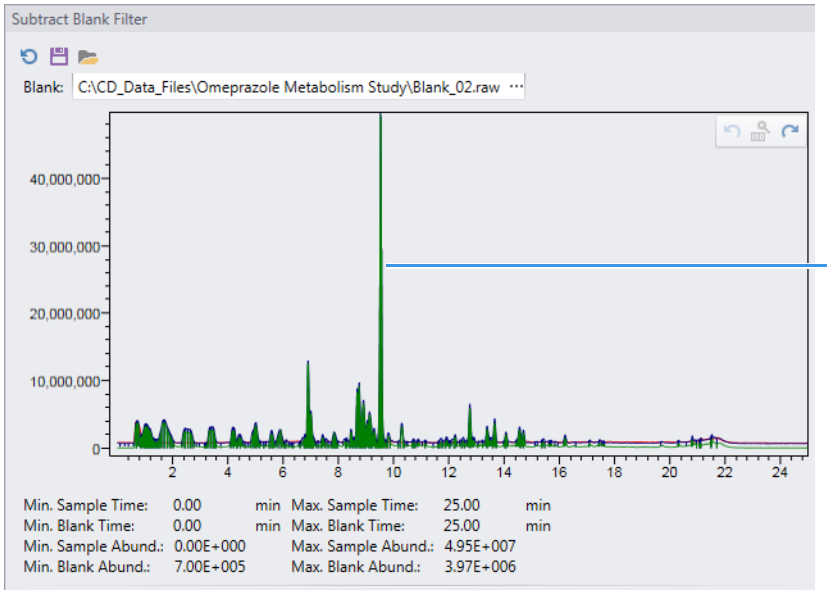
Figure 38. 0 minute shift and 0.75 scale



6. Use the preview pane to compare the relative retention times of the two chromatograms.
7. In the Parameters area, modify the background subtraction parameter settings as necessary.
8. Click **Preview**.

Figure 39 shows the background-subtracted trace for these two data files in the preview pane.

Figure 39. Preview pane with a background-subtracted trace in green



9. Adjust the settings as necessary.
10. Click **Accept**.

Subtract Blank Filter view parameters

Table 39. Subtract Blank Filter view parameter descriptions (Sheet 1 of 3)

Parameter or icon	Description
Reset Parameters to the Default State	Restores the parameter settings to their factory default values.
Save Parameters to a File	Saves the parameter settings to a .chpro_subtract file.
Load Parameters from a File	Loads the parameter settings from a .chpro_subtract file.
Blank	Specifies the data file from a blank sample (optimally acquired under the same conditions as the sample data). Accepts the following file formats: HCCX, MZML, or RAW
Preview pane	Displays the sample, blank, and background-subtracted chromatograms. The sample chromatogram appears in blue, the blank chromatogram appears in red, and the final background-subtracted trace appears in green. The information pane below the chromatogram plot displays additional data that can help you adjust the alignment parameters.

Table 39. Subtract Blank Filter view parameter descriptions (Sheet 2 of 3)

Parameter or icon	Description
Time Shift	
When the sample and blank profiles do not match, you can shift the time axis of the blank chromatogram and observe the effect of the adjustment in the preview pane.	
None or Manual	Selecting the Manual check box activates the Shift and Scale boxes. Default: None
Shift	Shifts the blank chromatogram along the *x* axis by the defined value to the right or left. Default: 0 min.; range: –2.50 to 2.50
Scale	Scales the time axis of the blank chromatogram. Default: 1 (no scaling); range: 0.75 to 1.25
Parameters	
Area Comparison Parameters	
These parameters compare the areas of all the identical ion profiles in the blank and sample chromatograms. If the areas are similar, then the sample removes the whole ion profile.	
Sample Signal Cut Off	Calculates the comparing areas based on the intensities of the ion profile higher than this specified relative intensity. Range: 0 to 100
Sample to Blank Ratio	Removes the ion profile from the sample chromatogram if the ratio of its area in the sample chromatogram to its area in the blank chromatogram is smaller than this setting. Range: 0 to 100
Ion Profile Comparison Parameters	
These parameters control the preprocessing of individual ion profiles before subtraction.	
Smoothing Half Length	Smooths each ion profile in the blank chromatogram by the Savitzky-Golay filter calculation. This parameter defines the half length of this filter. This means that the filter length is 2*(Smoothing Half Length) + 1.

Table 39. Subtract Blank Filter view parameter descriptions (Sheet 3 of 3)

Parameter or icon	Description
Remove Tolerance	Increases the intensity of the blank ion profile. When set to 0%, the application does not adjust the intensity of the blank ion profile. When set to 100%, the application doubles the intensity of the blank ion profile. Range: 0 to 500%
Salt & Pepper Filter Parameters	
The median filter processes each filtered ion profile. If the median value is zero, then the intensity is set to zero. This removes orphan peaks from the ion profile.	
Remove Salt & Pepper Noise	Applies the Salt & Pepper filter.
Noise Filter Half-Length	Defines the half-length of the median filter. The filter length is 2*(Smoothing Half Length) + 1. Range: 1 to 100
Buttons	
Preview	Displays the calculated chromatogram in the chromatogram view.
Accept	Applies the changes and closes the Subtract Blank Filter view.

Baseline correction and noise elimination filter

The baseline correction and noise elimination filter analyzes the ion profiles of the ions appearing in the spectra over the entire time range of a chromatogram. In contrast to threshold filters that independently analyze and modify the individual scans and their spectral peaks, the baseline correction and noise elimination filter analyzes and modifies the spectral peaks in a specified retention time range.

Supported file format: Baseline parameter file (.chpro_baseline)

To use the baseline correction and noise elimination filter, see these topics:

- Open the Baseline and Noise Elimination filter view
- Use the Wizard mode for baseline correction and noise elimination
- Use the Details mode for baseline correction and noise elimination

Open the Baseline and Noise Elimination filter view

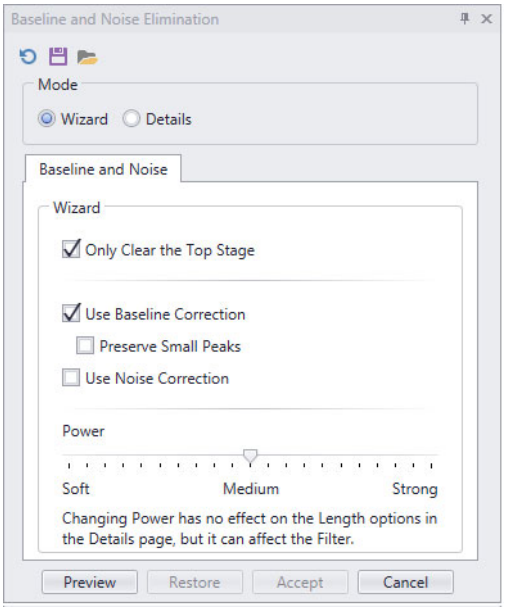
❖ To open the Baseline and Noise Elimination view

In Actions group of the Chromatogram Processor toolbar, click the **Apply baseline filter to all scans in the chromatogram** icon, .

The Baseline and Noise Elimination view has two modes: Wizard and Details. The view opens to the Wizard mode (Figure 40).

IMPORTANT The parameter settings on the Details page have no effect on the Wizard mode, but the Power setting on the Wizard page affects the Details mode.

Figure 40. Baseline and Noise Elimination view – Wizard mode



Use the Wizard mode for baseline correction and noise elimination

The Wizard mode provides a simplified set of the most important parameters.

❖ To run the Baseline Correction and Noise Elimination filter in the Wizard mode

1. Open the Baseline and Noise Elimination view. See "Open the Baseline and Noise Elimination filter view" on page 70.
2. Select the Wizard option if it is not already selected.
3. Optimize the parameter settings and click **Preview**.

Tip To reset the parameters to their factory default settings, click the **Reset** icon,

Table 40 describes the parameters for the Wizard mode of the Baseline Correction and Noise Elimination filter.

Table 40. Baseline and Noise Elimination – Wizard mode parameter descriptions

Parameter	Description
Only Clear the Top Stage	Processes only spectra in MS1 stage (full scan).
Use Baseline Correction	Applies one of the following methods to the chromatogram: • Top-Hat filter • Savitzky-Golay derivative filter (default) • LOESS derivative filter
Preserve Small Peaks	Preserves small peaks when using baseline correction.
Use Noise Elimination	Applies the following methods individually or simultaneously: • Counter filter to reduce chemical noise • Quantile filter to reduce electronic noise
Power	Specifies the mass difference for merging the spectral peaks into one *m/z* value. A low (soft) value results in more components (oscillating ions), and a high (strong) value results in fewer components (merging ions).

Tip If you previously optimized the settings for a similar experiment and saved the settings to a file, click the **Load** icon, , and select a Baseline Parameters File (chpro_baseline) to load the saved parameter settings.

4. After you optimize the settings, click **Accept**.
5. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a Baseline Parameters File (.chpro_baseline).
6. (Optional) To undo baseline correction, click **Restore**. To undo baseline correction and close the view, click **Cancel**.

Use the Details mode for baseline correction and noise elimination

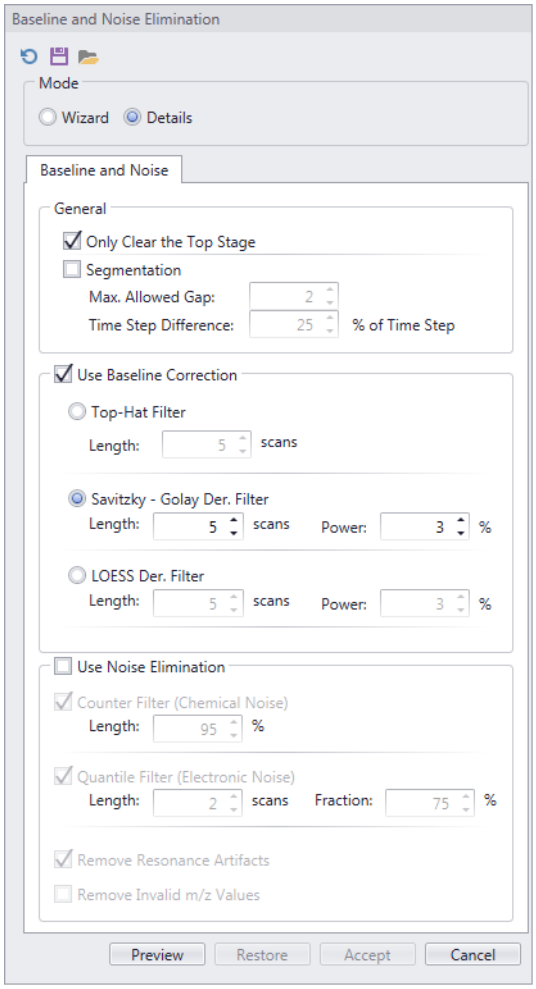
❖ To run the Baseline Correction and Noise Elimination filter in the Details mode

1. Open the Baseline and Noise Elimination view. See "Open the Baseline and Noise Elimination filter view" on page 70.
2. In the Wizard mode, optimize the Power setting, as this setting also affects the Details mode.

3. Select the **Details** option.

   The Details view opens.

   Figure 41. Baseline and Noise Elimination view – Details mode



4. Optimize the parameter settings and click **Preview**.

   Tip To reset the parameters to their factory default settings, click the **Reset** icon, ↺

   Table 41 describes the parameters for the Details mode of the Baseline Correction and Noise Elimination algorithm.

Table 41. Baseline and Noise Elimination – Details mode parameter descriptions (Sheet 1 of 3)

Parameter	Description
General	
Only Clear the Top Stage	Applies the filter only to top-stage scans. For most experiment types, the MS1 scans are the top-stage scans. For reaction monitoring (SRM) experiments, the MS2 scans are the top-stage scans.
Segmentation	Groups the scans that make up the chromatogram into different segments, and then processes the segments independently. Each group must have an equidistant retention time difference between the scans. The Max. Allowed Gap and Time Step Difference parameters control this distance. Segmentation provides better results if a chromatogram exhibits diverse shape, peak density, and baseline characteristics over the retention time scale. Default: Clear
	IMPORTANT Never use segmentation with the LOESS derivative filter. For the Top-Hat and Savitzky-Golay filters, only turn on segmentation if you obtain unacceptable results without segmentation.
Max. Allowed Gap	Specifies how many scans can exist between two scans in each group. For example, for a chromatogram from a polarity switching experiment (positive and negative), where the scans alternate between positive and negative, set the allowed gap to one (scan) so that the segmentation process creates two groups by skipping every other scan. Default: 2; range: 0 to 100

Table 41. Baseline and Noise Elimination – Details mode parameter descriptions (Sheet 2 of 3)

Parameter	Description
Time Step Difference	Specifies the maximum retention time difference between two scans consecutive scans as a percentage of the time step between the first scan and the scan that follows the second scan. When the difference exceeds this percentage, the application separates the first two scans into different groups. $\frac{S_2 - S_1}{S_3 - S_1}$ Where: • S_1 = retention time of the first scan • S_2 = retention time of the second scan • S_3 = retention time of the third scan Default: 25% of the Time Step; range: 0 to 100
Use Baseline Correction Use this area to specify the baseline correction settings.	
Use Baseline Correction check box	Enables the Baseline Correction filter. Default selection: Savitzky-Golay Derivative Filter
Top-Hat Filter	A morphological two-step filter. The algorithm searches the average opening of the signal on an area around the analyzed spectral peak (centroid), and then subtracts this value from the original analyzed peak. Use this filter to remove wide chromatographic peaks while preserving narrow chromatographic peaks. Increasing the filter length removes fewer wide chromatographic peaks. Default Length: 5; range: 1 to 1000 scans
Savitzky-Golay Derivative Filter	Defines the baseline by the point where the first derivation of a smoothed ion profile (values from Savitzky-Golay filter) is smaller than the Power parameter value. Subtracts the corrected baseline from the original ion profile in the time domain specified by the Length parameter. Default Length: 5; range: 2 to 1000 scans Default Power: 3%

Table 41. Baseline and Noise Elimination – Details mode parameter descriptions (Sheet 3 of 3)

Parameter	Description
LOESS Derivative Filter	Similar to the Savitzky-Golay Derivative Filter, but it does not require equidistant sampling. Default Length: 5; range: 2 to 1000 scans Default Power: 3%
Use Noise Elimination Use this area to identify and remove chemical and electronic noise peaks (centroids).	
Use Noise Elimination	Enables the Noise Elimination algorithm.
Counter Filter (Chemical Noise)	Counts the occurrence of non-zero spectral peaks in an ion profile. If the percentage of signal occurrence is higher than the Length setting, the ion is considered as chemical noise and the whole ion profile is removed. Default: 95%
Quantile Filter (Electronic Noise)	Counts the occurrence of non-zero peaks in the neighboring space on both sides (2×Length + 1 peak) of a spectral peak. If the percentage of signal occurrence is smaller than the Fraction setting, the filter removes the analyzed chromatographic peak. This filter removes narrow chromatographic peaks caused by electronic noise peaks (centroids). Default Length: 2 scans; default Fraction: 75%
Remove Resonance Artifacts	Removes FT residual peaks. See "Resonance peaks" on page 404.
Remove Invalid m/z Values	Removes spectral peaks that cannot be explained by any of the possible elemental compositions for common small organic molecules and their adduct ions.

Tip If you previously optimized the settings for a similar experiment and saved the settings to a file, click the **Load** icon, , and select a Baseline Parameters file (chpro_baseline) to load the saved parameter settings.

5. (Optional) To undo baseline correction, click **Restore**. To undo baseline correction and close the view, click **Cancel**.
6. After you optimize the settings, click **Accept**.
7. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a Baseline Parameters File (.chpro_baseline).

Contaminants Elimination Filter

The Contaminants Elimination Filter filters the chromatographic data for any known unwanted substances. Using either the provided set of known LC/MS contaminants or your user-created formulas or *m/z* values, you can create an exhaustive list to exclude these *m/z* values from the chromatographic data.

Supported file format: Exclude Ions parameter file (.chpro_exclude)

For details, see these topics:

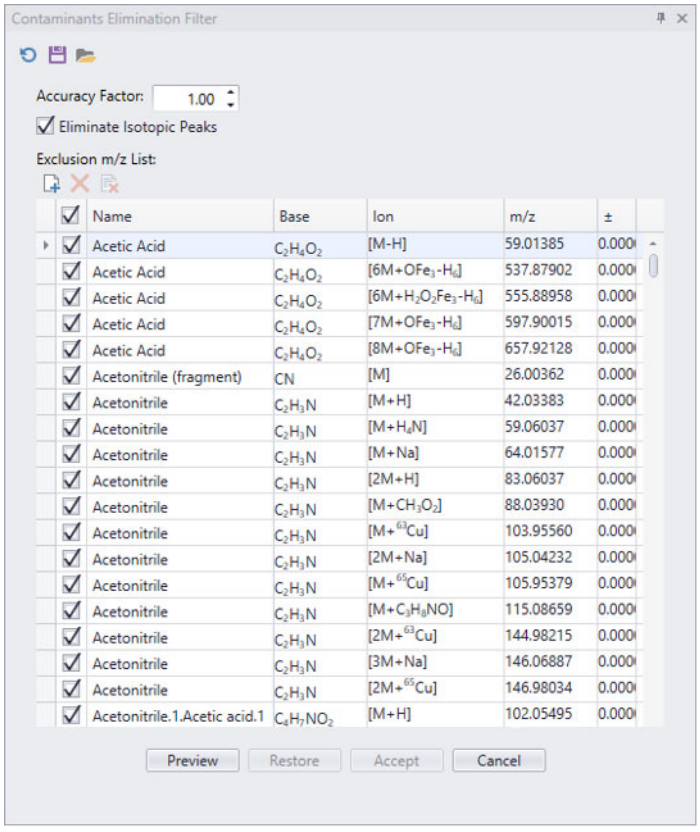
- Open the Contaminants Elimination Filter view
- Contaminants Elimination Filter parameters
- Add Custom Contaminant dialog box

Open the Contaminants Elimination Filter view

❖ To open the Contaminants Elimination Filter view

In the Action group of the Chromatogram Processor toolbar, click the **Remove contaminants from chromatogram** icon, .

Figure 42. Contaminants Elimination Filter view



Contaminants Elimination Filter parameters

Table 42. Contaminants Elimination Filter parameter descriptions

Parameter, icon, or button	Description
Icons	
Reset Parameters to the Default State	Reverts the user-changed parameters to the default state.
Save Parameters to a File	Saves the Exclude Ions parameters to a file (.chpro_exclude)
Load Parameters from a File	Loads the Exclude Ions parameters from a file (.chpro_exclude)
Parameters	
Accuracy Factor	Specifies the tolerance for matching the experimental *m/z* values with the theoretical *m/z* values defined in multiples of the experimental accuracy for the observed peak. See "Accuracy" on page 397. Default: 1
Eliminate Isotopic Peaks	Selects to remove relevant isotopic peaks.
Exclusion m/z List	Specifies the *m/z* values that you want to exclude.
Icons	
Add new custom contaminant	Adds new custom contaminant. The name, molecular formula, and charge specifications for the contaminant are mandatory. See "Add Custom Contaminant dialog box" on page 78.
Remove selected custom contaminant	Deletes selected user-defined contaminant from the list.
Remove all custom contaminants	Deletes all user-defined contaminants.
Buttons	
Preview	Begins the filtering process and shows the result in the chromatogram view.
Restore	Resets the chromatogram to its original (before filtering) state.
Accept	Accepts the filter and closes the Contaminants Elimination Filter window.
Cancel	Cancels the filter and closes the Contaminants Elimination Filter window.

Selecting or clearing the default and user-defined contaminants updates the *m/z* exclusion list.

Add Custom Contaminant dialog box

Clicking the Add New Custom Contaminant icon in the Contaminants Elimination Filter view opens the Add Custom Contaminant dialog box.

Figure 43. Add Custom Contaminant dialog box

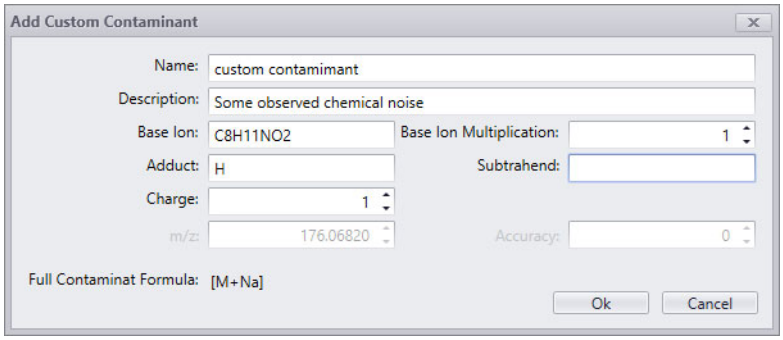


Table 43. Add Custom Contaminant dialog box parameter descriptions

Parameter	Description
Name	Name of the contaminant as it appears in the list.
Description	Description of the contaminant.
Base Ion	Molecular formula of the contaminant.
Adduct	Expected MS adduct of the contaminant.
Charge	Expected charge of the contaminant
Base Ion Multiplication	Number of base formulas that form a single adduct.
Subtrahend	Expected Neutral Loss on the contaminant.
m/z	When you define the base ion formula, the adduct, and the charge of a contaminant, the application automatically calculates the adduct ion's *m/z* value. Otherwise, you must enter the *m/z* value of the contaminant.
Accuracy	When you define the base ion formula, the application automatically calculates the accuracy of the contaminant's *m/z* value.

Peak Elimination filter

Use the Peak Elimination Filter view to remove spectral peaks that cannot be explained by any theoretical formula calculated from a subset of specified elements and their abundances.

Supported file format: Peak Elimination Filter parameter file (.chpro_pef)

For details, see these topics:

- Open the Baseline and Noise Elimination filter view
- Peak Elimination Filter parameters

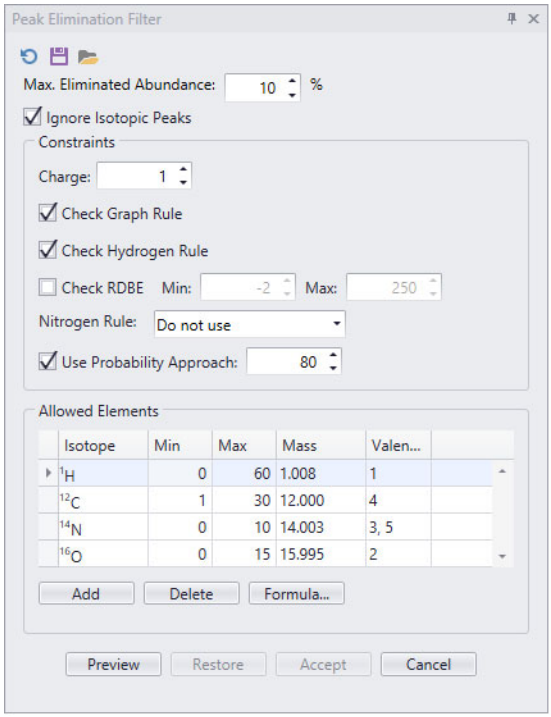
Open the Peak Elimination Filter view

❖ To open the Peak Elimination Filter view

From the Actions toolbar group of the Chromatogram Processor toolbar, click the **Peak elimination filter** icon, .

The Peak Elimination Filter view provides options to verify the elemental composition of the spectral peaks that make up the chromatogram. If a peak's *m/z* value cannot be defined by any combination of elements (such as a subformula), the filter removes it.

Figure 44. Peak Elimination Filter view



Peak Elimination Filter parameters

Table 44. Peak Elimination Filter view parameters (Sheet 1 of 2)

Icon, parameter, or button	Description
Reset parameters to the default state	Reverts the user-changed parameters to the default state.
Save parameters to a file	Saves the Peak Elimination Filter parameters to file (.chpro_pef).
Load parameters from a file	Loads the Peak Elimination Filter parameters from a file (.chpro_pef).
Max. Eliminated Abundance	Specifies the relative intensity limit above which peaks are not filtered.
Ignore Isotopic Peaks	Ignores isotopic peaks—that is, selecting this check box prevents the filter from removing isotopic peaks.
Constraints	
Charge	Charge applied to any generated formula.
Check Hydrogen Rule	Specifies whether to use an algorithm for the exclusion of implausible formulas with high numbers of hydrogen.
Check RDBE	Valid only for formulas having RDBE is within the minimum/maximum range. RDBE limits the calculated formulas to those that matches from a chemical perspective.
Nitrogen Rule	Specifies whether to use the nitrogen rule in the elemental composition calculation. The choices include: • Do not use • Even-electron ion (for example, radical-cation) • Odd-electron ion (for example, protonated)
Use Probability Approach	Use empirical rules to determine the likelihood of the generated formula based on the ratio of carbon versus hydrogens and carbon versus heteroatoms. The higher value increases the weight of the empirical threshold.
Allowed Elements table The Allowed Elements list displays a set of possible elements that you can use to generate possible formulas. The values in the Valence column are used to calculate the RDBE value.	
Add	Opens the Select Element dialog box.
Delete	Deletes the selected element.
Formula	Opens the Enter Molecular Formula dialog box.

Table 44. Peak Elimination Filter view parameters (Sheet 2 of 2)

Icon, parameter, or button	Description
Buttons at the bottom	
Preview	Begins the filtering process and shows the result in the chromatogram view.
Restore	Resets the chromatogram to its original (before filtering) state.
Accept	Accepts the filter and closes the Peak Elimination Filter window.
Cancel	Reverts the filter and closes the Peak Elimination Filter window.

Smoothing filter

The application performs smoothing to average ion profiles (not the total ion chromatogram) for every ion (*m/z* value) found in the data file with their neighbors in a time series. Smoothing can improve component detection by eliminating spikes that can cause false positive results.

Supported file format: Smoothing Filter parameter file (.chpro_smooth)

For details, see these topics:

- Use the Wizard mode for the smoothing filter
- Use the Wizard mode for the smoothing filter
- Use the Details mode for the smoothing filter

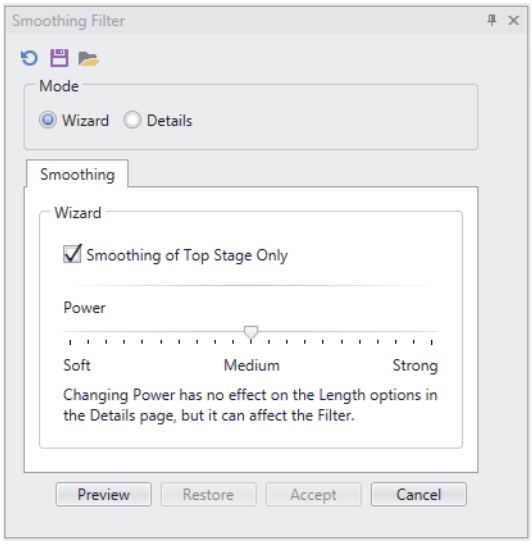
Open the Smoothing Filter view

❖ **To open the Smoothing Filter view**

From the Action group of the Chromatogram Processor toolbar, click the **Apply Smoothing Filters to All Scans in the Chromatogram** icon,  .

The view opens to the Wizard mode (Figure 45).

Figure 45. Smoothing Filter view – Wizard mode



Use the Wizard mode for the smoothing filter

❖ To use the Wizard mode for the smoothing filter

1. Open the Smoothing Filter view. See "Use the Wizard mode for the smoothing filter" on page 82.
2. (Optional) To reset the parameters to the factory default values, click the **Reset** icon, .
3. Specify whether to smooth only the MS1 scans or the scans from all the MS stages and the smoothing power.

> **Note** To load these settings from a file, click the **Load from File** icon, , and select the Smoothing Filter parameters file (.chpro_smooth) that you want to use.

Table 45. Smoothing Filter – Wizard mode parameter descriptions

Parameters	Description
Smoothing of Top Stage Only	Processes only the spectral peaks in the MS1 stage scans (full scans).
Power	Specifies the mass difference within which the algorithm merges spectral peaks into one *m/z* value. A low (soft) value might result in more components (oscillating ions) and a high (strong) value can result in fewer components (merging ions).

4. Click **Preview** to preview the effect of the parameter settings in the chromatogram view.
5. (Optional) To undo smoothing, click **Restore**. To undo smoothing and close the view, click **Cancel**.

6. After you optimize the settings, click **Accept**.
7. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a Smoothing Filter Parameters File (.chpro_smooth).

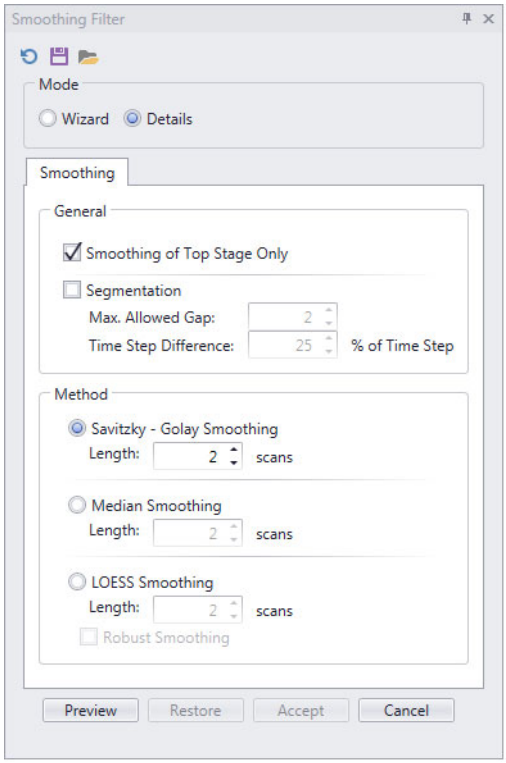
Use the Details mode for the smoothing filter

❖ To apply the smoothing filter in the Details mode

1. Open the Smoothing Filter view. See "Open the Smoothing Filter view" on page 81.
2. In the Wizard mode, specify the smoothing power. See "Use the Wizard mode for the smoothing filter" on page 82.
3. Select the **Details** option.

   The Details view opens.

   Figure 46. Smoothing Filter View – Details mode



4. Specify the advanced parameter settings for the smoothing filter (Table 46).

Table 46. Smoothing Filter – Details mode parameter descriptions (Sheet 1 of 2)

Parameter	Description
General	
Smoothing of Top Stage Only	Processes only the spectral peaks in the MS1 stage scans (full scans).
	Note The Smoothing of Top Stage Only check boxes for the Wizard and Details modes are synchronized.
Segmentation	Divides the chromatogram into discrete parts before applying the smoothing filter. This option can provide better results for a chromatogram that exhibits diverse shape, peak density, and baseline characteristics over the retention time scale. Avoid segmentation if you use a LOESS derivative filter or if the results obtained without segmentation are acceptable.
Method	
Savitzky-Golay Smoothing	Smooths each ion profile in the chromatogram using the Savitzky-Golay filter. The Length parameter determines the filter length. Default length: 2 scans; range: 1 to 100
	IMPORTANT This smoothing method requires equidistant time steps between scans. If the smoothed results are unacceptable, use another smoothing method, or select the Segmentation check box and specify the maximum allowed gap and time step difference.
Median Smoothing	Smooths each ion profile in the chromatogram using the Median filter. The Length parameter determines the filter length. Default length: 2 scans; range: 1 to 100

Table 46. Smoothing Filter – Details mode parameter descriptions (Sheet 2 of 2)

Parameter	Description
LOESS Smoothing	Smooths each ion profile in the chromatogram using the LOESS filter (locally weighted scatterplot smoothing). The Length parameter determines the filter length. This filter works in a similar way to the Savitzky-Golay filter, but it is slower and does not require equidistant time steps between scans. When Robust Smoothing is enabled (by selecting the check box), the smoothing is slower but more robust to outlier data in ion profiles. Default length: 2 scans; range: 1 to 100
Robust Smoothing (for the LOESS method)	Smooths using a robust version of the LOESS filter. This option is both less sensitive to outliers and slower than the LOESS Smoothing filter (without robust smoothing).

5. Click **Preview** to preview the effect of the parameter settings in the chromatogram view.
6. (Optional) To undo smoothing, click **Restore**. To undo smoothing and close the view, click **Cancel**.
7. After you optimize the settings, click **Accept**.
8. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a Smoothing Filter Parameters File (.chpro_smooth).

Threshold filter

Use the threshold filter to eliminate noise peaks or peaks from minor components that are not of interest. Or, use the filter to mark peaks that are less intense than the calculated threshold level.

Thresholding is a data processing method that analyzes every scan to reduce ion intensities or delete spectral peaks if algorithmic criteria have been met. For this filter to function properly, the intensity of the noise peaks must be lower than the intensities from the analyte peaks, and the noise peak count must be significantly higher than the signal peak count.

Supported file format: Threshold Filter parameter file (.chpro_threshold)

For details, see these topics:

- Open the Threshold Filter view
- Use the Wizard mode for the Threshold Filter
- Use the Details mode for the threshold filter

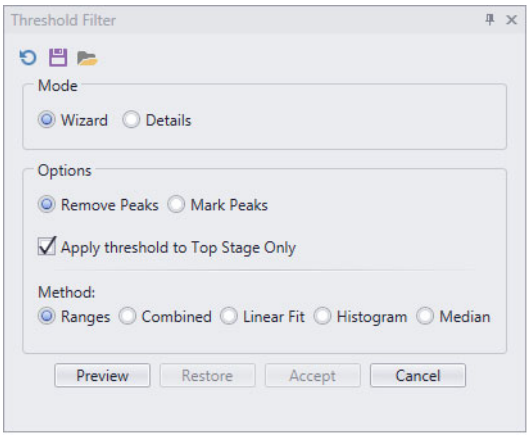
Open the Threshold Filter view

❖ To open the Threshold Filter view

From the Actions group of the Chromatogram Processor toolbar, click the **Apply threshold filter to all scans in the chromatogram** icon, .

The Threshold Filter view opens to the Wizard mode. The icons at the top of the view and the buttons at the bottom of the view are common to both processing modes.

Figure 47. Threshold Filter view – Wizard mode



Use the Wizard mode for the Threshold Filter

The Wizard mode provides a simplified set of the most important parameters.

❖ To use the Wizard mode for the Threshold Filter

1. Open the Threshold Filter view. See "Open the Threshold Filter view" on page 86.
2. (Optional) To reset the parameters to the factory default values, click the **Reset** icon, .
3. Specify the following:
   - Whether to remove low-intensity peaks or only mark the noise peaks in blue and the signal peaks in red.
   - Whether to apply the filter to all the scans in the chromatogram or only the MS1 stage scans.
   - The method for calculating the intensity threshold.

   Tip To load these settings from a file, click the **Load from File** icon, , and select the Threshold Filter parameters file (.chpro_threshold) that you want to use.
4. Click **Preview** to preview the effect of the parameter settings in the chromatogram view.

5. (Optional) To undo smoothing, click **Restore**. To undo smoothing and close the view, click **Cancel**.
6. After you optimize the settings, click **Accept**.
7. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a Smoothing Filter Parameters File (.chpro_threshold).

Table 47. Threshold Filter – Wizard mode parameter descriptions (Sheet 1 of 2)

Parameter	Description
Select one of these options: Remove Peaks or Mark Peaks.	
• Remove Peaks	Removes peaks below the calculated threshold intensity value.
• Mark Peaks	Marks peaks (in red) that are higher in intensity than the calculated threshold intensity value (the point where the application preserves peaks). The peaks that are below the intensity threshold remain blue—these peaks are more likely due to chemical or electronic noise. Does not remove any spectral peaks.
Apply Threshold to Top Stage Only	Specifies that only MS1 (full scan) scans are processed.
Method	
• Ranges	Creates a histogram from the abundances of the peaks outside of the combinatorially possible elemental compositions that are common for small organic molecules. Uses the smoothed maximum of these peaks as the threshold noise value. Applies only to spectra having more than five peaks.
• Combined	Calculates the linear fit and histogram thresholds, and then uses the smaller value from these two values as the threshold value.

Table 47. Threshold Filter – Wizard mode parameter descriptions (Sheet 2 of 2)

Parameter	Description
• Linear Fit	Determines the threshold noise value by applying a linear fit to the scan data occurs as follows: • Applies a linear fit [Intensity = a + b × (*m/z*)] to a scan, and then applies the test for outlier data (using Student's t-distribution) to each spectral peak. • Considers outlier peaks as signals and excludes them from noise. • Iteratively applies linear regression and exclusion of outlier peaks (signal) to each considered peak while finding no new outlier points. • Determines the threshold value as the intensity of the most intense noise peak (the highest peak that is not excluded from noise).
• Histogram	The application creates a histogram of abundance versus time from the abundances of all the spectral peaks in the analyzed scans, and then applies smoothing to the histogram. The application uses the abundance at the last data point or the minimum point between two peaks in the smoothed histogram as the threshold noise level.
• Median	Applies a median filter to the analyzed scans, and then uses the maximum filtered abundance as the threshold value.

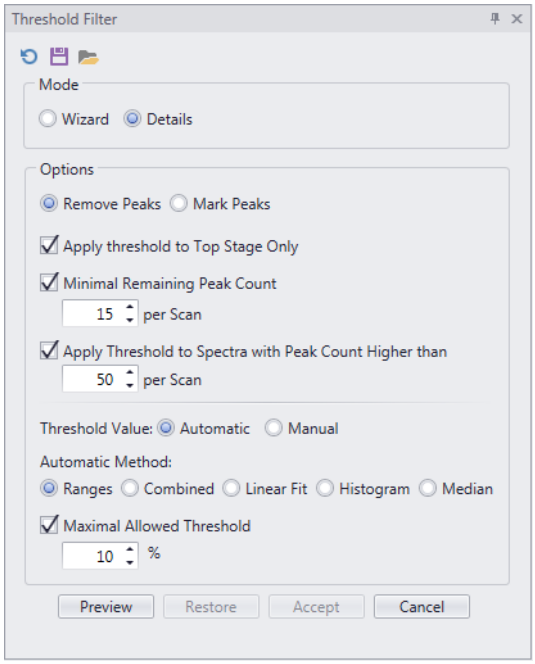
Use the Details mode for the threshold filter

❖ **To use the Details mode for the Threshold Filter**

1. Open the Threshold Filter view. See "Open the Threshold Filter view" on page 86.
2. Select the **Details** option.

   The parameters for the Details mode appear (Figure 48).

Figure 48. Threshold Filter view – Details mode



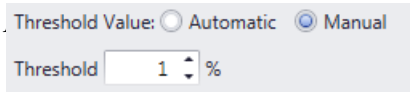
3. Specify the filter settings (Table 48).

Tip To load these settings from a file, click the **Load from File** icon, , and select the Threshold Filter parameters file (.chpro_threshold) that you want to use.

Table 48. Threshold Filter – Details mode parameter descriptions (Sheet 1 of 2)

Parameter	Description
Select one of these options: Remove Peaks or Mark Peaks.	
• Remove Peaks	Removes the peaks below the calculated threshold intensity value.
• Mark Peaks	Marks the threshold peaks in red that have a higher intensity than the threshold value (the point where the application preserves peaks). No peaks are removed.
Apply Threshold to Top Stage Only	Specifies that only MS1 (full scan) scans are processed.
Minimal Remaining Peak Count	Specifies the minimum number of peaks per scan that must remain after applying the filter. If a scan's peak count is equal to or less than this value, the filter does not remove any peaks. The filter removes the least intense peaks first. Default: 15 peaks per scan; range: 0 to 65 000

Table 48. Threshold Filter – Details mode parameter descriptions (Sheet 2 of 2)

Parameter	Description
Apply Threshold to Spectra with Peak Count Higher Than	Specifies that the threshold filter is applied only to scans with a higher number of peaks than the specified value. Default: 50 peaks per scan; range: 0 to 65 536
Threshold Value	Select one of these options: Automatic or Manual
Automatic Method for the Automatic option	See the descriptions for the five methods that you can select from to calculate the threshold intensity value in "Use the Wizard mode for the Threshold Filter" on page 86.
Maximal Allowed Threshold	Specifies the maximum allowed intensity threshold for the Automatic option.
Threshold	Specifies the intensity threshold for the Manual option. 

4. Click **Preview** to preview the effect of the parameter settings in the chromatogram view.
5. (Optional) To undo smoothing, click **Restore**. To undo smoothing and close the view, click **Cancel**.
6. After you optimize the settings, click **Accept**.
7. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a Smoothing Filter Parameters File (.chpro_threshold).

Component detection

The Mass Frontier application incorporates an automated system for detecting chromatographic components in complex LC/MS runs and extracting mass spectral signals from closely coeluting components (deconvolution).

To identify components by running a library search or to send a component to the Curator or Data Manager module, you must first apply one of the component detection algorithms to the LC/MS data.

Supported file formats: JCD parameter file (.chpro_jcd), TECD parameter file (.chpro_tcd), and DICD parameter file (.chpro_direct)

The component detection and spectra deconvolution system involves the combined use of the following procedures:

- Noise examination, signal filtering, and smoothing
- Baseline definition and demarcation of chromatographic peaks
- Background scan determination and background subtraction
- Component candidate detection and model ion selection (*m/z*)
- Model ion profile correlation and confirmation or rejection of component
- Spikes elimination
- Component retention time calculation
- Spectra deconvolution using linear algebra

You can use the component detection algorithms for broad types of chromatographic runs, for clean and noisy signals, and for simple or complex chromatograms from samples that include small- and medium-sized organic compounds. However, to optimize the detection of components for specific applications, you might need to adjust some of the parameter settings.

The application identifies components by using any of these algorithms:

- Joint Component Detection (JCD) algorithm
- Total Ion Extraction Component Detection (TECD) algorithm
- Direct Infusion Components Detection (DICD) algorithm

Joint Component Detection (JCD) algorithm

The Joint Components Detection (JCD) algorithm is based on the statistical analysis of ion profile maxima. The ion profiles with comparable shapes and maxima belonging to a limited time range are considered as a single component. The algorithm extracts individual mass spectral peak abundance profiles to produce a "purified" spectrum or spectral trees, and generates the peak shape of a representative component. For best results, use the JCD algorithm for chromatographic data from LC/MS runs, but be aware that it requires significant computer processing resources.

Tip To significantly reduce the computation time for large files, apply a background subtraction, threshold filter, or both to the chromatogram before running the JCD algorithm.

There are two parameter adjustment modes for the JCD algorithm: Wizard and Details. The settings for the two modes are mutually exclusive, so the application only applies the settings for the selected mode.

For details, see these topics:

- Use the Wizard mode for the JCD algorithm
- Use the Details mode for the JCD algorithm

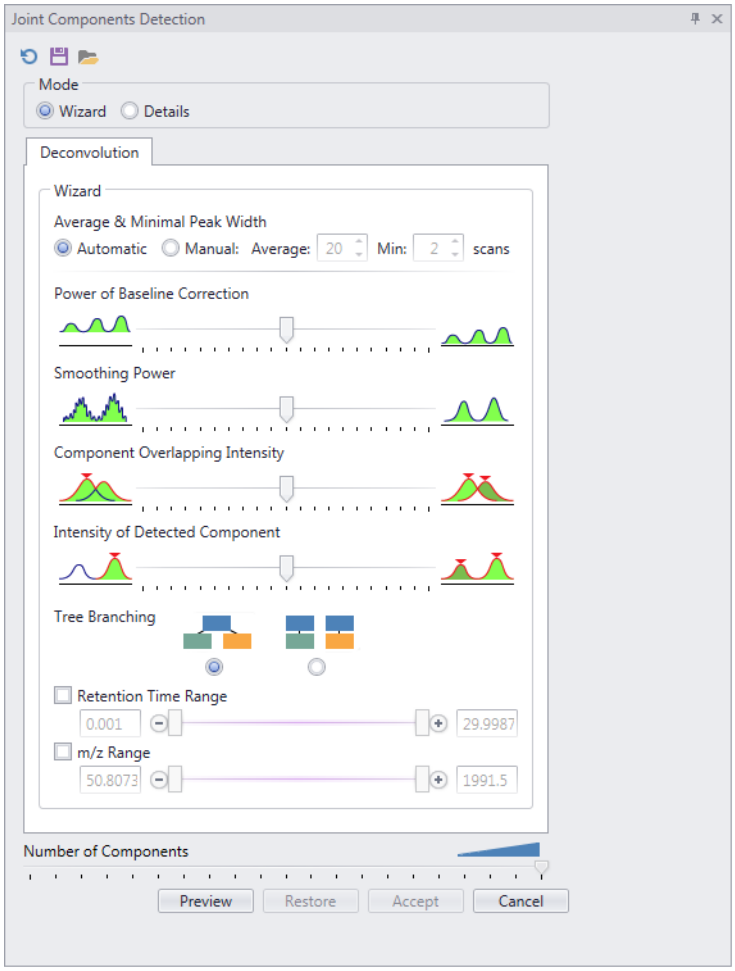
Use the Wizard mode for the JCD algorithm

❖ **To apply the Joint Components Detection algorithm in the Wizard mode**

1. Open a data file in a Chromatogram Processor window.
2. In the Actions group of the Chromatogram Processor toolbar, click **JCD**.

   The JCD view opens to the Wizard mode.

Figure 49. Joint Components Detection view in the Wizard mode



3. Optimize the parameter settings and click **Preview**.

Tip To reset the parameters to their factory default settings, click the **Reset** icon,

Table 49 describes the parameters for the Wizard mode of the JCD algorithm.

Table 49. JCD – Wizard mode – parameter descriptions

Parameter icon	Description
Average & Minimal Peak Width	Automatic—The application automatically calculates the average chromatographic peak width by analyzing the chromatogram. Manual—Activates the Average and Min boxes where you can specify the average chromatographic peak width by the number of scans. • Average—Default: 20 scans; range: 3 to 100 • Min.—Default: 2 scans; range: minimum peak width must be lower than the average peak width. If the peak width, in scans, is less than the specified minimum peak width, the algorithm considers the peak to be a spike rather than a chromatographic peak for a component. Error message that appears when the minimum peak width is greater than the average peak width: Minimal peak width must be lower than peak width.
Power of Baseline Correction	Specifies the power of the baseline correction within a low-to-high range.
Smoothing Power	Specifies the smoothing power applied to each ion profile within a low-to-high range.
Components Overlapping Sensitivity	Specifies the overlapping sensitivity within a low-to-high range.
Intensity of Detected Components	Specifies the intensity of detected components within a high-to-low range.
Tree Branching	Specifies one of the following: • Merge the parent ions into one component (). • Produce an individual tree for each parent ion ().
Retention Time Range	Specifies the retention time range to process. Does not process scans outside this time range. To adjust the retention time range, enter the start and end times in the boxes or use the sliders. Default: Entire acquisition run time read from the data file
m/z Range	Specifies the *m/z* range of the parent ions to process.

Tip If you previously optimized the component detection settings for a similar experiment and saved the settings to a file, click the **Load** icon, , and select the parameters file to load the saved parameter settings.

4. (Optional) To undo component detection, click **Restore**. To undo component detection and close the JCD view, click **Cancel**.
5. After you optimize the settings, click **Accept**.
6. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a JCD parameters file (.chpro_jcd).

Use the Details mode for the JCD algorithm

Use the Details mode to fine-tune the individual deconvolution parameter settings.

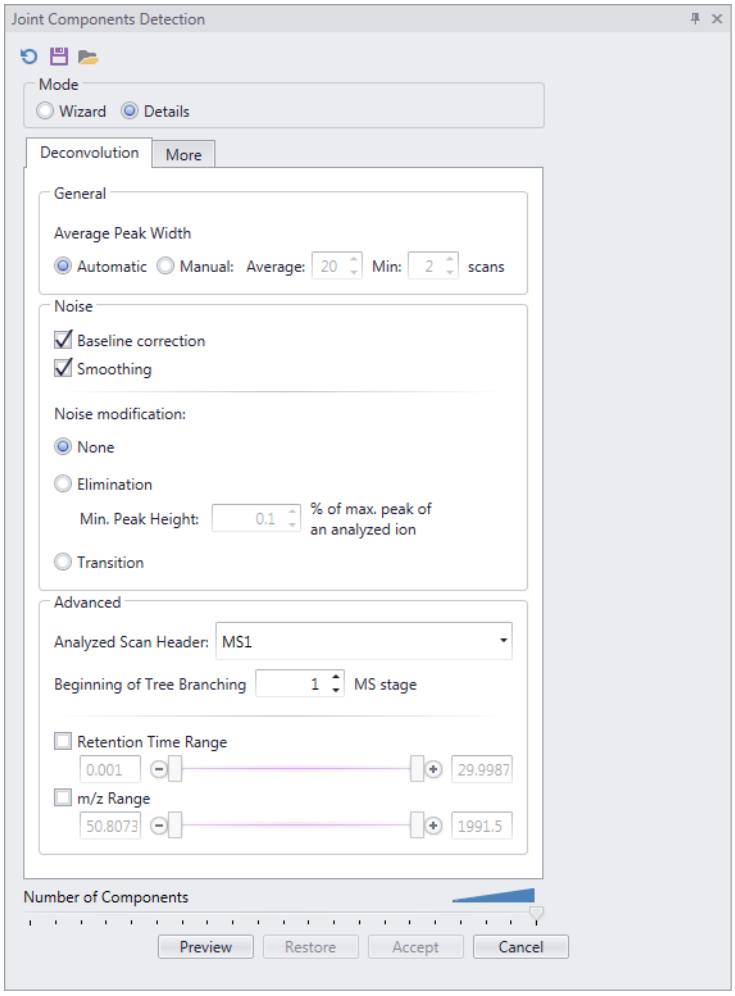
❖ To apply the Joint Components Detection algorithm in the Details mode

1. Open a data file in a Chromatogram Processor window.
2. In the Actions group of the Chromatogram Processor toolbar, click **JCD**.

   The JCD view opens to the Wizard mode.
3. Select the **Details** option.

   The Details mode opens to the Deconvolution page.

Figure 50. JCD view – Details mode – Deconvolution page



4. Optimize the parameter settings on the Deconvolution page.

Tip To reset the parameters to their factory default settings, click the **Reset** icon.

Table 50 describes the parameters for the Details mode of the JCD algorithm.

Table 50. JCD – Details mode – Deconvolution page parameter descriptions (Sheet 1 of 2)

Parameter	Description
General	
Average Peak Width	Specifies the chromatographic peak width in number of scans that the algorithm uses to identify a potential component. Select one of these options: Automatic or Manual. For the Manual option, enter the average chromatographic peak width in the Average box and the minimum number of scans across the peak in the Min scans box. • If you set the value too high, the application might ignore narrow chromatographic peaks. • If you set the value too low, the application might split a single component into two different components. The JCD algorithm only applies the Average Peak Width parameter to the scans that are specified in the Analyze MS Stages parameter. For example, if Analyze MS Stages is set to Top Stages, the Average Peak width is applied to MS1 scans only.
Noise	
Baseline Correction	Applies an automated baseline correction for each ion profile by using the Top-Hat algorithm. Select this check box only for chromatograms with an elevated baseline.
Smoothing	Performs the smoothing of each ion profile by using the Average Peak Width value and the LOESS algorithm. If the Average Peak Width parameter setting is too large or peaks are narrower than four to five scans, the smoothing might remove narrow chromatographic peaks.

Table 50. JCD – Details mode – Deconvolution page parameter descriptions (Sheet 2 of 2)

Parameter	Description
Noise Modification	Determines how to adjust the intensity values of spectral peaks with abundances comparable to the noise level. Select one of these options: • None—Does not alter the spectral peak intensities. • Elimination—Eliminates spectral peaks with intensities lower than the specified minimum peak height. The application applies the Min. Peak Height value to each ion profile. The specified minimum peak height is a percentage of the maximum analyzed ion profile. • Transition—Analyzes each ion profile and adds artificial mass peaks if zero peak intensity is detected. This option reduces the number of false positive detected peaks.
Advanced	
Analyzed Scan Header	Analyzes only scans with the selected scan header type. The selection list includes all the scan headers recognized in the raw data file or the HighChem chromatogram file. Default: MS1 (recommended) Selections: Scan headers read from the raw data file. **Tip** For AIF experiments with wide isolation widths, you might get useful results for the preliminary analysis of complex data by selecting an MS2 HCD scan header.
Beginning of Tree Branching	Determines the MS stage where a spectral tree is divided into two or more independent components based on the precursor ion. Default: 1
Retention Time Range	Processes scans from specified the retention time range. Default: The check box is not selected.
m/z Range	Processes only parent ions from the specified *m/z* range. Default: The check box is not selected.

5. Click the **More** tab.
6. Optimize the parameter settings on the More page of the Details mode.

Figure 51. JCD – Details mode – More page

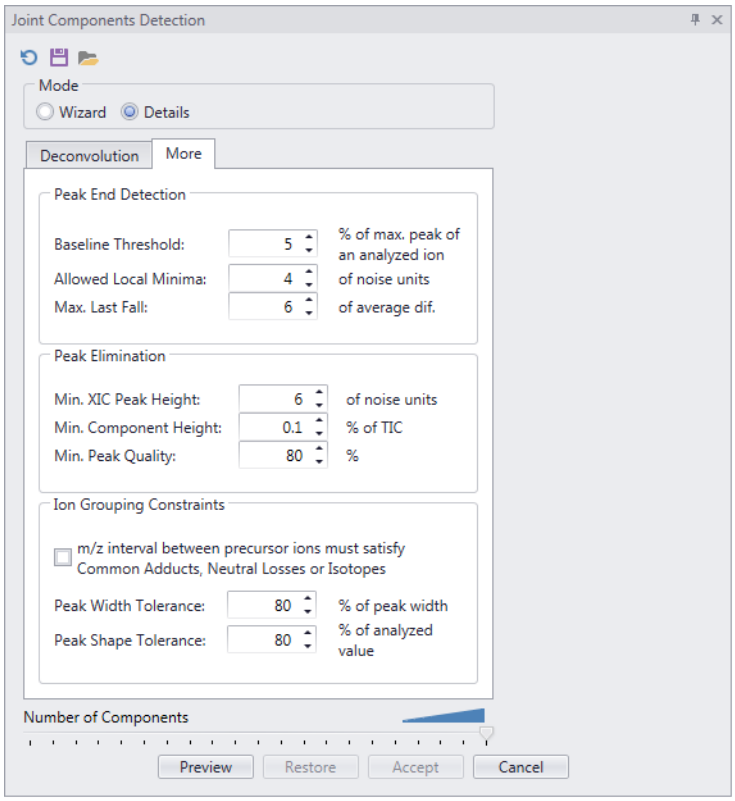


Table 51. JCD – More page – parameter descriptions (Sheet 1 of 2)

Parameter	Description
Peak End Detection	
Baseline Threshold	Specifies the peak baseline as a percentage of base peak height. Default: 5
Allowed Local Maxima	Specifies the sensitivity to local minima of ion profiles. Detects peak ends when the local minimum of the analyzed ion profile is greater than this value. This value is determined as a multiple of estimated noise. Default: 4 (four times the estimated noise level)

Table 51. JCD – More page – parameter descriptions (Sheet 2 of 2)

Parameter	Description
Max. Last Fall	Specifies the maximum allowed difference in intensity between the first and second scans at the beginning of a chromatographic peak and the penultimate and last scans at the end of a chromatographic peak. If the difference in intensity between the first two scans or between the last two scans is higher than this value, the first or last scan is removed from components, respectively. Default: 6 abundance units
Peak Elimination	
Min. XIC Peak Height	Specifies the minimum ion profile peak height to be considered as a potential component. This value is determined as a multiple of the estimated noise. Default: 6
Min. Component Height	Specifies the minimum intensity of component peaks. This value is determined as a percentage of the intensity of the total ion chromatogram at the position of the component's maxima. Default: 0.1% of TIC
Min. Peak Quality	Specifies the minimum quality of the chromatographic peak's shape. Default: 80% (triangular peak shape)
Ion Grouping Constraints	
m/z interval	Merges horizontal precursor ions only if the *m/z* difference is equal to one of the common adducts, neutral losses, or isotopes or any combination of the three.
Peak Width Tolerance	Specifies a limit for the time difference of the ion profiles' maxima. High values cause the merging of randomly coeluting components, and low values split a component into more false-positive components. The application merges ion profile peaks within this range into a single component. Default: 80% of the peak width
Peak Shape Tolerance	Specifies the degree (%) of similarity of ion profile shapes. If two ion shapes meet the specified percentage for sharpness tolerance, as well as other parameters, the algorithm merges the ions into a single component. Default: 80% of the analyzed value

7. Click **Preview**.
8. After you optimize the parameter settings, click **Accept**.
9. (Optional) To reuse the parameter settings for similar LC/MS data files, click the **Save** icon, , and save the settings to a JCD parameters file (.chpro_jcd).
10. (Optional) To undo component detection, click **Restore**. To undo component detection and close the JCD view, click **Cancel**.

Total Ion Extraction Component Detection (TECD) algorithm

The Total Extraction Components Detection (TECD) algorithm creates spectral trees for every section of a chromatogram that starts with an MS1 scan. Each generated tree is divided into subtrees based on the value of the Beginning of Tree Branching parameter. The algorithm merges the subtrees into components by using the precursor ion *m/z* value and the spectral tree match factor similarity score.

You can apply the TECD algorithm to data-dependent chromatograms only, as it creates components according to precursor values and spectra similarity only. It is not as advanced as JCD, but it is significantly faster.

Figure 52. Total Extraction Components Detection view with the default settings

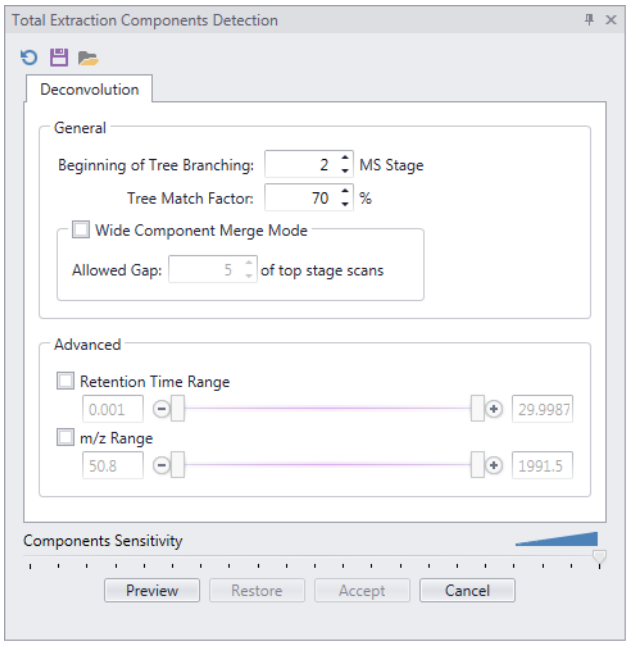


Table 52 describes the parameters for the TECD algorithm.

Table 52. Total Extraction Components Detection parameter descriptions (Sheet 1 of 2)

Parameters and icons	Description
Icons	
Reset Parameters to the Default State	Reverts user-changed parameters to the default state.
Save Parameters to a File	Saves the TECD parameters to a .chpro_tcd file.
Load Parameters from a File	Loads the TECD parameters from a .chpro_tcd file.
General	
Beginning of Tree Branching	Specifies the minimum number of tree sections the algorithm creates from the initial spectral tree. The setting determines the MS stage where a division of a spectral tree takes place.
Tree Match Factor	Specifies the minimum percentage that two spectral trees within adjacent tree sections must match before the algorithm considers the two spectral trees to be the same component. To be considered a match, two spectral trees must have identical precursors up to the level specified by the Beginning of Tree Branching setting and a Tree Match Factor value that exceeds the specified setting.
Wide Component Merge Mode	Enables a comparison of the spectral trees for potential matching and merging, not only in adjacent sections but also in nonadjacent sections up to the distance specified by the Allowed Gap value.
Allowed Gap	Specifies the maximum distance between nonadjacent tree sections when comparing the spectral trees for merging.
Advanced	
Retention Time Range	Processes only the scans within the specified retention time range.
m/z Range	Processes only the scans within the specified *m/z* range.
Bottom controls	
Components Sensitivity	Controls the number of detected components to be accepted. The slider bar is only enabled after the Preview action is completed. To detect fewer low-intensity components, move the slider bar to the left. Default: All (The slider is at the right end of the adjustment range.)

Table 52. Total Extraction Components Detection parameter descriptions (Sheet 2 of 2)

Parameters and icons	Description
Preview	Begins the component detection process and shows the results in the chromatogram view.
Restore	Resets the chromatogram to its original (before component detection) state.
Accept	Accepts the detected components and closes the TECD window.
Cancel	Cancels the detected components and closes the TECD window.

Direct Infusion Components Detection (DICD) algorithm

The Direct Infusion Components Detection algorithm is a spectral tree construction utility. Use this algorithm to create one or more spectral trees from a single raw file by reading various MSn scans acquired in one run, and constructing a tree according to their MS stage and precursor ion *m/z* values.

Figure 53. Direct Infusion Components Detection with the default parameter settings

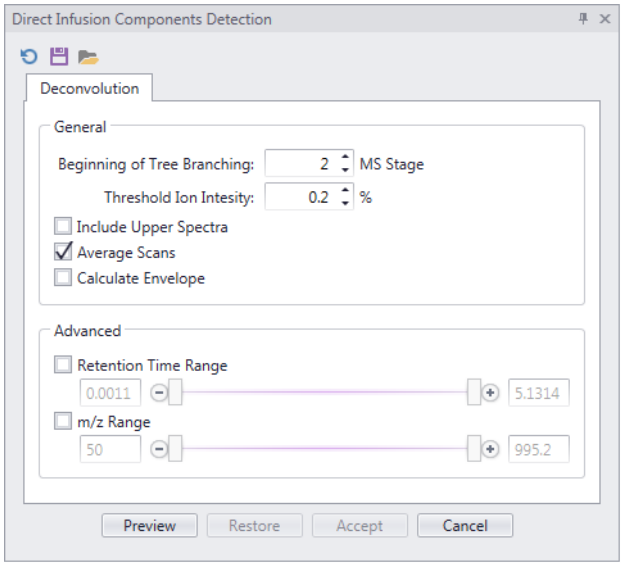


Table 53 describes the parameters for the DICD algorithm.

Tip To acquire spectral scans for a compound that you want to add to a custom user library, prepare a solution for that compound alone, and infuse the sample solution into a high-resolution accurate-mass MS.

To detect the component in the raw data file, Thermo Fisher Scientific recommends that you use the following DICD parameter settings:

- Beginning of Tree Branching: 1

  This setting preserves the original MS1 scans in the spectral tree.

- Threshold Ion Intensity: 0

  Use steps 3 and 4 of the Curator to remove noise and resonance peaks.

- Average Scans: Clear

  Use step 6 of the Curator to merge replicate spectra.

Table 53. Direct Infusion Components Detection parameter descriptions (Sheet 1 of 2)

Parameter	Description
Icons	
 Reset Parameters to the Default State	Reverts the user-changed parameters to the default state.
 Save Parameters to a File	Saves the DICD parameters to a .chpro_direct file.
 Load Parameters from a File	Loads the DICD parameters from a .chpro_direct file.
General	
Beginning of Tree Branching	Specifies the minimum number of tree sections the algorithm creates from the initial spectral tree. The value determines the MS stage where the division of the spectral tree takes place. Default: 2
Threshold Ion Intensity	Removes spectral peaks with an intensity that is lower than the specified threshold from each analyzed scan. Default: 0.2%
Include Upper Spectra	Adds actual scans in the stage above the level set in Minimal Tree Depth to the resulting spectral trees. When you clear this option, the spectra above the stage set in Minimal Tree Depth contain spectra with a single peak equal to the precursor ion of the product spectra.

Table 53. Direct Infusion Components Detection parameter descriptions (Sheet 2 of 2)

Parameter	Description
Average Scans	Specifies that every tree node only contains average spectra.
Calculate Envelope	When selected, the software calculates the ion profile (envelope) for each tree, displays abundance values for each component in the Components list, and displays a component's chromatogram in green in the chromatogram view.
Advanced	
Retention Time Range	Processes scans from the retention time range.
m/z Range	Processes only parent ions from the *m/z* range.
Buttons at the bottom	
Preview	Begins the component detection process and shows the result in the chromatogram view.
Restore	Resets the chromatogram to its original (before component detection) state.
Accept	Accepts the detected components and closes the DICD window.
Cancel	Reverts the detected components and closes the DICD window.

FISh analysis

Applying a FISh analysis (with the default parameter settings) to a chromatogram creates a marked peaks chromatogram made up of the spectral peaks for the defined FISh model fragments. You can use this feature to extract spectral peaks for structurally-related features, for example, compounds related to the parent drug in metabolite ID, API impurity ID, or compounds that share a common list of fragments. The FISh detection algorithm also applies the component detection algorithm that you specify: JCD or TECD.

Tip To get started with a FISh analysis, refer to the following tutorial:

Detecting Structurally-Related Compounds with a FISh Analysis

To access this tutorial, click the **Help** icon,  , at the top right of the application window, and then select the tutorial from the dropdown list.

Supported data formats:

- FISh parameter file (.fish2.par)
- Fragments for FISh detection (.sdf)

You can use the following opened windows as the source for the FISh reference spectrum:

- Spectrum in a Chromatogram Processor window
- Spectrum in a Data Manager window

You can use the following opened windows as the source for the FISh reference structure:

- Structure in a Curator window
- Structure in a Structure Editor window

You can open a structure file or use the structures from an opened Structure Grid as the source for the FISh fragments.

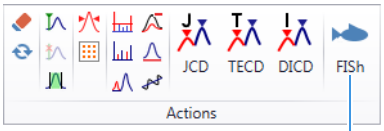
For details about running a FISh analysis and viewing FISh chromatograms, follow these topics in the order listed:

1. Create or select the FISh model for a FISh analysis
2. Specify the FISh detection options and start the analysis
3. View a FISh chromatogram

Create or select the FISh model for a FISh analysis

❖ **To create or select the FISh model**

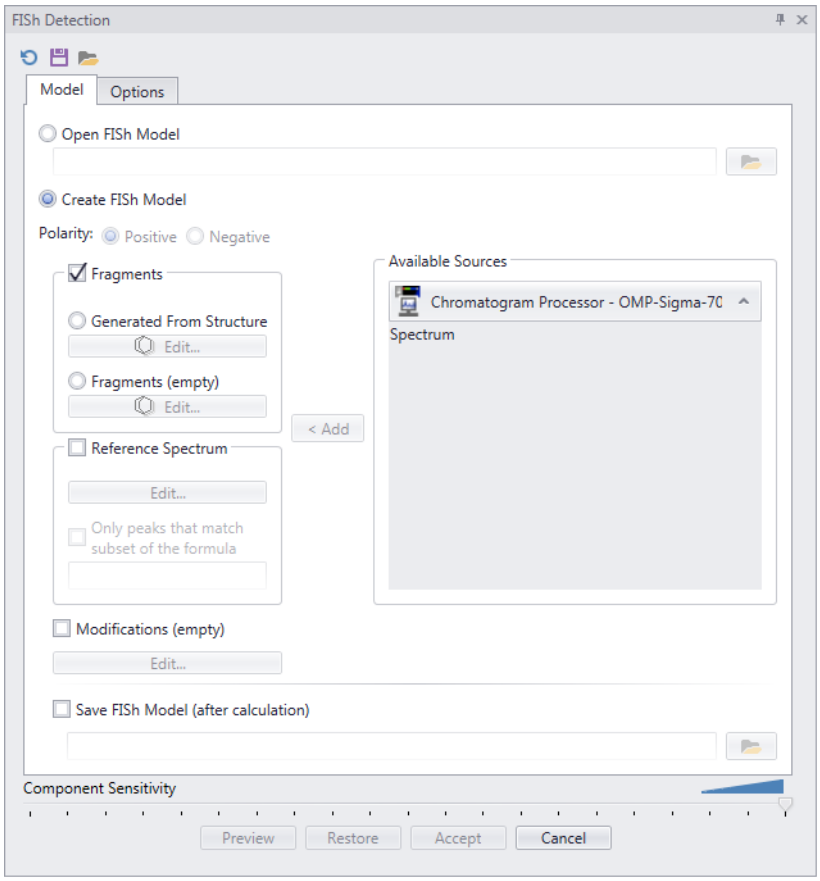
1. Open a raw data file.
2. In the Actions group of the Chromatogram Processor toolbar, click **FISh**.



FISh button

The Model page of the FISh Detection view opens to the right of the chromatogram and MS spectrum views.

Figure 54. FISh Detection view – Model page



3. Do one of the following to specify the FISh model:

   - Select the **Open FISh Model** option, and then browse to and select a FISh model file.

     All the other parameters on the Model page become unavailable. Go to "Specify the FISh detection options and start the analysis" on page 111.

   –or–

   - Select the **Create FISh Model** option.

     All the other parameters on the Model page become available. By default, the Fragments check box is selected.

4. To create the fragments set, do one or more of the following:

   - To generate the fragments from a structure, select the **Generated From Structure** option, and then select or draw the structure in the Structure Editor dialog box and click **OK**.

     When you run the analysis, you specify the reaction restrictions for the fragment generation process.

- To load a fragments list from a file, do the following:

  a. Click the **Fragments** option.

     The FISh filter: Input Fragments dialog box opens.

  b. Click the **Import** icon, .

  c. Select **Import File**, open the structure file that contains the fragment set, and click **OK**. Or, select another opened module that contains the fragment structures and click **OK**.

- To use a spectrum from the current raw data file or from another opened module as the source of the fragments' *m/z* values, do the following:

  a. Display a scan of interest in the MS spectrum view. In the Available Sources box, click **Spectrum** under the current instance of Chromatogram Processor. Then, click **Add**.

     Or, to use a spectrum from another opened module, click **Spectrum** under the source of interest in the Available Sources box, and then click **Add**.

     The application automatically selects the Reference Spectrum check box.

  b. In the Reference Spectrum area, click **Edit**.

  c. In the FISh Filter: m/z List dialog box, select, enter, or edit a list of *m/z* values and their accuracy, and then click **OK**.

  d. To filter out peaks for a specific formula, select the check box below in Edit button and enter the formula.

5. To search for spectral peaks that can be explained by various modifications of the fragment structures, do the following:

   a. Click the **Modifications** check box.

      The FISh Modifications dialog box opens.

   b. Select the check boxes for the modifications that you want to add, click **Add**, and then click **OK** to close the dialog box.

6. (Optional) To save the current FISh model, select the **Save FISh Model** check box, name the file in the Save As dialog box, and click **Save**.

After you specify the FISh model, go to "Specify the FISh detection options and start the analysis" on page 111.

Table 54. FISh Detection – Model page parameter descriptions (Sheet 1 of 3)

Parameter	Description
Icon	
 Reset Parameters to the Default State	Not applicable to the parameters on the model page.
 Save Parameters to a File	Saves the FISh parameters to a file (.fish2.par).
 Load Parameters from a File	Loads the FISh parameters from a file (.fish2.par).
Select the Open FISh Model option or the Create FISh Model Option.	
Open FISh Model	Uses the parameters from a saved FISh model (.fish2.par) file. When you select this option, the Create FISh Model parameters are unavailable. To use the parameters from a saved FISh model file, select the **Open FISh Model** option, and then browse to and select a FISh model file. The remaining parameters on the Model page are unavailable.
Create FISh Model	Creates a new FISh model. When you select this option, you cannot browse to and select a FISh model file.
Parameters for creating a FISh model	
Polarity	Select Positive or Negative polarity to add to the FISh model.
Fragments	Select this check box to define the template list of fragments or m/z values that are either removed and marked or conversely not removed and not marked from a chromatogram.
Generated from Structure	Select this option to open the Structure Editor dialog box for specifying the structure to fragment using the SledgeHammer module. The resulting fragments are the source of FISh fragments for annotation (building the FISh chromatogram).

Table 54. FISh Detection – Model page parameter descriptions (Sheet 2 of 3)

Parameter	Description
Fragments > Edit	Select this option to load fragments from an SDF file or to manually add or edit fragments. 1. To create the fragments list, click **Edit**. 2. In the FISh Filter: Input Fragments dialog box, do any of the following; • Click the **Import** icon, , click **Import File**, select a structure file, and click **Open**. • Click the **Add New** icon, . Then use the Structure Editor dialog box to specify the fragment structure.
Reference Spectrum	Use the parameters in this area and a spectrum from the list of Available sources to set up a list of *m/z* values for the FISh fragments. When you select a spectrum as the source of the FISh fragments and click Add, the application automatically selects the Reference Spectrum check box. Selecting this check box opens the FISh Filter: Input m/z List dialog box.
Reference Spectrum > Edit	Opens the Input m/z List dialog box where you can create a list of *m/z* values. Enter the *m/z* values and their tolerance in AMU. You can paste the data from a spreadsheet or from the Clipboard. The text content can contain either a single *m/z* column or an *m/z* column and an accuracy column.
Only Peaks that Match Subset of Formula	Select this check box to enter a reference formula that limits the fragments by their elemental composition. Uses only the spectral peaks (*m/z* values) that can explain any subset of the elemental composition that you enter.
Modifications	Select this check box to specify a modifications list.
Modifications > Edit	Opens the Modifications dialog box where you can create new modifications or select predefined modifications.

Table 54. FISh Detection – Model page parameter descriptions (Sheet 3 of 3)

Parameter	Description
Save FISh Model (after calculation)	Saves the active fragment source to the specified (.fish2.par) file. You can use this FISh model later without defining the fragments and modifications.
Available Sources	Lists the active windows that the application can use to create the FISh model (*m/z* values or fragment source). The available data types— Structure, Spectrum, or Fragments—appear under each available source. To add the respective source to the FISh model, select the type, and then click the **Add** button.

Specify the FISh detection options and start the analysis

❖ To specify the FISh options and start the analysis

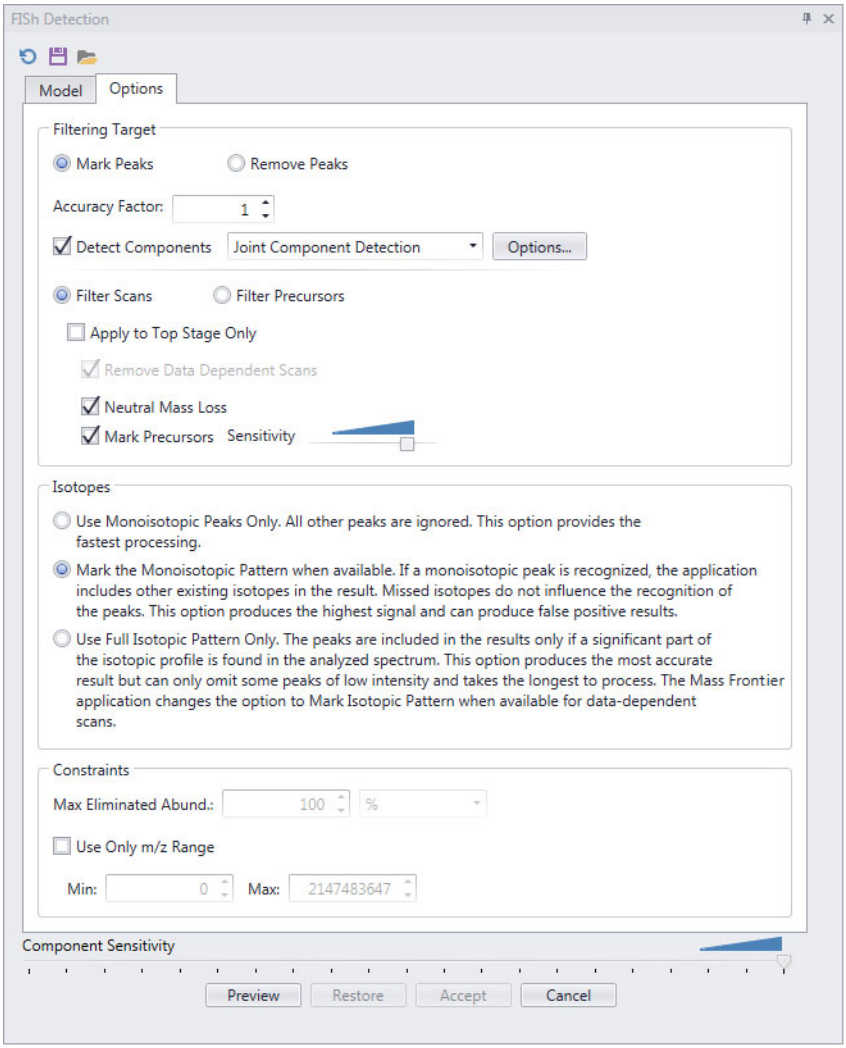
1. Click the **Options** tab to open the Options page of the FISh Detection view.
2. Click the **Reset** icon, , to reset the parameters on the Options page to the factory default settings.

   The factory default settings for the Options page are as follows:

   - The filtering target is set to mark peaks, detect components by using the JCD algorithm, and filter scans (mark the spectral peaks explained by the FISh model at all MSn stages).
   - The analysis includes the isotopic peaks when available.

   When the analysis finishes, you can view the marked peaks chromatogram in the chromatogram view, the explained spectral peaks on the Spectrum page of the MS spectrum view, and the fragment explanations for the spectral peaks on the FISh page of the MS spectrum view.

Figure 55. FISh Detection view – Options page



3. Modify the settings on the Options page as applicable (see Table 55).

Note For detecting known and unknown components, use the default settings:
- Mark Peaks: Selected
- Filter Scans: Selected
- Neutral Mass Loss (available after you set up the FISh model): Selected
- Mark Precursors: Selected

IMPORTANT The Filter Precursors feature excludes MS1 scans from the FISh analysis, which makes it incompatible with component detection.

4. Click **Preview**.
5. To accept the results, click **Accept**.

Use the Options page of the FISh Detection view to specify the component detection algorithm and the spectral peaks that make up the FISh chromatogram.

Table 55. FISh Detection – Options page parameter descriptions (Sheet 1 of 5)

Parameter	Description
Icon	
Reset	Resets the parameters on the Options page to their factory default state.
Filtering Target	
Select one of the options: Mark Peaks or Remove Peaks	
Mark Peaks	Marks those peaks in red (■) that are explained by any combination of the specified fragments—that is, peaks that can be explained by the specified fragments or their modifications appear in red in the MS spectrum view. These peaks also make up a marked peaks chromatogram and are part of the TIC chromatogram. This option does not remove any peaks from the MS2 scans. Default: Selected These selections are available for the Mark Peaks option: • Filter Scans • Filter Scans > Apply to Top Stage Only • Filter Scans > Mark Precursors • Filter Precursors • Filter Precursors > Filter Top Stage Scans
Remove Peaks	Removes spectral peaks that are not explained by any combination of the specified fragments or their modifications. Also, marks the peaks (see Marks Peaks in the previous table row). Default: Not selected These selections are available for the Remove Peaks option: • Filter Scans • Filter Scans > Mark Precursors • Filter Scans > Apply to Top Stage Only • Filter Scans > Apply to Top Stage Only > Remove Data Dependent Scans • Filter Precursors • Filter Precursors > Filter Top Stage Scans

Table 55. FISh Detection – Options page parameter descriptions (Sheet 2 of 5)

Parameter	Description
Accuracy Factor	Specifies the tolerance for matching the experimental *m/z* values with the ones from the theoretical calculations (fragmentation) and is defined in multiples of the experimental accuracy for the observed peak. Default: 1; range: 0.01 to 100
Detect Components	Specifies the component detection algorithm. Selections: Joint Component Detection or Total Extraction Components Detection
Options	Opens the dialog box where you specify the parameter settings for the selected component detection algorithm.
Note The Filter Scans and Filter Precursors options are mutually exclusive. The Filter Precursors option excludes MS1 scans from the FISh analysis, which makes it incompatible with component detection.	
Filter Scans	Specifies the filtering of spectral peaks in the scans. When this option is selected, the application does not remove entire scans; it only removes peaks from scans. For the Mark Peaks option, specifies the marking of explained spectral peaks. For the Remove Peaks option, specifies the removal of unexplained spectral peaks. These check boxes are available when the raw data includes data-dependent scans: Apply to Top Stage Only and Mark Precursors. You can select Apply to Top Stage Only or Mark Precursors.
Apply to Top Stage Only	When selected, the filter is applied only to top stage scans (usually MS1 scans). Data-dependent fragmentation scans are not affected. When the Mark Peaks option is selected, the Remove Data Dependent Scans, Neutral Loss, and Mark Precursors check boxes are unavailable. When the Remove Peaks Option is selected, the Neutral Loss and Mark Precursors check boxes are unavailable.

Table 55. FISh Detection – Options page parameter descriptions (Sheet 3 of 5)

Parameter	Description
Remove Data Dependent Scans	When selected, removes product scans if their precursor peaks or parent spectrum have been removed. If the Remove Peaks option is selected and the precursor peak in the parent spectrum is not explained by the FISh model, then the peaks in the data-dependent scan are removed. When cleared, applies the filter to data-dependent scans in the same manner as a top stage scan—that is, it removes individual spectral peaks from the product scans, but it does not remove the entire scan. Available when the Remove Peaks option, the Filter Scans option, and the Apply to Top Stage Only check box are selected.
Neutral Mass Loss	Specifies that all MS^n spectra are analyzed by the neutral mass loss. The neutral mass losses are calculated as the difference of the *m/z* values between the molecular ion and all the fragments structures provided in the FISh model. The fragments with the highest *m/z* value are used as the molecular ion. If the same value of the neutral loss is found between the precursor ion and any peak in the MS^n spectrum, the Spectrum page of the MS spectrum view shows the neutral loss. By default, MS^n peaks generated by a neutral loss are color-coded lime green ( ).

Table 55. FISh Detection – Options page parameter descriptions (Sheet 4 of 5)

Parameter	Description
Mark Precursors	When selected, the precursor peak that produces a product spectrum is included in the FISh chromatogram (even if it is not explained by the FISh model) if its product spectrum has a significant number of peaks explained by the FISh model (directly by any fragment from the fragments source, modification, or neutral loss). By default, product-explained peaks are color-coded purple ( ). The Sensitivity setting defines the relative summed intensity of the explained peaks in the product spectrum to the summed intensity of all the peaks in the product spectrum: • Moving the slider to the right increases the sensitivity and a smaller percentage of the product peaks must be explained to mark or remove the precursor peak in the parent spectrum. • Moving slider to the left decreases the sensitivity and a higher percentage of the product peaks must be explained to mark or remove the precursor peak in the parent spectrum. Therefore, decreasing the sensitivity setting typically returns fewer (structurally-related) components), as application uses the MS1 data for component detection. Selecting this option lets the FISh analysis explain a molecular ion of unknown modification when the FISh model and the metabolite produce the same fragments. Default: Selected
Filter Precursors	Removes fragmentation scans from the chromatogram when the precursor ion is not explained by the FISh model. Also removes the spectral peaks for unexplained precursor ions from the parent scans. This option is only available for data-dependent chromatograms. Filter Top Stage Scans: Modifies the top stage scans (usually MS1 scans). This check box is available when you select the Filter Precursors option. **IMPORTANT** The FISh analysis does not perform component detection when the Filter Precursors option is selected.

Table 55. FISh Detection – Options page parameter descriptions (Sheet 5 of 5)

Parameter	Description
Isotopes	
These options are available for data-dependent scans.	
Use Monoisotopic Peaks Only	Only includes monoisotopic peaks in the results. Ignores all other isotopes. This option uses the shortest computation time.
Mark Isotopic Pattern when Available	If the application recognizes a monoisotopic peak, it includes other existing isotopes in the result (as part of the total ion current). Missed isotopes do not influence the recognition of peaks for fragment ions that match the FISh model. This option produces the highest signal, but can also produce false positive results.
Use Full Isotopic Pattern Only	The peaks in an MS1 scan are included in the results only if a significant part of the isotopic profile is found in the analyzed spectrum. This option produces the most accurate result, but it tends to omit low-intensity peaks and consumes the most time. For data-dependent scans, the application changes the option to Mark Isotopic Pattern because the isolation width for MSn scans is usually to narrow to include all the isotopic peaks for a fragment ion. The FISh analysis checks for the presence of isotopic ions that are within the detected isolation width.
Constraints	
Maximum Eliminated Abundance	Peaks with intensity higher than this threshold value are not removed.
Use only m/z Range	Min/Max values of the FISh fragments.
Component Sensitivity slider	
Controls the number of detected components that are accepted. By default, moving the slider to the left removes the components starting from those with the lowest abundance.	
Buttons	
Preview	Runs the FISh analysis and shows the result in the chromatogram view.
Restore	Resets the chromatogram to its original state before the FISh analysis.
Accept	Accepts the detected components and closes the FISh Detection view.
Cancel	Cancels the analysis and closes the FISh Detection view.

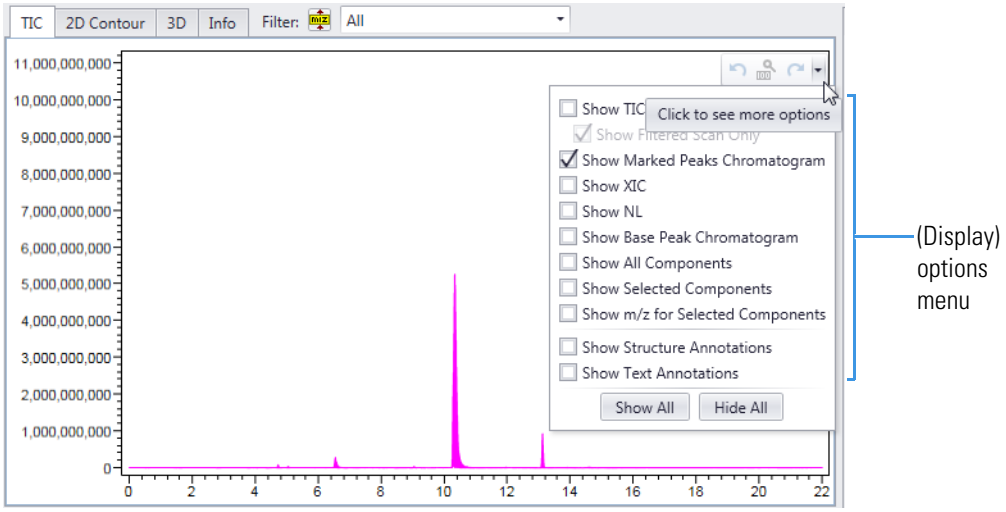
View a FISh chromatogram

You can inspect the FISh explained peaks in the chromatogram view and on the FISh page of the MS spectrum view. See "MS spectrum view – FISh page" on page 59.

❖ To view the FISh chromatogram from a FISh analysis

1. Open the options menu for the TIC page of the chromatogram view.
2. With the exception of the Show Marked Peaks Chromatogram check boxes, clear all the other check boxes.

Figure 56. Marked peaks chromatogram in the chromatogram view of the Chromatogram Processor



Component library searches

The Mass Frontier application processes MSn spectra in hierarchically consistent spectral trees that you can search in spectral libraries. A spectral tree represents a distinct component as detected by any of the component detection methods.

In the Component Search view, you can submit all the detected components for a library search using any of the predefined search methods or with user-defined parameter settings (custom profiles). The search results are displayed in the context of the whole component (all the scans associated with the component) in contrast to the Spectrum Search, which searches for a library spectrum that matches a single query spectrum.

Note The default spectral library for searching LC/MS data is the mzCloud Reference library.

See the following topics:

- Identify components by running a components library search
- Component Search toolbar
- Search types for a component search
- Search parameters for each search type
- Library parameter for a component search
- Search results for a component search
- Search Details window for a component search

Identify components by running a components library search

After you detect the components in a chromatogram, you can identify the components by running library searches in the Chromatogram Processor window.

❖ To identify components by running a components search

1. Open a Chromatogram Processor window. See "Open a Chromatogram Processor window" on page 24.
2. Run one of the component detection algorithms. See "Component detection" on page 91.

   A collapsible Components list appears in the chromatogram data view at the upper left of the Chromatogram Processor window.
3. Accept the detection results.

   The component detection view closes.

4. In the Search group of the Chromatogram Processor toolbar, click **Components Search**.

   The Component Search view opens at the right of the Chromatogram Processor window.

5. Select the search type. See "Search types for a component search" on page 123.

6. Select the mass spectral libraries that you want the application to search.

   IMPORTANT If you select an mzCloud library, make sure that your computer has access to the mzCloud mass spectral database. To check the connection, click **Online** in the upper right corner of the application window and choose **Connection Check**.

7. Do one of the following:

   - To run a library search on selected components, select the components in the Components list, and then click **Search Selected**.
   - To run a library search on all the components, click **Search All**.

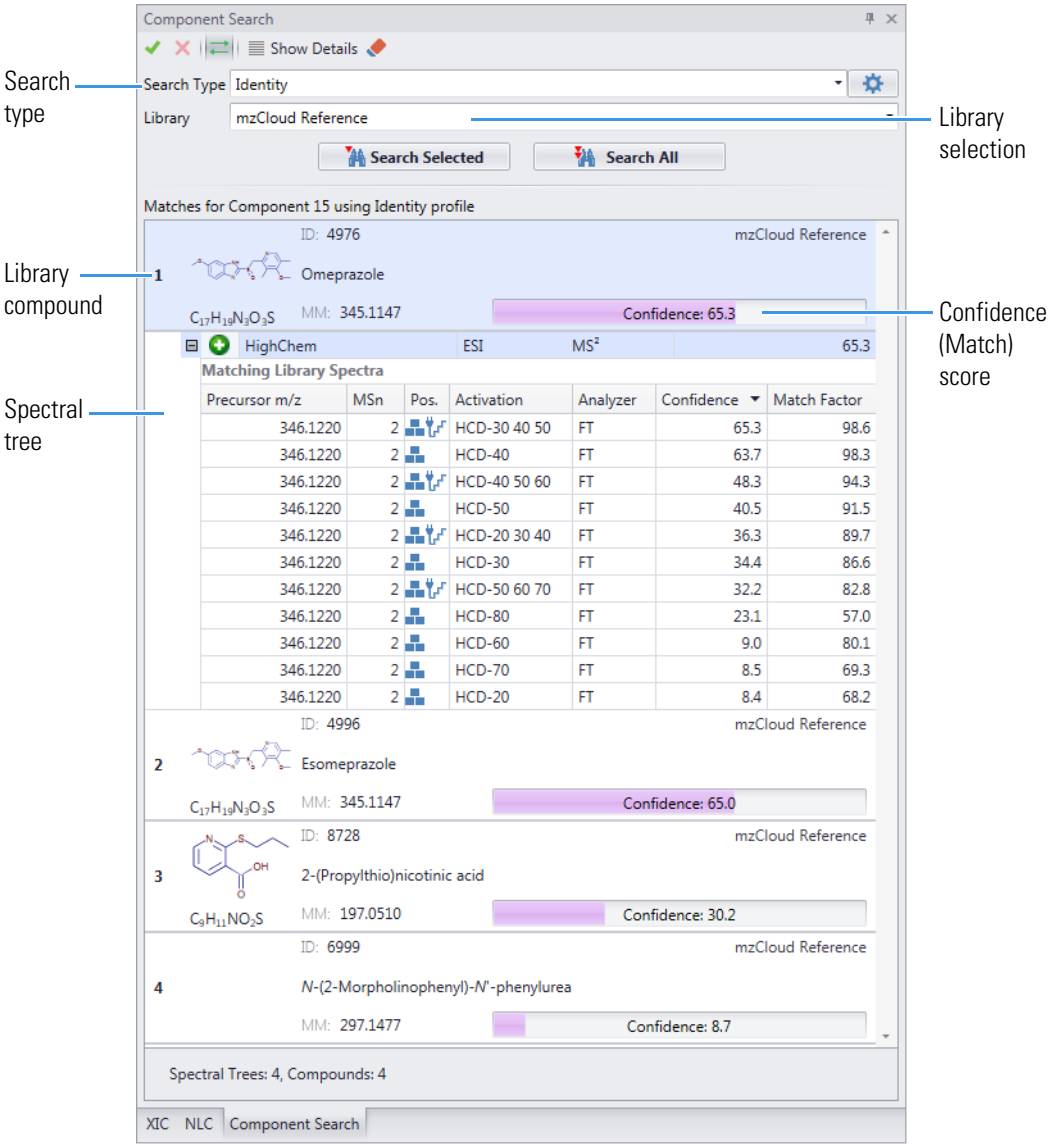
     Tip To minimize the processing time, consider deleting components that are of no interest from the Components list before running a components search on the entire list.

     To delete components, select them, and then click **Delete** in the Edit group of the Chromatogram Processor toolbar.

   The application searches the specified libraries for spectra that match the query spectra for the submitted components.

Figure 57 shows the Component Search view with a list of search results for a component from an Identity search.

Figure 57. Component Search view with search results from an Identity search



Component Search toolbar

Figure 58. Component Search view toolbar before you run a search

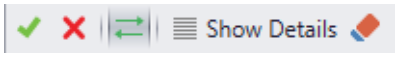


Table 56. Component Search toolbar

Icon or button		Description
Accept Structure		Accepts the selected library compound for the component. The library compound name and component match score appear in bold in the Components list in the chromatogram data view in the Chromatogram Processor window.
Accept All Suggested Structures		Accepts the top library compounds for the selected components. The library compound names and component match scores appear in bold in the Component list in the Chromatogram Processor window.
Clear Accepted Structure		Clears the accepted compound for the selected component.
Clear Accepted Structures for Selected Components		Clears all the accepted compounds for the selected components in the Components list.
Automatically Select Best Matching Spectrum Pair		In the Compare Spectra pane, when cycling through Component Spectral Tree (Query), always display the corresponding top matching spectra from library. When cycling through the spectra from library, always display the corresponding top matching query spectra.
Show Details	–	Opens a new page with the search results. See **Search Details**.
Clear		Clears all the preliminary search results from the Components list. Does not clear the library structures (annotations) that you have accepted.

Search types for a component search

Table 57 describes the search types for a component search.

Table 57. Search types for a component search (Sheet 1 of 2)

Selection	Description
Identity	A search method that compares only the MS^2 spectra for a component to any MS^2 spectra in the selected libraries. The primary algorithm is Confidence, which takes into account the dot product of both spectra, distribution of peaks, activation energy difference, and polarity, and uses a machine-learning Bayesian Network model to estimate the likelihood of a correct match. Additionally, the MS^2 precursor of the unknown and library trees must match.
Identity Substructure	A search method that compares the MS^n spectra for a component to any MS^n spectra in the selected libraries. The primary algorithm is Confidence, which takes into account the dot product of both spectra, distribution of peaks, activation energy difference, and polarity, and uses a machine-learning Bayesian Network model to estimate the likelihood of a correct match of the respective MS^n precursors.
Similarity Reverse	A search method that compares the MS^2 spectra for a component to any MS^2 spectra in the selected libraries. The primary algorithm is Confidence, which takes into account the dot product of the reverse search match, the distribution of peaks, and the ratio of the most intense matching peaks. A higher similarity score indicates the extent to which the library compound resembles the unknown component.
Similarity Forward	A search method that compares the MS^2 spectra for a component to any MS^2 spectra in the selected libraries.The primary algorithm is Confidence, which takes into account the dot product of the forward search match, the distribution of peaks, and the ratio of the most intense matching peaks. A higher similarity score indicates the extent to which the unknown component resembles the library compound.

Table 57. Search types for a component search (Sheet 2 of 2)

Selection	Description
Tree Search	A combined search method that calculates the largest overlap between the potentially large component spectral tree against the library. Individual spectral scores of precursor ions closer in value to the *m/z* value of the MS2 precursor ion contribute more to the overall ranking score than smaller fragments. Because this search method ranks the results within the same component, you can compare the absolute values of the score between different components only if the spectral trees are similarly constructed. Additionally, the MS^2 precursor of the unknown and library trees must match.
Subtree Search	A combined search method that calculates the largest overlap between the potentially large component spectral tree against the library. Because this search method ranks the results within the same component, you can compare the absolute values of the scores between different components only if their spectral trees are similarly constructed.
Custom Profile	Appears when any of the search options are modified.

Search parameters for each search type

Use the Search Parameters dialog box to specify the parameter settings for the various component search types.

To open the Search Parameters dialog box, click the settings icon,  , to the right of the Search Type list in the Component Search view.

Figure 59. Search Parameters dialog box

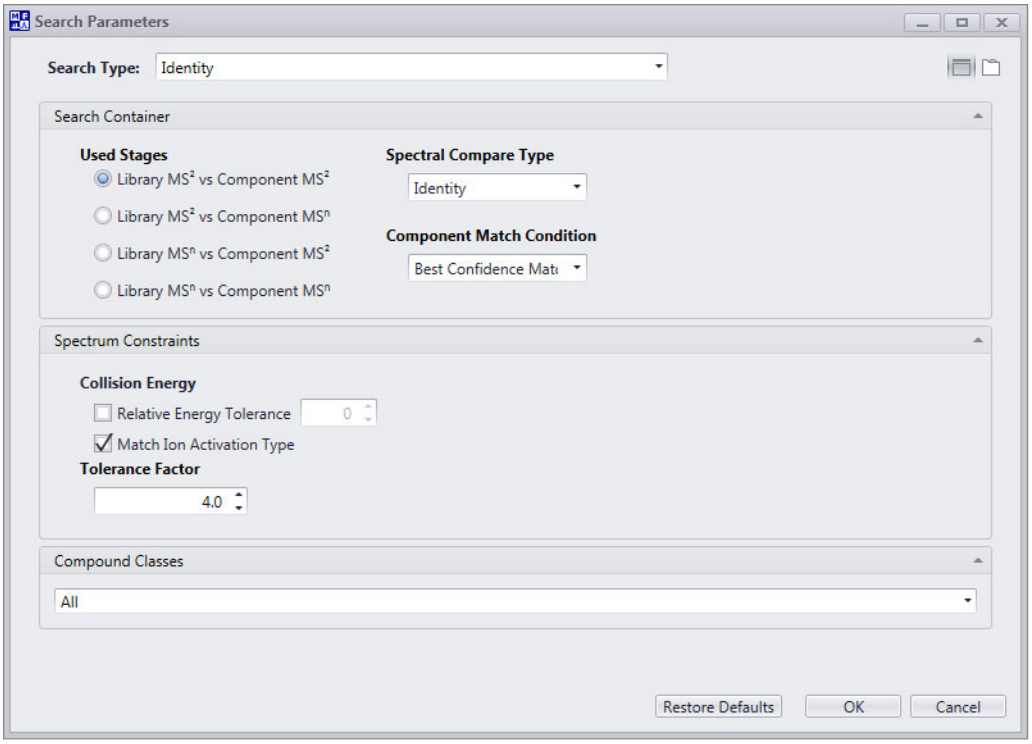


Table 58 describes the parameters in the Search Parameters dialog box.

Table 58. Search Parameters dialog box parameter descriptions (Sheet 1 of 3)

Parameter	Description
Search Type	Specifies the search type template as the basis for the custom search.

Table 58. Search Parameters dialog box parameter descriptions (Sheet 2 of 3)

Parameter	Description
Search Container	
Used Stages	Specifies the MS stages in the search. Selections: • Library MS^2 vs Component MS^2—Compares the MS^2 spectra only from the component with any MS^2 spectra in the selected libraries • Library MS^2 vs Component MS^n—Compares any MS^n spectra from the component with any MS^2 spectra in the selected libraries. • Library MS^n vs Component MS^2 —Compares the MS^2 spectra only from the component with any MS^n spectra in the selected libraries. • Library MS^n vs Component MS^n —Compares any MS^n spectra from the component with any MS^n spectra in the selected libraries.
Spectral Compare Type	Specifies the method for comparing individual query and library spectra. Selections: • Identity: The *m/z* values of the precursor ions for the query and library spectra must match. • Similarity Forward: Considers only those *m/z* peaks present in the query spectrum. • Similarity Reverse: Considers only those *m/z* peaks present in the library spectrum.

Table 58. Search Parameters dialog box parameter descriptions (Sheet 3 of 3)

Parameter	Description
Component Match Condition	Specifies the method used to calculate the component score. Selections: • Best Confidence Match— The highest confidence score from the library spectra. • Best Spectral Match— The highest spectral match from the library spectra. • Aggregated Tree Match— Combined search method, which calculates the largest overlap between the potentially large component spectral tree against the library. As the spectra of MS3 and higher fragmentation stages are compared between the trees, their contribution to the overall score diminishes relative to the *m/z* value of their precursor ion with respect to the original molecular ion—that is, the individual spectral scores of precursor ions that are closer to the *m/z* value of the MS^2 precursor ion contribute more to the overall ranking score than smaller fragments. Confidence is used in the overall tree match score calculation. • Aggregated Sub-Tree Match— A combined search method that calculates the largest overlap between the potentially large component spectral tree against the library. Confidence is used in the overall subtree match score calculation.
Spectrum Constraints	
Collision Energy	Relative Energy Tolerance—Specifies the relative ion activation energy tolerance for the library spectra with respect to the query spectra. Library hits outside the tolerance interval are ignored. Match Ion Activation Type —Considers only the library spectra with the same ion activation type. For example, the search does not compare an HCD query spectrum against CID library spectra.
Tolerance Factor	Specifies the Accuracy Factor used for both matching precursor ions and individual spectral peaks. Default: 4
Compound Classes	
Limits the library search to only the selected compound classes.	

Library parameter for a component search

This parameter specifies the libraries that you want to search. You can select any combination of user and *mz*Cloud libraries.

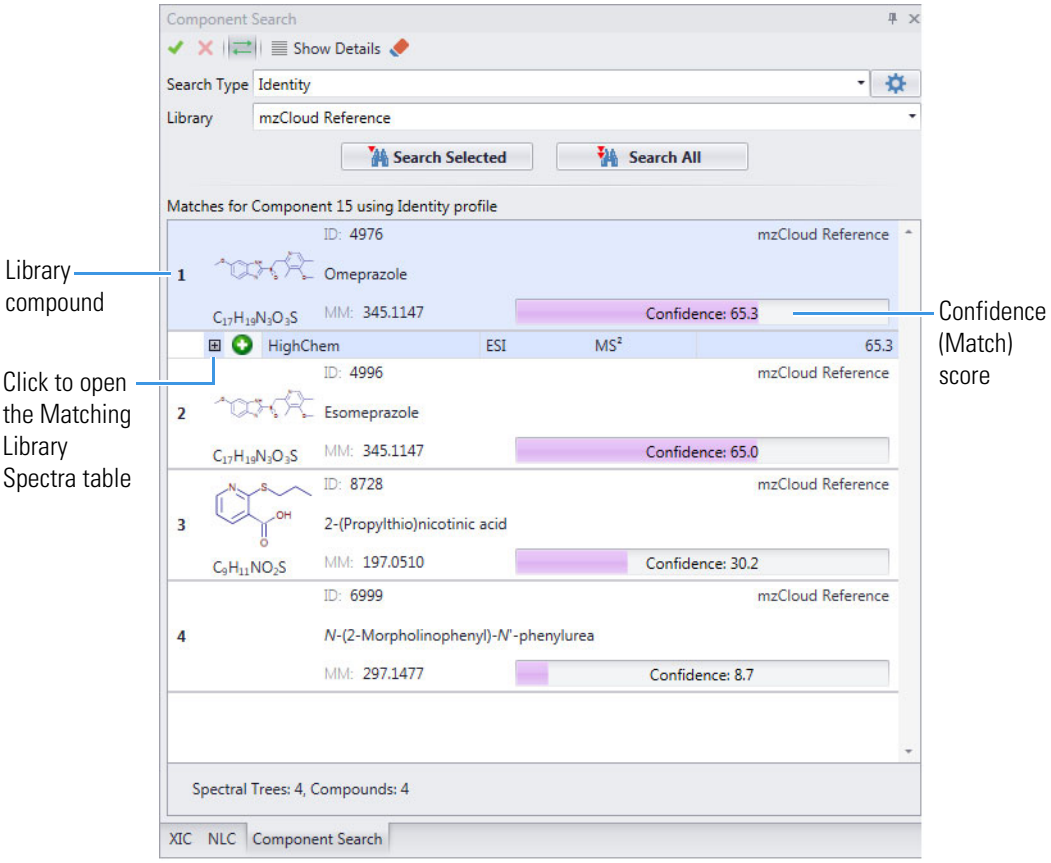
Search results for a component search

The Matches for Component *X* pane below the search parameters displays the library search results for the selected component in the Components list (chromatogram data view of the Chromatogram Processor). Based on the component match score, the matching compounds are sorted in descending order.

Note When you select more than one component in the chromatogram data view, the Component Search view displays the following message: Multiple components selected.

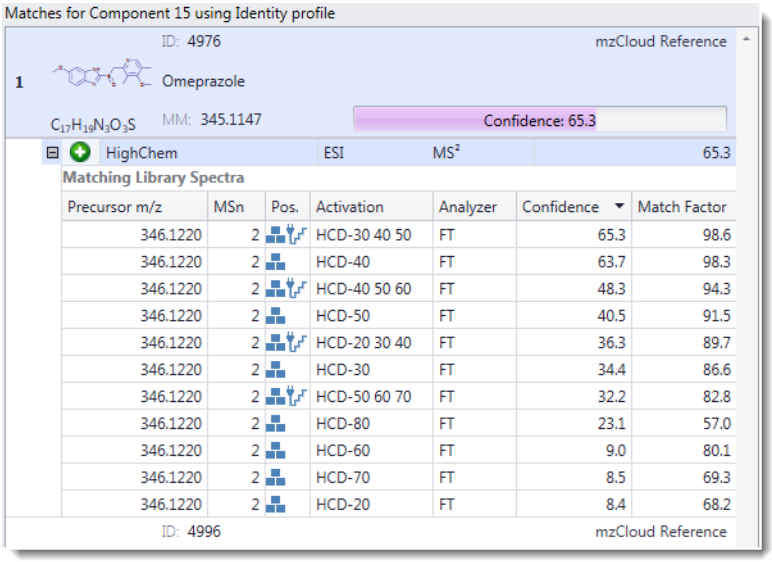
Clicking the expand icon, +, below the library entry displays the table of matching library spectra.

Figure 60. Component Search view with match results from an Identity search



The Matching Library Spectra table provides additional information about the library matches.

Figure 61. Matching Library Spectra table for omeprazole



Clicking a row in the Matching Library Spectra table displays the library spectrum on the Compare Spectra page in the MS spectrum view (Figure 62).

Figure 62. Spectra Compare page of the MS spectrum view

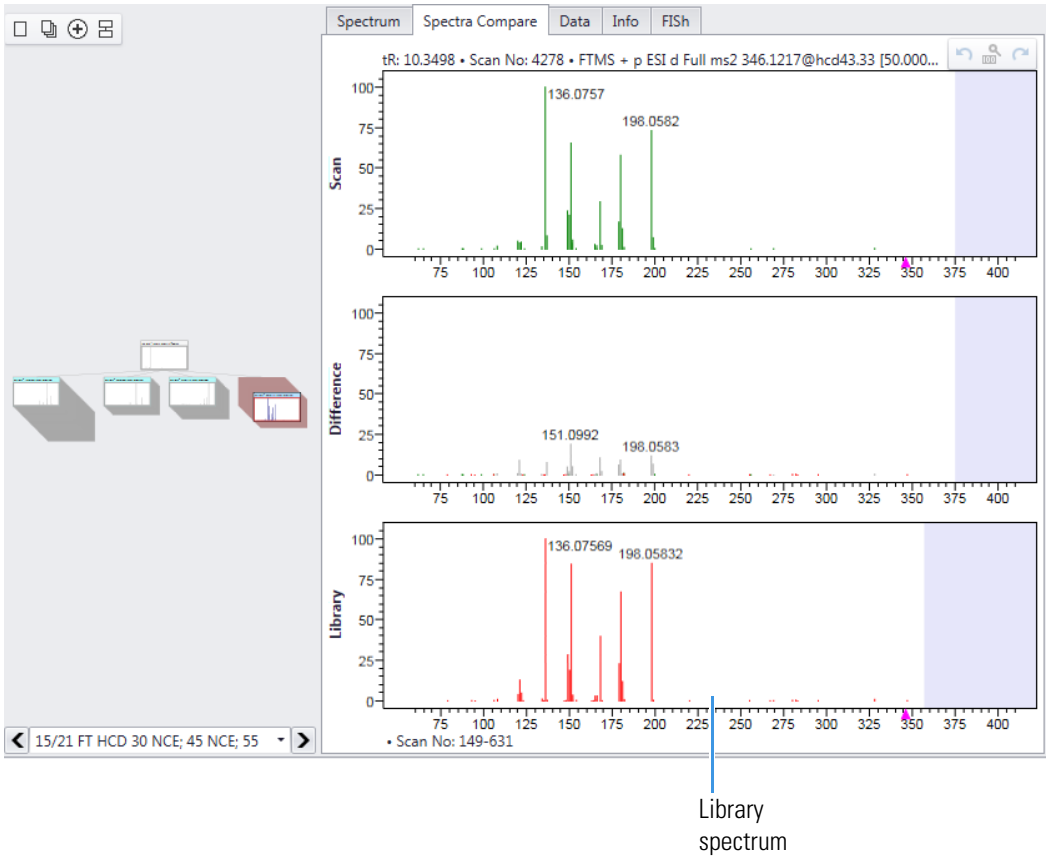


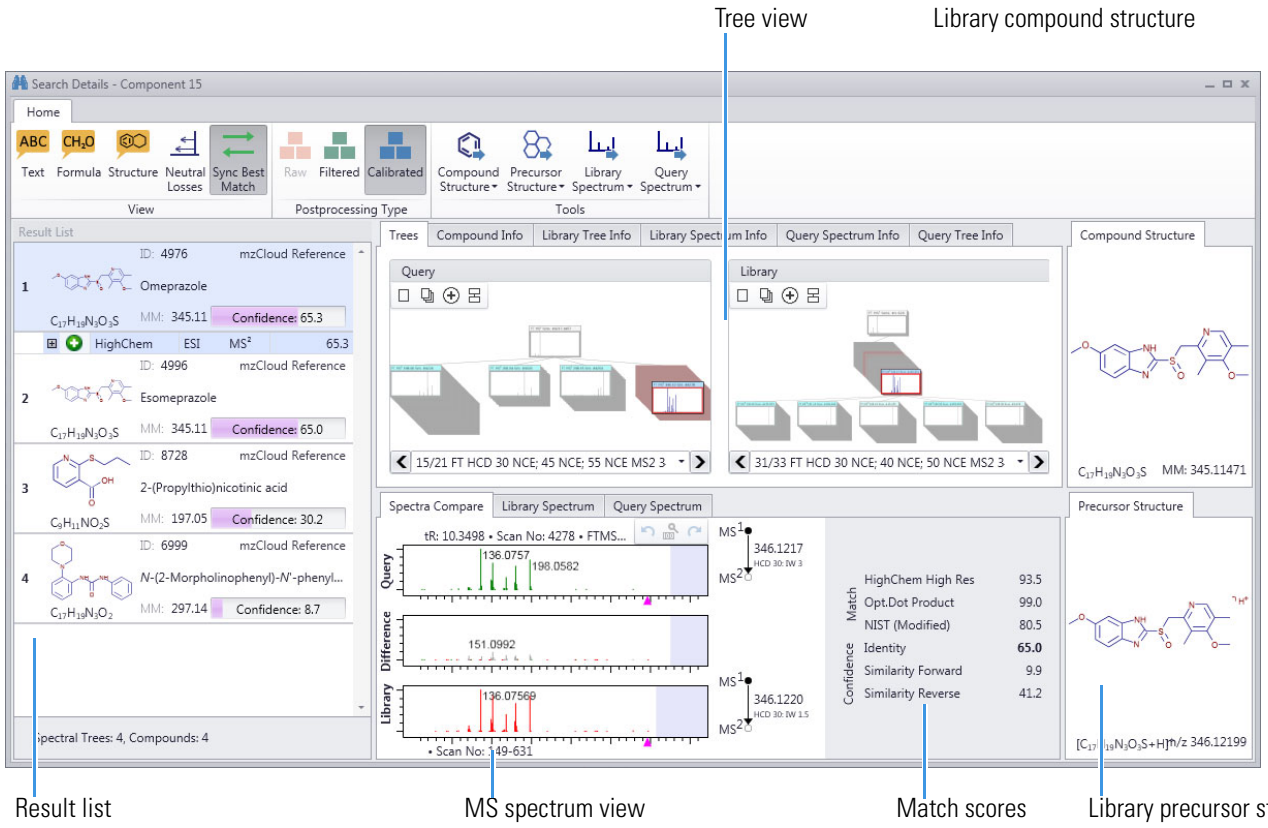
Table 59. Component Search results – Matching Library Spectra table column descriptions

Column	Description
Precursor *m/z*	*m/z* value of the precursor ion
MSn	MS stage
Pos.	Post-processing type of library spectrum. See "Post-processing" on page 406.
Activation	Ion activation type and relative energy
Analyzer	Mass analyzer
Confidence	Confidence score of the respective query-library spectrum pair
Match Factor	Dot product spectral match of the respective query-library spectrum pair

Search Details window for a component search

A Search Details window displays detailed information about the search results for a selected component.

Figure 63. Search Details window



For details about the Search Details window, see these topics:

- Search Details toolbar
- Result List in a Search Details window
- Match details pane of a Search Details window
- Scan pane of a Search Details window
- Compound Structure pane of a Search Details window
- Precursor Structure pane of a Search Details window

Open a Search Details window

❖ **To open the Search Details window for a result in the Component Search view**

Click **Show Details** in the Component Search view.

The Search Details window opens as a tabbed page in the application window. You can float the page as a separate window.

Search Details toolbar

Figure 64 shows the toolbar for the Search Details window that opens when you click Show Details for a search result from a component search.

Figure 64. Search Details toolbar

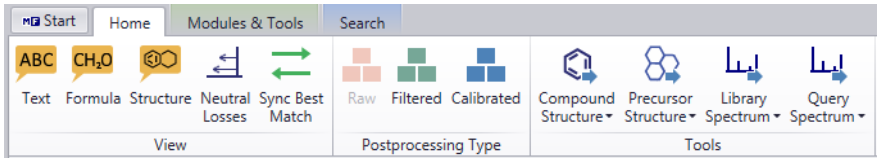


Table 60. Search Details toolbar (Sheet 1 of 2)

Name	Icon	Description
View		
Text		Displays text annotations in the library spectrum.
Formula		Displays formula annotations in the library spectrum.
Structure		Displays structure annotations in the library spectrum.
Neutral Losses		Displays neutral losses in the library and compare spectrum.
Sync Best Match		In the Compare Spectra pane, when cycling through Component Spectral Tree (Query), always display corresponding top matching Library spectra. When cycling through the Library spectra, always display corresponding top matching Query spectra.
Post-processing type		
Raw		Raw spectral tree without any filtering or recalibration.
Filtered		Filtered spectral tree with noise peaks and low-quality scans removed.
Calibrated		Filtered and recalibrated spectral tree.

Table 60. Search Details toolbar (Sheet 2 of 2)

Name	Icon	Description
Tools		
Compound Structure		Sends the library compound structure to New Curator, New SledgeHammer, New Sub/Structure Search, New Metabolika, New Structure Editor, or Structure Grid.
Precursor Structure		Sends the library precursor structure to New Curator, New SledgeHammer, New Sub/Structure Search, New Metabolika, New Structure Editor, or Structure Grid..
Library Spectrum		Sends the single library spectrum to New Data Manager, Spectrum Search or mzLogic Search.
Query Spectrum		Sends the single query spectrum to New Data Manager, Spectrum Search or mzLogic Search.

Result List in a Search Details window

The Result List displays matching spectra grouped by the library and compound structure, as in the Search Result pane.

Match details pane of a Search Details window

The match details pane of a Search Details window displays detailed information about the query and selected library record. There are several pages dedicated to different types of metadata.

- Trees—Displays the query component and library trees in the Spectral Tree panes. The red color saturation indicates the value of the match score in the corresponding spectra.
- Compound Info—Displays the metadata associated with the library compound.
- Library Tree Info—Displays the metadata associated with the library record, such as acquisition details, contributor, and so on.
- Library Spectrum Info—Displays the metadata associated with the library spectrum, such as scan numbers, isolation width, and so on.
- Query Spectrum Info—Displays the metadata associated with the query spectrum, such as scan numbers, isolation width, and so on, as in the Scan Window > Info page in Chromatogram Processor.
- Query Tree Info—Displays the metadata associated with the query component, such as chromatogram file name, component retention time, and intensity.

Scan pane of a Search Details window

To facilitate the inspection of the query-library spectral match, the scan pane provides a detailed view of the query spectrum, the library spectrum, and a difference spectrum of these two spectra. The match score pane displays several calculated spectral match scores using different criteria.

Compound Structure pane of a Search Details window

The Compound Structure pane displays the library compound structure.

Precursor Structure pane of a Search Details window

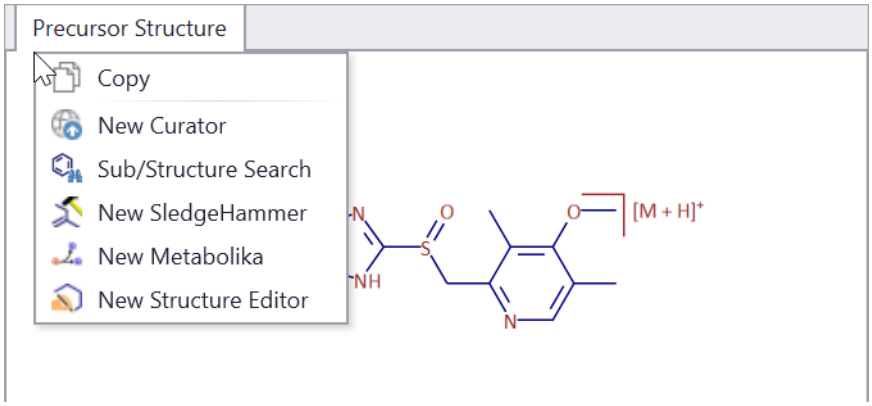
The Precursor Structure pane displays the library precursor structure.

Send the component search results to other modules

From the shortcut menu for a Compound Structure pane or a Precursor Structure pane in a Search Details window, you can send the compound structure or the precursor structure to the following:

- The Curator module for annotating the spectral tree
- The New SledgeHammer module to generate fragments
- The Structure Editor for editing
- The Structure Search dialog box for a Structure or Substructure Search
- A new Metabolika window for creating a new Metabolika Pathway

Figure 65. Precursor Structure pane in the Search Details window



From the Tools toolbar group in a Search Details window you can do the following:

- Send the compound structure or the precursor structure to any opened module that can receive structures.
- Run a Spectrum Search or an mzLogic Search on a selected query spectrum or library spectrum.

mzLogic searches

After running a component detection algorithm, use the mzLogic search feature for the putative identification and ranking of structural candidates for unknown components that are not present in the reference libraries—that is, use the mzLogic algorithm to identify components that you cannot identify with an mzCloud identity search. The mzLogic search algorithm provides a ranking score for the various database search results when an unknown compound has data-dependent MS2 scans and similarity results from an mzCloud similarity search.

The algorithm performs an MSn similarity forward and reverse search against the mzCloud library and a precursor *m/z* search against the MolGate structure database and/or any open Structure Grid page. Using the combinations of structure and spectral search results, the spectrum-structure match correlation algorithm assigns a score for each mzLogic candidate based on the structural overlap with the mzCloud similarity hits.

For details, see these topics:

- Use the mzLogic view with other modules
- Run an mzLogic component search
- mzLogic view parameters

Use the mzLogic view with other modules

From the mzLogic view, you can do any of the following:

- Use any open Structure Grid as a source for the mzLogic structure candidates
- Send all ranked mzLogic candidates to the opened Structure Grid or new Batch Fragmentation pages.
- Send selected mzLogic candidate to the Curator, Structure Editor, SledgeHammer, Sub/Structure Search or Structure Grid pages.

Run an mzLogic component search

Use the mzLogic view to run an mzLogic analysis to identify an unknown component. The unknown component must have data-dependent MS2 scans in its spectral tree.

❖ To run an mzLogic component search

1. Open a data file in a Chromatogram Processor window, and then apply a component detection algorithm.
2. (Optional) To filter out components without MS2 scans, do the following:
   a. In the chromatogram data view, right-click the MSn column heading and choose **Filter Editor**.

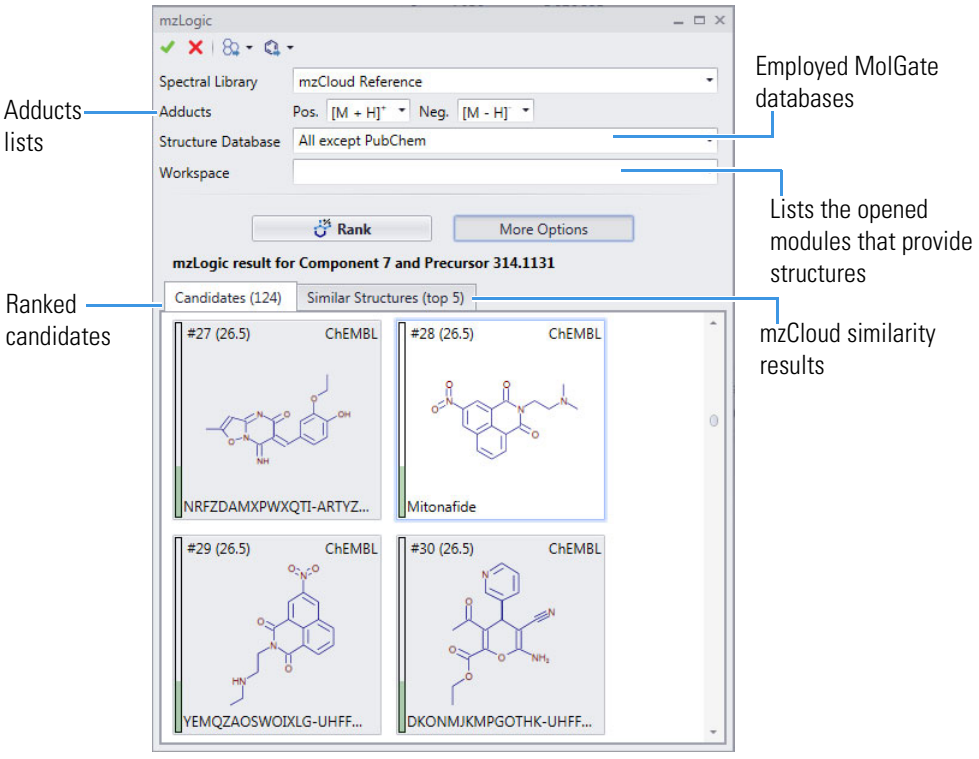
b. Set up the following filter and click **OK**.

MSn is greater than or equal to 2

3. (Optional) To determine which components cannot currently be identified with an Identity search, in the Search group of the Chromatogram Processor toolbar, click **Components Search** and run an Identity search on all the detected components.
4. In the chromatogram data view, select a component that you were unable to identify with an Identity search.
5. In the Search group of the Chromatogram Processor toolbar, click **mzLogic**.

The mzLogic view opens at the right of the application window.

Figure 66. mzLogic view



mzLogic view parameters

Table 61. mzLogic view toolbar and parameters (Sheet 1 of 2)

Icon or parameter	Description
Accept Structure	Accepts the selected mzLogic candidate as the component's matching name. The compound name, mzLogic score (match score), and search method (annotation source) appear in bold in the Components list of the chromatogram data view in the Chromatogram Processor window.
Clear Accepted Structure	Clears the accepted structure for the component—this is, clicking this icon clears the component's accepted structure and the component's matching name that you selected and accepted after running an mzLogic search.
Candidates	Sends all the mzLogic candidates to a Structure Grid window, a new Batch Fragmentation window, or a new Metabolika Pathway window.
Selected Candidates	Sends selected mzLogic candidate to the Curator, Structure Editor, Fragments & Mechanism, Sub/Structure Search or Structure Grid page.
Spectral Library	Specifies the mass spectral libraries for the similarity search. The mzLogic algorithm can search multiple libraries, including the mzCloud Reference and Autoprocessed libraries and any of the custom user libraries that you created with the Mass Frontier application.
Adducts	Specifies the positive and negative adducts for the precursor ions.
Structure Database	Specifies the structure databases to search for structure candidates that match the precursor *m/z* value of the selected data-dependent MS2 spectrum of an unidentified component. **Note** Including the PubChem database in the structure search significantly increases the processing time.
Workspace	Specifies an opened Structure Grid with potential structure candidates.
Rank	Starts the analysis.
More Options	Displays the Structure Database and Workspace parameters.

Table 61. mzLogic view toolbar and parameters (Sheet 2 of 2)

Icon or parameter	Description
Post-analysis results	
Status area	Displays information about the analysis in the following format: mzLogic result for Component *X* and Precursor *m/z value*.
Candidates page	Displays cards for the mzLogic structure candidates. The green bar indicates the mzLogic score.
Similar Structures page	Displays the top 5 similar structures that the analysis found in the selected spectral libraries.

Extracted Ion Chromatogram (XIC) filter

Use the Extracted Ion Chromatogram pane of a Chromatogram Processor window to display chromatograms for extracted ions in different colors in the chromatogram view. The extracted ion chromatogram (XIC) is also known as an individual, or single extracted ion chromatogram (EIC) or ion profile.

Supported data formats: XIC parameters file (.xic_par) and plain text file (.txt, .csv, .dat)

For more information about working with the Extracted Ion Chromatogram view, see these topics:

- Use the Extracted Ion Chromatogram pane with other modules
- Use extracted ion chromatograms for verifying component detection
- Open and pin the Extracted Ion Chromatogram pane
- Extracted Ion Chromatogram pane parameters

Use the Extracted Ion Chromatogram pane with other modules

You can use the structures in an opened Structure Grid window to specify the *m/z* values for the extracted ion chromatograms you want to display in the chromatogram view of a Chromatogram Processor window. The structures must include a charge.

See "Use a structure grid as an XICs source" on page 272.

View an extracted ion chromatogram for a detected component

❖ **To view an XIC trace for a detected component**

In the chromatogram data view of a Chromatogram Processor window, right-click the component and choose **Show XIC m/z**.

Use extracted ion chromatograms for verifying component detection

You can use an extracted ion chromatogram (XIC) to verify the automated component detection and spectra deconvolution results. An XIC for a model peak helps you to determine if an ion profile is a single compound or a mixture of compounds.

For the JCD algorithm, component detection is based on chromatographic retention time and peak shape. When you select the Merge the Parent Ions into One Component ( ) option, the XIC for a component might include multiple *m/z* values.

Note When a component is made up of multiple ions with the same retention time and peak shape, these ions are typically due to in-source fragments and various adduct ions of the same parent compound.

Figure 67 shows the chromatographic peak and the MS1 scan at the peak apex for a component detected in the plant sample.raw demonstration file. In the MS1 scan, the peak at *m/z* 741.2265 is probably the protonated ion of the parent compound, and the peaks at lower *m/z* values (for example, *m/z* 287.0558 and *m/z* 595.1676) are probably in-source fragments, as the fragmentation scans for these ions match those for similar substructures.

Figure 67. Component in the plant sample.raw file (precursor *m/z* 741.2265 and RT 14.577 min)

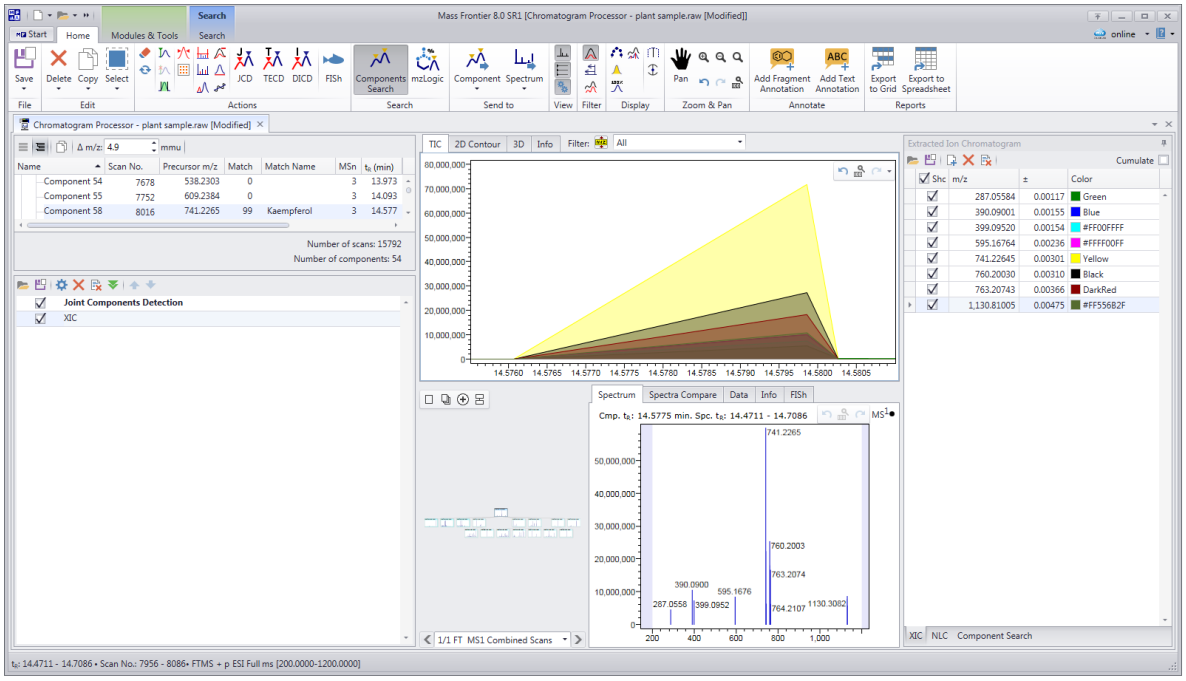
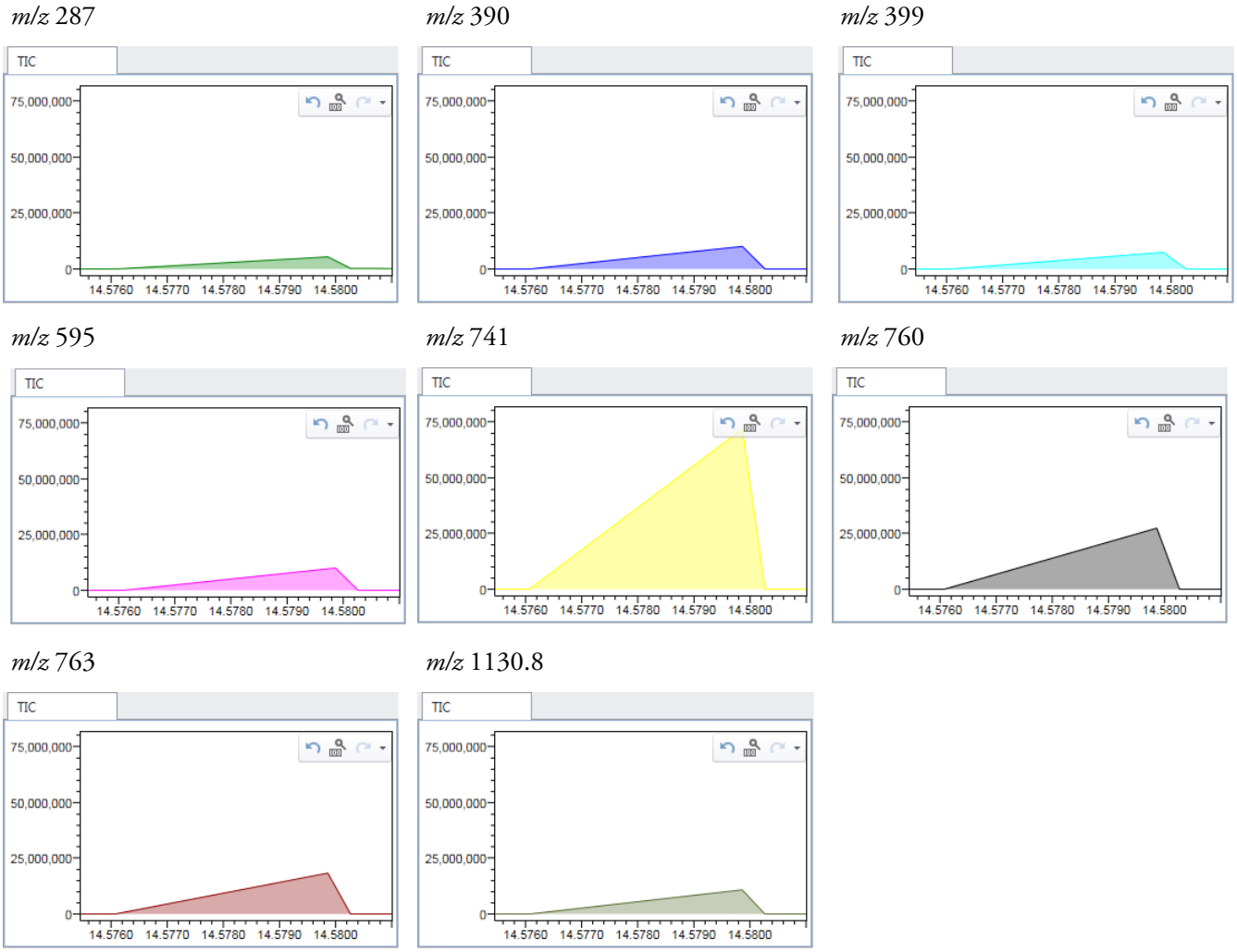


Figure 68 shows the individual chromatographic peaks for each *m/z* value.

Figure 68. Individual chromatographic peaks at 14.577 min for the selected component



Open and pin the Extracted Ion Chromatogram pane

In addition to the chromatogram data, chromatogram, command processor, and MS spectrum views, the Chromatogram Processor window includes two collapsible panes: XIC and NLC.

❖ To open the Extracted Ion Chromatogram (XIC) pane and keep it open

1. Do either of the following:
   a. Click the **XIC** tab at the upper right of the Chromatogram Processor window.
   b. To keep the pane open, click the pin icon.

   –or–

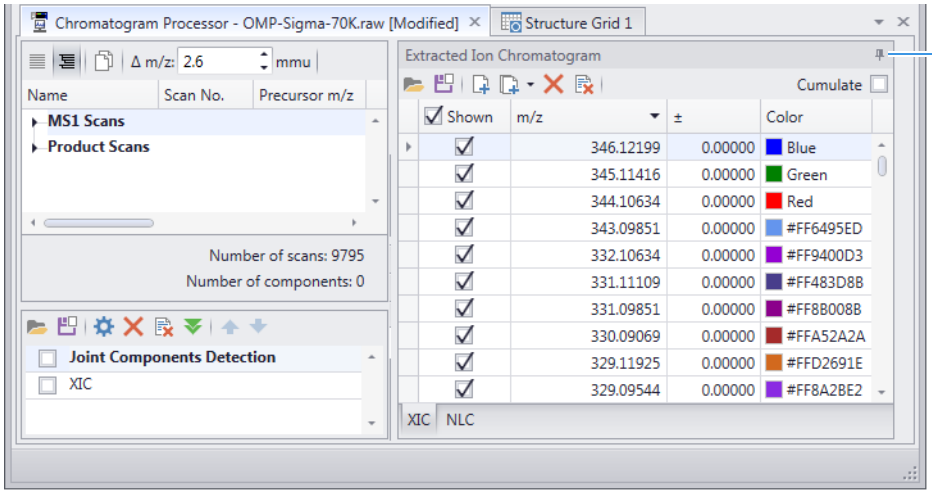
c. In the Filter group of the Chromatogram Processor toolbar, click the **Show Extracted Ion Chromatograms** icon, .

If a Structure Grid window is open, the **Ions** icon, , is available. After you add ions to the list, the Save and Remove icons become available.

2. To close the XIC pane, click the pin icon again.

Figure 69 shows a pinned Extracted Ion Chromatogram pane with a list of XICs from an opened Structure Grid.

Figure 69. Extracted Ion Chromatogram pane with XICs from a Structure Grid



Extracted Ion Chromatogram pane parameters

The Chromatogram Processor window includes a collapsible Extracted Ion Chromatogram pane for specifying the extracted ion chromatograms to display in the chromatogram view.

Use the pin icon at the upper right of the pane to control the pane's auto-hide feature.

Table 62. Extracted Ion Chromatogram pane parameter descriptions (Sheet 1 of 2)

Icon or column		Description
Toolbar		
Open XICs		Add XIC files from one of the supported files (.xic_par, .csv, .txt, .dat). If the data is in plain text format, the XIC list must be formatted as follows: one ion per line, the first column must specify the *m/z* value, and the optional second column must specify the *m/z* tolerance in AMU.
Save All XICs		Saves all XICs to an .xic_par file.

Table 62. Extracted Ion Chromatogram pane parameter descriptions (Sheet 2 of 2)

Icon or column	Description
Add New XIC	Adds a new XIC trace with an editable *m/z* value, tolerance, and display color.
Add Ions	Adds ions from an opened Structure Grid window.
Remove selected XIC	Deletes the selected XICs.
Remove all XICs	Deletes all the XICs in the table.
Cumulate check box	When clear, the application uses the intensity of the centroid peak nearest to the specified *m/z* value within the specified tolerance as the intensity of extracted ion chromatogram. When selected, the application uses all the centroid peaks for the specified *m/z* value within the specified tolerance as the intensity of extracted ion chromatogram. Default: Clear
	Tip To optimize the XIC trace, do the following: • To simulate an XIC trace for a nominal mass experiment by using high-resolution data, select this check box and set the mass tolerance to 0.5 Da. • To avoid dropped data points in the XIC caused by small noise peaks that are closer to the specified *m/z* value than the component peaks, select this check box and set the mass tolerance appropriately.
Table columns	
Shown	Specifies whether to display the XIC in the chromatogram view.
m/z	Specifies the *m/z* value of the XIC.
±	Specifies the *m/z* tolerance of the XIC.
Color	Specifies the display color of the XIC.

Display an XIC by selecting a spectral peak

You can display an extracted ion trace in the chromatogram view of a Chromatogram Processor window by selecting a peak in the MS spectrum view.

❖ **To add an XIC for a specific spectral peak to the chromatogram view**

Do either of the following on the Spectrum page of the MS spectrum view:

- Double-click any peak in the spectrum.

–or–

- Right-click any peak in the spectrum and choose **Show XIC *m/z***.

Neutral Loss Chromatogram (NLC) filter

In the chromatogram view of a Chromatogram Processor window, you can display a chromatogram for specified neutral losses in a different color.

The neutral loss is defined as the difference in the *m/z* value between the precursor ion and any peak in the product spectrum.

Supported data formats: NLC parameters file (.nlc_par) and plain text file (TXT, CSV, or DAT)

For details, see these topics:

- Neutral Loss Chromatogram pane
- Add and delete neutral loss masses

Neutral Loss Chromatogram pane

Set up a neutral loss chromatogram to show the in-source fragments in MS^1 scans (for example, typical glucose loss for flavonoids) or common neutral losses of the product spectra.

Tip Use the pin icon to control the NLC pane's auto-hide feature.

Table 63. Neutral Ion Chromatogram pane icons, parameters, and columns (Sheet 1 of 2)

Name	Icon	Description
Open NLCs		Adds a list of neutral masses from one of the supported files (.nlc_par, .csv, .txt, .dat). If the data is in plain text format (.txt), the NLCs must be formatted as one neutral loss per line. The first column specifies the *m/z* value, and the optional second column specifies the *m/z* tolerance in amu.
Save All NLCs		Saves the list of neutral loss masses to an NLC Parameters File (.nlc_par), which is a Mass Frontier specific file type.
Add New NLC		Adds a new blank row to the NLC table. Clicking the down arrow to the right of the Add icon displays a list of predefined neutral losses. See "Add and delete neutral loss masses" on page 144.
Remove selected NLCs		Deletes the selected NLCs.

Table 63. Neutral Ion Chromatogram pane icons, parameters, and columns (Sheet 2 of 2)

Name	Icon	Description
Remove all NLCs		Deletes all the NLCs.
Check All		Analyzes common neutral losses in the MS spectrum view. Only the common neutral losses are applied to the MS spectrum view. Custom neutral losses (manually added to the table) are not used in the MS spectrum view.
Peaks		Specifies the maximum number of peaks to label with their neutral losses in the MS spectrum view. Range: 1 to 255
Threshold		Specifies the intensity threshold in relative abundance for a peak to be labeled with its neutral loss in the MS spectrum view.

Add and delete neutral loss masses

When you open a Chromatogram Processor, the Neutral Loss Chromatogram pane appears as a collapsed tab at the right of the window.

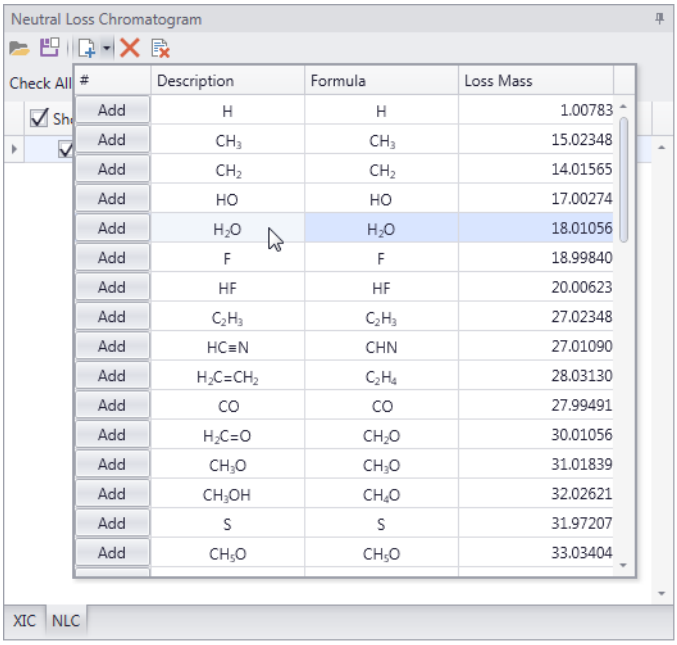
❖ To display neutral loss chromatograms in the chromatogram view

1. (Optional) To pin the Neutral Loss Chromatogram pane, do one of the following:
   - In the Display group of the Chromatogram Processor toolbar, click the **Show Neutral Loss Chromatogram** icon, .
   - Point to the NLC tab to open the pane, and then click the pin icon.
2. Specify the display options for the MS spectrum view:
   - Maximum number of spectral peaks to label
   - Minimum intensity threshold for the labeled spectral peaks
3. Add a neutral loss mass and its tolerance to the table in the Neutral Loss Chromatogram pane by doing either of the following:
   a. Click the **Add Neutral Loss** icon, , to add a new blank row to the table.
   b. Enter the Loss Mass and its tolerance and select a display color for the chromatogram view.

   –or–

   a. Click the down arrow to the right of the Add Neutral Loss icon to display a list of common neutral losses (Figure 70).

Figure 70. Predefined neutral losses



b. Click **Add** to the left of the common neutral loss of interest.

c. Enter the mass tolerance and select a display color for the chromatogram view.

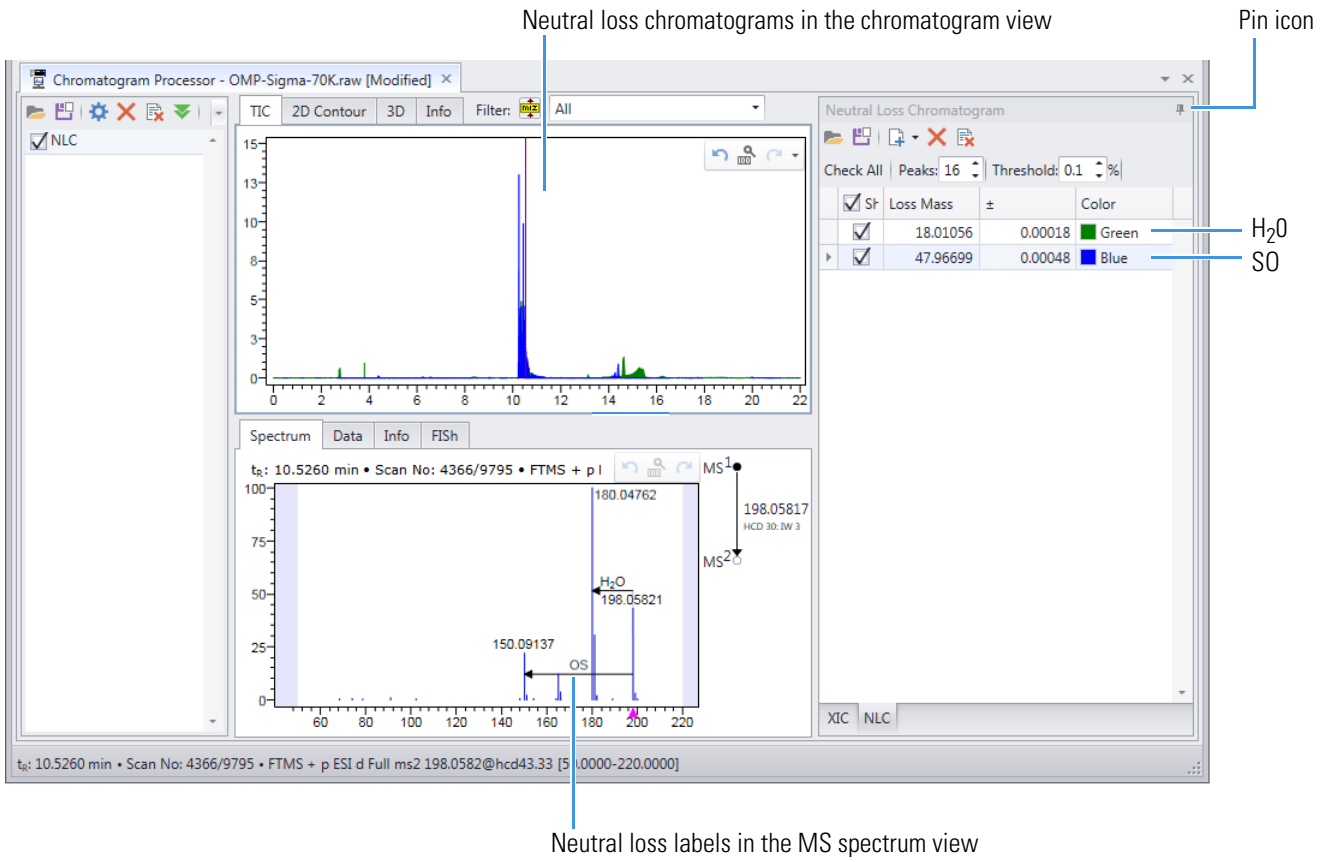
4. In the chromatogram view, set up the display options as follows:

   a. Open the display options menu by clicking the down arrow to the right of the Redo Zoom icon.

   b. Clear the **Show TIC** check box.

   c. Select the **Show NL** check box.

Figure 71. Chromatogram view with two neutral loss chromatograms



Generate reports

You can export the Components list with the accepted library compounds and active search results to several text formats, such as HTML, PDF, TXT, CSV, or XLSX.

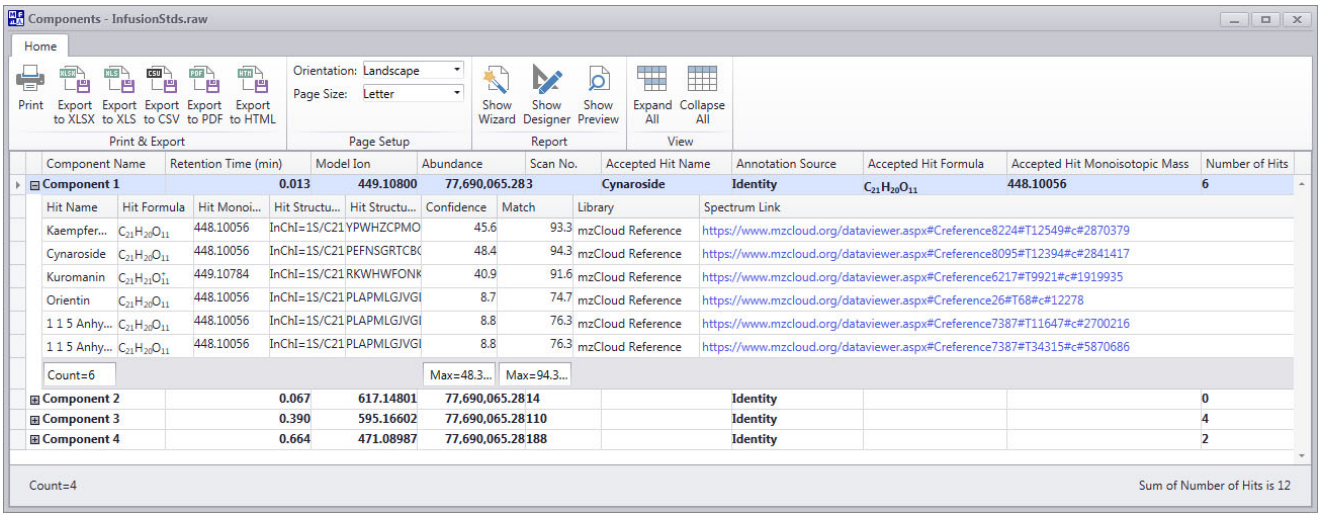
You can export the Components list in two distinct layouts:

- Grid layout
- Spreadsheet layout

Grid layout

Clicking Export to Grid in the Reports group of the Chromatogram Processor toolbar organizes components in collapsible groups of scans and corresponding search results for each component (Master/Detail view). This layout does not require further processing and provides a user-friendly interface of export actions with some basic data-processing functionality, such as sorting or filtering.

Figure 72. Grid layout for a report

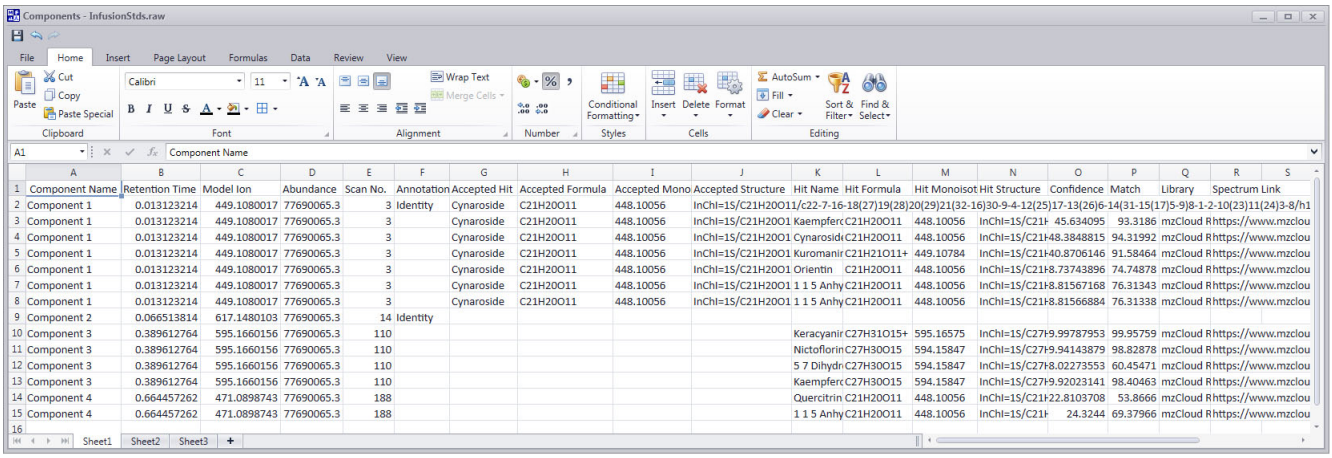


Spreadsheet layout

Clicking Export to Spreadsheet in the Reports group of the Chromatogram Processor toolbar exports all the scans and search results in separate rows, which provides flexibility in data post processing; however, you might require additional third-party data processing software, such as Excel to view the report.

The spreadsheet layout displays all search data in a single table and provides basic processing and editing actions.

Figure 73. Spreadsheet layout for a report



4

Data Manager module

The Data Manager module manages spectral and structural information in the connected Mass Frontier Library Service. It includes library maintenance utilities that let you create and organize spectral and fragmentation libraries with chemical structures. Because the Data Manager module supports ion structures (precursor ion structure) and spectral tree representation (copied from the Chromatogram Processor module), you can also use it to create library entries.

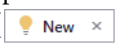
The Data Manager module provides a flexible set of tools to import, save, edit, or annotate spectral trees and fragmentation pathways to a common record format that you can save in a user-created library or export to an external file format for archiving.

The Data Manager module supports these spectral record formats: .dbbak, .ccomp, .dbbakx, .mfcdc. and .msp.

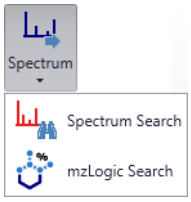
Contents

Use Data Manager with other modules

From a Data Manager window, you can work interactively with other modules as follows:

- Load components from a Chromatogram Processor window to a new (

) library in the Data Manager window.
- Send a compound structure and its spectral tree to a new Curator window.
- Send a compound structure or a precursor structure to the Structure Editor, the SledgeHammer, the Curator, the Structure Search, and the Metabolika module.
- Send a selected spectrum to a Spectrum Search or an mzLogic Search from the Tree view.



- Send a selected spectral peak to a Peak Search from the Tree view.

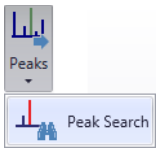


Table 64. Quick reference for sending data to and from the Data Manager window12

Task	Procedure
Load components from a Chromatogram Processor window.	1. Run a component detection algorithm in the Chromatogram Processor window. 2. Open a Data Manager window. 3. In the Chromatogram Components toolbar group, choose **Load From > Chromatogram Processor**.
Import a compound with a curated spectral tree into a user library.	1. Open the user library in a Data Manager window. 2. In a Curator window, curate a component's spectral tree. Then, from the Edit toolbar group, choose **Save > Save To Library**. 3. In the Save As Record dialog box, select the user library and click **OK**. 4. In the Data Manager window, click the **Notifications** tab. Click **Add**.

Table 64. Quick reference for sending data to and from the Data Manager window22

Task	Procedure
Run a Spectrum Search or an mzLogic Search.	1. In the Tree view of the Data Manager window, display the spectrum of interest on the Spectrum page of the spectrum view. 2. Then, in the Send To toolbar group, choose **Spectrum > Spectrum Search** or **Spectrum > mzLogic Search**.
Run a Peak Search.	1. In the Tree view of the Data Manager window, display the spectrum of interest on the Spectrum page of the spectrum view. 2. Select a peak, and then, in the Send To toolbar group, choose **Peaks > Peak Search**.
Send a structure to another module.	In the Tree view of the Data Manager window, select a user library, and then select the library compound. In the Send To toolbar group, choose **Structure > *Module***.

Open a Data Manager window

❖ To open a Data Manager window

Do any of the following:

- To open a new Data Manager window, click the **Modules & Tools** tab, and then click **Data Manager**. Or, from the Start menu, choose **New**, and then click **Data Manager**.
- To open an existing Data Manager window, from the Start menu, choose **Open**, click the **Data Manager** icon on the right, select a Data Manager file, and click **Open**.

There are two possible views for the upper portion of a Data Manager window: Tree view or Mechanism view. Use the Tree view to view the library entries in a mass spectral library and the Mechanism view to view fragmentation mechanisms. The tabs on the left side of the window control which view is visible.

Figure 74 shows the selection of a library entry in a Data Manager window where the Tree view is active.

Figure 74. Data Manager window with the Tree view

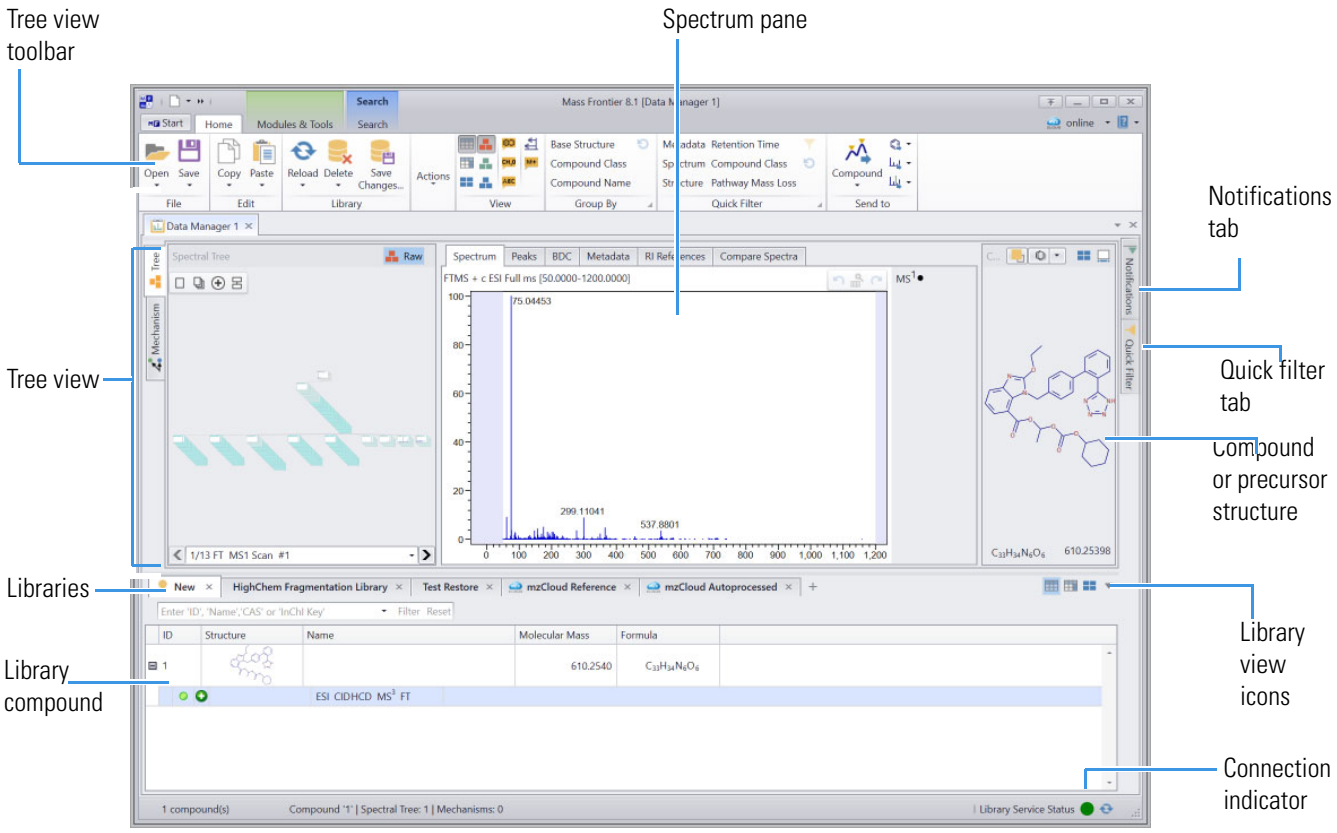
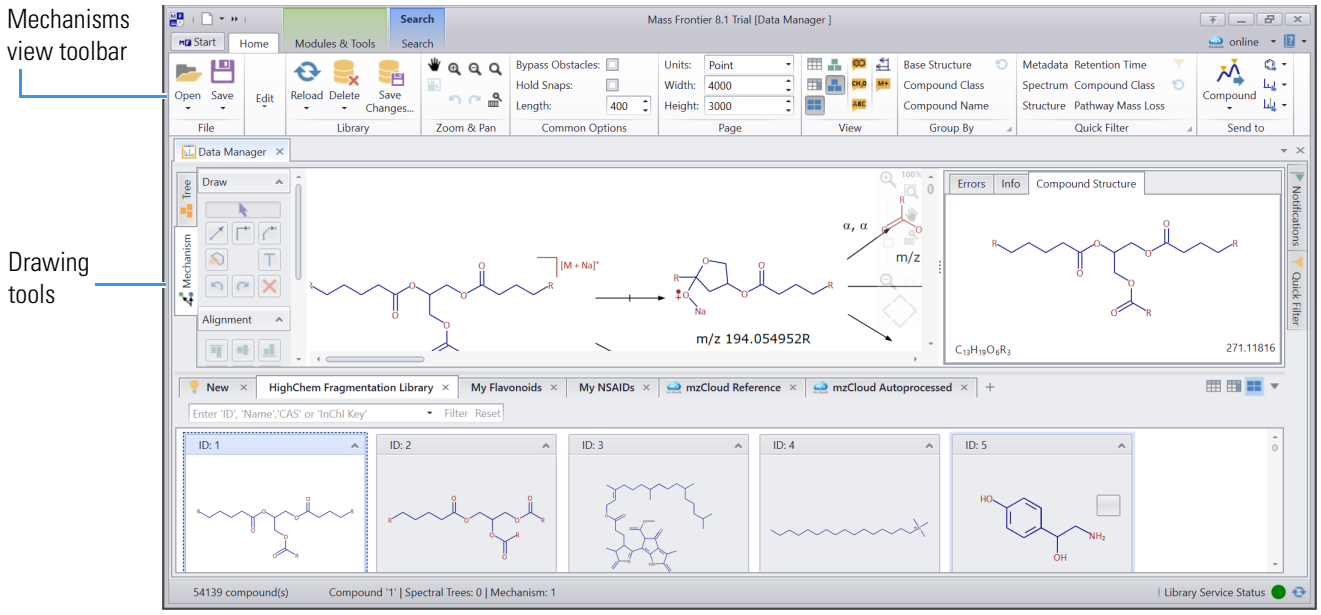


Figure 75 shows a Data Manager window with an active Mechanisms view.

Figure 75. Data Manager window with the Mechanisms view



Data Manager toolbar

The Data Manager toolbar contains the following groups:

File group

Table 65. File group buttons on the Data Manager toolbar12

Button	Description
Open 	Opens files these file types: • .dbbak • Mass Frontier Compound Data Container (MFCDC)—This file type contains the spectral tree and structure for an individual compound. You can create MFCDC files in the Data Manager or Curator windows. • .ccomp • .dbbakx - This file type contains multiple compounds with the spectral trees, and also fragmentation pathways for an individual compound. You can create .dbbakx files in the Data Manager • .msp
Open > Cancel Open File	Aborts the opening of a large file.

Table 65. File group buttons on the Data Manager toolbar22

Button	Description
Save > Compound	Saves selected compound and spectral tree into an MFCDC format to the specified location and file name.
Save > Multiple Compounds	Saves multiple compounds and spectral trees into an .dbbakx format to the specified location and file name.

❖ **To import/export multiple spectral trees and/or compounds into a single file**

1. Click **Open** and select one or more files.
2. Select **Save** > **Multiple Compounds**.

   A new window of **Select Compounds Data to File** opens.
3. Select the compounds from the list. Click the save button located on the top right side of the window and enter the file name and save.
4. Click **Save** on the **Select Compounds Data to File** window.

   A new file with multiple compounds is saved in.dbbakx format.

Edit group – Tree mode

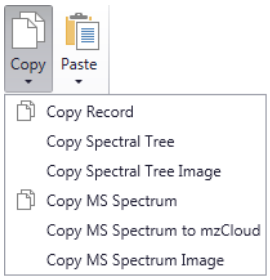


Table 66. Edit group on the Data Manager – Tree mode toolbar12

Command	Description
Copy > Copy Record	Copies the compound, selected spectral tree, and mechanism to the Clipboard.
Copy > Copy Spectral Tree	Copies spectral tree to the Clipboard.
Copy > Copy Spectral Tree Image	Copies spectral tree to the Clipboard as an image.
Copy > Copy MS Spectrum	Copies the MS spectrum to the Clipboard.

Table 66. Edit group on the Data Manager – Tree mode toolbar22

Command	Description
Copy > Copy MS Spectrum to Legacy mzCloud	Copies the MS spectrum to the Clipboard in a format that you can paste into mzCloud' s spectra search dialog as a query spectrum.
Copy > Copy MS Spectrum to New mzCloud	Copies the MS spectrum to the Clipboard in a format that you can paste into new mzCloud's spectra search dialog as a query spectrum.
Copy> Copy MS Spectrum Image	Copies the MS spectrum to the Clipboard as an image for presentation purposes. You can edit the image in a vector or raster drawing application.
Paste > Paste Record	Pastes the compound, selected spectral tree, and mechanism from the Clipboard as a new item.
Paste > Paste Spectral Tree	Pastes spectral tree from the Clipboard as a new item.
Paste > Paste MS Spectrum	Pastes the MS spectrum from the Clipboard as a new item.
Paste > Paste Mechanism CTRL+V	Pastes the mechanism from the Clipboard as a new item.

Edit group – Mechanisms mode

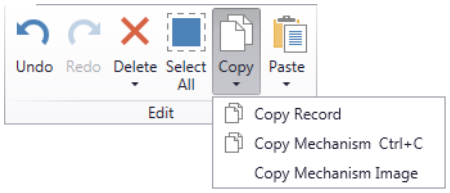


Table 67. Edit group on the Data Manager – Mechanisms mode toolbar12

Command	Description
Undo	Undoes the last action.
Redo	Reverts to the last undone action.
Delete > Delete Selection	Deletes the selected item.
Delete > Delete All	Deletes all the items.

Table 67. Edit group on the Data Manager – Mechanisms mode toolbar22

Command		Description
Select All		Selects all the items.
Copy > Copy Record		Copies the compound, selected spectral tree, and mechanism to the Clipboard.
Copy > Copy Mechanism		Copies the mechanism to the Clipboard.
Copy > Copy Mechanism Image		Copies an image of the mechanism to the Clipboard.
Paste > Paste Record		Pastes the compound, selected spectral tree, and mechanism from the Clipboard as a new item.
Paste > Paste Spectral Tree		Pastes spectral tree from the Clipboard as a new item.
Paste > Paste MS Spectrum		Pastes the MS spectrum from the Clipboard as a new item.
Paste > Paste Mechanism CTRL+V		Pastes the mechanism from the Clipboard as a new item.

Library group

Table 68. Library group on the Data Manager toolbar

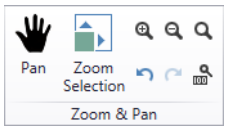
Command	Description
Reload > Reload Compounds	Reloads the compound records in the selected library.
Reload > Reload Spectral Tree	Reloads the spectral trees under the records in the selected library.
Reload > Cancel Import	Aborts importing the compound records in the selected library.
Delete > Delete Compound	Deletes the selected compound record from the library.
Delete > Delete Spectral Tree	Deletes the selected spectral tree from a compound record.
Delete > Delete Mechanism	Deletes the selected fragmentation mechanism.
Save Changes	Saves changes to the libraries. Also, saves the compound records to the new working page of the user libraries.

Actions group – Tree mode

Table 69. Actions group on the Data Manager toolbar

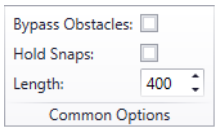
Icon	Description
Add Fragment Annotation	Opens the Structure Editor to draw and add the fragment annotation in the spectrum window
Add Text Annotation	Adds text annotation in the spectrum window
Auto Fragment Annotation	Loads from the SDF file or from the Fragments & Mechanisms module.
Edit Annotation	Edits the selected fragment or text annotation in the spectrum window.
Remove Annotation	Deletes the selected fragment, formula, or text annotation in the spectrum window.
Remove All Annotations	Deletes the fragment, formula, and text annotations.

Zoom & Pan group – Mechanisms view



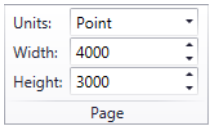
Use these tools to zoom and pan in the drawing area of the Data Manager window – Mechanisms view. See "Zoom & Pan group" on page 240.

Common Options group – Mechanisms view



See "Common Options group" on page 242.

Page group – Mechanisms view



See "Common Options group" on page 242.

View group

Table 70. View group on the Data Manager toolbar

Icon		Description
Grid View		Shows the database compounds in grid view.
Combined Grid View		Shows the database compounds in combined grid view.
Card View		Shows the database compounds as cards.
Show Raw Spectral Tree		Shows only raw trees.
Show Filtered Spectral Tree		Shows only filtered trees.
Show Calibrated Spectral Tree		Shows only calibrated trees.
Show Fragment Annotations		Shows/Hides the structure annotations.
Show Formula Annotations		Shows/Hides the assigned formula annotations.
Show Text Annotations		Shows/Hides the assigned text annotations.
Show Neutral Losses		Shows/Hides the neutral losses in spectrum.
Show Adducts		Show/Hides the adducts.

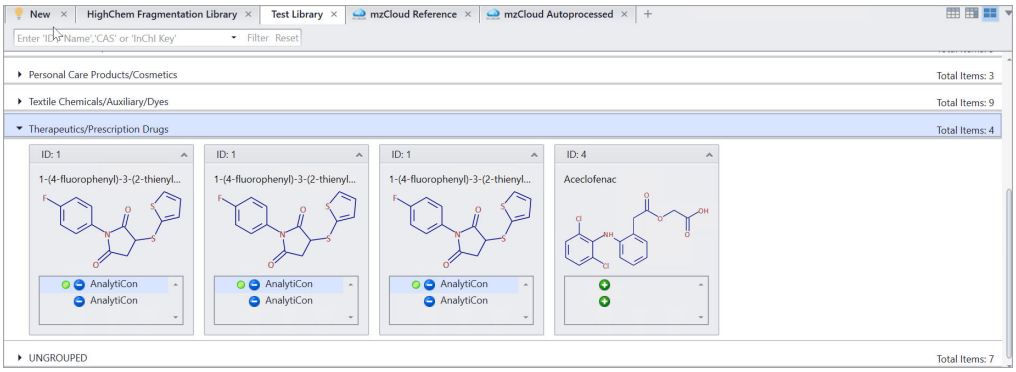
Group By

❖ To Open and group libraries

1. Open Data Manager window. See "Open a Data Manager window" on page 151.
2. Select library of interest from the Libraries Pane.
3. Click any one of the following icon from the Group By tools:
   - Base Structure
   - Compound Class
   - Compound Name

   All the records in the library will be arranged and grouped by base structure. The results displayed will have a header with number of records available for each of the group. The records with no defined base structure will be assigned to a common group, UNGROUPED.

Figure 76. Test Library grouped by Compound Class



4. Click  to cancel the grouping.

Table 71. Group By Tools on the Data Manager tool bar

Button	Description
Base Structure	Groups all the records in the selected library by Base Structure.
Compound Class	Groups records by Compound Class.
Compound Name	Groups records by Compound Name.

Quick Filter group

Table 72. Quick Filter group on the Data Manager toolbar

Button	Shortcut Key	Description
Metadata	Ctrl+Shift+I	Opens the Metadata tab in Quick Filter Pane of Data Manager.
Spectrum	Ctrl+Shift+S	Opens the Spectrum tab in Quick Filter Pane of Data Manager.
Structure	Ctrl+Shift+T	Opens the (Sub)Structure tab in Quick Filter Pane of Data Manager.
Retention Time	Ctrl+Shift+R	Opens the Retention Time & Index tab in Quick Filter Pane of Data Manager.
Compound Class	Ctrl+Shift+C	Opens the Compound Class tab in Quick Filter Pane of Data Manager.
Pathway Mass Loss	Ctrl+Shift+M	Opens the Pathway Mass Loss tab in Quick Filter Pane of Data Manager.

See “Quick filter pane” on page 176 for details on quick filter tool.S

Send To group

Table 73. Send To group on the Data Manager toolbar

Button		Description
Compound		Sends compound to a New Curator page.
Structure		Sends the structure to New Curator, SledgeHammer, Sub/Structure Search or Structure Editor, or any open Structure Grid page.
Spectrum		Sends the selected spectrum to Spectrum or mzLogic Search.
Peaks		Sends the selected peak to Peak Search.

Tree view

In the Tree view, the upper half of the Data Manager window has three panes:

- Spectral Tree pane
- Spectrum pane with the following pages:
  - Spectrum pane – Spectrum page
  - Copy Spectrum to Legacy mzCloud
  - Spectrum pane – BDC page
  - Spectrum pane – Metadata page
  - Spectrum pane – RI References page
  - Spectrum pane – Compare Spectra page
- Compound Structure or Precursor Structure pane

Related Topics

- Notifications pane
- Libraries pane
- Quick filter pane

Spectral Tree pane

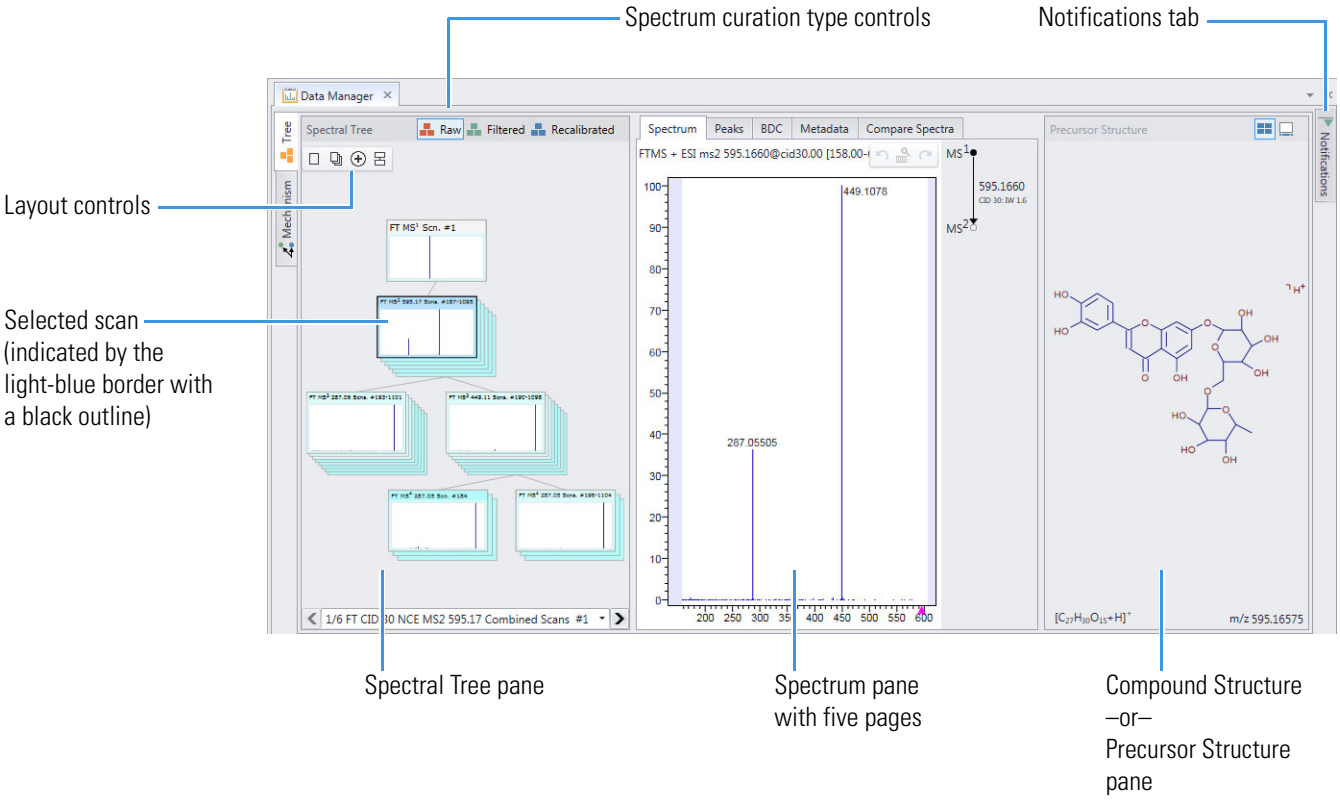
In a Data Manager window, the Spectral Tree pane is an interactive tool where you can visualize the hierarchical arrangement of the MSn data.

Selecting a scan in the tree does the following:

- Displays the spectrum in the spectrum pane to the right.
- Displays the structure of the library compound in the Compound Structure pane on the far right when you select an MS1 scan.
- Displays the precursor structure for the scan in the Precursor Structure pane on the far right when you select an MS2 or higher scan.

Figure 77 shows the Tree view with the selection of an MS^2 scan in the Spectral Tree pane.

Figure 77. Tree view in the Data Manager window



Use the icons in the upper left of the Spectral Tree pane to switch between the folded and unfolded tree layouts. The folded layout shows only the MSn precursor path and *m/z* value. The unfolded view shows each scan as a separate tile with the color-coded ion activation method and mass analyzer.

Table 74. Spectral tree layout controls

Icon	Description
Fold Whole Tree	Folds the entire tree.
Unfold Whole Tree	Unfolds the entire tree.
Compress Selected Node	Compresses the selected node and all of its product nodes to a single circle.
Expand Whole Tree	Expands the entire tree.

Use these computer keyboard and mouse controls to modify the tree layout or display various scans in the spectrum viewer:

- To fold or unfold a single selected node, double-click the selected nodes.
- To compress or expand a node, press CTRL and double-click the node. The compressed node appears as a small circle.
- To browse the respective scans in the selected node of the spectral tree, use the dropdown selection box in the lower section.

Table 75. Buttons in the upper right of the Spectral Tree pane

Icon	Description
Raw Tree	Shows the raw tree.
Filtered Tree	Shows the filtered tree. Available for a filtered spectral tree.
Recalibrated Tree	Shows the recalibrated tree. Available for a recalibrated spectral tree.

To open a shortcut menu, right-click the Spectral Tree pane.

Table 76. Spectral Tree pane shortcut menu

Command	Description
Copy Spectral Tree	Copies the spectral tree to the Clipboard.
Copy Spectral Tree Image	Copies the spectral tree to the Clipboard as an image.
Paste Spectral Tree	Pastes the spectral tree from the Clipboard as a new item.
Copy MS Spectrum	Copies the MS spectrum to the Clipboard.

Table 76. Spectral Tree pane shortcut menu

Command	Description
Copy MS Spectrum to Legacy mzCloud	Copies the MS spectrum to the Clipboard in a format that you can paste to the Legacy mzCloud Spectrum Search dialog box as a query spectrum.
Copy MS Spectrum to New mzCloud	Copies the MS spectrum to the Clipboard in a format that you can paste to the new mzCloud Spectrum Search dialog box as a query spectrum.
Copy MS Spectrum Image	Copies the MS spectrum to the Clipboard as an image for presentations or reports.
Paste MS Spectrum 	Pastes the MS spectrum from the Clipboard as a new item.
Remove Node 	Deletes the selected node.
Remove Spectrum 	Deletes a single selected spectrum.

Spectrum pane – Spectrum page

The Spectrum page of the spectrum pane in a Data Manager window displays the spectrum of the scan that you selected in the Spectral Tree pane.

Table 77. Spectrum page shortcut menu

Command	Description
Copy MS Spectrum	Copies the MS spectrum to the Clipboard in the Mass Frontier native format, a text format, or as a vector (WMF and EMF) image. The application uses the native format to exchange the MS spectrum between modules and Mass Frontier instances. Use the text format to copy and paste a spectrum to a word processing or spreadsheet application.
Copy MS Spectrum to Legacy mzCloud	Copies the MS spectrum to the Clipboard in a format that you can paste to the online Legacy mzCloud Spectrum Search dialog box as a query spectrum.
Copy MS Spectrum to New mzCloud	Copies the MS spectrum to the Clipboard in a format that you can paste to the online New mzCloud's Spectrum Search dialog box as a query spectrum.

Table 77. Spectrum page shortcut menu

Command	Description
Copy MS Spectrum Image (Data Manager window only)	Copies the MS spectrum to the Clipboard as a vector (WMF and EMF) drawing. Use the vector format to paste a high-quality image to a presentation or reporting software application.
Paste MS Spectrum	Pastes the MS Spectrum from the Clipboard to the root node of the spectral tree. This command is available only when you select the New library tab.
Copy Annotation	Copies the selected annotation to the Clipboard in the Mass Frontier native format. Use this command to exchange annotations between Mass Frontier modules or Mass Frontier instances.
Paste Annotation	Pastes an annotation from the Clipboard to the spectrum.
Absolute Abundance	Switches the *y*-axis scale between absolute abundance and relative intensity on the Spectrum page of the MS Spectrum pane.
Reset Zoom	Resets the zoom to 100%.
Show Fragment Annotations	Shows the fragment annotations on the Spectrum page of the MS spectrum pane.
Show Formula Annotations	Shows the formula annotations on the Spectrum page of the MS spectrum pane.
Show Text Annotations	Shows the text annotations on the Spectrum page of the spectrum pane.
Auto Annotation Layout	Rearranges the annotations on the Spectrum page of the spectrum pane.
Show Accuracy	Shows accuracy on the Spectrum page of the spectrum pane.
Show Resolution	Shows resolution on the Spectrum page of the spectrum pane.
Show Grid	Shows grid on the Spectrum page of the spectrum pane.
Show Adducts	Shows the adducts on the Spectrum page of the spectrum pane.
Show Neutral Losses	Shows the neutral losses on the Spectrum page of the spectrum pane.

❖ Copy Spectrum to Legacy mzCloud

1. Open Data Manager module. Select MS spectrum file and open.

2. Click Copy MS Spectrum to Legacy mzCloud.
3. Open online Legacy mzCloud page and search Spectrum.
4. Paste the MS Spectrum copied from the Mass Frontier 8.1 application in the spectrum search dialog box.

   MS spectrum copies to the Legacy mzCloud page as a query spectrum.

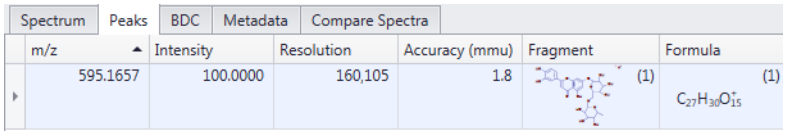
❖ **Copy Spectrum to New mzCloud**

1. Open Data Manager module. Select MS spectrum file and open.
2. Click Copy MS Spectrum to New mzCloud.
3. Open online New mzCloud page and search Spectrum.
4. Paste the MS Spectrum copied from the Mass Frontier 8.1 application in the spectrum search dialog box.

   MS spectrum copies to the New mzCloud page as a query spectrum.

Spectrum pane – Peaks page

The Peaks page of the spectrum pane in a Data Manager window lists the spectral peaks in a table format, with one row per peak. The table includes the following information about each peak: *m/z* value, relative intensity, resolution, accuracy, fragment structure annotation, and formula annotation.



Spectrum pane – BDC page

The Breakdown curve (BDC) page of the spectrum pane in a Data Manager window displays the breakdown graphs of individual ions in the spectra of the selected node as a function of activation energy.

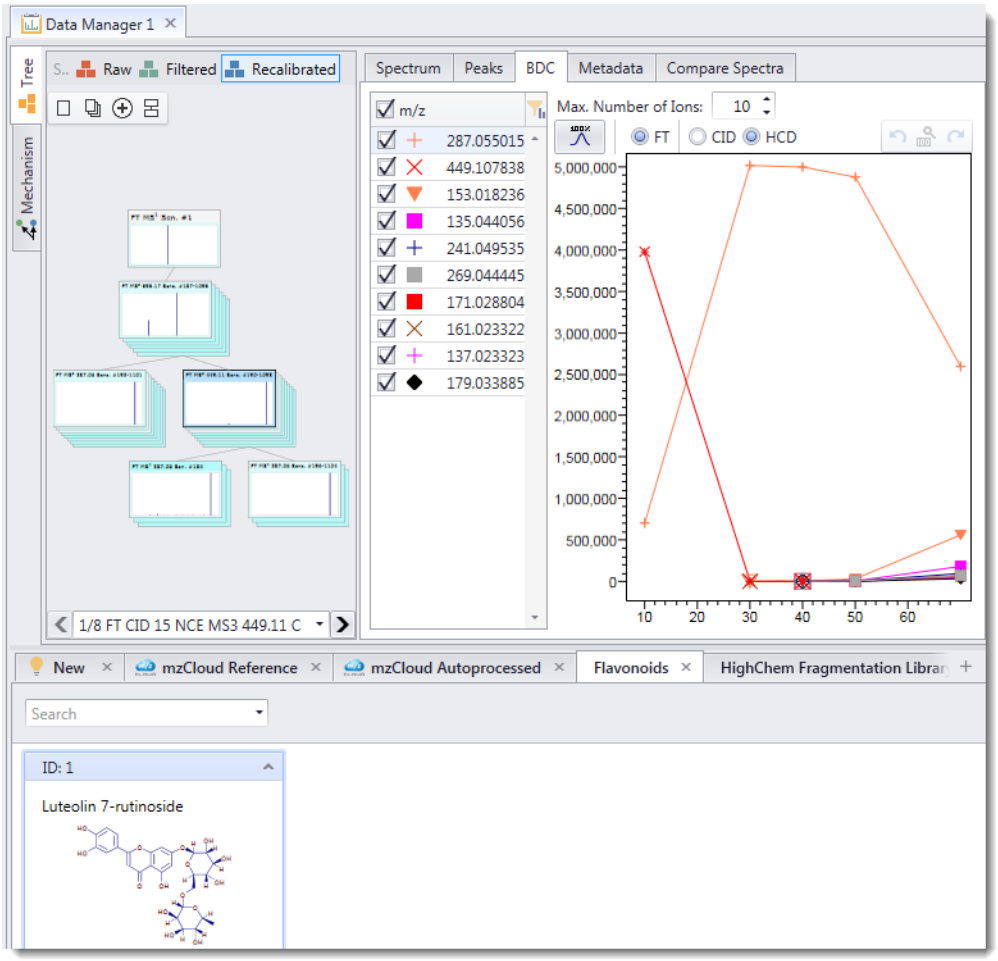
Table 78. BDC page parameter descriptions

Parameter or icon	Description
Max. Number of Ions	Specifies the maximum number of ions for which the breakdown profiles are displayed, sorted by their respective intensity.
Set order by ascending or descending *m/z* value 	Specifies whether to display the *m/z* values in ascending or descending order by intensity.

Table 78. BDC page parameter descriptions

Parameter or icon	Description
Show Absolute Intensities	Shows the absolute or relative intensities of the ions.
Analyzers (FT/IT)	Displays whether the scans are from an FT or IT mass analyzer.
Ion activation (CID/HCD)	Specifies whether to display the breakdown curves for CID or HCD scans.

Figure 78. BDC page of the spectrum pane in a Data Manager window



Spectrum pane – Metadata page

The Metadata page of the spectrum pane in a Data Manager window displays additional information about the compound entry.

Spectrum pane – RI References page

The RI Reference page of the spectrum pane in a Data Manager window provides literature references for the compound. You can add details of compound such as retention index, retention time, column name, standard set and description

❖ **To add Literature references**

1. Open Data Manager window. See "Open a Data Manager window" on page 151.
2. Click  on the RI References page of Spectrum pane.

   A list of options below the literature references opens as a drop down menu.
3. Double click on the text box for any of the following options. Enter the details and click **Update.**
   - RI (Retention Index)
   - RT (Retention Time)
   - Column Name
   - Standard Set
   - Description
4. Click Add new optional item  to add additional details to the literature references.

   Enter the caption and the value to update.
5. Click  to cancel the current literature.

Figure 79. RI References pane

Note You can add one or more literature references to the RI References page. Each literature reference is denoted by a number in an increasing order.

You can remove one or more literature references added to the RI References page.

Spectrum pane – Compare Spectra page

The Compare Spectra page of the spectrum pane in a Data Manager window provides a detailed view of the difference between the query spectrum and the library spectrum. The confidence/match area displays several calculated spectral match values using different criteria.

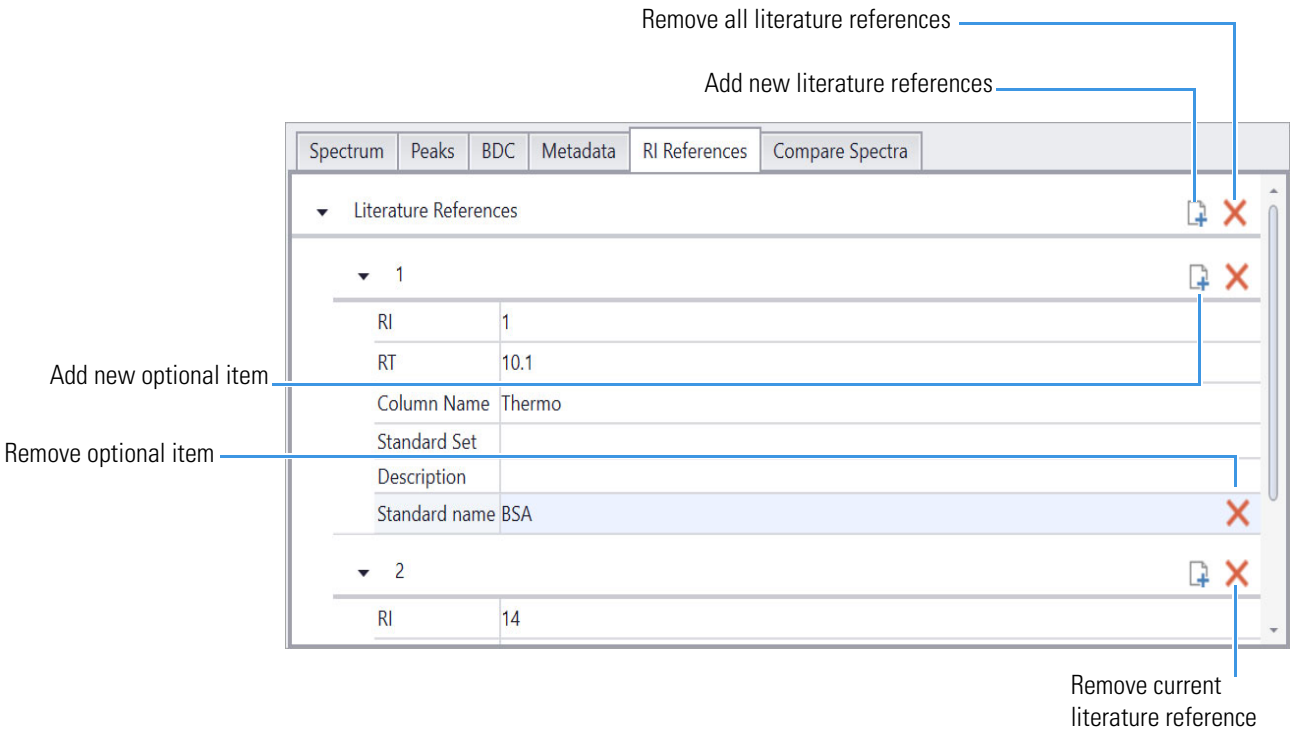


Table 79. Compare Spectra page parameter descriptions

Parameter or Button	Description
Button	
Pin Current	Copies the spectrum from the Query plot to the Library plot.
Paste	Pastes spectrum to the Query plot from the Clipboard.
Display area	
Query area	Displays the query spectrum.
Difference area	Displays a plot of the difference between the query spectrum and the library spectrum.
Library area	Displays the library spectrum.
Confidence/match score area	Displays various confidence and match scores.
Shortcut menu	
Copy MS Spectrum	Copies the MS spectrum to the Clipboard.
Reset Zoom	Resets the zoom level of the plot.

Compound Structure or Precursor Structure pane

In the Tree view, the pane on the far right of the Data Manager window is either a Compound Structure pane or a Precursor Structure pane, depending on the MS stage you select in the Spectral Tree pane.

In the Tree view, the pane on the far right of a Data Manager window displays the following:

- Compound Structure Pane
- Precursor Structure pane

Compound Structure Pane

When you select an MS1 scan in the Spectral Tree pane, the Compound Structure pane displays the structure of the library compound. You can add, edit, remove, copy or paste a base compound in the compound structure pane.

Figure 80. Compound Structure pane

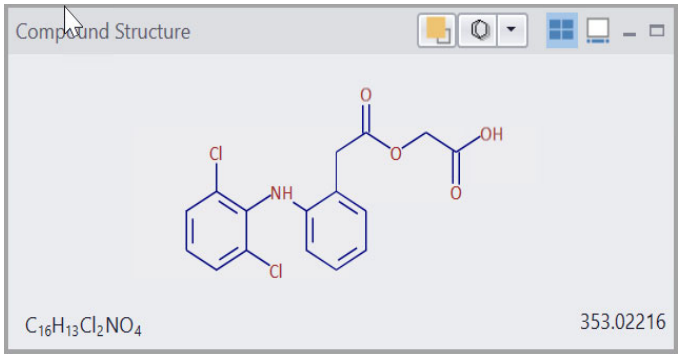


Table 80. Compound Structure pane toolbar

Icon	Description
Switch View	Turns on/off the view of base structure over the compound structure.
Add or edit base structure	Add or Edit the base structure in the Structure Editor window.
Drop down menu	Add, edit, remove, copy, paste and link the base structure in the Structure Editor window.
Grid View	If there are multiple structures assigned to a compound or precursor, clicking this icon arranges them in a grid layout.
List View	If there are multiple structures assigned to a compound or precursor, clicking this icon hides all but one of the structures. To view a specific structure, you must select it from the dropdown list.

❖ To Add, Edit, Remove, Copy, Paste and Link Base Compound

1. Click the drop down menu on the compound structure pane, or, right click on the structure pane.
2. Select any one of the following command from the drop down menu:
   - Add Base Structure
   - Edit Base Structure
   - Remove Base Structure
   - Copy Base Structure
   - Paste Base Structure
   - Link Base Structure

Table 81. Structure pane shortcut menu commands

Command	Description
Add Base Structure	Opens an empty Structure Editor window for adding another structure.
Edit Base Structure	Opens a Structure Editor window with the assigned structure.
Remove Base Structure	Deletes the assigned structure.
Copy Base Structure	Copies the assigned structure to the Clipboard.
Paste Base Structure	Pastes a structure from the Clipboard.
Link Base Structure	Link and overlay the Base Structure. The common features in the compound structure and base structure gets highlighted with color.

3. Click **Ok**.

Note For information about using Structure Editor window see "Structure Editor module" on page 249.

Right click on the Compound Structure pane to view and use Add, Edit, Remove, Copy, Paste and Link Base commands.

WARNING The action of adding structure gets canceled with a warning message, if you add an empty structure or the same structure as the query compound

Precursor Structure pane

When you select an MS2 or higher scan in the Spectral Tree pane, the Precursor Structure pane displays the precursor structure for the scan.

Figure 81. Precursor Structure pane

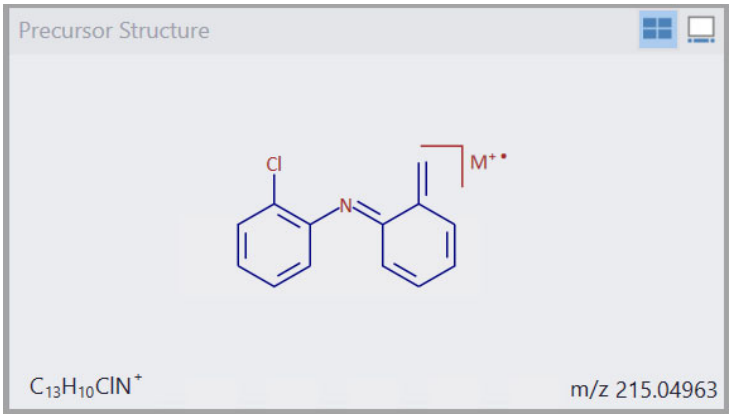


Table 82. Compound or Precursor Structure pane

Icon		Description
Grid View		If there are multiple structures assigned to a compound or precursor, clicking this icon arranges them in a grid layout.
List View		If there are multiple structures assigned to a compound or precursor, clicking this icon hides all but one of the structures. To view a specific structure, you must select it from the dropdown list.

Mechanism view

Use the Data Manager's Mechanism view to enter and edit fragmentation reactions.

Table 83. Mechanism view toolbar13

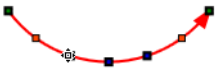
Icon		Description
Base mode		Returns to base mode where no tool is selected.
Straight/Arc		Adds a straight/arc connector or converts a connector to a straight/arc connector. This connector has the shape of a line or arc.  

Table 83. Mechanism view toolbar23

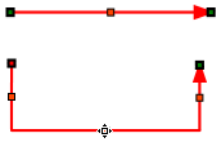
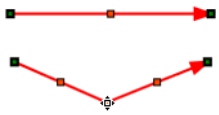
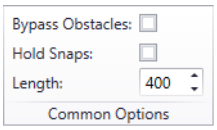
Icon		Description
Orthogonal		Adds an orthogonal connector or converts a connector to an orthogonal connector. An orthogonal connector has the shape of an orthogonal polyline. 
Polyline		Adds/converts to polyline connector. This connector has the shape of a polyline. 
Structure		Opens the Structure Editor for adding and editing structures.
Text		Adds/edits a text block.
Undo		Reverts the last modification of the pathway. You can undo up to six actions.
Redo		Repeats the last modification of the pathway. You can redo up to six actions.
Delete selection		Deletes the selected part of the pathway.
Top alignment		Aligns to the top.
Middle alignment		Aligns to the middle (vertical center).
Bottom alignment		Aligns to the bottom.
Left alignment		Aligns to the left.
Center alignment		Aligns to the horizontal center.
Right alignment		Aligns to the right.
Boundary snap		Snaps to boundary box. The start and end points of the reaction (snap points) are snapped to a rectangular box around the structure.
Envelope snap		Snaps to envelop only the mechanism.

Table 83. Mechanism view toolbar33

Icon		Description
Check		Verifies the scheme for errors such as elemental consistency, unconnected reaction or structures, uncharged fragments, fragments with different polarities, or more unconnected pathways.
Lock handle		Locks the position of the connector's handle. The relative position of the connector's handle is fixed to the structure if the Hold Snaps check box in the Common Options group of the Data Manager – Mechanism view toolbar is clear. 
Select All		Selects the entire pathway.
Rectangular Selection		Shows a rectangular shape.selection
Lasso Selection		Shows Lasso selection; the arbitrary shape of the selection.

Notifications pane

The Notifications pane displays notifications from the Mass Frontier Library Service—that is, when you save a compound entry to a user library, delete a library entry, create a new library, install, remove, or uninstall a library, the service sends a notification to all the opened Data Manager windows. If the notification message represents an unsuccessful action, the service writes a detailed record to the error log.

Note If no Data Manager window is open, the application automatically saves the new entry to the selected library without sending a notification to the Notifications tab.

Note To view the notifications, you might need to click the down arrow at the right end of the Modification bar.

Figure 82. Notification for adding a compound to a Data Manager window that is currently open

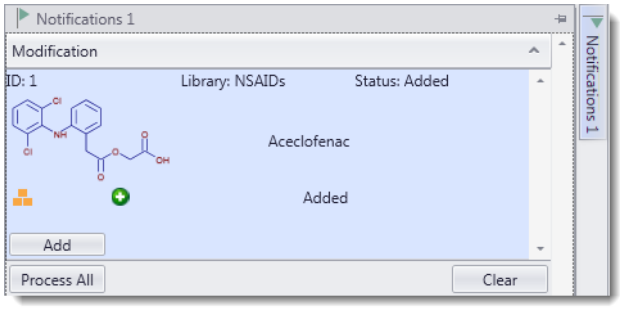
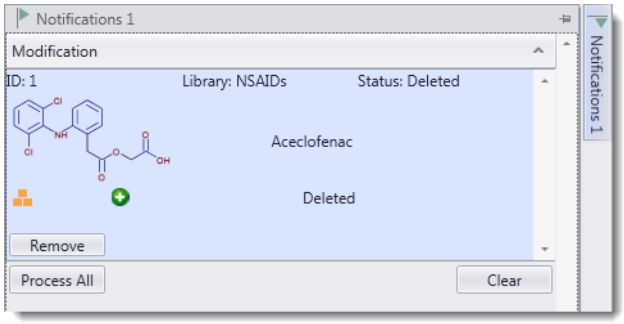


Figure 83. Notification to complete the removal of a library entry



Libraries pane

In the Libraries pane, you can browse all the libraries that are accessible to your Mass Frontier installation.

There are three categories of libraries:

- New—An Active temporary library that does not persist data when closing the Data Manager page. You can save the content of the new library to any of the user libraries or files.
- User Libraries—These are the libraries that you create by using Server Manager on a connected Mass Frontier Library Service.
- Pre-installed libraries —These are the read-only libraries provided as part of the installation:
  - The HighChem Fragmentation Library contains fragmentation templates extracted from the literature. This library is part of the Mass Frontier Library Service installation.
  - The mzCloud Reference library provides direct access to the mzCloud spectral library. The processing computer must have an Internet connection to access the library.
  - The mzCloud Auto processed library

When a library contains are large number of compounds, use the Search box below the library tabs bar to search for specific compounds. You can search by the compound's name or ID.

You can display library compounds in three different layouts based on your preferences.

Table 84. Data Manager libraries tab bar icons

Icon	Description
	Default Tile view: Compound ID, Compound Name, Structure, and every mechanism or spectral tree entry with the corresponding contributor name and polarity are displayed.
	Condensed list view: Compound ID, Compound Name, Structure, Molecular Mass, and formula are displayed in rows.
	Detailed list view: Compound ID, Compound Name, Structure, Molecular Mass, formula, and every mechanism or spectral tree entry with corresponding contributor name and polarity are displayed in rows.

Copy library records

Use the Copy/Paste record functionality in Data Manager to directly combine the records in locally installed libraries. The libraries must be installed on a connected Mass Frontier Library Service.

Tip See "Migrate spectral libraries" on page 390 if the library files are not installed on the computer with the connected Mass Frontier Library Service, or see Transferring Spectral Libraries if the library files are on different computers.

Note The Copy/Paste functionality works only between libraries installed on the same Mass Frontier Library Service. To exchange records between different Mass Frontier Library Services, export the records to an MFCDC file from the source service, and import it to the target service.

❖ To copy/paste spectral records

1. Open the Mass Frontier 8.1 application and click **Data Manager**.
2. Select the mass spectral library with the record.
3. In the Edit toolbar group, click **Copy > Copy Record**.

   Tip Large library records take a significant time to copy. The green indicator on the spectral library tab indicates activity on the library. You must wait until the green indicator disappears to continue working with the libraries
4. Click the target spectral library tab and select the location to copy the record.
5. In the Edit toolbar group, click **Paste > Paste Record**.

6. At the prompt, click **Yes** to confirm adding the new record.
7. From the Library toolbar group, click **Save Changes**.
8. In the Save Changes dialog box, select the check box for the record that you want to save, and then click **Save**.

   The newly added record appears in the target library.

Quick filter pane

Use the quick filter tool in Data Manager to directly filter the records from locally installed libraries using different functions.

❖ **To open Quick Filter tool**

1. Open the Mass Frontier 8.1 application and click **Data Manager**.
2. Select the mass spectral library with the record.
3. In the Quick Filter toolbar group, click one of the function to filter records from the available libraries. Or, Click **Quick Filter** pane available just below the notification pane on the Data Manager window. See "“To Open and group libraries” on page 158" for shortcut keys of different functions.

There are different panels in the quick filter tool bar:

- Metadata
- Compound Class
- Spectrum
- (Sub)Structure
- Retention Time & Index
- Pathway Mass Loss

Metadata

Use this option to search and filter the libraries using specific compound metadata property.

❖ **To filter using compound metadata**

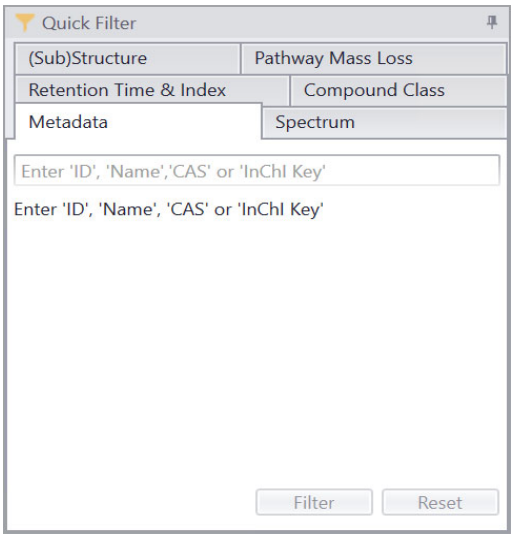
1. Open Quick Filter tool. See “To open Quick Filter tool” on page 176

2. Select the Metadata panel. Enter any one of the metdata property in the search bar to search and filter:

Table 85. Metadata panel with different functions

Property	Description
ID	Enter the unique compound Identifier in the search bar to search and filter from the libraries.
Name	Enter compound name in the search bar to search and filter from the libraries.
CAS	Enter the Chemical Abstracts Service (CAS) number in the search bar to search and filter from the libraries.
InChIKey	International Chemical Identifier (InChIKey) is 27-characters long unique text that denotes a chemical compound. Enter InChIKey in the search bar to search and filer from the libraries.

Figure 84. Quick filter by Metdata



3. Click **Filter.**

   Search results will be displayed in the Library tab of the Data Manager window.

4. Click **Reset** to cancel the filter search.

Note Reset the filter after every search to cancel and start a new filter search.

Compound Class

Use this option to search and filter the libraries using the class of the chemical compounds.

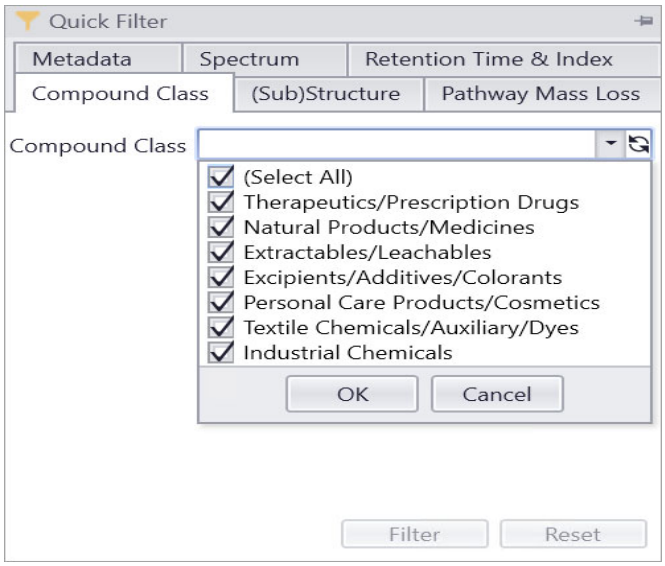
❖ To filter using the class of compounds

1. Open Quick Filter tool. See "To open Quick Filter tool" on page 176
2. Select the Compound Class panel.
3. Enter one or more class of compound in the search bar to search and filter from the libraries.

-or-

Check one or more compound classes from the drop-down to search and filter from the libraries.

Figure 85. Quick filter by Compound Class



4. Click **Filter.**

   Search results will be displayed in the Library tab of the Data Manager window.

5. Click **Reset** to cancel the filter search.

Note Reset the filter after every search to cancel and start a new filter search.

Spectrum

Use this option to search and filter the libraries using specific spectrum of the compounds.

❖ To open and filter spectrum of interest

Do any one of the following to open spectrum of interest in the quick filter pane:

1. From the application window, import the spectrum of interest.

2. Choose Quick Filter tool. See "To open Quick Filter tool" on page 176.Select the spectrum panel in the Quick Filter pane.
3. Click **Pin Current** on the spectrum panel of quick filter to pin the spectrum.

   -or-

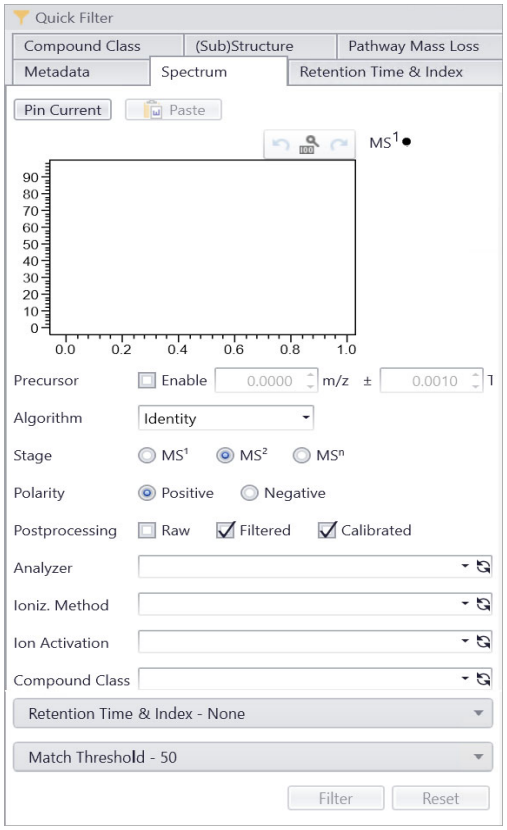
   Right click on the spectrum of interest in the spectrum tab of Data Manager window. Choose **Copy MS Spectrum** from the options to copy the data to clipboard. Click **Paste.**

4. Select the spectrum search parameters. See "Spectrum filter parameters" on page 180.
5. Click **Filter.**

   Search results will be displayed in the Library tab of the Data Manager window. See "" on page 183.
6. Click **Reset** to cancel the filter search.

Note Reset the filter after every search to cancel and start a new filter search.

Figure 86. Quick Filter by Spectrum



❖ Spectrum filter parameters

Use spectrum filter parameters to filter the query spectrum from the libraries.

Table 86. Spectrum filter parameters and description

Parameter or button	Description
Paste Spectrum	Pastes spectrum from the Clipboard.
Pin Current	Pins the query spectrum to the filter.
Precursor	When enabled, constrains the search to library spectra with matching precursor ion m/z.
Query Spectrum	

Table 86. Spectrum filter parameters and description

Parameter or button	Description
Search Algorithm	Specifies an Identity search or a Similarity search. **Match:** Displays the match between query compound spectrum and the compound spectrum from library search. • Cosine: Dot product algorithm with fragment intensities weighted by 0.75 and no weighting on fragment m/z values. • HighChem High Res: Use for high resolution data. Determines whether the application computes the score calculated by a proprietary algorithm that indicates how well the library spectrum and the query spectrum match. • Opt Dot Product: Use Opt Dot product algorithm to Determines whether the application displays the score calculated by an optimized dot product algorithm that indicates how well the library spectrum and the query spectrum match. • NIST algorithm: Determines whether the application computes the score calculated by a National Institute of Standards and Technology algorithm that indicates how well the library spectrum and the query spectrum match. **Note** When you select mzCloud libraries, the Cosine algorithm will be disabled as this is not supported in mzCloud.

Table 86. Spectrum filter parameters and description

Parameter or button	Description
Search Algorithm	**Confidence**: Displays the confidence of the match. Considers dot product of both spectra, distribution of peaks, activation energy difference, and polarity. Uses a machine-learning Bayesian Network model to estimate the likelihood of a correct match. • Identity (Default): Considers all the peaks in the query spectrum and the library spectrum. Select one of the following methods to calculate the spectral match score. • Similarity Forward: Conducts a forward search, which searches the query spectrum against each library spectrum. If the query spectrum includes a peak that is not in a specified library spectrum, the match score is negatively affected. Use a forward search when the query spectrum is of high quality—that is, when it has good fragmentation and few low-intensity background peaks. • Similarity Reverse: Searches for a match between the fragmentation scans in the mzCloud database and the best fragmentation scan for a compound (across the input file set).
Stage	Select the MS^1, MS^2 option or the MS^n option. MS^1— Compares the query spectrum with any MS^1 spectra in the selected libraries. MS^2— Compares the query spectrum with any MS^2 spectra in the selected libraries. MS^n—Compares the query spectrum with any MS^n spectra in the selected libraries.
Ionization Methods	Searches only for spectra from the specified ionization method. Default: All Select one or more Ionization Methods from the drop down menu and click **Ok**.
Mass Analyzers	Searches only for spectra from the specified mass analyzer. Default: All Select one or more Mass Analyzers from the drop down menu and click **Ok**.

Table 86. Spectrum filter parameters and description

Parameter or button	Description
Ion Activation	Searches only in the spectra of the specified ion activation. Default: All Select one or more Ion Activation method from the drop down menu and click **Ok**.
Retention Time and Index Filter	Selections: • None—Does not constrain the search results by the retention time of the library spectrum. • Range—Filters the results by setting retention time limits in minutes (decimal). • Search in Literary sources— When enabled, filters the results by searching in the literature. • Column Name- Select the column name from the drop down to filter. • Standard Set- Select the standard set from the drop down to filter.
Compound Class	Use this to filter the search by Compound Class. Select one or more Compound Class from the drop down menu and click **Ok**.
Match Threshold	Use to specify and filter the threshold of match for the query spectrum from library. Range: Minimum: 0 -Maximum: 100% Default: 50%. Drag and select the percentage of threshold and click **Filter**. Reset the filter after every search to cancel and start a new filter search.

Note The .'msp' spectrum format is not recommended as a query spectrum in the Data Manager. Using this format as query spectrum can lead to an incorrect result.

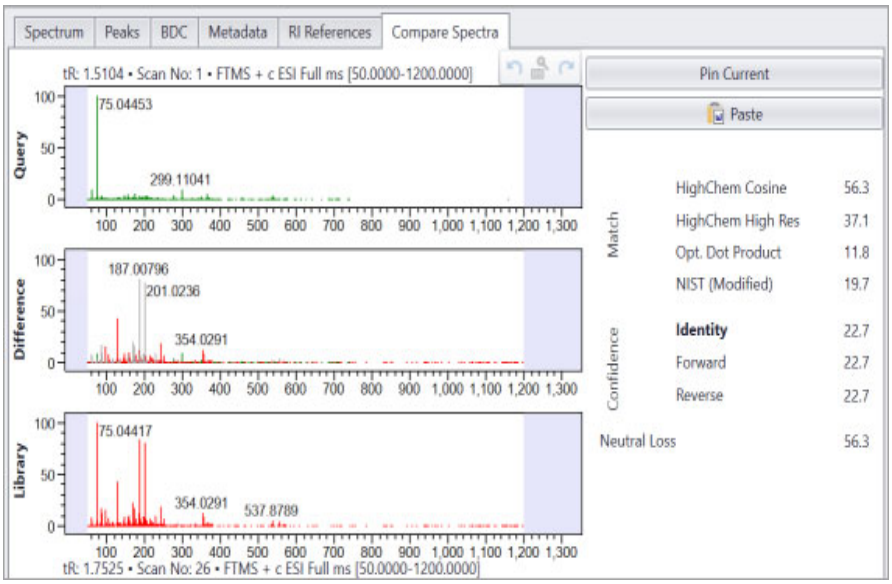
In the quick filter spectrum search, the spectrum with unknown polarity isn't displayed in the options dialog.

❖ Spectrum filter results window

The filter results appear in a new window, Compare Spectra. Confidence and match score is provided in the compare spectra tab of Data Manager window.

- Confidence score: The Confidence score is a probability-based library searching and scoring algorithm, primarily developed to reduce the likelihood of a false positive identification. In essence, it is a measure of the probability of a correct compound identification calculated from a Bayesian network extensively trained on searching against the mzCloud database.
- Match score: The match score provides the level of match between the query spectrum and the library database.

Figure 87. Spectrum filter result window



In the library tree, the color and share of spectrum's border modifies based on the match score; for example, a dark red border indicates a relatively high match score, a light pink border indicates a relatively low match score, and a gray border indicates that the library spectrum does not match the query spectrum.

(Sub)Structure

Use this option to search and filter the libraries based on the structure of the compounds.

❖ To open and filter query compound

Do any one of the following to open query compound structure in the quick filter pane:

1. From the application window, import the structure of query compound.

2. Choose Quick Filter tool. See "To open Quick Filter tool" on page 176.Select the (Sub)Structure panel in the Quick Filter pane.
3. Click **Pin Current** on the (Sub)Structure panel of quick filter to pin the structure of query compound.

   -or-

   Right click on the structure of query compound in the Compound Structure tab of Data Manager window. Choose **Copy** from the options and Click **Paste** in the Sub(Structure) quick filter tab.

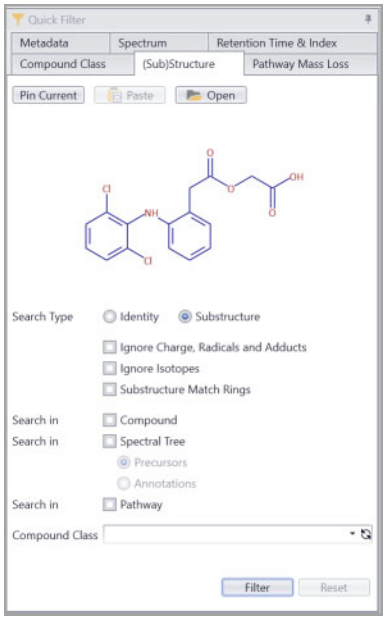
   -or-

   Click **Open** in the (Sub)Structure panel of the Quick Filter pane. A new file explorer window will open. Select the file of interest.
4. Select the search parameters. See "Spectrum filter parameters" on page 180.
5. Click **Filter.**

   Search results will be displayed in the Library tab of the Data Manager window. See "" on page 183.
6. Click **Reset** to cancel the filter search.

Note Reset the filter after every search to cancel and start a new filter search.

Figure 88. Quick Filter by Structure



❖ Structure filter parameters

Use structure search parameters to filter the query compound structure from the libraries.

See "Structure Search dialog box parameters" on page 360.

Retention Time & Index

Use this option to search and filter the libraries based on the retention time and retention index of the query compounds.

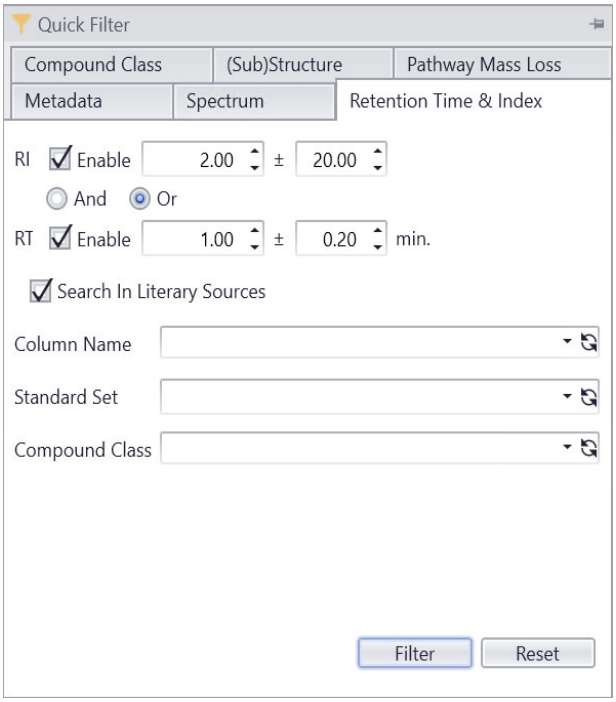
❖ To filter using retention time and/or retention index

1. From the application window, import the file of interest.
2. Choose Quick Filter tool. See "To open Quick Filter tool" on page 176.Select the Retention Time & Index panel in the Quick Filter pane.
3. Enable Retention time (RT) and/or Retention Index (RI). Input values in the text space provided. You can also select other parameters like Compound Class, standard set, match threshold and perform search filter.
4. Click **Filter.**

   Search results will be displayed in the Library tab and Metadata tab of the Data Manager window.
5. Click **Reset** to cancel the filter search.

Note Reset the filter after every search to cancel and start a new filter search.

Figure 89. Quick Filter by Retention Time & Index



Pathway Mass Loss

Use this option to search and filter the query compound from the HighChem Fragmentation Library.

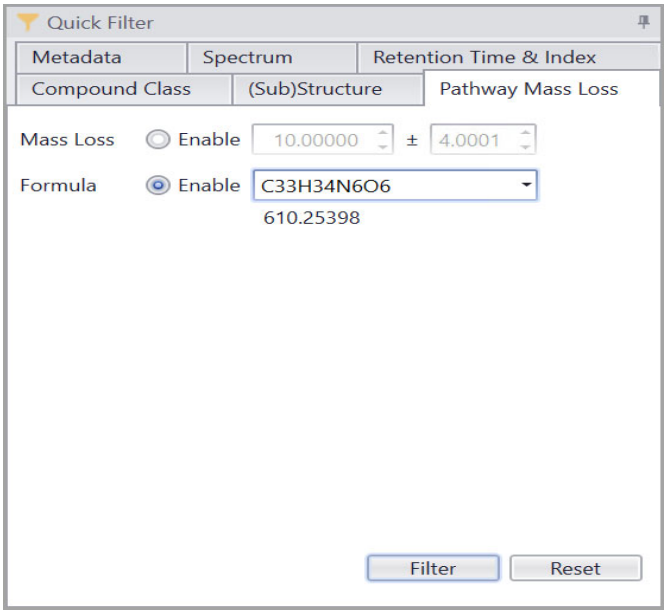
❖ To open and filter query compound

1. From the application window, import the file of interest.
2. Choose Quick Filter tool. See "To open Quick Filter tool" on page 176.Select the Pathway MAss Loss panel in the Quick Filter pane.
3. Enable Mass Loss option or Formula and input the test value in the text space provided.
4. Click **Filter.**

   Search results will be displayed in the Library tab of the Data Manager window.
5. Click **Reset** to cancel the filter search.

Note Reset the filter after every search to cancel and start a new filter search.

Figure 90. Quick Filter by Pathway Mass Loss



Note Use Pathway Mass Loss quick filter to search and filter the query compound the query compound from any user library containing mechanisms.

Use mzCloud Reference Library, mzCloud Autoprocessed library or user library to filter the query spectrum using Metadata, Retention time & Index, Compound Class, Spectrum and Structure Quick Filters. When these filters are used, HighChem Fragmentation Library search will not show any results.

5

Curator module

Use the Curator module to create high-quality library entries for identified components of interest. The guided processing workflow includes filtering, mass recalibration, annotation, and metadata compilation. The output of the curation process is a collection of raw, filtered, and calibrated spectral trees that you can save either to a custom user library or to a file for future reference.

To get started with creating user libraries of curated compound entries, refer to the *Mass Frontier 8.1 tutorial to Curate spectra for a user library.*

Contents

Use the Curator module with other modules

To curate the spectral tree for a component, the Curator module needs the component's proposed structure and the component's spectral tree.

In the Curator module, you work with other modules as follows:

- Receive components with spectral trees, associated structures, or both from the Chromatogram Processor module.

- Receive structures from any opened module with structures, such as Structure Editor, mzLogic results, and others.
- Receive fragments for annotation and mass recalibration from any opened Structure Grid or SledgeHammer window.
- Save compounds with curated spectral trees to a user library.

Opening a new Curator window

You can open the Curator module directly from the Start menu or the Modules & Tools toolbar or indirectly by sending a component for curation from the Chromatogram Processor module.

When you open the Curator module, it opens as a tabbed page in the application window. You can float the page as a separate window or dock the page to another tab position in the application window.

❖ To open a new Curator window

Do any of the following:

- From the Start menu, choose **New**, and then click **Curator** in the New Document view.
- On the Modules & Tools toolbar, click **Curator**.

  Note When you open a new Curator window from the Start menu or the Modules & Tools toolbar, you must import a spectral tree from a file or paste a spectral tree from the Clipboard. You can copy a spectral tree to the Clipboard from a Data Manager window.

- From the Chromatogram Processor module, do the following:

  a. Run component detection.

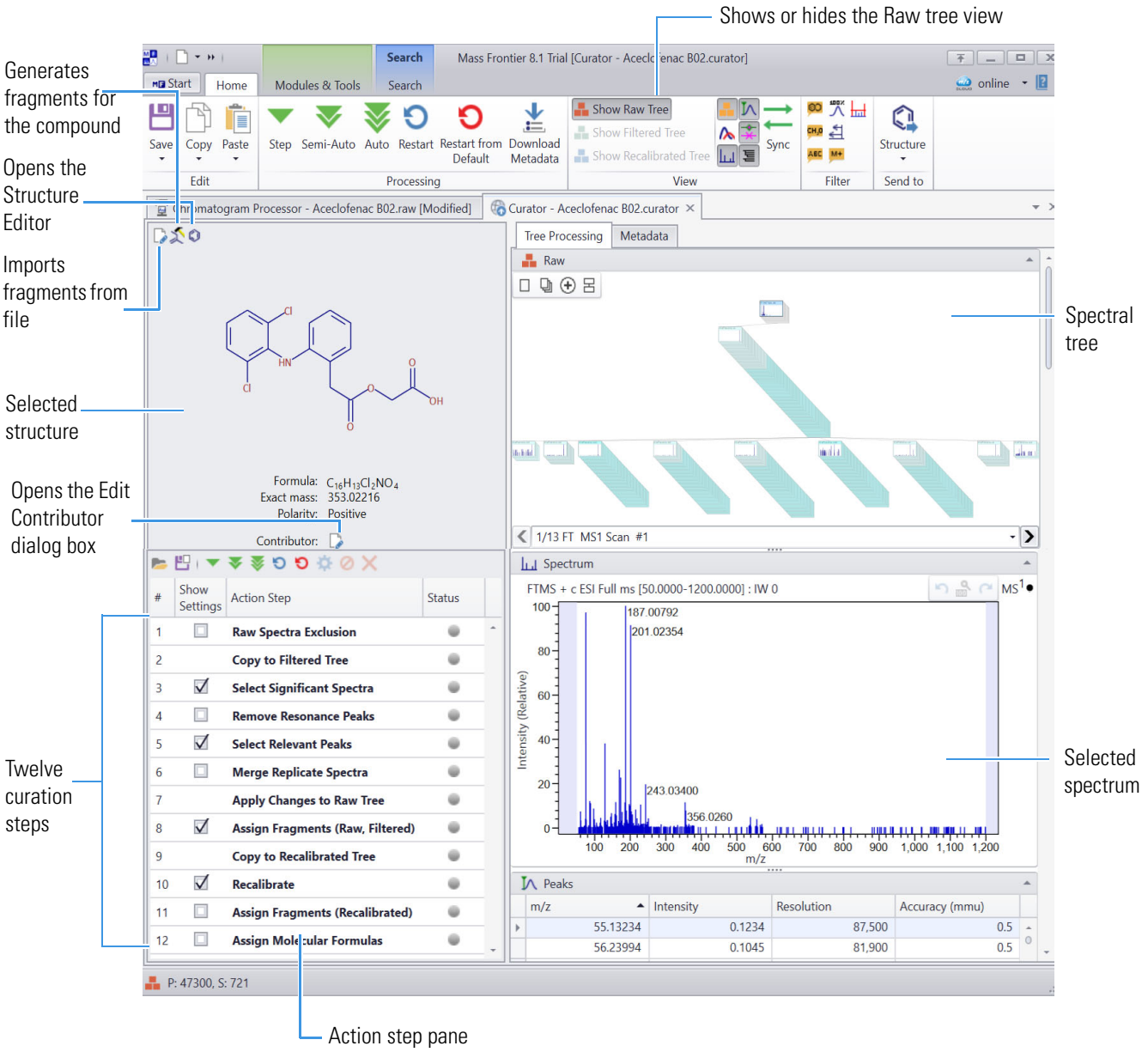
  b. Select a component in the chromatogram data view, click **Component** in the toolbar, and then select **New Curator**.

     If the component is unannotated, the application prompts you to open a structure file.

  c. At the prompt in the new Curator window, open a structure for the component.

     In the Curator window, the selected structure appears in the upper left pane, and the raw spectral tree for the selected component appears in Raw tree view on the upper right (Figure 91).

Figure 91. Curator window with the structure and spectral tree for the selected component



Curator – Spectral Tree pane – Tree Processing page

When you open a new Curator window from the Start menu or the Modules & Tools toolbar, the Tree Processing page does not contain a spectral tree; it contains only two buttons that you can use to import the tree from a file or paste the tree from the Clipboard. When you open a new Curator window by sending an unannotated component from a Chromatogram Processor window, the application prompts you to specify the component's structure.

Table 87. Spectral Tree pane buttons that are available until you open a spectral tree

Button	Description
Import Tree	Imports the spectral tree from a file. Supported file formats are MFCDC, Curator, CCOMX or DBBAK (MF7 format).
Paste Tree	Pastes the tree from the Clipboard.

As you curate a component's spectral tree, you can display trees for each of the post-processing types: RAW, Filtered, and Recalibrated. The RAW (tree) view is always available unless you hide it. If you are curating a raw data file, the Filtered (tree) view appears after action step 3, and the Recalibrated (tree) view appears after action step 9.

The Tree Processing page has the following collapsible groups:

- Spectral Tree group
- Breakdown Curves (BDC) group
- Spectrum group
- Peaks group
- TIC Profiles of Parallel Spectra group
- Spectrum Info group

Tip You can show or hide any of the groups on the Tree Processing page by clicking the small triangle on the right end of the group's title bar. You can also show or hide any of these groups by clicking the appropriate icon in the View group of the Curator toolbar.

Spectral Tree group

On the Tree Processing page of a Curator window, use the spectral tree group to visualize the hierarchical scan arrangement of the MSn data. During the curation process, the following panes appear in the spectral tree group: Raw, Filtered, and Recalibrated.

You can modify the tree layout or browse the node items (scans) as follows:

- To change the display between the schematic and full view, click the **Fold Whole Tree** and **Unfold Whole Tree** icons (or press the **SHIFT** key and double-click the tree).

The schematic view displays only the MSn precursor path and *m/z* value. The full view shows every scan visualized separately with its color-coded ion activation method and the mass analyzer.

- To fold or unfold a specific node, double-click the node.
- To compress or expand a node, press the **CTRL** key, and then double-click the node. The compressed node appears as a small circle.
- To browse the respective scans in the selected node of the spectral tree, use the dropdown selection box in the lower section.

For additional commands, right-click the pane to open the shortcut menu.

Table 88. Spectral tree group shortcut menu commands

Command	Description
Mark Node	Marks the selected node.
Delete Node	Deletes the selected node.
Delete Spectrum	Deletes the selected spectrum.
Delete Spectra	Opens pane, where multiple spectra can be chosen to delete.
Delete Child Nodes	Deletes the child nodes.

Breakdown Curves (BDC) group

Use the panes in the Breakdown Curves group on the Tree Processing page of a Curator window to visualize the effect of collision energy.

Table 89. Breakdown Curves (BDC) group parameter descriptions

Parameters and icons	Description
Max. Number of Ions	The maximum number of ions shown.
Acc. Factor	Accuracy factor used to calculate the breakdown curves.
Set ordering by default 	Lists the *m/z* values by their maximum intensity in descending order.
Show Absolute Intensities 	Sets the *y*-axis to display the absolute or relative intensities of the peaks.
Analyzers (FT/IT)	Mass resolution based on the scan filter (IT or FT).
Ion activation (CID/HCD)	A technique based on the scan filter.
Shortcut menu commands	
Reset Zoom	Resets the zoom to full size
Show Grid	Shows the graph grid

Spectrum group

Use the panes in the Spectrum group on the Tree Processing page of a Curator window to view a node item's spectrum.

Table 90. Spectrum pane shortcut menu commands

Command	Description
Reset Zoom	Resets the zoom action.
Show Grid	Shows graph grid.
Show Absolute Intensities	Shows the absolute/relative intensities of the peaks in Spectrum and Peaks Groups.
Show Fragment Annotations	Shows or hides the structure annotations assigned during the curation process.
Show Formula Annotations	Shows or hides the formula annotations assigned during the curation process.
Show Text Annotations	Shows or hides the assigned text annotations.
Show Neutral Losses	Shows or hides neutral losses in the individual spectra.
Show Adducts	Shows or hides adducts.
Show the Selected Peak in Grid	To automatically scroll to the information for a specific peak, point to the peak until a yellow triangle appears above the peak, and then right-click the peak and choose this command to automatically scroll to the row for the selected peak in the Peaks group table below the spectrum. A black triangle to the left of a table row indicates the current selection. To automatically scroll to the information for a specific peak in the Peaks table, you can also hold the SHIFT key on the computer keyboard and point to the peak until a yellow triangle appears above the peak. 
Copy MS Spectrum	Copies the selected single spectrum to the Clipboard.
Isotope Pattern	Opens the Isotope Pattern tool and shows the formula explanation and theoretical isotope pattern for the peak that you selected. Available following curation action step 5, where you select the relevant adducts ions for the component's spectral tree.

Peaks group

The Peaks group on the Tree Processing page of a Curator window contains tables for the Raw, Filtered, and Recalibrated spectral trees that list all the peaks and their properties in the selected spectrum.

Table 91. Peaks table column descriptions

Column	Description
m/z	Measured *m/z* value of the peak.
Intensity	The measured intensity of the peak.
Resolution	Measured resolution of the peak.
Accuracy (mmu)	The measured accuracy of the peak.
Additional columns for the Filtered Peaks pane	
Formula Explanation	Explains peak with appropriate formula.
Formula m/z	Theoretical formula's *m/z* value.
Delta (ppm)	Difference between the theoretical *m/z* (calculated from the formula explanation) and the measured *m/z*.
Accuracy Factor	Accuracy factor needed for the formula explanation.
Is Explained	Peak has a formula explanation. Peak does not have a formula explanation.

Table 92. Peaks group shortcut menu commands

Command	Icon	Description
Show peaks in BDC View		Adds peak to the list of displayed ions in the BDC Group. Multi-selection of peaks is available.
Delete Peaks (Filtered Peaks pane)		Deletes peak from the spectrum and from the Peak Group. Multi-selection of peaks is available.
Show Absolute Intensities		Shows absolute/relative intensities of the peaks in the Spectrum and Peaks Groups.
Isotope Pattern (Filtered Peaks pane)		Shows selected peaks formula explanation in the Isotope Pattern tool.

TIC Profiles of Parallel Spectra group

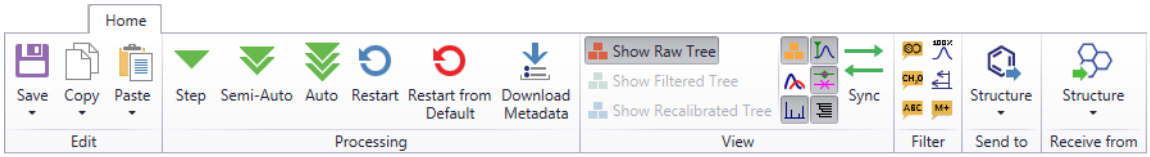
The TIC Profiles group on the Tree Processing page of a Curator window displays the total ion current profile of the parallel spectra. You can apply analyzer and ion activation filters.

Spectrum Info group

The Spectrum Info group on the Tree Processing page of a Curator window shows additional information related to each spectrum saved in the data file. Each file format contains a different list of items that describe the sample, instrument, experimental conditions, and so on.

Curator toolbar

Figure 92. Curator toolbar



The Curator toolbar has five groups:

- Edit group
- Processing group
- View group
- Filter group
- Send to group
- Receive From group

Edit group

Table 93. Edit group on the Curator toolbar (Sheet 1 of 2)

Toolbar command	Icon	Description
Save > Save		Saves the current spectral tree to a Mass Frontier Data Container file (.mfcdc) or a Curator file (.curator). Does not warn you about overwriting a previous version.
Save > Save As		Saves the current spectral tree to a Mass Frontier Data Container file (.mfcdc) or a Curator file (.curator). Warns you when you attempt to overwrite an existing file.
Save > Save to Library		Saves the current spectral tree to the specified local library.
Copy > Copy Tree		Copies the spectral tree to the Clipboard.
Copy Raw Spectrum		Copies selected raw spectrum to the Clipboard.

Table 93. Edit group on the Curator toolbar (Sheet 2 of 2)

Toolbar command	Icon	Description
Copy Filtered Spectrum		Copies selected filtered spectrum to the Clipboard.
Copy Calibrated Spectrum		Copies selected calibrated spectrum to the Clipboard.
Copy Structure		Copies the structure to the Clipboard.
Paste Tree		Pastes the tree from the Clipboard to a tree.
Paste Structure		Pastes the structure from Clipboard to a tree.

Processing group

The Processing group buttons on the Curator toolbar are associated with the action steps in the command processor pane.

Table 94. Processing group of the Curator toolbar (Sheet 1 of 2)

Button	Image	Description
Step		Runs the next step in the curation process. Use this mode to manually perform each step and apply manual threshold filtering and peak editing in the filtered tree. The application prompts you to review and set the parameters settings at each of the selected steps.
Semi-Auto		(Recommended) Runs all the remaining action steps while allowing you to set specific parameter settings during the curation process. Use this mode to review and optimize the parameter settings at each of the selected steps during the curation process. Each settings dialog box has an Apply & Continue in Semi-Auto mode button.

Table 94. Processing group of the Curator toolbar (Sheet 2 of 2)

Button	Image	Description
Auto		Automatically runs the remaining action steps in the curation process using the previously set parameter settings until the application reaches action step 7 and opens the Reaction Restrictions dialog box. To continue the curation process, click **Generate**. Use this mode to automatically run through the curation process with the currently defined settings. **Note** Thermo Fisher Scientific recommends that you preview and optimize the parameter settings for each action step before using the Auto mode. After you optimize the settings, you can save the parameter settings to a .curator_act file for future use.
Restart		Restarts the curation process and parameters for each action step.
Restart from Default		Restarts the curation process with default parameters.
Download Metadata		Downloads metadata (Names, Synonyms, and Identifiers) from the selected databases by using the component's structure and compound name.

View group

The icons in the View group collapse or expand the grouped panes on the Tree Processing page of the Curator window.

Figure 93. Collapsed groups on the Tree Processing page of a Curator window

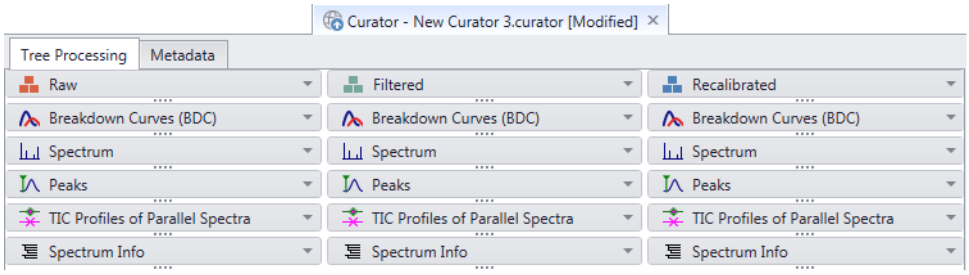


Table 95. View group of the Curator toolbar (Sheet 1 of 2)

Button or icon		Description
Button		
Show Raw Tree		Shows the Raw (tree) pane.
Show Filtered Tree		Shows Filtered (tree) pane.

Table 95. View group of the Curator toolbar (Sheet 2 of 2)

Button or icon		Description
Show Calibrated Tree		Shows Recalibrated (tree) pane.
Icon		
Show/Hide Tree Group		Shows or hides the tree panes group on the Tree Processing page.
Show/Hide BDC Group		Shows or hides the breakdown curves group on the Tree Processing page.
Show/Hide Spectrum Group		Shows or hides the Spectrum group on the Tree Processing page.
Show/Hide Peaks Group		Shows or hides the Peaks list on the Tree Processing page.
Show/Hide TIC Group		Shows or hides the TIC Profiles of Parallel Spectra group on the Tree Processing page.
Show/Hide Common Info Group		Shows/hides common information group of spectrum in the Tree Processing view.
Sync		Synchronizes selection on Raw, Filtered, and Recalibrated trees.

Filter group

Table 96. Filter group of the Curator toolbar

Icon		Description
Show Fragment Annotations		Shows/hides structure annotations assigned during the processing workflow.
Show Formula Annotations		Shows/hides formula annotations assigned during the processing workflow.
Show Text Annotations		Shows/hides assigned text annotations.
Show Absolute Intensities		Shows absolute/relative intensities of the peaks in the Spectrum Group and Peaks Group.
Show Neutral Losses		Shows/hides neutral losses in individual spectra.
Show Adducts		Shows/hides adducts.
Show Threshold		Shows/hides threshold in the Spectrum.

Send to group

Table 97. Send To group of the Curator toolbar

Button	Description
Structure >	Sends structure to a New Curator, New SledgeHammer, New Metabolika, Sub/Structure Search, or New Structure Editor window.

Receive From group

Table 98. Receive From group of the Curator toolbar

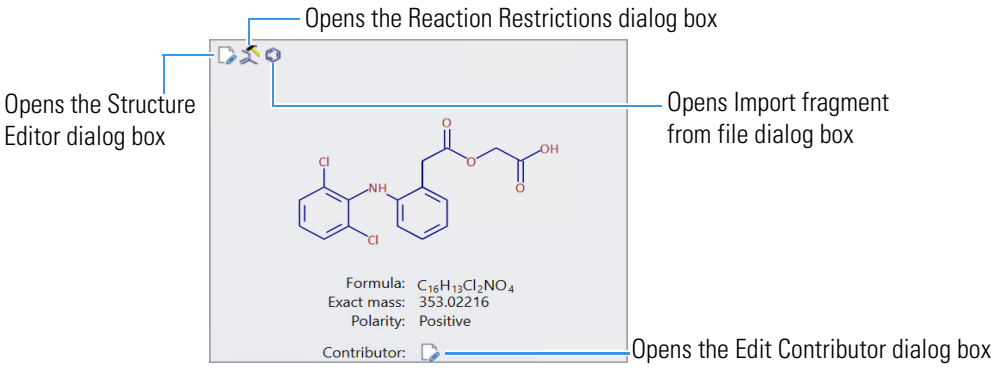
Button	Description
Structure	Receives structures from any opened module that can hold structures and adds them in the Assign Fragments parameter dialog box. Available when another structure module is open.

Curator structure pane

The structure pane at the upper left of a Curator window shows the structure assigned to the spectral tree and if defined, the structure's contributor. Without a structure, the application cannot calculate the formulas for the spectral peaks.

Tip Double-clicking the structure or clicking the edit icon, , in the upper left corner opens the Structure Editor dialog box. Clicking the edit icon, , to the right of Contributor opens the Edit Contributor dialog box.

Figure 94. Structure pane of a Curator window



Related Topics

- Structure Editor module (to edit compound structure).

- Batch Fragment Generation module (to import fragments from file).
- SledgeHammer module (to generate fragments for the compound structure).
- Edit the contributor text (to edit the text that describes the contributor in the structure pane).

Edit the contributor text

Use the Edit Contributor dialog box to edit the text that describes the contributor in the structure pane.

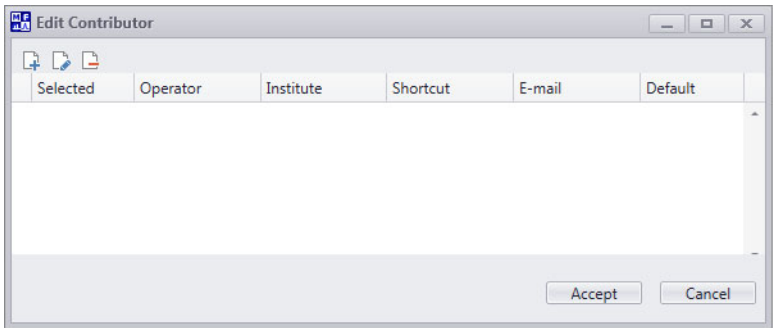
Tip You can also define and modify the list of contributors in the Tree group on the Metadata page in a Curator window.

❖ To edit the contributor text

1. Click the edit icon, , to the right of Contributor in the structure pane.

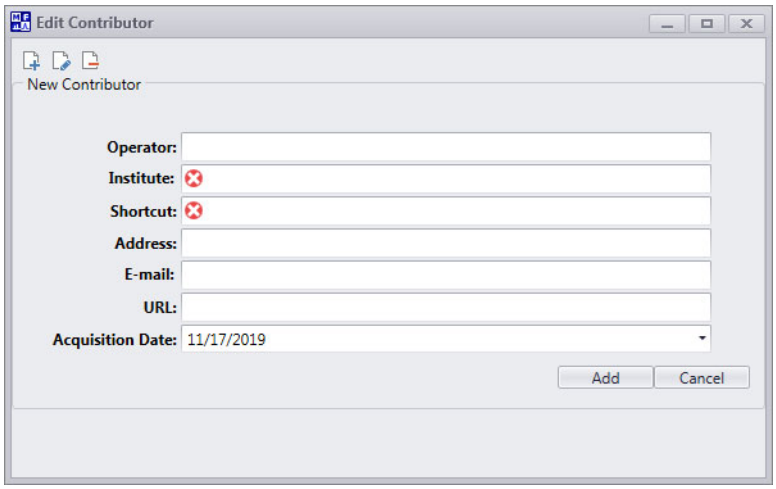
   The Edit Contributor dialog box opens.

   Figure 95. Edit Contributor dialog box



2. Click the **New Contributor** icon, , to open the New Contributor view of the Edit Contributor dialog box.

Figure 96. Edit Contributor dialog box showing the New Contributor view



3. Enter the institute and shortcut information and other information as appropriate.

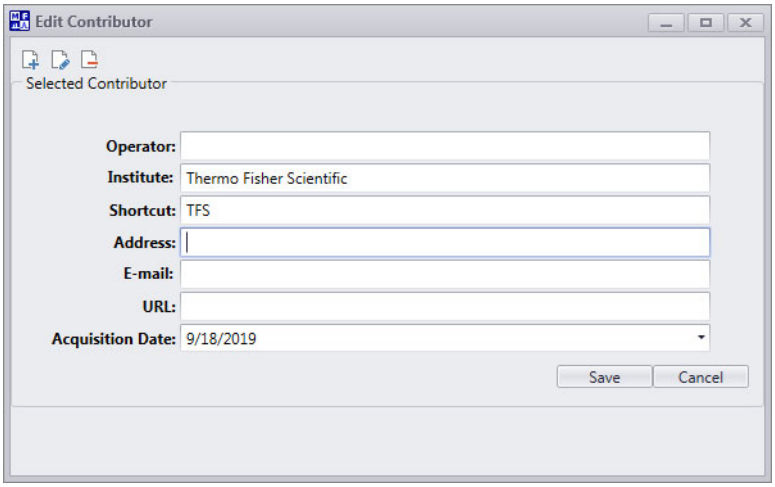
Table 99. Edit Contributor dialog box

Tooltip	Icon	Description
New Contributor		Opens a new view to define the contributor.
Edit Selected Contributor		Opens the Selected Contributor view for editing the information about the selected contributor.
Delete Contributor from List		Deletes the selected contributor.
Default Contributor		Specifies the default contributor.
Set this Contributor as Default		Specifies that the contributor is not the default contributor.

4. In the Edit Contributor dialog box, do any of the following:

- To add a row to the contributor list, click the **New Contributor** icon, .
- To edit a row in the contributor list, double-click the row or select the row and click the **Edit Selected Contributor** icon, . Then, make the appropriate edits in the Selected Contributor view.

Figure 97. Selected Contributor view



- To delete a row in the contributor list, select the row and click the **Delete Contributor from List** icon, .

Curator command processor pane

The command processor pane of the Curator window includes a toolbar and a table of twelve action steps that you can apply to the selected spectral tree.

For information about each action step, see "Curator action steps" on page 206.

For information about the toolbars, shortcut menus, and icons, see these topics:

- Curator action step pane toolbar
- Toolbar for each curator action step
- Curator action step pane shortcut menu
- Action step status

Curator action step pane toolbar

This is the toolbar for the action step pane that includes 12 action steps.

Table 100. Curator – Command Processor pane toolbar descriptions (Sheet 1 of 2)

Tooltip	Icon	Description
Load actions from a file		Loads the actions from a .curator_act file.
Save actions to a file		Saves the actions to a .curator_act file.

Table 100. Curator – Command Processor pane toolbar descriptions (Sheet 2 of 2)

Tooltip	Icon	Description
Apply next action step to compound		Runs the next step in the curation process. Use this mode to manually perform each step and apply manual threshold filtering and peak editing in the Filtered tree.
Apply all the remaining steps in the semi-auto mode		(Recommended) Runs all the remaining action steps while allowing the user to set specific parameter settings during the curation process. Use this mode to be prompted to review and set parameter settings for each selected step in the curation process.
Apply all the remaining steps in the auto mode		Runs the remaining action steps in the curation process automatically using previously set parameter settings. Use this mode to automatically run through the curation process with the currently defined settings.
Restart post-processing workflow with actual settings		Restarts the curation process and parameters for each action step.
Restart post-processing workflow with default commands		Restarts the curation process and reverts the parameters settings to the default state.
Change parameters of the selected action		Opens the parameter settings window for the selected action step. You can open multiple parameter settings windows by using this icon.
Turn off/on the selected action		Turns off/on the selected action.
Remove the selected action		This icon appears when you apply any of these commands from the shortcut menu for a spectral tree: Delete Spectra, Delete Selected Spectrum, or Delete Node. When you apply any of these commands, the command appears in the action step list. Click this icon to undo the action step.

Toolbar for each curator action step

The following toolbar is located in the upper right of each action step dialog box.

Table 101. Common action step toolbar

Icon	Action
Load From	Opens a dialog box for selecting a parameter settings file for the current action step.
Save As	Saves the parameter settings to a curator file. The file extension is specific to the action step. See "Supported file formats in Curator" on page 8.
Reset to Default	Resets the parameter settings to their factory default values.

Curator action step pane shortcut menu

To select additional processing modes, right-click the action step in the action step pane of the Curator window.

Table 102. Shortcut menu for the Curator actions steps

Command	Description
Run Semi-Auto until the selected action	Runs Semi-Auto tree processing until the selected action step.
Run Auto until the selected action	Runs Auto tree processing until the selected action step.
Turn off/on the selected action	Turns the selected action off or on.

Action step status

Table 103 describes the possible states of the action steps.

Table 103. Action step status indicator

Status indicator	Meaning
	The action step is unprocessed.
	The action step successfully completed.

Table 103. Action step status indicator

Status indicator	Meaning
	Changes to the parameters settings for this action step have not been applied.
	The action step failed.
	You have changed the parameter settings for an action step that was already successfully completed, invalidating the action step. Restart the whole curation process.

Curator action steps

Use the curation wizard in the lower left of the Curator window to curate a component's spectral tree.

The curation wizard includes twelve action steps (listed in order):

1. Raw spectra exclusion
2. Copy to filtered tree
3. Select significant spectra
4. Remove resonance peaks
5. Select relevant peaks
6. Node item color showing saturated shades of red
7. Apply changes to raw tree
8. Assign fragments (raw, filtered)
9. Copy to recalibrated tree
10. Recalibrate
11. Assign fragments (recalibrated)
12. Assign molecular formulas

Raw spectra exclusion

Curation action step 1, Raw Spectra Exclusion, removes non-supported types of spectra from a raw tree.

Figure 98. Raw Spectra Exclusion dialog box for action step 1

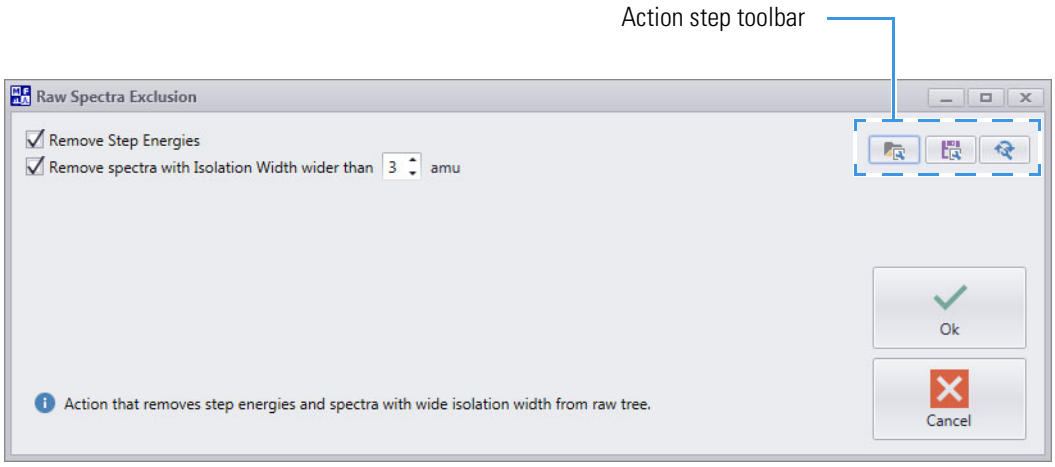
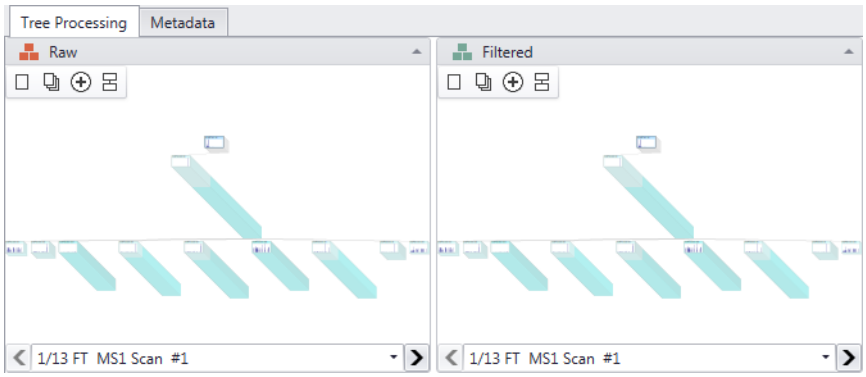


Table 104. Raw Spectra Exclusion step parameter descriptions

Parameter	Description
Remove Step Energies	Removes scans with step energies.
Remove Spectra with Isolation Width Wider Than	Removes scans that have an isolation width wider than the set value.

Copy to filtered tree

Curation action step 2, Copy to Filtered Tree, creates a copy of the raw tree from the Raw Spectra Exclusion step and adds this copy to the Filtered view of the Tree group on the Tree Processing page of the Curator window.



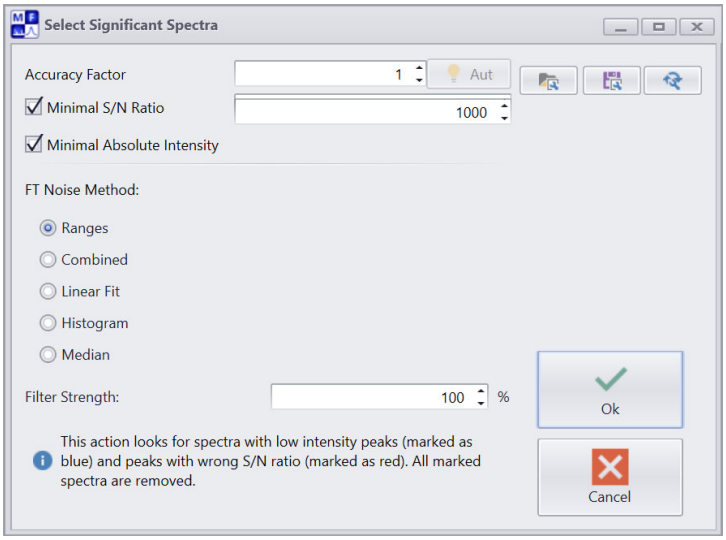
Select significant spectra

Curation action step 3, Select Significant Spectra, looks for and removes scans that contain only low-intensity peaks below the specified intensity threshold or only peaks below the specified signal-to-noise ratio.

❖ To remove spectra below a specified S/N ratio or intensity level

1. In the Select Significant Spectra dialog box, specify the parameter settings.

   Figure 99. Select Significant Spectra dialog box for action step 3



2. Click **Preview**.
3. Review the Raw tree view and the Filtered tree view on the Curator page.

   On the Tree Processing page, a red or blue border indicates that a spectrum meets the removal criteria:

   - A blue border indicates that the spectrum's base peak is below the specified minimum intensity level.

   - A red border indicates a that the signal-to-noise level for the spectrum is below the specified threshold.

Table 105. Select Significant Spectra parameter descriptions (Sheet 1 of 3)

Parameter	Description
Accuracy Factor	Specifies the factor applied to each peak in a spectra. Default: 1 (means no correction).
Minimal S/N Ratio	Specifies the minimum signal-to-noise ratio for every spectrum.
Minimal Absolute Intensity	Specifies the minimum absolute intensity of the highest peak in each spectrum.

Table 105. Select Significant Spectra parameter descriptions (Sheet 2 of 3)

Parameter	Description
FT Noise Method	Select one of these options: Ranges, Combined, Linear Fit, Histogram, or Median.
Ranges	A histogram is created from abundances for peaks outside of the combinatorially possible elemental compositions common for small organic molecules. The smoothed maximum of such peaks is then considered as the threshold value. Applies only for spectra having more than 5 peaks.
Combined	Calculates the Linear Fit and Histogram thresholds. Uses the lower of these two values as the threshold value.
Linear Fit	The application determines the threshold noise value by applying a linear fit as follows: 1. Applies intensity = a + b × (*m/z*) to a scan, and then applies the test for outlier data (using Student's t-distribution) to each scan peak. 2. Considers outlier peaks as signals and excludes them from noise. 3. In iterative form, applies a linear regression and exclusion analysis of outlier peaks (signal) to each considered peak while finding no new outlier points. 4. Determines a threshold intensity value as the intensity of the most intense noise peak (the highest peak that is not excluded from noise).
Histogram	A histogram is created from abundances of all peaks in analyzed scans, and then each histogram is smoothed and the end of the smoothed trace or the minimum between two trace peaks is considered as the threshold value.

Table 105. Select Significant Spectra parameter descriptions (Sheet 3 of 3)

Parameter	Description
Median	The median filter is applied to an analyzed scan. The maximal filtered abundance is used as the threshold value.
Filter Strength	For these methods —Ranges, Combined, Linear Fit, or Histogram—the application multiplies the calculated threshold value by the Filter Strength setting. For the Median method, the application uses the Filter Strength setting to calculate the filter length. A soft value can result in more individual *m/z* dimensions and a strong value can result in merging more ions into one *m/z* value.

Remove resonance peaks

Curation action step 4, Remove Resonance Peaks, identifies and removes resonance peaks from each spectrum in the filtered tree. Resonance peaks are residual peaks from the Fourier Transform (FT) or Orbitrap analyzer. See "Resonance peaks" on page 404.

Figure 100. Remove Resonance Peaks dialog box for action step 4

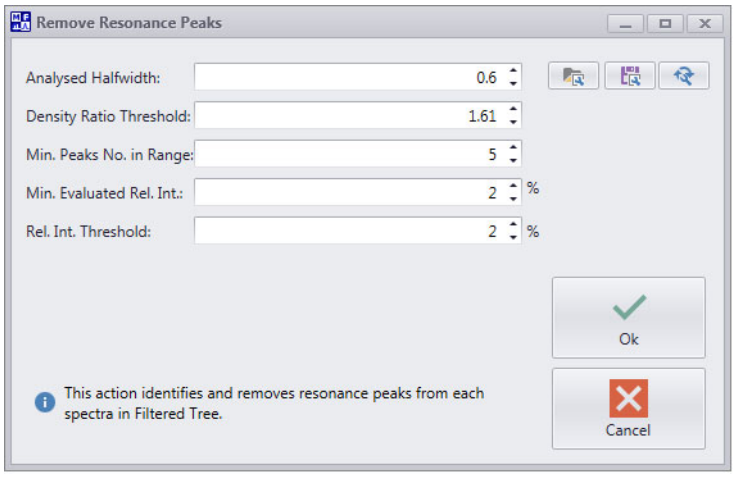


Table 106. Remove Resonance Peaks parameter descriptions

Parameter	Description
Analyzed Half Width	± *m/z* area used in detecting resonance. Default: 0.6
Density Ratio Threshold	A resonance peak is detected if the signal density in the vicinity of the analyzed peak is higher than the average peak density in the spectrum multiplied by the Density Ratio Threshold. Default: 1.61
Min. Peaks No. in Range	Specifies the minimum number of peaks in the validated peak vicinity. Default: 5 peaks
Min. Evaluated Rel. Int.	Specifies the minimum relative intensity of a valid peak. Default: 2%
Rel. Int. Threshold	Specifies the relative intensity threshold for Resonance Peaks (relative to validated peak, not spectrum). To be considered a resonance peak, the intensity of the peak must be less than this parameter setting. Default: 2%

Select relevant peaks

Curation action step 5, Select Relevant Peaks, uses a set of mathematical methods to generate all the possible theoretical *m/z* values for the component's structure. If a spectral peak does not match any of these values, this step deletes it from the spectrum.

During the curation process, the application automatically analyzes the spectral tree and selects suitable adduct ions. You do not need to click Auto Suggest unless you change other parameter settings. Clicking Advanced displays the advanced view with additional parameters.

Figure 101 shows the auto suggested MS1 adducts for the component detected in the Aceclofenac B02.raw file provided in the application's Demo folder.

Figure 101. Select Relevant Peaks step (with auto detected adducts ions for the specific tree)

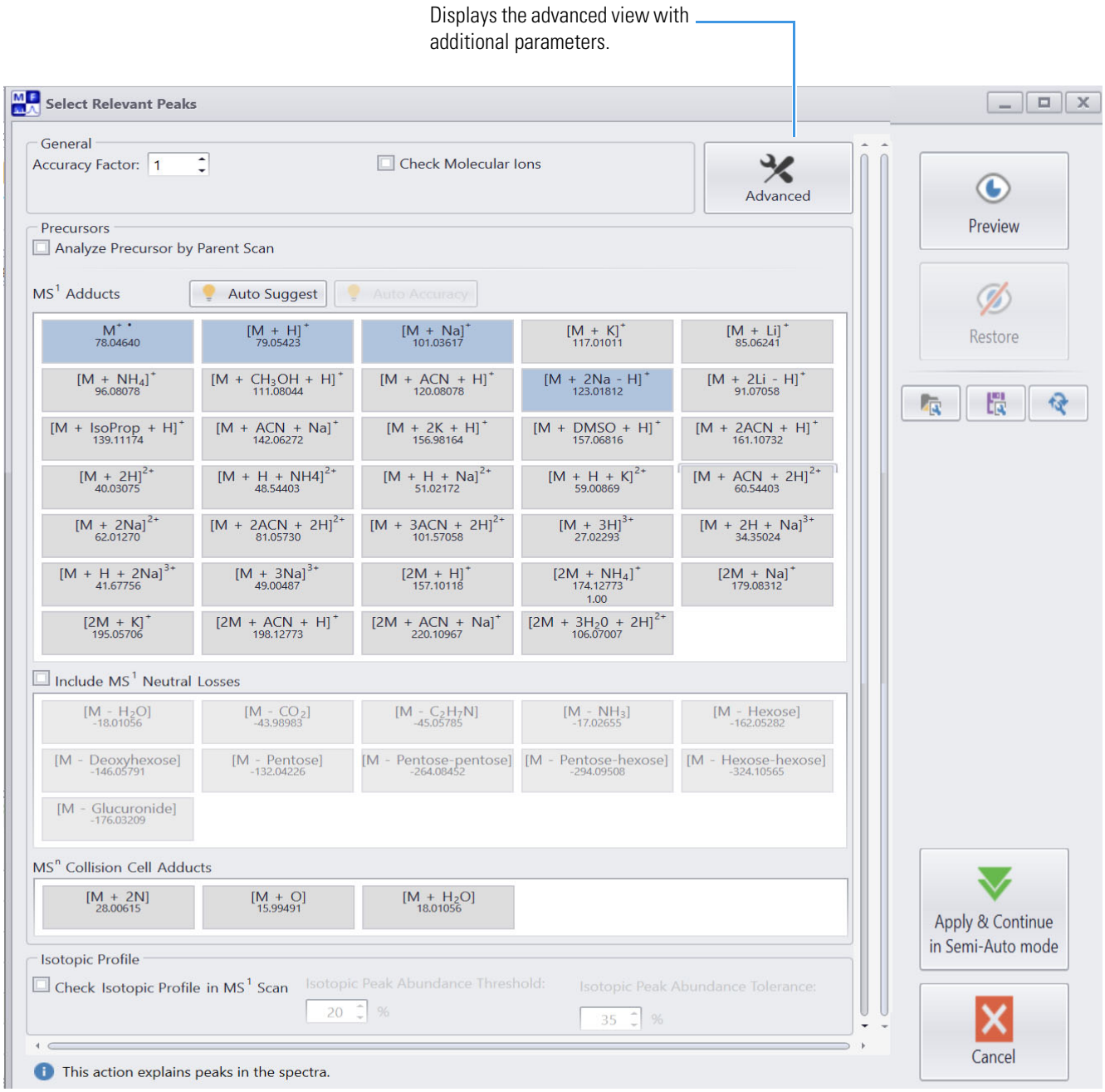


Table 107 describes the parameter settings in the Select Relevant Peaks dialog box.

Table 108 describes the color-coding of spectral peaks.

Table 109 describes the color-coding of the node items in the spectral tree.

Table 107. Select Relevant Peaks parameter descriptions (Sheet 1 of 4)

Parameter	Description
General (Basic)	
Accuracy Factor	Modifies the accuracy specified in the raw file. Specifies a mass window for each observed peak for comparing the peak's *m/z* value to the possible *m/z* values for the component's adduct ions. If the accuracy factor is too low, the mass window might be too narrow, causing the step to remove relevant peaks. If the accuracy factor is too high, the mass window might be too wide, allowing irrelevant peaks or multiple formulas for one peak. The presence of irrelevant peaks or multiple formulas for each peak can affect the recalibration step. Default: 1.0 (no correction); range: 1.0 to 3.0
Check Molecular Ions	Checks whether the MS1 spectra include the molecular ion. If an MS1 spectrum does not include an explained peak for a molecular ion, the curation process deletes the spectrum. Default: Selected
General (Advanced)	
Show Threshold Warnings	Shows a specific color (default is orange) that indicates unexplained peaks whose intensity is higher than the specified value. Based on the number of such peaks, the background color of the spectrum appears in saturated shades of red. Default: Selected and set to 50%
Remove Noise spectra	Removes spectra that do not contain an explained peak above the noise level calculated in the Select Significant Spectra step. Default: Selected
Precursors (General)	
Analyze Precursor by Parent Scan	Only use the precursors that exist in the parent scan. When this check box is clear, the application merges the precursors from parallel spectra into one parent node. Default: Clear
Precursors (Advanced)	
Minimal Intensity of MS^1 Adduct	Specifies the minimum relative intensity of each adduct ion in the MS1 spectrum. Default: 1%

Table 107. Select Relevant Peaks parameter descriptions (Sheet 2 of 4)

Parameter	Description
Precursor selections	
Auto Suggest (button)	Automatically analyzes the component's tree to select suitable molecular ions (MS1 adducts and MS1 neutral losses) and estimates the accuracy factor. To apply the neutral loss suggestions, you must select the Include MS1 Neutral Losses check box. When you run the wizard, the Select Relevant Peaks step automatically runs the auto suggest analysis. If you change the selections in the MS1 Adducts and MS1 Neutral Losses areas, click **Auto Suggest** to revert to the automatic selections.
Auto Accuracy (button)	This button is inactive if there are no fragments generated in advance. If the fragments are generated, then this button becomes active. This function analyzes the presence of in-silico generated fragments in MS1 spectra. If option "Check Isotopic profile for MS1" is selected, the match of the isotopic profile is also analyzed.
MS1 Adducts table	List of possible adducts that can appear in the MS1 spectrum with the calculated *m/z* values. Click the adduct icons to select them.
Include MS1 Neutral Losses check box and table	Includes/excludes possible neutral losses that can appear in the MS1 spectrum. Default: Clear
MSn Collision Cell Adducts table	Includes/excludes possible adducts formed in the collision cell that can appear in the MSn spectra. Default: Clear
Isotope Profile (General)	
Check Isotopic Profile in MS1 Scan	Checks whether the MS1 spectrum contains the full isotope pattern for the molecular ion if the theoretical intensity of the isotopic peaks is above a predetermined threshold. If the isotope profile for the molecular ion does not meet the criteria for the presence of isotopic ions, the molecular ion is not explained and is later deleted. If no molecular ion is explained in a spectrum, the whole spectrum is deleted. Default: Clear

Table 107. Select Relevant Peaks parameter descriptions (Sheet 3 of 4)

Parameter	Description
Isotopic Peak Abundance Threshold	Specifies the theoretical relative intensity for an isotopic peak below which the application can ignore it when searching for an expected isotope pattern in the spectrum. If the expected abundance for an isotopic peak is above this threshold, the isotopic peak must be present in the spectrum to meet this criterion for the isotope profile. Available when the Check Isotopic Profile in MS1 Scan check box is selected. Default: 20%
Isotopic Peak Abundance Tolerance	Specifies the relative allowed difference in abundance between the theoretical and observed abundances of the isotope peaks. If the relative abundance of an isotopic peak is outside this tolerance, the isotope profile does not meet this criterion for the isotope profile. Available when the Check Isotopic Profile in MS1 Scan check box is selected. Default: 35%
Isotope Profile (Advanced)	
Check Isotopic Profile in MSn Scan	Checks if other isotopes exist in the spectrum. Considers the product spectra isolation widths of all the precursors. If the *m/z* values of the theoretical isotopic peaks do not fall within the experimental isolation width, the application ignores this criterion. The application checks the relative abundance of observed isotopic peaks above the specified abundance threshold for the theoretical isotope pattern. If the isotope profile for the molecular ion does not meet the criteria for the presence of isotopic ions, the molecular ion is not explained and is later deleted. If no molecular ion is explained in a spectrum, the whole spectrum is deleted. Default: Clear

Table 107. Select Relevant Peaks parameter descriptions (Sheet 4 of 4)

Parameter	Description
Ignore MS^n Isotopes	In the MSn spectra, analyzes only the most intense peak in the isotope pattern and ignores the other peaks in the pattern. Default: Clear
Formula Generator (Advanced)	
Check Graph Rule	Specifies whether to exclude formulas if the atoms cannot connect in any way using valences common in organic chemistry. Default: Selected
Check Hydrogen Rule	Specifies whether to use the hydrogen rule for the exclusion of implausible formulas with improbably high numbers of hydrogens. Default: Selected
Check RDBE	Specifies the formulas for which RDBE is within the From-To range. RDBE limits the calculated formulas to those that make sense from a chemical perspective. Default: Selected; default range: –2 to 250
Nitrogen Rule	Specifies whether to use the nitrogen rule in the elemental composition calculation. Selections: • Do not use • Even-electron ion (for example, radical-cation) • Odd-electron ion (for example, protonated) Default: Not Used
Probability Approach and Threshold	Use only formulas where the combination of elements corresponds to the combination of elements common in nature. Considers the combination of carbon versus hydrogen and carbon versus common heteroatoms. A higher threshold percentage eliminates more formulas. Default: Selected; threshold: 80%

Figure 102 shows a color-coded MS1 spectrum for the Select Relevant Peaks action step.

Figure 102. Filtered spectrum for the aceclofenac component in the demo data

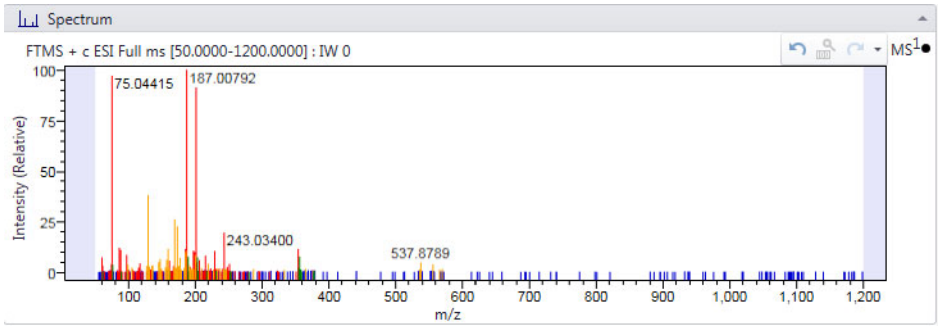


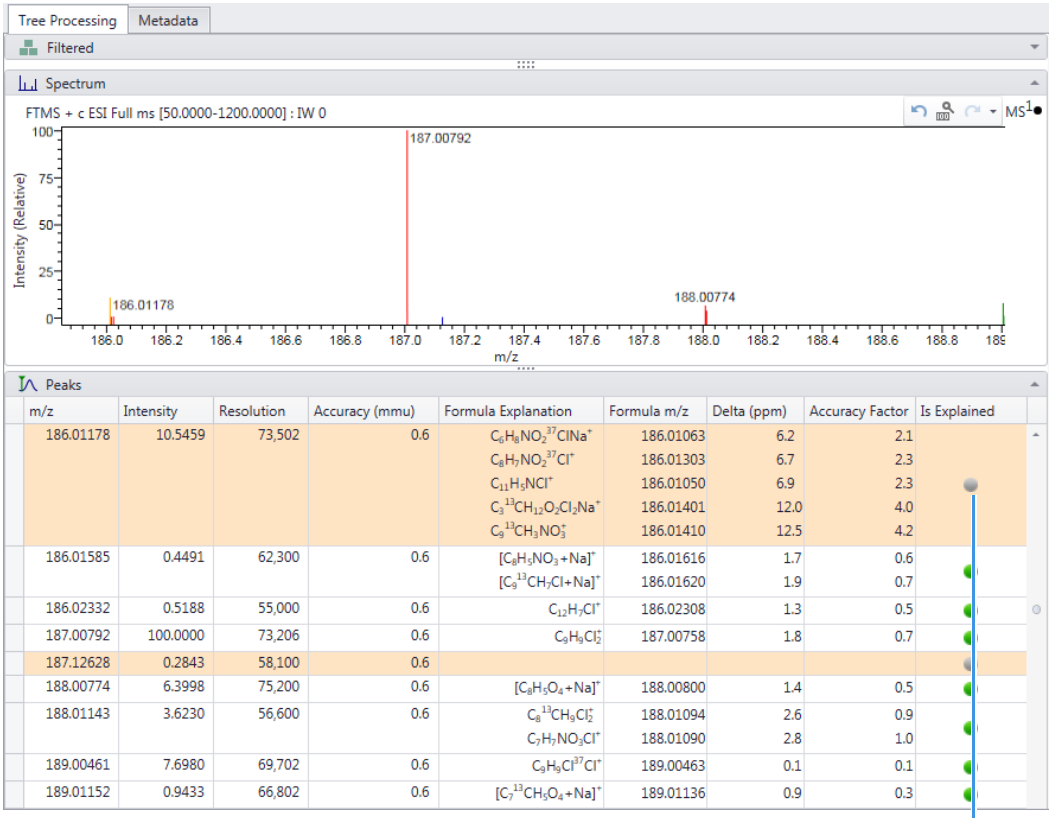
Table 108. Color-coded spectral peaks

Color	Meaning
Red	The peak has a formula explanation (explained).
Blue	The peak has no formula explanation (unexplained).
Orange	The peak is unexplained and has a relative intensity above the value specified for Show Threshold Warnings.
Green	The peak has a formula explanation that includes at least one isotope.

Figure 103 shows the 186 to 189 *m/z* range with color-coded peaks:

- The orange peak at *m/z* 186.01178 is unexplained and has a higher relative intensity (10.55%) than the specified threshold. None of the formula explanations for this peak fall within the specified mass tolerance.
- The red peak at *m/z* 187.00792 has one formula explanation.
- The blue peak at 187.12628 is unexplained and has a lower relative intensity (0.28%) than the specified threshold.
- The green peak at *m/z* 189.00461 has a formula explanation with one ^{13}C atom.

Figure 103. *m/z* range from 186 to 189 for the filtered MS1 spectrum of aceclofenac with default accuracy factor (with corresponding Peaks table)



(Optional) Do the following to explain the orange or blue peaks from the peaks table

- Select the orange or blue peaks form the peaks table with suggested formula explanation and accuracy factor. For instance in the Figure 103 on page 218, the orange peak with *m/z* value 186.01178 has formula explanations and accuracy factor of 2.1.
- Open the Select Relevant Peaks dialog box and enter the suggested accuracy factor (2.1) for the orange or blue peak and then click **Preview.**

  The change in accuracy factor to the suggested value will make the blue or orange peaks to be explained and then the color will turn to red. No shades only 4 color can have peaks (blue, orange, green and red).

Figure 104. *m/z* 186.01178 peak (red) explained with accuracy factor 2.1 in aceclofenac.

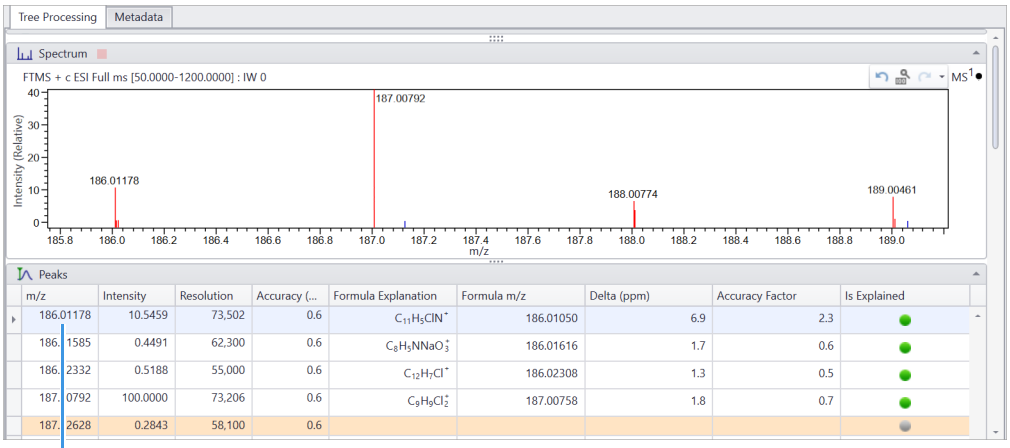
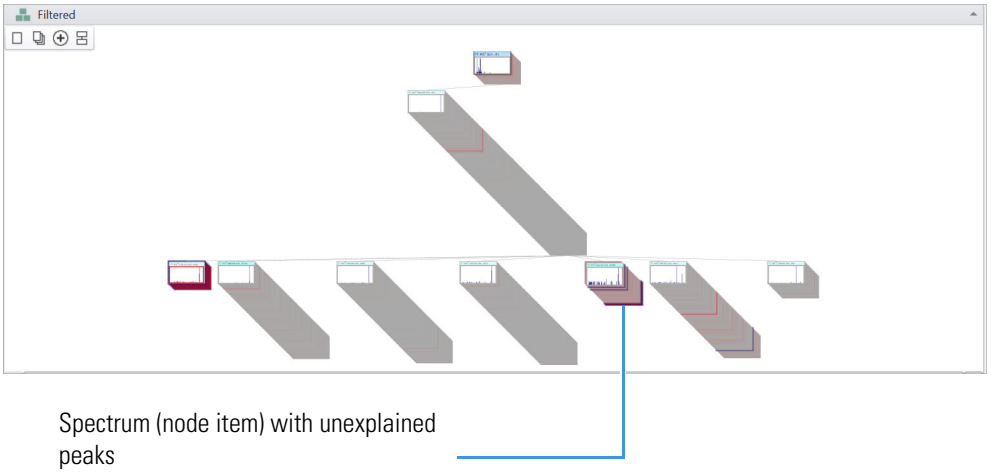


Table 109. Node item color

Color	Meaning
Saturated shades of red	The spectrum contains unexplained peaks. When a spectrum (node item) contains a number of unexplained peaks with a relatively high-intensity, the spectrum's border turns a shade of red. As the number of unexplained peaks increases, the saturation of the red color increases.
Blue	The peaks in the spectrum do not meet the condition of the Remove Noise Spectra parameter. The spectrum is deleted.

Figure 105. Node item color showing saturated shades of red



Merge replicate spectra

Curation action step 6, Merge Replicate Spectra, merges replicate spectra in the filtered tree with the same experimental conditions (mass range, mass analyzer, ion activation type and energy, and isolation width) into one spectrum. It combines replicate spectra to create a single averaged spectrum. During merging, intensities of the same peaks (same *m/z* value within the mass tolerance) are additive.

Figure 106. Merge Replicate Spectra action step

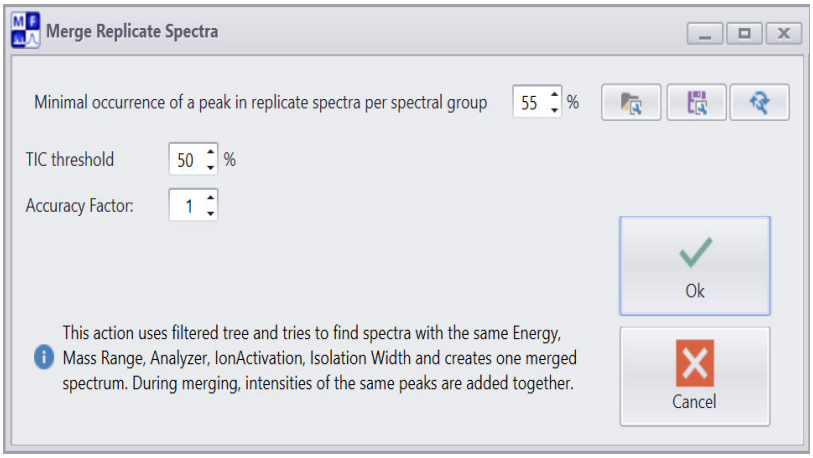


Table 110. Merge Replicate Spectra parameter description

Parameter	Description
TIC threshold	Specifies threshold for the mass spectral peak intensities. Default: 50%
Accuracy Factor	Specifies the factor applied to each peak in a spectra. Default: 1 (means no correction).
Minimal occurrence of a peak in replicate spectra per spectral group	Minimum occurrence of the same peak in replicate spectra (in %). Default: 55%

Apply changes to raw tree

Curation action step 7, Apply Changes to Raw Tree, removes the irrelevant spectra in the raw tree.

Assign fragments (raw, filtered)

Curation action step 8, Assign Fragments (Raw, Filtered), lets you to import previously calculated compound fragments and assigns them to peaks in the Raw and Filtered Tree. You can import fragments from an SDF file or from the SledgeHammer module.

Note During a semi-auto or auto curation process, the application pauses at this step and waits for you to import a set of fragments.

Figure 107. Assign Fragments (Raw, Filtered) step

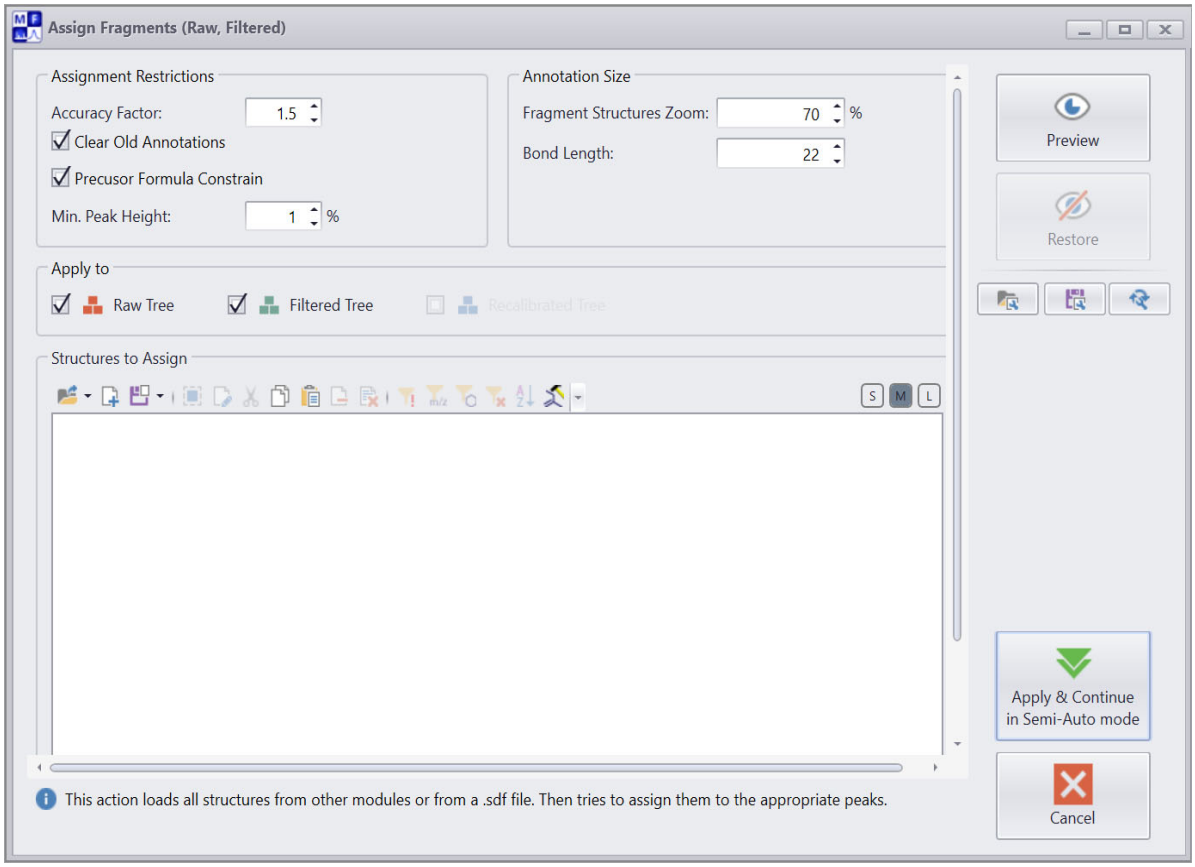


Table 111. Assign Fragments (Raw, Filtered) parameter descriptions (Sheet 1 of 3)

Parameter	Description
Assignment Restrictions	
Clear Old Annotations	Clears existing annotations in the spectral tree. Default: Selected
Precursor Formula Constrain	All annotations in a spectrum are subformulas of its precursor structure. Default: Selected

Table 111. Assign Fragments (Raw, Filtered) parameter descriptions (Sheet 2 of 3)

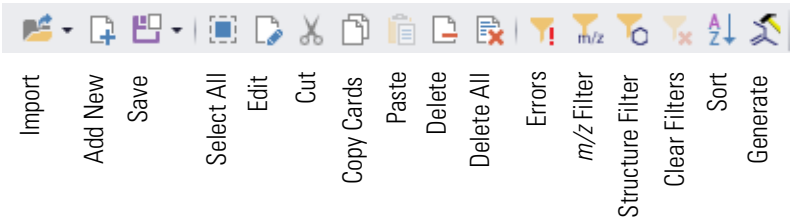
Parameter	Description
Min. Peak Height	Specifies the minimum relative intensity of an annotated peak. Default: 1%
Annotation Size	
Fragment Structures Zoom	Specifies the relative size of the fragment structure annotations in the Spectrum panes on the Tree Processing page of the Curator window. Default: 70%
Bond Length	Specifies the bond length of the fragment structure annotations.
Apply to	
Raw Tree	Applies parameters to the raw tree.
Filtered Tree	Applies parameters to the filtered tree.
Recalibrated Tree	Applies parameters to the recalibrated tree. Applies only when annotating the recalibrated tree.
Structures to Assign	
	
Import > Import File	Imports fragments from an SDF file, or from the SledgeHammer module.
Add New	Adds new card where a new structure you can draw.
Save > Save Selection	Saves selected structure to a MOL file.
Save > Save All	Saves all structures to an SDF file.
Select All	Selects all visible cards.
Edit	Opens the Structure Editor where you can edit the structure from the selected card.
Cut	Removes selected card.
Copy Cards	Copies one or more structures from the selected cards to the clipboard.
Paste	Pastes structure from the clipboard.

Table 111. Assign Fragments (Raw, Filtered) parameter descriptions (Sheet 3 of 3)

Parameter	Description
Delete	Deletes selected card.
Delete All	Deletes all cards.
Errors	Shows only structures with errors.
m/z Filter	Shows structures with the defined *m/z* value.
Structure Filter	Shows structures with the specified structural pattern.
Clear Filters	Clears all the filters.
Sort	Sorts the structures by their *m/z* values.
Generate	Generates fragments for the selected structure card.

Copy to recalibrated tree

Curation action step 9, Copy to recalibrated Tree, creates a copy of the filtered tree in the recalibrated tree pane on the Tree Processing page of the Curator window.

Recalibrate

Curation action step 8, Recalibrate, improves the mass accuracy for each peak by using a calibration curve to recalibrate each peak's *m/z* value. The calibration curve is built from the molecular formula annotations and structure annotations assigned to the peaks. This step does not include peaks with more than one formula annotation in calculating the calibration curve.

Figure 108 shows the calculated calibration curve for the MS1 spectrum of aceclofenac—the compound used in the *Mass Frontier 8.1 tutorial to Curate Spectral Trees for Spectral Libraries* . By default, the dialog box displays the Calibration page. To view the recalibrated peaks in a table format, click the Table tab.

The calibration curve indicates the best fit of the selected calibration model across the annotated peaks in the spectrum.

Figure 108. Recalibrate dialog box for action step 10

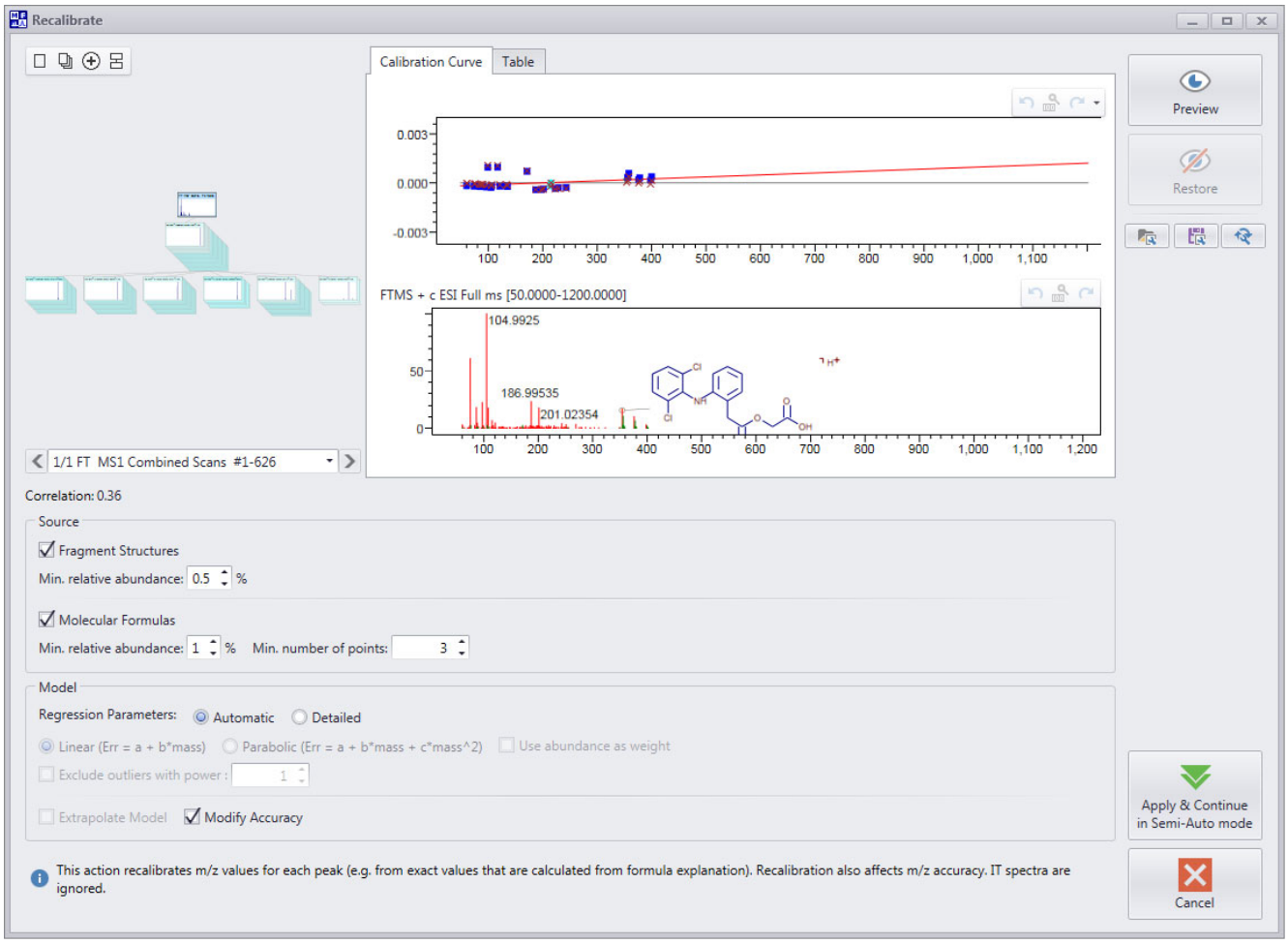


Figure 109 shows the Table page with the calibration data for the peaks in the MS1 spectrum of aceclofenac.

Figure 109. Table page of the Recalibrate dialog box

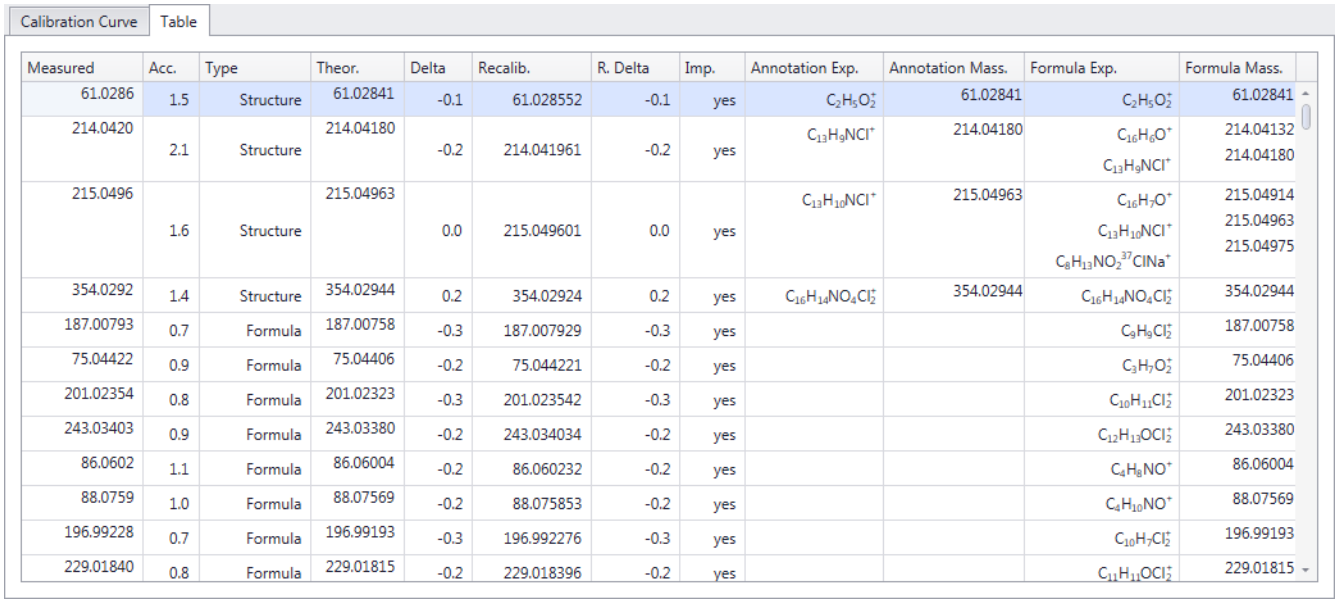


Table 112. Recalibrate action step parameter descriptions (Sheet 1 of 3)

Parameter	Description
Source	
Fragment Structures	Specifies whether to consider peaks with fragment annotations in the calculation of the calibration curve.
Min. relative abundance	Specifies the minimum relative abundance of peaks with fragment annotations that are used to calculate the calibration curve.
Molecular Formulas	Specifies whether to consider peaks with formula annotations in the calculation of calibration curve.
Min. relative abundance	Specifies the minimum relative abundance of peaks with formula annotations that are used to calculate the calibration curve.
Min. number of points	Specifies the minimum required number of calibration curve points (annotated peaks)

Table 112. Recalibrate action step parameter descriptions (Sheet 2 of 3)

Parameter	Description
Model	
Regression Parameters	Determine how the regression parameters are determined: Automatic: Uses a linear regression fit that minimizes the difference between the recalibrated and theoretical m/z value for each peak, weights each peak by its abundance, excludes outliers, and does not recalibrate peaks outside the range of the calculated calibration curve. Detailed: Applies the user defined parameter settings to all the spectra in the spectral tree. The Detailed option lets you select the curve fit: Linear or Parabolic. It also lets you specify whether to weight the data points by their abundance, exclude outliers, and extrapolate the calibration model to peaks outside the range of the calibration points.
Linear	Specifies a linear regression fit for the calibration curve.
Parabolic	Specifies a parabolic regression fit for the calibration curve. Available when the Detailed option is selected.
Use Abundance As Weight	Specifies whether to weight the calibration points by the abundances of the peaks. Available when the Detailed option is selected.
Exclude Outliers with Power	Analyzes outliers and excludes them from the calibration model. Available when the Detailed option is selected.

Table 112. Recalibrate action step parameter descriptions (Sheet 3 of 3)

Parameter	Description
Extrapolate Model	Applies the calibration model even to peaks outside the range of the calibration points. Available when the Detailed option is selected.
Modify Accuracy	1. Calculates the *m/z* values for all the formulas and fragments that are connected with the recalibrated centroid peaks (difference between *m/z* of peak and formula or between *m/z* of peak and fragment is within used tolerance). 2. Calculates the *m/z* differences between the observed peak and its annotations (Abs (*m/z* of centroid peak minus *m/z* of formula); Abs (*m/z* of centroid peak minus *m/z* of fragment)) 3. Assigns the largest difference as the accuracy of the recalibrated centroid peak.

Assign fragments (recalibrated)

Curator action step 11, Assign Fragments (Recalibrated) assigns the previously calculated fragments for the compound to the spectral peaks in the recalibrated tree.

For information about the parameter settings, see "Assign fragments (raw, filtered)" on page 221.

Assign molecular formulas

Curator action step 12, Assign Molecular Formulas, assigns appropriate molecular formulas to each peak in the tree. It uses the peak explanations from the Select Relevant Peaks action step.

Figure 110. Assign Molecular Formulas dialog box for action step 12

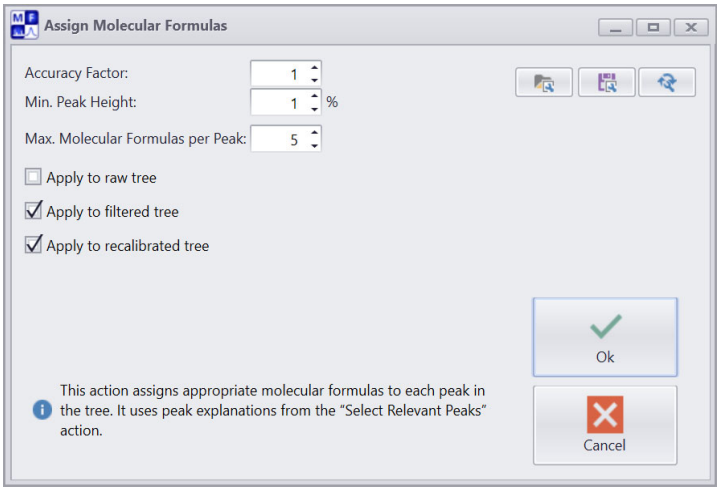
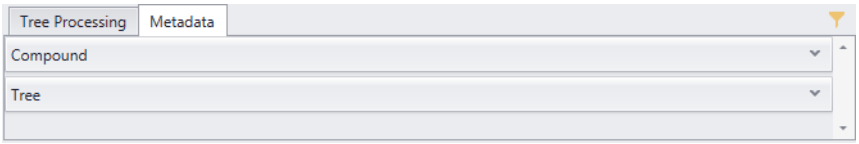


Table 113. Assign Molecular Formulas step parameter descriptions

Parameter	Description
Accuracy Factor	Specifies the factor applied to each peak in a spectra. Default: 1 (means no correction).
Minimum Peak Height	Specifies the minimum height for a peak to be annotated.
Max. Molecular Formulas per Peak	Specifies the maximum number of molecular formula annotations per peak. Default: 5
Apply to Raw Tree	Specifies whether to annotate the Raw tree. Default: Clear
Apply to Filtered Tree	Specifies whether to annotate the Filtered tree. Default: Selected
Apply to Recalibrated Tree	Specifies whether to annotate the Recalibrated tree. Default: Selected

Curator – Spectral pane – Metadata page

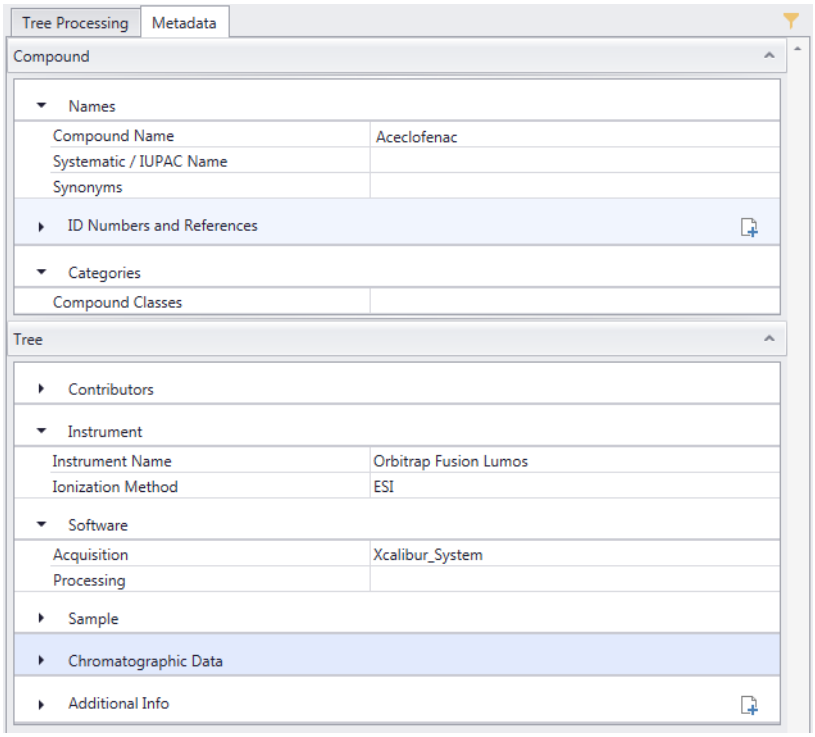
Use the Metadata page of the Curator window to add metadata to a compound. The Metadata page has two collapsible groups: Compound and Tree.



The Compound group contains Names, Synonyms or Identifiers, which can be downloaded automatically from selected data sources or entered manually.

The Tree group shows additional information extracted from the data file. Each file format contains a different list of items that describe the sample, instrument, experimental conditions, data acquisition software, and so on. The Tree Group can also contain information about the analyst who contributed the raw data, and the mass spectrometry specialist who curated the compound's spectral tree.

Figure 111. Metadata page of a Curator window



Download metadata for a compound

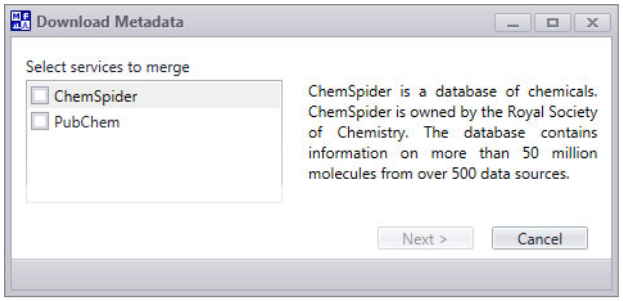
The Download Metadata option imports supplementary compound metadata from public data sources like ChemSpider and PubChem. The Search is based on the compound's structure and name.

❖ To download metadata from public data sources

1. In the Processing group of the Curator toolbar, click **Download Metadata**.

   The Download Metadata wizard opens.

   Figure 112. Download Metadata wizard

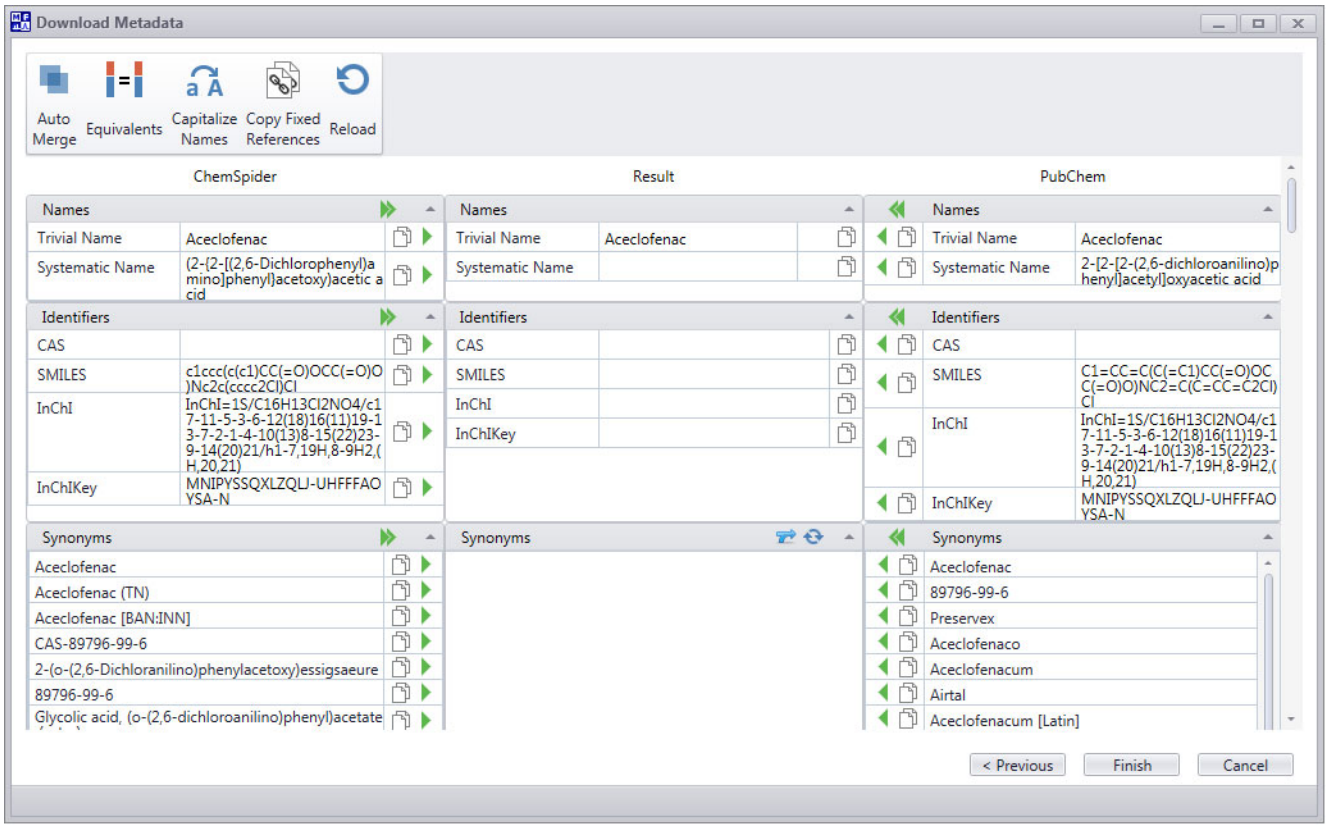
2. In the Download Metadata wizard, select the check boxes for the data sources of interest and click **Next**.

   A list of matching database entries appears.

3. In the list, select the entry of interest**,** and then click **Next.**

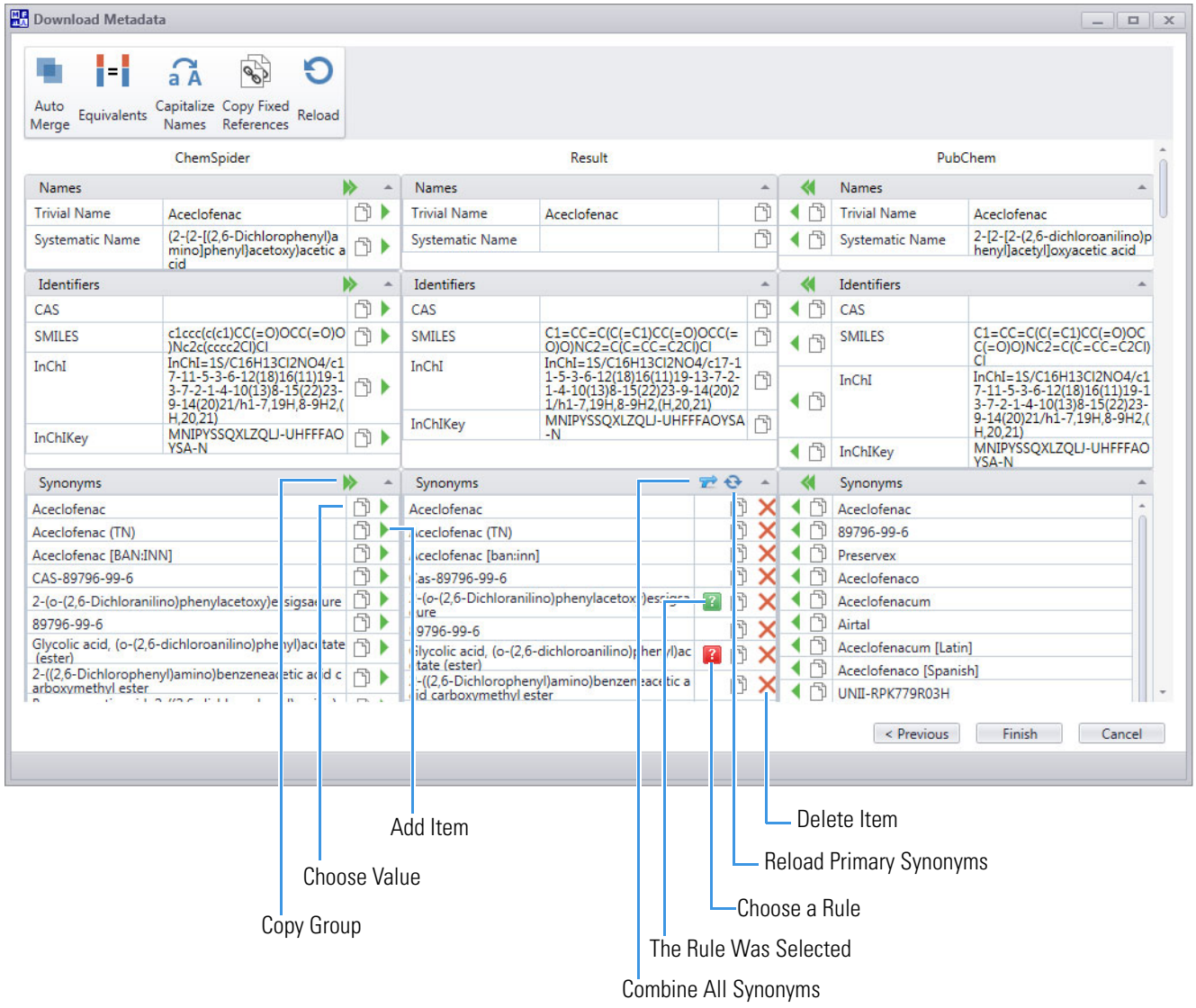
   The final page of the wizard appears.

Figure 113. Final page of the Download Metadata wizard



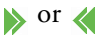
4. To copy an item from the *public source* column to the Result column, click the **Add Item** icon, .

Figure 114. Result column with added items



5. To import the metadata into your library entry, click **Finish**.
6. To skip searching for additional KEGG references, click **No** at the prompt.
7. To skip searching for additional Wikipedia references, click **No** at the prompt.
8. On the Curator page, click the **Metadata** tab to open the Metadata page and view the imported data.

Table 114. Download Metadata dialog box toolbar

Button or icon		Description
Auto Merge		Automatic merging. Finds the same hits in databases (names, identifiers, synonyms, references) and fills the Result column with them.
Equivalents		Finds similar synonyms in the Result column to avoid duplicates.
Capitalize Names		Capitalizes names and synonyms in the Result column.
Copy Fixed References		Copies default references from data sources in the Result column
Reload		Discards all changes and reverts the Result column to the initial state.
Add Item		Manually adds selected item from the data source to the Result column
Copy Group		Manually adds the whole group from the selected data source to the Result column
Combine All Synonyms		Fills the Result column with all the synonyms found in the data sources.
Reload Primary Synonyms		Discards all changes in the Result column of the Synonyms group.
Choose a rule		Opens window for choosing the rules of italicization according to IUPAC. Batch Change for All Names is possible.
The rule was selected		Rules of italicization according to IUPAC were selected.
Copy Value		Copies content of the selected row to the Clipboard.
Delete item		Deletes the selected row.

6

Metabolika Pathways module

The Metabolika module supports creating, visualizing, storing, exporting, and processing metabolic pathways. A Metabolika pathway drawing consists of structures interconnected by arrows, representing metabolic reactions or a series of reactions. You can send pathway structures individually or in a batch to other Mass Frontier modules. The other modules use the structures for data curation, substructure search, fragment generation, and so on. You can add and edit structures, connect them with various connector types, and annotate them with custom text. You can import structures from supported file types or draw them in the internal structure editor.

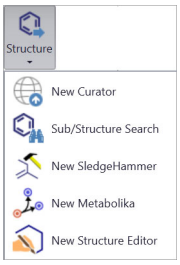
The supported file types for the Metabolika Pathways module are the Metabolika Drawing Format (.metabolikadrw) and the Metabolika Data Format (.metabolika).

Contents

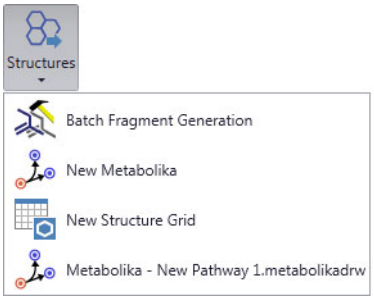
Use the Metabolika module with other modules

From the Metabolika window, you can work interactively with the other modules as follows:

- Send single structures to the Curator, SledgeHammer, Sub/Structure Search, or Structure Editor modules.



- Send multiple structures to the Batch Fragment Generation, Metabolika, and Structure Grid modules.



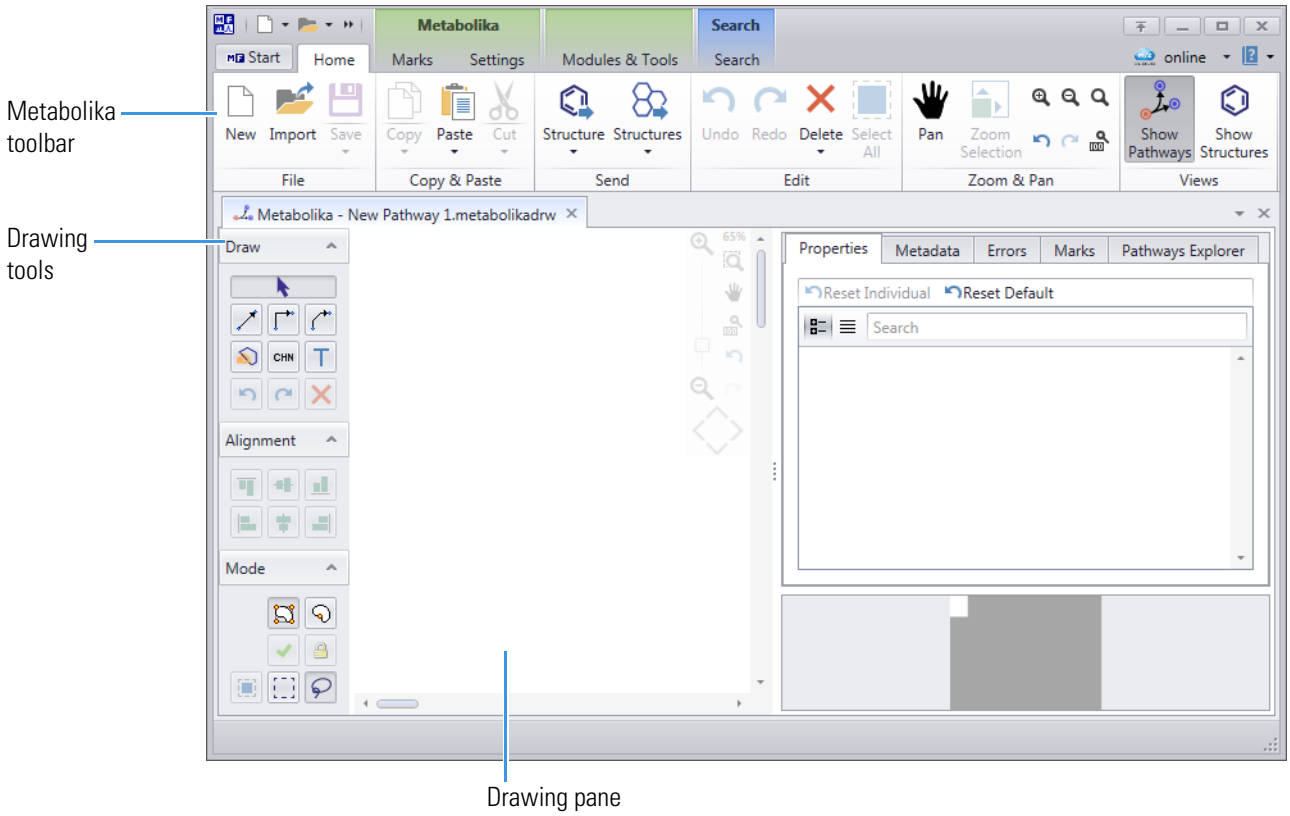
- Import external metabolika drawing files.
- Open structure files by clicking the Add/Edit Structure icon, , to open the Structure Editor, and then opening a structure file in the Structure Editor.

Open the Metabolika window

❖ **To open the Metabolika window**

From the Mass Frontier startup Window, under Modules & Tools, click **Metabolika**.

Figure 115. New Metabolika window



Metabolika Home toolbar

These topics describe the buttons and icons on the Metabolika Home toolbar:

- File group
- Copy & Paste group
- Send group
- Edit group
- Zoom & Pan group
- Views group

File group

Table 115. File group on the Metabolika toolbar

Button	Description
New Metabolika Pathway	Opens a new empty Metabolika document.
Import Pathway from File	Opens an existing Metabolika document (.metabolikadrw or .metabolika file format) from a specified location and adds the pathway to the drawing area of the active Metabolika window.
Save	Saves the open Metabolika document (.metabolikadrw or .metabolika file format), overwriting the last saved version.
Save As	Saves the open Metabolika document to a new file in a user-specified location.

Copy & Paste group

Table 116. Copy & Paste group on the Metabolika toolbar

Button	Description
Copy	Copies either the entire scheme in workspace (if nothing is selected) or the selected structure to the Clipboard.
Copy Scheme	Copies the entire scheme to the Clipboard.
Copy Selection	Copies the actual selection to the Clipboard.
Copy Structure	Copies the selected structure to the Clipboard.
Copy Style	Copies style of the selected element to the Clipboard.
Paste	Pastes the current content of the Clipboard to a scheme, if applicable.
Paste Scheme	Pastes a scheme previously copied to the Clipboard to a scheme.
Paste Structure	Pastes a structure previously copied to the Clipboard to a scheme.
Paste Bitmap	Pastes a bitmap image previously copied to the Clipboard to a scheme.
Paste Style	Pastes the style of selected elements to the style previously copied to the Clipboard.

Table 116. Copy & Paste group on the Metabolika toolbar

Button		Description
Cut		Deletes the selected elements of the scheme.
Cut Scheme		Deletes the entire scheme.

Send group

Table 117. Send group on the Metabolika toolbar

Command		Description
Send Structure > New Curator		Sends individually selected structure to a newly opened Curator module.
Send Structure > New SledgeHammer		Sends individually selected structure to a newly opened SledgeHammer module.
Send Structure > Sub/Structure Search		Performs Sub/Structure Search with the selected structure.
Send Structure > New Structure Editor		Sends individually selected structure to a newly opened Structure Editor.
Send Structures		Sends multiple selected structures to one of the two following modules: Batch Fragment Generation and Structure Grid.
Send Structures > Batch Fragment Generation		Sends multiple selected structures to Batch Fragment Generation.
Send Structures > New Structure Grid		Sends multiple selected structures to a newly opened Structure Grid document.

Edit group

Table 118. Edit group on the Metabolika toolbar (Sheet 1 of 2)

Button		Description
Undo		Reverts the last pathway modification.
Redo		Repeats the last pathway modification.
Delete > Delete Selection		Deletes the selected pathway part.

Table 118. Edit group on the Metabolika toolbar (Sheet 2 of 2)

Button		Description
Delete > Delete All		Deletes the entire pathway.
Select All		Selects the entire pathway.

Zoom & Pan group

Use these tools to zoom and pan in the drawing area of the Metabolika window and the Data Manager window – Mechanisms view.

Table 119. Zoom & Pan group on the Metabolika and Data Manager – Mechanisms toolbars

Button or icon		Description
Pan		Clicks and holds to move the entire pathway.
Zoom Selection		Zooms to select the elements of the pathway.
Zoom In		Zooms in the center of the pathway.
Zoom Out		Zooms out the center of the pathway.
Zoom To Area		Zooms the cursor position.
Zoom Undo		Reverts the last zoom action.
Zoom Redo		Repeats the last zoom action.
Zoom Reset		Resets the zoom level to 100% and move to the upper left corner of the pathway.

Views group

Table 120. Views group on the Metabolika toolbar

Button		Description
Show Pathways		Shows the entire pathway including the structure and connectors (reactions).
Show Structures		Displays only the structures from the pathway in a table form.

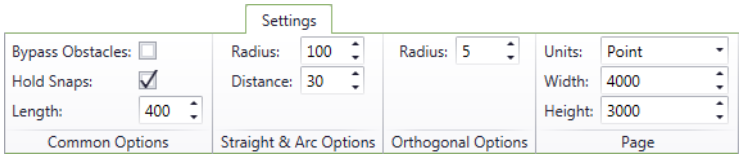
Metabolika Marks toolbar

Marks are a special form of highlighting/marking structures in Metabolika. The marks can have different shapes and colors. The marks are not saved to the file.

Table 121. Marks toolbar buttons

Button		Description
Mark Selection		Highlights (change the background of) the currently selected structures.
Mark Redundant Structures		Highlights the identical structures occurring multiple times in the pathway.
Mark Structures		Highlights the structures from a selected list; populates a list of imported structures in valid formats (.sdf, .mol, .mcs, or .tml) or from an existing pathway (.metabolika and .metabolikadrw).
Unmark Selection		Unmark the currently selected structures (change their background to default).
Unmark All		Unmark the structures (change their background to default).
Zoom to Selected Item		Zooms the view that the selected items in the Mark pane are visible. You can select multiple items in the Mark pane. You can access this action from the context menu on the Mark pane grid.
Highlight Selected Item		Highlights the selected items in the Mark pane as a green rectangle. You can select multiple items in the Mark pane. You can access this action from the context menu on the Mark pane grid.

Metabolika Settings toolbar



See these topics:

- Common Options group
- Straight & Arc Options group

Common Options group

Use the parameters in the Compound Options group of the Metabolika – Settings toolbar to modify the connector properties.

Table 122. Common Options group on the Settings toolbar

Setting	Description	Default value/setting
Bypass Obstacles	Bypasses colliding structures for straight/arc connector.	No (The check box is clear.)
Hold Snaps	Holds the fixed position of the snap-on connector.	Yes (The check box is selected.)
Length	Default connector's length.	400

Straight & Arc Options group

Table 123. Straight & Arc Options group on the Settings toolbar

Setting	Description	Default value/setting
Radius	Default radius of the arc	100
Distance	Default arc distance	30

Orthogonal Options group

Table 124. Orthogonal Options group on the Settings toolbar

Setting	Description	Default value/setting
Radius	Default radius of corners	5

Page group

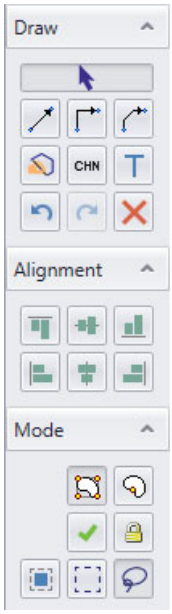
These settings affect the size of the drawing area.

Table 125. Page group on the Settings toolbar

Setting	Description	Default value/setting
Units	Units of page dimensions	Point
Width	Page width	4000
Height	Page height	3000

Metabolika drawing tools

The drawing tools are located on the left side of the drawing pane in a Metabolika window.



See these topics:

- Draw group
- Alignment group
- Mode group

Draw group

Use the icons in the Draw group to add structures, connectors, and formulas to the drawing pane.

Table 126. Draw group on the drawing tools toolbar (Sheet 1 of 2)

Icon	Description
	Returns the pointer to the base mode.
	Adds/converts the connector to a connector that has a line or arc shape.
	Adds/converts the connector to a connector that has an orthogonal polyline shape.
	Adds/converts the connector to a connector that has a polyline shape.
	Adds/edits structures.

Table 126. Draw group on the drawing tools toolbar (Sheet 2 of 2)

Icon	Description
	Adds/edits formulas.
	Reverts the last action.
	Repeats the last action.
	Deletes the selection

Alignment group

To align the selected items of the pathway, use the icons in the Alignment group. You must select one reaction arrow or two items (structure or text block) of the pathway to activate the icons.

Table 127. Metabolika alignment tools

Icon	Description
	Align to the top
	Align to the middle
	Align to the bottom
	Align to the left
	Align to the center
	Align to the right

Mode group

To change the selection mode, use the icons in the Mode group of the Metabolika Home toolbar.

Table 128. Metabolika selection mode tools (Sheet 1 of 2)

Icon	Description
	Snaps to boundary box. The start and end points of the reaction (snap points) are snapped as a rectangular box around the structure.
	Snaps to envelop. The start and end points of the reaction (snap points) are snapped as an envelope surrounding the bonds of the structure, not the boundary box.

Table 128. Metabolika selection mode tools (Sheet 2 of 2)

Icon	Description
	Verifies the scheme for errors such as elemental consistency, unconnected reactions or structures, or more unconnected pathways.
	Locks the relative position of connector's handle even if the Hold Snap check box is clear. This allows holding the relative position of the connector's handle for selected connectors independently from settings. This attribute is saved in the file and restored after reopening.
	Selects the elements of the pathway.
	Rectangular selection shape.
	Lasso selection; the arbitrary shape of the selection.

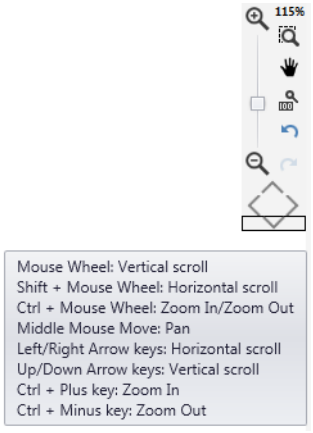
Metabolika Drawing and Viewing pane

See these topics:

- Zoom and pan tools
- Drawing pane shortcut menu

Zoom and pan tools

Use the zoom and pan tools in the upper right of the drawing pane in the Metabolika window to zoom and pan.



Drawing pane shortcut menu

Right-clicking the drawing pane in the Metabolika window opens a shortcut menu.

Table 129. Metabolika shortcut menu (Sheet 1 of 2)

Command	Description
Add Arrow	Adds an arrow to the drawing area.
Add Structure	Opens the Structure Editor dialog box for adding a structure.
Add Formula	Opens the Formula dialog box for adding a chemical formula.
Add Standard Text	Opens the plain text dialog box for adding formatted text.
Draw Straight Arrow	Activates the straight arrow pointer.
Merge Text and Arrow	Merges a selected standard text string to a selected arrow.
Is Reversible	Adds arrowheads to both ends of the connector.
Edit Structure	Opens the Structure Editor dialog box for editing the selected structure.
Edit Standard Text	Use to edit standard text, which is text that is not associated with a structure or an arrow.
Edit Arrow Caption	Use to edit the text associated with a connector. Available when you select an arrow.
Reshape	Reshapes the selected connector.
Is Locked	–
Show Structure As	Use to change the structure format. Options: Structure, Formula, or Description
Zoom and Pan > Hand	Activates the hand pointer for panning.
Zoom and Pan > Zoom to Box	Activates the magnifier pointer for selecting a rectangular area to magnify.
Copy > Selection	Copies the selection to the Clipboard.
Copy > Fragmentation Scheme	Copies the contents of the drawing area to the Clipboard.
Copy > Structure	Copies the selected structure to the Clipboard.
Copy > Style	–
Paste > Fragmentation Scheme	Pastes a fragmentation scheme from the Clipboard.
Paste > Structure	Pastes a structure from the Clipboard.
Paste > Bitmap	Pastes a bitmap image from the Clipboard.
Paste > Style	–
Delete > Unconnected Snaps	Deletes a portion of a connector.

Table 129. Metabolika shortcut menu (Sheet 2 of 2)

Command	Description
Delete > Selection	Deletes the selected items.
Delete > Fragmentation Scheme	Deletes all the items in the drawing area.
Select All	Selects all the items in the drawing area.

Properties page

The Properties page contains properties of selected pathway elements. The properties differ for different element types (connectors, structures, text blocks, and so on). For instance, the properties of a structure (shown below) contain various categories, such as Label (font settings of the label), Label Position (location of the label with respect to the given structure), Structure (selection of the color of atoms and bonds), and Tinge (width, style, and color of the given structure outline). You can either adjust or reset the properties to default values.

Metadata page

The Metadata page in the Metabolika window contains Metadata information for individual objects of the pathway. You can add and edit the metadata information.

Errors page

The Errors page automatically verifies elemental consistency, unconnected reaction or structures, or more unconnected metabolic pathways. The Errors view displays the error list.

Marks page

The Marks page lists the highlighted structures and graphical properties of individual highlights (colors, borders, and shape). You can adjust the properties and sort and delete the highlighted elements.

See these topics:

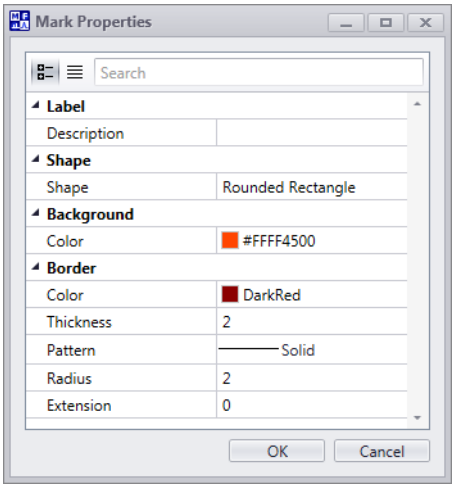
- Mark Properties dialog box
- Define Structure and Mark dialog box

Mark Properties dialog box

❖ To open the Mark Properties dialog box

Select one or more structures in the drawing pane, click the **Marks** tab, and then click **Mark Sel.** on the Marks page.

Figure 116. Mark Properties dialog box with the default settings



Define Structure and Mark dialog box

❖ To open the Define Structure and Mark Properties dialog box

Select one or more structures in the drawing pane, click the **Marks** tab, and then click **Mark Struct** on the Marks page.

Pathways Explorer page

The Pathways Explore view lets the user browse and open existing pathways either from the library of Mass Frontier or from user-specified sources.

7

Structure Editor module

Structural information is essential for interpreting and investigating structure-spectra relationships because mass spectra reflect the structural features of molecules. Use the Structure Editor module to edit, import, export, and verify chemical structures.

Note Structure Editor module supports these formats to import a file:

- Molecular Design Ltd. molecular structure files with the MOL extension.
- HighChem Maximal Compressed Structure format with the MCS extension.
- HighChem Compressed Template Structure format with the TML extension.
- International Chemical Identifier (InChI).
- Simplified molecular-input line-entry system (SMILES).

To save a file, the only supported file format is standard MDL mol file (version V2000).

Contents

Use the Structure Editor with other modules

From the Structure Editor, you can send a structure to the following modules:

- The Curator module, which uses structures to annotate spectral trees.
- The SledgeHammer module, which generates possible fragments of a structure.
- The Structure Grid module, to create, edit, and organize fragments or compounds from a suspect list, an mzLogic search, or a Metabolika Pathway.

You can also copy a compound structure generated in the Structure Editor and paste it in any window where the structure of a compound can be imported.

Open the Structure Editor Module

The Structure Editor module has a toolbar at the top and a set of drawing tools on the left.

- The toolbar provides options to open a structure file, edit the drawing, save the drawing to a structure file, and send the structure to other modules.
- The drawing tools (such as Draw, Atom properties, Bond properties, charge and so on) the left provides drawing tools can be used to create a structure and it's properties.

Do any of the following to open the Structure Editor module:

❖ To open a new Structure Editor module

- Open the Mass Frontier 8.1 application.
- Go to the Start menu, choose **New,** and then click the **Structure Editor** icon on the right.
- Select a Structure Editor file, and click **Open**.

-or-

- Open the Mass Frontier 8.1 application.
- Click the **Modules & Tools** tab, and then click **Structure Editor**.

❖ To open an existing Structure Editor module

- Go to the Start menu, choose **Open**.
- Click the **Structure Editor** icon on the right.
- Select a Structure Editor file, and then click **Open**.

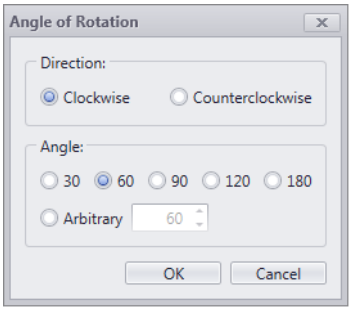
Note

Structure Editor Home toolbar

Table 130. Structure Editor Home toolbar (Sheet 1 of 2)

Button		Description
File group		
Open		Opens the Open Structure File dialog box for selecting a structure file. Supported file formats are MOL, MCS, or TML.
Save		Saves the current structure as an MOL, MCS, or TML file in the current location. **Note** Does not issue a warning when overwriting the file.
Save As		Saves the current structure as an MOL, MCS, or TML file to the specified location with the specified file name.
Edit group		
Undo		Reverts the most recent change in the Structure Editor.
Redo		Repeats the most recent change.
Delete		Deletes the selection.
Select all		Selects everything present in the active Structure Editor page.
Cut		Removes the selection and saves it to the Clipboard.
Copy		Copies the selection to the Clipboard.
Paste		Pastes the structure from the Clipboard, either from a structure or an InChI string.
Export InChI & SMILES		Generates an InChI string, InChI key representation, and SMILES from the structures on the active page.
Import InChI or SMILES		Converts an InChI string to a molecular structure representation. You can also directly copy a valid InChI string, or SMILES from the Clipboard. See "Import/Export InChI or SMILES" on page 253
Selection group		
Lasso		Lasso-like selection method.
Rectangle		Rectangular selection method.
Modify group		
Resize		Lets you resize the selected structure by using the mouse.

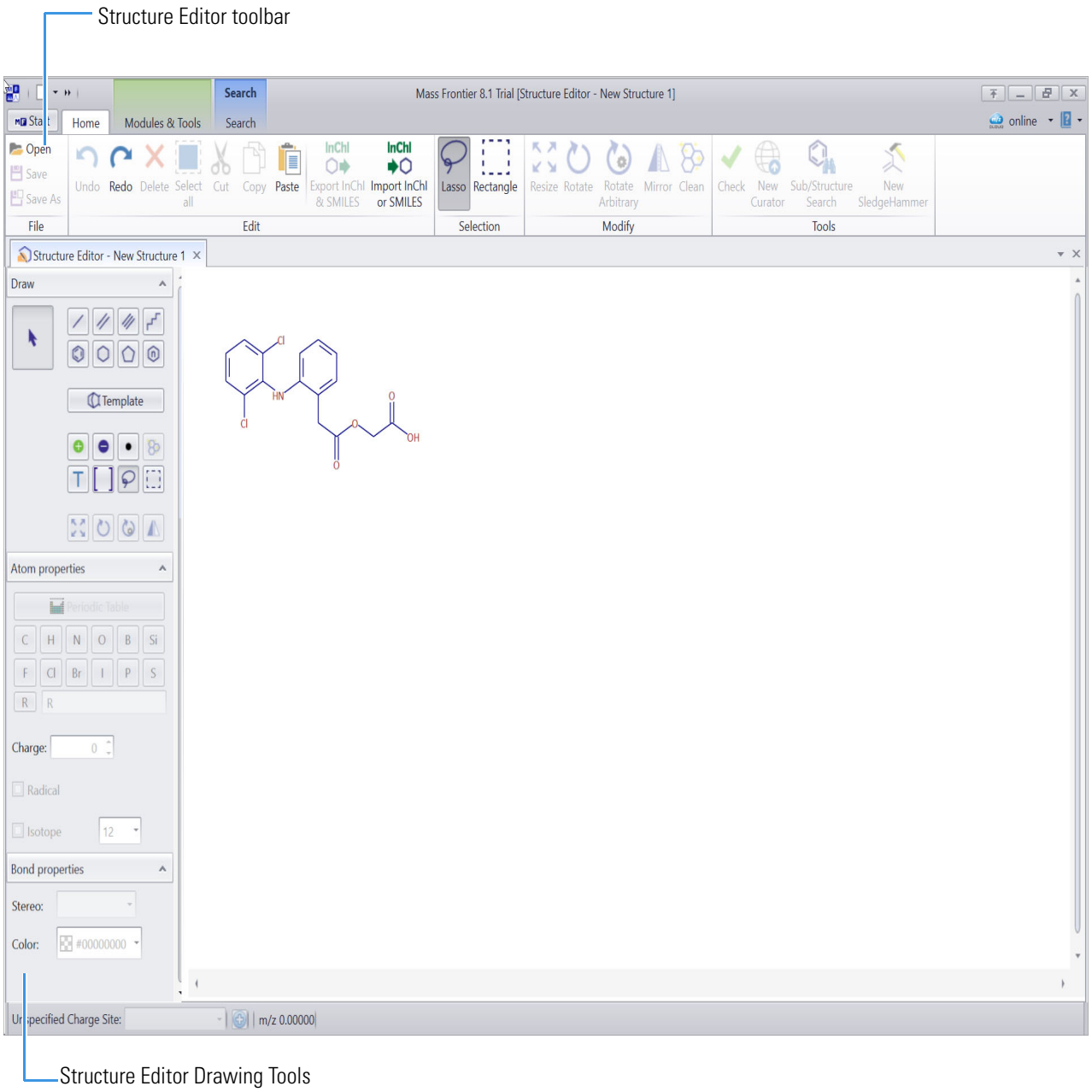
Table 130. Structure Editor Home toolbar (Sheet 2 of 2)

Button		Description
Rotate		Lets you rotate the structure by using the mouse.
Rotate Arbitrary		Opens the Angle of Rotation dialog box where you can specify the structure's angle of rotation. 
Mirror		Creates a mirror image of the structure.
Clean		Automatically adjusts the bond lengths and angles of the selected substructure. This functionality aligns with NCDK.
Tools group		
Check		Verifies if the structure is correct. Applies standard stoichiometric and bond-order rules.
New Curator		Creates a new instance of the Curator module with the created structure.
Sub/Structure Search		Initiates a Sub/Structure search using the active structure.
New SledgeHammer		Opens a new SledgeHammer page and predicts fragments.

Note

The Clean function cannot determine the stereo chemistry of the structure.

Figure 117. Structure Editor window



Import/Export InChI or SMILES

❖ **Import a compound structure from SMILES or InChI**

1. Open the Structure Editor module. See "Open the Structure Editor Module" on page 250.

2. Copy the valid InChI or SMILES of a known compound structure from another reliable source.
3. Click InChI on the Structure Editor toolbar.

   The InChI/SMILES dialog box opens.
4. Paste the copied InChI/SMILES in the text box and click **OK**.

   The known compound structure is generated in the workspace of Structure Editor.

Note You cannot import a compound structure from InChI Key.

❖ **Export SMILES or InChI of a compound structure**

1. Open the Structure Editor module. See "Open the Structure Editor Module" on page 250.
2. Create a structure. See "Create or Edit Structure" on page 259.
3. Click InChI on the Structure Editor toolbar.

   The InChI/SMILES dialog box opens with the InChI, InChI key, and SMILES generated.
4. Copy the InChI/InChI key/SMILES from the text box to clipboard and export it to different modules or save it as MOL file.

You can export the InChI, InChI key, and SMILES of the compound to the Structure Editor, the Structure Grid, the Structure Search, the SledgeHammer, the Curator, or any other module where a compound structure can be copied.

Drawing tools and atom and bond properties pane

The Structure Editor contains several tools that you can use to draw and modify chemical structures. Some of the most common actions are as follows:

- To select or clear atoms or bonds, press and hold SHIFT.
- To change an element to an R group, press the R key on the keyboard.
- To change an element to another element that is represented by a single-character symbol—H, B, C, N, O, F, P, S, K, V, Y, I, W, or U—press the corresponding key on the keyboard
- To change an element to chlorine (Cl) or bromine (Br), press SHIFT+C or SHIFT+B, respectively.

Table 131 describes the drawing and properties tools at the left of the Structure Editor window.

Table 131. Drawing tools at the left in the Structure Editor window (Sheet 1 of 4)

Icon		Description
Draw pane		
Default Mode		Activates the Select mode. To select a group of adjacent atoms, drag the cursor to form a rectangle around the atoms. To select a group of atoms that are not adjacent, hold the SHIFT key and select individual atoms. To move selected atoms, click, drag, and release the selection.
Single Bond		Draws a single bond. Click and release the selection to create a new bond with the standard length and orientation. Click, drag, and release the selection to connect two atoms at an arbitrary length.
Double Bond		Draws a double bond. Click and release the selection to create a new bond with the standard length and orientation. Click, drag, and release the selection to connect two atoms at an arbitrary length.
Triple Bond		Draws a triple bond. Click and release the selection to create a new bond with standard length and orientation. Click, drag, and release to connect two atoms at an arbitrary length.
Chain		Draws an alkaline chain at arbitrary length.
Benzene Ring		Draws a benzene ring. Drawing a ring over an existing atom or bond creates fused molecular structures.
Six-Membered Ring		Draws a cyclohexane ring. Drawing a ring over an existing atom or bond creates fused molecular structures.
Five-Membered Ring		Draws a cyclopentane ring. Drawing a ring over an existing atom or bond creates fused molecular structures.
n-Membered Ring		Creates a saturated n-membered cycloalkane ring, where n must be an integer from 3 to 25.
Template		Opens the Templates dialog box for you to choose from over 200 predefined structure templates.
Positive Charge		Assigns a positive formal charge to the selected atom. Limit: 0 to +9

Table 131. Drawing tools at the left in the Structure Editor window (Sheet 2 of 4)

Icon		Description
Negative Charge		Assigns a negative formal charge to the selected atom. Limit: 0 to -5. See "Create or Edit Structure" on page 259.
Radical		Converts the selected atom to a radical. You can add one or more radicals to the structure.
Clean		Creates precise structures by automatic adjustments of bond lengths and angles.
Text		Adds a text annotation to the page.
Markush Bracket		Creates a Markush Bracket to indicate the number of repeats of a substructure.
Lasso		Switches to the lasso selection mode.
Rectangle		Switches to the rectangular selection mode.
Resize		Lets you resize the selected structure by using the mouse.
Rotate		Lets you rotate the structure by using the mouse.
Rotate Arbitrary		Opens the Angle of Rotation dialog box where you can specify the angle of rotation.
Mirror		Creates a mirror image of the structure.
Atom Properties pane **Note** You must select the corresponding atoms or bonds to activate the buttons in the Atom properties pane.		
Periodic Table		Opens the Periodic Table tool where you can specify the element symbol of the selected atom.
C, H, N, O, B, Si, F, Cl, Br, I, P, and S		Shortcuts to the most common element selections.
R-Substituent		Adds a R group to the selected atom in the compound structure. You can specify the R substituent in the text box beside the R button. **Note** While performing the Sub/Structure Search in the SledgeHammer, R-Substituent will match any element or isotope and displays the results.

Table 131. Drawing tools at the left in the Structure Editor window (Sheet 3 of 4)

Icon	Description
Charge	Specifies the selected charge (positive or negative) for the selected atom. See "Create or Edit Structure" on page 259.
Radical check box	Converts the selected atom to a radical.
Isotope check box	Converts the atom to an isotope with the corresponding number of neutrons. For any selected atom, naturally occurring isotopic numbers are highlighted in an Isotope combo box. You can enter any positive integer number in the text box of an isotope.The mass of this artificial isotope remains same as the natural isotope.
Bond Properties pane	
Stereo	A wedge and hash projection. Provides a selection of different bond styles that can be used to draw a three-dimensional structure of a compound. **Note** The effect is only visual and does not affect the structure processing algorithms, such as SledgeHammer and Sub/Structure Search.
None 	Represent a bond that is in the plane of the screen.
Wedge: Up 	Represents a bond that is in front of the plane of the screen. Atoms or groups are located at the wider end of the wedge and are projecting out towards the viewer.
Wedge: UpReverse 	Represents a bond that is in front of the plane of the screen. Atoms or groups are located at the narrower end of the wedge and are projecting out towards the viewer.
Hash: Down 	Represents a bond that is behind the plane of the screen. Atoms or groups are located at the wider end of the hashed wedge and are projecting away from the viewer.

Table 131. Drawing tools at the left in the Structure Editor window (Sheet 4 of 4)

Icon		Description
Hash: DownReverse		Represents a bond that is behind the plane of the screen. Atoms or groups are located at the narrower end of the hashed wedge and are projecting away from the viewer.
Color		Provides a selection of different bond colors. You can add any color to the bonds in the structure. **Note** The effect is only visual and does not affect the structure processing algorithms, such as New SledgeHammer and Sub/Structure Search.

Add Custom Adduct

In the Structure Editor window, you can add an adduct to the compound.

❖ **To add a custom adduct**

1. Open the Structure Editor window.
2. Create a structure.
3. Click  at the bottom of the Structure Editor window just beside the Unspecified Charge Site.

   The Add Custom Adduct dialog box opens.
4. Input the values in each of the options to create an adduct. See Table 132.
5. Click **OK** to add the adduct to the compound structure.

Table 132. Options to create a custom adduct

Options	Description
Caption	Displays the final adduct created.
Mass change	Displays the change in the mass of the molecule that was caused by the addition or removal of an adduct.
Base Mass Multiplication	Multiplies the mass of the molecule by an integer. Default: 1 Range: 1 to 16
Charge	Lets you input the charge to the molecule. Default: 1 Range: -5 to +9

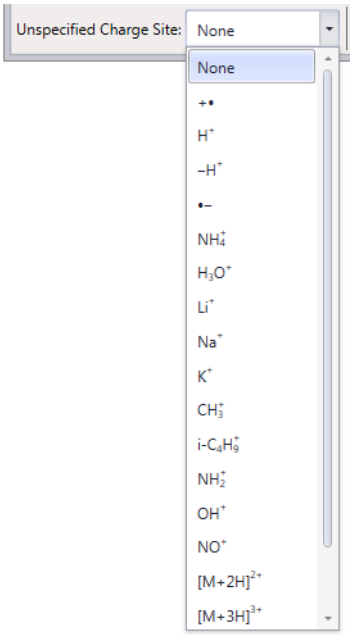
Table 132. Options to create a custom adduct

Options	Description
Adduct	Lets you input the atom to form the adduct. Default: H
Neutral loss	Lets you input the loss of low-mass neutral fragments. Default: H_2O

Assign an unspecified charge site to a structure

❖ **To assign an unspecified charge site to a structure**

1. Open a structure from a structure file or draw the structure in the Structure Editor window. See "Create or Edit Structure" on page 259
2. From the Unspecified Charge Site dropdown list at the bottom left of the Structure Editor window, select the unspecified charge type. See "Unspecified charge site" on page 406.



Create or Edit Structure

You can create or edit a structure in the Structure Editor module and can save, import, and export the structure files to other modules.

The Structure Editor module supports following format of molecule structure:

- Structure with a maximum number of atoms (heavy atoms plus explicit hydrogens) as 1000.
- Structure can contain charge on any atom (not only at one) and value of charge can be from value -5 to +9.
- Structure can contain radical on more than one atom.
- Structure can contain any mass number (isotope), not just naturally occurring.
- Structure can contain any unspecified charge.
- Any atom and bond can have any color.
- Full support for stereo down bond.
- Add label to the molecule.

❖ To create a new structure in the Structure Editor

1. Open a Structure Editor window.
2. Create a new structure using the drawing tools. See " Drawing tools at the left in the Structure Editor window" on page 255.
3. In the Tools area of the Structure Editor tool bar, click **Check** on the tools group of Structure Editor tool bar.

   A Check Structure window opens with a message. Click **OK**.
4. In the File area of the Structure Editor tool bar, click Save As to save the file.

   The file is saved as an MOL file.

❖ To edit the Structure in Structure Editor

1. Open a Structure Editor window.
2. From the file group of Structure Editor tool bar, click **Open.**
3. Select the query structure from the list of molecules.
4. Click **Open**.
5. Edit the structure of the molecule using the drawing tools.
6. In the Tools area of the Structure Editor tool bar, click **Check** on the tools group of Structure Editor tool bar.

   A Check Structure window opens with a message. Click **OK**.
7. In the File area of the Structure Editor tool bar, click Save As to save the file.

   The file is saved as an MOL file.

Note If the structure created or edited is not correct, then a Structure Check Results dialog box opens with an error message. This dialog box highlights the exact position the of error in the structure of the molecule.

Tip Multiple localized positive or negative charges (-7 to +7) can be added on individual atoms in a structure. The structure check considers the combinations of localized and non- specific charges into consideration.

For any molecule with modifications, the new monoisotopic mass and *m/z* value are calculated and displayed at the bottom of the Structure Editor window beside the Unspecified Charge Site area.

Structure Editor limitations

The Mass Frontier application deals primarily with small organic structures, not peptides or other biological molecules.

- The Structure Editor only accepts single structures; that is, it does not accept two or more structures that are not connected by bonds. The application registers multiple non-connected structures as mixtures.
- The Structure in SledgeHammer must follow any of the following rules for fragmentation:
    - The structures must contain a charge (+1 or -1) on only one atom.
    - The structure should be neutral.
    - The structure must contain one of the supported unspecified adducts. The following are the unspecified adducts:
        - M+●
        - [M+H]+
        - [M-H]-
        - [M+H3O]+
        - [M+NH4]+
        - [M+Li]+
        - [M+Na]+
        - [M+K]+

    If the structure doesn't not follow rules mentioned, an error message appears in the Structure Editor and the structure cannot be sent to the SledgeHammer for fragmentation.

- The Structure Editor module does not support the conversion of InChI, and InChI key to a structure.

8

Structure Grid module

The Structure Grid module stores chemical structures and provides a functional links to other modules. In a Structure Grid window, you can create, edit, and organize fragments or compounds from a suspect list, an mzLogic search, or a Metabolika Pathway.

Note Supported structure formats: SDF, MOL, MCS, and TML.

Contents

Use Structure Grid module with other modules

From the Structure Grid, you can select and send single structures to the following modules:

- The Curator module (for annotating a spectral tree).
- The SledgeHammer module (to generate fragments).
- The Structure Editor (for editing).

- The Metabolika pathway module (as an unconnected structure).
- The (Sub)Structure Search module (to search for the structure in the online mzCloud library and your local libraries).

You can send multiple structures to a Batch Fragmentation module or a Metabolika module or copy them to another Structure Grid window.

You can also use the structures in a Structure Grid window for the following:

- To display XIC's in a Chromatogram Processor window
- As the structure candidates for an mzLogic search
- To annotate spectral peaks in a Data Manager window
- To annotate spectral peaks in a Curator window
- As the fragments for an FISh analysis

You can load structures to a Structure Grid from any opened window that holds structures (for example, windows in the Curator, mzLogic Details, Data Manager, Metabolika, and Structure Editor modules).

Open a structure grid

Follow one of the procedures to open Structure Grid module:

❖ **To open an empty Structure Grid, do one of the following:**

In the Modules & Tools toolbar, Click **Structure Grid**.

–or–

From the Start menu, choose **New** > **Structure Grid**.

❖ **To open a Structure Grid with structures from a structure file, do the following:**

- From the Start menu, choose **Open** > **Structure Grid**.
- In the Open Structures dialog box that opens, select a structure file and then click **Open**.

Add structures to a structure grid

❖ **To add structures to a structure grid**

1. Open the Structure Grid. See Open a structure grid.
2. Do any of the following:
   - To add structures from a file:

– Click **Open**.

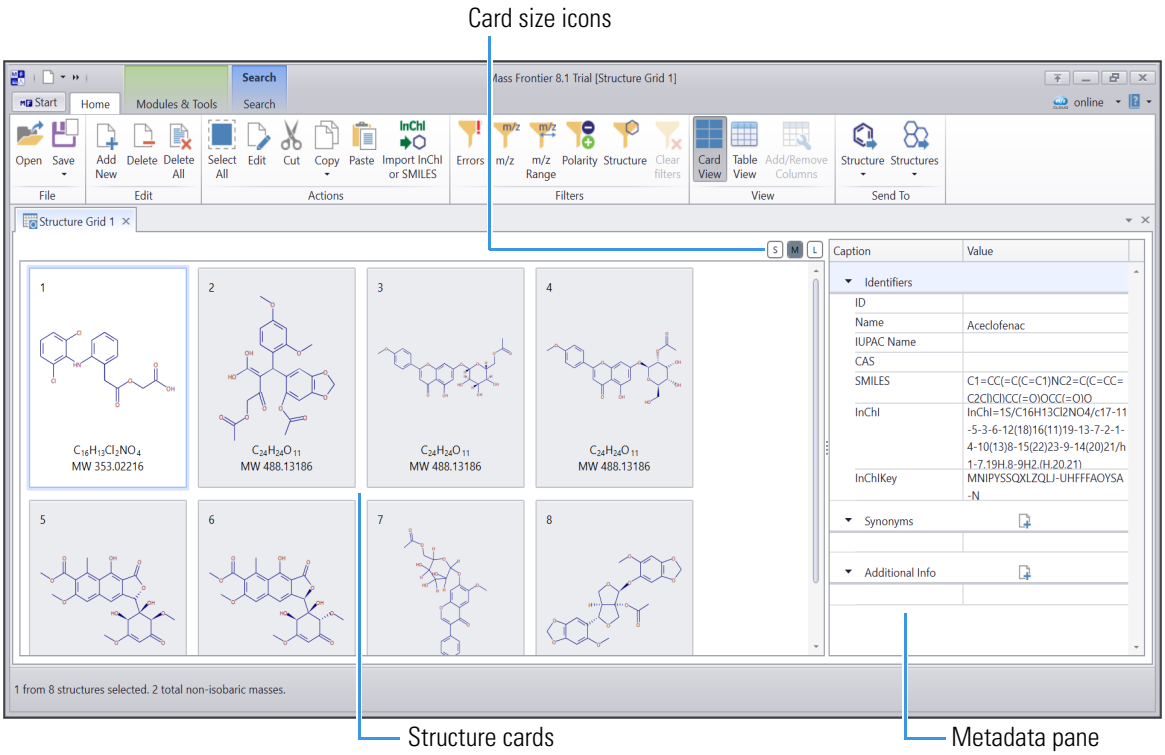
– Select a structure file and then click **Open**.

-or-

- Paste a structure from the Clipboard to the Structure Grid.

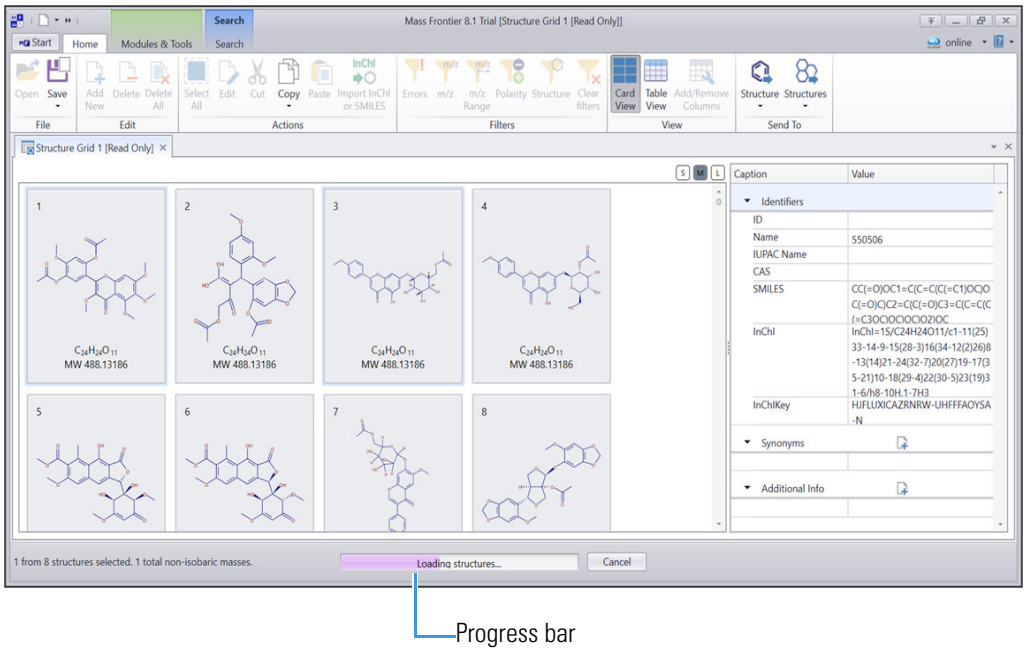
The selected files open. If the application cannot load a file, it will display a warning message that includes the reason for the failed loading of the file.

Figure 118. Structure Grid window set to the Card view



3. (Optional) If you need to cancel the opening or saving of a large SDF file, click **Cancel** on the progress bar.

Figure 119. Structure Grid window with progress bar.



Filter the structures in a structure grid

Use the command buttons in the Filters group of the Structure Grid toolbar to display a subset of the structures.

❖ To filter the structures in a Structure Grid

1. Do any of the following:

   - Filter structures by a specific *m/z* value as follows:

   – Click **m/z**.

     The Filter Structures by m/z dialog box opens.

   – Enter the m/z value and its tolerance and then click **OK**.

   - Filter structures by an *m/z* range as follows:

   – Click **m/z range**.

     The Filter Structures by m/z Range dialog box opens.

   – Enter the *m/z* range and then click **OK**.

   - Filter structures by their polarity as follows:

   – Click **Polarity**.

     The Polarity dialog box opens.

– Select a polarity (Negative, Positive, or Neutral) and then click **OK**.

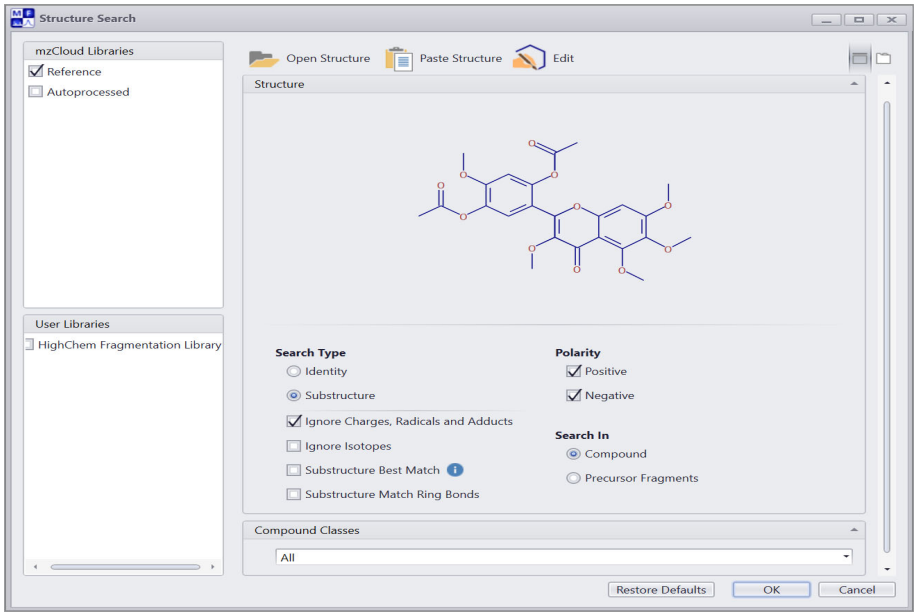
• Show only the structures with a specific structural pattern (substructure) as follows:

– Click **Structure**.

The (Sub)structure Search dialog box (Figure 120) opens.

– Specify the structure for the search and the search parameters, and then click **OK**.

Figure 120. (Sub)Structure Search dialog box



2. (Optional) To clear the filters, click **Clear Filters.**

Structure Grid toolbar

Table 133. Structure Grid toolbar (Sheet 1 of 3)

Buttons		Description
File group		
Open		Opens structures from selected SDF, MOL, MCS, or TML files.
Save (All)		Saves all the structures in the Structure Grid to an SDF file.
Save (Selection)		Saves selected structures in the Structure Grid to an SDF file.
Edit group		
Add New		Opens the Structure Editor dialog box.

Table 133. Structure Grid toolbar (Sheet 2 of 3)

Buttons		Description
Delete		Deletes the selected structure card.
Delete All		Deletes all the structure cards.
Actions group		
Select All		Selects the displayed structures on the page.
Edit		Opens the Structure Editor dialog box for editing the structure in the selected structure card.
Cut		Removes one or more selected structure cards and pastes the structures to the Clipboard.
Copy (Cards)		Copies one or more structures from the selected structure cards and pastes them to the Clipboard.
Copy (Rows)		Copies the values from one or more selected rows in the Table view.
Copy (Table)		Copies the values from all the rows in the Table view.
Paste		Pastes one or more structures to new structure cards.
Import InChI or SMILES		Converts an InChI string to a molecular structure representation. You can also directly copy a valid InChI string or SMILES from the Clipboard. See "Import a compound structure from SMILES or InChI" on page 253
Filters group		
Errors		Displays structure cards that violate the standard stoichiometric rules. See "Create or Edit Structure" on page 259.
m/z		Displays structures with the defined *m/z* value and tolerance.
m/z Range		Displays structures within the defined *m/z* range.
Polarity		Displays structures with the defined polarity.
Structure		Displays structures with the specified structure pattern.
Clear Filters		Clears filters and displays the structure cards.

Table 133. Structure Grid toolbar (Sheet 3 of 3)

Buttons		Description
Views group		
Card View		Switches the display to the Card View.
Table View		Switches the display to the Table View.
Add/Remove Columns		Opens the Add/Remove Columns dialog box for customizing the columns in the Table View.
Send To group		
Structure		Sends the structure from the selected structure card to any of the other modules (New Curator, Sub/Structure Search, New SledgeHammer, New Metabolika, New Structure Editor). See "Structure search" on page 358 to use Sub/Structure search.
Structures		Sends structures from the Structure Grid module to the Batch Fragment Generation, New Metabolika, New Structure Grid modules.
Receive From group		
Structure		Loads a single structure that is selected in another open window.
Structures		Loads all structures from another open window.

Metadata pane

The Metadata pane at the right of a Structure Grid window displays additional metadata corresponding to the selected structure card. By default, InChI, InChIKey, and SMILES are generated directly from the structure. If the structures are imported from an SDF file, the corresponding metadata is also imported from the SDF file.

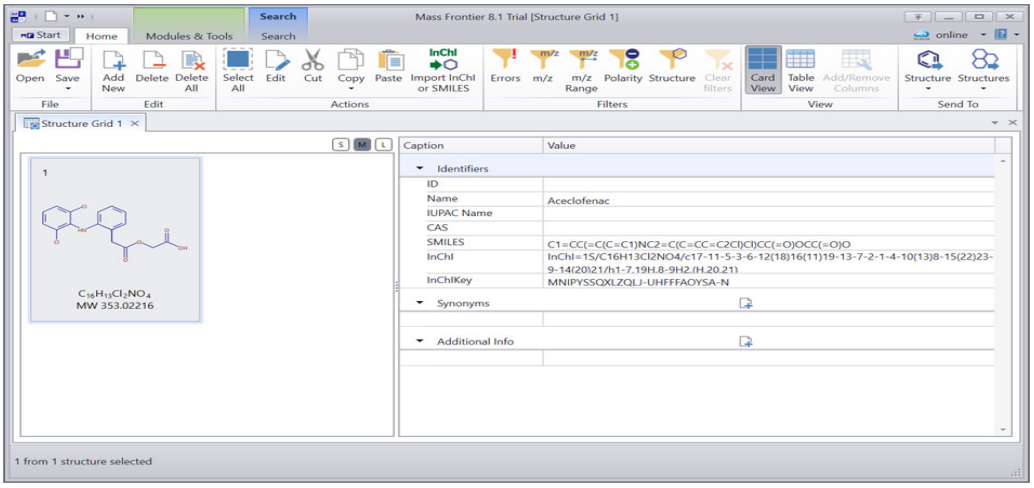
You can do following in Metadata pane:

- Edit the fields and add additional information.
- Save the changes to an SDF file.

Note You cannot save changes to a MOL file.

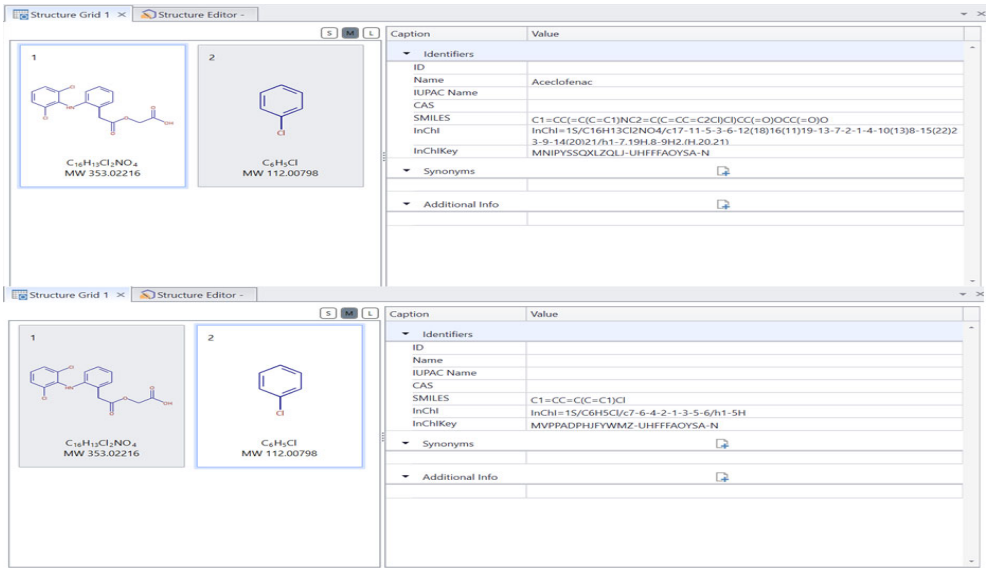
- Import the information from the pane when copying or sending structure cards to other modules.

Figure 121. Structure Grid module with Metadata of a compound



If you select a part of the compound structure, copy it, and create a new card in the Structure Grid module, the Metadata pane automatically generates the new InChI, InChI key, and SMILES for the selected part of the compound that retains the metadata for the original compound structure.

Figure 122. Structure Grid module with new Metadata of a part of a compound.



Note When a InChI, InChI key, and SMILES string of a compound structure or a part of a compound structure is generated, it can be copied/exported to other modules.

Card view

The Card view is the default layout in the Structure Grid window. Each imported structure appears on a separate structure card, along with the structure's molecular formula and exact mass (or *m/z* value if the structure is an ion). Double-clicking a card opens the structure in a Structure Editor window.

Table view

The Table view layout for a Structure Grid displays additional metadata for each structure card, such as the compound name, average mass, formula, and *m/z* values of the selected adducts.

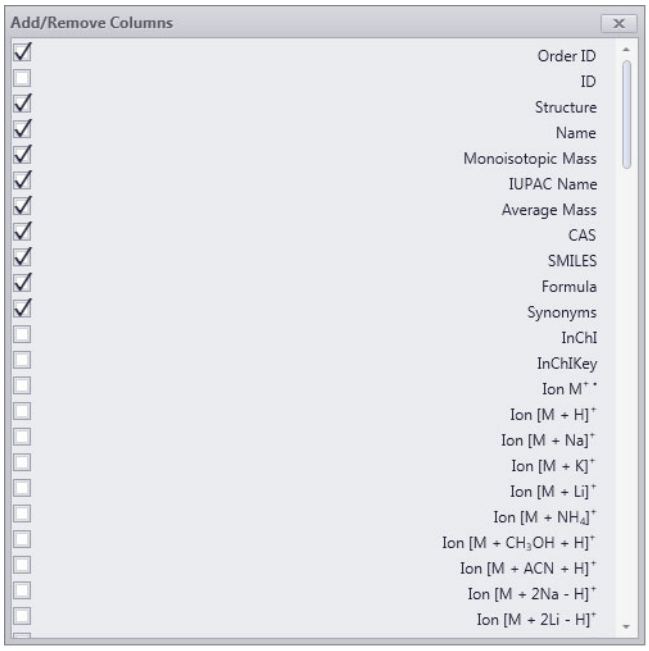
You can also customize the columns that are displayed in the Table view.

❖ To customize the columns in the Table view for a Structure Grid window

1. Open the Structure Grid. See "Open a structure grid" on page 264.
2. In the Structure Grid toolbar, click **Table View**.
3. (Optional) To add or delete table columns, click **Add/Remove Columns** in the Structure Grid toolbar.

   The Add/Remove Columns dialog box opens.

   Figure 123. Add/Remove Columns dialog box for the table view of a Structure Grid

4. Select or clear the check box for the column that you want to add or remove, respectively.

Use a structure grid as an XICs source

❖ **To use a structure grid as a source of XICs in a Chromatogram Processor window**

1. Open a Structure Grid window that contains one or more charged structures.
2. Open a file in a Chromatogram Processor window.
3. Open the **Extracted Ion Chromatogram** pane. See "Open and pin the Extracted Ion Chromatogram pane" on page 140.
4. In the Extracted Ion Chromatogram pane, click the **Add Ions** icon,  , and select the opened Structure Grid from the dropdown list.

   The *m/z* values for the charged structures in the Structure Grid populate the XIC table.

Note If you have specified specific adducts and neutral losses for the structures in the Structure grid, the application uses these entries to calculate the *m/z* values of the ions.

Use a structure grid as a source of structure candidates for an mzLogic search

You can use the structures in one or more Structure Grid windows as the structure source for an mzLogic search. If a structure is neutral, the application calculates the expected *m/z* value by using the selected adduct in the mzLogic search window. For details, see "MolGate Search tool" on page 328.

Note The multiple charged molecule search is not supported by mzCloud libraries.

Batch Fragment Generation module

The Mass Frontier application supports batch processing for fragment prediction. Use the Batch Fragmentation module to generate fragments without mechanisms for multiple input structures in a single batch.

Supported file formats: MOL, TML, MCS, or SDF

Contents

Start the Batch Fragment Generation wizard

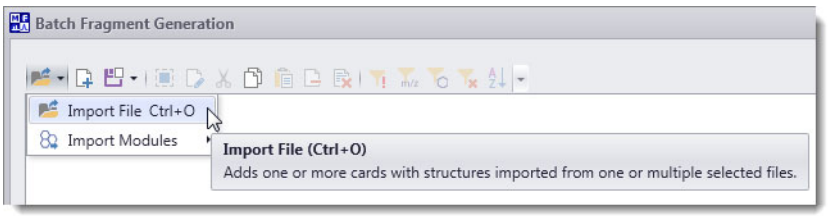
❖ **To use the Batch Fragment Generation wizard**

1. In the Modules & Tools toolbar, click **Batch Fragment Generation**.

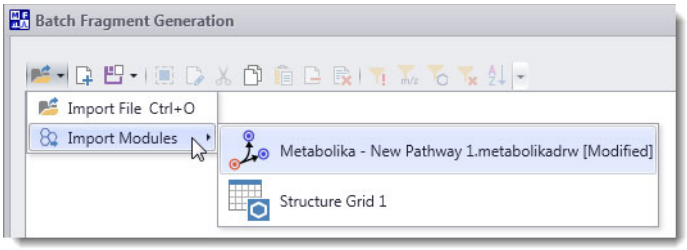
   The Batch Fragment Generation dialog box opens.

2. Do any of the following:

   - Click the **Import** icon, , choose **Import File**, and select a structure file.



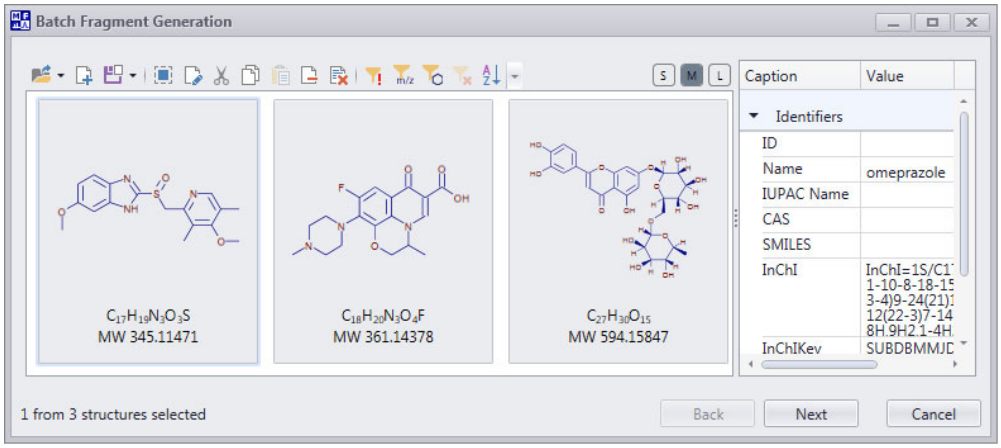
- Click the **Import** icon, , choose **Import Modules**, and select the opened module that contains the structures of interest.



- Click the **Add New** icon, , and select a structure file.
- Paste structures from the Clipboard.

The structures appear as cards.

Figure 124. Batch Fragment Generation wizard with three structures



Note Use the toolbar buttons to add, delete, sort, or filter the structures. See "Batch Fragment Generation toolbar" on page 275.

3. Click **Next**.

The wizard's reaction restrictions step opens. See "Specify the reaction restrictions for a batch fragmentation" on page 276.

Batch Fragment Generation toolbar

Use the toolbar buttons to manage the structures.

Table 134. Batch Fragment Generation toolbar

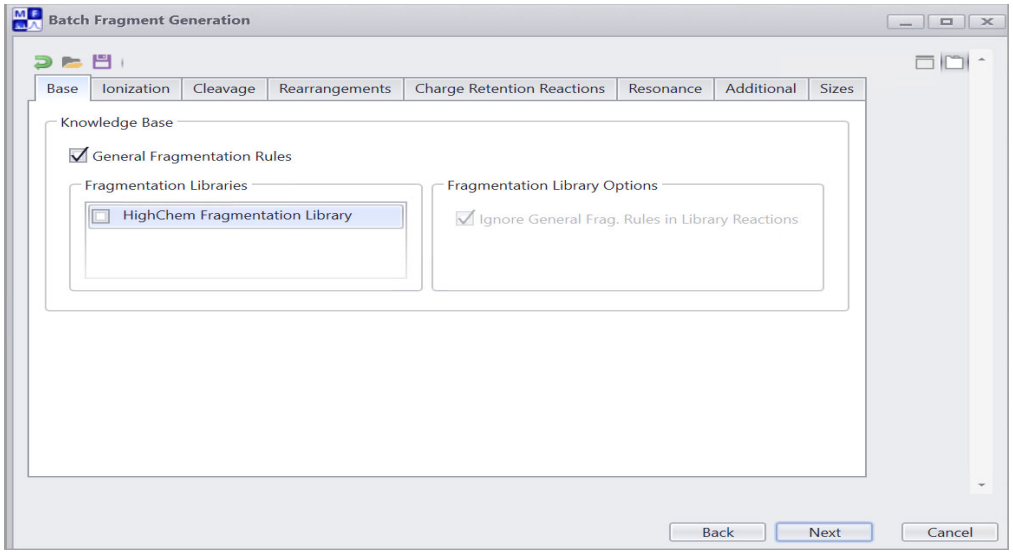
Button		Description
Import		Imports one or more structures from a file (.mol, .mcs, .tml, or .sdf) or from an open page with structures.
Add New		Opens a new Structure Editor window where you can draw the structure you want to use.
(Save) Selection		Saves the selected structure(s).
(Save) All		Saves all structures.
Select All		Selects all structures displayed on the page.
Edit		Edits the structure in Structure Editor.
Cut		Removes one or more selected structures and places into the clipboard.
Copy Cards		Copies one or more structures to the clipboard.
Paste		Pastes one or more structures.
Delete		Deletes selected structures.
Delete All		Deletes all structures.
Errors		Shows only the structures that violate the standard stoichiometric rules. See "Create or Edit Structure" on page 259.
m/z Filter		Shows only structures with the defined *m/z* and tolerance.
Structure Filter		Opens a new (Sub)Structure Search window where you can draw a (sub)structure to filter your structures.
Clear Filters		Clears all the filters applied.
Sort		Sorts the structures by *m/z* value.

Specify the reaction restrictions for a batch fragmentation

Use the reaction restriction page (second step) of the Batch Fragment Generation wizard to define the common fragmentation parameters shared among the fragmented structures.

For information about these pages, see "Reaction Restrictions dialog box" on page 286.

Figure 125. Batch Fragment Generation wizard – Reaction restriction pages



After you specify the parameters settings, click **Next** to define the path to save the files with generated fragments. See "Generate the output files for a batch fragmentation" on page 277.

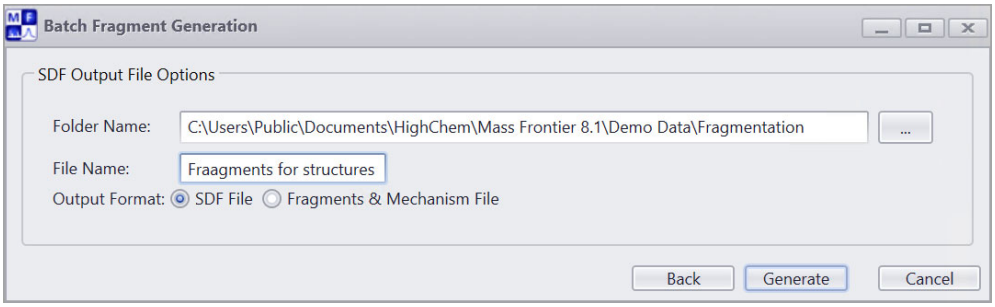
Generate the output files for a batch fragmentation

Use the SDF Output File Options page of the Batch Fragment Generation wizard to specify the folder and file name for the SDF file or SledgeHammer file for the generated fragments.

❖ To generate the fragments and save them to a file

1. In the SDF Output File Options area, select the folder where you want to store the structure files.

   Figure 126. SDF Output File Options page of the Batch Fragment Generation wizard



2. Type a base file name for the files.
3. Select the file type.
4. Click **Generate**.

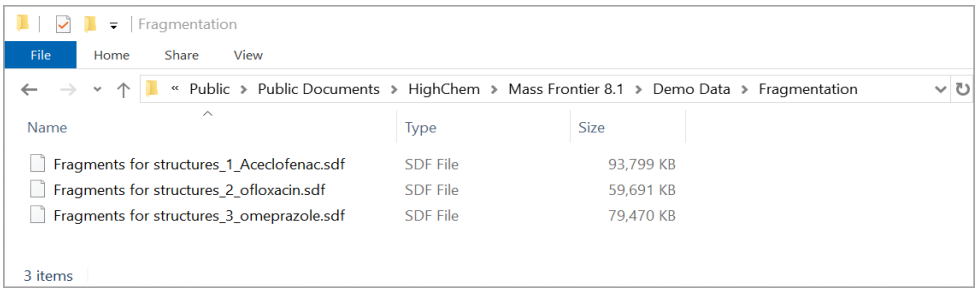
   The application creates an SDF or Mechanisms file for each input structure:

   SDF files contain generated fragments in the same order as they are generated. If a fragment has multiple possible resonance forms, only the structure of its statistically dominant resonance form is saved.

   Mechanisms files contains full fragmentation pathways. You can open this file type in the SledgeHammer module.

   The file name is a combination of the user-specified file name, ID number of the structure in the input structure grid, and the structure name (if defined).

   Figure 127. Output folder for a batch fragmentation



10

SledgeHammer module

The SledgeHammer module automatically predicts the fragments and detailed fragmentation and rearrangement mechanisms for chemical structures. This module uses a mathematical approach to simulate the unimolecular ion-decomposition reactions that take place in a mass spectrometer, and also includes a comprehensive set of known reactions and library mechanisms that support this automated prediction.

You can use the generated fragments and corresponding mechanisms to do the following:

- Check the consistency between a chemical structure and its mass spectrum
- Confirm library search identifications
- Recognize the structural differences between the spectra of closely related compounds
- Interpret the spectra of isotopically labeled compounds
- Illustrate the structure-spectra relationship for educational purposes

The SledgeHammer module supports the following structure formats: MOL, SDF, MCS, and TML.

Contents

Assumptions for generating the fragmentation and rearrangement pathways

The SledgeHammer module uses a mathematical approach to simulate unimolecular ion-decomposition reactions. This systematic approach, which generates possible fragmentation and rearrangement pathways, is based on these assumptions:

Table 135. Assumptions for generating the fragmentation and rearrangement pathways

Assumption	Description
General Fragmentation and Rearrangement Rules	The systematic approach optionally predicts reaction pathways by using a set of general fragmentation and rearrangement rules. This approach does not include compound-specific mechanisms that cannot be generally applied. At first, this might seem to be a disadvantage; however, you can use this approach in combination with a substructure search for identifying specific compound classes. For details, see "Unexplained spectral peaks from compound-specific fragmentation reactions" on page 314.
Fragmentation Library Mechanisms	The system optionally accesses an intelligent fragmentation mechanism knowledge base for predicting unimolecular decomposition reactions. HighChem Fragmentation Library currently contains approximately 226,895 individual mechanisms. You can also include your own mechanisms in fragmentation prediction. For details, see "Reaction Restrictions – Base page" on page 288.
Charge Localization Concept	Every ion-decomposition reaction that is generated is based on the charge localization concept. The application determines exactly where the charge site in all precursor and product ions is located. The system internally generates resonance reactions, which are not displayed by default. These reactions can move charge sites to distant locations and, in some complicated structures, the charge localization concept might appear to have been violated. If a reaction step is not clear, you can set up the system to display mechanisms along with resonance reactions. You can, however, use an unspecified charge location that is internally transformed to all combinatorial structures with a localized charge. For details, see "Reaction Restrictions – Charge Retention Reactions page" on page 295

Table 135. Assumptions for generating the fragmentation and rearrangement pathways

Assumption	Description
Unimolecular Linear Reaction Mechanisms	The application generates only unimolecular reactions. The reaction pathways are displayed as linear reaction mechanisms, which incorporate one intermediate site on the left and one intermediate site on the right for each reaction step. The application includes only ionic products in reaction pathways; it does not display neutral fragments.
Bond Creation	Using the General Rules, the application now supports bond creation between the heavy atoms. The system now includes ring contractions, skeletal and heave atom rearrangement.
Ionization Methods	The application supports the following ionization methods: electron impact, protonation, deprotonation, cluster ion formation, alkali metal adducts, and chemical ionization. For details, see "Reaction Restrictions – Ionization page" on page 290.
Formally Possible Solutions	The mechanisms generated by the application contain formally possible reaction steps. The system does not determine the stability of product ions from thermodynamic data or rates of reaction. When evaluating generated mechanisms, keep this rule in mind: Short and uncomplicated reaction pathways are more favorable than complex mechanisms involving complicated, multi-step hydrogen rearrangements.

Generate fragments and mechanisms

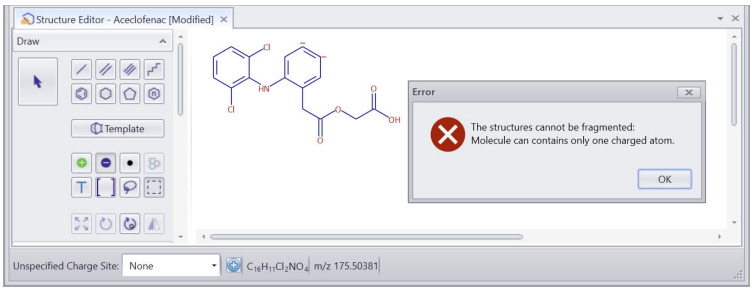
You can generate fragmentation and rearrangement pathways from any structure you supply, including ions and isotopically labeled compounds. The structure can originate from a structure file or any of these windows: Data Manager, Structure Editor, Structure Grid, Curator, Metabolika, or another SledgeHammer window.

Before the application starts the fragment generation, it checks the input structure for errors. The Structure in SledgeHammer must follow any of the following rules for fragmentation:

- The structures must contain a charge (+1 or -1) at only one atom.
- The structure should be neutral.
- The structure must contain one of the supported unspecified adducts.

If it finds any errors, a message box displays the errors and cancels the generation.

Figure 128. Error message for the fragmentation of acecelofenac structure with charge on two atoms



You can start the fragment generation process in several ways:

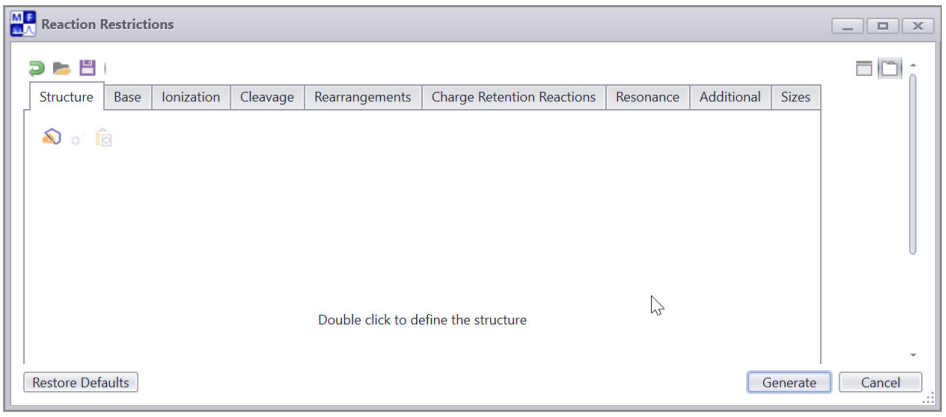
- When you start a generation from a Data Manager window, the application automatically links the generated fragments in the SledgeHammer window with the corresponding spectrum in the Data Manager window. The application also highlights peaks that have the same *m/z* value as that of the generated fragments in red. Selecting a highlighted (red) peak reveals all the pathways leading to it.
- When you start the generation of fragments and mechanisms from a module that can export a structure, such as the Structure Editor, Structure Grid, Curator, Metabolika, or SledgeHammer modules, the application assumes that the complete structure is intended as input. You can manually link the generated fragments in the SledgeHammer window with any opened Data Manager window. The application highlights peaks that have the same *m/z* value as the generated fragments in red. Selecting a highlighted (red) peak reveals all the pathways leading to it.

❖ To start the generation of possible fragmentation and rearrangement pathways

1. Do one of the following:
   - To open the Reaction Restrictions dialog box and specify the structure for the fragment generation process, go to step 2.
   - To open the Reaction Restrictions dialog box by sending a structure from another module, go to step 3.
2. To open the Reaction Restrictions dialog box and define the structure, do the following:
   a. In the **Modules & Tools** toolbar, click **SledgeHammer**.

      The Reaction Restrictions dialog box opens to the Structure page.

Figure 129. Structure page of the Reaction Restrictions dialog box without a structure



b. To define the structure, do one of the following:

- At the upper left of the Structure page, click the **Structure Editor** icon, , and then draw a structure or open a structure file.
- At the upper left of the Structure page, click the **Paste Structure from Clipboard** icon, , to paste a structure from the Clipboard.

c. Go to step 4.

3. To open the Reaction Restrictions dialog box by sending a structure from another module, do one of the following:

- In a Curator window that includes a defined structure, choose **Structure > New SledgeHammer** from the Send To group of the Curator toolbar.
- In a Metabolika window, select a structure, and then choose **Structure > New SledgeHammer** from the toolbar.
- In a Structure Editor window, click **New SledgeHammer** in the toolbar.
- In a Structure Grid window, select a structure. Then, in the Send To toolbar group, choose **Structure > New SledgeHammer**.
- In the Tree view of a Data Manager window, select a library entry, and then choose **Structure > New SledgeHammer** from the toolbar.

4. Specify the appropriate parameter settings on the pages of the Reaction Restrictions dialog box. Or, click the **Open Parameters from a File** icon, , to open a file (.hammer) with the stored settings for the reaction restrictions.

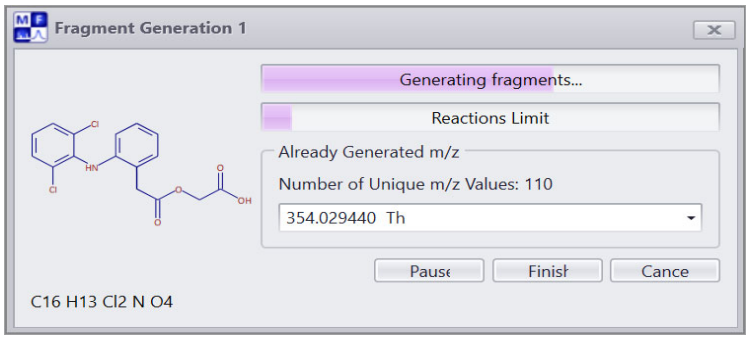
Tip Selecting only the General Fragmentation Rules check box on the Base page decreases the processing time. However, these rules do not support negative ionization data.

For information about the parameters in the Reaction Restrictions dialog box, see "Reaction Restrictions dialog box" on page 286.

5. Click **Generate**.

   The Fragment Generation dialog box displays the progress as the application checks for structure errors (Figure 130). See "Fragment Generation dialog box" on page 285.

   Figure 130. Fragment Generation dialog box



   When the generation finishes, the SledgeHammer window opens.

For information about the SledgeHammer window, see "Work in a SledgeHammer window" on page 301.

Fragment Generation dialog box

After you start the generation of fragments and mechanisms, the Fragment Generation dialog box opens.

Table 136. Fragment Generation dialog box parameter descriptions

Parameter	Description
Generation of Reactions	Displays the current task. The status bar displays the relative completion state of the current check.
Reactions Limit	Indicates approximately how many temporary internal reactions have been generated from a particular structure. Large and structurally complicated molecules can produce an enormous number of reactions but consume a large amount of memory. This memory consumption limits the number of temporarily generated reactions. If the reactions limit is reached, the generation stops, and the application displays a warning message. The application also displays the fragments and mechanisms generated up to that point. The most important fragments are generated first, so even if a generation stops, the most important fragments have likely been generated. However, if you are missing an important fragment because the generation was interrupted, you can increase the reactions limit. For details, see "Reaction Restrictions – Sizes page" on page 298.
Already Generated m/z	Generates a list of *m/z* values of the ions that have already been generated, and the total number of ions that have already been generated.
Pause	Temporarily interrupts generation to redirect processing power either to other processes that might be simultaneously running in the application or to other Windows applications.
Finish	Stops the current generation. The application displays fragments and any production mechanisms that are generated up to that point.
Cancel	Cancels the current generation. The application does not display any generated fragments. It can take several seconds before the window closes.

Reaction Restrictions dialog box

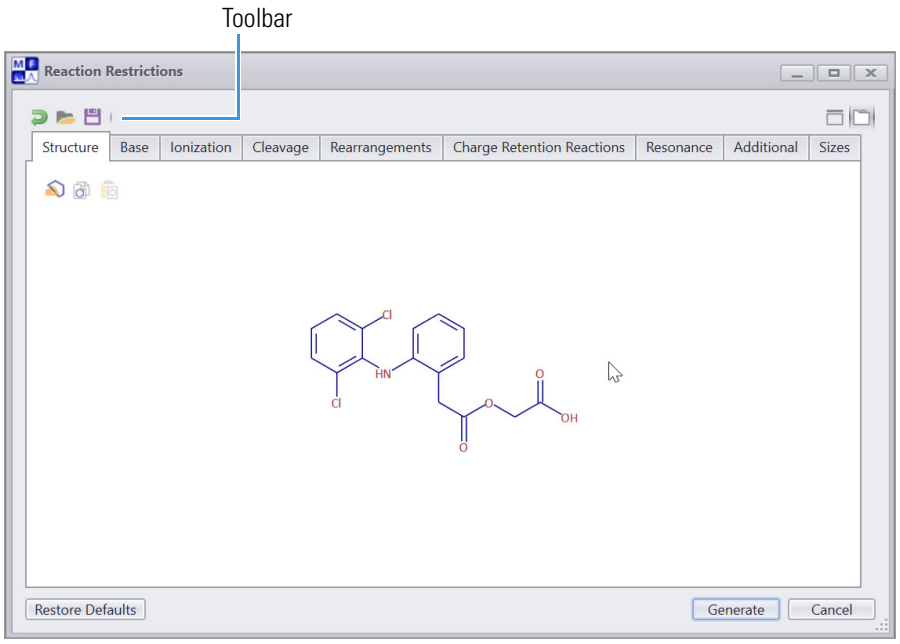
Use the Reaction Restrictions dialog box to select a different ionization method for the fragment generation process, to modify the basic fragmentation and rearrangement mechanisms of the fragment generation process, or both.

The Reaction Restrictions dialog box automatically opens when you start the fragment generation process. This dialog box retrieves its parameter settings from the corresponding Reaction Restrictions view in the Global Settings dialog box. Changing the parameter settings in the "local" Reaction Restrictions dialog box automatically changes the settings in the Global Settings dialog box—that is, the application automatically synchronizes the settings for the reaction restriction parameters. Clicking the Restore Default button restores the factory default settings.

Note For information about opening the Reaction Restrictions dialog box and starting the fragment prediction for a selected structure, see "Generate fragments and mechanisms" on page 281.

The Reaction Restrictions dialog box has a toolbar at the top, nine pages of parameter settings (or collapsible parameter groups), and three command buttons at the bottom.

Figure 131. Reaction Restrictions dialog box with a structure



For details about working with the Reaction Restrictions dialog box and specifying the parameter settings for fragment generation, see these topics:

- Reaction Restrictions dialog box toolbar and command buttons
- Reaction Restrictions – Structure page

- Reaction Restrictions – Base page
- Reaction Restrictions – Ionization page
- Reaction Restrictions – Cleavage page
- Reaction Restrictions – Charge Retention Reactions page
- Reaction Restrictions – Additional page
- Reaction Restrictions – Sizes page
- Save reaction restrictions to a file
- Import reactions restrictions from a file

Reaction Restrictions dialog box toolbar and command buttons

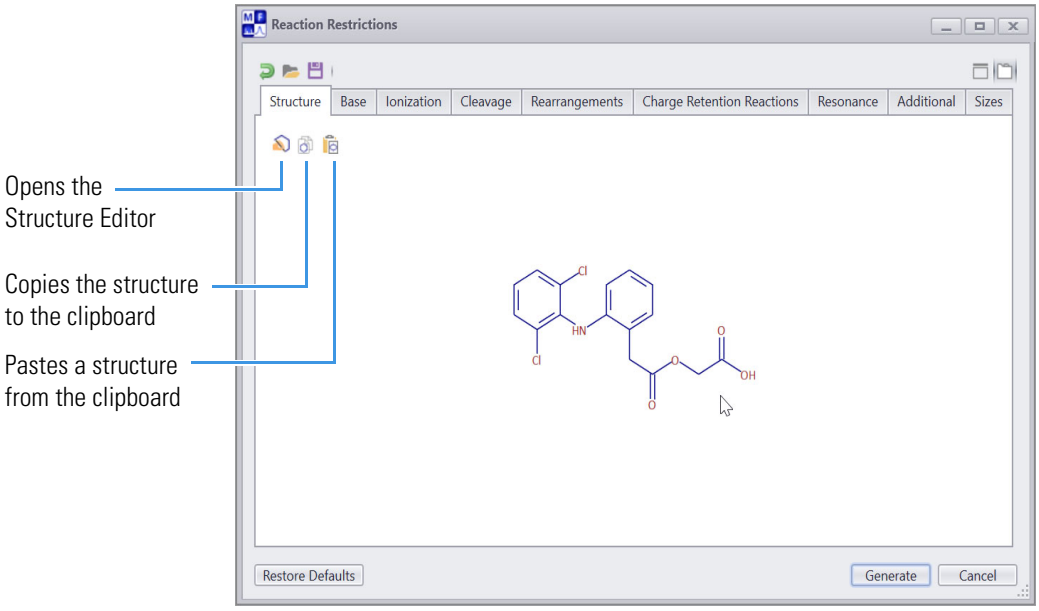
Table 137. Reaction Restrictions dialog box icons and buttons

Icon or button	Description
Restore All Default Parameter Values	Restores the default values in the Reaction Restrictions dialog box.
Open Parameters from a File	Imports parameters from a file (.hammer).
Save Parameters to a File	Saves current parameters to a file (.hammer).
Restore Defaults	Restores the factory default parameter settings.
Generate	Starts the fragment generation process.
Cancel	Cancels the fragment generation process and closes the dialog box.

Reaction Restrictions – Structure page

For information about sending a structure to the Structure page from other modules, see "Generate fragments and mechanisms" on page 281. After you add a structure, you can edit it or select a different structure by opening the Structure Editor.

Figure 132. Structure page of the Reaction Restrictions dialog box



Reaction Restrictions – Base page

Use the Base page to specify the type of knowledge base that the application uses for predicting fragmentation pathways.

The SledgeHammer module contains general mechanisms and can predict fragments using the General Rules or the HighChem Fragmentation Library either in positive mode or negative mode (negative mode produces three times more fragments than positive mode).

In the Base page of Reaction Restrictions, you can choose HighChem Fragmentation Library option over the general fragmentation rules to increase the speed of fragmentation. (This is applicable in common cases). The Library Rules can be reserved for cases where General Rules are not able to predict fragments.

Selecting Fragmentation Library options is best in conjunction with small user-defined libraries of specific reaction mechanisms.

Tip Structures with an unspecified charge site in the fragmentation library or in the starting structure slow the generation process. When possible, avoid using unspecified charge sites in the fragmentation library. This is because, when you use an unspecified charge site, the system must consider a large number of combinations for each step.

Figure 133. Reaction Restrictions – Base page

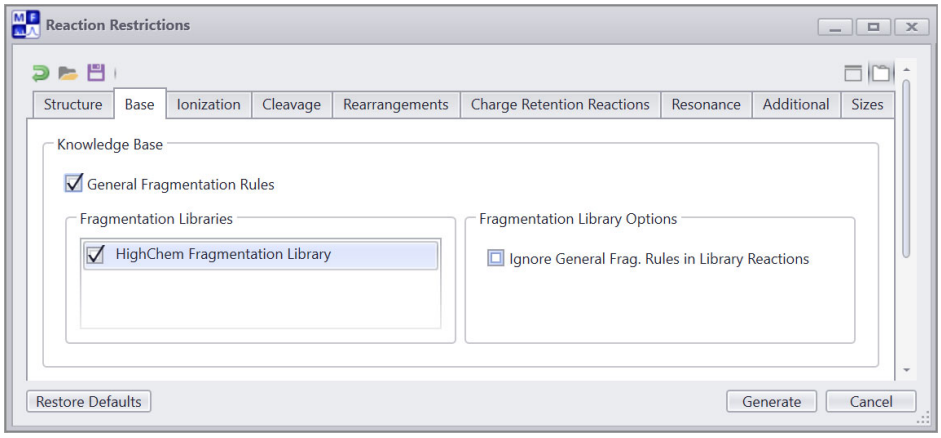


Table 138. Reaction Restrictions – Base page parameter descriptions (Sheet 1 of 2)

Parameters	Description
General Fragmentation Rules	Uses the general rules for generating fragments and mechanisms. Using only these rules provides the fastest method of generating fragments.
Fragmentation Libraries	
HighChem Fragmentation Library	Uses both the general rules and the specific rules for fragmentation collected in the extensive HighChem Fragmentation Library. The HighChem Fragmentation Library contains approximately 226,895 mechanisms, so calculation times are significantly longer when you select this library. The local HighChem Fragmentation Library contains only extracted fragmentation mechanisms. **Note** If user fragmentation libraries have been created they are listed here, and can be used during fragmentation. These libraries can have specific mechanisms that are not covered by HighChem Fragmentation Library or small subset of mechanisms. The fragmentation with this small library is faster than fragmentation with whole HighChem Fragmentation Library. See "HighChem Fragmentation Library" on page 399. Default: Unelected (Select this option if General Rules are unable predict your fragments).

Table 138. Reaction Restrictions – Base page parameter descriptions (Sheet 2 of 2)

Parameters	Description
Fragmentation Library Options	Available when you select the Use HighChem Fragmentation Library check box.
Ignore General Frag. rules in Library Reactions	Selecting this check box excludes from HighChem Fragmentation Libraries mechanisms that can be predicted by General Rules. This increase speed of fragmentation without any significant loss of predicted fragments.

Reaction Restrictions – Ionization page

Use the Ionization page of the Reaction Restrictions dialog box to specify the ionization method.

When comparing the fragments and mechanisms generated for a compound with the mass spectrum of that compound, select the ionization method that was used to acquire the mass spectral data.

Figure 134. Ionization page of the Reaction Restrictions dialog box

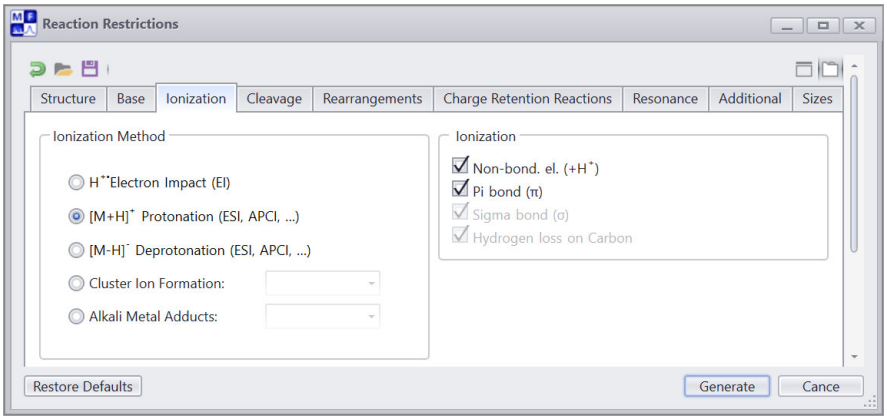


Table 139. Ionization page parameter descriptions (Sheet 1 of 2)

Parameter	Description
Ionization Method	
Specifies the ionization method.	
$H^{+\bullet}$ Electron Impact (EI)	Electron Impact (EI) mode that produces $M^{+\bullet}$ ions.
$[M+H]^+$ Protonation (ESI, APCI, ...)	Positive ionization. The protonation mode represents soft ionization techniques such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), fast atom bombardment (FAB), and other techniques.

Table 139. Ionization page parameter descriptions (Sheet 2 of 2)

Parameter	Description
$[M–H]^-$ Deprotonation (ESI, APCI, ...)	Negative ionization $[M–H]^-$. The deprotonation mode represents soft ionization techniques such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), fast atom bombardment (FAB), and other techniques.
Cluster Ion Formation	Ionizes the compounds with either an ammonium ion or a hydronium ion to give an $[M+NH_4]^+$ peak or an $[M+H_3O]^+$ peak, respectively.
Alkali Metal Adducts	The alkali metals like Lithium, sodium, and potassium react with the molecules to give an $[M+Li]^+$, $[M+Na]^+$, $[M+K]^+$ adduct ions respectively.
Ionization	
Non-Bond el. ($-H^+$)	Performs ionization on non-bonded electrons.
Pi Bond [π]	Performs ionization on π bonds. Available only when either the Electron Impact method or the Protonation method is selected.
Sigma Bond [σ]	Performs ionization on σ bonds. Available only when the Electron Impact ionization method is selected.
Hydrogen Loss on Carbon	Ionizes carbon atoms by removing one of their hydrogen atoms.

Reaction Restrictions – Cleavage page

Use the Cleavage page of the Reaction Restrictions dialog box to specify the cleavage mechanisms

Figure 135. Cleavage page of the Reaction Restrictions dialog box

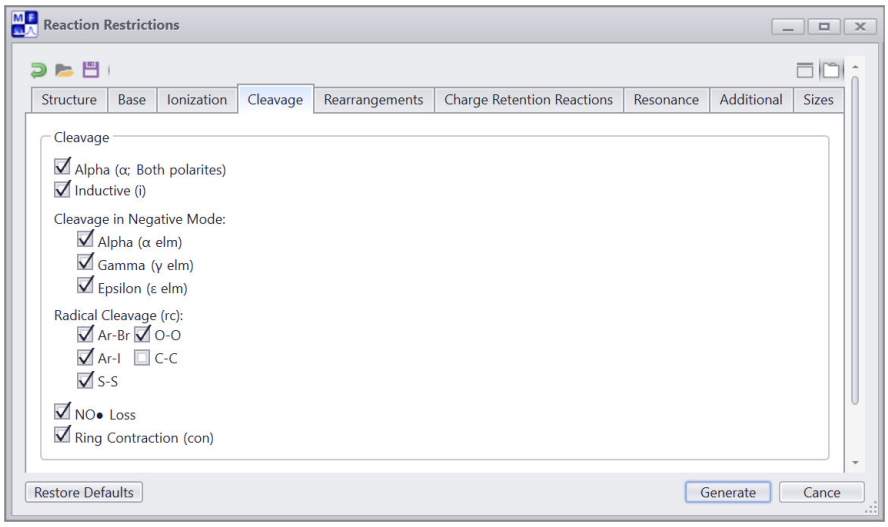


Table 140. Ionization & Cleavage page parameter descriptions (Sheet 1 of 2)

Parameter	Description
Alpha [α; Both Polarities]	Cleavage of the carbon-carbon bond that is adjacent to the carbon bearing a specified functional group. Available only when the Electron Impact ionization method is selected.
Inductive [i]	Cleavage that neutralizes the positive charge at the ionization site by transferring the positive charge to an adjacent atom.
Cleavage in Negative mode	
Alpha (α elm)	Refers to alpha elimination, the molecular ion is derived by the cleavage of bond linking the carbon to the atom with negative charge occupying an alpha position.
Gamma (γ elm)	Refers to gamma elimination, where the molecular ion is derived by the cleavage of bond linking the carbon to the atom with negative charge occupying a gamma position.
Epsilon (ε elm)	Refers to epsilon elimination, where the molecular ion is derived by the cleavage of bond linking the carbon to the atom with negative charge occupying an epsilon position.
Radical Cleavage (rc)	
Ar-Br	Loss of bromine (Br•) from an aromatic ring.
Ar-I	Loss of iodine (I•) from an aromatic ring.
O-O	Cleavage between an O-O bond in an aliphatic chain.

Table 140. Ionization & Cleavage page parameter descriptions (Sheet 2 of 2)

Parameter	Description
C-C	Cleavage between a C-C bond in an aliphatic chain. By default this option is cleared.
S-S	Cleavage between a S-S bond in an aliphatic chain.
NO•Loss	Loss of nitric oxide (NO•) from aromatic nitroso compounds through a charge retention mechanism.
Ring Contraction (con)	Rearrangement reaction that leads to the formation of a new ring with one or more fewer carbon atoms than the original molecule.

Reaction Restrictions – H-Rearrangements page

Use the H-Rearrangement page to specify the restrictions for the four basic hydrogen rearrangements.

Table 141. Hydrogen rearrangements

Abbreviation	Description
rH_A	Radical site rearrangement
rH_B	Charge site rearrangement (α, β)
rH_C	Charge site rearrangement (γ)
rH_R	Charge remote rearrangement

Radical site rearragement is the default rearrangement type for hydrogen transfer from a sterically optimal atom, usually from a γ-atom (McLafferty rearrangement).

Note By default, hydrogen atoms are set to shift to an adjacent atom [$rH_{1,2}$]. This setting cannot be deactivated.

There are two reasons for changing the default setup of rearrangements:

- You are missing an important peak and you suspect an unusual rearrangement.
- You might want to simplify a mechanism by deactivating rearrangements that cause redundant reaction steps.

Figure 136. H-Rearrangement page of the Reaction Restrictions dialog box

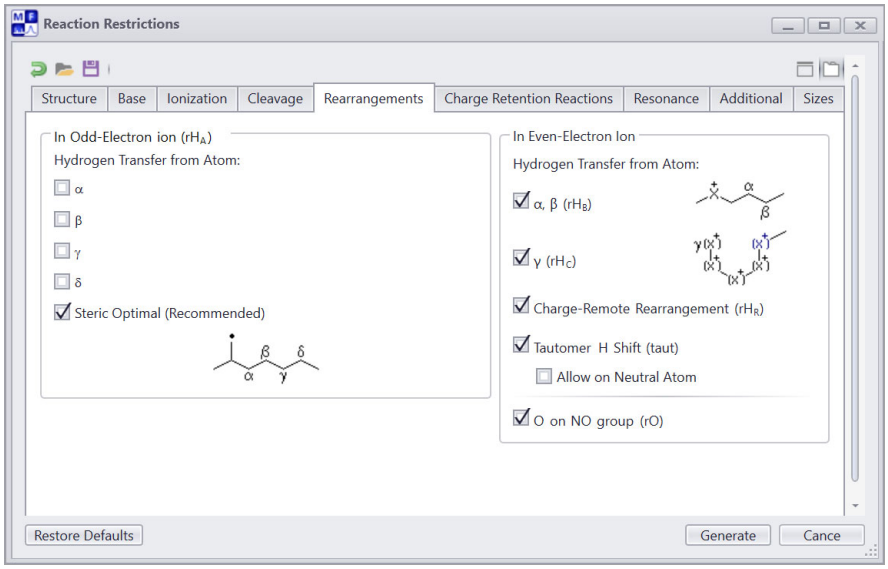


Table 142. H-Rearrangement page parameter descriptions

Parameter	Description
In Odd-Electron Ion [rH_A]/ Hydrogen Transfer from Atom	Specifies one of the following hydrogen transfer methods: • α • β • γ • δ • Steric Optimal
In Even-Electron Ion/Hydrogen Transfer from Atom	Specifies one of the following hydrogen transfer methods: • α, β, [rH_B] • γ [rH_C] • Charge-Remote Rearrangement [rH_R] • Tautomer H Shift (taut) • Allow on Neutral Atom: If you select this option, the application can apply this mechanism to both charged atoms and neutral atoms. If you clear this option, the application can only apply this mechanism to charged atoms. • O on NO group [rO]: Oxygen atom of an NO group or NO_2 group on an aromatic ring.

Reaction Restrictions – Charge Retention Reactions page

Use the Charge Retention Reactions page to define the reactions that are allowed on neutral parts of the structure of interest.

Figure 137. Charge Retention Reactions page of the Reaction Restrictions dialog box

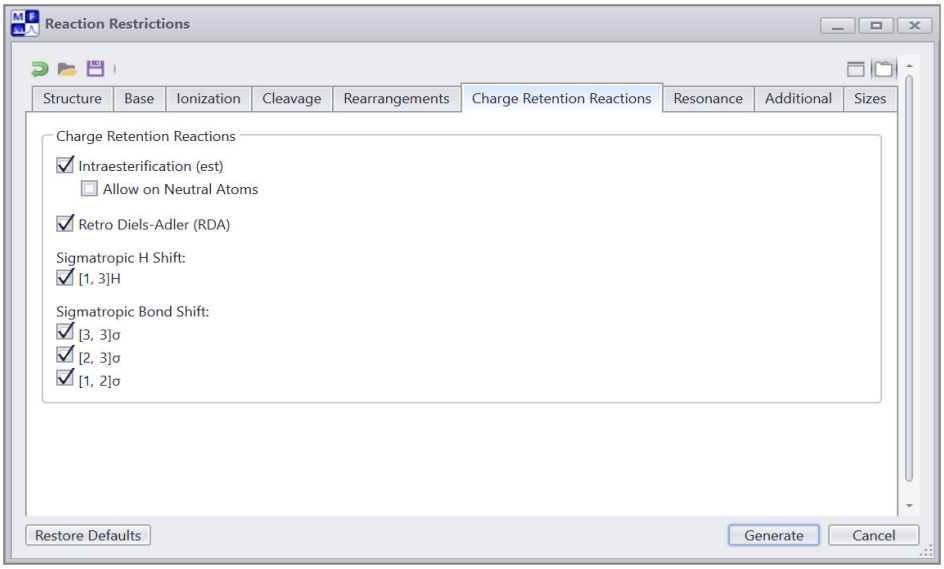


Table 143. Charge Retention Reactions parameters

Parameter	Description
Intraesterification [est]	Esterification between two groups on the same ring.
Allow on Neutral Atoms:	Choose this option to allow esterification on neutral atoms.
Retro Diels-Adler [RDA]	Use this option to perform a RDA type of charge retention reaction.
Sigmatropic H Shift	Use this option to perform a sigmatropic [1, 3]H shift type of charge retention reaction.
Sigmatropic Bond Shifts	Use this option to perform a sigmatropic bond shifts between the following atom positions: • [3, 3]σ • [2, 3]σ • [1, 2]σ

Reaction Restrictions – Resonance page

The application generates fragmentation and rearrangement mechanisms along with electron shift reactions (resonance reactions). Because these reactions can cause a large number of by-products (even for relatively small structures), by default, the resonance reactions are not depicted.

To keep the reaction network simple, the application reduces reaction complexity by not displaying resonance reactions. As a result, elementary reaction steps that include resonance reactions are merged into a single step.

(Optional) If you want to display all resonance reactions instead of the reduced mechanisms, choose **Yes** in the Display Resonance Reactions section of Resonance page.

Figure 138. Resonance page of the Reaction Restrictions dialog box

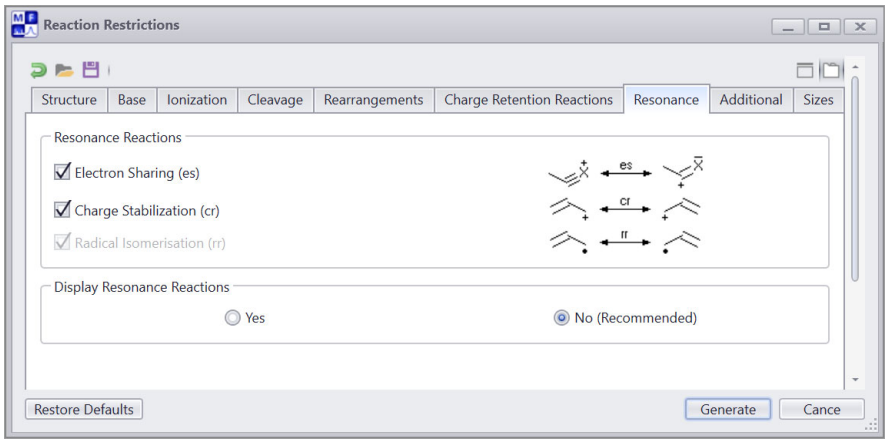
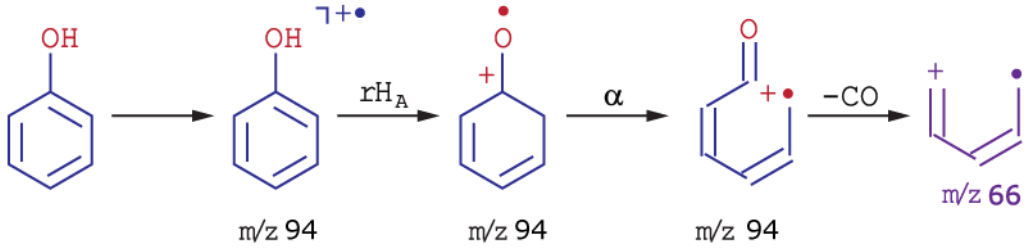


Table 144. Resonance page parameter descriptions

Parameter	Description
Resonance Reactions	
Specifies any of the following reactions:	
Electron Sharing [es]	Available for all ionization methods.
Charge Stabilization [cr]	Available for all ionization methods.
Radical Isomerization [rr]	Available only for the electron impact ionization method.
Display Resonance Reactions	
Yes	Displays all resonance reactions.
No (Recommended)	Does not display resonance reactions.

Reaction Restrictions – Additional page

By default, the application does not activate mechanisms that cleave the bonds of aromatic rings. However, you might activate the bond cleavage of aromatic rings when working with small aromatic compounds. For example, when you fragment phenol, a fragment with an m/z of 66 can result. However, this fragment only forms if the bonds of the phenol aromatic ring are cleaved.



By default, the cleavage of aromatic systems is deactivated because of the large quantity of fragments that can be generated from large aromatic compounds, and because the aromatic bonds are very strong.

Figure 139. Additional page of the Reaction Restrictions dialog box

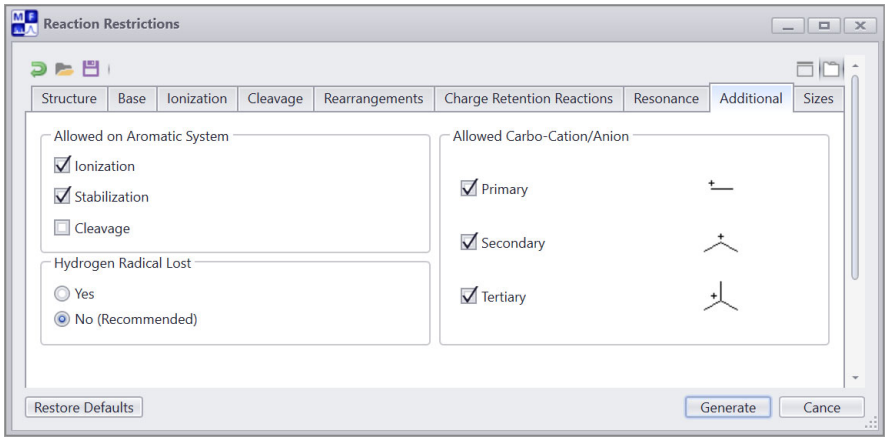


Table 145. Additional page parameter descriptions (Sheet 1 of 2)

Parameter	Description
Allowed on Aromatic System	
Specifies any of the following reactions: • Ionization • Stabilization • Cleavage	
Hydrogen Radical Lost	

Table 145. Additional page parameter descriptions (Sheet 2 of 2)

Parameter	Description
Yes	Allows hydrogen radical lost.
No (Recommended)	Does not allow hydrogen radical lost.
Allowed Carbo-Cation/Anion	
Specifies any of the following: • Primary • Secondary • Tertiary	

Reaction Restrictions – Sizes page

On the Sizes page in the Reaction Restrictions dialog box, you can limit the size and complexity of a generated reaction pathway.

Increasing the maximum number of reaction steps can exponentially increase the number of fragments produced for a specified reaction path.

Tip Generally, keep this number small, and if you must generate additional fragments, you can select individual fragments to use as starting points for additional reactions.

Large and structurally complicated molecules can produce an enormous number of reactions, but generating all of these possible reactions consumes a lot of memory. This memory consumption limits the number of reactions that the application can temporarily generate. If the maximum number of generated reactions is reached, the generation stops and the application displays an warning message, but the fragments and mechanisms generated up to that point are displayed. The most important fragments are generated first, so even if a generation stops, the most important fragments have likely been generated.

Note If you are missing an important fragment because the generation was interrupted, you can increase the reactions limit.

Figure 140. Sizes page of the Reaction Restrictions dialog box

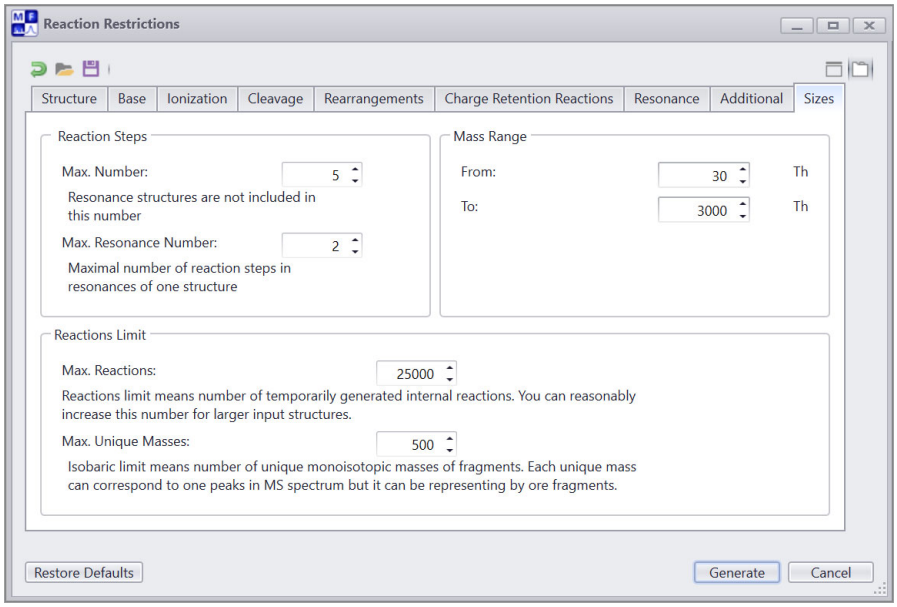


Table 146. Sizes page parameter descriptions (Sheet 1 of 2)

Parameter	Description
Reaction Steps	
Max. Number	Specifies the maximum number of cascaded fragment reactions. Default: 5
Max. Resonance Number	Specifies the maximum number of reaction steps that involve the resonance structures of a single structure. Default: 2
Reactions Limit	
Max. Reactions	Specifies the maximum number of temporarily generated internal reactions. Default: 25 000; range 1 to 100 000
Max. Unique Masses	Specifies isobaric means number of unique monoistopic masses of fragments. Each unique mass can correspond to one peak in an MS spectrum but it can be representing by more fragments. Default: 500

Table 146. Sizes page parameter descriptions (Sheet 2 of 2)

Parameter	Description
Mass Range	
Specifies the size range of the generated fragments.	
From	Specifies the lower end of the *m/z* range. Default: 30; range: 30 to 3 000 Th
To	Specifies the upper end of the *m/z* range. Default: 3 000; range: 30 to 3 000 Th

Save reaction restrictions to a file

❖ **To save the reaction restrictions for the fragment generation process to a file**

1. Specify the restrictions for all the pages of the Reaction Restrictions dialog box.
2. Click the **Save** icon,  .

   The Save Reaction Restrictions dialog box opens.
3. Name the file and click **Save**.

Note The extension for a reaction restrictions file is .hammer.

You can import these restriction settings when you generate fragments for similar compounds that were acquired with the same ionization method.

Import reactions restrictions from a file

❖ **To import the reaction restrictions for the fragment generation process from a file**

1. Click the **Open Parameters from a File** icon,  .

   The Open Reaction Restrictions dialog box opens.
2. Select a reaction restrictions file (.hammer) and click **Open**.

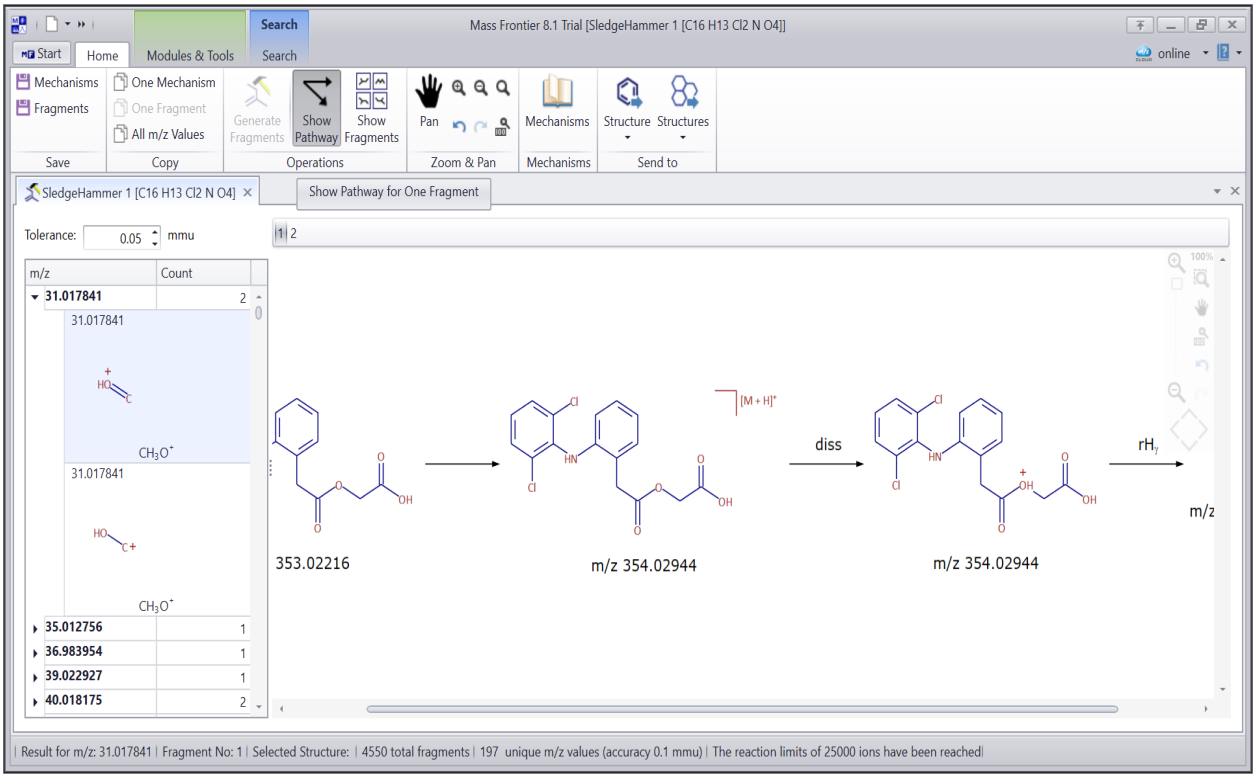
   The application overwrites all the parameter settings in the Reaction Restrictions dialog box with the settings in the file.
3. (Optional) If you are importing the reaction restrictions into the Reaction Restrictions view of the Global Settings dialog box, click **OK**.

   All changes in the Reaction Restrictions view take effect after the regeneration of fragments and mechanisms in any opened Reaction Restrictions dialog boxes and affect all subsequent generations.

Work in a SledgeHammer window

When the application finishes a reaction-generating process, a SledgeHammer window opens. You cannot directly open this window from any menu or toolbar. A SledgeHammer window displays either the complete mechanisms of the ion-decomposition reactions (**Show Pathway**) or only the resulting fragments (**Show Fragments**).

Figure 141. SledgeHammer window



To work with the SledgeHammer window, see these topics:

- SledgeHammer toolbar
- Display the fragmentation pathways or isobaric fragments for an m/z value
- Customize the list of isobaric m/z values
- Save fragments to a file
- Copy fragments or mechanisms to the Clipboard
- Start the fragmentation of a selected fragment
- Send fragments to other modules from the SledgeHammer module

SledgeHammer toolbar

Figure 142. SledgeHammer window toolbar

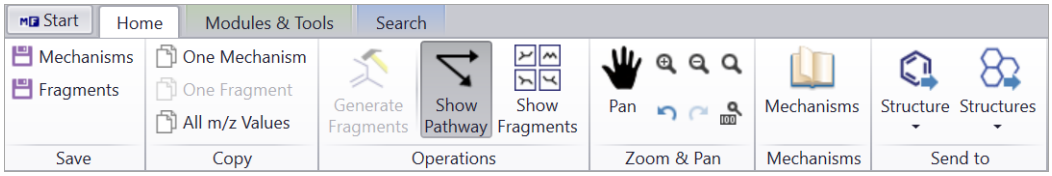


Table 147. SledgeHammer toolbar descriptions (Sheet 1 of 2)

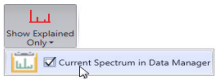
Button or icon		Description
Save		
Mechanisms		Saves the selected mechanism to a file (.mechanisms).
Fragments		Saves the fragments to an SDF file.
Copy		
One Mechanism		Copies the selected mechanism in both graphic format (Enhanced Windows Metafile) and data format.
One Fragment		Copies the selected fragment in graphic format (Enhanced Windows Metafile) and internal data format, MDL, and MOL data format, that can be pasted in another program that supports this format.
All m/z Values		Copies all unique *m/z* values.
Operations		
Generate Fragments		Generates fragments from the selected fragment.
Show Pathway		Shows the pathways for the selected fragment.
Show Explained Only > Current Spectrum in Data Manager		Displays only the fragments that are explained in the currently selected spectrum. See "Eliminate generated fragments that are not present in a spectrum" on page 312. Available when a Data Manager window is opened.
Show Fragments		Shows all isobaric fragments.
Zoom & Pan		
Pan		Pan

Table 147. SledgeHammer toolbar descriptions (Sheet 2 of 2)

Button or icon		Description
Zoom in		Zoom in
Zoom out		Zoom out
Zoom to area		Zoom to area
Reset zoom		Reset zoom
Undo		Undo
Mechanisms		
Mechanisms		Opens a new Reaction Mechanisms Overview window for viewing the general fragmentation rules.
Send To		
Structure		Sends a single selected structure to the selected module.
Structures		Sends multiple selected structures to the selected module.

Display the fragmentation pathways or isobaric fragments for an *m/z* value

The application can generate several possible isobaric fragments for a particular *m/z* value. When the application generates more than one fragment for an *m/z* value, it displays each numbered fragment separately.

The application sorts isobaric fragments according to the simplicity of their fragmentation mechanism, so fragment 1 is produced by the simplest (shortest) mechanism. The isobaric fragments are usually isomers of the same fragments with a different charge, radical, or π bond location.

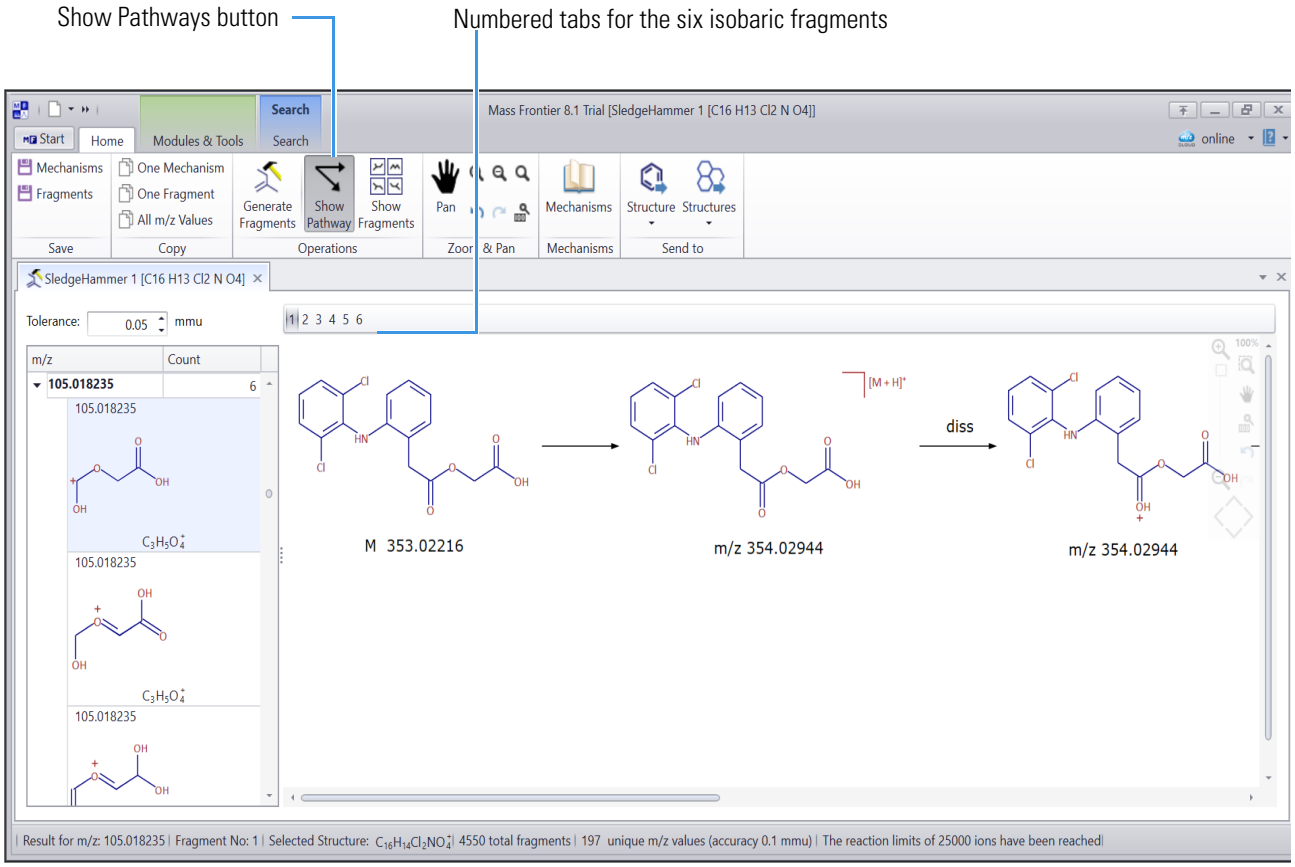
❖ To display the fragmentation pathways or isobaric fragments for an *m/z* value

1. Click **Show Pathways** in the SledgeHammer toolbar.
2. In the left pane of the SledgeHammer window click the row for the *m/z* value of interest.
3. To view the individual pathways for the isobaric fragments, do either of the following:

- In the left pane, click the down arrow to the left of the *m/z* value.
- A list of isobaric fragments appears.
- Click a fragment to display its pathway.

- Click through the numbered tabs at the top left of the display pane.

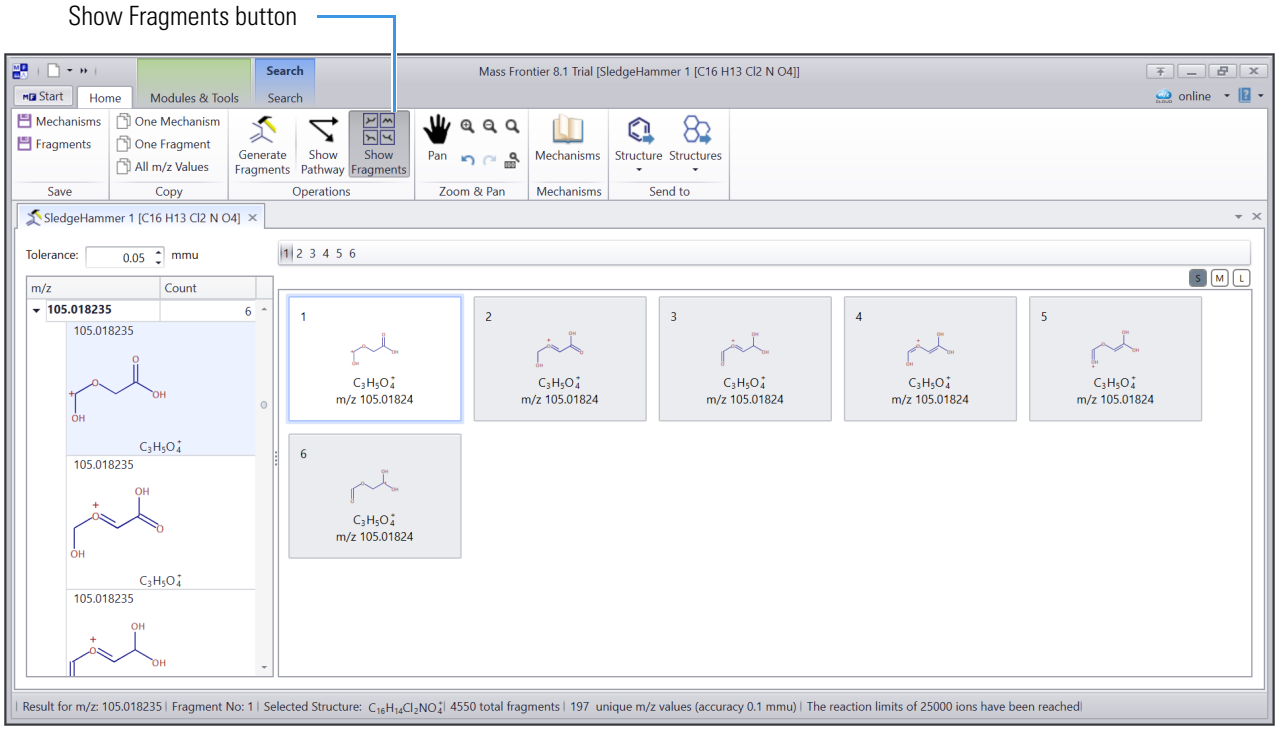
Figure 143. Pathway for an *m/z* value with six possible isobaric fragments



4. To display the isobaric fragments for an *m/z* value, click **Show Fragments** in the SledgeHammer toolbar.

   The isobaric fragments appear as cards in the display pane.

Figure 144. Pathway for an *m/z* value with six possible isobaric fragments



Customize the list of isobaric m/z values

The fragments are grouped in the table on the left side of the SledgeHammer window by the defined tolerance. If you change the tolerance value, the application automatically regroups the fragments using the new value.

An isobaric group can contain any number of fragments. However, to avoid a slow software response, a maximum of 256 fragments (first 256) are displayed. The fragments in an isobaric group are sorted based on the *m/z* value (depending on the defined tolerance, multiple *m/z* values can be in one isobaric group) and by the depth of graph (the distance between the final fragment and the root). The fragments that are closer to the root are more probable and are preferred.

Save fragments to a file

In a SledgeHammer window, you can save all the fragments to an SDF file. You can open an SDF file in any Mass Frontier module that accepts a structure file or in any Windows application that accepts this structure file type.

❖ To save all the fragments to an SDF file

1. In the SledgeHammer window, click **Fragments** in the Save toolbar group.
2. Select the folder where you want to store the file, name the file, and then click **Save**.

Save mechanisms to a file

In a SledgeHammer window, you can save the mechanisms to a .mechanisms file. You can open this file type only in a SledgeHammer window.

❖ To save mechanisms to a file

1. In the SledgeHammer window, click **Mechanisms** in the Save toolbar group.
2. Select the folder where you want to store the file, name the file, and then click **Save**.

Copy fragments or mechanisms to the Clipboard

❖ To copy fragments or mechanisms to the Clipboard

Do any of the following:

- To copy a mechanism to the Clipboard, display the mechanism in the Fragments & Mechanisms window, and then click **One Mechanism** in the Copy group of the SledgeHammer toolbar.

  Tip You can paste this image to a Microsoft Office document as an editable image or to a vector drawing application as a vector drawing. To edit the drawing in a Microsoft office document, right-click the image and choose **Edit Picture**. At the prompt, click **Yes**.

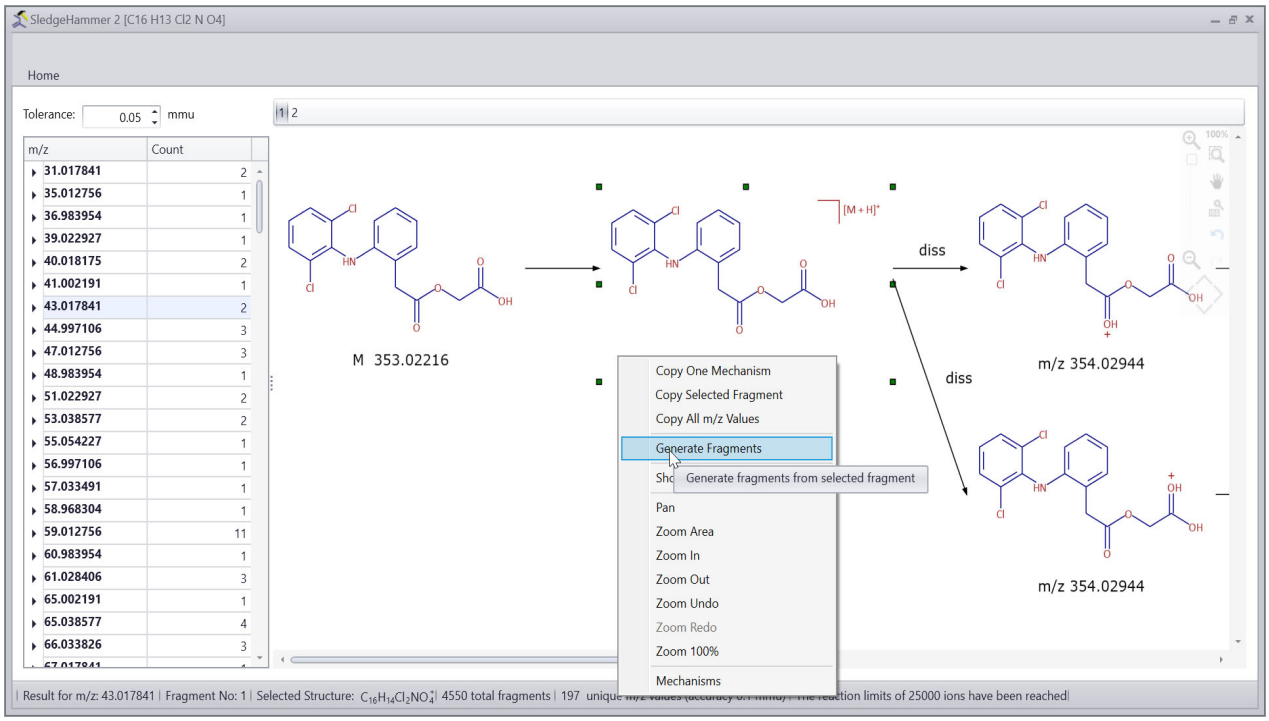
- To copy a single fragment to the Clipboard, select the fragment, and then click **One Fragment** in the Copy group of the SledgeHammer toolbar.
- To copy all the fragments listed in the SledgeHammer window, click **All m/z Values** in the Copy group of the SledgeHammer toolbar.

Start the fragmentation of a selected fragment

To start the fragmentation of a selected fragment in a SledgeHammer window

1. In the SledgeHammer window, right-click the fragment and choose **Generate Fragments**.

Figure 145. Shortcut menu for the SledgeHammer window



The Reaction Restrictions dialog box opens.

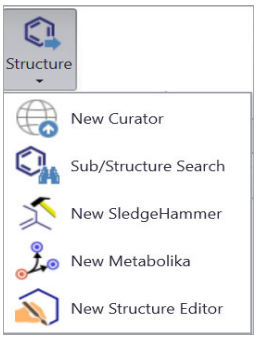
2. To generate fragments, do one of the following:
   - Specify the appropriate parameter settings on the pages of the Reaction Restrictions dialog box. See "Reaction Restrictions dialog box" on page 286.
   - Click the **Open Parameters from a File** icon, , to open a file (.hammer) with the stored settings for the reaction restrictions.
3. Click **Generate**.

Send fragments to other modules from the SledgeHammer module

You can send a selected fragment or all the generated fragments to another module that is able to process them.

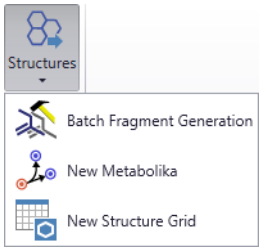
From a SledgeHammer window, you can send a single structure to the following:

- A new Curator window
- A new SledgeHammer window (to generate fragments)
- A Sub/Structure Search
- A new Metabolika window
- A new Structure Editor window (for editing)



You can send multiple structures to the following modules:

- The Batch Fragment Generation dialog box (for additional fragmentation)
- A new Metabolika window
- A new Structure Grid window



In addition, you can copy one mechanism, one fragment, or all the *m/z* values to the Clipboard.

Preview unimolecular reactions

The Mass Frontier application uses general unimolecular reactions for the prediction of fragmentation, rearrangement, and resonance mechanisms.

❖ To preview unimolecular reactions

Do one of the following:

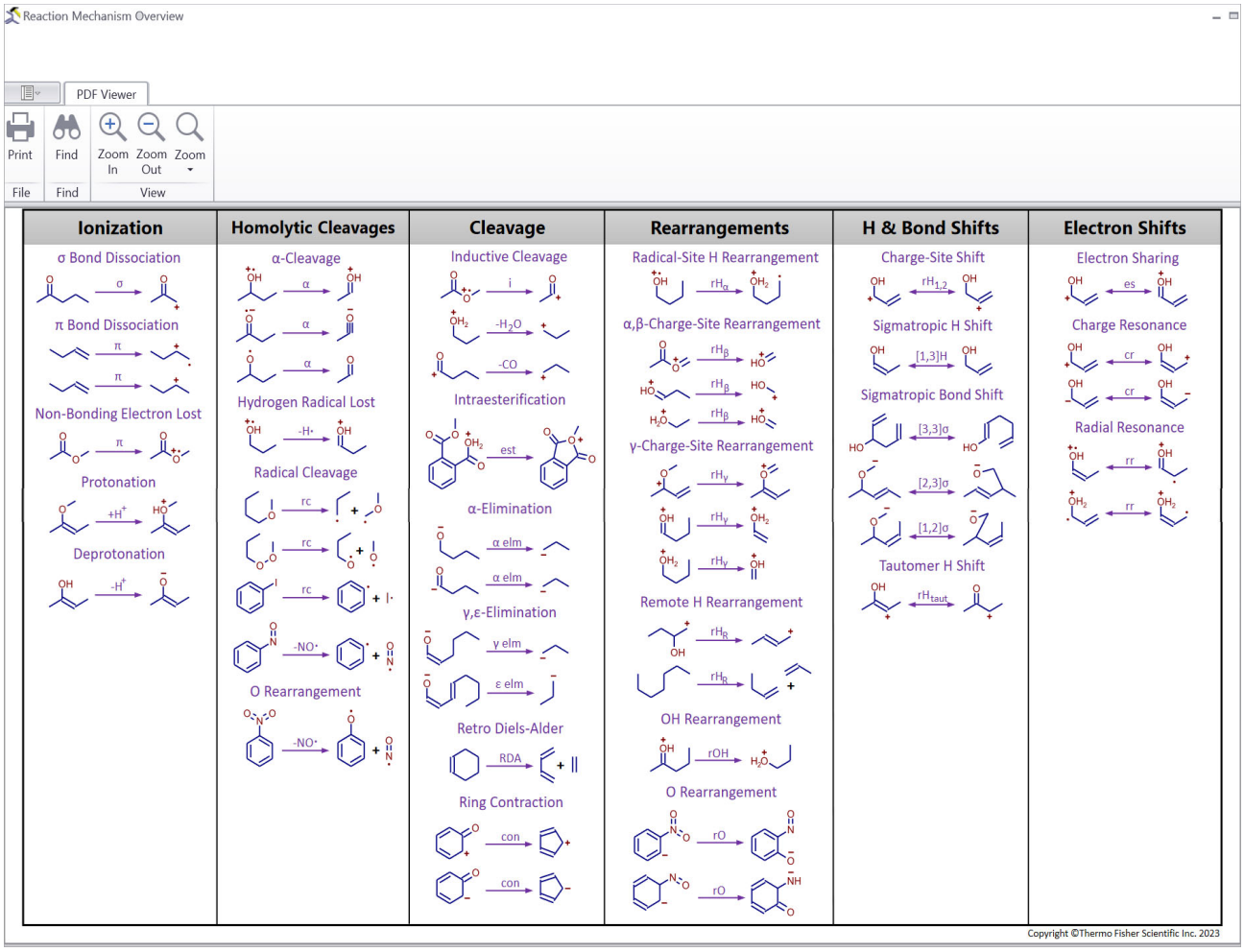
- In a SledgeHammer window, click **Mechanisms** in the toolbar.

–or–

- Click the **Modules & Tools** tab. Then, click **Reaction Mechanism Overview**.

The Reaction Mechanisms Overview window opens.

Figure 146. Reaction Mechanism Overview window



Reaction formalism

Table 148 describes the reaction formalism (description of mathematical or logical terms) used in the Mass Frontier application.

Table 148. Reaction formalism (Sheet 1 of 3)

Symbol	Convention
Ionization	
σ	Sigma bond dissociation
π	Pi bond dissociation
$+H^+$	Protonation
$-H^+$	Protonation

Table 148. Reaction formalism (Sheet 2 of 3)

Symbol	Convention
Homolytic Cleavages	
α	Alpha cleavage
–H•	Hydrogen radical loss
rc	Radical Cleavage
–NO•	Radical Cleavage on NO_2 or NO group
Cleavage	
i	Inductive cleavage
-H_2O	Inductive cleavage (Loss of water molecule)
-CO	Inductive cleavage (Loss of carbon monoxide)
est	Intraesterification
α elm	Alpha elimination
γ elm, ε elm	Gamma elimination, Epsilon elimination
RDA	Retro Diels-Alder reaction
con	Ring contraction
Rearrangements	
rH_α	Radical-Site H rearrangement
rH_β	α,β Charge site rearrangement
rH_γ	γ Charge site rearrangement
rH_R	Remote H rearrangement
rOH	OH rearrangement
rO	O rearrangement
H & Bond shifts	
$rH_{1,2}$	Hydrogen shift to adjacent position
[1,3]H	[1,3] Sigmatropic H shift
[3,3]σ	[3,3] Sigmatropic Bond shift
[2,3]σ	[3,3] Sigmatropic Bond shift in negative mode
[1,2]σ	[3,3] Sigmatropic Bond shift in negative mode
rH_{taut}	Tautomer H shift
Electron Shifts	
es	Electron sharing

Table 148. Reaction formalism (Sheet 3 of 3)

Symbol	Convention
cr	Charge resonance
rr	Radical resonance

Work with generated fragments

The SledgeHammer module automatically generates fragments and detailed fragmentation and rearrangement mechanisms that you can use to annotate your chemical structures. For information about opening the SledgeHammer window, see "Generate fragments and mechanisms" on page 281.

See these topics:

- Link generated fragments with a spectrum
- Automatically assign generated fragments to a user library entry
- Eliminate generated fragments that are not present in a spectrum
- Simulate fragmentation processes in MS/MS experiments

Link generated fragments with a spectrum

You can link generated fragments to the peaks in a mass spectrum as follows:

If you start the generation of fragments and mechanisms from a Data Manager window, the generated fragments are automatically linked to the peaks in the selected spectrum according to their *m/z* values. After processing finishes, explained peaks appear in a different color—by default, red—in the original mass spectrum. Selecting an explained peak reveals all the mechanisms leading to the fragment that the peak represents. In addition, you can automatically assign generated fragments.

- If you start fragment generation from another module that can export structures (for example, Structure Editor, Curator, Metabolika, or Structure Grid) or directly from the Reaction restrictions dialog box, you can manually link the generated fragments in the SledgeHammer window to a mass spectrum in any opened Data Manager window. The application highlights peaks (by default, in red) that have the same *m/z* values as the generated fragments. Selecting a highlighted peak reveals all the pathways leading to the fragment that the peak represents.

Automatically assign generated fragments to a user library entry

❖ **To automatically assign generated fragments to a mass spectrum**

1. Generate fragments (See "Generate fragments and mechanisms" on page 281.) and leave the SledgeHammer window open.
2. Open a Data Manager window.
3. In the Libraries tab bar, select the user library that contains the entry you want to annotate.
4. In the user library, select the entry that you want to annotate.
5. In the Data Manager toolbar, click **Auto Fragment Annotation** and then select the appropriate **SledgeHammer** window from the dropdown list.

When an unexplained peak is likely to be an isotopic peak of an explained peak, it is depicted in a third color—by default, green. Selecting such a peak reveals all the mechanisms leading to the fragment that can produce that isotopic profile.

IMPORTANT The fragment predictability range is usually between 50 and 90 percent.

The application cannot predict energies and barriers in ionized molecules, which prevents the prediction of some of the peaks in a mass spectrum. In addition, some fragments are the result of compound-specific mechanisms that cannot be applied generally when proposing fragmentation and rearrangement pathways.
Tip If you suspect a compound-specific mechanism is responsible for fragment formation, verify your assumption by conducting a substructure search where you can compare the explained and unexplained peaks in the spectra retrieved by the substructure search.

Eliminate generated fragments that are not present in a spectrum

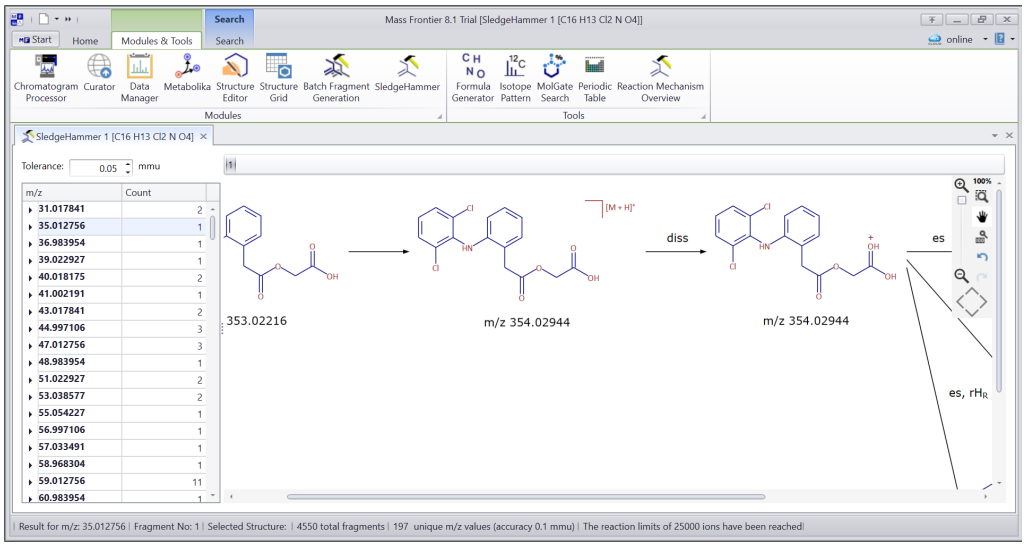
The SledgeHammer window shows all *m/z* values that have been generated with the specified Reaction Restrictions settings. When the generated fragments are linked to a spectrum because they were generated from a Data Manager window that contains the specified spectrum, you can eliminate the *m/z* values that do not have corresponding peaks in the spectrum. In some cases, the application generates a large number of theoretically possible fragments with a variety of *m/z* values; therefore, the application shows only those fragments that can be linked with a spectrum (explained peaks).

❖ **To eliminate *m/z* values that cannot be linked with peaks in a spectrum**

1. Generate a list of fragments to display a SledgeHammer window (Figure 147).

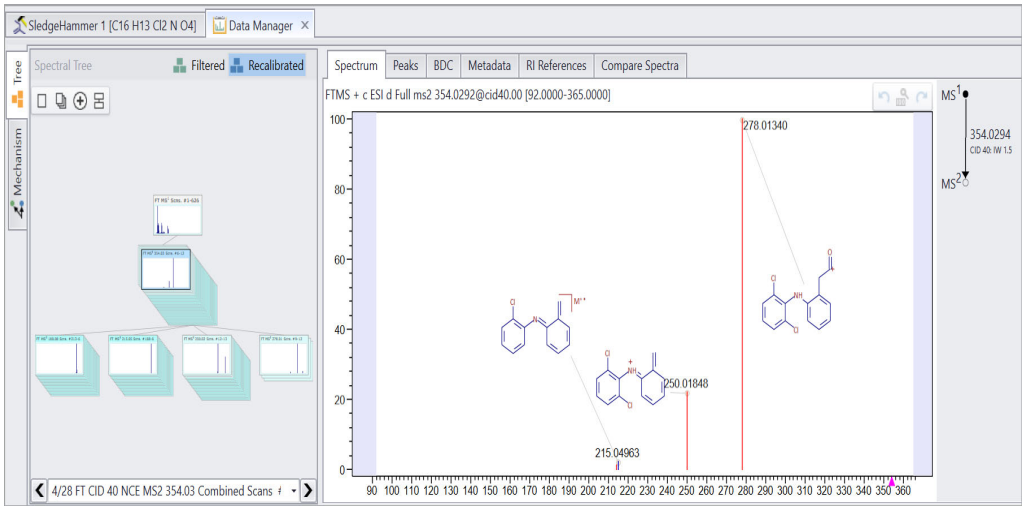
   For information about opening the SledgeHammer window, see "Generate fragments and mechanisms" on page 281.

Figure 147. SledgeHammer window from the fragmentation of aceclofenac



2. Open a Data Manager window, and then select the spectrum of interest.

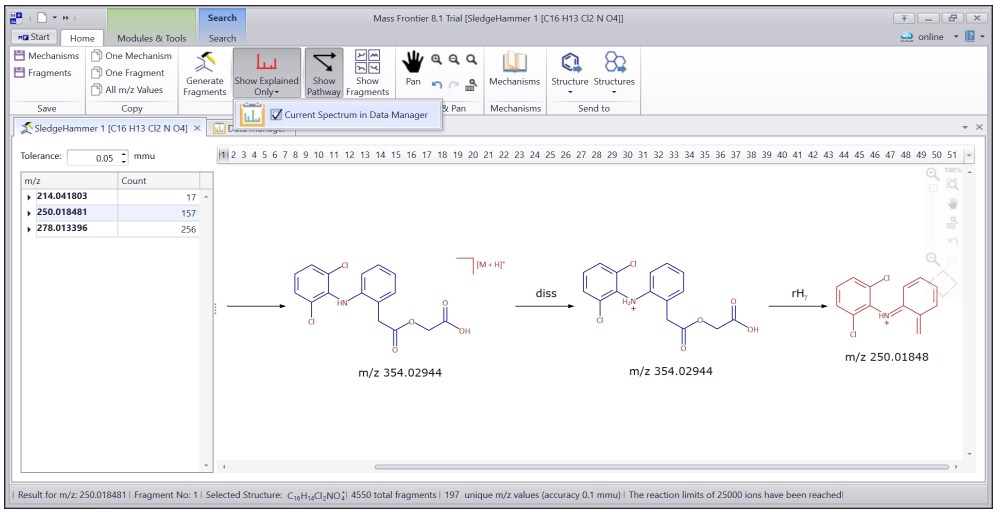
Figure 148. MS2 spectrum for aceclofenac with three explained peaks



3. In the SledgeHammer toolbar, click **Show Explained Only**, and then select the appropriate Data Manager window from the dropdown list.

   The fragments list on the left lists the fragments that are explained in the selected spectrum (Figure 149).

Figure 149. Fragments explained in the selected spectrum



Tip When you eliminate *m/z* values that do not correspond to peaks in a spectrum, these values are not permanently deleted. To restore the original display, click **Show Explained Only** and clear the Current Spectrum in Data Manager check box.

Simulate fragmentation processes in MS/MS experiments

To simulate fragmentation processes in MSn experiments, you can generate the fragments and mechanisms from neutral compounds or from ions. You can also select a product fragment (parent ion) in a SledgeHammer window and start the generation from there. You can simulate an unlimited number of consecutive secondary ion decomposition reactions.

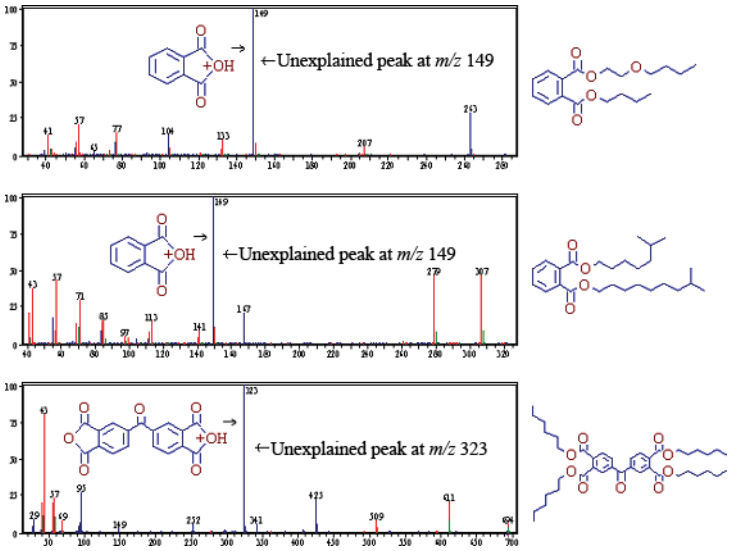
Unexplained spectral peaks from compound-specific fragmentation reactions

An unexplained, prominent peak that is present in the spectra of structurally similar compounds can be a strong indicator of a compound-specific fragmentation process. In some cases, the fact that the application cannot explain a spectral peak (because the corresponding fragment was probably formed by a compound-specific mechanism) can help in identifying the characteristic substructure that gives rise to the peak.

For example, phthalates—which are common contaminants from elasticized polymers—produce a characteristic ion at *m/z* 149, which is formed by a highly specific mechanism. The application is not able to explain this peak because its corresponding fragment is formed by an unusual sequential hydrogen rearrangement-cyclization reaction, which the application does not support.

After the generation of fragments and mechanisms of the retrieved examples, the prominent peak corresponding to the phthalate group remains unexplained in the majority of cases. For example, the prominent peak for a phthalate with a functional group at position 3, 4, 5, or 6 is shifted to a higher mass by a value that is equal to the mass of this functional group.

Figure 150. Mass spectra for phthalates with unexplained fragment peaks



To distinguish between a randomly unexplained peak and a compound-specific peak, consider running a substructure search on the spectrum to retrieve compounds that can fragment and produce the substructure that gives rise to the peak to the peak under investigation.

11

Tools

The Mass Frontier tools are functional blocks that provide additional analytical or interpretation functionality. The Formula Generator, Isotope Pattern, MolGate Search, Periodic Table, and Reaction Mechanism overview tools work in context with the data in the current active module. For example, if you open more than one instance of the Chromatogram Processor module, the tool processes the data from the active page (or floating window). You can also use the Formula Generator and Isotope Pattern tools independently of the application's modules by manually entering the required input.

Contents

Formula Generator tool

The Formula Generator tool calculates a list of theoretical molecular formulas that fit an *m/z* value. Because the number of possible molecular formulas for a specified *m/z* value is closely related to the mass tolerance, the elements used, and the maximum allowed number of atoms for each isotope, carefully evaluate these parameters before starting the formula generation. You can either manually enter the *m/z* value or have the software automatically retrieve the value from any spectral peak in the MS spectrum view of a Data Manager window or a Chromatogram Processor window. From the Formula Generator tool, you can also initiate the calculation of the isotopic pattern for any generated formula through the Isotope Pattern tool.

For details, see these topics:

- Use the Formula Generator tool
- Formula Generator parameters
- Molecular Formula Settings dialog box

Formula Generator tool with other modules

To automatically fill the m/z and Tolerance fields in the Formula Generator, select any peak in the spectra of the active module (Curator, Chromatogram Processor, or Data Manager).

To open the Isotopic Pattern tool with the simulated isotope pattern for a formula, right-click any formula in the formulas table in the Formula Generator view and choose **Isotope Pattern**.

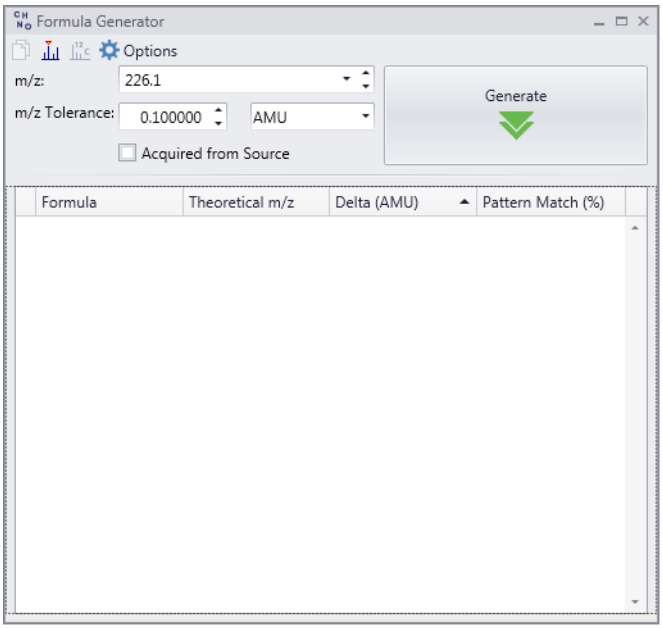
Use the Formula Generator tool

❖ To determine the possible formulas for a spectral peak or an m/z value

1. Open the Formula Generator tool as follows:
   a. In the application tab bar, click the **Modules & Tools** tab.
   b. In the Tools group of the Modules & Tools toolbar, click **Formula Generator**.

      The Formula Generator view opens to the left of the tabbed module pages.

      Figure 151. Formula Generator tool default parameter settings



2. (Optional) To modify the options for calculating the possible formulas, do the following:
   a. Click **Options**.

      The Molecular Formula Settings dialog box opens.
   b. Modify the settings as applicable.
3. To generate a list of the possible formulas do one of the following:
   a. To generate a list of formulas for a specific m/z value, type or paste the value in the m/z box.

b. Specify the mass tolerance for the calculation in the m/z Tolerance box.

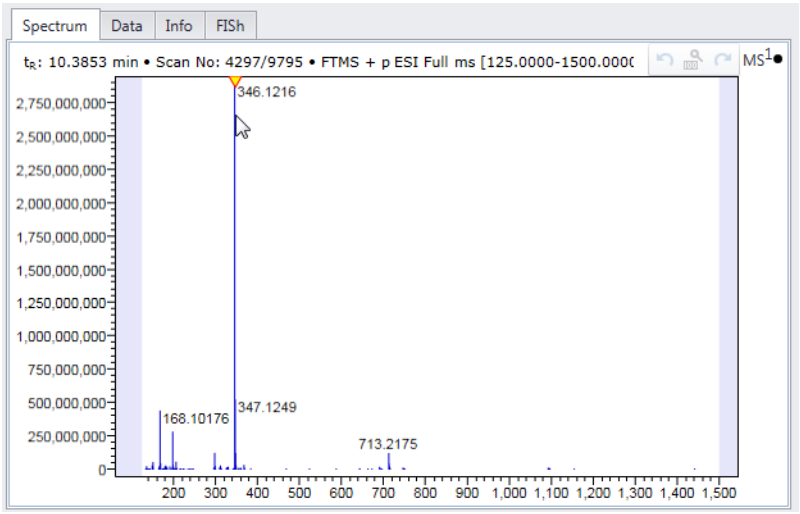
c. Click **Generate**.

–or–

a. To generate the possible formulas for a spectral peak, select the **Acquired from Source** check box to use the mass tolerance from the instrument. Or, clear this check box, and instead, type the mass tolerance in the m/z Tolerance box and select the units from the dropdown list to the right.

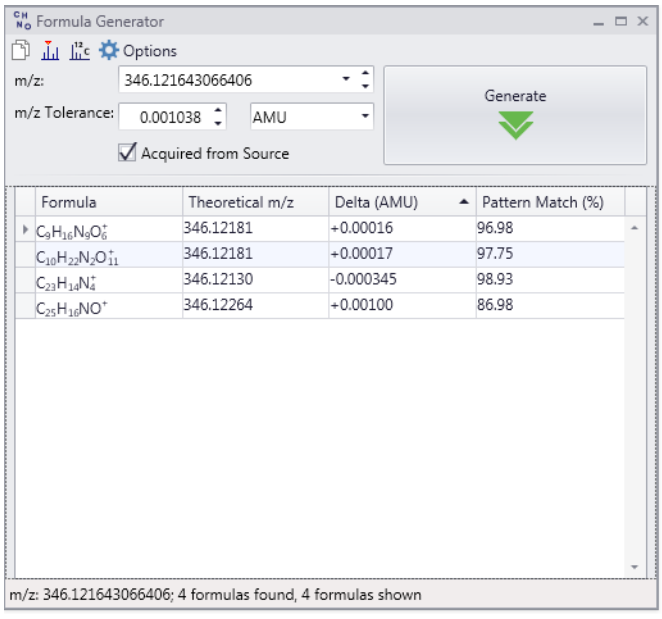
b. Click the **Pick Peak** icon,  .

c. Click the peak of interest in the MS spectrum view.



The application calculates the possible formulas for the selected peak.

Figure 152. Formulas for a selected peak on the Spectrum page of the MS spectrum view



4. To copy the result to the Clipboard, select the rows of interest in the formula table. Then, right-click the table and choose **Copy**.
5. To view the isotope pattern for any of the possible formulas, select the formula, and then click the Isotope Pattern icon,  .

   The Isotope Pattern view opens and displays the isotope pattern for the selected formula.

Formula Generator parameters

Table 149. Formula Generator parameter descriptions

Icon or parameter		Description
Copy Result		Copies the result to the Clipboard.
Pick Peak		Activates the Pick Peak feature for selecting a peak in any MS spectrum view.
Isotope Pattern		Opens the Isotope Pattern tool and populates the Formula box with the selected formula.
Options		Opens the Molecular Formula Settings dialog box. See "Molecular Formula Settings dialog box" on page 320. Use this dialog box to change the parameter settings for calculating the possible formulas.
m/z		Specifies the *m/z* value of the unknown formula.
m/z Tolerance		Specifies the tolerance value and units for the calculation of possible formulas.
Acquired from Source		When selected, the *m/z* value and tolerance are automatically retrieved from the characteristics of the selected peak.
Generate		Starts the calculation.

Molecular Formula Settings dialog box

Use the Molecular Formula Settings dialog box to speed up calculations, restrict the number of possible formulas, or set the charge, state, and polarity for the Formula Generator tool. See "Use the Formula Generator tool" on page 318.

Figure 153. Molecular Formula Settings dialog box

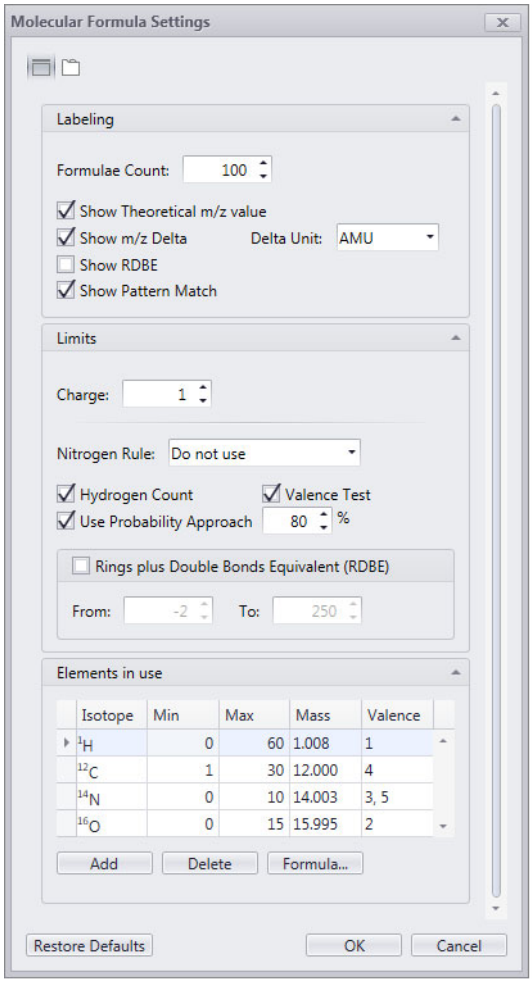


Table 150. Molecular Formula Settings dialog box parameter descriptions (Sheet 1 of 3)

Parameters	Description
Labeling	
Formula Count	Limits the number of formulas displayed in the formula list. Default: 100 Limit: 1 to 255
Show Theoretical m/z value	Displays the Theoretical *m/z* column in the formula list.
Show *m/z* Delta and the units of display (Delta Units)	Displays the Delta (units) column in the formula list. Units: AMU, MMU, PPM, Resolving Power
Show RDBE	Displays the Ring Double Bond Equivalents (RDBE) column in the formula list.

Table 150. Molecular Formula Settings dialog box parameter descriptions (Sheet 2 of 3)

Parameters	Description
Show Pattern Match	Displays the Pattern Match (%) column in the formula list. The Formula Generator calculates the pattern match if you select an *m/z* value by using the Pick Peak function. This value represents the similarity between the formula's theoretical isotopic profile and the mass spectrum where you picked the mass peak (see Figure 154 on page 324).
Limits	
Charge	Specifies the charge state of the ion. Default value: +1 Limit: -7 to +8
Nitrogen Rule	Specifies whether to use the nitrogen rule in the elemental composition calculation. The selections include: • Do not use • Even-electron ion (for example, radical-cation) • Odd-electron ion (for example, protonated)
Hydrogen Count	Specifies whether to use an algorithm for the exclusion of implausible formulas with improbably high numbers of hydrogens.
Valence Test	Specifies whether to exclude formulas if the atoms cannot connect using valences common in organic chemistry.
Probability Approach	Use empirical rules to determine the likelihood of the generated formula based on the ratio of carbon versus hydrogen and carbon versus heteroatoms. A higher value increases the weight of the empirical threshold. Default value: 80%
Ring plus Double Bond Equivalent (RDBE)	Displays only formulas for which RDBE is within the From-To range. RDBE limits the calculated formulas to those that make sense from a chemical perspective.
Elements in Use	
Specifies whether the application considers isotopes of particular elements with a maximum number when calculating formulas.	

Table 150. Molecular Formula Settings dialog box parameter descriptions (Sheet 3 of 3)

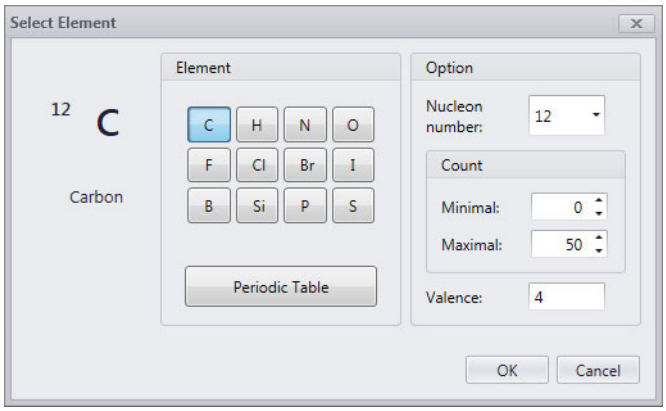
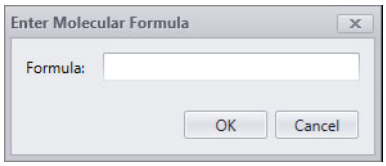
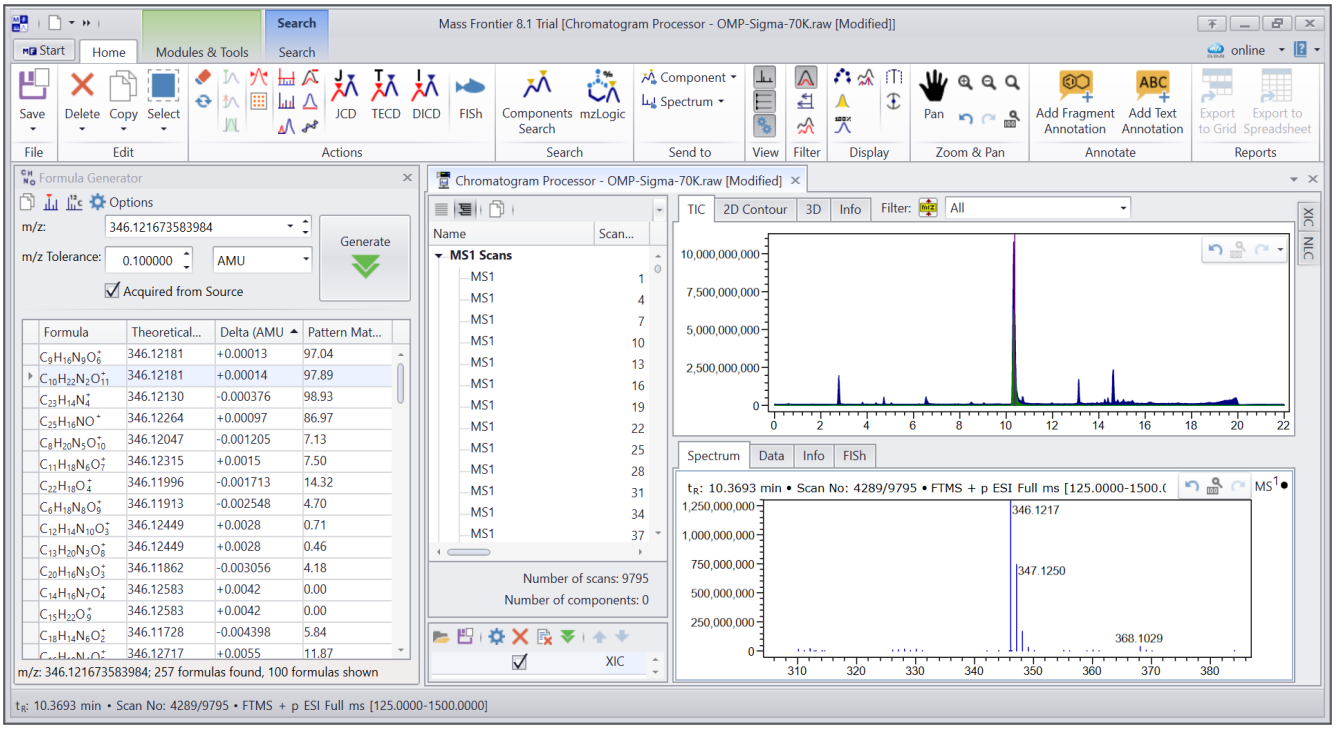
Parameters	Description
Add	Opens the Select Element dialog box for specifying a new element. The application uses the new element when calculating formulas. 
Delete	Removes the selected element from the Elements in Use list.
Formula	Opens the Enter Molecular Formula dialog box where you can specify a new formula. The application uses the new formula to derive upper limits for individual elements and considers these limits when it calculates formulas. 

Figure 154 shows the results for the peak from the protonated ion of omeprazole in scan 4272 of the OMP-Sigma-70K.raw example file.

Figure 154. Results for the peak picked in scan 4272of the OMP-Sigma-70K.raw example file



Isotope Pattern tool

The Isotope Pattern tool simulates the relevant isotopic profiles for a molecular formula.

You can choose to display up to three different pattern simulations:

- Pattern: Theoretical MS spectrum based on the mathematically exact calculation of the *m/z* distribution.
- Profile: MS spectrum for the isotope pattern based on the profile acquisition mode with the defined tolerance settings.
- Centroid: MS spectrum for the isotopic pattern based on centroided data with the defined tolerance settings.

The tool also displays the isotope pattern in a tabular format with individual *m/z* values and relative intensities corresponding to the isotopic formula.

Supported data formats:

- Plain text *.txt to export profiles in a table format
- Plain text *.csv file that contains a list of data to export to the Clipboard

- A generic*.dat file

For details, see these topics:

- Isotope Pattern tool with other modules
- Use the Isotope Pattern tool
- Isotope Pattern parameters

Isotope Pattern tool with other modules

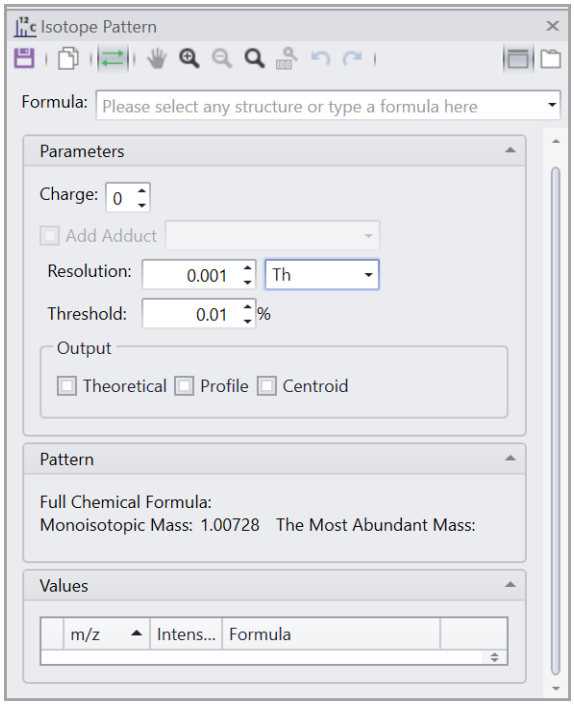
The application automatically populates the Formula box with the formula of the selected structure in an active module (Curator, Structure Editor, Chromatogram Processor, Metabolika, Structure Grid, and Data Manager). You can also populate the Formula box by right-clicking a formula in the Formula Generator tool and choosing Isotope Pattern.

Use the Isotope Pattern tool

❖ To display the isotope pattern for an ion

1. Do one of the following:
   - In the Modules & Tools toolbar, click **Isotope Pattern**, and then enter the formula in the Formula box.

     Figure 155. Isotope Pattern tool without a structure or formula



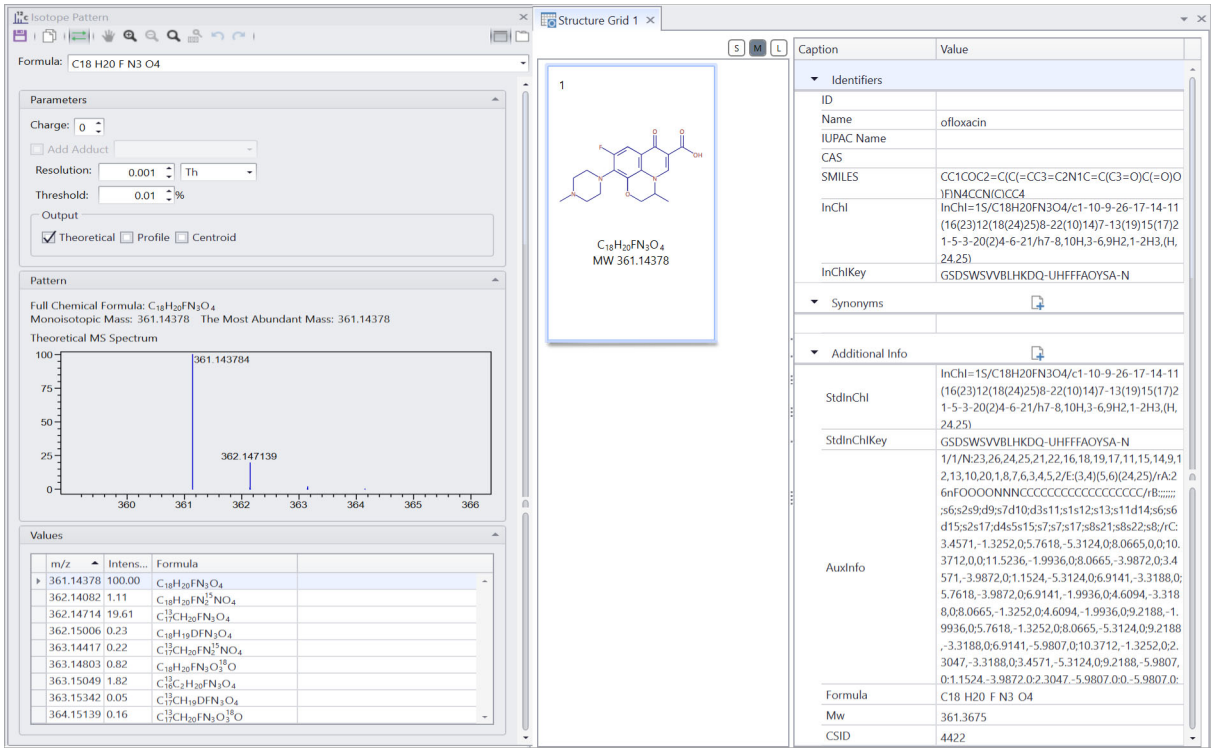
- In the Formula Generator tool, right-click a formula and choose **Isotope Pattern**. Or, in any of the modules where you can select a structure, select the structure of interest, and then click **Isotope Pattern** in the Modules & Tools toolbar. If the application does not automatically synchronize with the structure in the active module, click the **Synchronize Isotope Pattern with the Active Source** icon, .

  The application automatically populates the Formula box.

2. To specify the adduct ion, select the **Add Adduct** check box, and then select the adduct from the list to the right.
3. Specify the resolution and intensity threshold for the isotope pattern.
4. In the Output area, select the pattern types that you want to display.

   Figure 156 shows the isotope pattern for the oxoflaxcin.

   Figure 156. Isotope pattern for the oxoflaxcin



5. To copy the picture or the table do the following:
   a. To copy the plotted pattern to a Microsoft Office document, click the **Copy Data to Clipboard** icon, , and choose the plot type.
   b. Open the document and choose **Paste > Paste Special**.
   c. In the Paste Special dialog box, select **Picture (Enhanced Metafile)** and click **OK**.

   Note You can also copy the image to a vector drawing application.

a. To copy the table to a Microsoft Office document, click the **Copy Data to Clipboard** icon, , and choose **Copy Table**.

b. Open the document and choose **Paste**.

Isotope Pattern parameters

Table 151. Isotope Pattern tool parameter descriptions (Sheet 1 of 2)

Parameter or icon	Description
Save	Converts the Pattern, Profile, and Centroid isotopic profiles to a tabular form and saves this information to a plain text file (.txt) or CSV file or DAT file.
Copy	Copies the output profiles as a vector drawing to the Clipboard. Or, copies the Values table with individual isotopic formulas to the Clipboard. Use Paste Special to paste the image to a Microsoft Office document.
Refresh	Recalculates the isotopic pattern if the active structure is changed.
Pan	Switches your cursor into pan mode.
Zoom in	Zooms in the spectra with predicted patterns.
Zoom out	Zooms out the spectra with predicted patterns.
Zoom to area	Zooms into a specified rectangular area in the spectra with predicted patterns.
Reset zoom	Resets the scale.
Undo	Reverts last zoom operation.
Redo	Restores last zoom operation.
Charge	Overrides the formal charge of the formula. Selecting a value enables the Add Adduct check box and the adducts list to the right.
Resolution	Defines the value and unit for the resolution used to simulate the profile and centroid spectra.
Threshold	Defines the minimum relative intensity of the simulated peaks.

Table 151. Isotope Pattern tool parameter descriptions (Sheet 2 of 2)

Parameter or icon	Description
Output	Select one or more of these check boxes to display the MS spectrum of the isotope pattern. • Theoretical • Profile • Centroid
Values	Displays the *m/z* values, intensities, and formulas of the isotopic peaks in the spectrum.

MolGate Search tool

Use the MolGate Search tool to create a list of structural candidates for a specific *m/z* value that you can then send to other modules, such as a Structure Grid, Metabolika, or Batch Fragment Generation window.

❖ **To run a MolGate search for structure candidates and send these candidates to a structure grid**

1. In the Modules & Tools toolbar, click **MolGate Search**.

   The mzLogic MolGate Tool opens.

2. Type an *m/z* value in the m/z box between 50.00000 to 2000.00000.
3. Specify the accuracy between 0 to 30 ppm or the *m/z* value.
4. Select the adducts from the drop down and click **OK**.
5. Select the public databases and any other opened modules with structures that you want the tool to search from the drop down menu and click **OK**.

   -or

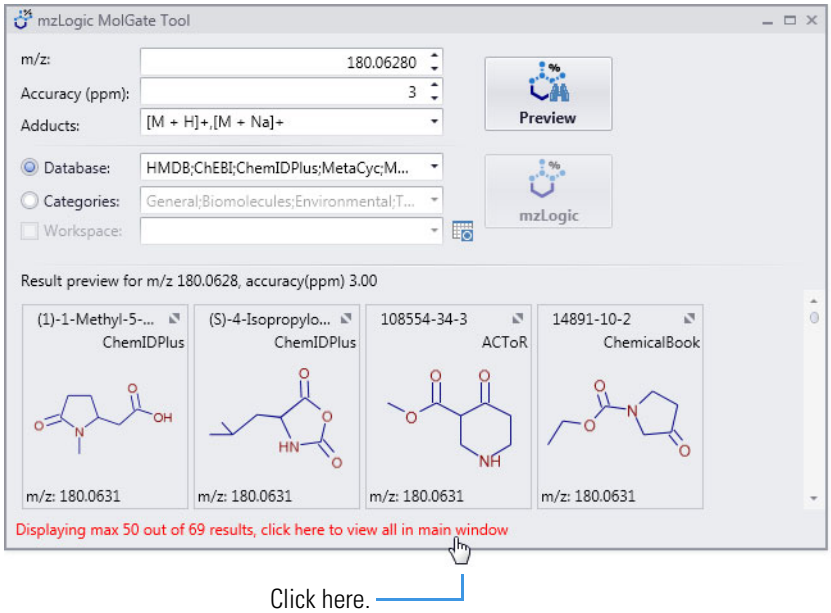
   Select one or more Categories from the drop down menu and click **OK**.

6. Click **Preview**.

   The tool searches the databases and retrieves up to 50 structure candidates.

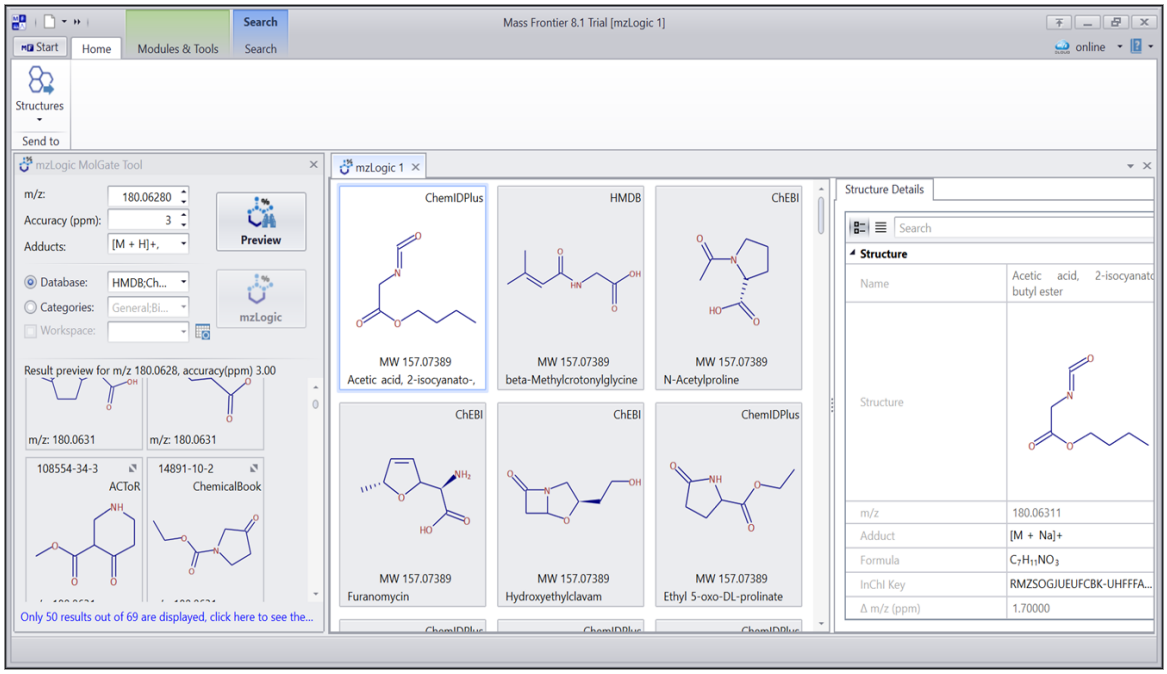
7. To review the search results, click the link at the bottom of the window (Figure 157).

Figure 157. Clicking the link at the bottom of the window



The search results appear on a tabbed mzLogic page in the application window.

Figure 158. mzLogic page with structure candidates



8. To send the structure candidates to a New Structure Grid or New Metabolika or Batch Fragment Generation, click Structures in the toolbar, and then choose **New Structure Grid** or **New Metabolika** or **Batch Fragment Generation**.

   A new Structure Grid that includes the structure candidates opens as a tabbed page.

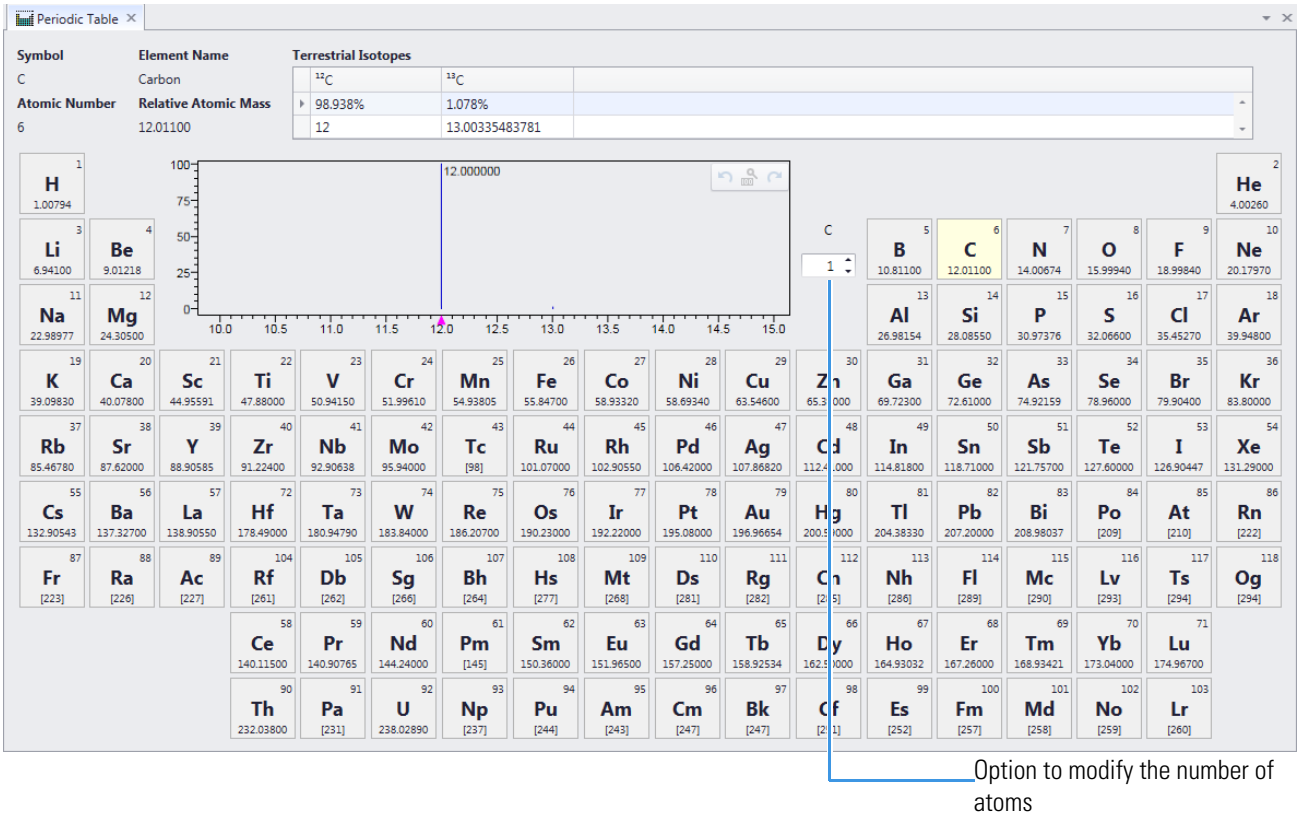
Periodic Table tool

The Periodic Table tool displays the terrestrial isotopic abundance of elements and their multi-atomic isotopic profiles.

❖ To open the Periodic Table tool

In the Modules &Tools toolbar, click **Periodic Table**.

Figure 159. Periodic Table tool



You can modify number of atoms for a specific element in the periodic table. With change in the number of atoms, the spectrum and the terrestrial isotope values modifies. When you select an element that does not occur naturally, the Terrestrial Isotopes section displays a message "**X is not a naturally occurring element**", where X stands for the name of element.

Figure 160. Terrestrial Isotope section highlighting Polonium as a unnatural element

In the spectrum profile, the purple triangle always indicates a mono-isotopic peak.

Reaction Mechanism Overview tool

The Mass Frontier application uses general Reaction Mechanism Overview tool for the prediction of fragmentation, rearrangement, and resonance mechanisms. To open and view reaction mechanism overview, see "Preview unimolecular reactions" on page 308.

Reaction mechanism overview parameters

Table 152. Reaction mechanism overview parameter description

Parameter or icon	Description
File > Print 	Allows you to print the unimolecular reactions.
Find > Find 	Allows you to search for specific molecular reaction from the list available.  You can enter the keyword in the search box provided.
Find > Settings	Whole Words Only: This setting, when selected, will search for the keyword irrespective of its letter case. Case Sensitive: This setting, when selected, will search for the keyword specific to the letter case.
Find > Previous	Helps you navigate to the previous search result.
Find > Next	Helps you navigate to the next search result.
Zoom in 	Zooms in the molecular reaction page. Shortcut key: Ctrl+OemPlus
Zoom out 	Zooms out the molecular reaction page. Shortcut key: Ctrl+OemMinus
Zoom 	Allows you to change the zoom level of the document.
Reaction mechanism overview page	This page details the reactions mechanisms available in the Mass Frontier application. See "Reaction formalism" on page 309.

12

Independent library searches

Independent of the application modules, the Mass Frontier 8.1 application features several library search functions for retrieving spectra, trees, or structures from the spectral libraries. You can simultaneously search multiple libraries using these search options.

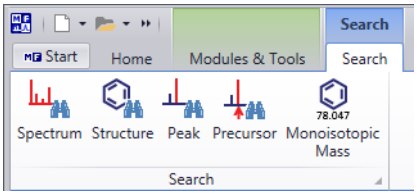
Contents

Run a library search from the Search toolbar

❖ **To run any of the independent library searches**

1. From the Mass Frontier window, click the **Search** tab.

   Figure 161. Library search options on the Search toolbar



2. To search a set of spectral libraries for library entries that meet the following criteria, do the following from the Search toolbar:

   - Click **Monoisotopic Mass** to search for library entries where the neutral compound or the precursor ion at any MS stage in the spectral tree matches a specified monoisotopic mass. Enter the mass and mass tolerance for the search and other search constraints as appropriate. For details, see "Query pane for a monoisotopic mass search" on page 336.
   - Click **Precursor** to search for library entries where the precursor ion at any MS stage matches the specified precursor *m/z* value. Enter the *m/z* value and mass tolerance for the search. Select whether to search only the MS^2 spectra or all MS^n stages, and then specify other search constraints as appropriate. For details, see "Precursor Search parameter settings" on page 345.
   - Click **Peak** to search for library entries where the spectra include any of the spectral peaks (*m/z* values) that you specify. Enter the *m/z* values and intensity cutoffs for the peaks. Specify the *m/z* tolerance for the search, the MS stages for the search, whether to limit the search to spectra for a specific precursor *m/z* value, and other search constraints as appropriate. For details, see "Peak search parameter settings" on page 340.
   - Click **Structure**, and then select a structure and whether the library compound entry or a substructure of the compound entry must match the structure. For details, see "Structure search" on page 358.
   - Select a spectrum in any of the Mass Frontier spectrum views (in any of the modules or search result windows), and then click **Spectrum** to search for library entries with spectra that match the selected query spectrum. When you select an mzCloud library, select a fragmentation spectrum (MS^2 or higher). For details, see "Spectrum searches" on page 348.

3. In the mzCloud Libraries pane and the User Libraries pane, select the libraries for the search.
4. Click **OK** to start the search.

   When the search finishes a search window opens. See "Search result window toolbar and panes" on page 362 and "Search result toolbar" on page 363.

5. To review the search query or modify the query and rerun the library search, click **Edit Search Query** in the search result window toolbar.

Libraries panes for any of the search types

The mzCloud Libraries and User Libraries panes specify the spectral libraries for the search. You can select any combination of user and mzCloud libraries.

Related Topics

- Select the Mass Frontier Library Service

Compound Classes pane for any of the search types

Use the Compound Classes list to restrict the library search to one or more compound classes.

Tip If you have not defined the compound classes for the compound entries in your user libraries, do not constrain library searches of your user libraries to specific compound classes.

Monoisotopic mass search

The monoisotopic mass search option retrieves all the library compounds that match the queried monoisotopic mass within the defined mass tolerance. The search ranks the matching library entries according to their closeness to the queried molecular mass.

For details, see these topics:

- Open the Monoisotopic Mass Search dialog box
- Query pane for a monoisotopic mass search
- Monoisotopic search result window

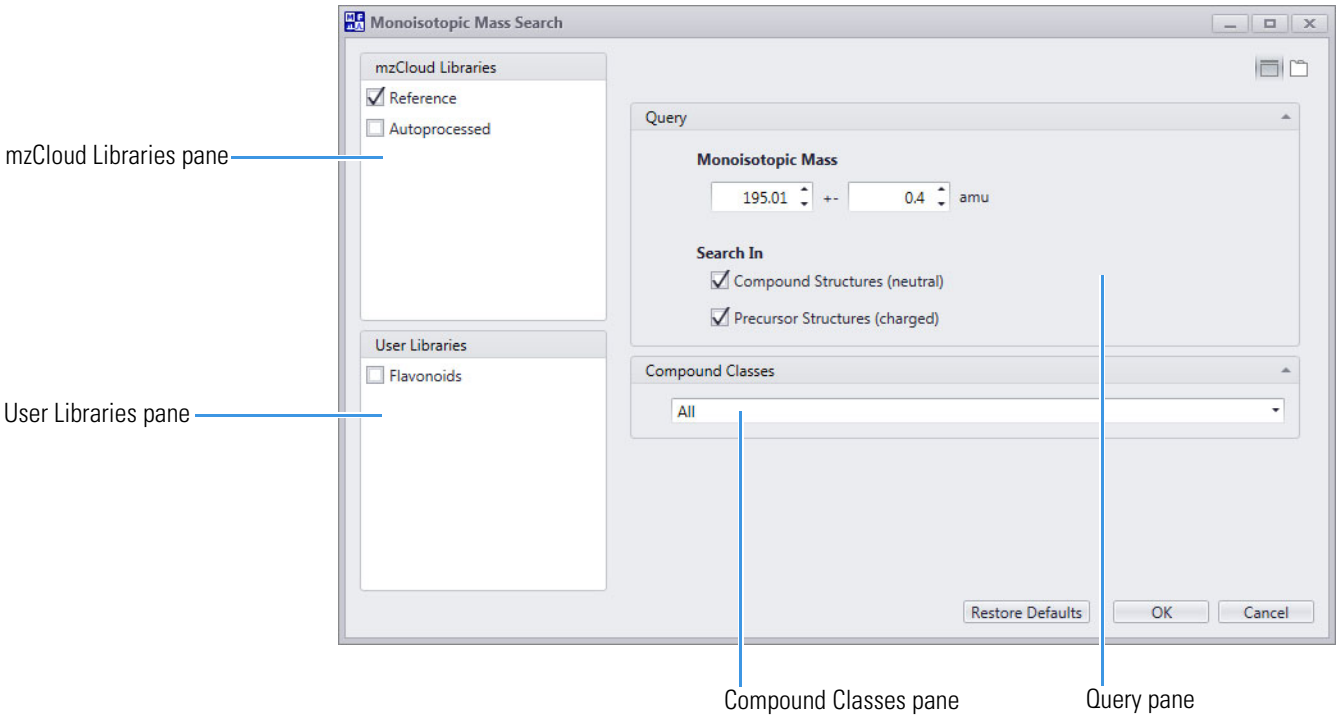
Open the Monoisotopic Mass Search dialog box

Use the Monoisotopic Mass Search dialog box to search the spectral libraries by running a monoisotopic mass query.

❖ To open the Monoisotopic Mass Search dialog box

1. From the application window, click the **Search** tab.
2. On the Search toolbar, click **Monoisotopic Mass**.

Figure 162. Monoisotopic Mass Search dialog box with the default settings



Query pane for a monoisotopic mass search

Use the Monoisotopic Mass Search dialog box to search the mass spectral libraries for the monoisotopic mass of a compound structure or a precursor structure.

Table 153. Query parameters for a monoisotopic mass search

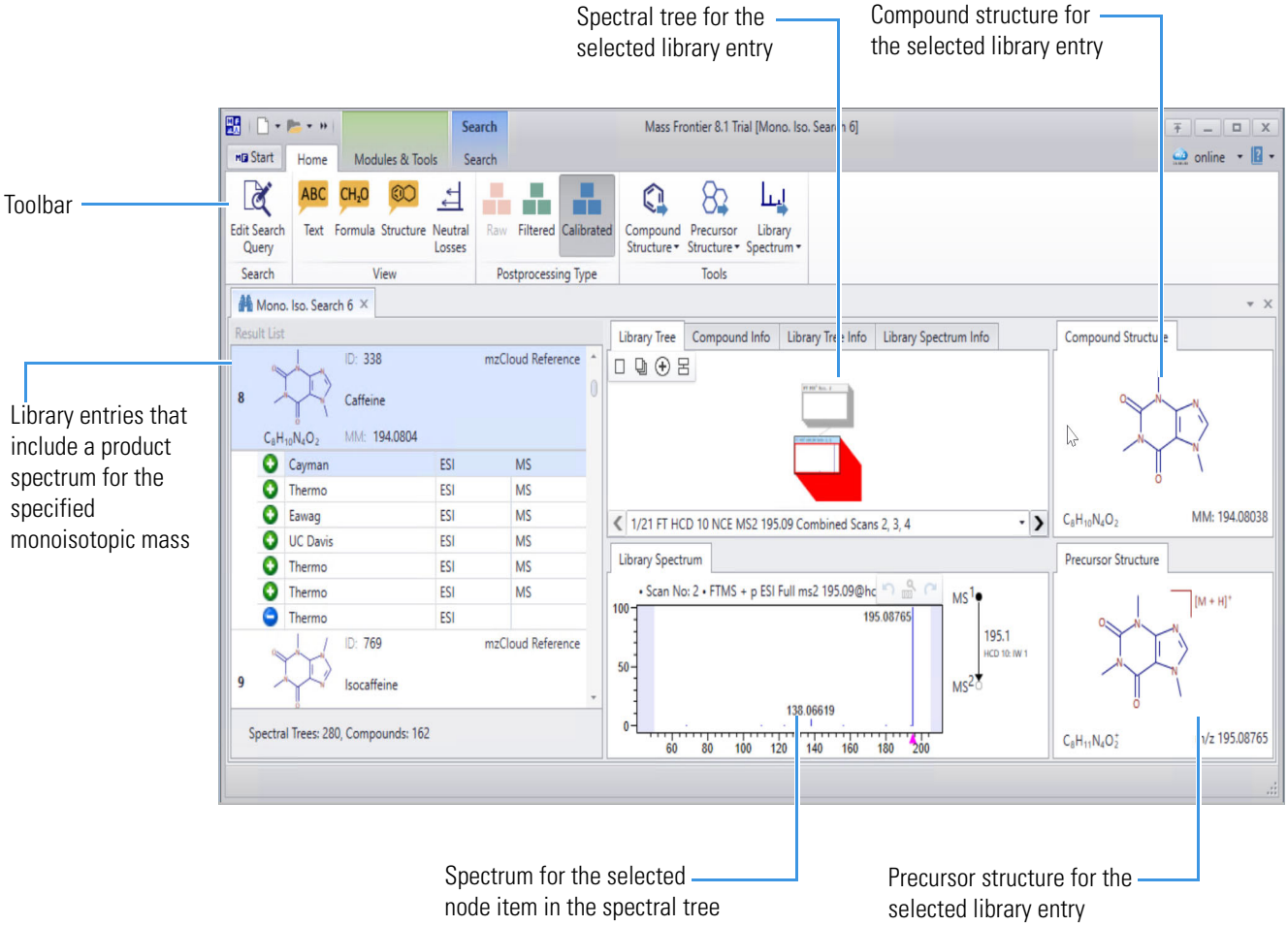
Parameter	Description
Monoisotopic Mass	Specifies the *m/z* value and accuracy of the searched monoisotopic mass.
Search In	You can select one or both of these check boxes: • Compound Structures (neutral)—Searches for the compound structure. • Precursor Structures (charged)—Searches for the precursor structure.

Monoisotopic search result window

After you run a library search, the search results appear in a new window where you can review the library compounds that the search returned.

In the spectral tree for a library entry, a red border on a spectrum (node item) indicates that the spectrum's precursor ion matches the specified monoisotopic *m/z* value.

Figure 163. Monoisotopic search result window



Related Topics

- Monoisotopic mass search
- Search result window toolbar and panes
- Search result toolbar

Peak search

A peak search retrieves all library entries with spectra that include the queried peak's *m/z* value within the defined mass tolerance.

For details, see these topics:

- Open the Peak Search dialog box
- Peak search parameter settings
- Peak search results window

Open the Peak Search dialog box

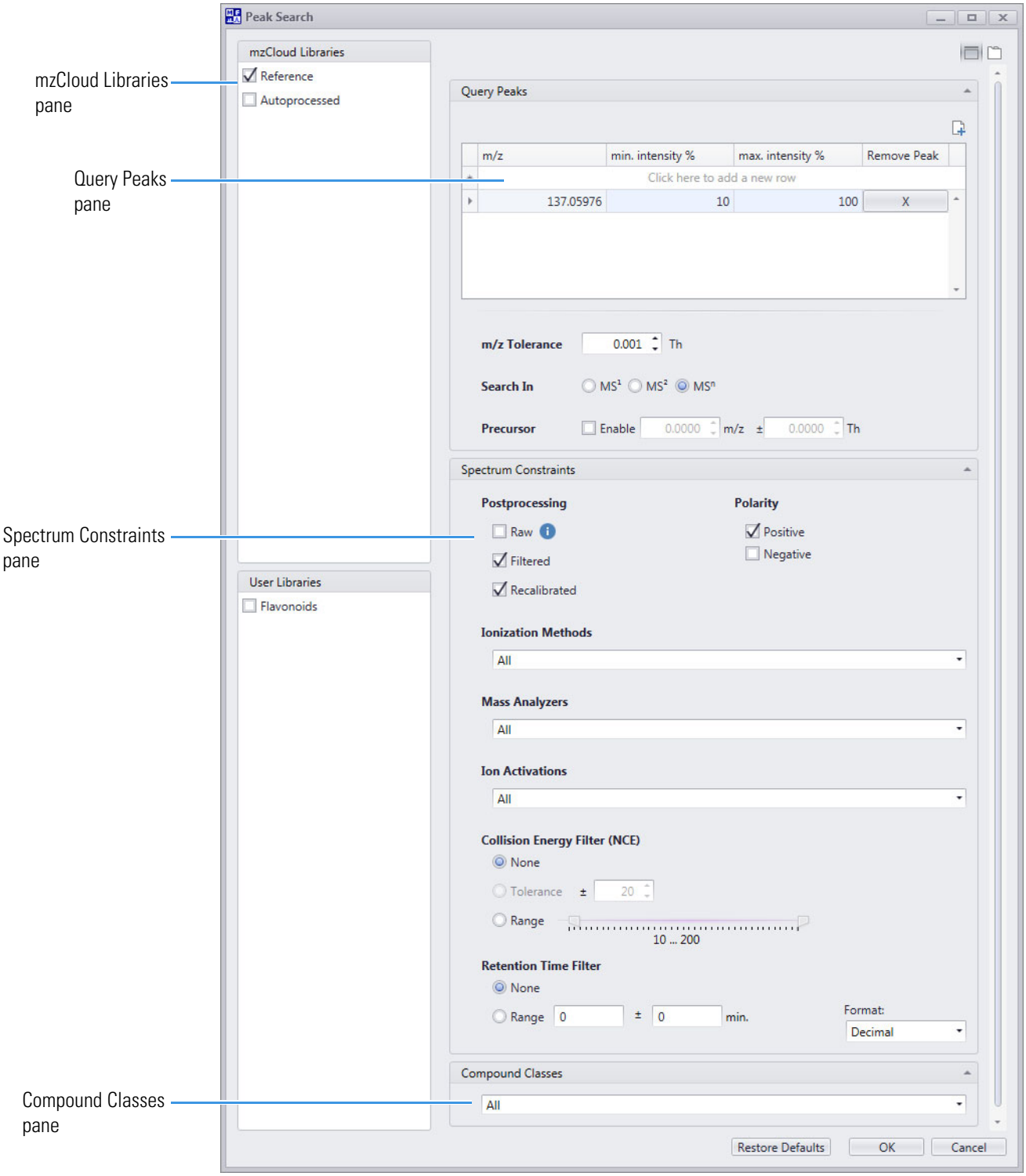
❖ To open the Peak Search dialog box

1. From the application window, click the **Search** tab.
2. On the Search toolbar, click **Peak**.

–or–

- Right-click any displayed spectrum and choose **Peak Search**.

Figure 164. Peak Search dialog box with the default parameter settings



Peak search parameter settings

Use the Peak Search dialog box to search the library spectra for matching peaks (*m/z* values with various relative intensities).

Table 154. Peak search parameter settings (Sheet 1 of 2)

Parameter	Description
Query Peaks	
Add Peak icon,	Adds a row to the peak list.
m/z column	Specifies the *m/z* value of the searched ion.
Min. Intensity% column	Specifies the minimum relative intensity of a peak in the searched library spectrum.
Max. Intensity% column	Specifies the maximum relative intensity of a peak in the searched library spectrum.
Remove Peak icon	Removes the peak from the list.
m/z Tolerance	Specifies the accuracy of the searched peaks.
Search In	Specifies the spectrum type for the search: • MS1— Search peaks in MS1 spectra only • MS2 —Search peaks in MS2 spectra only. • MSn— Search peaks in any MSn spectra.
Precursor	Activates or deactivates precursor search and sets the *m/z* value and accuracy of the searched precursor. Selecting the Enable check box constrains the search to spectra with the matching precursor ion *m/z* value.
Spectrum Constraints The Spectrum Constraints options define additional constraints for the library search.	
Post-processing	Searches only for spectra of the selected post-processing type.
Polarity	Searches only for spectra of the selected polarity. By default, the application selects the polarity of the query spectrum.
Ionization Methods	Searches only for spectra from the specified ionization method. Default: All
Mass Analyzers	Searches only for spectra from the specified mass analyzer. Default: All
Ion Activations	Searches only in the spectra of the specified ion activation. Default: All

Table 154. Peak search parameter settings (Sheet 2 of 2)

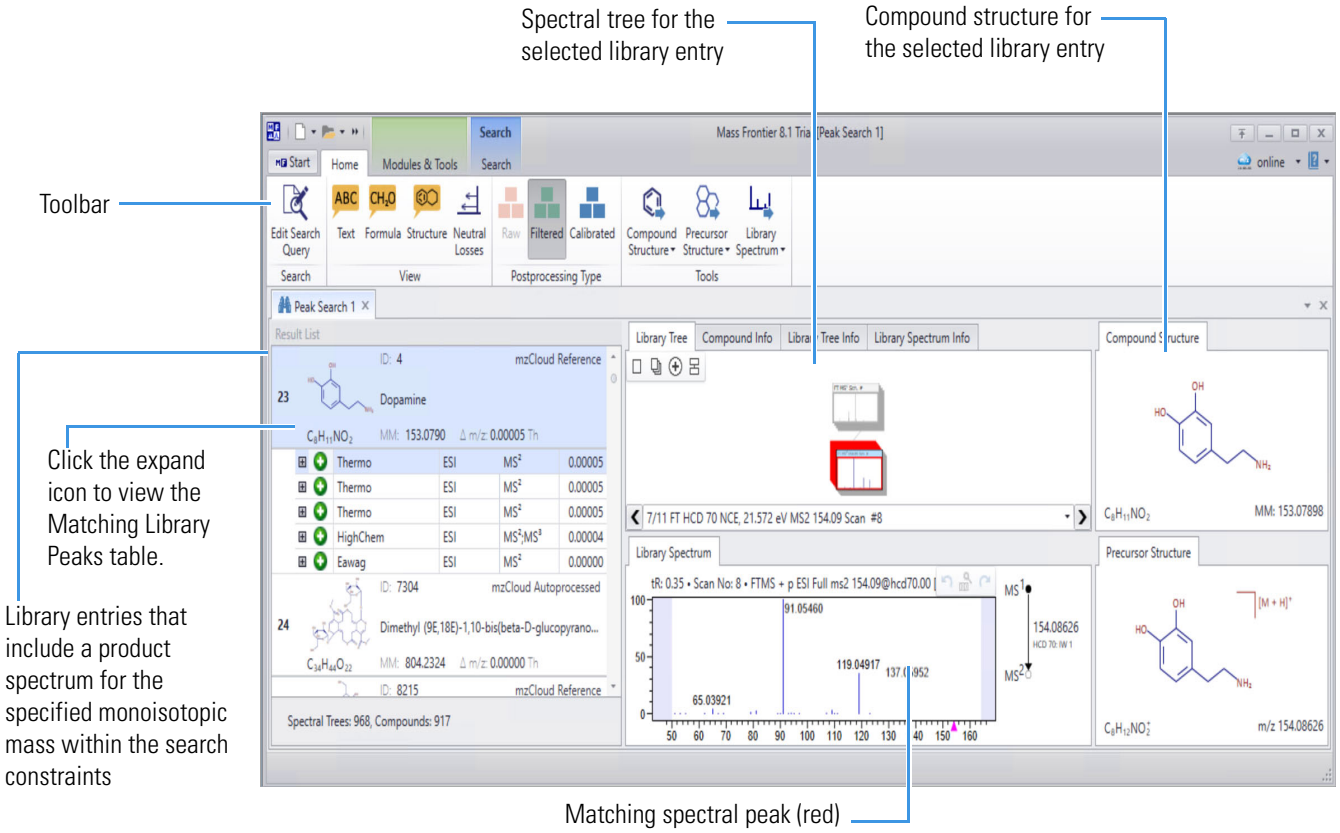
Parameter	Description
Collision Energy Filter (NCE)	Selections: • None—Searches for spectra with any collision energy. • Tolerance—Searches for spectra within the specified collision energy interval. The interval is relative to the relative collision energy of the query spectrum. • Range—Searches for spectra within the specified collision energy interval. Default: None
Retention Time Filter	Selections: • None—Does not constrain the search results by the retention time of the library spectrum. • Range—Filters the results by setting retention time limits in minutes or 24-hour clock format. – Minutes (Range 0 ± 0 min.) –or– – 24-hr clock (Range 0 : 0 : 0 +/- 0 : 0 : 0)
Compound Classes	Searches only for the spectra of the specified compound class. Default: All

Peak search results window

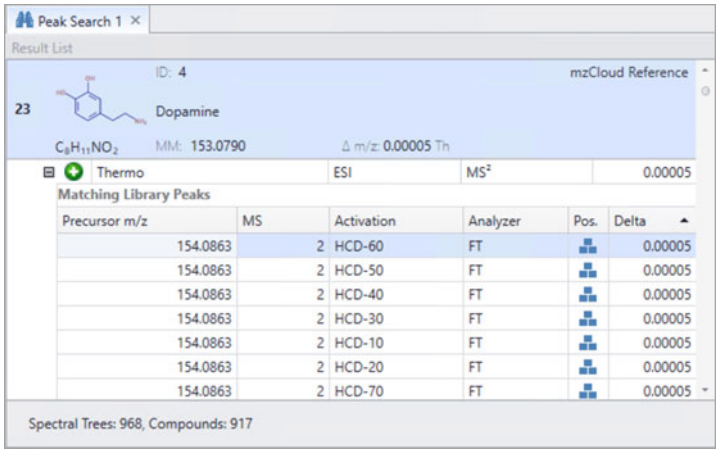
After you run a library search, the search results appear in a new window where you can review the library entries that the search returned.

On the Library Tree page of the library entry pane, a red border on a spectrum (node item) in the spectral tree indicates that the spectrum includes one or more of the query peaks. In the Library Spectrum pane, the red peaks in the spectrum match the query peaks.

Figure 165. Peak search result window



Clicking the expand icon below the compound entry opens the Matching Library Peaks table.



The columns in the Matching Library Peaks table display additional information about the library entry.

Table 155. Matching Library Peaks table for a matching library entry from a library peak search

Column	Description
Precursor *m/z*	*m/z* value of the spectrum's precursor ion.
MSn	MS stage of the spectrum.
Pos.	Library spectrum's post-processing type. See "Post-processing" on page 406.
Activation	Ion activation type and relative energy.
Analyzer	Mass analyzer use to acquire the spectrum.
Delta	*m/z* difference between the query peak and the peak in the library spectrum.

Precursor search

The Precursor Search dialog box provides options to search library spectra by matching the *m/z* value of the precursor ion.

For details, see these topics:

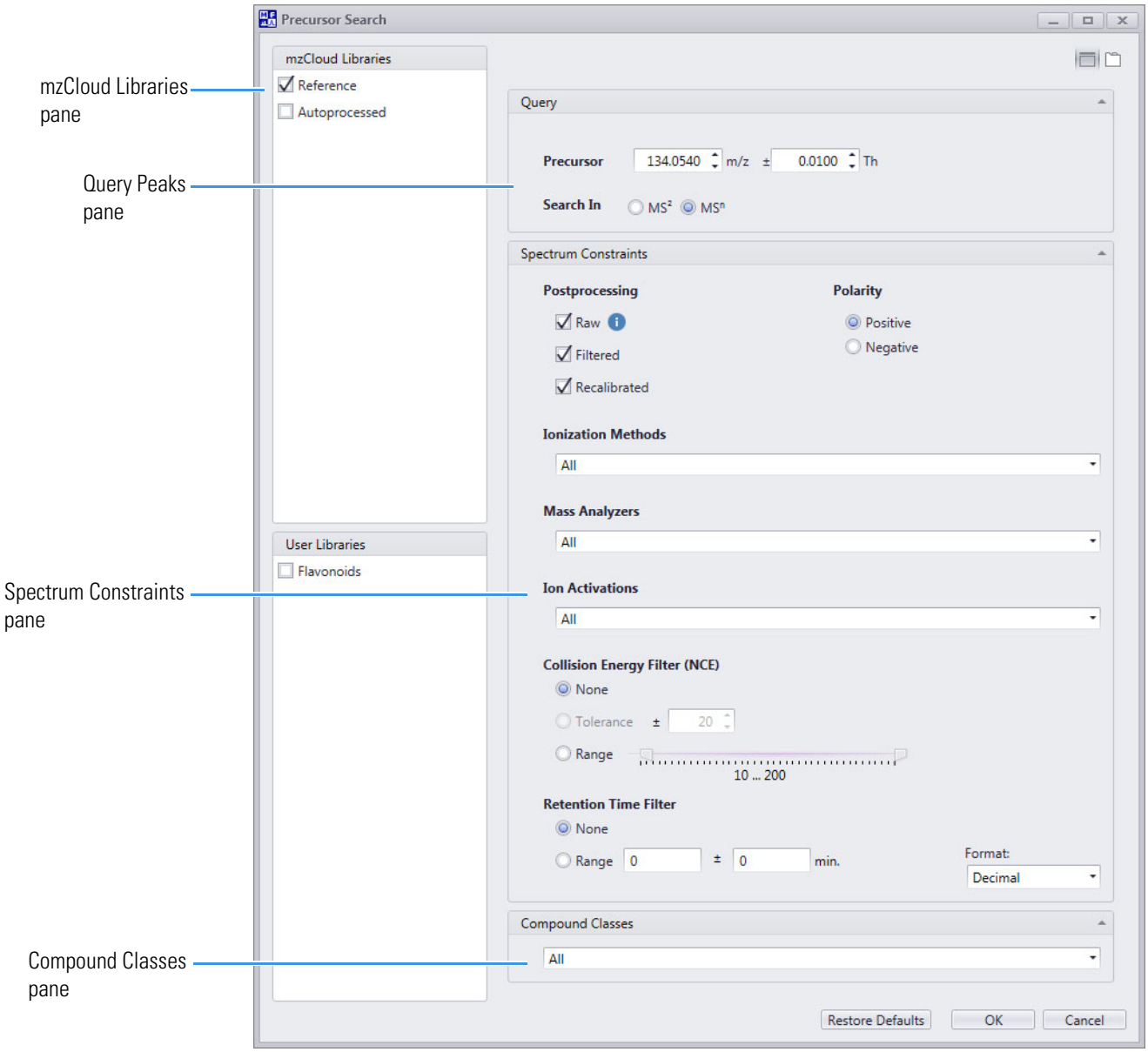
- Open the Precursor Search dialog box
- Precursor Search parameter settings
- Precursor Search results

Open the Precursor Search dialog box

❖ **To open a Precursor Search dialog box**

1. From the application window, click the **Search** tab.
2. In the Search toolbar, click **Precursor**.

Figure 166. Precursor Search dialog box



Precursor Search parameter settings

Use the Precursor Search dialog box to search for library spectra by their precursor *m/z* value.

Table 156. Precursor search parameter settings (Sheet 1 of 2)

Parameter	Description
Query	
Specifies the precursor *m/z* value for the search.	
Precursor	Specifies the *m/z* value and accuracy of the searched precursor.
Search In	Specifies whether to search MS^2 spectra only or all MS^n stage spectra. • MS^2—Search in MS2 spectra only. • MS^n—Search in any MSn spectra.
Spectrum Constraints	
Defines additional constraints for library searching	
Post-processing	Searches only for spectra of the selected post-processing type.
Polarity	Searches only for spectra of the selected polarity. By default, the application selects the polarity of the query spectrum.
Ionization Methods	Searches only for spectra from the specified ionization method. Default: All
Mass Analyzers	Searches only for spectra from the specified mass analyzer. Default: All
Ion Activations	Searches only in the spectra of the specified ion activation. Default: All
Collision Energy Filter (NCE)	Selections: • None—Searches for spectra with any collision energy. • Tolerance—Searches for spectra within the specified collision energy interval. The interval is relative to the relative collision energy of the query spectrum. • Range—Searches for spectra within the specified collision energy interval. Default: None

Table 156. Precursor search parameter settings (Sheet 2 of 2)

Parameter	Description
Retention Time Filter	Selections: • None—Does not constrain the search results by the retention time of the library spectrum. • Range—Filters the results by setting retention time limits in minutes (decimal) or 24-hour clock format.
Compound Classes	Searches only for the spectra of the specified compound class. Default: All

Precursor Search results

After you run a library search, the search results appear in a new window where you can review the library entries that the search returned.

In the library tree for an entry, a library spectrum has a red border if its *m/z* value matches the query's s precursor *m/z* value.

Figure 167 shows the library search results for the following query: precursor *m/z* 195.0877 in MS^2 spectra only.

Figure 167. Precursor Search window

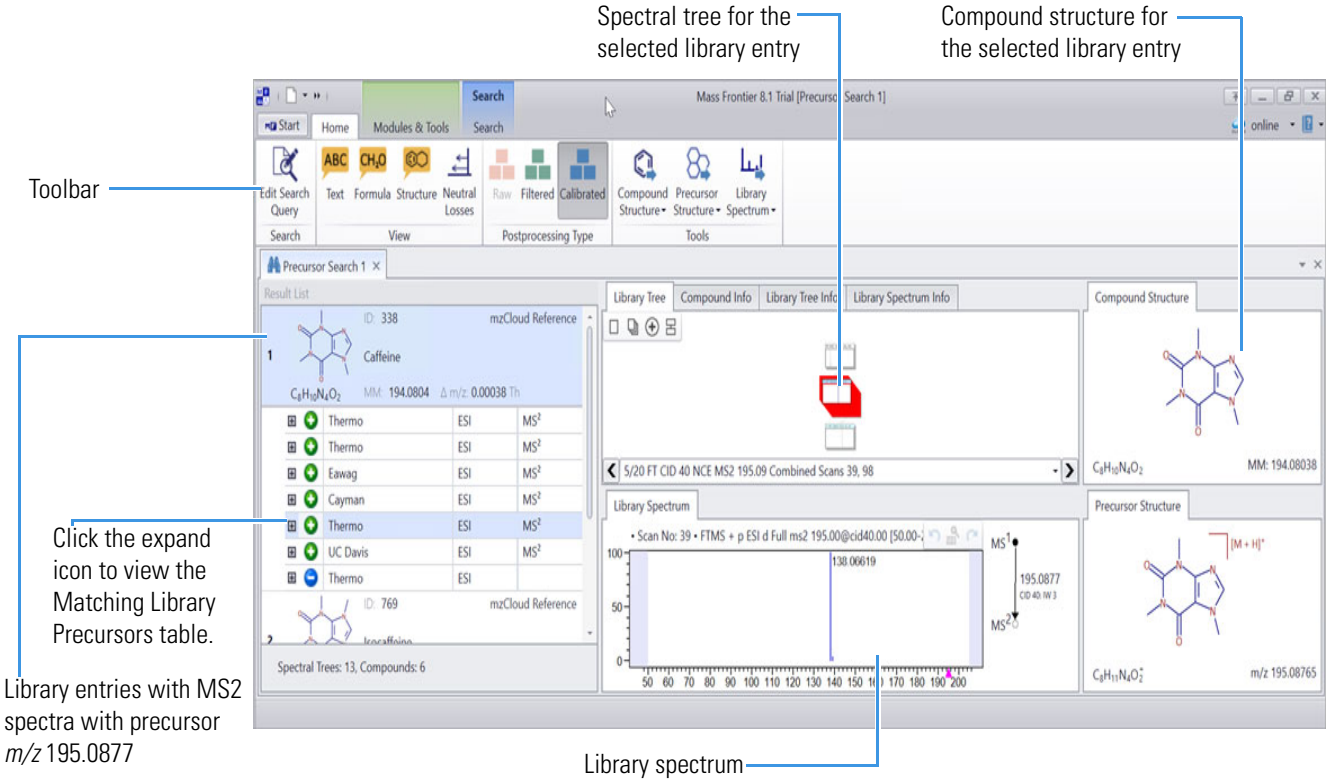
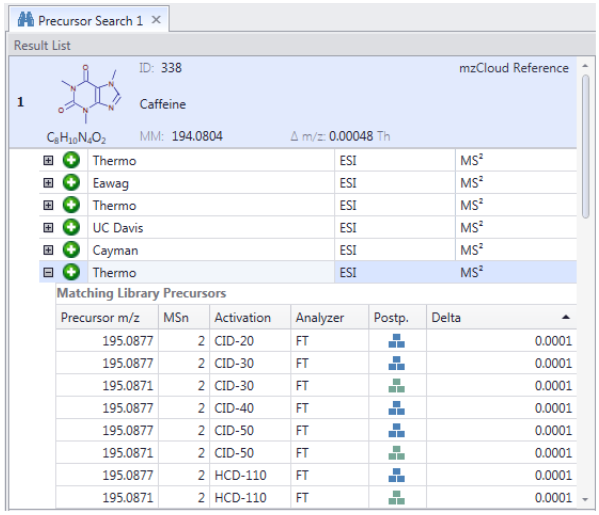


Figure 168. Matching Library Precursors table for the caffeine entry in the mzCloud library



Spectrum searches

A spectrum search searches for library spectra that match an unknown query spectrum.

For details, see these topics:

- Open the Spectrum Search dialog box
- Spectrum Search parameters
-
- Query Spectrum Peak Filter dialog box
- Spectrum Search results window

Open the Spectrum Search dialog box

❖ **To open the Spectrum Search dialog box from the application window:**

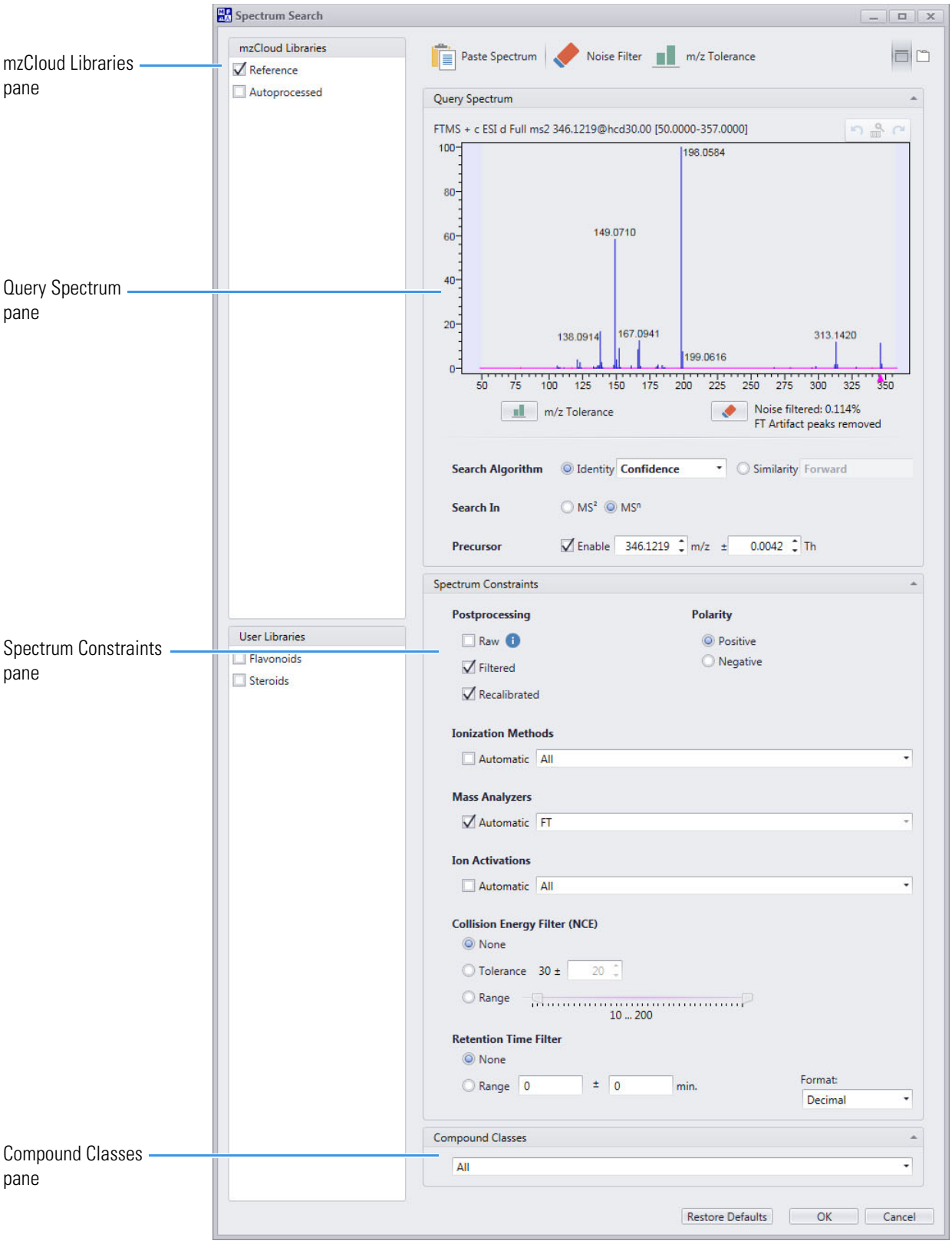
- From the application window, click the **Search** tab.
- Then, on the Search toolbar, click **Spectrum**. Use the Paste Spectrum command to import the query spectrum of interest from the Clipboard.
- (Optional) You can send the spectrum to this dialog box from another module.

❖ **To open the Spectrum Search dialog box from the Chromatogram Processor or the Data Manager module:**

- In a Chromatogram Processor window, display the query spectrum on the Spectrum page of the MS spectrum view.
- Right-click on the spectrum and choose **Spectrum Search**.
- (Optional) In a Chromatogram Processor module or the Data Manager module, from the **Send To** toolbar group, click **Spectrum** > **Spectrum Search**.

Figure 169 shows the Spectrum Search dialog box.

Figure 169. Spectrum Search dialog box



Spectrum Search parameters

Use the Spectrum Search dialog box to define the parameter settings for a spectrum search.

Table 157. Spectrum Search dialog box parameter settings and toolbar (Sheet 1 of 3)

Parameter or button	Description
Paste Spectrum 	Pastes spectrum from the Clipboard.
Noise Filter 	Opens the Query Spectrum Peak Filter dialog box for adjusting the noise filter. See "Query Spectrum Peak Filter dialog box" on page 352.
m/z Tolerance 	Opens the Select Peak Tolerance dialog box for setting the tolerance for the matching *m/z* value. See "" on page 352.
Query Spectrum	
Search Algorithm	Specifies an Identity search or a Similarity search. Identity: Considers all the peaks in the query spectrum and the library spectrum. Select one of the following methods to calculate the spectral match score: • Confidence (Default): Considers dot product of both spectra, distribution of peaks, activation energy difference, and polarity. Uses a machine-learning Bayesian Network model to estimate the likelihood of a correct match. • HighRes algorithm: Based on the weighted distance between the spectral peaks. • LowRes algorithm: Based on the optimized dot-product function and an additional term based on the ratios of the peak intensities. • NIST algorithm: Developed by the National Institute of Standards and Technology (NIST). Similarity: Considers only peaks that exist either in the query spectrum or the library spectrum: • Forward: Considers only those *m/z* peaks in the library spectrum that are also present in the query spectrum. • Reverse: Considers only those *m/z* peaks in the query spectrum that are also present in the library spectrum.

Table 157. Spectrum Search dialog box parameter settings and toolbar (Sheet 2 of 3)

Parameter or button	Description
Search In	Select the MS^2 option or the MS^n option. MS^2— Compares the query spectrum with any MS^2 spectra in the selected libraries. MS^n—Compares the query spectrum with any MS^n spectra in the selected libraries.
Precursor	When enabled, constrains the search to library spectra with matching precursor ion *m/z*.
Spectrum Constraints	
Post-processing	Searches only for spectra of the selected post-processing type.
Polarity	Searches only for spectra of the selected polarity. By default, the application selects the polarity of the query spectrum.
Ionization Methods	Searches only for spectra from the specified ionization method. Default: All
Mass Analyzers	Searches only for spectra from the specified mass analyzer. Default: All
Ion Activation	Searches only in the spectra of the specified ion activation. Default: All
Collision Energy Filter (NCE)	Selections: • None—Searches for spectra with any collision energy. • Tolerance—Searches for spectra within the specified collision energy interval. The interval is relative to the relative collision energy of the query spectrum. • Range—Searches for spectra within the specified collision energy interval. Default: None

Table 157. Spectrum Search dialog box parameter settings and toolbar (Sheet 3 of 3)

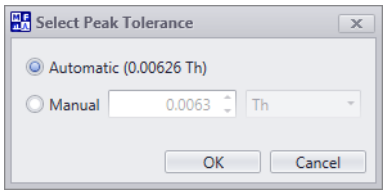
Parameter or button	Description
Retention Time Filter	Selections: • None—Does not constrain the search results by the retention time of the library spectrum. • Range—Filters the results by setting retention time limits in minutes (decimal) or 24-hour clock format.
Compound Classes	Searches only for the spectra of the specified compound class. Default: All

Note The .'msp' spectrum format is not recommended as a query spectrum in the Data Manager. Using this format as query spectrum can lead to an incorrect result.

Select Peak Tolerance dialog box

Clicking the m/z Tolerance button in the Spectrum Search dialog box opens the Select Peak Tolerance dialog box.

Figure 170. Select Peak Tolerance dialog box



The Select Peak Tolerance dialog box specifies the *m/z* tolerance used in the peak matching algorithm. By default, it calculates the mass tolerance automatically from the spectrum.

Related Topic

- Open the Spectrum Search dialog box

Query Spectrum Peak Filter dialog box

Clicking the Noise Filter button in the Spectrum Search dialog box opens the Query Spectrum Peak Filter dialog box.

The Query Spectrum Peak Filter dialog box defines the noise filtering method. Use the options to control the calculated threshold level in the spectrum pane.

Figure 171. Query Spectrum Peak Filters dialog box

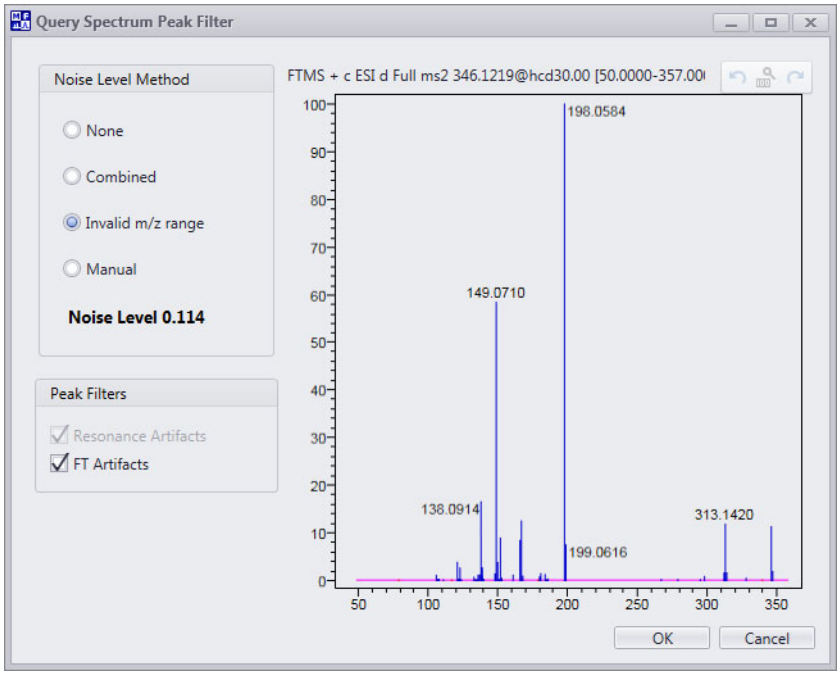


Table 158. Query Spectrum Peak Filter parameter settings (Sheet 1 of 2)

Parameter	Description
Noise Level Method	
The noise level method defines the algorithm for calculating the threshold line height.	
None	No filtering.
Combined	The application calculates the noise level by using both the linear fit and histogram methods, and then uses the lower calculated value for the noise threshold.
Invalid *m/z* range	(Default) A histogram is created from abundances for peaks outside of the combinatorially possible elemental compositions for the elements that are common for small organic molecules. The smoothed maximum of such peaks is considered as the threshold value. Applies only to spectra having more than five peaks.
Manual (option and box)	Selecting this option enables the manual noise level box where you enter the noise level filter in relative abundance. Default: 10%
Peak Filters	
The peak filters control the filtering of individual peaks in the spectrum. See "Resonance peaks" on page 404.	

Table 158. Query Spectrum Peak Filter parameter settings (Sheet 2 of 2)

Parameter	Description
Resonance Artifacts	Removes resonance artifacts. Selectively removes peaks from the invalid *m/z* range.
FT Artifacts	Removes peaks from the invalid *m/z* ranges.

Related Topic

- Open the Spectrum Search dialog box

Spectrum Search results window

After you run a library search, the search results appear in a new window where you can review the library entries that the search returned.

In the library tree, the color and shade of a spectrum's border spectrum corresponds to the spectrum's match score; for example, a dark red border indicates a relatively high match score, a light pink border indicates a relatively low match score, and a gray border indicates that the library spectrum does not match the query spectrum.

Figure 172. Spectrum Search results window

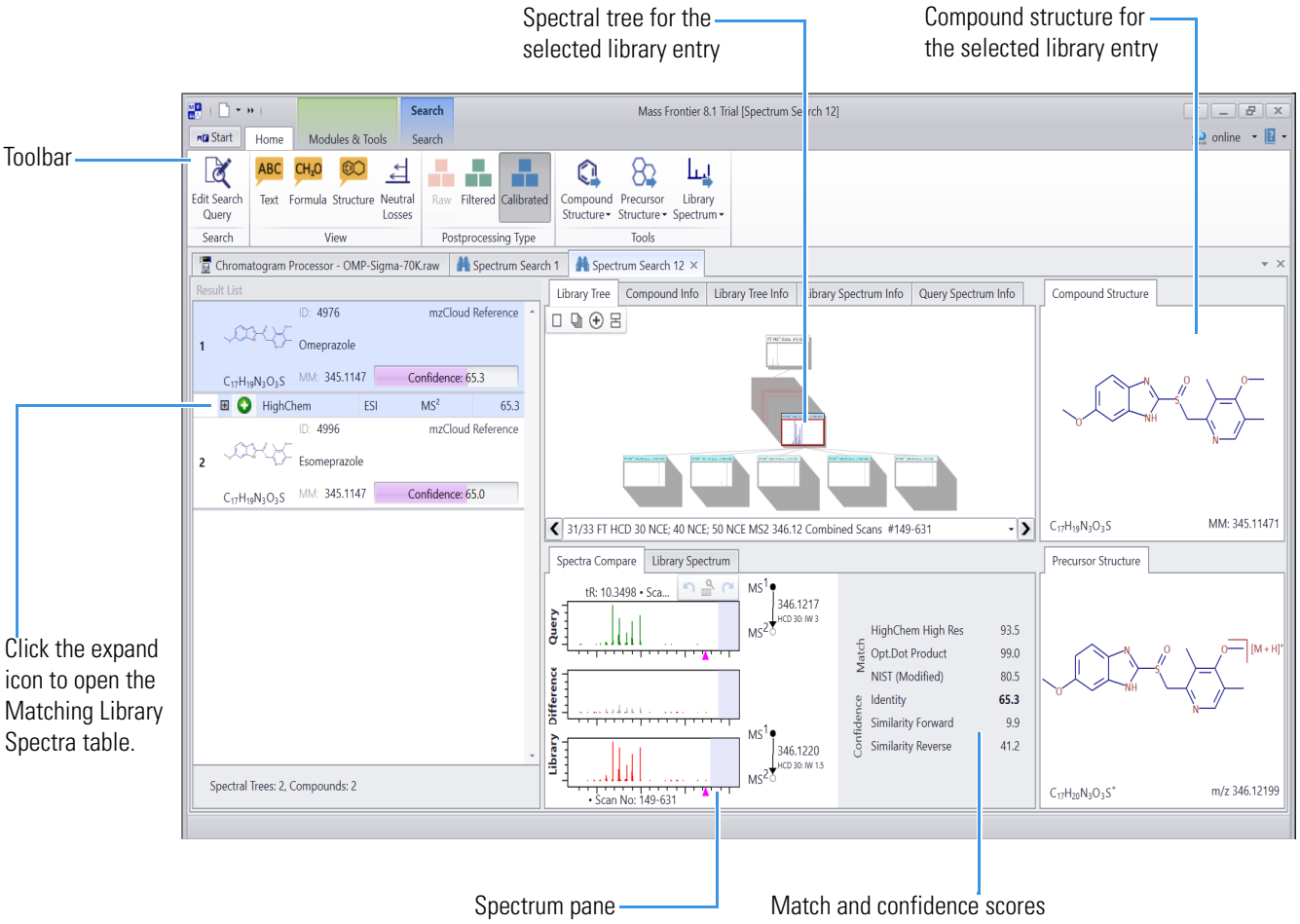
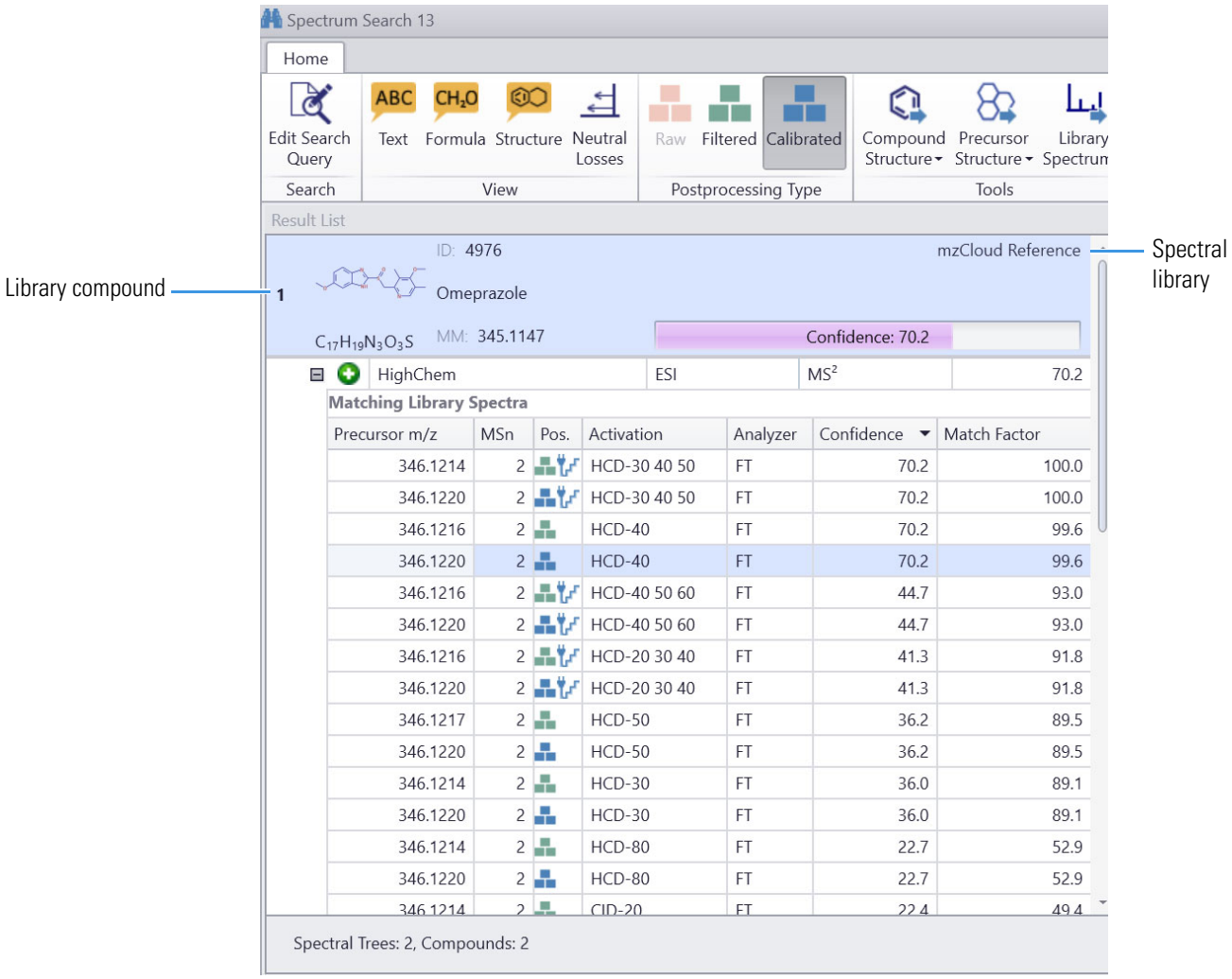


Figure 173 shows the Matching Library Spectra table for the second library entry that the search returned.

Figure 173. Result List pane with the table of matching library spectra for the second search result



The columns in the Matching Library Spectra display additional information about the library entries.

Table 159. Column descriptions for the Matching Library Spectra table (Sheet 1 of 2)

Column	Description
Precursor *m/z*	*m/z* value of the precursor ion.
MSn	MS stage.
Pos.	Post-processing type of the library spectrum. See "Post-processing" on page 406.
Activation	Ion activation type and relative energy.
Analyzer	Mass analyzer.

Table 159. Column descriptions for the Matching Library Spectra table (Sheet 2 of 2)

Column	Description
Confidence	The confidence score of the respective query-library spectrum pair.
Match Factor	Dot product spectral match of the respective query-library spectrum pair.

Figure 160 describes the panes of the Spectrum Search window.

Table 160. Spectrum Search window panes

Pane	Description
Result List	Provides detailed information about the library compounds that the search returns.
Library Tree	The Library Tree pane contains displays detailed information about the selected library record. Pages: • Library Tree—Displays the spectral tree for the library compound. • Compound Info—Displays the metadata associated with the library compound • Library Tree Info—Displays information about the contributors, the acquisition instrument, the data system and processing application, and the chromatographic method (when the contributor supplies this information) • Library Spectrum Info—Displays the metadata associated with the library spectrum, such as scan numbers, isolation width, and so on. • Query Spectrum Info—Displays the metadata associated with the library spectrum, such as scan numbers, isolation width, and so on.
Spectrum pane	Displays a detailed view of the query, library, and difference spectra to facilitate the inspection of the query-to-library spectral match. The confidence and match scores appear on the right side of the Spectra Compare page.
Compound Structure	Displays the library compound structure.
Precursor Structure	Displays the library precursor structure.

Structure search

Because the rules of systematic nomenclature do not necessarily lead to a unique name for each compound, a name search can be ineffective in many cases. A compound's CAS number is more specific than its name, but determining a compound's CAS number might require a separate database search. By contrast, a structure search, where you draw or import the query structure, is the most straightforward method for finding compounds in a spectral library.

While a structure search (Identity search) provides an exact match of the query and library structures, a substructure search retrieves compounds that contain a common structural subset, called a substructure. The exact substructure must be embedded in each molecule retrieved.

The exact match in structure and substructure searches has a notable exception—it ignores stereo bonds because optical activity does not play a significant role in mass spectrometry. All other structural features such as bond multiplicity, atom state, and skeletal arrangement must match exactly. You have the option to ignore charges, radicals, and unspecified charge sites, and the option to disregard isotopes.

For details about a structure search, see these topics:

- Start a structure search
- Structure Search dialog box parameters
- Structure Search results

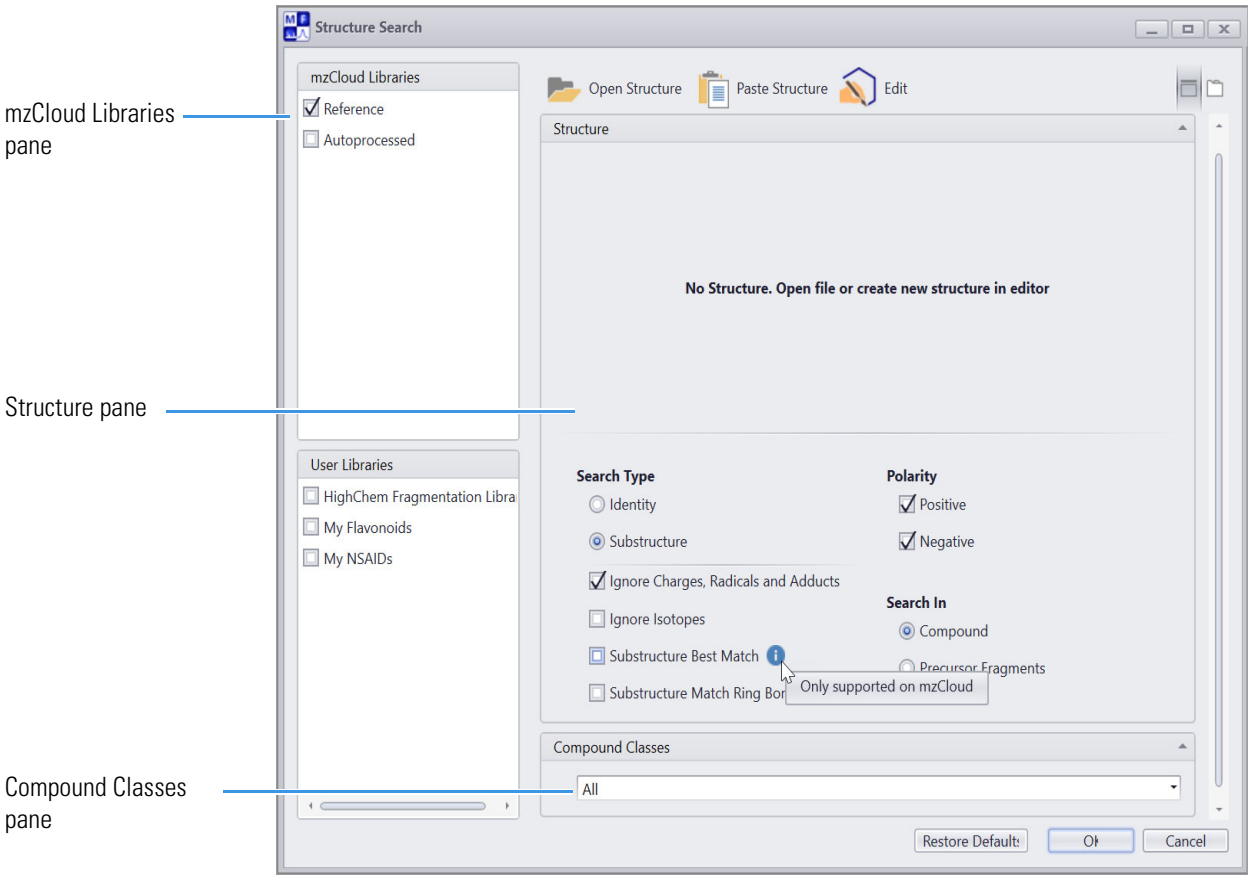
Start a structure search

❖ To start a structure search

1. From the application window, click the **Search** tab.
2. On the Search toolbar, click **Structure**.

   The Structure Search dialog box opens.

Figure 174. Structure Search dialog box



3. Do the following:
   - Click **Open Structure** and choose the query structure file. Or, if you copied a structure to Clipboard, click **Paste Structure**.

     The selected structure appears in the Structure pane.
   - (Optional) If you need to edit the structure, click **Edit**.
4. Specify the parameter settings for the search.
5. Click **OK**.

Structure Search dialog box parameters

Table 161. Structure search parameter descriptions (Sheet 1 of 2)

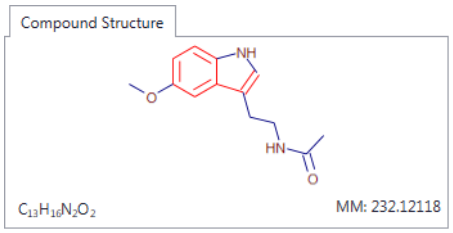
Parameter or button	Description
Open Structure,	Opens the structure from a file.
Paste Structure	Pastes the structure from Clipboard.
Edit	Opens the Structure Editor dialog box for editing the selected structure or drawing a new structure.
Search Type	Specifies an Identity search or a Substructure search. • Identity—Locates a library spectrum that closely matches an unknown. Provides an exact match of query and library structure. • Substructure—Retrieves compounds that contain a common structural subset, called a substructure.
Additional search type options	
Ignore Charges, Radicals, and Adducts	Ignores charges, radicals, and adducts in the substructure search.
Ignore Isotopes	Ignores isotopes in the substructure search.
Substructure Best Match	Available only with the Substructure option. A substructure can sometimes fit at several locations of a larger structure. The best matching substructure appears in red on the Compound Structure pane of the Structure Search results window. Using this option lengthens the calculation time. **Note** This option is valid only for mzCloud libraries. This figure shows the compound structure for melatonin with its styrene substructure drawn in red. 

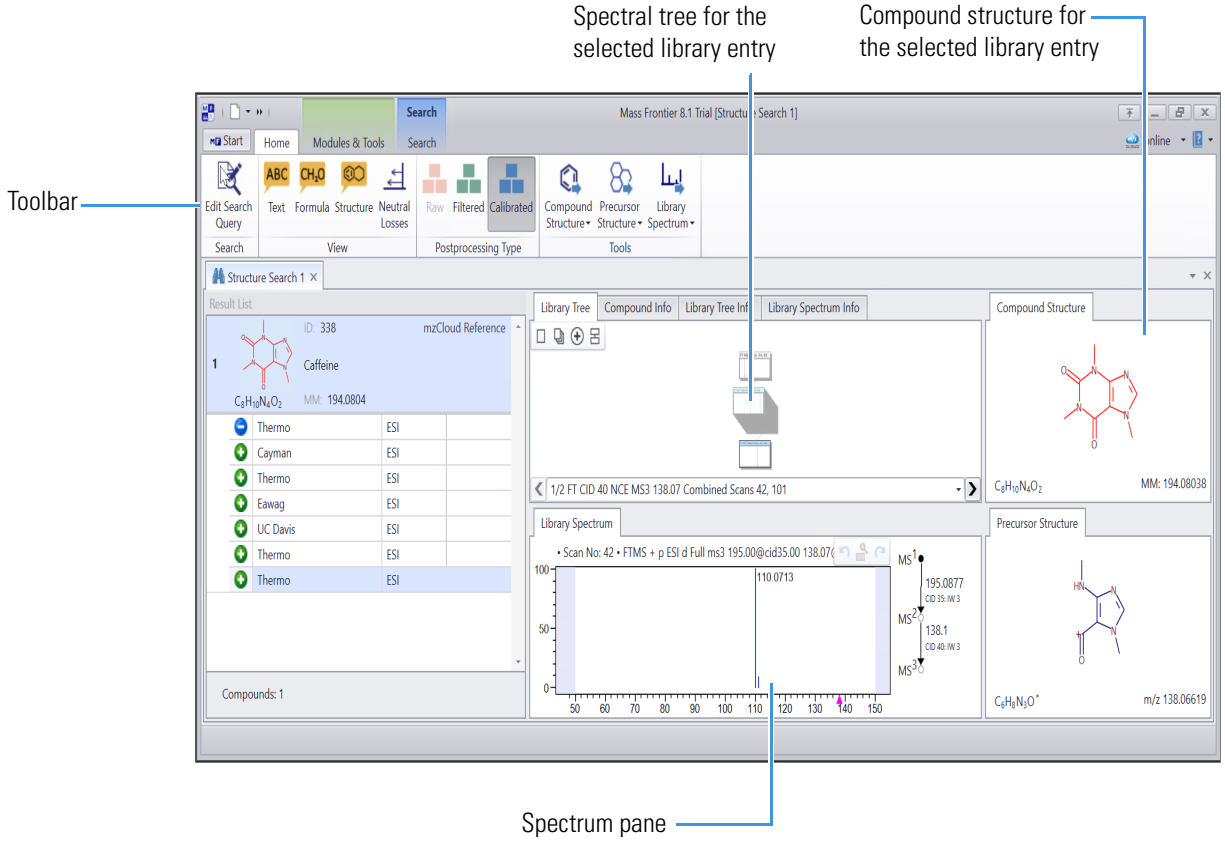
Table 161. Structure search parameter descriptions (Sheet 2 of 2)

Parameter or button	Description
Substructure Match Ring Bonds	Available only with the Substructure option. Because fragmentation mechanisms on rings significantly differ from acyclic moieties, searching substructures that exactly match the ring membership is useful for each bond. Using this option lengthens the calculation times. This options is valid only for non-aromatic bonds. Aromatic bonds must always match between substructure and structure.
Polarity	Searches only ions with the specified polarity: Positive or Negative.
Search In	Compound—Searches only the top-level tree (full scan, source CID). Precursor Fragments—Searches the entire tree.
Compound Classes	Constrains the library search to the selected compound classes.

Structure Search results

After you run a library search, the search results appear in a new window where you can review the library entries that the search returned.

Figure 175. Result from a structure search for caffeine



Search result window toolbar and panes

The search result windows (Structure Search, Peak Search, Precursor Search, or Mono. Iso. Search) contains a toolbar and several panes:

- Search result toolbar
- Result List pane
- Library entry information pane
- Library Spectrum pane
- Compound Structure pane
- Precursor Structure pane

Search result toolbar

When you run a library search from the Search toolbar, a search result window opens as a tabbed page and the search result toolbar replaces the search toolbar.

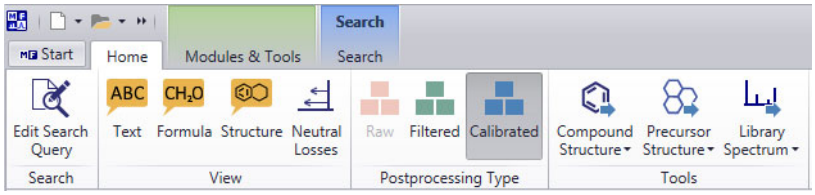
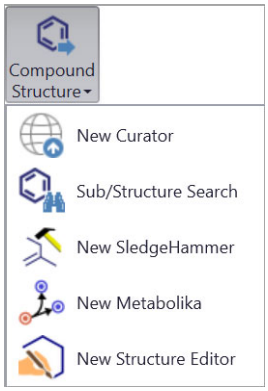
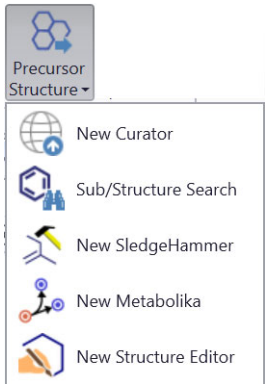
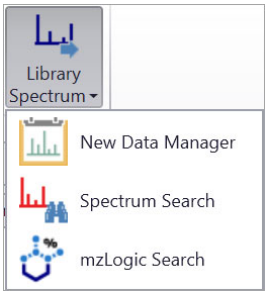


Table 162. Search results toolbar button descriptions (Sheet 1 of 2)

Name	Icon	Description
Search		
Edit Query Search		Opens the respective search dialog box for restarting the search with different parameter settings.
View		
Use the View group of the search result toolbar to view the test, formula, structure, or neutral loss annotations for the library entries that your library search found.		
Text		Displays text annotations in the library spectrum.
Formula		Displays formula annotations in the library spectrum.
Structure		Displays structure annotations in the library spectrum.
Neutral Losses		Displays neutral losses in the library spectrum and the comparison spectrum.
Post-processing Type		
Use the Post-processing Type group on the search results toolbar to filter the results by the spectrum type: raw, filtered, or calibrated.		
Raw		Original unmodified spectra.
Filtered		Original data with applied threshold filter.
Calibrated		Original data with applied threshold filter and mass recalibration.

Table 162. Search results toolbar button descriptions (Sheet 2 of 2)

Name	Icon	Description
Tools		
Use the Tools group on the search results toolbar to send the compound structure, precursor structure, or selected spectrum to other modules (new or active).   		
Compound Structure		Sends the library compound structure to any of these (new or active) modules: Curator, SledgeHammer, Sub/Structure Search, Structure Editor, Metabolika, or Structure Editor. Or, to an active Structure Grid.
Precursor Structure		Sends the library precursor structure to any of these (new or active) modules: Curator, SledgeHamer, Sub/Structure Search, Metabolika, or Structure Editor. Or, to an active Structure Grid.
Library Spectrum > Data Manager		Sends the selected library spectrum to the new or active Data Manager window.
Library Spectrum > Spectrum Search		Sends the selected library spectrum to the Spectrum Search dialog box.
Library Spectrum > mzLogic Search		Opens the mzLogic MolGate Tool pane and runs the search when you submit or enter the appropriate precursor information.

Result List pane

The Result List pane lists the library entries that the search returns. The library entries include a subtable for the compound's spectral tree.

Library entry information pane

The library entry information pane displays detailed information about the selected library record on the following pages:

- The Library Tree page displays the library tree for the entry that you select in the Result List pane.
- The Compound Info page displays the metadata associated with the library compound.
- The Library Tree Info page displays the metadata associated with the library record, such as acquisition details, contributor, and so on.
- The Library Spectrum Info page displays the metadata associated with the library spectrum, such as scan numbers, isolation width, and so on.

Library Spectrum pane

The Library Spectrum pane displays the library spectrum for the spectrum that you selected in the Library Tree pane.

Compound Structure pane

The Compound Structure pane displays the library compound structure. Match with the query structure is highlighted in red.

Precursor Structure pane

The Precursor Structure pane displays the library precursor structure.

13

Global Settings

This chapter describes the configuration options for the user interface that are available from the Global Settings dialog box.

Contents

Configure the application

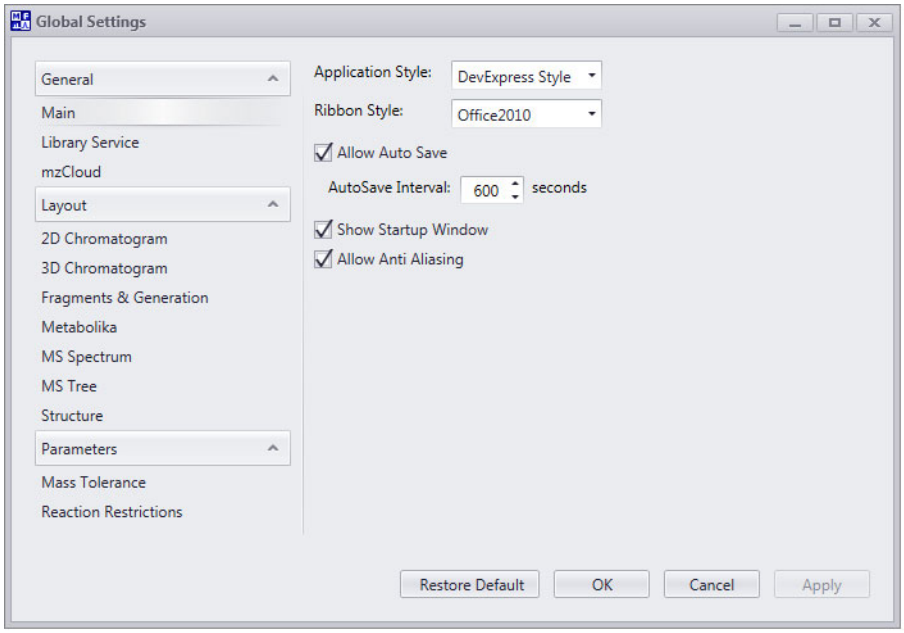
Use the Global Settings dialog box to configure the Mass Frontier 8.1 application.

❖ **To specify the configuration settings**

1. From the application's Start menu, click **Global Settings**.

   The Global Settings dialog box opens.

Figure 176. Global Settings dialog box



2. Do any of the following:

   - To restore the factory default settings for a specific view, open the view by selecting it in the left pane, and then click **Restore Default**.
   - To undo all the new settings, click **Cancel**.
   - To apply the new settings without closing the dialog box, click **Apply**.
   - To apply the new settings and close the dialog box, click **OK**.

General configuration settings

Table 163 describes the settings in the Global Settings dialog box that are located under General.

Table 163. Global Settings dialog box – General area parameter descriptions (Sheet 1 of 2)

Parameter	Description
Main	
Application Style	Specifies the color scheme and style for Mass Frontier. Default: DevExpress Style Options: • DevExpress Style: Black text on a light gray background. • Seven: Black text on a white background. The tab for the current module has a light blue background. • Office 2019 Colorful • Office 2019 Colorful Touch • Metropolis Dark: White or orange text on a dark background. The color of the text that describes the current location is orange. • Metropolis Light: Black or orange text on a light gray background. The color of the text that describes the current location is orange. • Deep Blue: Black text on a blue background. The tab for the current module has a dark blue background and white text. • Light Gray: Black or white text on a white or light gray background. The tab for the current module has a light blue background and black text. • Visual Studio 2010: Black text on a light gray background. The tab for the current module has a light-yellow background.
Ribbon Style	Specifies the ribbon style for the application window. Default: Office2010 Options: • Office2007 • Office2010 • TabletOffice
Allow Auto Save	Enables the auto-save feature, and specifies the interval between each auto save (in seconds). Default: 600 s; range: 120–99 999 999 s
Show Startup Window	Displays the startup window at launch.

Table 163. Global Settings dialog box – General area parameter descriptions (Sheet 2 of 2)

Parameter	Description
Allow Anti Aliasing	Enables anti aliasing. **Tip** If the screen refreshes too slowly, clear this check box. This option must be selected to display the 3D chromatogram on the 3D page of the Chromatogram view in a Chromatogram Processor window. By default, this option is enabled.
Library Service	
Use Localhost Service	The application uses the local Mass Frontier Library Service. To test the connection, click the **Test Connection** icon,  .
Auto-Detect Network Service	To detect the library services on your network, click the **Start Auto Detection** icon,  . If the search returns multiple hosts, select the appropriate host.
Set Service Manually	Select this option to manually set the library service. Specify the computer's IP address.
mzCloud	mzCloud is a mass spectral database that assist in identifying compounds even when they are not present in the library. Uncheck it to make this option unavailable. **Note** Ther user can enter their mz Cloud credentials.

Layout configuration settings

Table 164 describes the parameters in the Global Settings dialog box that are located under Layout.

Table 164. Layout settings (Sheet 1 of 7)

Parameter	Description
TIC and 2D Chromatogram	
Signal Line Width	Specifies the thickness of the signal lines in the 2D chromatogram. Default: 1; range: 1.0–no upper limit
Colors	Specifies the colors for each profile or peak in the chromatogram. Displays the changes at the bottom of the window in a demo chromatogram.

Table 164. Layout settings (Sheet 2 of 7)

Parameter	Description
Time Axis Notation	Specifies the notation format for the time axis. Options: • Decimal (for example, 1.750 min) • Hexadecimal (24-hour clock, for example, 1:45:0 = 1 hour, 45 minutes, and 0 seconds).
Annotation Box	Specifies the font family, font style, font size, background color, and rendering timeout for the text annotation.
General Font	Specifies the font family, font size, and font color used for the axis labels.
Background	Specifies the background color for the view.
Pane	Specifies the color of the plot area in a chromatogram view.
Border Style	Specifies the border style between the background and chromatogram.
Border Color	Specifies the color of the selected border.
Show Grid	Enables the grid in the chromatogram.
Grid Color	Specifies the color of the selected grid.
3D Chromatogram	
Scan Color	Specifies the color of the scans.
Peak Width	Specifies the width of the peaks.
Time Axis Notation	Specifies the notation format for the time axis. Options: • Decimal (for example, 1.750 min) • Hexadecimal (24-hour clock, for example, 1:45:0 = 1 hour, 45 minutes, and 0 seconds).
Font	Specifies the font family, font style and font size, background color, and rendering timeout for the text annotation.
Background	Specifies the background color for the view.
Ground Color	Specifies the ground color.
Show Grid	Enables the grid in the chromatogram.
Wall Color	Specifies the color of the wall.
Grid Color	Specifies the color of the selected grid.

Table 164. Layout settings (Sheet 3 of 7)

Parameter	Description
Grid Line Width	Specifies the width of the grid. Default value: 1
Rendering Timeout	Specifies the rendering timeout.
Fragments & Generation	
Arrow	Specifies cosmetic changes to the arrow.
Shape	Specifies if the arrow is filled or open.
Width	Specifies the width of the arrow (in pixels).
Height	Specifies the height of the arrow (in pixels).
Min. Length	Specifies the minimum length of the arrow (in pixels).
Pen Color	Specifies the color of the pen.
Pen Width	Specifies the width of the pen (in pixels).
Arrow Label (Font)	Specifies the font, font size, font style and font color of the arrow label.
Text Box	Enables a text box for the arrow. You can specify a color for the text box.
Standard Text	Specifies cosmetic changes to the standard text.
Background Color	Specifies the background color for the view.
Border Color	Specifies the color of the border.
Border Width	Specifies the width of the border (in pixels).
Border Margin	Specifies the size of the border margin.
Radius of Border	Specifies how circular the corners of the borders based on their radii.
Font	Specifies the font, font size, font style and font color of the text.
Miscellaneous	
Background Color	–
Result Fragment Color	Specifies the color of the resulting fragment.
Metabolika	
Arrow	Specifies cosmetic changes to the arrow.
Shape	Specifies if the arrow is filled or open.
Width	Specifies the width of the arrow (in pixels).

Table 164. Layout settings (Sheet 4 of 7)

Parameter	Description
Height	Specifies the height of the arrow (in pixels).
Min. Length	Specifies the minimum length of the arrow (in pixels).
Pen Color	Specifies the color of the pen.
Pen Width	Specifies the width of the pen (in pixels).
Arrow Label (Font)	Specifies the font, font size, font style and font color of the arrow label.
Text Box	Enables a text box for the arrow. You can specify a color for the text box.
Standard Text	Specifies cosmetic changes to the standard text.
Background Color	Specifies the background color for the view.
Border Color	Specifies the color of the border.
Border Width	Specifies the width of the border (in pixels).
Border Margin	Specifies the size of the border margin.
Radius of Border	Specifies how circular the corners of the borders based on their radii.
Font	Specifies the font, font size, font style and font color of the text.
Miscellaneous	–
MS Spectrum	
Peak Line Width	Specifies the thickness of the peak line (in pixels).
Colors	These check boxes specify the colors of various spectral peaks, depending on the ion type.
Annotation Box	These options specify the appearance of text annotations.
General	See 2D Chromatogram of layout settings. The parameter settings under 2D Chromatogram and MS Spectrum are not independent of each other.
MS Tree	
Colors	Specifies the colors for each individual part of the MS Spectrum. The changes are displayed in real time at the bottom of the window in a demo tree.
Caption	Specifies the font, font size, and font color used for the captions.

Table 164. Layout settings (Sheet 5 of 7)

Parameter	Description
Spacing	Specifies the spacing between parallel spectra within a single node. The number in each Spacing category corresponds to the spectrum count in the node. For instance, Spacing 3 defines spacing in nodes comprising up to three parallel spectra.
Width of Node	Specifies the width of the tree node (in pixels).
Height of Node	Specifies the height of the tree node (in pixels).
Vertical Distance between Nodes	Specifies the vertical distance between tree nodes (in pixels).
Horizontal Distance between Nodes	Specifies the horizontal distance between tree nodes (in pixels).
Width of Collapsed Node	Specifies the width of collapsed tree nodes (in pixels).
Height of Collapsed Node	Specifies the height of collapsed tree nodes (in pixels).
Structure	You can change the design of the structures and other objects displayed in the drawing area of the Structure Editor. Use the Structure parameters to create graphics that suit your report or publication needs. The structure settings apply to all structures in the application. Any change in the Structure settings affects the structures in other modules.
Atom	
Font	Specifies the font, font size, and font color used for the atoms.
Show Carbons	Displays carbon symbols in the structure.
Charge Marging	Displays the distance between the position of charge in a structure. Default value: 1 Range: 1 to 15
Charge Size	Displays the size of the charge in a compound structure. Default value: 6 Range: 3 to 15
Radical Size	Displays the size of a radical. Default value: 5 Range: 3 to 15

Table 164. Layout settings (Sheet 6 of 7)

Parameter	Description
Charge Thickness	Displays the thickness of the charge in a compound structure. Default value: 1 Range: 1 to 7
Show Hydrogens	Displays hydrogen symbols in the structure. By default, the Structure Editor displays hydrogen symbols for heteroatoms only. It does not display the symbols for hydrogen atoms attached to carbon atoms.
Bond	
Length	Specifies the length of the bonds (in pixels).
Thickness	Specifies the thickness of the bonds. Options: • Thin • Middle • Thick
Color	Specifies the color of the bonds. (For black-and-white print only) If you have set bright colors for bonds or atoms, the lines and fonts might appear blurry. To solve this issue, specify dark colors for structural items, including spectra, chromatograms, and mechanisms.
Bond space	Displays the distance between the bonds in a compound structure. Default value: 2 Range: 2 to 10
Stereo Bond Width	Displays the width of the setero bonds in a compound structure. Default value: 5 Range: 3 to 20
Additional	
Selected atom(s) Colors	Displays the color of one or more selected atoms.
Font	Specifies the font family, font style, font size, and font color used for the atoms

Table 164. Layout settings (Sheet 7 of 7)

Parameter	Description
Text box	
Guided Bond Drawing	Enables guided bond drawing. When drawing a bond, you can point the mouse to the structure to preview different suggested placements of the new bond and select the preferred position.
(Drawing) Thickness	Specifies the thickness of the guided bond drawing preview.
(Drawing) Color	Specifies the color of the guided bond drawing preview.
Bracket Color	Displays the color of the Markush bracket.
Bracket Thickness	Displays the thickness of the Markush bracket. Default value: 1 Range: 0.5 to 5
Bracket Arm	Displays the arm length of the Markush bracket. Default value: 0.05 Range: 0.01 to 0.5

Mass accuracy and precision configuration settings

Use the Mass Tolerance view of the Global Setting dialog box to change the mass tolerance values of selected scans. When processing spectra with different mass tolerance settings from different origins, you might want to force (locally or globally modify) the tolerance values to create comparable data. The application uses tolerance values when comparing two *m/z* values. See "Accuracy" on page 397.

Table 165. Mass Tolerance parameter settings (Sheet 1 of 2)

Parameter	Description
Mass Accuracy of Experimental Data	
Determine from Source (Recommended)	Makes the application use the tolerance data as it is recorded in the source data.
User Defined	Overrides the tolerance data read from the source data.
Orbitrap/Fourier	Specifies the accuracy for an Orbitrap/FT mass analyzer.
Ion Trap	Specifies the accuracy for an ion trap mass analyzer.
Quadrupole	Specifies the accuracy for a Quadrupole mass analyzer.
TOF	Specifies the accuracy for a TOF mass analyzer.

Table 165. Mass Tolerance parameter settings (Sheet 2 of 2)

Parameter	Description
Sector	Specifies the accuracy for a sector mass analyzer.
Others	Specifies the accuracy for other mass analyzer.

Use the Accuracy settings to help you differentiate between adjacent peaks, the m/z values of spectra, and the fragments of a fragments list. Delta*M* specifies the smallest m/z value that can separate two peaks without the peaks being combined into a single peak. Two peaks that are separated by an m/z value that is smaller than the Delta*M* value are merged into a single peak with a single m/z value.

Table 166. Mass Precision (Decimals) parameter settings

Parameter	Description
From Accuracy	Makes the application use the accuracy value that is defined in the Mass Accuracy area to determine the precision.
Number of Decimal Digits	Specifies the precision for experimental data.
Number of Decimal Digits for exact data	Specifies the precision for theoretical data, for example, the mass of the formula.

Use Precision settings to display the m/z values of the peaks on the x-axis of an MS spectrum. In contrast to Mass Tolerance settings, the application ignores precision settings in calculations and always uses the highest possible precision. To ensure the correct differentiation of peaks from the depicted *m/z* values, you must set the precision to a value that is the same as the resolution or higher (number of decimal digits).

Reaction Restrictions configuration settings

You can use the Reaction Restrictions view of the Global Settings dialog box to specify the Reaction Restriction parameter settings. These settings affect the SledgeHammer and Batch Fragmentation modules.

Tip You can edit the reaction restriction parameter settings in any of these three locations:

- The Reaction Restrictions dialog box from SledgeHammer.
- The wizard from Batch Fragment Generation.
- The Reaction Restrictions view of the Global Settings dialog box.

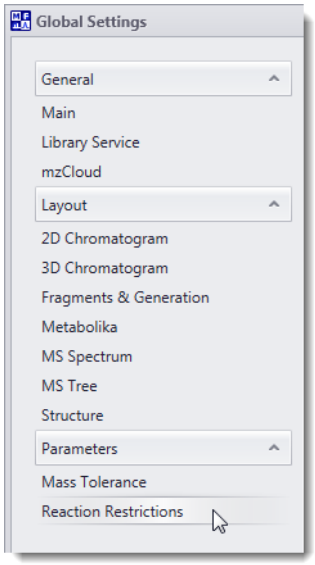
Changing the settings in one of these locations changes the settings in all three locations.

For details about specifying the reaction restriction settings, see "Reaction Restrictions dialog box" on page 286.

❖ To open the Reaction Restrictions view of the Global Settings dialog box

1. On the application tab bar, click the **Start** tab to open the Start menu.
2. On the Start menu, choose **Global Settings**.

   The Global Settings dialog box opens.
3. In the left pane, click **Reaction Restrictions**.



14

Create and manage spectral libraries

Use the Mass Frontier Server Manager to create and manage spectral libraries. This chapter describes how to create, import or export, backup or restore, merge, migrate, and share spectral libraries.

Note For information about adding, viewing, or copying library records, see Chapter 4, "Data Manager module."

Contents

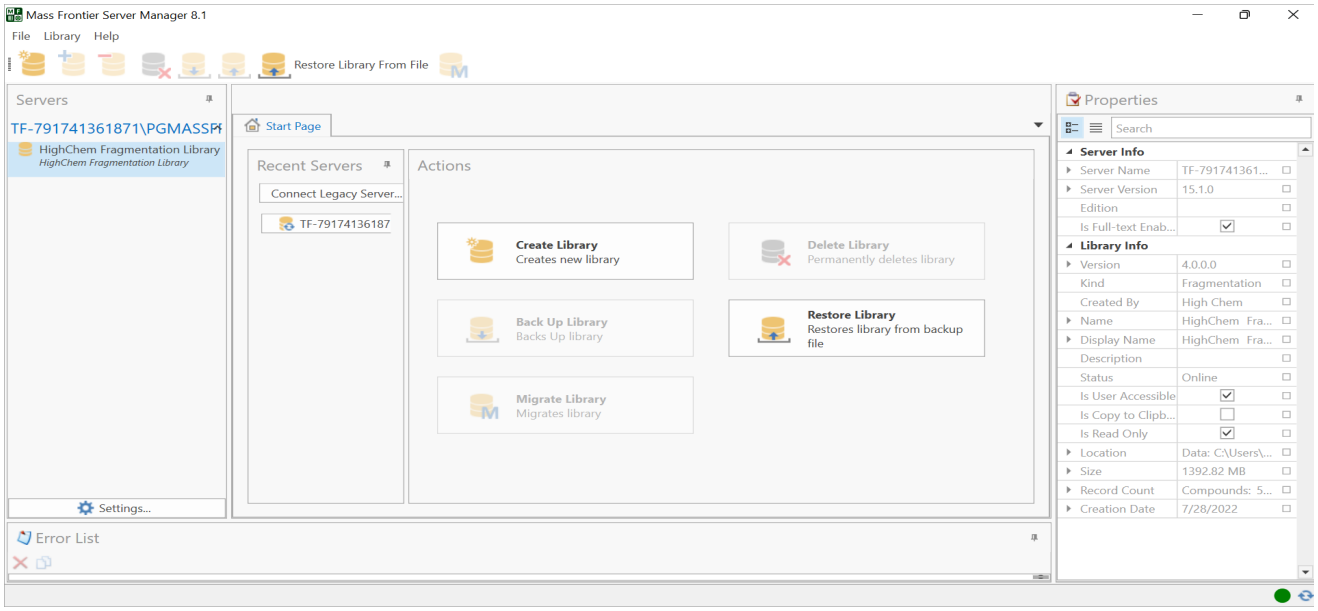
Select the Mass Frontier Library Service

❖ **To select the Mass Frontier library service:**

1. Open the Mass Frontier Server Manager 8.1 application by doing one of the following:
   - From the Windows Start menu, choose **(All) Programs > Thermo Mass Frontier 8.1 > Mass Frontier Server Manager 8.1**.

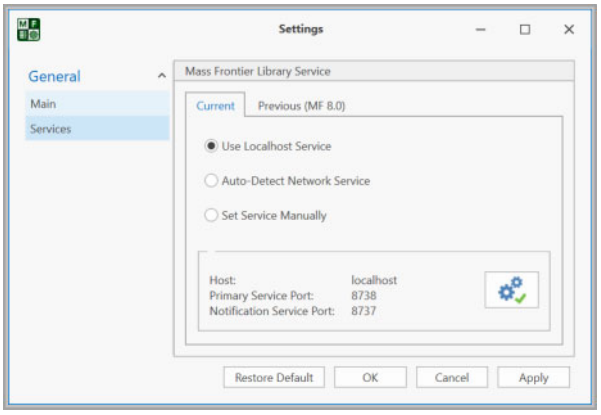
- From the Windows 10 Start menu, select High Chem **Mass Frontier 8.1 > Mass Frontier Server Manager 8.1**.
- From the desktop, double-click the **Mass Frontier Server 8.1** icon, .
- (Optional) If the connection fails, do the following:
  - Open the (Windows) Services dialog box.
  - In the Services (Local) list, right-click Mass Frontier Service 8.1, and then choose **Stop**.
  - Right-click on **postgre15massfrontier81** and then choose **Restart**.
  - After the service gets restarted, right-click Mass Frontier Service 8.1, and then choose **Start**.

Figure 177. Mass Frontier Server Manager 8.1 window



2. Open the **Settings > Services** option and do the following:
   a. At the bottom left of the Mass Frontier Server Manager window, click **Settings**.
   b. In the General pane of the Settings window, click **Services**.

Figure 178. Services view of the default Settings window



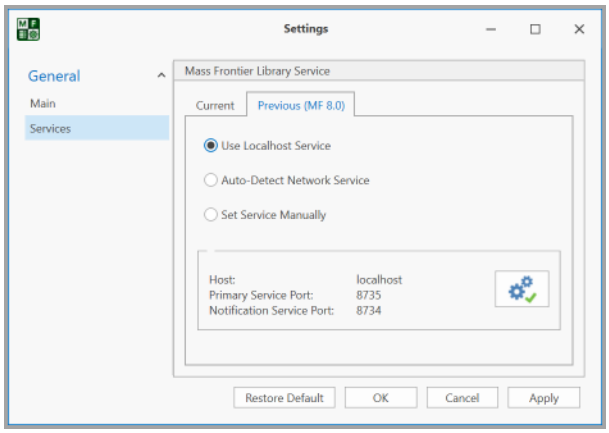
By default, the Current tab opens on the right hand side of the Mass Frontier Server Manager window.

Click **Previous** (**MF 8.0**) page to configure to Mass Frontier 8.0 version.

c. (Optional) To select a library service from the previous version of the Mass Frontier Server Manager (version 8.0), click the Previous (MF 8.0) tab.

   The Previous (MF 8.0) page opens.

Figure 179. Services view of the Previous (MF 8.0) tab



d. Select one of the service options (See Table 167 for descriptions of these options).

Table 167. Mass Frontier Library Service settings

Services	Description
Use Localhost Service	Select this option if you have installed the Mass Frontier application as a local installation, where both the application and the library server reside on your computer.
Auto-Detect Network Service	Select this option if you want to create a user library on a different computer. See "Connect the client-only Mass Frontier application to the library service" on page 384.
Set Service Manually	Select this option if the name of the computer does not appear automatically. Specify the computer's IP address.

3. To test the connection to the Mass Frontier Library Service installed on your computer, click the **Test connection to selected service** icon, .
4. At the prompt, click **OK**.
5. Click **OK** to close the Settings window.

Connect the application to the library service

Before you change the Mass Frontier Library Service connection to the Mass Frontier 8.1 application, close all the opened modules.

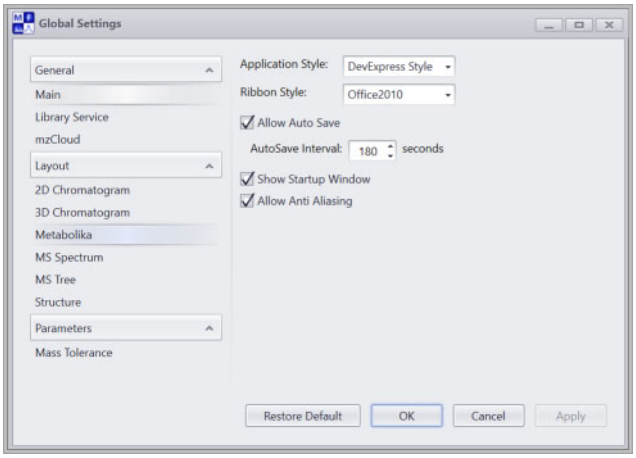
❖ **To connect the application to the library service**

1. Open the Mass Frontier 8.1 application.

2. From the Start menu, choose **Global Settings**.

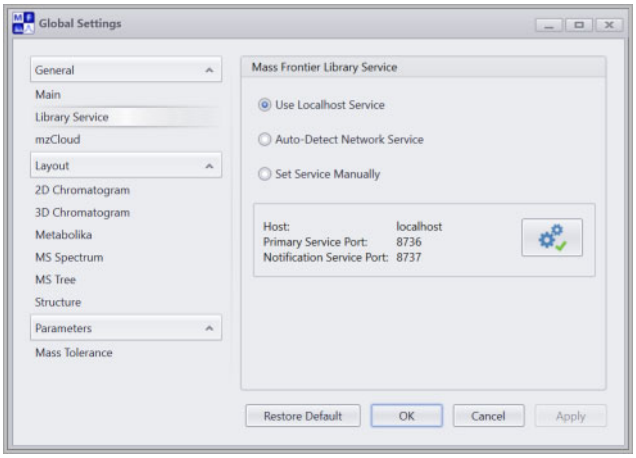
   The Global Settings dialog box opens.

   Figure 180. Global Settings dialog box



3. In the Global Settings dialog box, under General, click **Library Service**.

   Figure 181. Library Service view



4. Select the **Use Localhost Service** option if you have a Mass Frontier Library Service installed.

   Tip To connect to a Mass Frontier Library Service running on a different computer, see "Share spectral libraries using the client/server installation."

5. Click **Apply**, and then click **OK**.

The Mass Frontier application connects to the Mass Frontier Library Service and to the newly created library on your local computer. This completes the local libraries setup.

Tip To view newly created libraries, open a Data Manager window in the Mass Frontier application.

Share spectral libraries using the client/server installation

The Mass Frontier application supports the sharing of libraries on a local network. You can connect multiple Mass Frontier 8.1 client applications to a single Mass Frontier Library Service and work on the same set of spectral records.

The following definitions explain the terms used in this topic:

- Client—A computer in the local network with a client-only Mass Frontier 8.1 installation that is connected to the Mass Frontier Library Service.
- Server—A computer in the local network with the Mass Frontier Library Service installed. The setup and management of the libraries are identical to the default installation, as documented in "Select the Mass Frontier Library Service" on page 379.

For details, see these topics:

- Connect the client-only Mass Frontier application to the library service
- Create a new user library
- Restore a server library
- Delete a server library
- Back up spectral libraries
- Restore spectral libraries
- Migrate spectral libraries

Connect the client-only Mass Frontier application to the library service

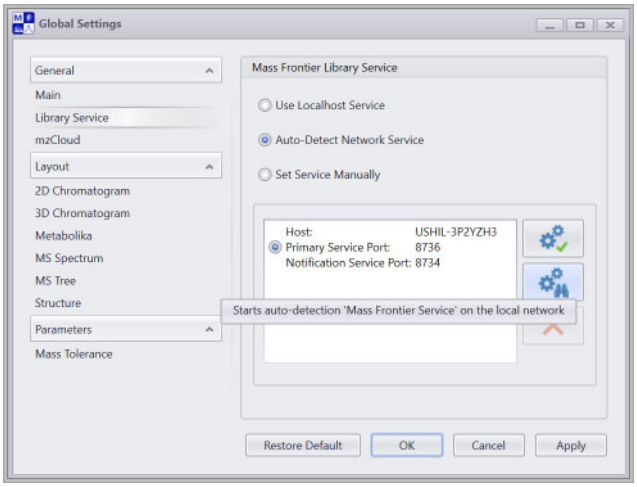
Before you change the Mass Frontier Library Service connection in the Mass Frontier 8.1 application, you must close all opened modules.

❖ To connect a client-only computer to the server

1. Start the Mass Frontier 8.1 application.
2. From the Start menu, choose **Global Settings**.
3. In the Global Settings dialog box, under General, click **Library Service**.
4. Select the **Auto-Detect Network Service** option.
5. To find all the available servers on the local network, click the auto-detect icon,  .

Tip The server uses ports 8733 and 8734 for communication with other clients. Adjust the server's firewall configuration to allow traffic from the clients.

Figure 182. Library Service view of the Global Settings dialog box



6. After the server name appears, click ✕ to cancel the auto-detect service.
7. To test the connection, select the server and click the **Test Connection to Selected Service** icon, .
8. When the success message appears, click **OK**.
9. Click **Apply** and **OK**.

The client-only Mass Frontier 8.1 application connects to the Mass Frontier Library Service on the server. The new libraries installed on the server are visible to the client in a Data Manager window.

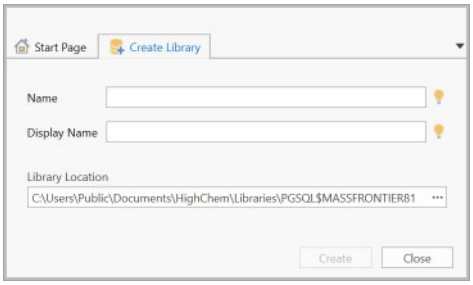
Create a new user library

To create a new user library, do the following:

- Select the Mass Frontier Library service. See "Select the Mass Frontier Library Service" on page 379.
- On the Start page of the Mass Frontier Server Manager window, click **Create Library**.

  The Create Library page opens.

  Figure 183. Create Library page of the Mass Frontier Server Manager



10. On the Create Library page, do the following:
    - In the Name box, type a name for the library.
    - (Optional) In the Display Name box, type the text string that you want the application to display in the Data Manager window.

      Note If you leave this box empty, the application displays the text that you entered in the Name box in the Data Manager window.

    - In the Library Location box, browse to the directory where you want to store the library.
11. Click **Create**.

    The name of the newly created library appears in the Servers pane on the left side of the Mass Frontier Server Manager window.

Restore a server library

When you create, or restore a new library on the server, it is visible to all the connected clients. It is also available in the Data Manager and for other functions, such as a library search or fragmentation generation.

Delete a server library

You can delete a server library in Mass Frontier 8.1 server manager.

❖ **Delete a server library which is not in use by client**

- Choose a library from the list of service libraries available on the server.
- Click **Delete Library** from the actions.

  A Delete Library page opens.

❖ Click **Delete**.

Figure 184. Delete library which is not in use by client

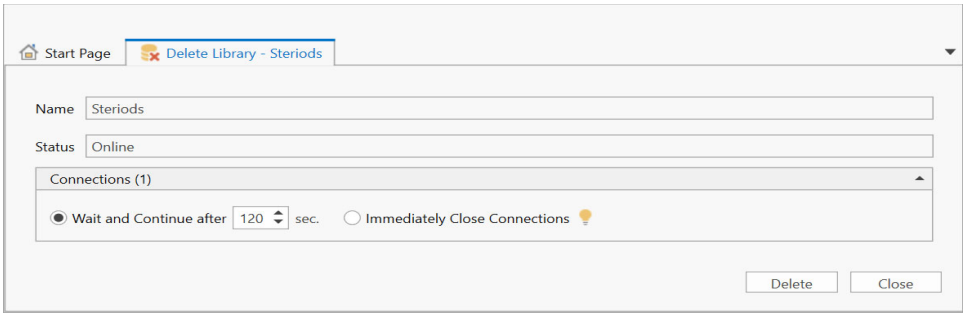
❖ Delete a server library which is in use by client

When you choose to delete a server library, Mass Frontier Server Manager 8.1 displays the clients currently connected to the Mass Frontier Library Service.

- Choose a library from the list of service libraries available on the server.
- Click **Delete Library** from the actions.

  A Delete Library page opens.
- Choose Wait and Continue after 120 s to disconnect the library service after a predefined grace period and then click **Delete**.
- Choose Immediately Close Connections to disconnect the library service immediately and then click **Delete**.

Figure 185. Delete library which is in use by client



If you delete a compound entry from a user library, a flashing green icon appears in the Library tab to notify you that the application is removing the record. In addition, the application sends a notification to the notifications pane of the opened Data Manager window. To view the notification, you might need to click the down arrow at the right end of the Modification bar in the Notifications pane.

Back up spectral libraries

Periodic backups of your production library prevents the accidental loss of important data.

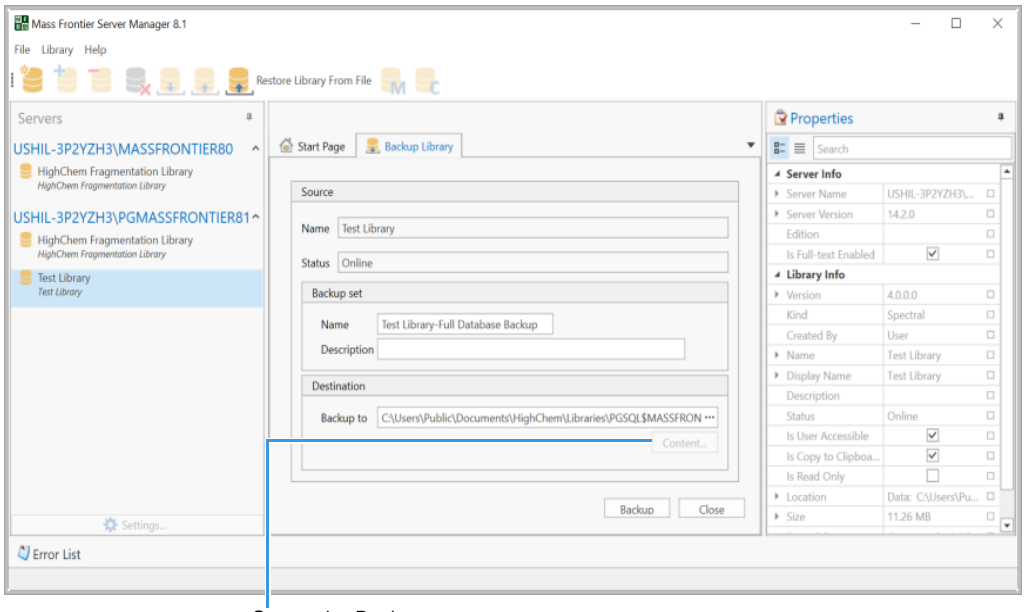
Tip You can use this procedure to exchange spectral libraries between different computers, as an alternative to transferring spectral libraries.

❖ To back up spectral libraries

1. Open Mass Frontier Server Manager 8.1.
2. Select the spectral library.
3. On the Start page, click **Back Up Library**.

The Backup Library page opens.

Figure 186. Backup Library page



Opens the Backup History dialog box

4. In the Destination area, do the following:
   a. Verify the path to the backup folder for all the library backups.
   b. (Optional) To view the backup history of all the files saved in the folder, click **Content**.

      The Backup History dialog box opens.
   c. (Optional) Review the backup history, and then close the dialog box.

IMPORTANT To avoid creating inconsistent backup files that cannot be restored, do not save a backup library to a backup file that holds a backup of another spectral library. In addition, make sure that the backup file name matches the spectral library name.

5. Click **Backup**.

Tip The default location of the spectral library backup files is Users\Public\Documents\HighChem\Libraries\MSSQL$MASSFRONTIER81\Backups.

To prevent data loss in case of disk failure, you can also copy the files to another storage medium such as a USB or network drive.

Restore spectral libraries

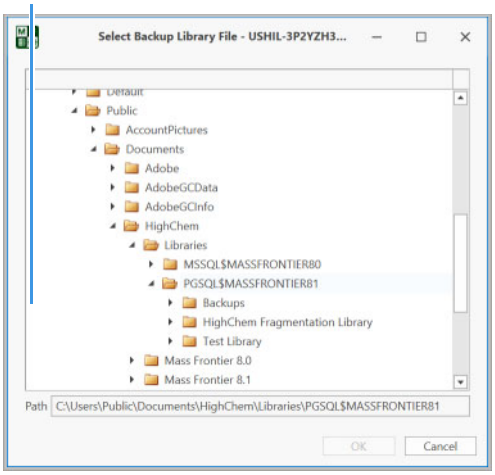
You can restore a spectral library and fragmentation database from a backup file to a new database or library using the function restore spectral libraries.

❖ To restore a spectral libraries

1. Open the Mass Frontier Server Manager 8.1.
2. Click **Restore Library** from the list of actions.

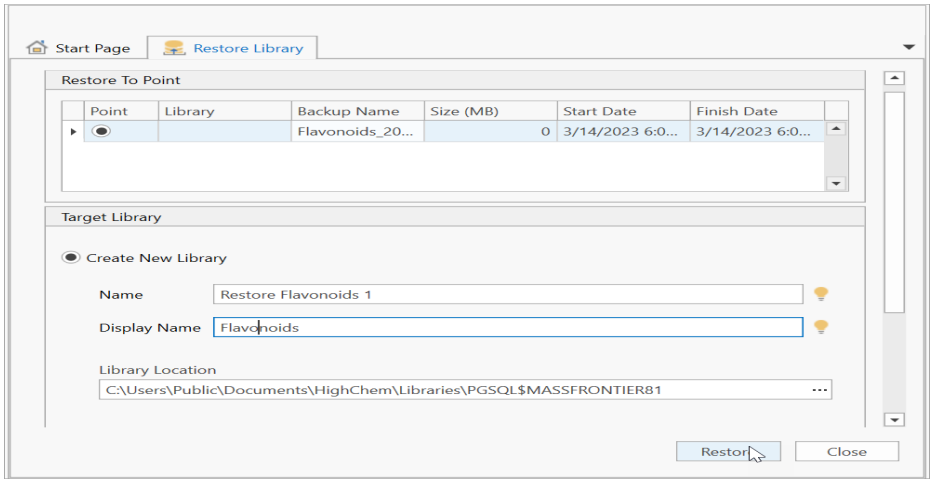
   The Select Backup Library File dialog box opens.

   Figure 187. Select Backup Library File dialog box



3. Select the backup library file (.bak file) from the folder where it is located and click **OK**.
4. Choose the restore point in the top region of restore library page.
5. Enter the new library name and display name in the target library section.

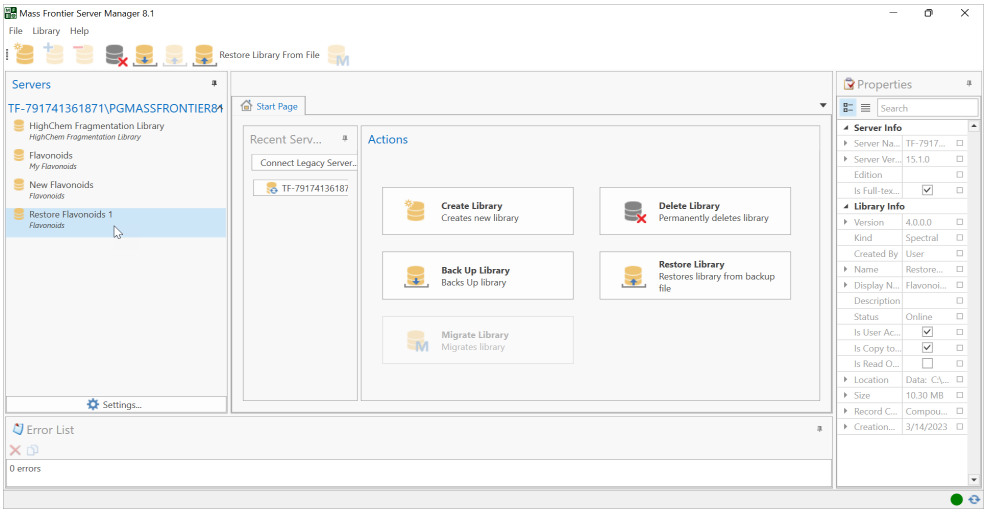
Figure 188. Restore Library page



6. Click **Restore**.

The backup spectral library restores with a new library name and display name. You can view the restored library in the servers pane of Mass Frontier Server Manager 8.1.

Figure 189. Restore Flavonoids 1 library in Server pane



Restored library

Migrate spectral libraries

You can import the spectral trees and fragmentation mechanisms created in the Mass Frontier 8.0 user libraries to the Mass Frontier 8.1 user libraries using the Migrating Spectral Libraries feature.

IMPORTANT Despite using the same file suffix (.mdf), the Mass Frontier 8.1 library files use different data formats. To import Mass Frontier 8.0 libraries to Mass Frontier 8.1, you must have installed the Mass Frontier 8.0 server and respective libraries, and they must be available for service. Refer to the *Mass Frontier 8.0 User Guide* for more details.

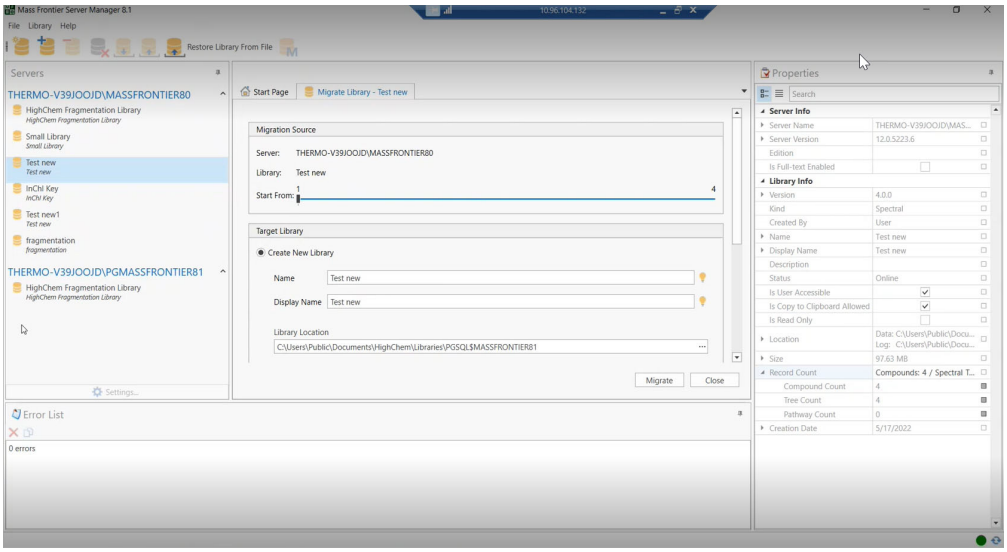
❖ To migrate spectral libraries

1. Open Mass Frontier Server Manager 8.1.
2. From the settings option, select the Mass Frontier 8.0 (Previous version) library service. See "Select the Mass Frontier Library Service" on page 379.

   You can view all the available libraries for Mass Frontier 8.0 and 8.1 in servers pane.
3. Choose the library to migrate from the Mass Frontier 8.0 library list and then click **Migrate Library** from the action pane**.**

The migration page opens.

Figure 190. Migrate Test new library



4. In the new Migrate Library page, adjust the parameters for the library migration as follows:

   a. In the Migration Source, select the range of library entries you want to import.

   b. In the Target Library, select the destination for the imported records.

   c. In the Migration Options, specify the action for imported records with unknown polarity.

   IMPORTANT The Mass Frontier 8.0 Library Service does not support unknown or unspecified polarity records. The polarity property must be correctly defined during the import of such records.

5. Click **Migrate**

   A green bar indicates the progress of migration. The newly converted library appears in the left pane under MASSFRONTIER81 and is available for use in the Mass Frontier 8.1 application.

Connect to the Mass Frontier 7.0 libraries

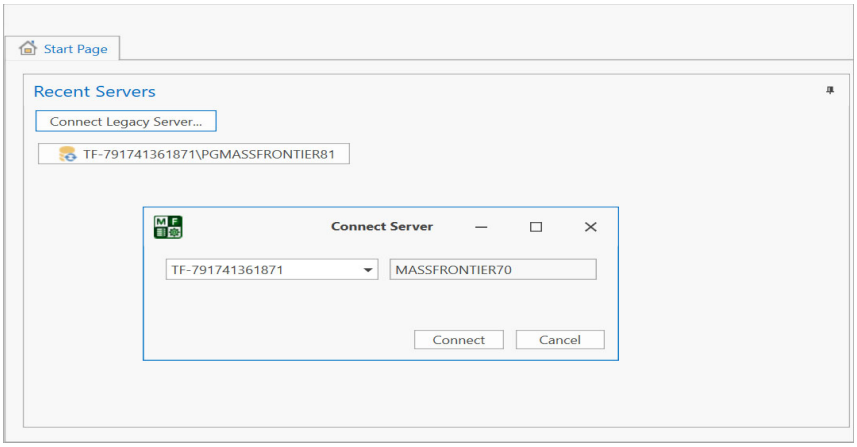
You can connect to the Mass Frontier 7.0 libraries if Mass Frontier 7.0 software (Legacy software) is installed in your computer.

Do the following to connect and view the libraries:

1. In the Start page of Mass Frontier Server Manager 8.1, click **Connect Legacy Server.**

The Connect server dialog box opens.

Figure 191. The Start page with connect server dialog box open



2. In the Connect Server dialog box, select the computer with Mass Frontier 7.0 libraries.

   Tip You can also search for available services in the local network from the dropdown list.

3. Click **Connect**.

   The Mass Frontier 7.0 libraries appear in the Servers pane. You can view more information about the selected library in the Properties pane, on the right.

Limitations to the Mass Frontier Server Manager 8.1

1. The Mass Frontier 8.1 does not support the migration of the Mass Frontier 7.0 libraries. However you can connect and view the Mass Frontier 7.0 libraries if you have the Mass Frontier 7.0 software installed on your computer or network.
2. The Mass Frontier Server Manager cannot recognize a folder with extended charset if your default language settings of computer is not English. The Server Manager does not show this folder and does not allow to install a user library in this folder.

Troubleshooting

Refer to the following table to troubleshoot common errors while using the Mass Frontier 8.1 and Mass Frontier Server Manager 8.1 applications.

Table 168. Troubleshooting (Sheet 1 of 4)

Issue	Cause	Remedy
The SledgeHammer module is too slow.	The module predicts too many fragments.	On the Sizes page of the Reaction Restrictions dialog box, decrease the Reaction Limit value to decrease the number of fragments. The factory default value is 25,000.
	The computer's memory is full.	Close other RAM consuming programs or restart the computer.
	The processing computer has insufficient RAM.	Upgrade the RAM in your processing computer.
The mzCloud library is not accessible, or the mzCloud library does not show up in the Data Manager module or in the Component Search view.	The connection to the mzCloud library has failed.	Try the following: • Check the Internet connection. From the Start menu, choose **Connection Check**. In the Connection Check dialog box, click **Run** to identify the network issue. • Verify that the system time is synchronized. An incorrect system time causes the mzCloud server to reject requests. • Restart the Mass Frontier 8.1 application. • Restart the computer.
The Missing Scan Data dialog box appears when you open a data file in the Chromatogram Processor module.	The Chromatogram Processor could not extract some relevant data from the input file, such as accuracy, precursor accuracy, and isolation width. The Log page lists all the affected scans.	For a missing accuracy value, you can either enter the value manually or set the application to use the predefined value in the Mass Tolerance view of the Global Settings dialog box.

Table 168. Troubleshooting (Sheet 2 of 4)

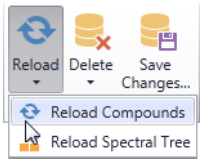
Issue	Cause	Remedy
The File Access Denied message appears, and you cannot copy a library.	You must activate the administrator privileges to copy the file from the default location.	1. In the Windows Search Programs and Files box, type **cmd**. 2. In the Programs list, select **cmd.exe**. 3. In the command prompt dialog box, type: **net user administrator /active:yes** and press the **ENTER** key. 4. Copy the library.
The compound you saved to the user library from New tab or from Curator is not displayed in the user library in Data Manager.	The Data Manager needs refreshing.	Do the following: In the Data Manager window, select the user library where you saved the compound. In the Data Manager toolbar, choose **Reload > Reload Compounds**.  The newly added compound appears in the library.

Table 168. Troubleshooting (Sheet 3 of 4)

Issue	Cause	Remedy
Auto Annotation: some predicted fragments are not annotated on the spectrum	By default, the application reads the mass accuracy for each spectral peak from the raw data. If the mass accuracy of the raw data is too low, the m/z value of the predicted fragment could be outside of the valid range of the peak's *m/z* value.	You can change the global setting to a user-defined mass accuracy or force the mass accuracy to a specific value in the Chromatogram Processor module. ❖ **To globally use the user-defined mass accuracy** 1. From the MF Start menu**,** choose **Settings**. 2. In the Global Settings dialog box, choose **Mass Tolerance**. 3. In the Mass Accuracy area, select the **User Defined** option. Then, enter the appropriate mass accuracy for your data. ❖ **To apply a user-defined mass accuracy to the current raw data** 1. In the Chromatogram Processor toolbar, click the **Force Accuracy** icon, . 2. Enter the appropriate value in the Force Tolerance dialog box, click **Preview**, and then click **Accept**.
Mass Frontier is slow when changing zoom or browsing the scans in the chromatogram.	The application has to complete the anti-aliasing process before it redraws the plots.	❖ **To turn off anti-aliasing** 1. Open the **Start** menu and choose **Global Settings**. 2. In the left pane of the Global Settings dialog box, click **Main**. 3. Clear the **Allow Anti-Aliasing** check box. 4. Restart the application. With anti-aliasing turned off, the application should be more responsive, as the graphics are less detailed.
IMPORTANT For the 3D page in the chromatogram view of the Chromatogram Processor module, anti-aliasing must be enabled. When you turn off anti-aliasing, the 3D graph is unavailable.		

Table 168. Troubleshooting (Sheet 4 of 4)

Issue	Cause	Remedy
While opening files, you see a black square on the screen, but it disappears within a few seconds.	This error usually indicates an issue with the rendering of the graphics.	Wait a few seconds to allow the program to continue. If the problem persists, reinstall the latest version of the graphics card driver.
The Chromatogram Processor window is not responding and cannot be closed even if no action is in progress.	A previous action might not have ended properly.	Try performing another action (for example, Threshold Filter, Smoothing Filter, or Remove Contaminants) to terminate the previous task. Then, reload the page.
The Component Search yields no results.	No Library is selected.	Choose a library from the dropdown Library menu in the Chromatogram Processor > Component Search view.
The Component Search Details window does not show library spectra.	The mzCloud library is temporarily disconnected.	Close and reopen the Component Search Details window from the Component Search view.
You cannot paste a spectrum to Data Manager.	Pasting spectra to the Data Manager window is only supported in a New (library) tab.	❖ **To paste a spectrum from the Clipboard** 1. In the Data Manager window, select the **New** tab. 2. Right-click the Spectrum page of the MS spectrum view and choose **Paste MS Spectrum**. Or, right-click the New library compound list and choose **Paste MS Spectrum**.
There are no entries in the library compound list in Data Manager.		When the Mechanism view is active, library compound lists are not displayed below the library tab bar. Toggle between the Mechanism and Tree views in the Data Manager window.
The connection to the Mass Frontier Service fails.	The service is not started.	If the connection fails, do the following: • Open the (Windows) Services dialog box. • In the Services (Local) list, right-click "Mass Frontier Service 8.1", and choose **Stop**, then right-click postgre15massfrontier81, and choose **Restart**. • Again right-click "Mass Frontier Service 8.1", and choose **Start**.

A

Glossary

Contents

Accuracy

The Mass Frontier application uses two parameters associated with the *m/z* value: accuracy and resolution. Resolution is the smallest difference in the *m/z* value of two centroid peaks that a detector can distinguish, and accuracy is the estimated difference between the observed and theoretical *m/z* values of a peak. Accuracy is a critical parameter for most of the spectra-processing algorithms such as Library Search, Isotope Pattern Prediction, Component Detection, FISh, Neutral Loss Chromatogram, Extracted Ion Chromatograms, and so on.

The Mass Frontier application usually determines the accuracy and resolution of the mass spectral data from the information in the raw data file. Some data formats such as mzML might not have accuracy defined. In the Chromatogram Processor module, when you open a data file where the accuracy of the data is not defined, a Missing Scan Data dialog box prompts you to specify the accuracy. In the Settings dialog box, you can set the application to override the accuracy value in imported data and, instead, use an accuracy value that you specify.

Component

In the Mass Frontier application, the definition of component (for the identification of a unique compound) depends on the component detection algorithm:

- For the JCD algorithm (for LC/MS data), a component consists of the ion profiles with comparable shapes and maxima belonging to a limited time range.

  When the Tree Branching – merge parent ions option is selected (), the component can consist of multiple XIC traces for various adduct ions or in-source fragments of the parent compound. For the precursor m/z value, the algorithm selects the *m/z* value of the most intense peak in the MS1 spectrum, after ruling out the following peaks:

  - Peaks from in-source fragments due to neutral losses
  - Peaks for possible adduct ions
  - Isotopic peaks

  When the Tree Branching – one tree per parent ion option is selected (), each adduct ion for a compound is considered as a separate component.

- For the TECD algorithm (for LC/MS data), a component consists of a spectral tree that is derived from the matching data-dependent scans for each precursor ion in the MS1 scans in the specified time range.
- For the DICD algorithm (for infusion data), a component consists of a spectral tree that is derived from the data-dependent scans for each MS2 precursor ion in the data file. When Beginning of Tree Branching is set to MS Stage 1, the full MS1 node of the spectral tree consists of the original MS1 scans or a combined MS1 scan if Average Scans is selected. When Beginning of Tree Branching is set to MS Stage2, the full MS1 node of the spectral tree includes only the MS2 precursor ion.

Related Topics

- Joint Component Detection (JCD) algorithm
- Total Ion Extraction Component Detection (TECD) algorithm
- Direct Infusion Components Detection (DICD) algorithm
- Use extracted ion chromatograms for verifying component detection

HighChem Fragmentation Library

Thermo Fisher Scientific has collated and uploaded the fragmentation mechanisms published in the print media dedicated to mass spectrometry. This library collection, HighChem Fragmentation Library, serves as a knowledge base for predicting the fragmentation pathways of your structures. Each reaction, along with the related chemical structures, was manually drawn in Pathway Editor and saved in the library. The fragmentation library currently contains approximately 220 000 individual reactions. The fragmentation pathways contain complementary information, such as title, authors, and information source.

There are two versions of the HighChem Fragmentation Library:

- A speed and size-optimized version for fast fragments generation. This library does not provide mechanism explanations or links to the published sources of the fragmentation templates. The default location of this library file is as follows:

  drive:\ProgramData\HighChem\Mass Frontier 8.0\Fragmentation\HighChem.FragmentationLibraries.db

- A full-size fragmentation library with all annotations and links to the published sources of the fragmentation templates. The generation of fragments is slow.

Tip When you select the fragmentation libraries for fragments generation, select the **Use HighChem Fragmentation Library** check box to use the optimized fragmentation library or the **Explain Mechanisms (slower)** check box to use the full-size fragmentation library.

Note Both HighChem Fragmentation Library versions are read only and copy protected. The copy and edit functionalities are disabled for these libraries.

To ensure high-quality data, fragmentation mechanisms have been evaluated in two stages: manual and automatic. The manual evaluation includes accuracy and plausibility assessments of reaction mechanisms and consistency checking between fragment masses and peak *m/z* values if the spectrum is available. The automatic evaluation includes simple element, charge, and radical consistency checks on both sides of the reaction and newly developed algorithms for complex electron mapping that have revealed formerly erroneous mechanisms. Both stages have uncovered numerous problems and errors regarding mechanisms, and HighChem has either made the appropriate corrections or excluded these mechanisms from the library.

Table 169. Overview of source journals used to construct the HighChem Fragmentation Library (Sheet 1 of 2)

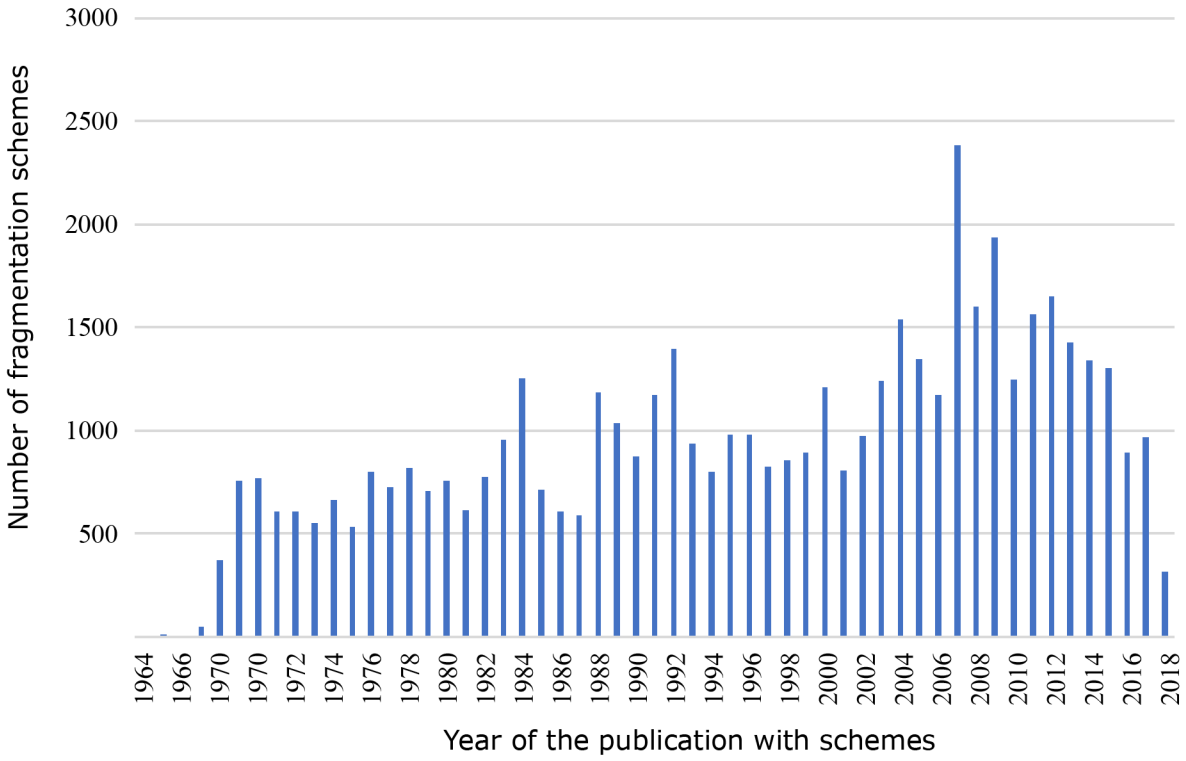
Journal	Volumes	Years	Publications	Fragmentation Schemes
Journal of the American Society for Mass Spectrometry	1–29	1990–2018	668	3897
International Journal of Mass Spectrometry and Ion Physic	1–53	1968–1983	92	346

Table 169. Overview of source journals used to construct the HighChem Fragmentation Library (Sheet 2 of 2)

Journal	Volumes	Years	Publications	Fragmentation Schemes
International Journal of Mass Spectrometry and Ion Processes	54–175	1983–1998	275	1117
International Journal of Mass Spectrometry	176–430	1998–2018	473	2235
Rapid Communications in Mass Spectrometry	1–31	1987–2017	1683	8808
Journal of Mass Spectrometry	30–53	1995–2018	951	5750
Organic Mass Spectrometry	1–29	1968–1994	2366	13480
Journal of the Mass Spectrometry Society of Japan	11–64	1964–2016	155	735
Mass Spectrometry Reviews	1–30	1981–2011	129	4028
European Journal of Mass Spectrometry in Biochemical, Medicine, and Environmental Research	1–2	1980–1982	12	47
Journal of Chromatography A	628–1477	1993–2016	573	2561
Journal of Chromatography B	670–1086	1995–2018	977	3652
Journal of Chromatography	181–536	1980–1991	103	282
Biomedical Mass Spectrometry	1–23	1974–1994	355	1345
Biomedical and Environmental Mass Spectrometry	13–19	1986–1990	142	635
European Journal of Mass Spectrometry	1–22	1995–2016	343	2165
Analytical Chemistry	70–86	1998–2014	3	5
International Union of Pure and Applied Chemistry		1991	1	2
Total			**9301**	**51 090**

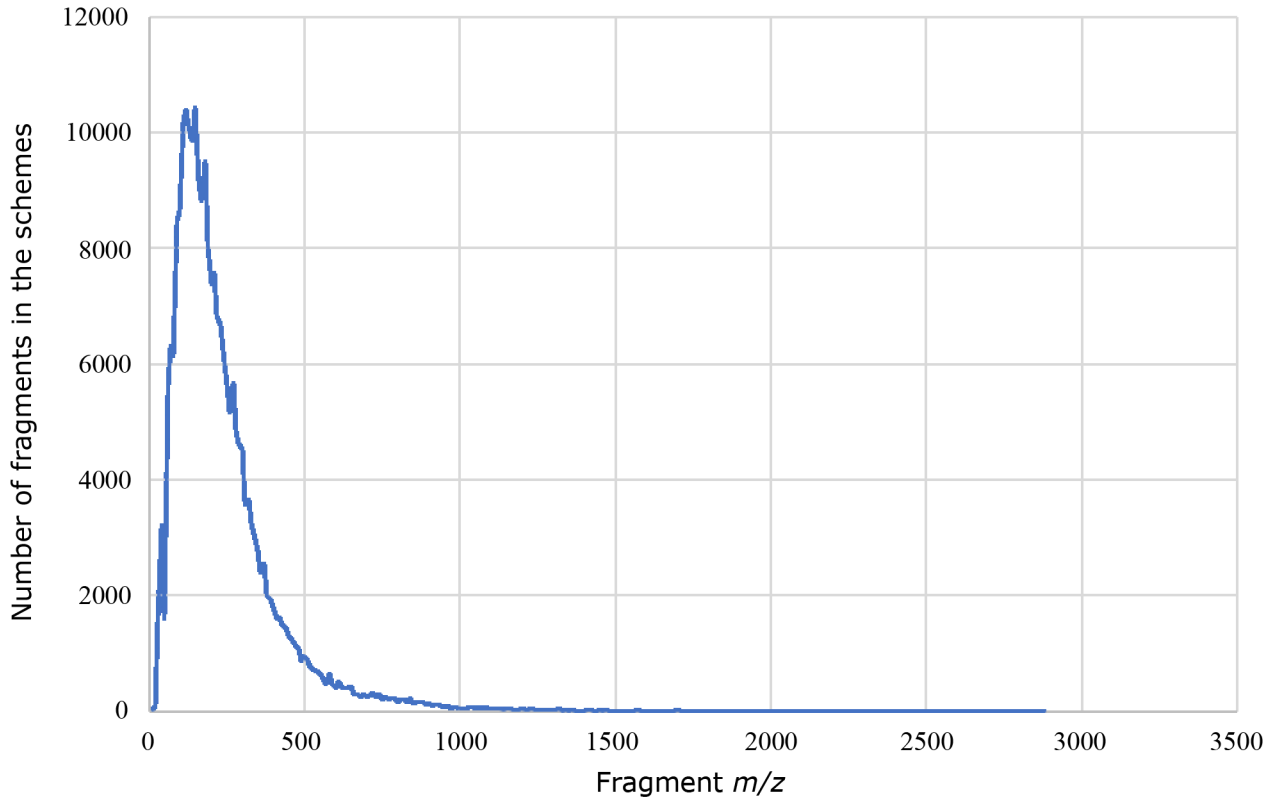
The collection of fragmentation data in HighChem Fragmentation Library is an ongoing project intended to provide up-to-date knowledge in mass spectrometry. Figure 192 illustrates the number of new schemes released each year from 1994 to 2018.

Figure 192. Overview of the fragmentation library publication dates



HighChem Fragmentation Library is primarily a small-molecule LC/MS library focusing on molecules with *m/z* values < 1000. Figure 193 displays the *m/z* distribution of the individual fragments in fragmentation schemes, with a maximum distribution between 0–500.

Figure 193. Distribution of the fragment *m/z* values in the schemes



Ion profile

An ion profile is an extracted ion chromatogram that uses the mass tolerance from the raw data or the mass tolerance set by the user.

MolGate structure aggregator

MolGate™ is an online collection of over 58 M chemical structures and metadata acquired from reputable public sources for specific usage in *m/z* structure search. You can query the structures by using the mzLogic Search tool or mzlogic Component Search from within the Mass Frontier 8.0 SR1 application. All structures come with the appropriate links to the database source. The online database is hosted on the mzCloud server address.

Table 170. Overview of compounds and sources accessible by MolGate (Sheet 1 of 2)

Acronym	Database	Number of Compounds
Kegg	Kyoto Encyclopedia of Genes and Genomes	14 911
ChEBI	Chemical Entities of Biological Interest (ChEBI)	32 286
HMDB	The Human Metabolome Database	41 011

Table 170. Overview of compounds and sources accessible by MolGate (Sheet 2 of 2)

Acronym	Database	Number of Compounds
Tox21	Tox21 - National Toxicology Program	6221
Phenol	Database on Polyphenol Content in Foods	905
MetaCyc	MetaCyc: Metabolic Pathways From all Domains of Life	10 920
WebBook	NIST Chemistry WebBook	39 407
FDA	Substance Registration System - Unique Ingredient Identifier (UNII)	30 410
ChemIDPlus	ChemIDplus Advanced	210 384
UNPD	Universal Natural Product Database	225 041
ECMD	E coli Metabolome Database	3548
ChEMBL	ChENBL, European Bioinformatics Institute	1 361 893
PubChem	PubChem, National Center for Biotechnology Information	58 991 624
WikiPathways	WikiPathways	2387
Drugs@FDA	Drugs@FDA - FDA Approved Drug Products	912
HSDB	Hazardous Substances Data Bank	4166
LactMed	Drugs and Lactation Database	1519
MeSH	Medical Subject Headings	50 983
CTD	Comparative Toxicogenomics Database	4371
DailyMed	DailyMed, US National Library of Medicine	1519
MedlinePlus	Medline Plus, US National Library of Medicine	1734
Pillbox	Drug Identification and Image Display	738
DSLD	Dietary Supplement Label Database	589
FooDB	Food Component Database	25 437
DrugBank	DrugBank, University of Alberta and The Metabolomics Innovation Centre	6678
ChemBank	ChemBank	1434
ACToR	ACToR	338 036
T3DB	T3DB	2321
Chemical Book	Chemical Book	26 818

The current implementation of the MolGate collection in the Mass Frontier 8.0 application has the following limitations:

- Structure search by *m/z* value of any of the supported adduct types only
- Structure searches of radicals and naturally charged compounds are not supported.

mzCloud spectral database

mzCloud is a state of the art mass spectral database that assists analysts in identifying compounds in areas such as life sciences, metabolomics, pharmaceutical research, toxicology, forensic investigations, environmental analysis, food control, and various industrial applications. mzCloud™ features a freely searchable collection of high resolution/accurate mass spectra using a new third generation spectra correlation algorithm.

mzCloud is an extensively curated database of high-resolution tandem mass spectra that are arranged into spectral trees. MS/MS and multi-stage MSn spectra were acquired at various collision energies, precursor *m/z* values, and isolation widths using collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD). Each raw mass spectrum was filtered and recalibrated giving rise to additional filtered and recalibrated spectral trees that are fully searchable. Besides the experimental and processed data, each database record contains the compound name with synonyms, its chemical structure, computationally and manually annotated fragments (peaks), identified adducts, multiply charged ions, molecular formulas, predicted precursor structures, detailed experimental information, peak accuracies, mass resolution, InChi, InChiKey, and other identifiers. mzCloud is a fully searchable library that allows spectra searches, tree searches, structure and substructure searches, monoisotopic mass searches, peak (*m/z* value) searches, precursor searches, and name searches.

Resonance peaks

Resonance peaks are residual peaks from the Fourier Transform (FT) or Orbitrap analyzer.

Figure 194 shows an unfiltered MS1 spectrum for a theophylline sample analyzed with an Orbitrap MS.

Figure 194. Unfiltered spectrum of theophylline

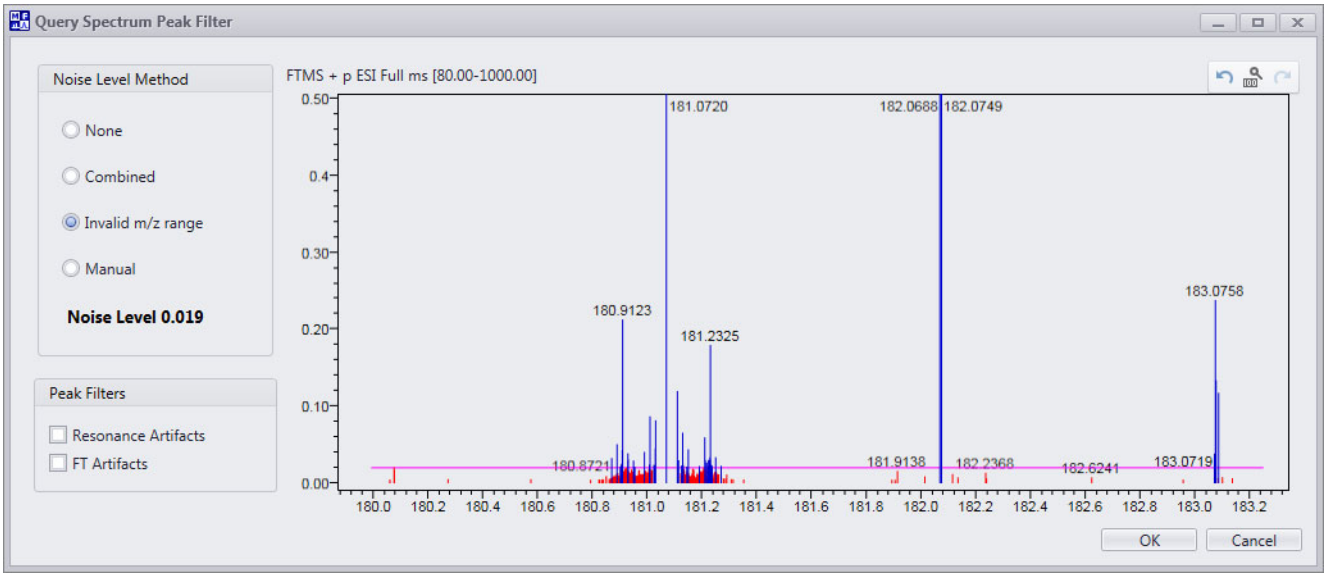


Figure 195 shows the filtered spectrum with the FT artifacts removed.

Figure 195. Spectrum of theophylline with the FT artifacts removed

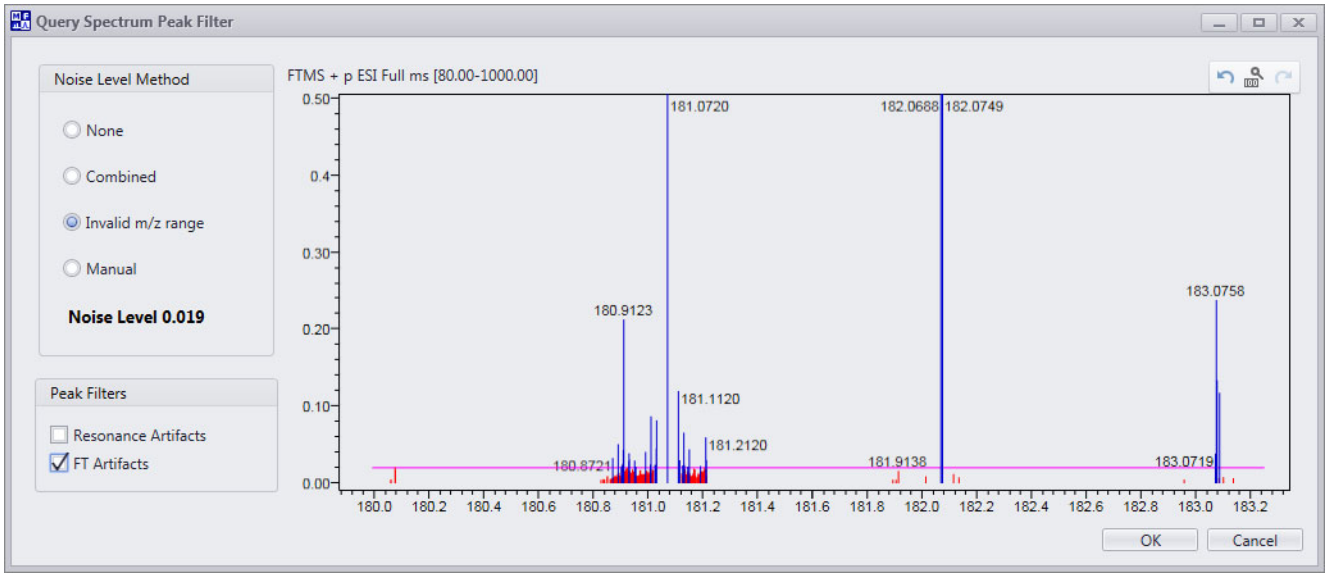


Figure 196 shows the filtered spectrum with the FT artifacts and resonance peaks removed.

Figure 196. Spectrum of theophylline with the FT artifacts and resonance peaks removed

Spectral tree

A spectral tree is a data structure that represents the hierarchical dependencies of the spectra acquired in MSn experiments.

Soft ionization and CID techniques can generate spectra whose appearance depends on the experimental conditions and sample preparation. To manage and search diverse product spectra with an identical precursor ion for a single chemical entity, the application uses spectral trees that can contain nodes with node items. The node item stands for any product or calculated spectrum of an identical precursor *m/z* value or *m/z* range (node spectra).

Node product spectra represent spectra that were acquired at various collision energies and isolation widths or by using Wideband Activation™. They can also be ZoomScan™ spectra, source CID spectra, or any other spectra that provide spectral signatures for correct compound identification (similar to identifying a person by using several fingerprints instead of a single fingerprint). The spectral node strategy strengthens the robustness of all the mathematical processing methods, and compared to spectral averaging, the node strategy reflects the physical principles behind the fragmentation.

Unspecified charge site

The application can process structures with unspecified charge sites. This type of molecular representation is important when using ionic structures because the favored ionization site or explicit charge site during fragmentation reactions is often unclear. You can choose from several pre-defined ion types when drawing structures in the Structure Editor module.

You must be aware of the potential complications when using an unspecified charge site with fragment prediction. If you attempt to generate fragments from a structure with an unspecified charge location, the system internally predicts all the relevant combinations of the structures by using an explicit charge location. This process significantly slows the calculations and increases the number of predicted fragments. When possible, avoid unspecified charge sites in predicting fragments and mechanisms.

Post-processing

In library searching and data curation, post-processing defines the methods that the mass spectrometry specialist (curator) used to create the library record. You can use the Curator module to generate library entries with raw, filtered, or calibrated spectral trees.

Additionally, the mzCloud online spectral database can use virtual stepped energy spectra, which are synthetically constructed averages of reference spectra. These are returned as standard library hits when searching stepped energy spectra and are the activation energies of the individual reference spectra that correspond to the individual energy steps of the query.

Table 171. Post-processing types for spectra

Type	Icon	Description
Raw		Original unmodified spectra. Some spectra from the spectral trees might have been removed during the curation process.
Filtered		Original data with filtering applied to eliminate noise peaks and low-quality scans. In addition, replicate scans are merged. Allows for better data compression.
Calibrated		Filtered data with applied mass recalibration. Allows for better search reproducibility.
Virtual Step Energy Spectrum		Average of reference spectra with activation energies corresponding to the energy steps of the query spectrum.

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