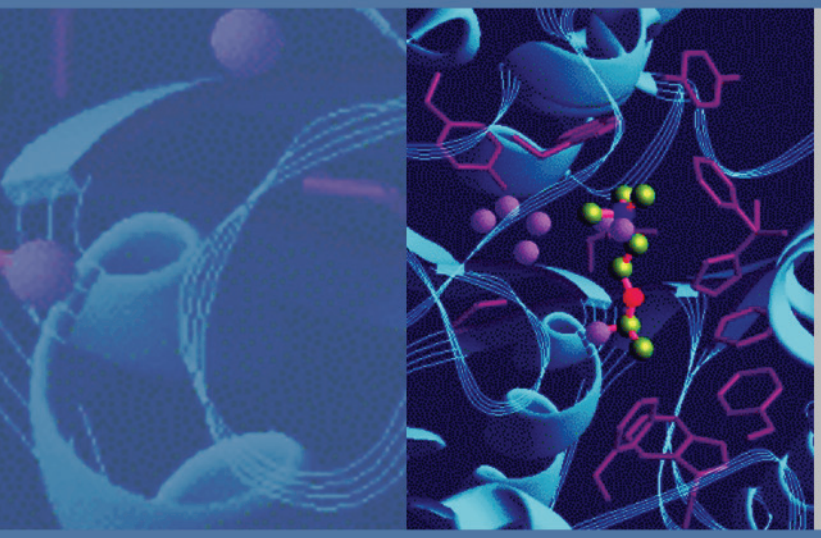

Mass Frontier

Version 7.0

User Guide

XCALI-97349 Revision A February 2011

DOCUMENTATION SURVEY

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

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Release history: Release A, February 2011

Software version: Mass Frontier 7.0

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

Contents

Preface

This guide describes how to use Thermo Scientific Mass Frontier™ for the management, evaluation, and interpretation of mass spectra.

Contents

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

Related Documentation

Mass Frontier includes Help and these manuals as PDF files:

- Mass Frontier User Guide
- Mass Frontier Quick Start Guide

❖ **To view product manuals**

Go to **Start > Programs > HighChem Mass Frontier 7.0.**

❖ **To open Help**

From the Mass Frontier window, choose **Help > Contents Index**.

For more information, visit www.thermo.com. You can find application notes at www.thermo.com\appnotes.

Special Notices

Make sure you follow the precautionary statements presented in this guide. The special notices appear in boxes.

Special notices include the following:

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.

System Requirements

Mass Frontier requires a license. In addition, your system must meet these minimum requirements.

System	Requirements
Hardware	• Computer with Pentium™ III-compatible or later processor, with a minimum of 600 MHz • 512 MB of RAM (1 GB or more recommended) • SVGA or higher resolution color monitor • 2 GB or more hard disk space
Software	In addition to the software included on the Mass Frontier CD, you must have the following applications: • Windows 7, Windows Vista™, Windows XP (SP 2), or Windows 2000 (SP 4) • Microsoft™ Office XP (recommended) • Internet Explorer™ 7.0 (SP 1) or later • Xcalibur™ 2.0 installed with local user access (recommended)

Getting a License

When you first start the Mass Frontier application, the activation window opens. The system generates a unique serial number that you must send to Thermo Fisher Scientific Technical Support. When contacting Technical Support, include your name, company, e-mail or fax number, the serial number, and the type of license you need. Thermo Fisher Scientific recommends copying and pasting the serial number and sending it by e-mail.

You will receive an Activation Key that you enter in the Activation Key box. When you click Activation, the program is activated according to the type of authorization your license gives you. You must run the Mass Frontier 7.0 application with administrator rights when entering the activation key.

Three types of licenses are available:

- 60-Day Evaluation Version (free of charge)
- Full Version Single License
- Upgrade from 5.0 Single License

The evaluation version is full-featured and automatically expires 60 days after activation. Any attempt to set back the system date automatically terminates this version. You can purchase and then activate the full version of the Mass Frontier application at any time, during or after the free evaluation, without reinstalling the software. The activation window with the serial number is located under **Help > About > Activation**.

Each Activation Key is only valid for a single license. Any additional installation generates a different serial number that requires a different Activation Key.

For questions regarding activation, contact Thermo Fisher Scientific Technical Support in San Jose, CA, United States:

- E-mail: ThermoMSLicensing@thermofisher.com
- Fax: [1] (408) 965-6120

When your license is activated, go to www.highchem.com and download the latest service release.

For technical questions or installation problems, contact HighChem Technical Support:

- E-mail: ms@highchem.com

  Use **Mass Frontier** in the e-mail subject line.

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To get local contact information for sales or service

Go to www.thermoscientific.com/wps/portal/ts/contactus.

❖ To copy manuals from the Internet

Go to mssupport.thermo.com, agree to the Terms and Conditions, and then click **Customer Manuals** in the left margin of the window.

❖ To suggest changes to documentation or to Help

- Fill out a reader survey online at www.surveymonkey.com/s/PQM6P62.
- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

Introducing Mass Frontier

The Mass Frontier application is a software package for the management, evaluation, and interpretation of mass spectra. Because of the large volume of information a mass spectrometer can produce, management capabilities are essential in mastering your analytical workloads. The Mass Frontier application provides tools for processing and organizing mass spectral and chromatographic data. To read more about HighChem Ltd., refer to the HighChem Web site.

Use the Mass Frontier application to review collected data and efficiently interpret mass spectra. The Mass Frontier application can interpret mass spectra even when an unknown is not found in your library or a commercially available library. The software package includes the HighChem Fragmentation Library™ and a library of Spectral Trees™.

Contents

Overview

The Mass Frontier application consists of multiple modules that are displayed as separate windows:

- Server Manager
-  Structure Editor
-  Database Manager
- Fragmentation Library
-  Chromatogram Processor
-  Components Editor
-  Spectra Classifier
-  Spectra Projector
-  Neural Networks
-  Fragments Comparator
-  Isotope Pattern
-  Periodic Table
-  Formula Generator
-  Fragments & Mechanisms
-  Report Creator

All the modules are located in the Mass Frontier application window. Four of the modules (Fragments & Mechanisms, Neural Networks, Spectra Projector, and Components Editor) are generated from your input data; they cannot be directly opened from the application window.

Although each of the modules is independent, the application can automatically establish a link between several modules (see "Exchanging Mass Spectral Data Between Modules" on page 65). For example, the Mass Frontier application can make a link between the Database Manager module and the Fragments & Mechanisms module. In this case, the application links the peaks in a mass spectrum in the Database Manager window with mass-to-charge ratios in the Fragments & Mechanisms window. You can also link records in the Database Manager window with objects in the Spectra Classifier and Spectra Projector windows.

Server Manager

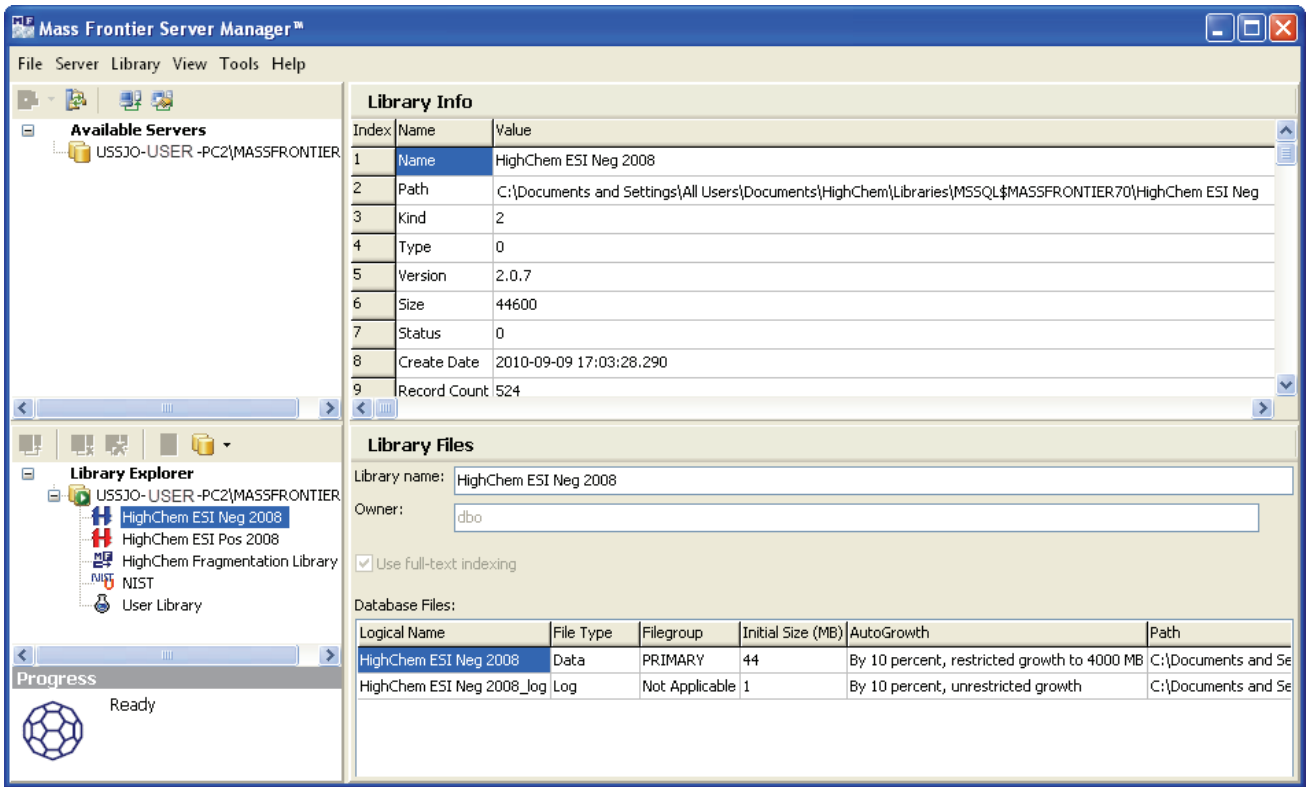
The Mass Frontier application includes the Mass Frontier Server Manager, a stand-alone application for management and administration of libraries on a Microsoft SQL Server™ relational model database server. Using the Server Manager, you can access libraries over a computer network. The SQL Server stores libraries that you can access from individual instances of the Mass Frontier application. Using the Server Manager, you create, install, remove, delete, import, convert, back up, and restore spectral and fragmentation libraries in one location for all instances of the application.

You can create libraries only on the same computer where the SQL Server is located. For example, if your SQL Server is located on a network computer, you cannot create a library on your local computer.

To access the Server Manager from the Mass Frontier main menu, choose **Library > Server Manager**. This menu item is accessible only if the Server Manager application is installed on the same computer as the Mass Frontier application. If the Server Manager is installed on a network computer, you must start it from this location.

For a complete description of the features in the Server Manager window, see Chapter 5, "Library Utilities."

Figure 1. Server Manager window

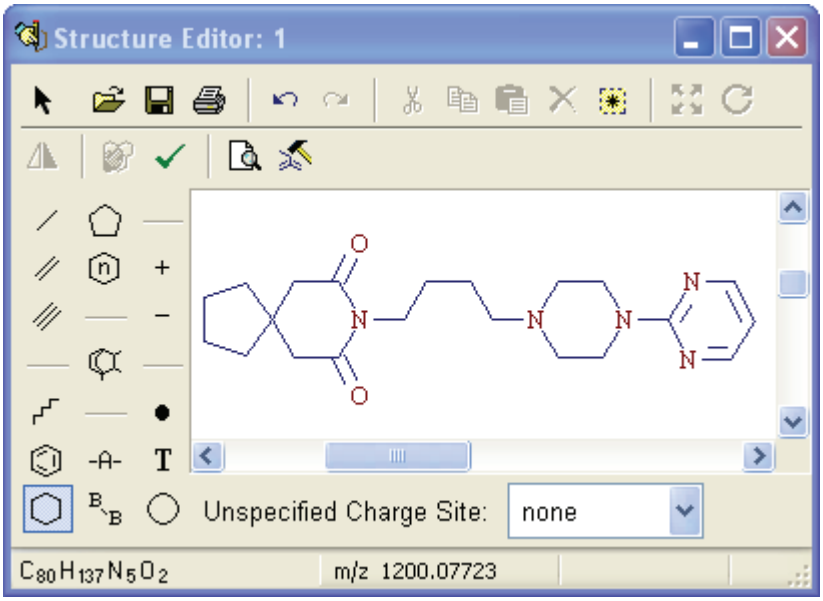


Structure Editor

The Structure Editor module is a structure drawing tool that automatically calculates the mass of a selected fragment and the corresponding loss. You can use the chemical structures that you created in this module throughout the Mass Frontier application.

For a complete description of the features in the Structure Editor window, see Chapter 4, "Structure Editor Module."

Figure 2. Structure Editor window

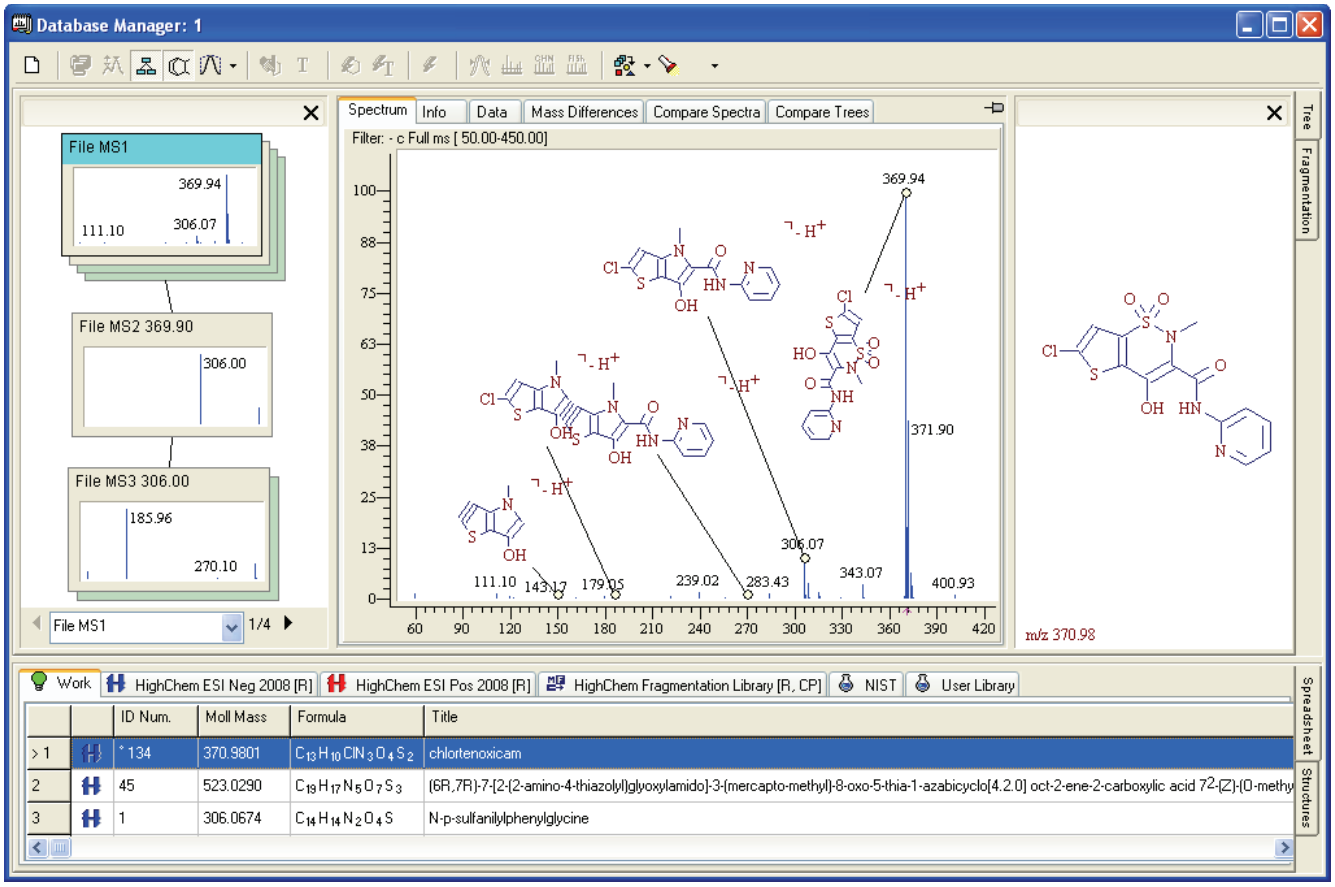


Database Manager

The Database Manager module provides several ways to organize and process mass spectra, chemical structures, and libraries, and include the data in a spreadsheet format. This module supports data exchange with the Microsoft Excel™ application. You can use the advanced database query and search features of the module to access information needed for rapid compound identification. You can also create your own libraries containing spectra, MS^n spectral trees, chromatograms, chemical structures, and extensive compound characteristics.

For a complete description of the features in the Database Manager window, see Chapter 2, "Database Manager Module."

Figure 3. Database Manager window



Fragmentation Library

Use the Fragmentation view on the Database Manager window to create and manage fragmentation mechanism databases. The Fragmentation view contains a full-featured graphical editor for entering fragmentation reactions that you can store in a database, together with complementary information for the reaction. You can query all the fields of the database, for example: authors, ionization method, or molecular mass. All the library structures from the reactions are also fully searchable.

For a complete description of the features in the Fragmentation view, see Chapter 7, "Fragmentation Library."

The Fragmentation Library contains an expert system that automatically extracts a decomposition mechanism for each fragmentation reaction in the database and determines the compound class range where you can apply the mechanism. This expert system applies database mechanisms to your structure and automatically predicts the fragmentation reactions for a specified compound.

The Mass Frontier application includes approximately 31 000 fragmentation schemes that contain approximately 130 000 individual reactions collected from mass spectrometry literature. The collected mechanisms are stored in the HighChem Fragmentation Library.

Figure 4. Fragmentation Library mechanisms

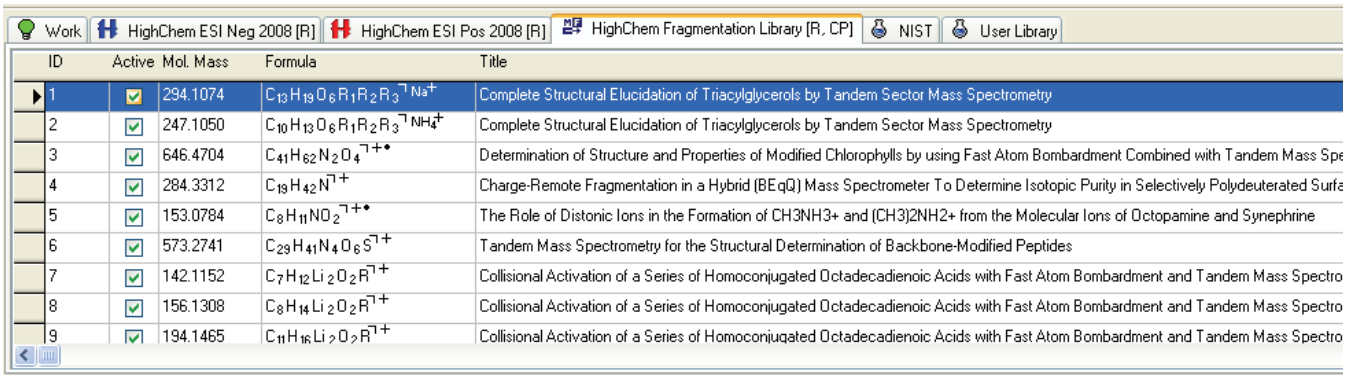


Figure 5. Reaction page of the Fragmentation view

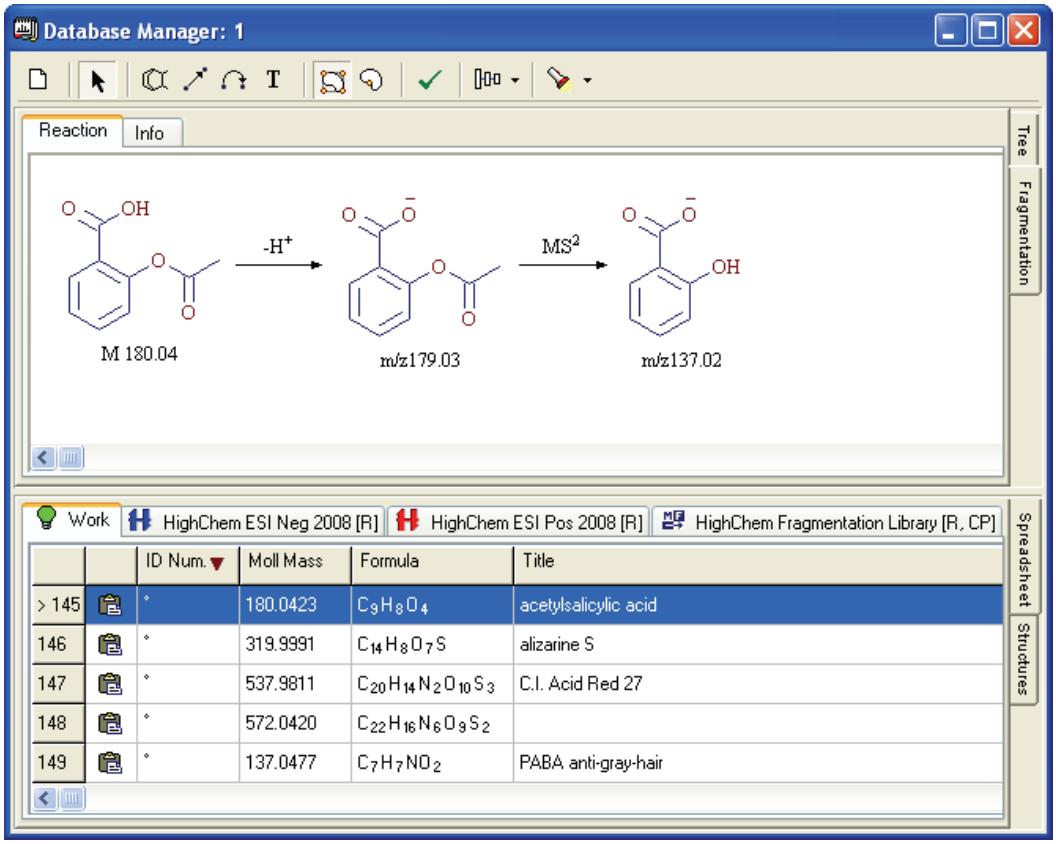
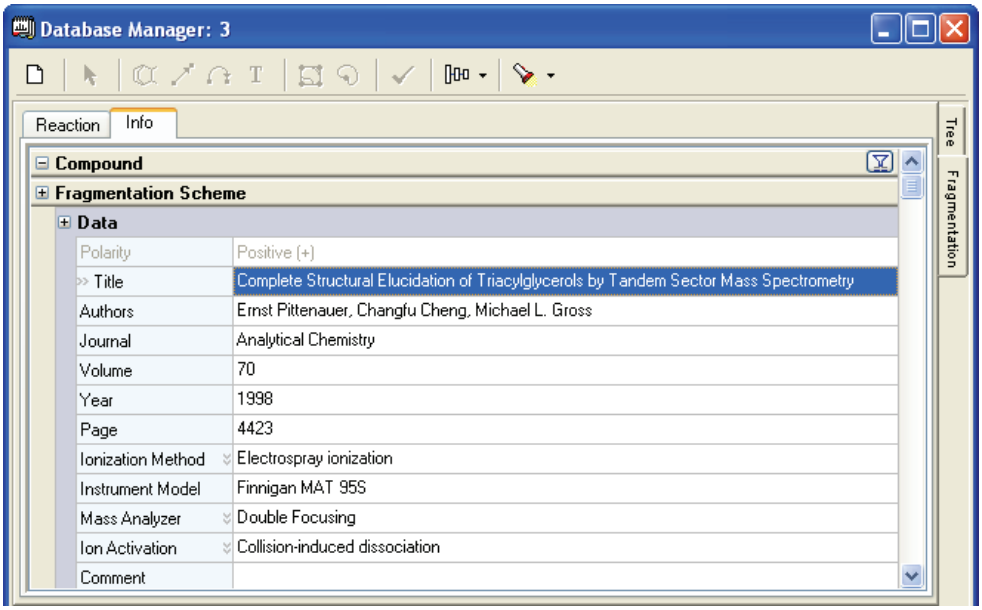


Figure 6. Info page of the Fragmentation view

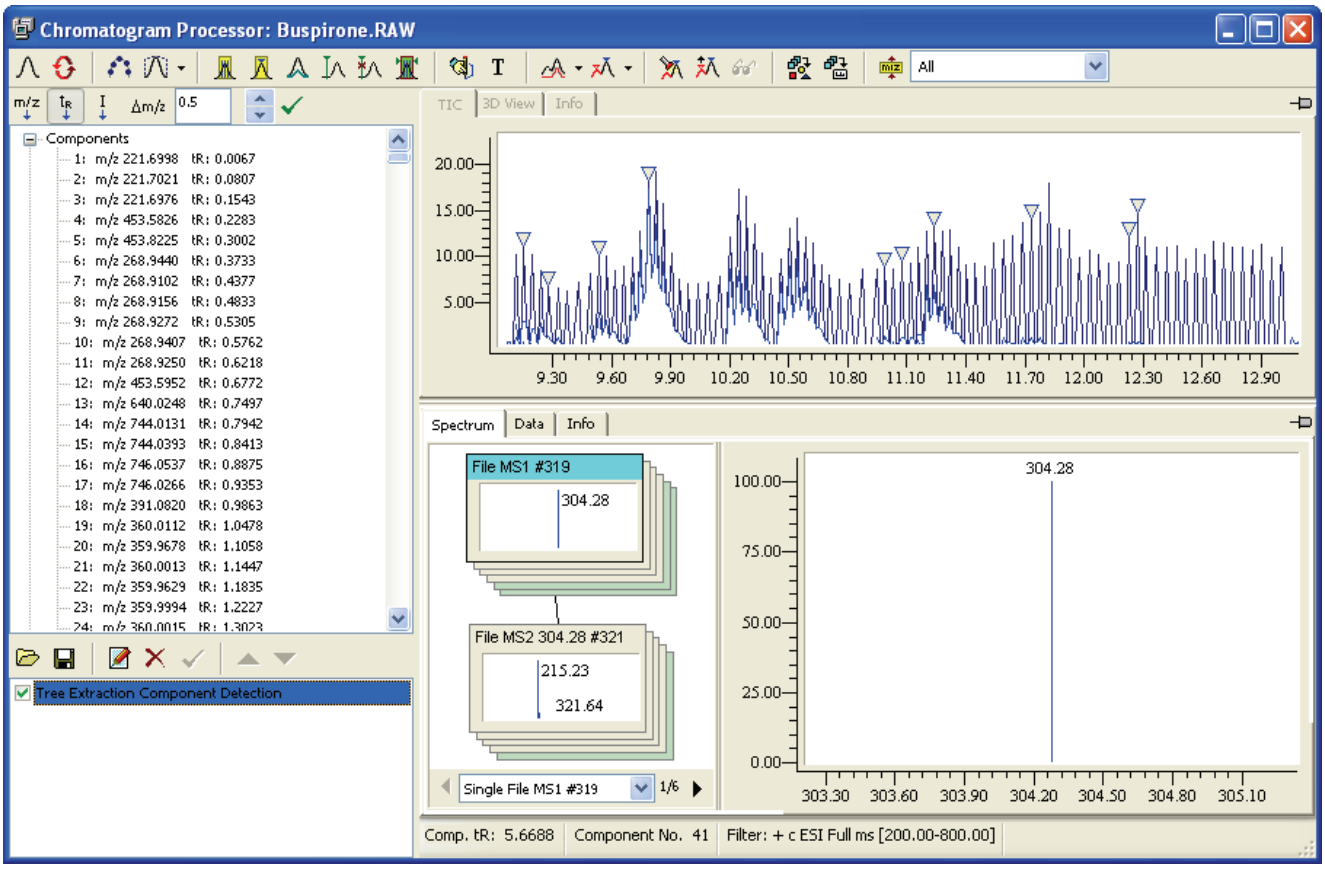


Chromatogram Processor

The Chromatogram Processor module extracts and processes mass spectral data, including GC/MS or LC/MS. By using the component detection and spectra deconvolution algorithms, you can automatically extract individual spectra or spectral trees from complex chromatographic data files. This module provides visual tools for selecting particular spectra from MS^n experiments.

For a complete description of the features in the Chromatogram Processor window, see Chapter 8, "Chromatogram Processor Module."

Figure 7. Chromatogram Processor window



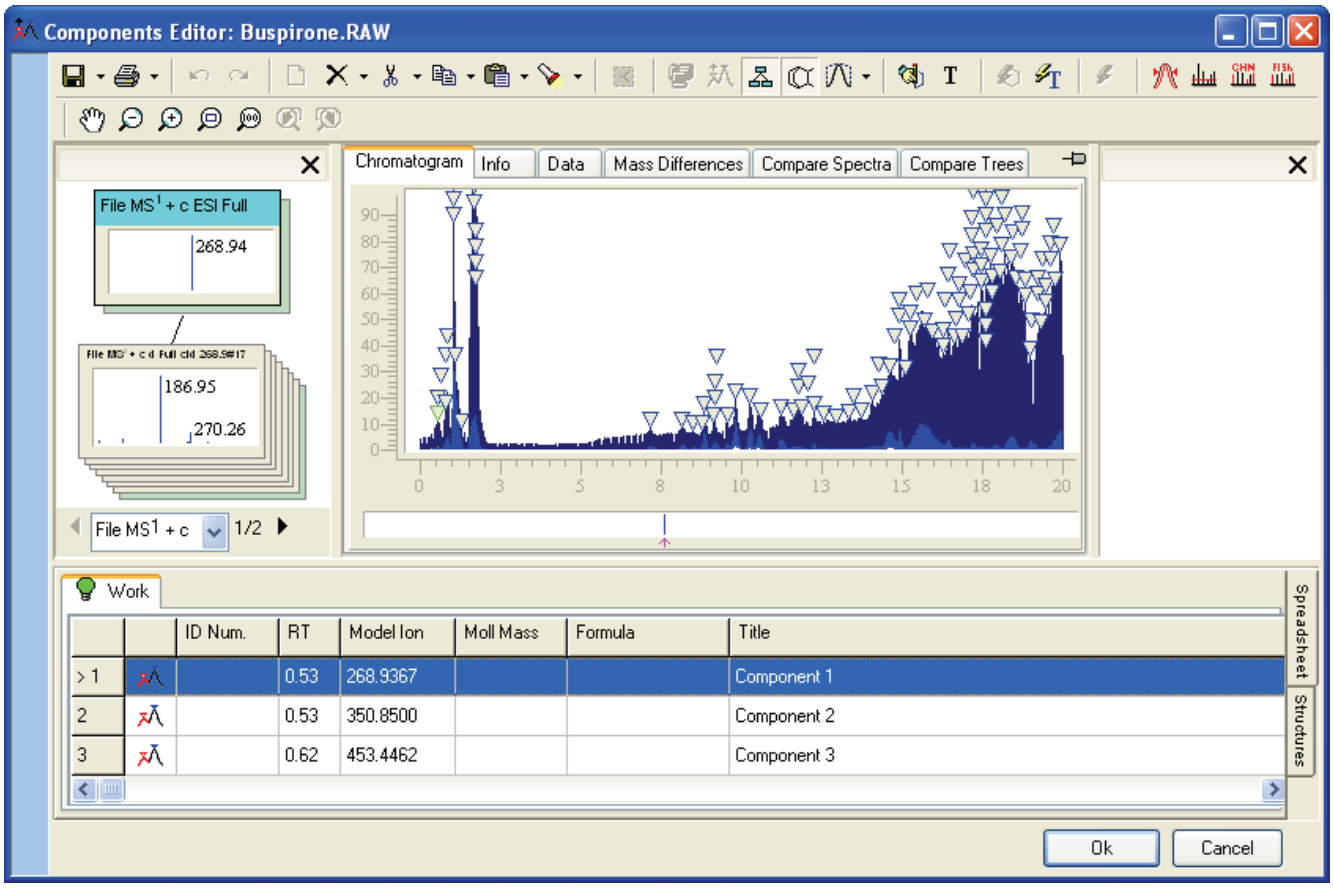
Components Editor

In the Components Editor module, you can edit, search, and organize chromatographic components that are stored in a library or generated by the Components Detection and Spectra Deconvolution feature. This module includes management tools that you can use to delete unrelated components, add chemical structures, edit extensive data fields, process spectral trees, annotate spectral peaks, or sort search hit lists for every component in a processed chromatogram.

For a complete description of the features in the Components Editor window, see Chapter 9, "Components Editor Module."

You cannot open the Components Editor module directly from the Mass Frontier application window. You must generate this module from your data in the Chromatogram Processor window. For additional information, see Chapter 8, "Chromatogram Processor Module."

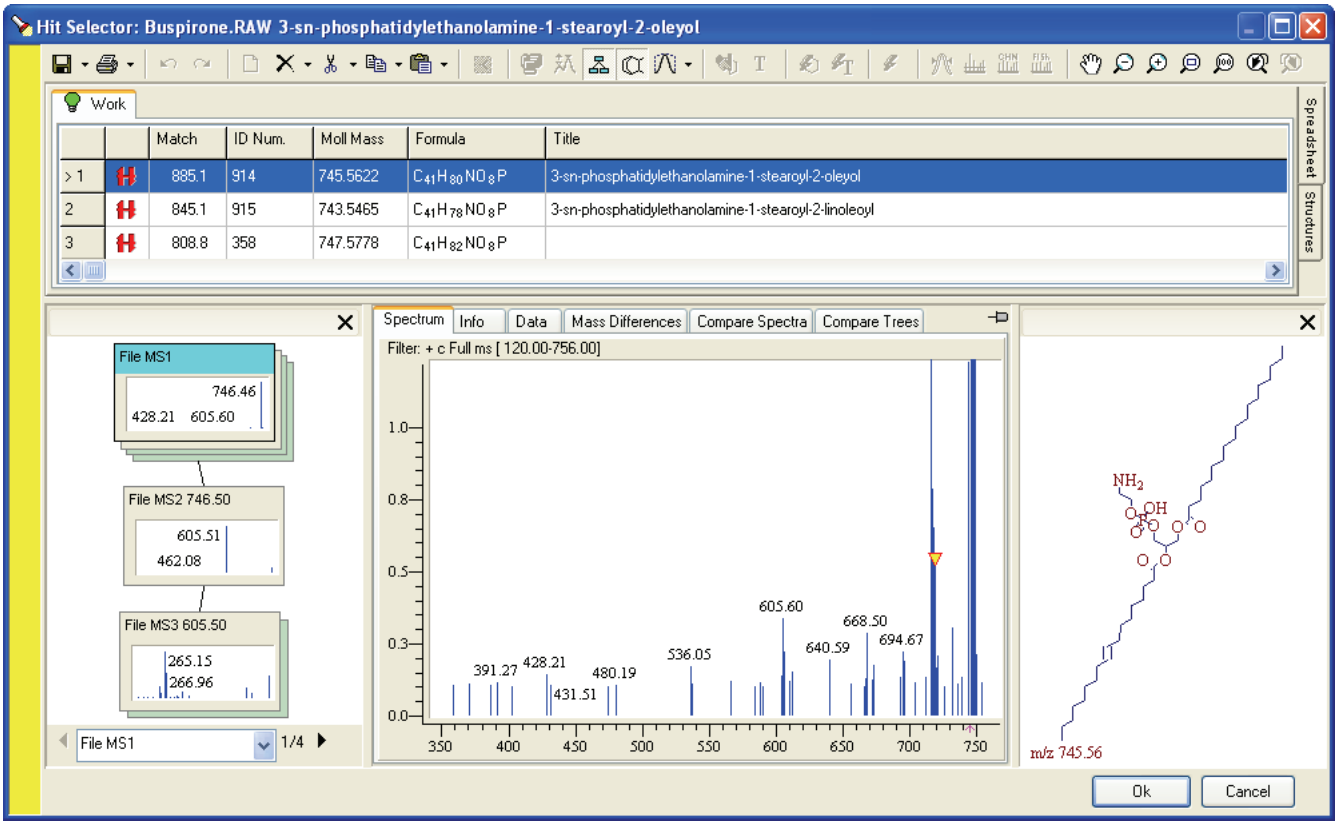
Figure 8. Components Editor window



Hit Selector

Use the Hit Selector feature to list the best matches found during a library search in the Components Editor window. For additional information, see "Searching Components" on page 334.

Figure 9. Hit Selector window

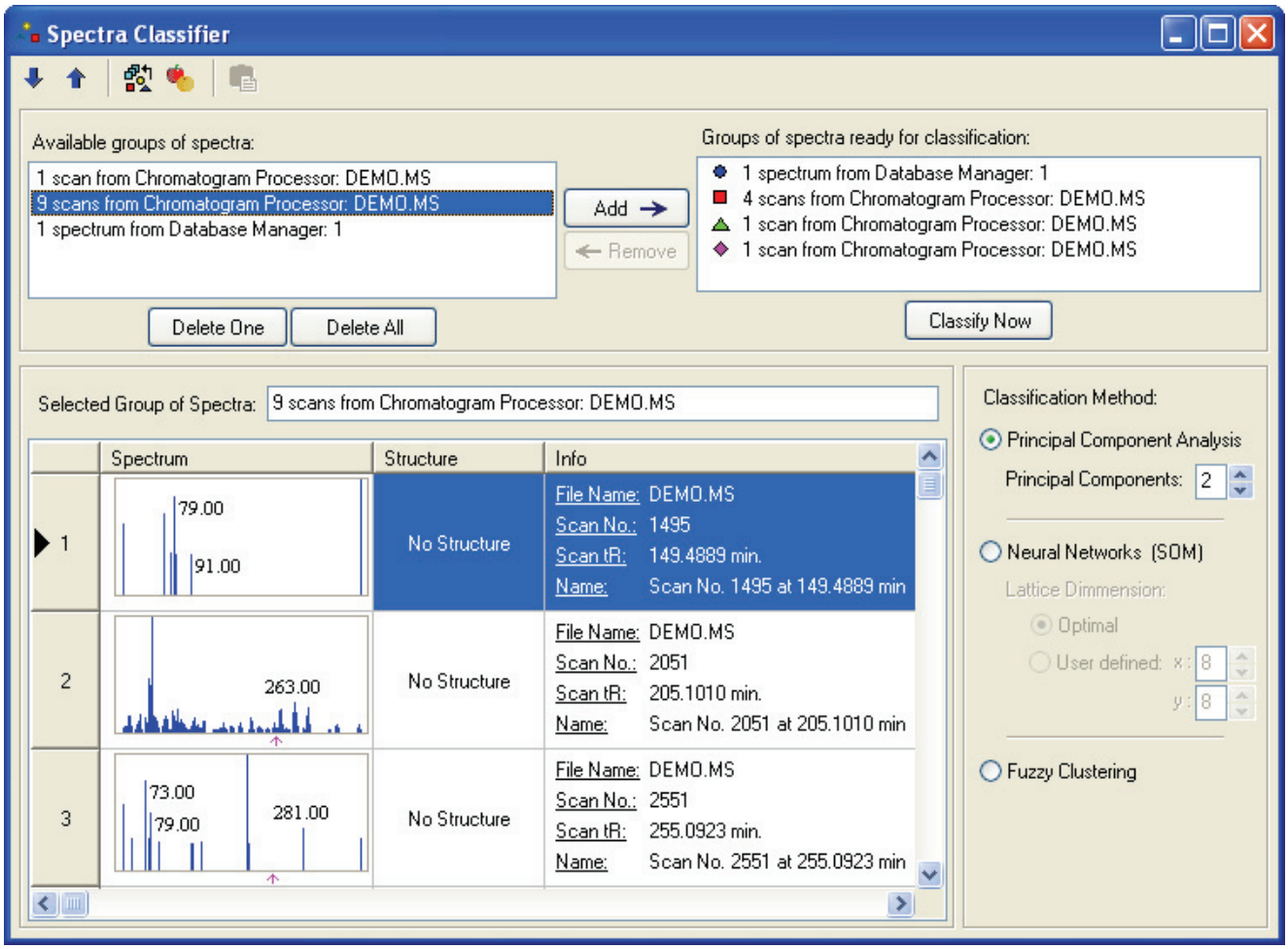


Spectra Classifier

The Spectra Classifier module retrieves and organizes spectra intended for classification. Because you can classify spectra according to various criteria (by structural, physical, and other properties), organizing the spectra into different groups can be useful. You can visually represent these groups of spectra in different ways (using colors, symbols, and numbers) to highlight similarities or dissimilarities among the spectral groups.

For a complete description of the features in the Spectra Classifier window, see Chapter 10, "Spectra Classifier Module."

Figure 10. Spectra Classifier window



Spectra Projector

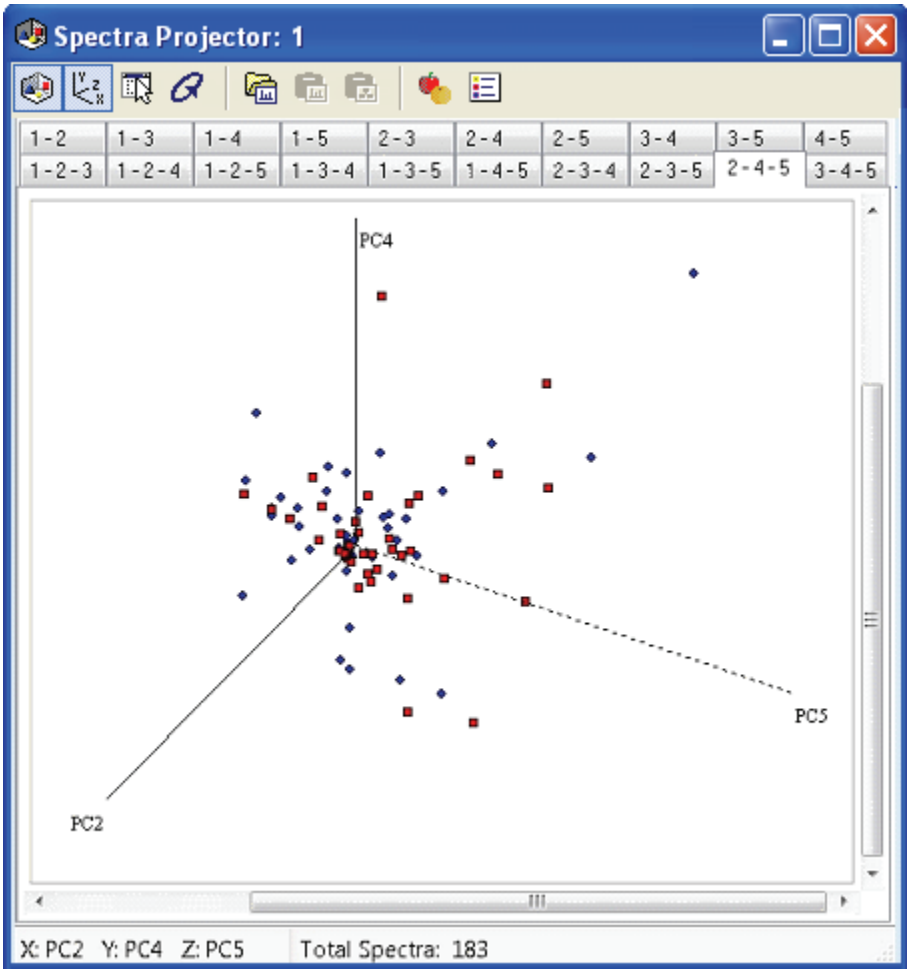
The Spectra Projector module displays the results of the Principal Component Analysis (PCA) and Fuzzy Clustering classification methods. You can classify mass spectral data by using 2-D or 3-D projections where each point represents a spectrum. When you want to determine the class membership of an unknown spectrum, open or paste it into an existing projection.

You cannot open the Spectra Projector module directly from the Mass Frontier application window. You must generate this module from the Spectra Classifier window using data from the Database Manager or Chromatogram Processor module.

For a complete description of the features in the Spectra Projector window, see Chapter 11, "Spectra Projector Module."

For additional information, see Chapter 10, "Spectra Classifier Module."

Figure 11. Spectra Projector window



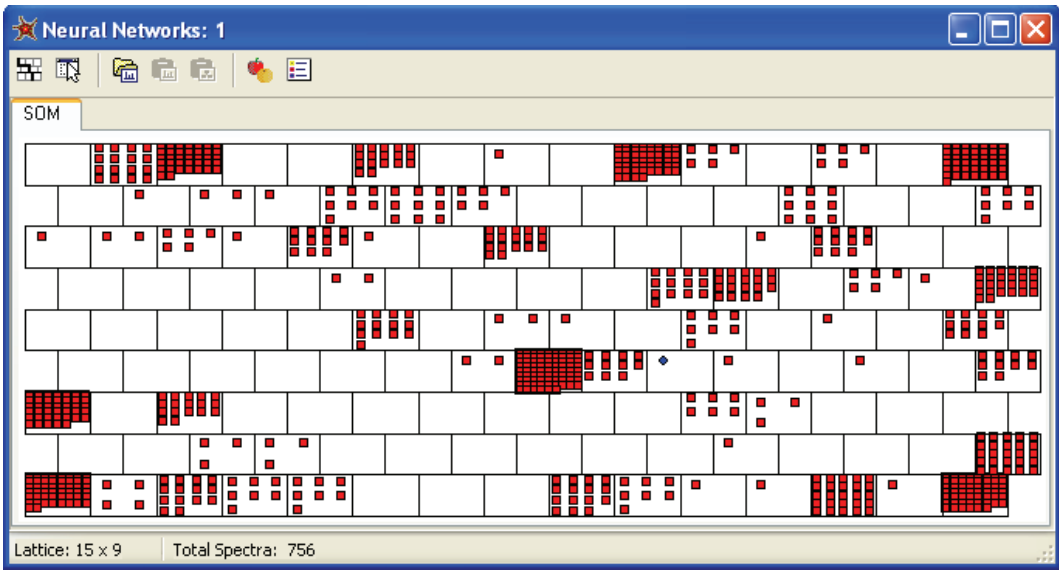
Neural Networks

The Neural Networks module displays the results of the Neural Networks classification method. Mass spectra are classified using the Self-Organizing Maps (SOM) method, which is a special class of neural network. If two or more spectra activate the same neuron, the corresponding compounds will likely exhibit similar physical or chemical properties, or biological activities. For additional information, see "Self-Organizing Maps" on page 343.

You cannot open the Neural Networks module directly from the Mass Frontier application window. You must generate this module from the Spectra Classifier window using data from the Database Manager or Chromatogram Processor module.

For a complete description of the features in the Neural Networks window, see Chapter 12, "Neural Networks Module."

Figure 12. Neural Networks window



Fragments Comparator

The Fragments Comparator module displays a series of fragments in a table format. The columns in the table represent individual compounds, and the rows display either mass-to-charge ratios or the structures of fragments. This module provides an effective comparison of the product ions of analogous molecules.

For a complete description of the features in the Fragments Comparator window, see "Fragments Comparator Window" on page 241.

Figure 13. Table page of the Fragments Comparator window

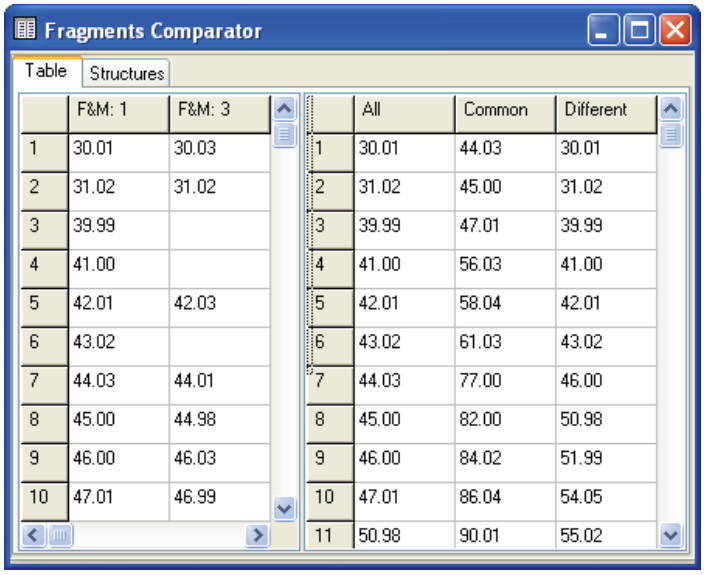
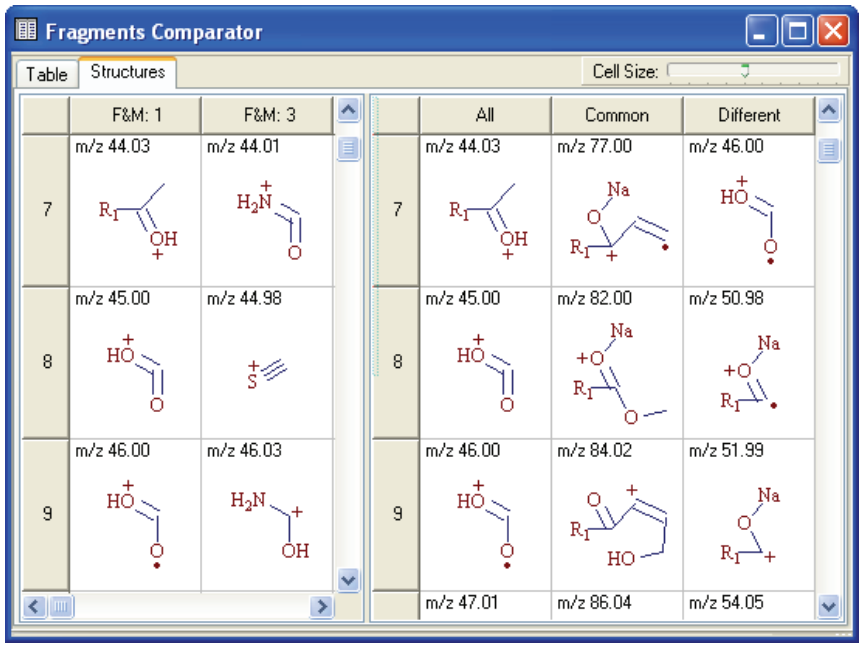


Figure 14. Structures page of the Fragments Comparator window

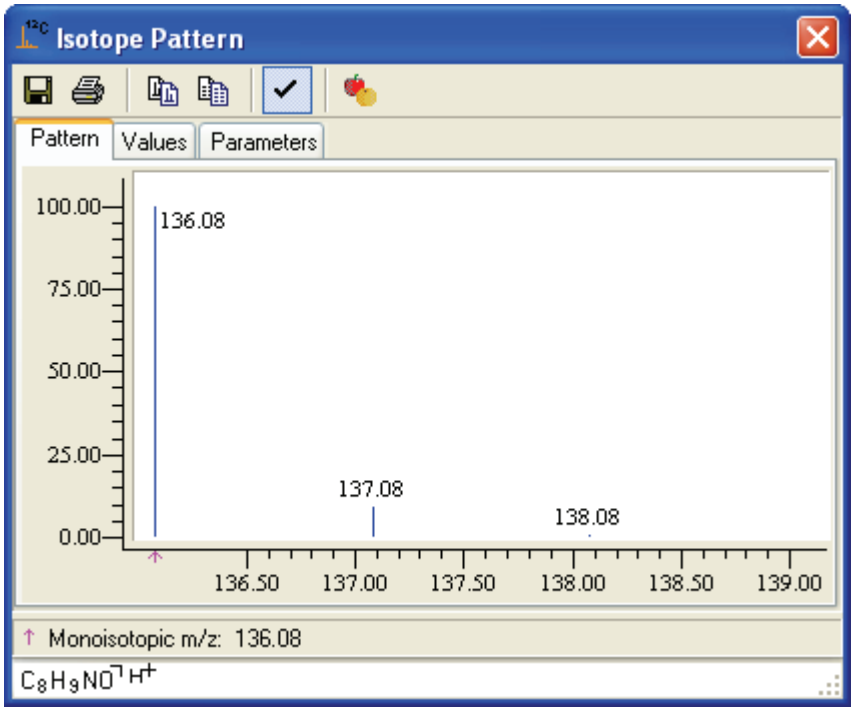


Isotope Pattern

The Isotope Pattern module displays the relevant isotopic profile when you select a structure or fragment in the Mass Frontier application. You can also calculate the isotope pattern from your molecular formula.

For a complete description of the features in the Isotope Pattern window, see Chapter 2, "Database Manager Module."

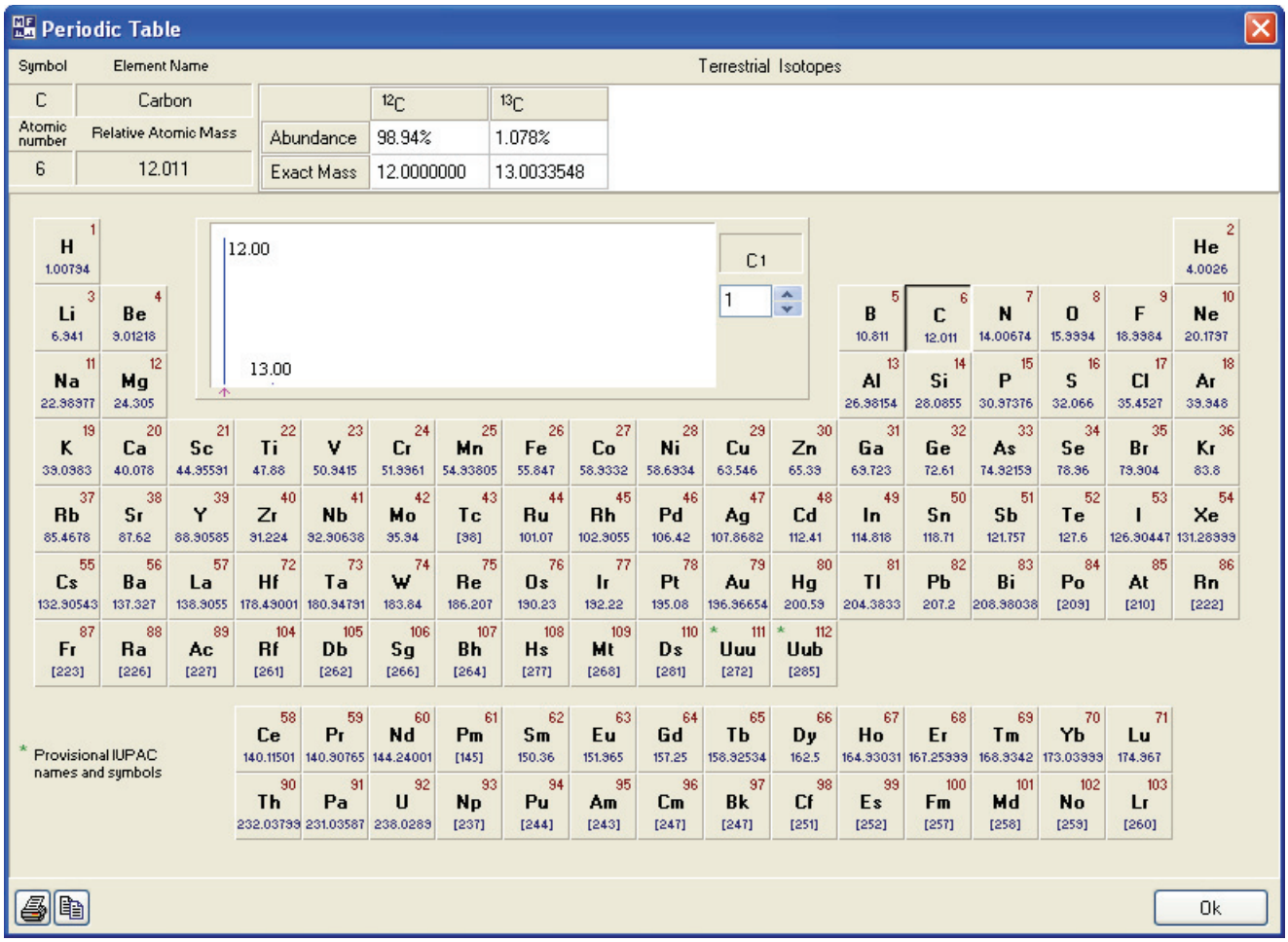
Figure 15. Isotope Pattern window



Periodic Table

The Periodic Table module displays the terrestrial isotopic abundance of elements and their multi-atomic isotopic profiles.

Figure 16. Periodic Table window

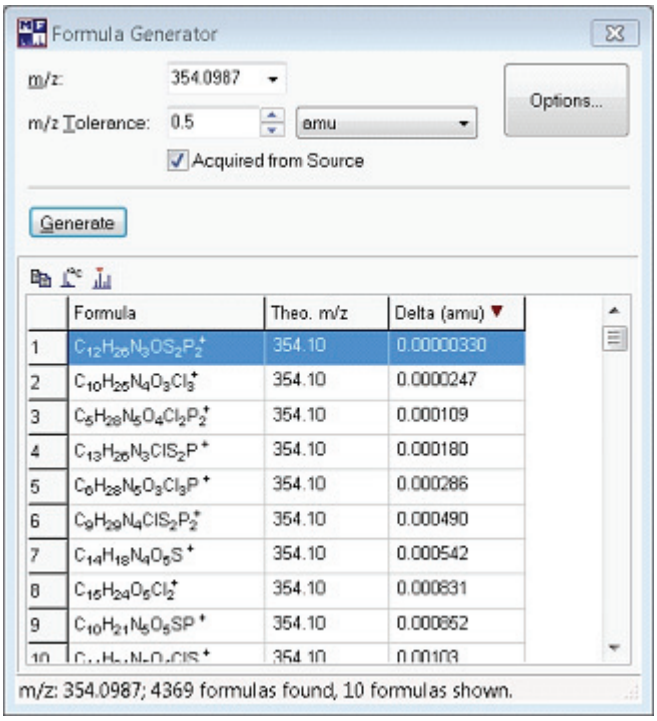


Formula Generator

The Formula Generator module calculates a list of theoretical molecular formulas that best fit an *m/z* value.

For a complete description of the features in the Formula Generator window, see Chapter 13, "Formula Generator Module."

Figure 17. Formula Generator window



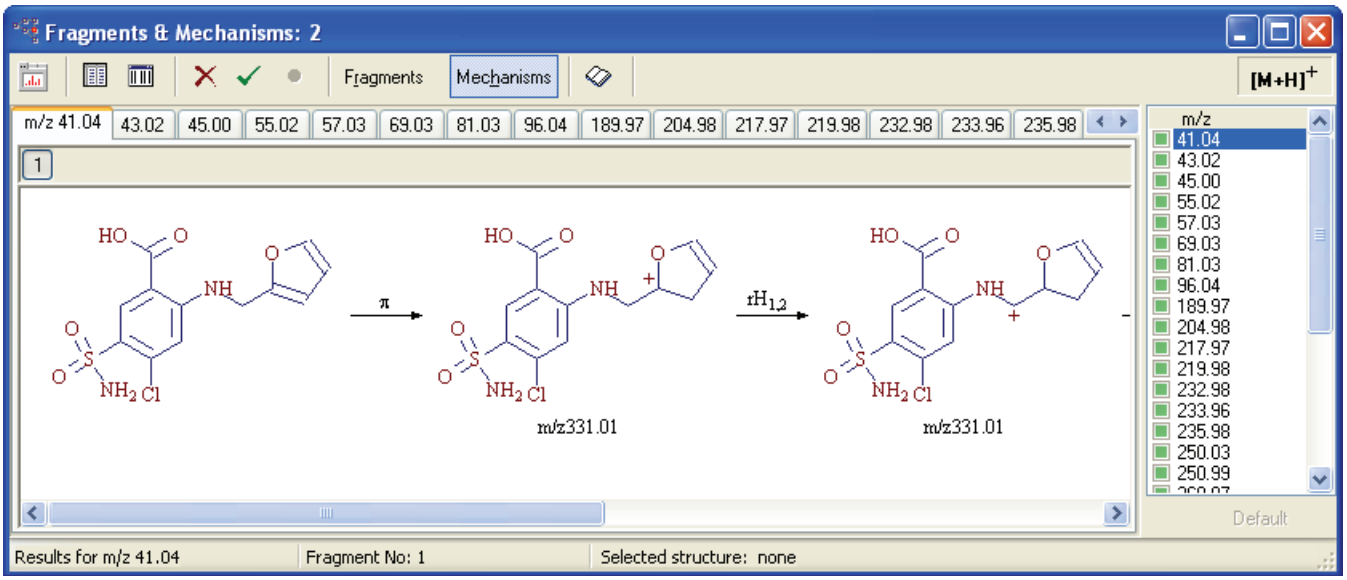
Fragments & Mechanisms

The Fragments & Mechanisms module automatically generates fragments and detailed fragmentation and rearrangement mechanisms from your chemical structure. This module includes a comprehensive set of known reaction mechanisms and library mechanisms that support automated prediction.

For a complete description of the features in the Fragments & Mechanisms window, see Chapter 6, "Fragments and Mechanisms Module."

You cannot open the Fragments & Mechanisms module directly from the Mass Frontier application window. You must generate this module from your data in the Structure Editor or Database Manager module. For additional information, see Chapter 4, "Structure Editor Module," or Chapter 2, "Database Manager Module."

Figure 18. Fragments & Mechanisms window



Report Creator

Use the Report Creator module to create customizable reports from modules displayed on the screen. You can print or export reports as PDF files.

For a complete description of the features in the Report Creator window, see Chapter 15, "Report Creator Module."

Figure 19. Report Creator window

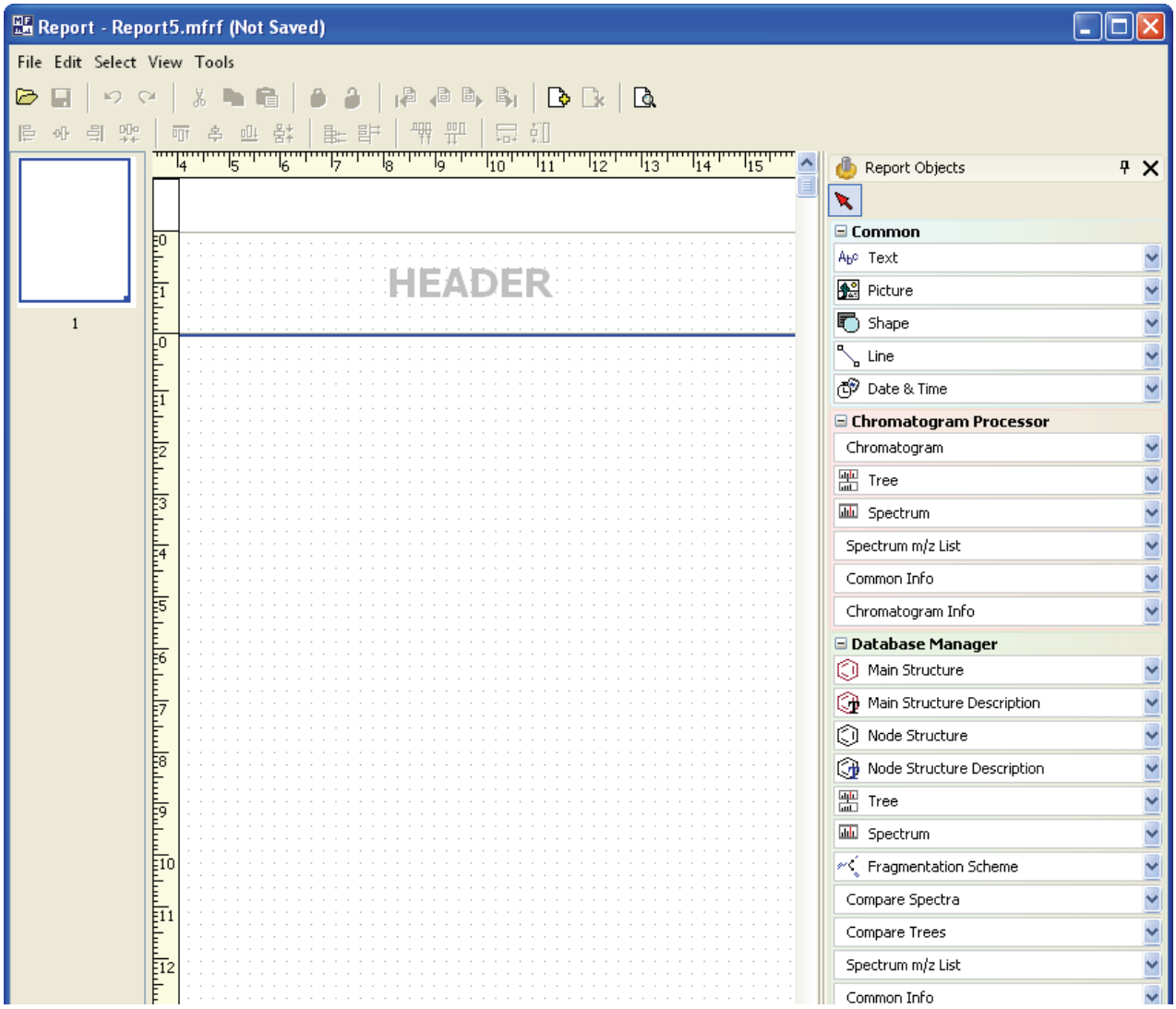
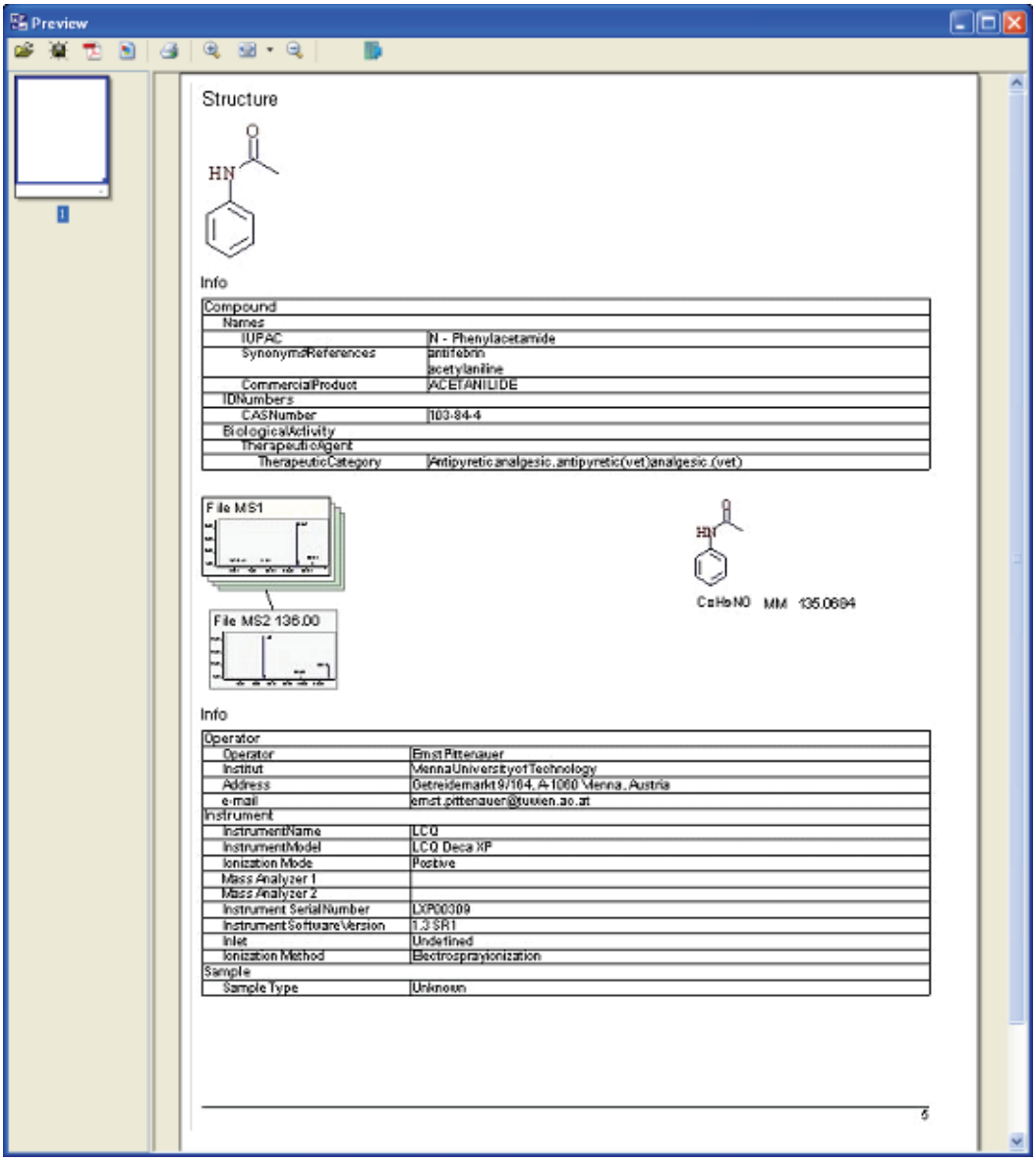


Figure 20. Report Preview window



Installing the Mass Frontier Application

To install the Mass Frontier application with its components, you must have access to an administrator account on the Windows Vista operating system or an account with administrator rights on the Windows XP operating system. You also have the option to install the Xcalibur data system with local user access before installing the Mass Frontier 7.0 application.

From the installation CD, you can install the Mass Frontier application and libraries as a local installation or as a client/server installation.

IMPORTANT Because of the high variability of Windows versions and service packs, you might be prompted to install additional Microsoft system components that are in the Setup Launcher.

Local Installation

The Mass Frontier application and libraries reside on the same computer. To run the Mass Frontier application locally, this computer must have the following applications:

- Microsoft SQL Server 2005 Express Edition SP3
- Mass Frontier Server Manager
- Mass Frontier

Note The Mass Frontier 7.0 application is bundled with Microsoft SQL Server 2005 Express Edition SP3 and the Mass Frontier Server Manager software.

❖ To install Mass Frontier 7.0 as a local installation

1. Click **SQL Server Express 2005**.

   The installation of Microsoft SQL Server 2005 Express Edition begins. The installation can take several minutes.

2. Click **Server Manager**.

   The installation of the Mass Frontier Server Manager 2.0 application begins. This module manages the creation, installation, conversion, and removal of libraries.

   Note If you do not have the Microsoft Backward Compatibility Components installed on the computer, you will be prompted to install them.

3. Click **MSFileReader**.

   The installation of the Thermo MSFileReader begins. This module manages access to Thermo Scientific raw data files.

4. Click **Mass Frontier**.

5. The installation of the Mass Frontier application begins.

   Note If you do not have the Microsoft SQL Native Client installed on the computer, you will be prompted to install it.

Client-Server Installation

In a client/server installation, you can share libraries over the network.

To run Mass Frontier as a client/server application, the libraries reside on a designated computer (server). This server must have the following applications:

- Microsoft SQL Server 2005 Express Edition SP3
- Mass Frontier Server Manager

Note The Mass Frontier 7.0 application is bundled with Microsoft SQL Server 2005 Express Edition SP3 and the Mass Frontier Server Manager software.

Other network computers (clients) must have the following applications:

- Mass Frontier
- Thermo MSFileReader

❖ To install components on the server

1. Click **SQL Server Express 2005.**

   The installation of Microsoft SQL Server 2005 Express Edition SP3 begins. The installation can take several minutes.

2. Click **Server Manager.**

3. The installation of the Mass Frontier Server Manager 2.0 application begins.

   This module manages the creation, installation, conversion, and removal of libraries.

   Note If you do not have the Microsoft Backward Compatibility Components installed on the computer, you will be prompted to install them.

❖ To install components on a client computer

1. Click **MSFileReader**.

   The installation of the Thermo Fisher Scientific MSFileReader application begins.

2. Click **Mass Frontier**.

   The installation of the Mass Frontier application begins.

   Note If you do not have the Microsoft Backward Compatibility Components and the Microsoft SQL Native Client installed on the computer, you will be prompted to install them.

New Features in Mass Frontier 7.0

The Mass Frontier 7.0 application includes new features in the Database Manager, Chromatogram Processor, and Report Creator modules.

New Database Manager Features

The Database Manager module has new features in its tree comparison and automatic fragment annotation algorithms.

- Tree Comparison

  The Mass Frontier tree comparison function has added these new features:

  – You can insert the left tree from the current record, Clipboard, or searched tree (if the current record is the result of a tree search). The right tree is the current record.

  – The first group of options (Type, Method, Match Stage, and Ignore Top Stage) affects the calculation of the match factor. The meaning of the parameters is the same as for the tree search in previous versions of the Mass Frontier application.

  – Sync Best Match: If you select a spectrum in the left or right tree, the most similar spectrum is selected in the opposite tree. If no spectrum matches, the text "No sync match found" appears under this check box.

  – Total Best Match: The two spectra with the highest match factors are selected in both trees.

  – Copy to Compare Spectra: You can show selected spectra in the Compare Spectra tab.

- Automatic Fragment Annotation

  The automatic fragment annotation algorithm now includes the following new features:

  – Improvements that consider all precursors within the range of the isolation width.

  – Modifications of the explained peaks affecting the precursor fragments of all the dependent nodes.

  – The ability to annotate peaks under the specified threshold.

For information about the new Database Manager features, see Chapter 2, "Database Manager Module."

New Chromatogram Processor Features

The Chromatogram Processor module in the Mass Frontier 7.0 application includes changes to the FISh algorithm, thresholding, baseline correction, smoothing, joint component detection, action handling, component search, and chromatogram access.

- FISh algorithm

  Enhancements to the FISh algorithm include these features:
  - Use of the *m/z* list as a model.
  - Extending the model by mass shifts.
  - The ability to perform FISh with neutral loss analysis for MS/MS scans.
  - Detection of integrated components.
  - The ability to automatically localize the site of modification through color coding of fragments common to the parent component.
  - A more intuitive graphical user interface with flexible, comprehensive options and the ability to define the FISh model with one click.

- Thresholding, correcting baselines, and smoothing

  A new wizard is available for thresholding, baseline correction, and smoothing.

- Joint component detection

  A new wizard is available for joint component detection.

- Tools

  The Chromatogram Processor module includes two new tools:
  - Slide bars to help you visualize processing parameters.
  - Actions that you can apply to chromatograms (base peak chromatograms, selected ion chromatograms, forced mass tolerance, thresholding, FISh, baseline correction, smoothing, and component detections) in the Chromatogram Processor window.

    You can save these actions to a file and load them. You can also drag them and drop them between Chromatogram Processor windows. In addition, you can activate and deactivate them, and reorder, modify, and remove them.

- Components search

  You can directly access the component search and the hit selector from the Chromatogram Processor window. The results of the component search appear in short form in the ToolTips.

- Save/open chromatogram

  You can save and load chromatograms with components, extracted ion chromatograms (XICs), selections, and actions in the new format.

For information about the new Chromatogram Processor features, see Chapter 8, “Chromatogram Processor Module.”

New Report Creator

With this new module, you can create comprehensive, flexible, reusable reports and then customize them.

For complete information about the Report Creator, see Chapter 15, "Report Creator Module."

Application Limitations

Although the Mass Frontier application is fully featured, there are application limitations.

- The application deals primarily with small organic structures rather than peptides and other biologically-related molecules. The Structure Editor and other modules dealing with structures have a limit of 199 non-hydrogen atoms per structure. When you exceed this number, a message appears, which reminds you of this limitation.
- The application is designed for pure substances only. It does not accept mixtures. The system considers a mixture to be two or more structures, depicted in the same window, which are not connected by a bond (represented as a line). If you try to generate fragments and mechanisms from a mixture, a message alerts you that this action is not permitted. The Check Structures option will also detect mixtures as an error. For additional information, see Chapter 4, "Structure Editor Module."
- However, library utilities support mixtures to ensure backward compatibility with commercial libraries. You may add mixtures to a user library, but the Fragmentation Library does not support mixtures.
- New aspects of the Mass Frontier system have greatly extended the automated generation of fragments and mechanisms, but some restrictions still remain. For additional information, see "Previewing Unimolecular Reactions" on page 193. The Mass Frontier application offers the ability to select reagent gases for chemical ionization. However, it cannot modify the relative ionization potentials of reagent gases. Negative ionization (deprotonation) is only supported using the Fragmentation Library. For additional information, see Chapter 7, "Fragmentation Library." There are no general rules for negative ionization. Because "soft" ionization techniques are mainly low energetic experiments, which often yield complex skeletal and "random" rearrangements, the predictability of these fragmentation and rearrangement processes is not as high as what can be attained by electron impact ionization. You can improve the degree of predictability by using compound specific fragmentation mechanisms in the Fragmentation Library module.
- The Mass Frontier application is primarily designed for neutral and single charged molecules. As a consequence you can attach the charge symbol (+ or –) to only one atom. If the charge multiplicity is described, in general you can use the unspecified charge location option in the Structure Editor window. None of the modules support biradicals.
- This application supports high resolution mass spectra with an *m/z* range of 1–3000 mass units. Classification modules allow only 800 peaks per spectrum. If a spectrum contains more than 800 peaks, the classification procedure selects the 800 most prominent peaks. For additional information, see Chapter 10, "Spectra Classifier Module."
- All spectral modules support spectral trees except Spectra Classifier, which uses total composite spectra generated from spectral trees. For additional information, see Chapter 3, "Spectral Tree Module."

Database Manager Module

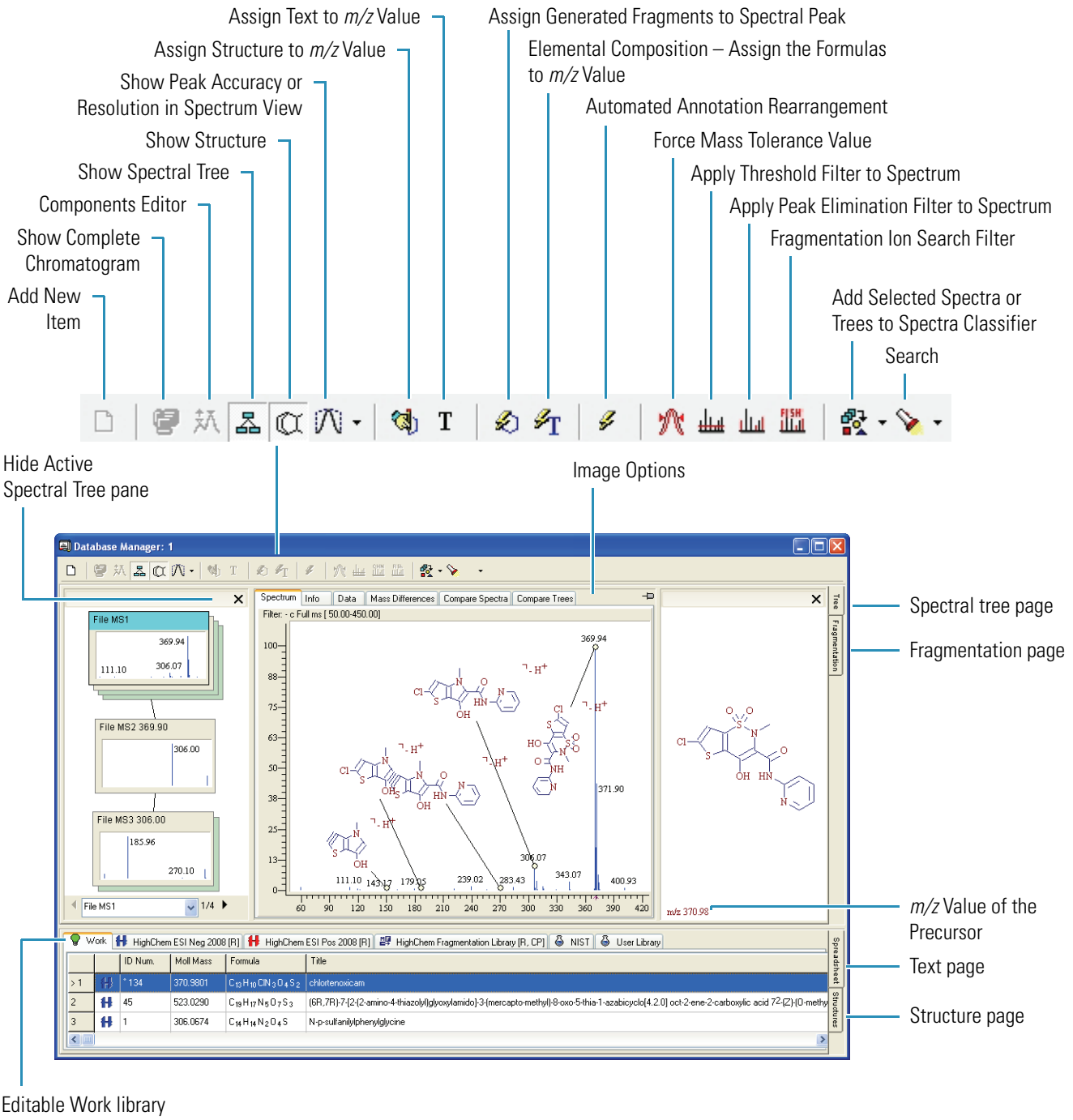
The Database Manager module manages spectral and structural information in a SQL Express database.

Contents

The Database Manager module includes library maintenance utilities that let you create and organize spectral and chromatographic libraries with chemical structures. Because the Mass Frontier application supports ion structures and spectral tree representation, you can also create true MS^n libraries. Advanced library query and search features provide access to the information you need to identify compounds and can help you interpret unknown spectra. The Database Manager module provides a flexible set of search restrictions to target your search results.

The following figure identifies key toolbar buttons, panes, and tabs in the Database Manager window.

Figure 21. Database Manager window



Using the Database Manager Window

Structural and spectral data are organized in spreadsheet-like pages with a variety of supplementary information. To organize your libraries, search results, and other data, you can move spectra-structure pairs from one Database Manager window to another.

The Database Manager module provides a customizable tool for creating reports that you can either print directly or copy to a word processor to create more advanced reports.

For each record in the Database Manager window, you can view the mass spectrum, a list of peaks, or the compound identification information and the neutral losses spectrum (when the molecular mass is available). You can also compare two spectra.

This section includes the following topics:

Opening a Database Manager Window

The number of Database Manager windows that you can simultaneously open is limited only by your system resources so that you have unconstrained flexibility in handling spectral and structural data.

Follow these procedures:

❖ To open an empty Database Manager window

Do one of the following:

Click the **Database Manager** button,  , in the Mass Frontier toolbar.

–or –

Choose **Tools > Database Manager** from the Mass Frontier main menu.

An empty Database Manager window opens.

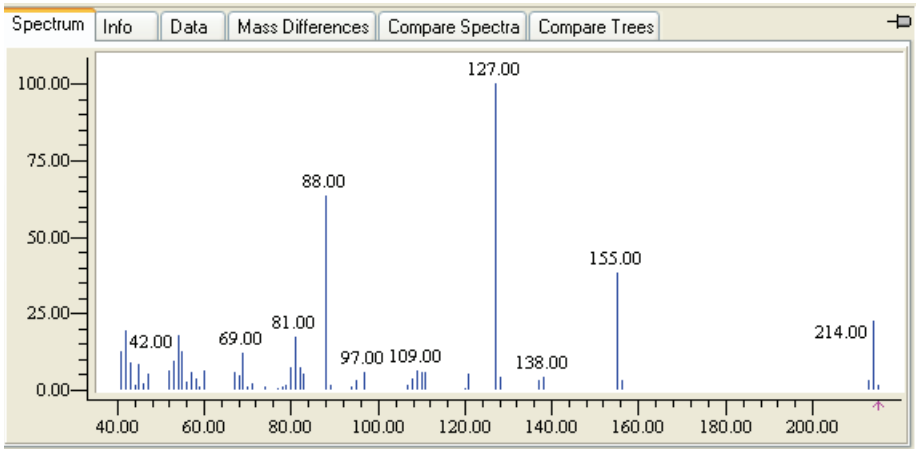
❖ To load mass spectra into the Database Manager window

1. Choose **File > Open > Mass Spectrum** from the Mass Frontier main menu.

   The Open Spectrum dialog box opens.

2. Select an .msp or .jdx file and click **Open**.

   The mass spectra file opens on the Spectrum page.

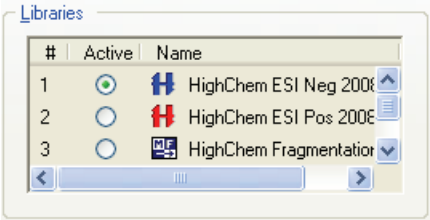


❖ To load search results in a Database Manager window

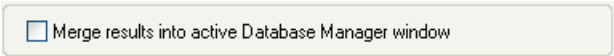
1. Choose **Search > *any criteria*** from the Mass Frontier main menu.

   The Library Search dialog box opens for the selected type of search. Each dialog box contains parameters specific to the type of criteria you are searching, but all Library Search dialog boxes include a Libraries area and a merge results option.

2. In the Libraries area, select the Active option for all the libraries you want to search.



3. Select or clear the **Merge Results into Active Database Manager Window** option.



   - When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet.
   - When cleared, the application displays the search results in a new Database Manager window.

4. Click **OK** in the Library Search dialog box.

   The application searches the selected libraries and adds the search results to the Work spreadsheet in the specified Database Manager window.

❖ To zoom and pan in the graphical display

- Click the **Hand** button, , in the Mass Frontier toolbar and drag the hand to pan horizontally or vertically.
  Similarly, hold down the H keyboard key or the Spacebar to change the cursor to the hand.
- Click the **Zoom Out** button, , in the Mass Frontier toolbar.
- Click the **Zoom In** button, , in the Mass Frontier toolbar.
- Click the **Zoom to Box** button, , in the Mass Frontier toolbar and describe a rectangle.
  Similarly, hold down the Z keyboard key and describe a rectangle.
- Click the **Zoom Reset** button, , in the Mass Frontier toolbar to return to full scale.
- Click the **Zoom Undo** button, , in the Mass Frontier toolbar to undo the last zoom operation.
- Click the **Zoom Redo** button, , in the Mass Frontier toolbar to restore the last zoom operation.
- Use the scroll wheel on the mouse to zoom the entire display.

Using the Detachable Panes

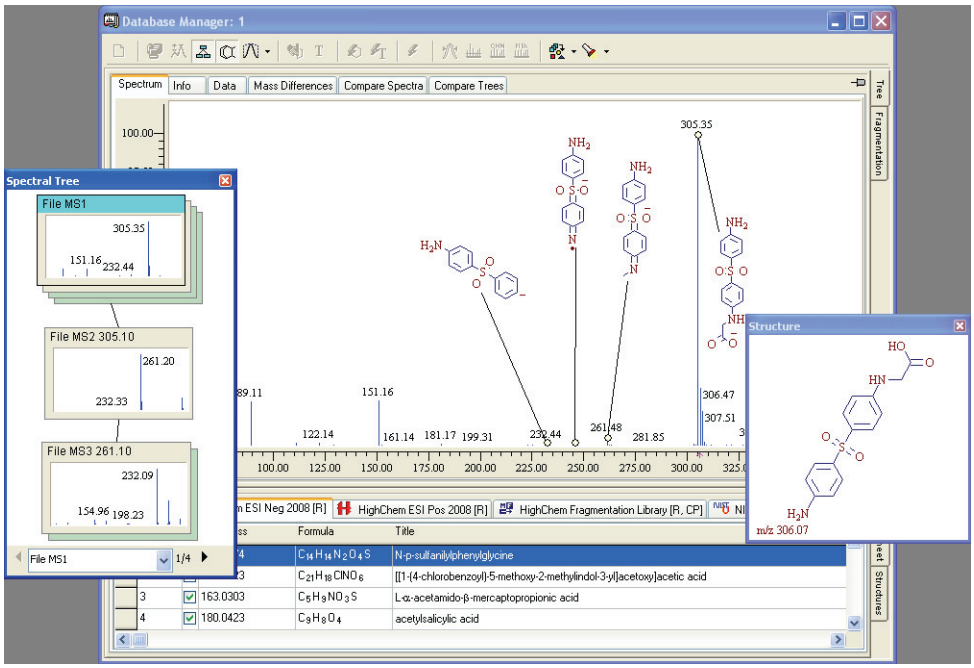
Spectral tree and structure panes are detachable windows that you can place anywhere inside or outside of a Database Manager window.

Follow these procedures:

- To detach the spectral tree or structure pane
- To turn the spectral tree or structure panes off and on

❖ To detach the spectral tree or structure pane

1. Click the header of the spectral tree or structure pane.
2. Drag the panes to a new location anywhere on your desktop.



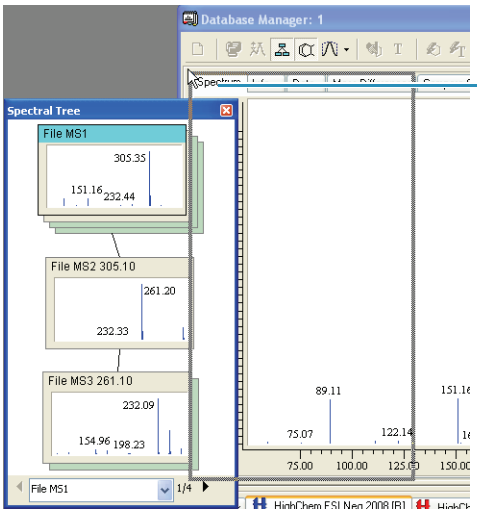
At any time, you can reattach the pane to the Database Manager window.

3. To reattach a pane, do the following:
   a. Click the header of the pane.
   b. Move your cursor to the left or right edge of the upper pane of the Database Manager window.

      When the pane is ready to reattach, the frame snaps to the location reserved for it in the Database Manager window.

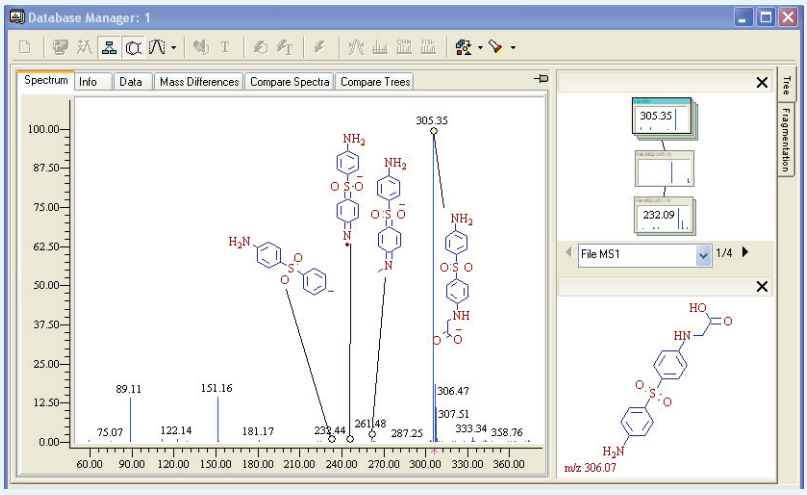
> **Tip** The snap is triggered by the location of the cursor on the edge of the upper pane, not the location of the detached pane.

c. Release the mouse.



When the cursor touches the edge, the detached pane snaps into place.

Tip You do not have to attach the Spectral Tree pane to the left side of the window and the Structure pane to the right side (the default locations). You can swap sides or attach both panes to one side of the Database Manager window.



❖ To turn the spectral tree or structure panes off and on

1. Click the **Show Spectral Tree** button,  , to turn on the Spectral Tree pane.

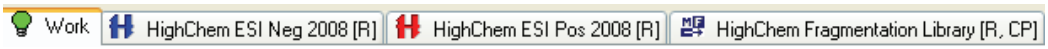
   This is a useful feature when, for example, you are dealing with single stage spectra (not MS/MS) and do not need a spectral tree pane.

2. Click the **Show Structure** button,  , to turn on the Structure pane.

These toggle features work even when the panes are detached from the Database Manager window.

Using Libraries in the Database Manager Window

In the Database Manager window, all installed libraries are represented as tabbed pages in the lower pane.



To add a library to the Database Manager window, use the Install Library feature in the Server Manager window. Similarly, to remove a library, use the Uninstall Library feature in the Server Manager window. The Mass Frontier software package includes the HighChem Spectral Tree Library (electrospray ionization (ESI) positive and negative) and the HighChem Fragmentation Library.

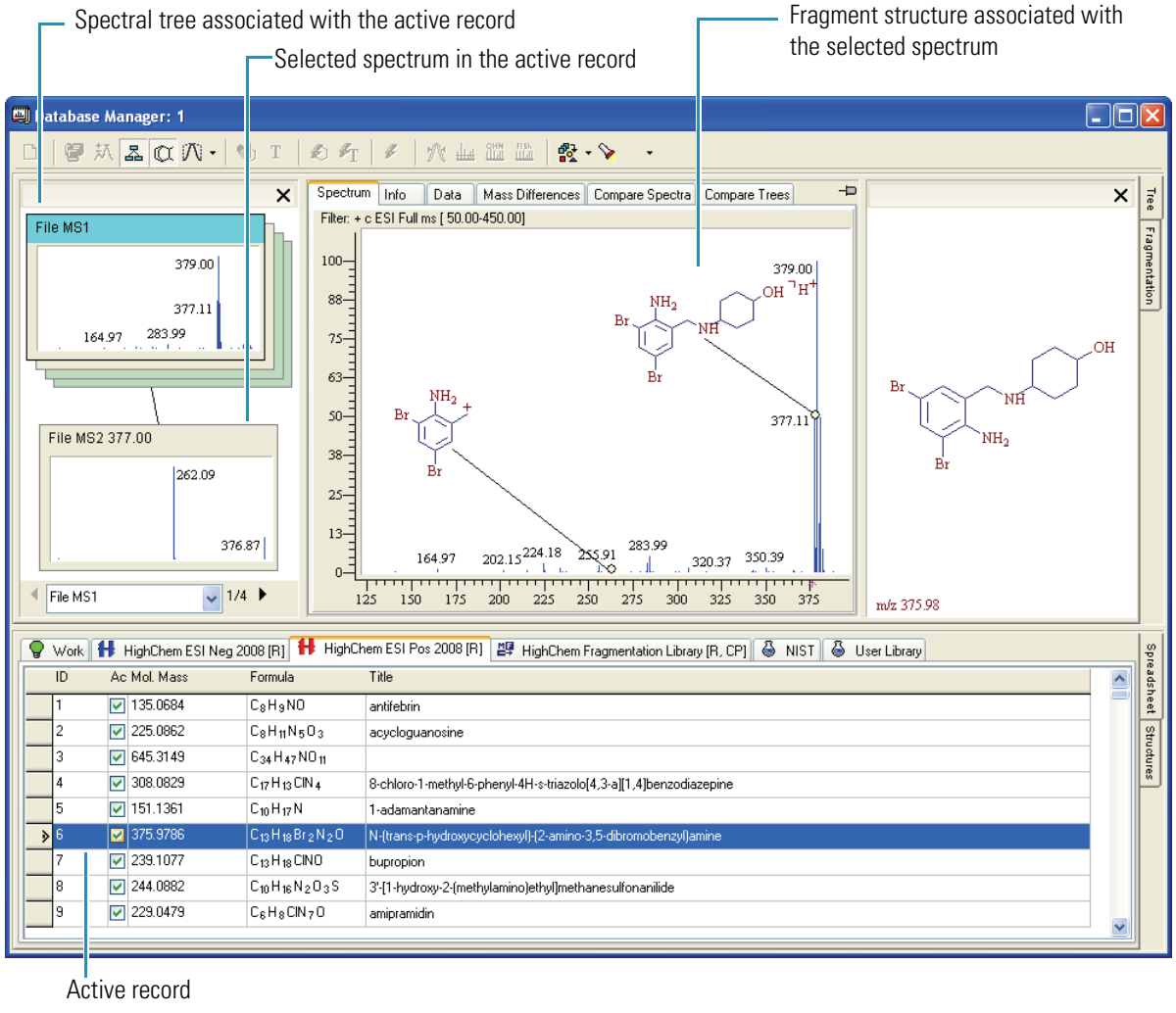
To open a library, click the library tab in the Database Manager window. To switch between spectral and fragmentation displays, click the **Tree** or **Fragmentation** tabs on the right side of the upper pane.

Using Records in the Database Manager Window

A single record in the Database Manager window contains one spectrum with a structure (when available) or one spectral tree with associated structures (when available) and complementary information associated with the record. See "Using the Info Page" on page 38.

Each record is visually represented as a single row in the Spreadsheet view. Triangular symbols, ▶ or ⯈, point to the active record. The structure, spectrum, spectral tree, and any other available information are displayed for the active record in the upper pane of the Database Manager window. See "Using the Library Pane" on page 66.

Figure 22. Displaying an active record in the Database Manager window



ID	Ac Mol. Mass	Formula	Title
1	135.0684	C_8H_9NO	antifebrin
2	225.0862	$C_8H_{11}N_5O_3$	acycloguanosine
3	645.3149	$C_{34}H_{47}NO_{11}$	
4	308.0829	$C_{17}H_{13}ClN_4$	8-chloro-1-methyl-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine
5	151.1361	$C_{10}H_{17}N$	1-adamantanamine
6	375.9786	$C_{13}H_{18}Br_2N_2O$	N-(trans-p-hydroxycyclohexyl)-(2-amino-3,5-dibromobenzyl)amine
7	239.1077	$C_{13}H_{18}ClNO$	bupropion
8	244.0882	$C_{10}H_{16}N_2O_3S$	3'-[1-hydroxy-2-(methylamino)ethyl]methanesulfonanilide
9	229.0479	$C_6H_8ClN_7O$	amipramidin

Using the Info Page

In addition to the mass spectrum or spectral tree and the chemical structure, each record on the Info page can include compound identifications, property, and origin information. The Info page contains editable fields organized by groups and subgroups. You cannot edit fields that display information automatically calculated from a chemical structure (formula, molecular mass, and so on) or acquisition settings adopted from a file. Text in noneditable fields is grayed out.

Note Both the Tree and the Fragmentation pages have Info tabs. The contents are identical.

The information on the Info page is divided into four groups:

- Compound

  Names, ID numbers, physical properties, and biological activity information

- Tree (associated with the entire tree)

  Comments, operator, instrument, and sample information

- Node (associated with a tree node [allows one chromatogram per tree node])

  Chromatographic data

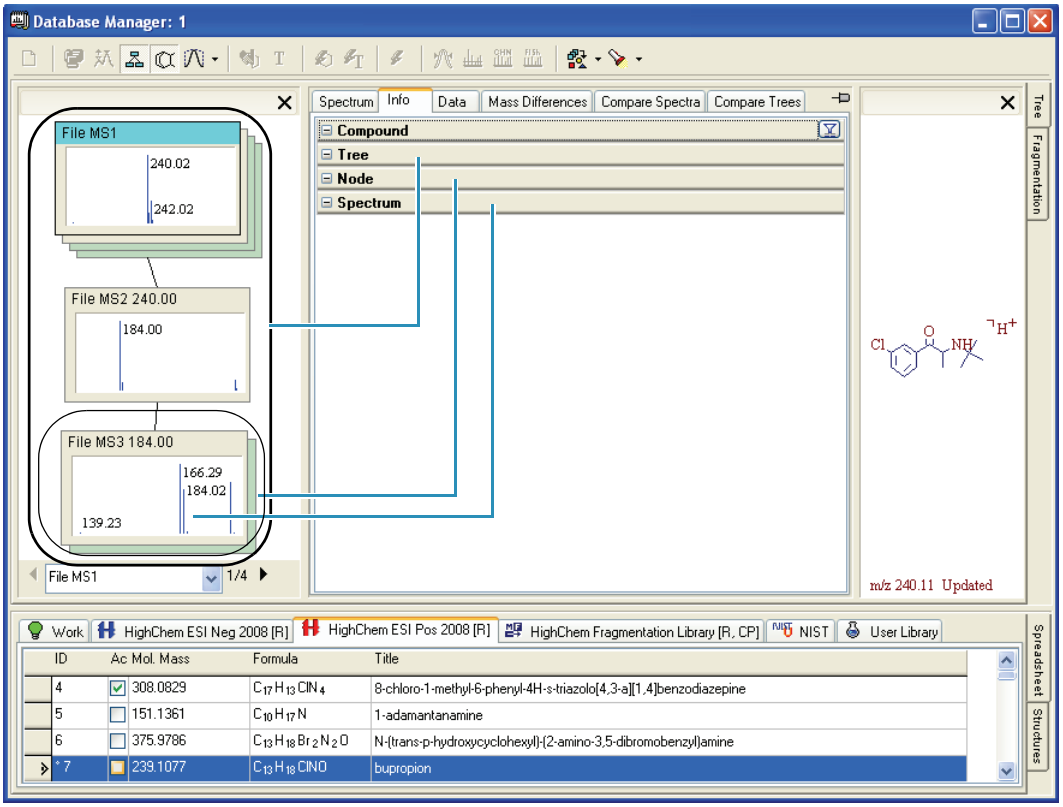
- Spectrum (associated with the selected parallel spectrum)

  File, MS/MS, and scan information

Make sure you understand the connection between data on the Info page and the spectral tree hierarchy.

Each parallel spectrum contains its own data in the Spectrum group because you can acquire parallel spectra under different experimental conditions (collision energy, isolation width, and so on) that you must store independently. In the Mass Frontier application, you can select items that you want displayed on the Info page for each library. See "Using the Info Page" on page 38.

Figure 23. Info page



When a record is saved in a library, all data on the Info page is automatically stored in the SQL database. To permanently store any change, update, or deletion on the Info page, save the corresponding record in the library.

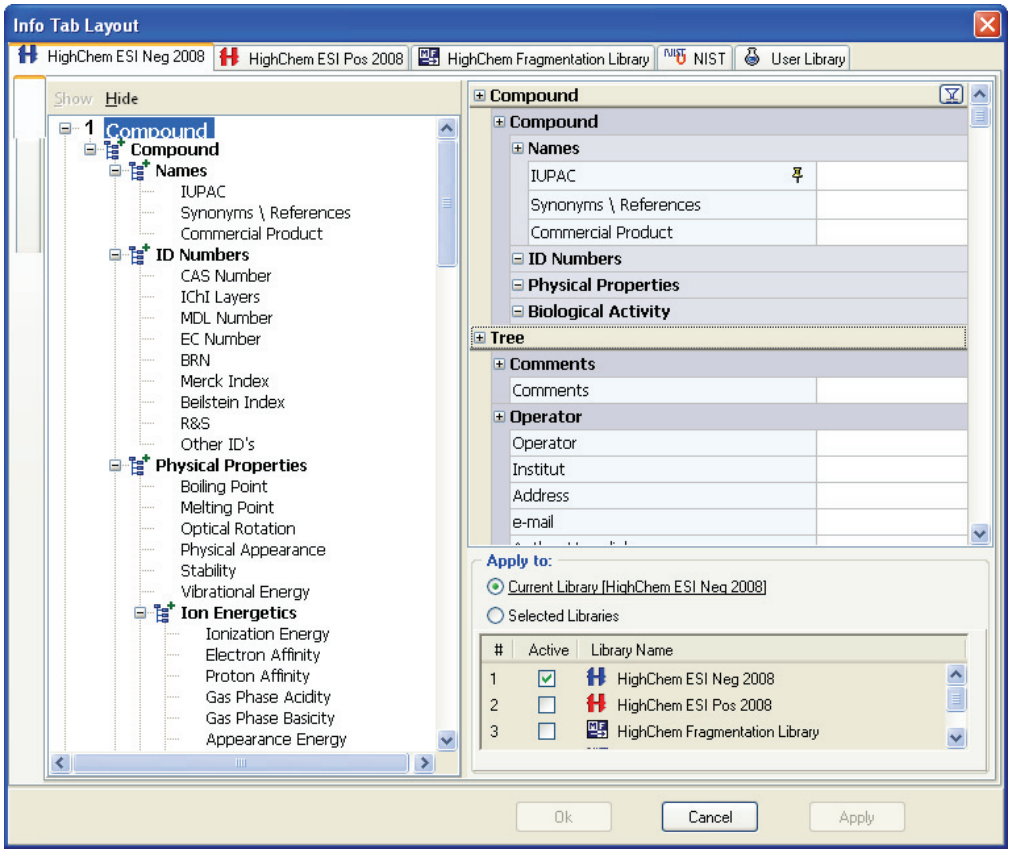
The list of information associated with a record might contain a large number of items that remain largely unused.

You can select items to appear on the Info page for each library.

❖ To exclude items from the information list

1. Choose **Options > Info Tab Layout** from the Mass Frontier main menu.

   The Info Tab Layout dialog box opens. The left pane lists all the available information groups and items that you can display in the Database Manager window. The right pane mirrors the current Database Manager display for the selected library.



2. Click the tab for the library you want to edit.
3. To hide any item of information, do the following:
   a. Select the item in the left pane.
   b. Right-click and choose **Hide** from the shortcut menu or click **Hide** at the top of the pane.

   The item is not available in the left pane. Until you click Apply, the items are still represented in the right pane.

> **Note** To show a hidden item, repeat step 3, clicking Show instead of Hide.

4. To hide an entire group of information, do the following:
   a. Select the group heading in the left pane.
   b. Right-click and choose **Hide** from the shortcut menu, or click **Hide** at the top of the pane.

   All items in the group are not available in the left pane. Until you click Apply, the items are still represented in the right pane.

   Note To show a hidden group, repeat step 4, clicking Show instead of Hide.

5. In the Apply To area, do one of the following:
   a. Select the **Current Library** option.
   b. Click **Apply**.
   c. Verify your changes in the right pane.
   d. To make changes to another library, repeat step 2 through step 5.

   –or–

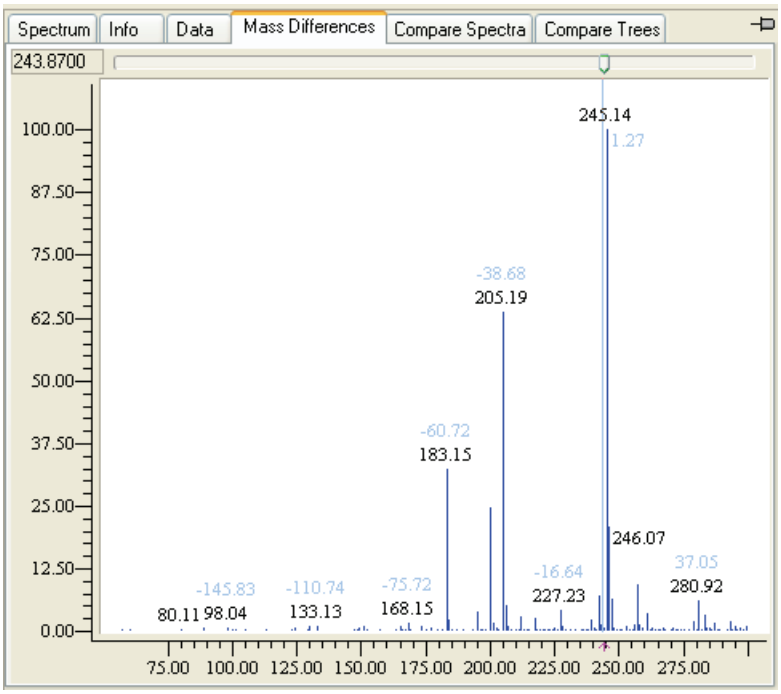
   a. Select the **Selected Libraries** option.
   b. Select the check box for each library that you want to apply the changes to.
   c. Click **Apply**.
   d. Verify your changes for each library in the right pane.

6. To make changes to another libraries, repeat step 2 through step 5.
7. Click **OK**.

Using the Mass Differences Page

Use the Mass Differences page in the Database Manager window to select any peak and view the mass differences to the right and left (possible precursor-product ions). You can move the *m/z* scale using the track bar at the top of the page. When molecular mass information is available, the zero value of the *m/z* scale automatically starts at the molecular mass—that is, a standard neutral loss spectrum is displayed. In this case, a blue selection bar in the track bar marks the shift of the *m/z* scale with respect to molecular mass. You can enlarge the graphic to see the number for less prominent peaks when there are a large number of peaks close to each other.

Figure 24. Mass Differences page



Using the Compare Spectra Page

Use the Compare Spectra page in the Database Manager window to compare two spectra.

- The bottom spectrum is from the active record currently selected in the Spreadsheet view.
- The middle spectrum displays the difference between the top and bottom spectrum.
- The top spectrum is initially blank.

You can add the currently selected record to the top spectrum or paste a record from the Clipboard.

After a spectrum search, the first spectrum in the results list is automatically pasted to the top pane so that you can view the peak differences of spectra in the active spectrum and the queried spectrum.

The peaks in the top and bottom spectra have different colors. For the middle spectrum, the application derives the difference peak color from the spectrum that has a more abundant peak at a particular *m/z* value.

Figure 25. Compare Spectra page

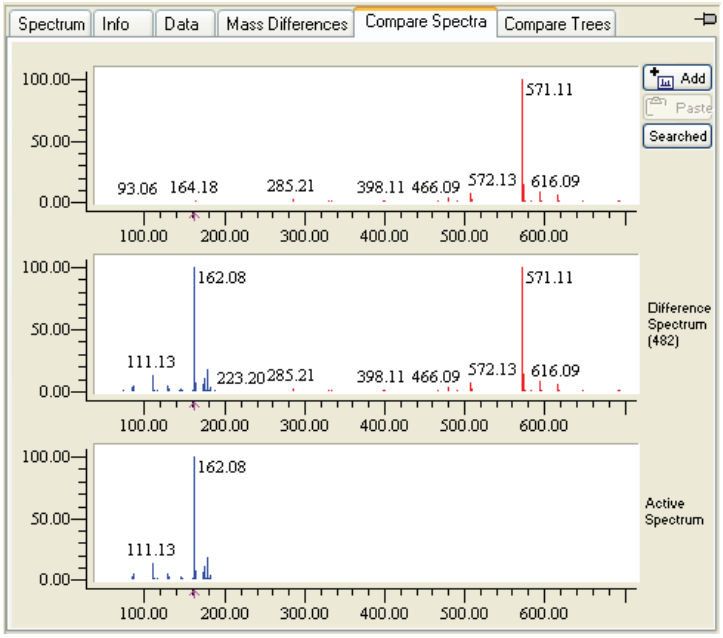


Table 1. Compare Spectra parameters

Parameter	Description
Add	Adds the currently selected spectra to the top pane.
Paste	Pastes the spectra on the Clipboard to the top pane.
Searched	Adds the queried spectrum to the top pane.
Difference Spectrum	Displays the difference between the top and bottom spectrum.
Active Spectrum	The active record currently selected in the Spreadsheet view.

Using the Compare Trees Page

Use the Compare Trees page in the Database Manager window to compare trees from different Database Manager windows.

Figure 26. Compare Trees page

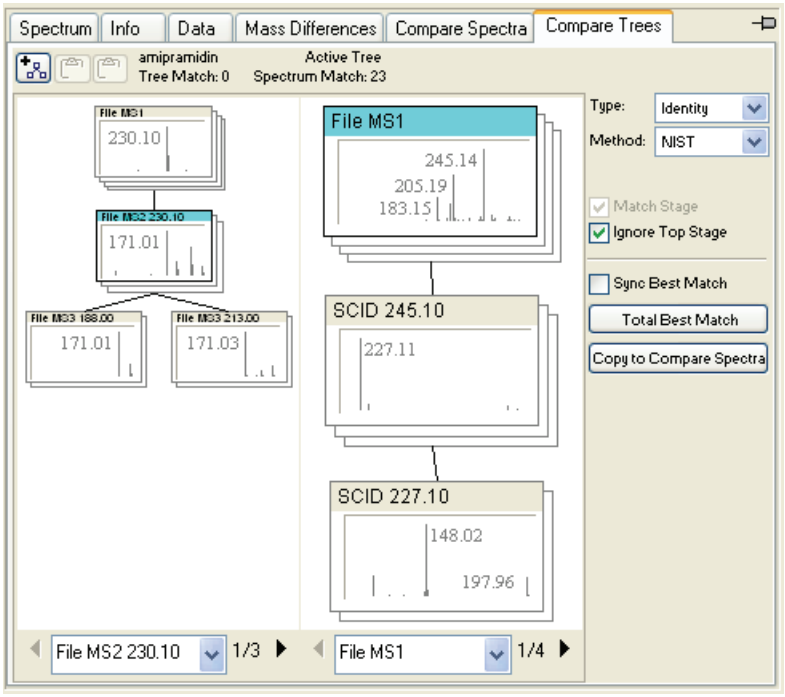


Table 2. Compare Trees parameters

Parameter	Description
Type	Similarity or Identity
Method	Specifies a HC Low, NIST, or HC Low-High peak comparison method.
Match Stage	Uses an identity search with the MS^n stage of the tree spectra. The stage of both nodes must be the same.
Ignore Top Stage	Excludes the top-level tree (full scan, source CID). Use this only when you select Match Stage.
Sync Best Match	Select the most similar spectrum in the second tree.
Total Best Match	Select the most similar spectrum in both trees.
Copy to Compare Spectra	Copies these to the Compare Spectra page. See "Using the Compare Spectra Page" on page 43.

Working with Spectral Records

In the Database Manager window, you can modify the records in a user library by copying records from other libraries, importing records from Microsoft Excel spreadsheets, or modifying the structures for the records.

You can exchange mass spectra between the Database Manager window and the Excel spreadsheet by using the export and import features. For additional information, see "Exporting Data to an Excel Spreadsheet" on page 388 or "Importing Spectra from an Excel Spreadsheet" on page 391.

This section includes the following topics:

Managing Spectral Records

You can copy or move records to different locations in the Spreadsheet view in the same Database Manager window. This lets you organize and maintain your experimental data or search results.

Follow these procedures:

- To open a mass spectrum from a file
- To save one or more spectra
- To select multiple records in the Work spreadsheet
- To select multiple records in a database spreadsheet
- To copy records
- To cut records
- To paste records to the Work spreadsheet
- To copy spectra from the Chromatogram Processor

❖ To open a mass spectrum from a file

1. Do one of the following:

   Click the **Open** button,  , in the Mass Frontier toolbar and choose **Mass Spectrum** from the menu.

   –or–

   Choose **File > Open > Mass Spectrum** from the Mass Frontier main menu.

   The Open Spectrum dialog box opens. By default, both NIST MSP and JCAMP-DX file types are displayed.

2. Select a file and click **Open**.

   The application adds the records in the selected file to the spreadsheet in the Work library.

❖ To save one or more spectra

1. Select one or more records that contain the spectra you want to save.

   See "To select multiple records in the Work spreadsheet" on page 47.

2. Do one of the following:

   Click the **Save** button,  , in the Mass Frontier toolbar and choose **Spectrum** from the menu.

   –or–

   Choose **File > Open > Spectrum** from the Mass Frontier main menu.

   The Save MS Spectrum dialog box opens.

3. Select the data format in the Save as Type list.
4. Type the file name in the File Name box and click **Save**.

> **Tip** Mass Frontier is a 32-bit application, so you can use long names to save structures. You can save spectra by their actual names, such as 1-Amino-2-hydroxyindane.mol.
>
> To save a spectrum with a long name, use CTRL+C to copy the name from the Info page, and then paste the name in the File Name box.

❖ To select multiple records in the Work spreadsheet

Do one of the following:

Select the first record, hold down the SHIFT key, and select the last record.

–or–

Drag the cursor from the first record to the last record.

❖ To select multiple records in a database spreadsheet

Hold down the CTRL key while you select multiple records.

> **Note** In a read-only database library spreadsheet, you cannot use the SHIFT key or drag the cursor to select a range of records.

❖ To copy records

1. Select the records in the Database Manager spreadsheet.

> **Note** You cannot copy records from the copy-protected HighChem Fragmentation Library.

2. Do one of the following:

   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Selected Rows** from the menu.

   –or–

   Choose **Edit > Copy > Selected Rows** from the Mass Frontier main menu.

   –or–

   Right-click and choose **Copy > Selected Rows** from the shortcut menu.

When you copy a record, the application copies the graphic of the selected mass spectrum to the Clipboard. When you select more than one record, the application copies only the graphic of the spectrum in the first record to the Clipboard. You can then paste this graphic into any Windows application.

❖ To cut records

1. Select the records in the Database Manager spreadsheet.

   You can cut only from an editable library.

2. Do one of the following:

   Click the **Cut** button,  , in the Mass Frontier toolbar and choose **Selected Rows** from the menu.

   –or–

   Choose **Edit > Cut > Selected Rows** from the Mass Frontier main menu.

   –or–

   Right-click and choose **Delete > Selected Rows** from the shortcut menu.

When you cut a record, the graphic of the selected mass spectrum is copied to the Clipboard. When you select more than one record, only the graphic of the spectrum in the first record is copied to the Clipboard. You can then paste this graphic into any Windows application.

❖ To paste records to the Work spreadsheet

1. Select the Work spreadsheet.

2. Do one of the following:

   Click the **Paste** button,  , in the Mass Frontier toolbar and select **Rows** from the menu.

   –or–

   Choose **Edit > Paste > Rows** from the Mass Frontier main menu.

   –or–

   Right-click and choose **Paste > Rows** from the shortcut menu.

❖ To copy spectra from the Chromatogram Processor

1. In the Chromatogram Processor window, right-click the Spectrum page and choose **Copy > Copy MS Spectrum** from the shortcut menu.

2. In the Database Manager window, right-click the Spectrum page and choose **Paste > Spectrum** from the shortcut menu.

   You can now extract spectral scans and move them to the Database Manager window for further processing.

Exporting Data to an Excel Spreadsheet

You can export four types of data from the Database Manager window to an Excel spreadsheet: text tables, graphics, mass spectra in numerical table format, and spectral trees (see "Copying and Pasting a Spectral Tree" on page 116). Because you can access all these types at the same time, you must specify the type of data you want to move to the Excel spreadsheet.

Follow these procedures:

- To export data records
- To export mass spectrum or mass differences graphics
- To export a mass spectrum in numerical table format
- To use an Excel spreadsheet as an editor

❖ To export data records

1. Select one or more records on the Spreadsheet page in the Database Manager window.
2. Do one of the following:

   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Selected Rows** from the menu.

   –or–

   Choose **Edit > Copy > Selected Rows** from the Mass Frontier main menu.
3. Click the Excel spreadsheet to make it active.
4. Right-click the Excel spreadsheet, and choose **Paste** from the shortcut menu.

   The application pastes the records into the spreadsheet.

	A	B	C	D	E
1					
2		*	229.0479	C6H8ClN7O	amipramidin
3		*	244.0882	C10H16N2O3S	3'-[1-hydroxy-2-(methylam
4		*	239.1077	C13H18ClNO	bupropion
5					

❖ To export mass spectrum or mass differences graphics

1. Choose **Microsoft Office > New Microsoft Excel Worksheet** from the Mass Frontier main menu.
2. Do one of the following in the Database Manager window:

   Click the **Spectrum** tab.

   –or–

   Click the **Mass Differences** tab.

   The mass spectrum or mass differences spectrum must be visible before you can copy it. For detailed information about the content of the spectrum and mass differences pages, see Chapter 2, "Database Manager Module."
3. Do one of the following:

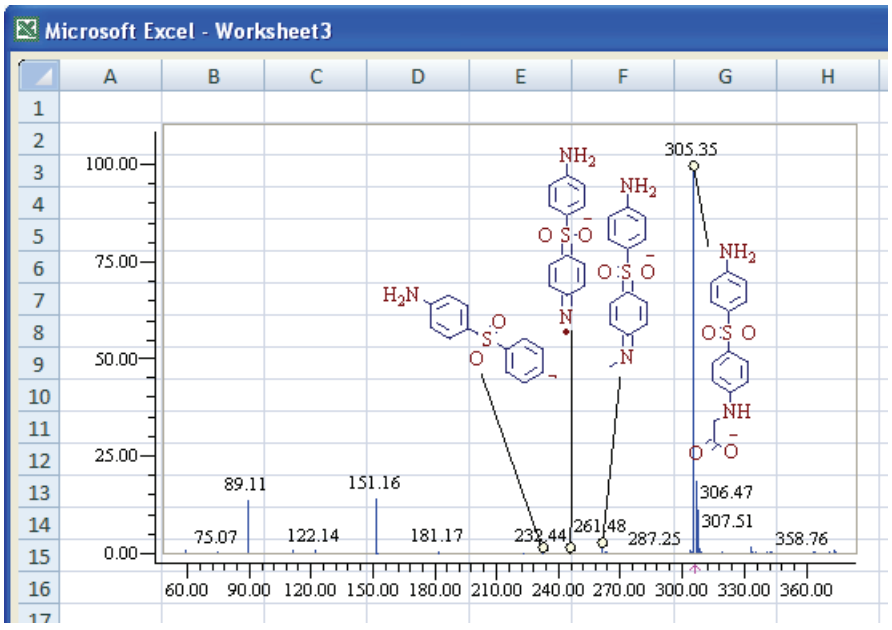
   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Spectrum** from the menu.

   –or–

   Choose **Edit > Copy > Spectrum** from the Mass Frontier main menu.
4. Click the Excel spreadsheet to make it active.
5. Right-click the Excel spreadsheet, and choose **Paste Special** from the shortcut menu.

   The Paste Special dialog box opens.
6. Select **Picture (Enhanced Metafile)** and click **OK**.

   The application pastes the spectra graphics into the spreadsheet.



❖ To export a mass spectrum in numerical table format

1. In a Database Manager window, click the **Data** tab to make the *m/z* and abundance table visible.
2. Do one of the following:

   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Spectrum** from the menu.

   –or–

   Choose **Edit > Copy > Spectrum** from the Mass Frontier main menu.
3. Click the Excel spreadsheet to make it active.
4. Right-click the Excel spreadsheet, and choose **Paste** from the shortcut menu.

   The application pastes the record data into the spreadsheet.

Microsoft Excel - Worksheet1

	A	B	C	D
1	m/z	Abundance	Accuracy	Resolution
2	33.09	35493672	0.5	0.5
3	39.11	15956602	0.5	0.5
4	47.27	37226953	0.5	0.5
5	60.46	141307891	0.5	0.5
6	61.29	16480493	0.5	0.5
7	65.42	84593373	0.5	0.5
8	94.39	23626432	0.5	0.5
9	136.27	13775699456	0.5	0.5
10	137.3	1340118840	0.5	0.5
11	138.34	88444076	0.5	0.5
12	143.17	144339023	0.5	0.5
13	149.28	13902487	0.5	0.5
14	153.22	52275005	0.5	0.5
15	158.13	1096194728	0.5	0.5
16	159.12	109676827	0.5	0.5

❖ To use an Excel spreadsheet as an editor

Export a spectrum or tree to an Excel spreadsheet, edit it, and then import the edited spectrum or tree into the Database Manager window.

Using the Excel application's editing features is useful when, for example, you have an experimental spectrum with several noise peaks at high *m/z* values that you want to delete or you want to extract part of a spectrum that is important to your report or presentation. For obvious reasons, do not add, delete, or alter prominent peaks.

Importing Spectra from an Excel Spreadsheet

You can import mass spectra or spectral trees stored in an Excel spreadsheet to the Database Manager window (see "Copying and Pasting a Spectral Tree" on page 116). You can organize spectral tables horizontally or vertically.

Note The Mass Frontier application supports standard tables with separated numbers, so you can also import spectra from other applications.

❖ To import spectra from an Excel spreadsheet

1. Make sure your table contains mass spectra.
2. Select the table you want to export into the Mass Frontier application.
3. Right-click the Excel spreadsheet, and choose **Copy** from the shortcut menu.
4. Choose **Edit > Paste > Spectrum** in the Mass Frontier main menu.

❖ To correctly interpret *m/z* values and abundance

Follow one of these conventions:

- When the spectral table is vertical, the first column must be the *m/z* value and the second must be abundance.
- When the spectra are vertically oriented, the first column is abundance, and the second column is the *m/z* value, label the first row of the first column "Abundance" and the first row of the second column "*m/z.*"
- When the spectral table is horizontally oriented, the first row must be the *m/z* value and the second row must be abundance.
- When the spectra are oriented horizontally, the first row is abundance, and the second row is the *m/z* value, label the first column of the first row "Abundance" and the first column of the second row "*m/z.*

IMPORTANT The third column can be accuracy in AMU. When the accuracy column is not present, the application uses the default value from the Tolerance Settings on the Options dialog box. See "Specifying Mass/Abundance Options" on page 381.

"

Adding Structures to a Record

The Database Manager module uses chemical structures at every stage, and you can add these chemical structures to a corresponding record. You can create user libraries with structures including isotopes, ions, radicals, and optically active compounds. You can use structures in the Database Manager window in connection with the Fragments & Mechanism window to check the consistency of a mass spectrum and chemical structure. You can use the Database Manager features to perform structural elucidation by modifying the input structure and regenerating product fragments.

Follow these procedures:

- To add a structure to a record that contains a tree
- To add a structure to a peak
- To paste a structure to a record
- To import structures into records
- To assign a structure to a peak
- To add formatted text to a peak
- To add an annotation to a peak

❖ To add a structure to a record that contains a tree

1. Select the appropriate node by clicking any spectrum in that node.

   When dealing with spectral trees, structures are associated with nodes and not with an entire tree.

2. Assign fragments for product spectra.

   This is useful for elucidation and database maintenance of collision-induced dissociation (CID) spectra.

The top level node, MS^1, holds structural information about the neutral compound. A structure displayed in the Database Manager window is associated with a selected node. You can add a structure not only to a spectrum or tree node but also to any peak.

❖ To add a structure to a peak

1. Click the **Assign Structure to *m/z* Value** button,  , in the Database Manager toolbar.

   Note Do not confuse this button with its generic equivalent in the Mass Frontier toolbar, which opens a Structure Editor window that does not automatically connect the fragment to its spectral peak.

   The Structure Editor – Database Manager dialog box opens.

2. Draw a fragment or open a structure.

   For detailed instructions about using the Structure Editor, see "Structure Editor Module" on page 125.

3. When you finish the fragment, click **OK**.

   Based on the fragment's *m/z* value, the application automatically connects your structure to its spectral peak in the Spectrum pane.

4. To connect the fragment to a different peak, drag the connecting circle at the baseline to any other peak.

5. To resize the drawn fragment, click the fragment and drag the handles.

Note Structures assigned to spectral peaks are not searchable.

❖ To paste a structure to a record

1. Copy the structure to the Clipboard.

   You can copy a structure into the Database Manager window from anywhere in the Mass Frontier application.

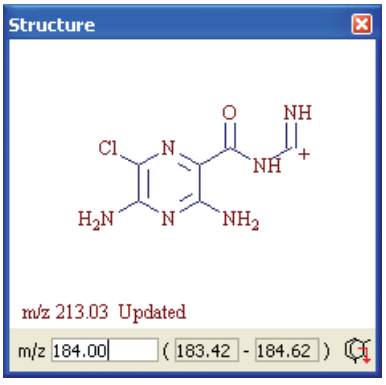
2. In the Database Manager spreadsheet, select the record you want to paste the structure into.

3. When processing a tree, select the node you want to paste the structure into.

4. Choose **Edit > Paste > Structure** from the Mass Frontier main menu.

When you add a structure to a record or replace an existing structure, the word "Updated" appears at the bottom of the structure pane. When you change anything in the record, including the structure, a small circle is displayed in the ID Number column in the spreadsheet. After you have added or changed a structure, the application automatically calculates and updates the molecular formula and molecular mass.

When you add a structure to a tree node or replace an existing structure, the Restore Precursor *m/z* button,  , at the bottom of the structure pane becomes active, if the molecular mass of the structure differs from the existing precursor *m/z* value. Using this button, you can set the precursor *m/z* value of the active product spectrum according to the molecular mass of the drawn structure.



❖ To import structures into records

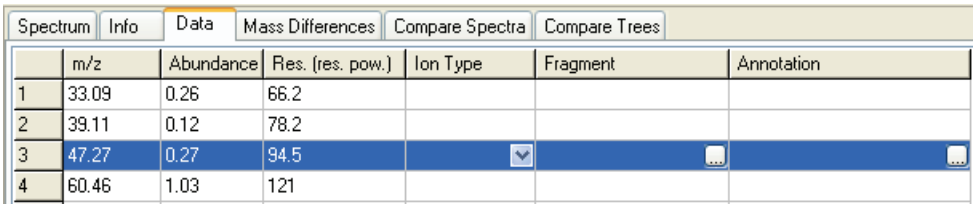
1. In the Database Manager spreadsheet, select the records you want to add structures to.
2. Choose **File > Open > Structure** from the Mass Frontier main menu.
3. Select a .mol, .mcs, or .tml file and click **Open**.

❖ To assign a structure to a peak

1. In the Database Manager window, click the **Data** tab.

   The Data page lists all peaks in the spectrum.

2. Select the row of the peak.

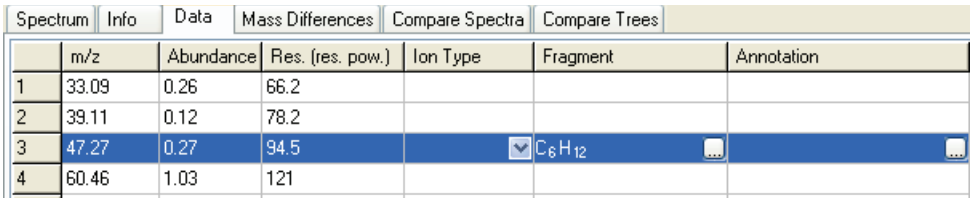


3. Click the **Structure Editor** button, [...], in the Fragment column.

   The Structure Editor opens.

4. In the Structure Editor, create the structure you want to assign to the selected peak, and click **OK**.

   The structure is added to the peak.



   For details about using the Structure Editor, see Chapter 4, "Structure Editor Module."

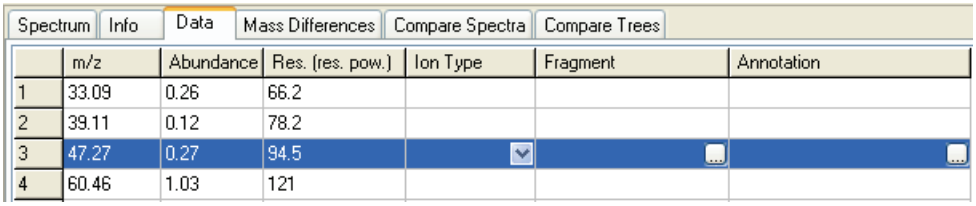
Note You cannot search structures assigned to spectral peaks. Structures are assigned to the *m/z* value so the annotation does not require a peak to be present in a spectrum.

❖ To add formatted text to a peak

1. In the Database Manager window, click the **Data** tab.

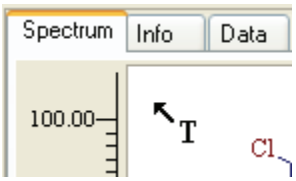
   The Data page lists all peaks in the spectrum.

2. Select the row of the peak.



3. Click the **Spectrum** tab.
4. Click the **Assign Text to *m/z* Value** button, T .
5. Move the cursor to the Spectrum page.

   The cursor changes to a special text cursor.



6. Click the Spectrum page where you want to place the text.

   The Annotation dialog box opens.
7. Enter your text.
8. To draw a line connecting the peak to the annotation, select the **Show Connection Line** check box.
9. Click **OK**.

   The application places the text on the Spectrum page.

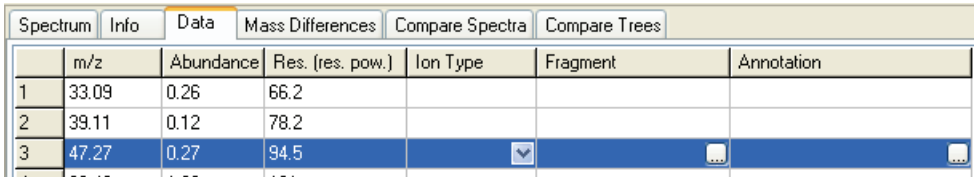
Note Text is assigned to the *m/z* value, so the annotation does not require that a peak be present in a spectrum.

❖ To add an annotation to a peak

1. In the Database Manager window, click the **Data** tab.

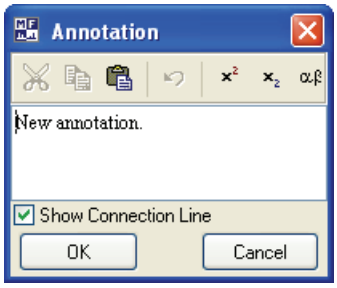
   The Data page lists all peaks in the spectrum.

2. Select the row of the peak.



3. Click the **Annotation** button, …, in the Annotation column.

   The Annotation dialog box opens.



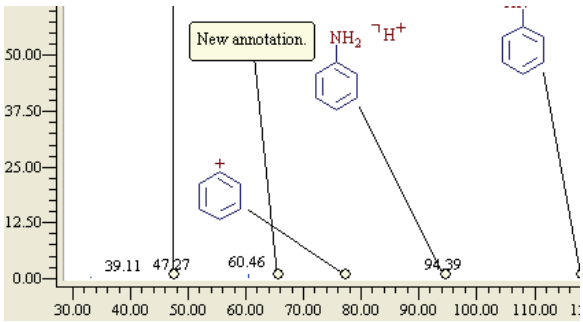
4. Enter your text.

5. To draw a line connecting the peak to the annotation, select the **Show Connection Line** check box.

6. Click **OK**.

7. To view the annotated text, click the **Spectrum** tab.

   The Spectrum page displays the annotated text with the optional connecting line.



Displaying Isotope Patterns

The Isotope Pattern module displays the relevant isotopic profile when you select a structure or fragment in the Mass Frontier application. You can also calculate the isotope pattern from a your molecular formula.

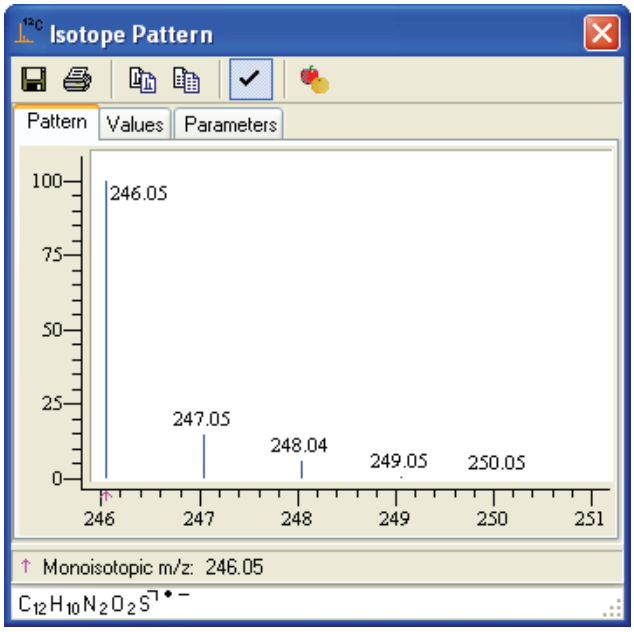
Follow these procedures:

- To display the isotope pattern for a structure
- To display the mass, abundance, and accuracy values
- To display the mass tolerance settings
- To save the spectrum as a NIST MSP file
- To zoom and pan in the graphical display

❖ To display the isotope pattern for a structure

1. Select a structure in the Database Manager window.
2. Choose **Tools > Isotope Pattern** from the Mass Frontier main menu.

   The Pattern page of the Isotope Pattern window opens.

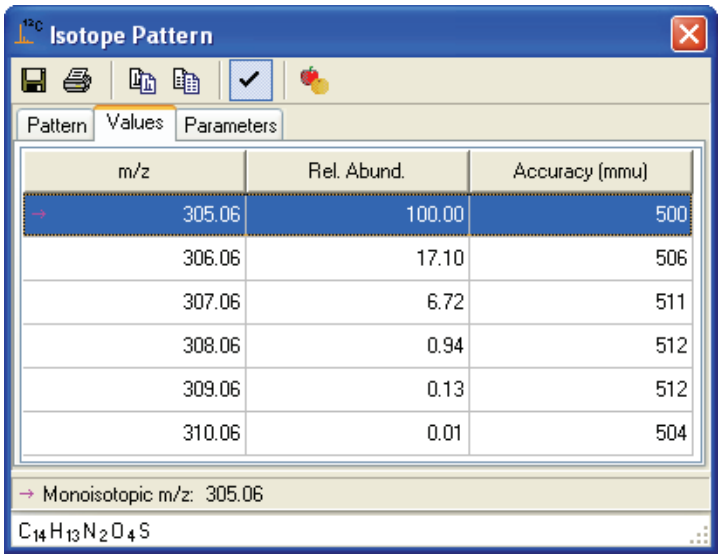


3. To copy the spectra image, click the **Copy as Image** button, .

❖ To display the mass, abundance, and accuracy values

1. Click the **Values** tab.

   The Values page of the Isotope Pattern window opens.

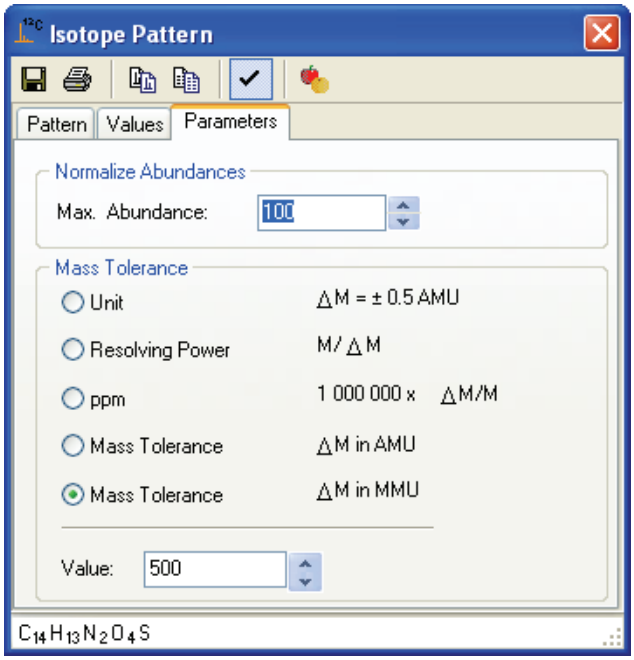


2. To copy the data, click the **Copy as Data** button, .

❖ To display the mass tolerance settings

1. Click the **Parameters** tab.

   The Parameters page of the Isotope Pattern window opens.



2. Enter a maximum abundance value.

3. Select a mass tolerance value.

   The application supports these tolerance types:

   - **Unit**
   - **Resolving power** M/ΔM
   - **ppm** 1 000 000 x ΔM/M
   - **Mass Accuracy** (ΔM in AMU or ΔM in MMU)

   where:

   M = *m/z* (mass-to-charge ratio)

   $\Delta M = M_2 - M_1$ (M_1, M_2 are two adjacent peaks)

   Peaks (*m/z* values) that fall into the ΔM band are merged into a single peak (*m/z* value).

4. To open the Tolerance Settings dialog box where you can specify additional tolerance parameters, click the **Program Settings** button,  .

❖ To save the spectrum as a NIST MSP file

1. From any page of the Isotope Pattern window, click the **Save** button,  .

   The Save MS Spectrum dialog box opens.

2. Type a name for the NIST MSP file (.msp), and click **Save**.

   The application saves the report in the …\HighChem\Mass Frontier 7.0 \Spectra folder.

❖ To zoom and pan in the graphical display

- Click the **Hand** button,  , in the Mass Frontier toolbar and drag the hand to pan horizontally or vertically.
  Similarly, hold down the H keyboard key or the Spacebar to change the cursor to the hand.
- Click the **Zoom Out** button,  , in the Mass Frontier toolbar.
- Click the **Zoom In** button,  , in the Mass Frontier toolbar.
- Click the **Zoom to Box** button,  , in the Mass Frontier toolbar and describe a rectangle.
  Similarly, hold down the Z keyboard key and describe a rectangle.
- Click the **Zoom Reset** button,  , in the Mass Frontier toolbar to return to full scale.
- Click the **Zoom Undo** button,  , in the Mass Frontier toolbar to undo the last zoom operation.
- Click the **Zoom Redo** button,  , in the Mass Frontier toolbar to restore the last zoom operation.
- Use the scroll wheel on the mouse to zoom the entire display.

Assigning Fragments to Spectral Peaks

Use the Mass Frontier application for the automated prediction of fragments from a structure. If you start the generation of fragments from Database Manager window and the active record contains a structure (an MS^1 structure for Spectral Tree), the application automatically links the generated fragments with peaks in the spectra according to their *m/z* values. For additional information, see "Linking Generated Fragments with a Spectrum" on page 216.

Follow these procedures:

- To predict fragments from a structure
- To automatically assign generated fragments to peaks
- To manually assign a fragment structure to a peak

❖ To predict fragments from a structure

1. Choose **Tools > Fragments and Mechanisms** from the Mass Frontier main menu.

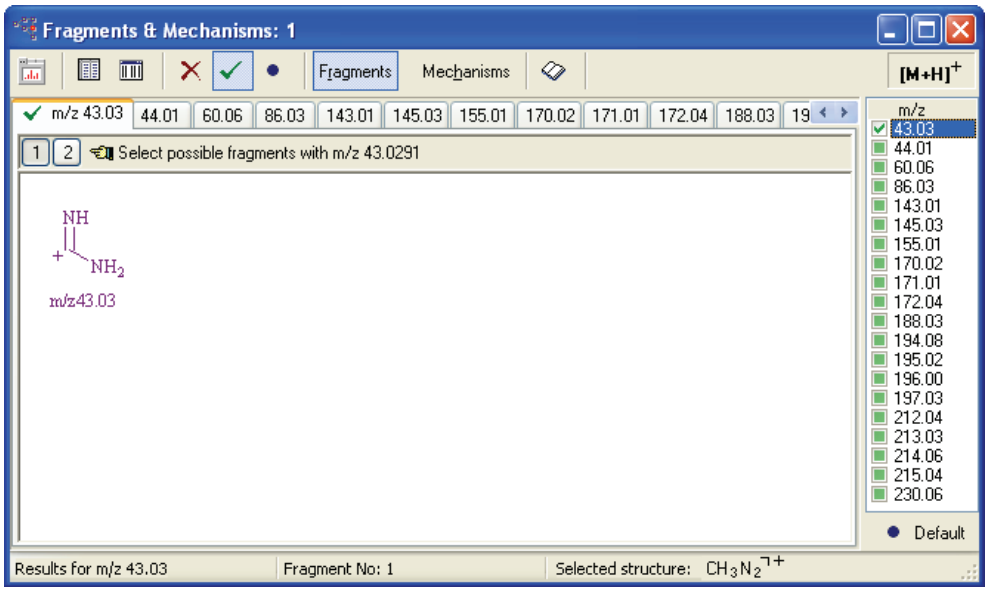
   Note The selected record must contain an MS^1 structure in the spectral tree.

   The Reaction Restrictions dialog box opens.

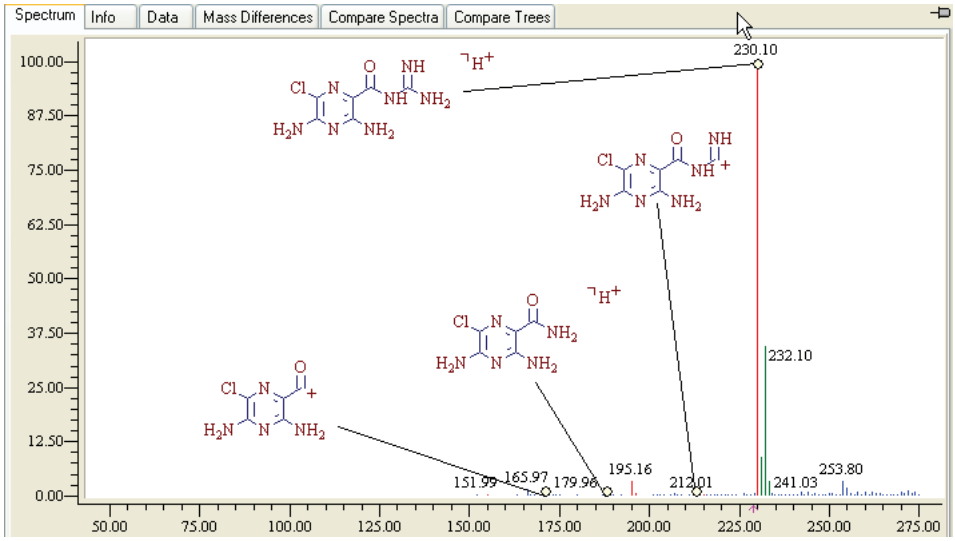
2. Specify your criteria and click **OK**.

   For details about using the Reaction Restrictions dialog box, see Chapter 6, "Fragments and Mechanisms Module."

   The Fragments & Mechanisms window displays possible fragments for each explained peak.



The application links the generated fragments with peaks in the spectrum (spectra in the spectral tree) according to their *m/z* values. On the Spectrum page, the explained peaks are highlighted in red. See "Linking Generated Fragments with a Spectrum" on page 216.



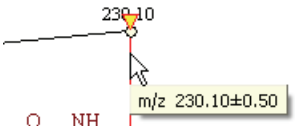
3. To display the mechanisms leading to an explained peak, click **Mechanisms** in the Fragments & Mechanisms window and then do one of the following:

   In the Fragments & Mechanisms window, select the *m/z* value of a peak.

   –or–

   a. On the Spectrum page of the Database Manager window, hold your cursor over a highlighted peak.

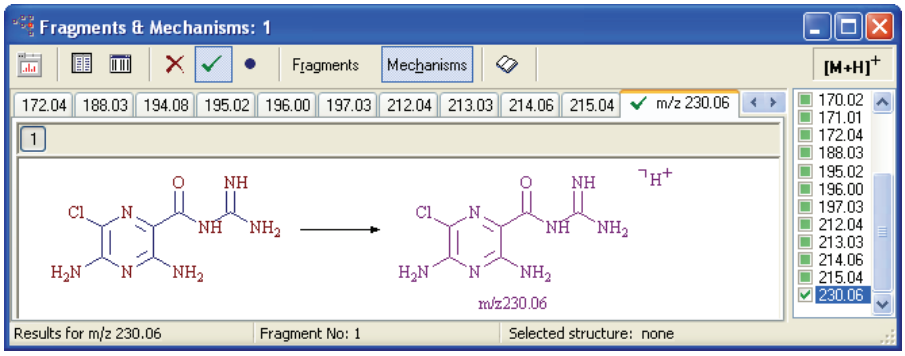
      A yellow triangle indicates the active peak.



   b. Click the peak.

The Fragments & Mechanisms window displays all mechanisms leading to the selected peak.

Figure 27. Fragments & Mechanisms window



❖ To automatically assign generated fragments to peaks

1. Choose **Tools > Fragments and Mechanisms** from the Mass Frontier main menu.

   The Reaction Restrictions dialog box opens.

2. Specify your criteria and click **OK**.

   For details about using the Reaction Restrictions dialog box, see Chapter 6, "Fragments and Mechanisms Module."

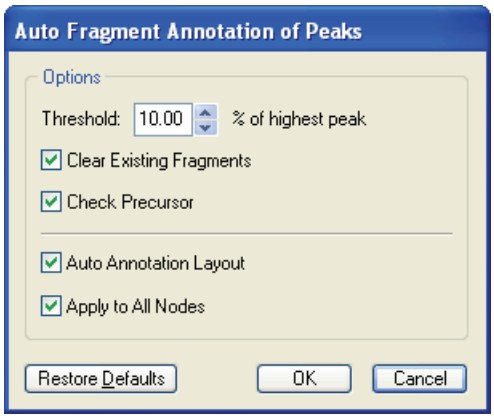
   The Fragments & Mechanisms window opens. This window must remain open to assign the fragments to peaks in the Database Manager window.

3. In the Database Manager window, click the **Spectrum** tab.

4. Right-click the Spectrum page and choose **Auto Annotation > Generated Fragments** from the shortcut menu.

   Note This command is not available unless the Fragments & Mechanisms window remains open.

   The Auto Fragment Annotation of Peaks dialog box opens.



5. Specify your criteria and click **OK**.

   The application reports the number of new fragments that are added to the spectrum.

❖ To manually assign a fragment structure to a peak

1. Click the **Assign Structure to *m/z* Value** button,  , in the Database Manager toolbar.

   Note Do not confuse this button with its generic equivalent in the Mass Frontier toolbar, which opens a Structure Editor that does not automatically connect the fragment to its spectral peak.

   The Structure Editor – Database Manager dialog box opens.

2. Draw a fragment or open a structure.

   For detailed instructions for using the Structure Editor, see "Structure Editor Module" on page 125.

3. When you finish the fragment, click **OK**.

   Based on the fragment *m/z* value, the application automatically connects your structure to its spectral peak in the Spectrum pane.

4. To connect the fragment to a different peak, drag the connecting circle at the baseline to any other peak.

5. To resize the drawn fragment, click the fragment and drag the handles.

Note You cannot search structures assigned to spectral peaks.

Exchanging Mass Spectral Data Between Modules

From the Database Manager window, you can import mass spectra directly from a file, the Chromatographic Processor, an Excel spreadsheet, or the Xcalibur data system. You can open as many Database Manager windows as your system allows and exchange mass spectra between these windows. The Mass Frontier application can automatically establish a link between Database Manager records and other modules that need to access spectral or structural data.

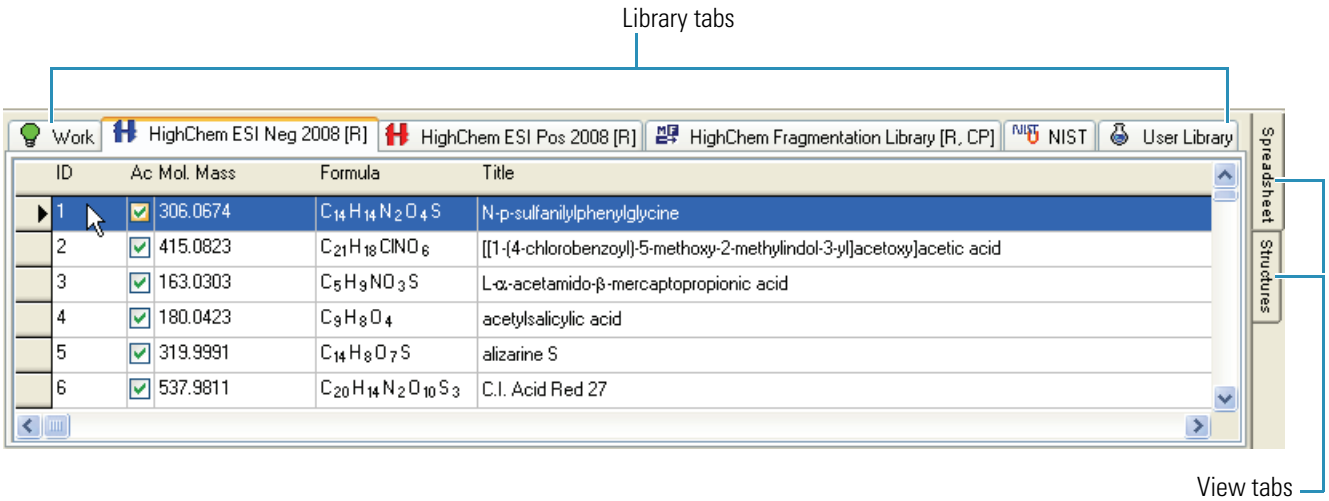
You can link equivalent spectral information to access the original data that was supplied as input for one of the many interpretation methods available in the application.

The direct feedback between source (records and mass spectra) and results (mechanisms, bar code spectra, classes, and projections) helps you keep track of all the modules that originate from a single source. This feature makes the simultaneous use of multiple modules easier, further supporting more sophisticated problem-solving approaches.

For additional information, see "Linking Generated Fragments with a Spectrum" on page 216, "Bar Code Spectra" on page 220, or "Accessing Spectra from a Spectra Projector Window" on page 367.

Using the Library Pane

The Library pane in the Database Manager window includes vertical tabs for the Spreadsheet and Structures views and horizontal tabs for the installed libraries.



This section includes the following topics:

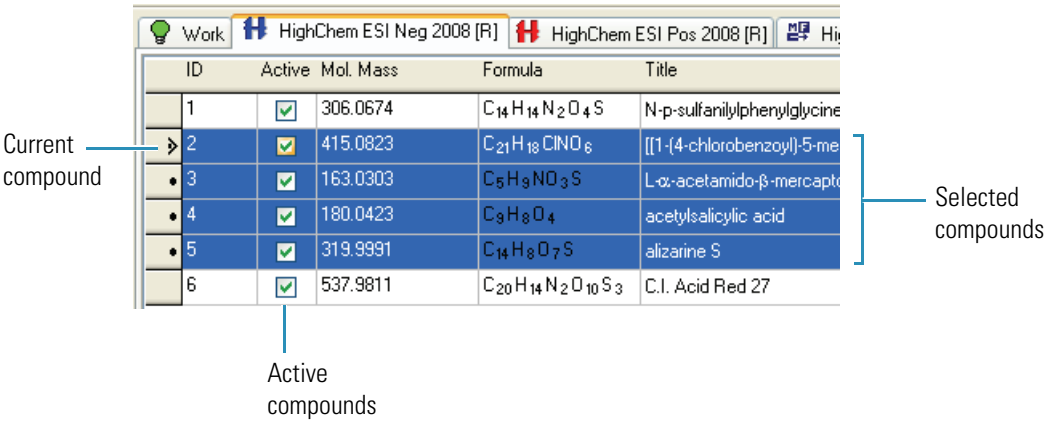
- Spreadsheet View
- Structures View

Spreadsheet View

The Spreadsheet view is organized with each record represented by a single row. The columns contain supplementary record information. To move a row, use the cut-and-paste functions. The Database Manager window does not support dragging rows. To sort a column, click the column header.

When you open a Database Manager window, the Work spreadsheet is empty. You can add records to the Work spreadsheet by conducting a search, opening spectra, or pasting records or stand-alone spectra. A Work spreadsheet can contain a maximum of 32 000 records. For the current record, the application displays the associated spectrum or tree and structure (optional) in the upper pane. An arrow, ⮞, in the first column indicates which record is current. You can select more than one record, but the row with the triangle symbol is always the current one.

Figure 28. Spreadsheet view



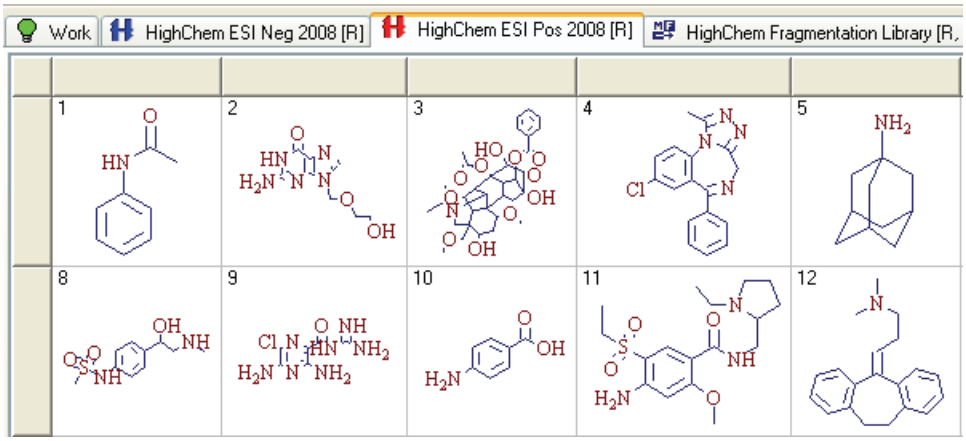
Structures View

The Structures view displays the library compounds in graphical format.

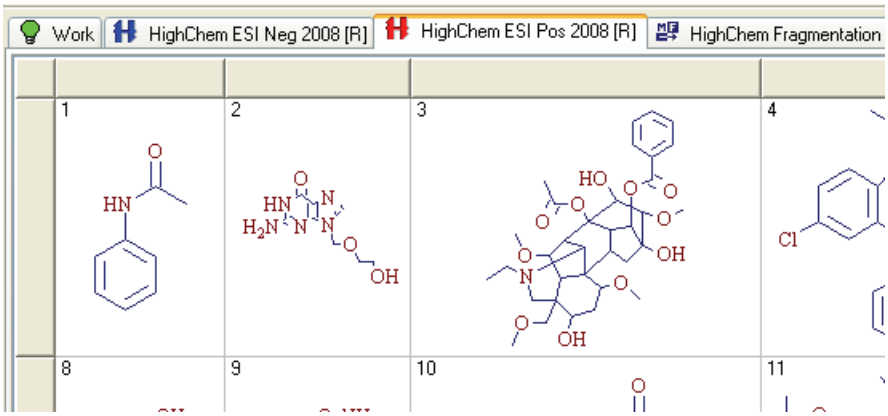
❖ To preview all structures

1. Click the **Structures** tab.

   The structures are organized in numbered cells.



2. To resize a structure, drag the column or row dividers.



Note In the Structures view, you can select only a single record (structure).

Processing Data-Reduced Chromatograms

Library storage of data-reduced chromatograms is not a replacement for the standard certified archiving procedures that the FDA (21 CFR Part 11) requires, but rather an accompaniment to them.

Follow these procedures:

- To import a data-reduced chromatogram from the Chromatogram Processor
- To edit or search components in data-reduced chromatograms
- To preview a chromatogram in the Chromatogram Processor window
- To access the original chromatographic data set

❖ To import a data-reduced chromatogram from the Chromatogram Processor

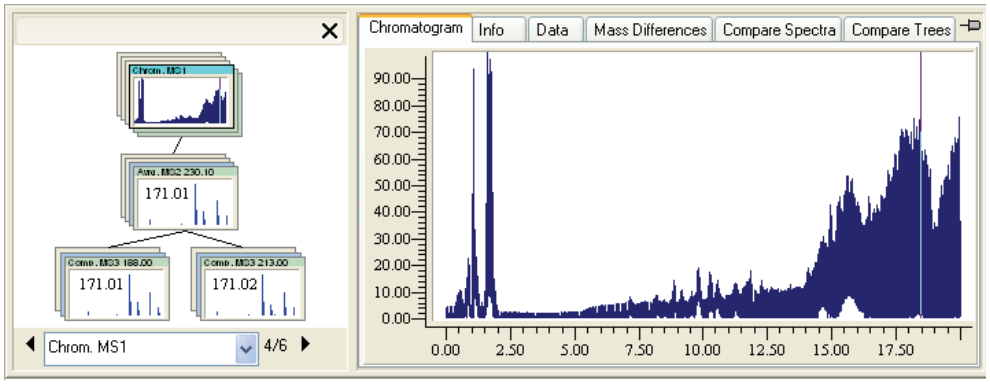
1. In the Chromatogram Processor, right-click any of the panes and choose **Copy > Copy Chromatogram** from the shortcut menu.

   For detailed information about using the Chromatogram Processor module, see Chapter 8, "Chromatogram Processor Module."

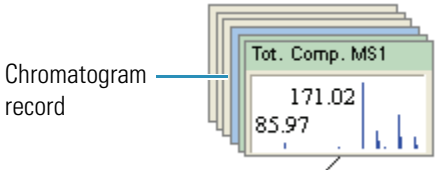
2. In the Database Manager window, select the appropriate level in the Spectral Tree pane.

3. Right-click and choose **Paste > Chromatogram** from the shortcut menu.

   The application adds the chromatogram to the tree and replaces the Spectrum tab with a Chromatogram tab.



Chromatograms are highlighted in blue in the Spectral Tree to easily distinguish them from other records.



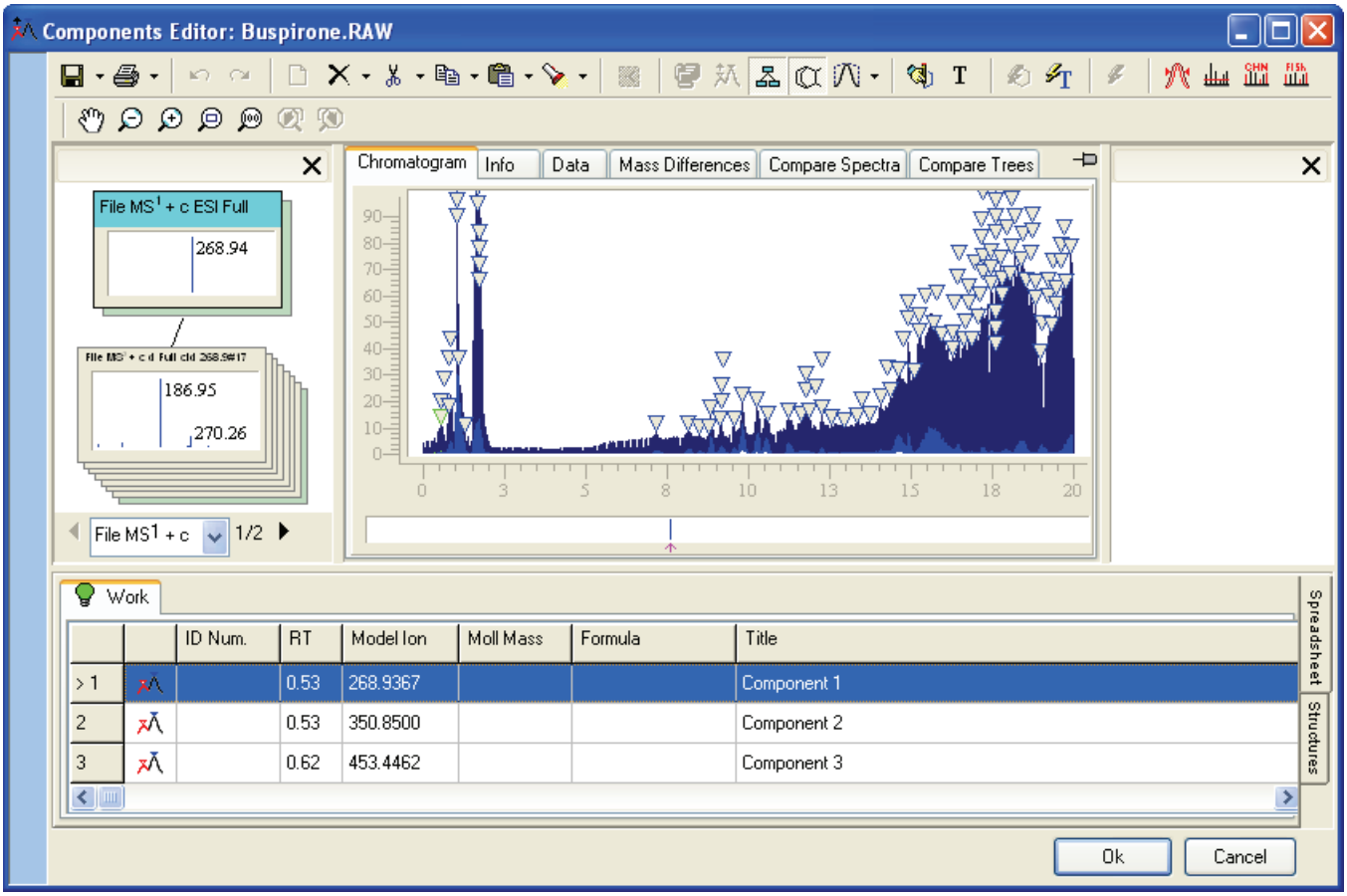
You can save data-reduced chromatograms in the Database Manager window to any library using the same procedure as for saving spectra or trees.

❖ To edit or search components in data-reduced chromatograms

1. In the Chromatogram Processor, click the **Components Editor** button, .

> **Note** This feature is enabled only after you have generated components by using one of the Components Detection and Spectra Deconvolution features in the Chromatogram Processor.

The Components Editor window opens.



2. To search trees, do the following:
   a. Choose **Search > Tree** from the Mass Frontier main menu.

      The Library Search dialog box opens.

   b. In the Libraries area, select the libraries to search.

   c. Click **OK**.

      For additional information about the Library Search parameters, see "Spectral Tree Search" on page 74.

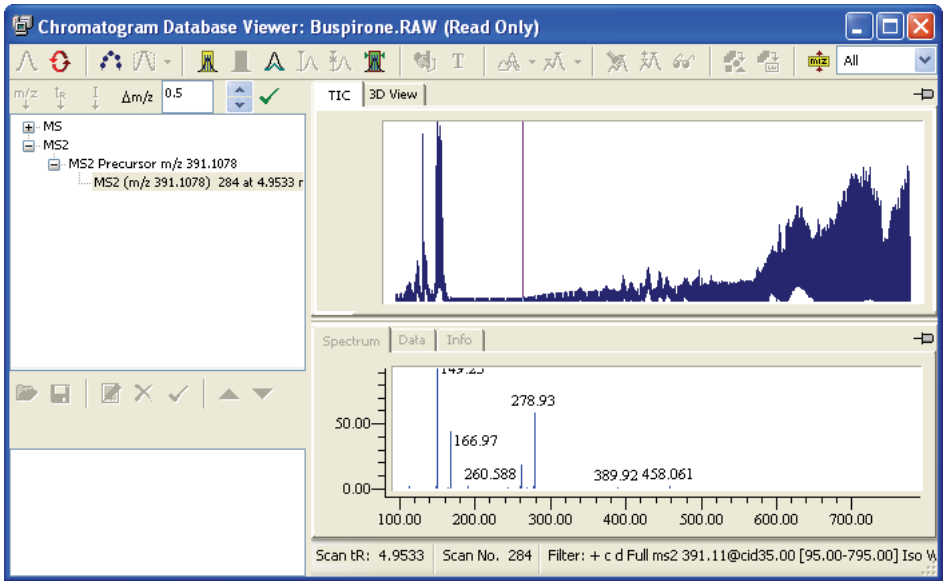
3. To search chromatographic scans, do the following:
   a. Choose **Search > Tree** from the Mass Frontier main menu.

      The Library Search dialog box opens.
   b. In the Libraries area, select the libraries to search.
   c. Click the **Advanced** tab.
   d. In the Search In area, select the **Chromatograms** check box.
   e. Click **OK**.

❖ To preview a chromatogram in the Chromatogram Processor window

1. In the Spectral Tree pane, select the chromatogram you want to preview.
2. Click the **Chromatogram Processor** button,  , in the Database Manager toolbar.

   The Chromatogram Database Viewer opens.



   The application recalls the file path to the original chromatographic data for every chromatographic library entry.

3. To preview other chromatograms, click the record in the Spectral Tree pane.

❖ To access the original chromatographic data set

1. In the Database Manager window, locate the record in the library.
2. Click the **Chromatogram Processor** button,  , in the Database Manager toolbar.

   The Chromatogram Database Viewer opens.
3. Click the **Reload Original Data File** button,  , in the Chromatogram Database Viewer toolbar.

   The original data opens in a new Chromatogram Processor window.

Using the Search Utilities

The Mass Frontier application features several query and search functions available for retrieving spectra, trees, chromatograms, or structures from libraries. You can simultaneously search every library using these search options.

Search menu commands

Search option	Description
Tree	Searches for the library spectral tree most closely matching an unknown spectral tree or subtree. For additional information, see "Spectral Tree Search" on page 74.
Spectrum	Searches for the library spectra most closely matching an unknown spectrum. For additional information, see "Spectrum Search" on page 79.
(Sub)Structure	Searches for an exact match for the query structure (structure search), or searches for an exact match for the structure subset (substructure search). For additional information, see "(Sub)Structure Search" on page 84.
Name	Incremental name search. For additional information, see "Name Search" on page 91.
Formula	Searches for compounds with a specified molecular formula. For additional information, see "Formula Search" on page 92.
Molecular Mass	Searches for compounds with a specified molecular mass. For additional information, see "Molecular Mass Search" on page 94.
ID Number	Searches for library entries with a specified ID number. For additional information, see "ID Number Search" on page 96.
CAS Number	Searches for one or more compounds with a specified Chemical Abstract Service registry number. For additional information, see "CAS Number Search" on page 97.
Fragmentation Data	Searches for fragmentation data associated with a compound. For additional information, see "Fragmentation Data Search" on page 98.
Retention Time	Searches for library entries with a range of specified retention times. For additional information, see "Retention Time Search" on page 101.

❖ To perform a search

1. Choose **Search** > ***your specific search type***.

   The Search <*search type*> dialog box opens.

2. Select the library or libraries to be searched.

3. Specify additional search criteria.

   For detailed explanations of search criteria by search type, see the appropriate section:

   - Spectral Tree Search
   - Spectrum Search
   - (Sub)Structure Search
   - Name Search
   - Formula Search
   - Molecular Mass Search
   - ID Number Search
   - CAS Number Search
   - Fragmentation Data Search
   - Retention Time Search

4. Do one of the following:

   In the Library Search dialog box, select the **Merge Results into Active Database Manager Window** check box.

   The application adds the search results to the end of the Work library in the Spreadsheet view in an active Database Manager window.

   –or–

   Clear the **Merge Results into Active Database Manager Window** check box.

   The application opens a new Database Manager window to display the search results.

   > **Note** If the **Merge Results into Active Database Manager Window** check box is not available, there is no active Database Manager window. The application opens a new Database Manager window to display the search results.

5. Click **OK**.

When the search is successful, the application stores the results in the Spreadsheet view in the current Database Manager window or a new Database Manager window.

Spectral Tree Search

The Mass Frontier application processes MS^n spectra in hierarchically consistent spectral trees that you can search in spectral or chromatographic libraries. The Advanced page in the Spectral Tree Search dialog box contains options for spectral tree searching. See "Advanced page of the Spectral Tree Search dialog box" on page 77.

Each node in spectral trees can consist of four types of spectra (average, composite, parallel, and source CID) and you can specify the type of spectra to search.

Use the Source CID Spectra option when dealing with source CID spectra. When you have a library that consists exclusively of source CID spectra and your unknown spectrum is also a source CID, you should exclude other spectra types from the search. If no such library is available, you can search source CID spectra in product CID spectra, but pay careful attention to the search results. Source CID spectra might contain fragmentation products from all the ions present in the source, including adduct or cluster ions, while product CID spectra are preferably generated from protonated or deprotonated ions.

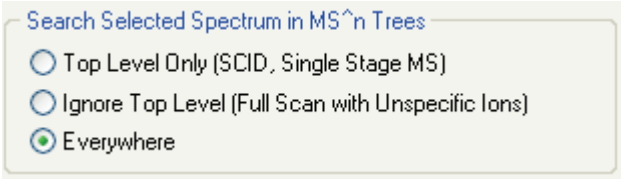
Two combinations relate to the tree search:

- Search a single spectrum in library trees

  See "Spectrum Search" on page 79.

- Search a tree in library trees

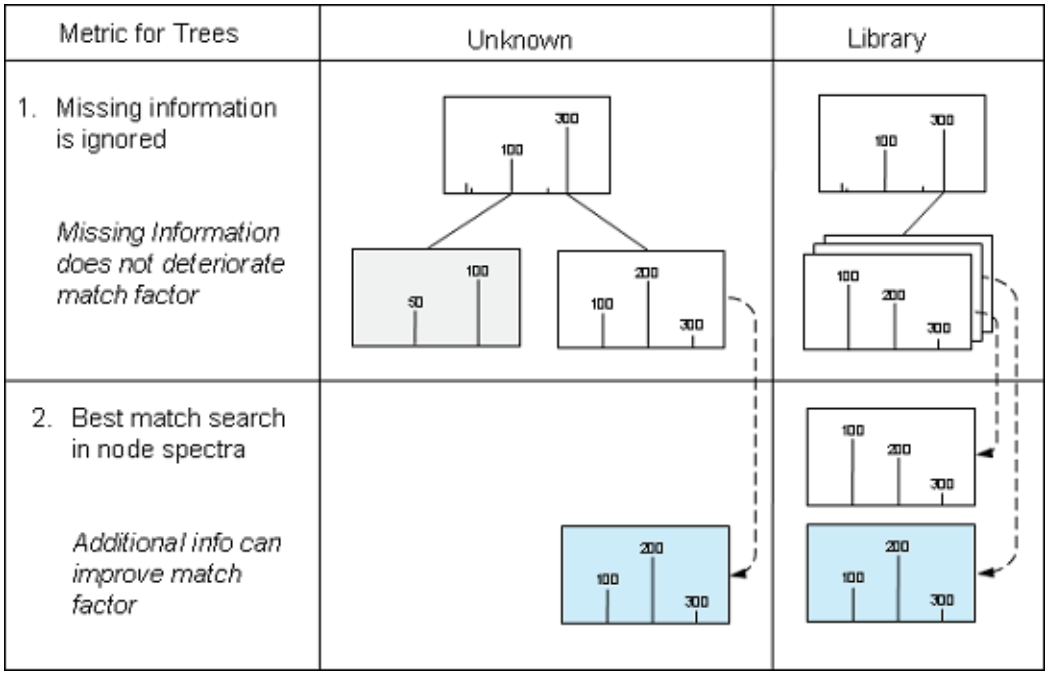
When you search a single spectrum in library trees, the Mass Frontier application compares the spectrum to every spectrum in the tree hierarchy and individually calculates the match factor. You can search a single spectrum in the top level only (full scan, source CID), everywhere except for the top level (first stage) or everywhere in the tree.



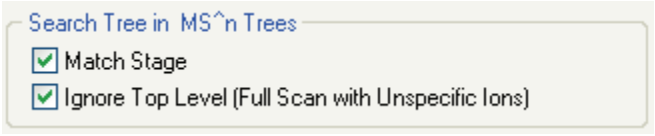
When you search a tree (unknown) against spectral trees in a library, the application compares the spectrum according to a special logic. See "Searching an unknown against a library" on page 75. It compares the corresponding spectra on an identical level (MS^n stage) with a common precursor *m/z* by using an algorithm based on the optimized dot-product. The application ignores a spectrum that appears on one side only, which does not adversely affect

the search result. If there are several corresponding spectra (single node with average, composite, parallel, or other spectra), the Mass Frontier application accepts the best match (optimistic approach). The total match factor is calculated from all the non-zero match factors.

Figure 29. Searching an unknown against a library



You can search a spectral tree using two options. You can include or exclude the top tree level (full scan, source CID) from a search, and the MS^n stage of the tree spectra can be identical (identity search) or not identical (subtree search):



The Spectral Tree Search dialog box includes the following pages:

- Tree page of the Spectral Tree Search dialog box
- Advanced page of the Spectral Tree Search dialog box

Figure 30. Tree page of the Spectral Tree Search dialog box

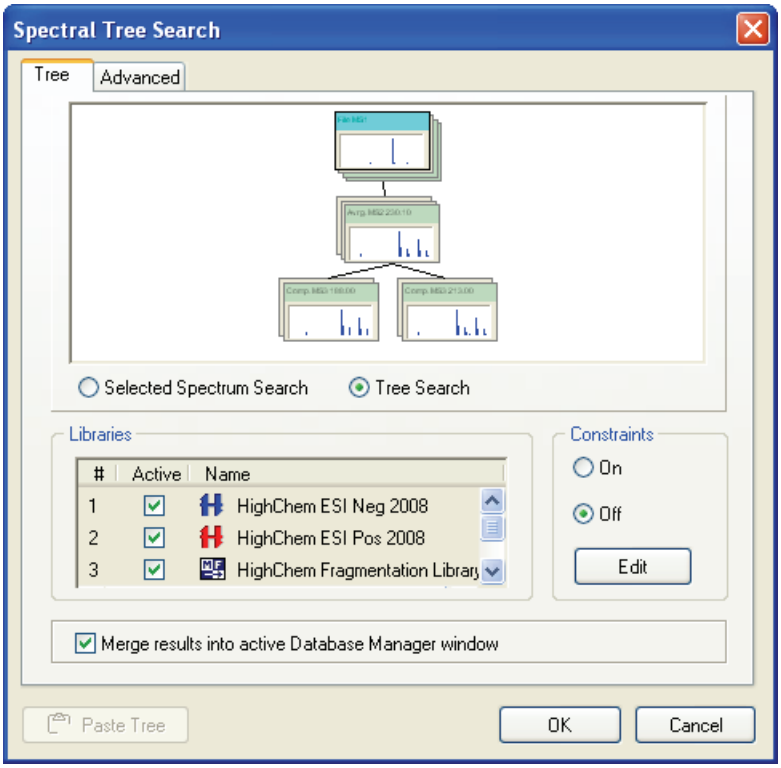


Table 3. Tree parameters

Parameter	Description
Selected Spectrum Search	Changes this search to a Spectrum search. See "Spectrum Search" on page 79.
Tree Search	Default search type.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.
Paste Tree	Available when you have a copied spectral tree on the Clipboard. Lets you search for a spectral tree other than the currently selected spectral tree in the Database Manager window.

Figure 31. Advanced page of the Spectral Tree Search dialog box

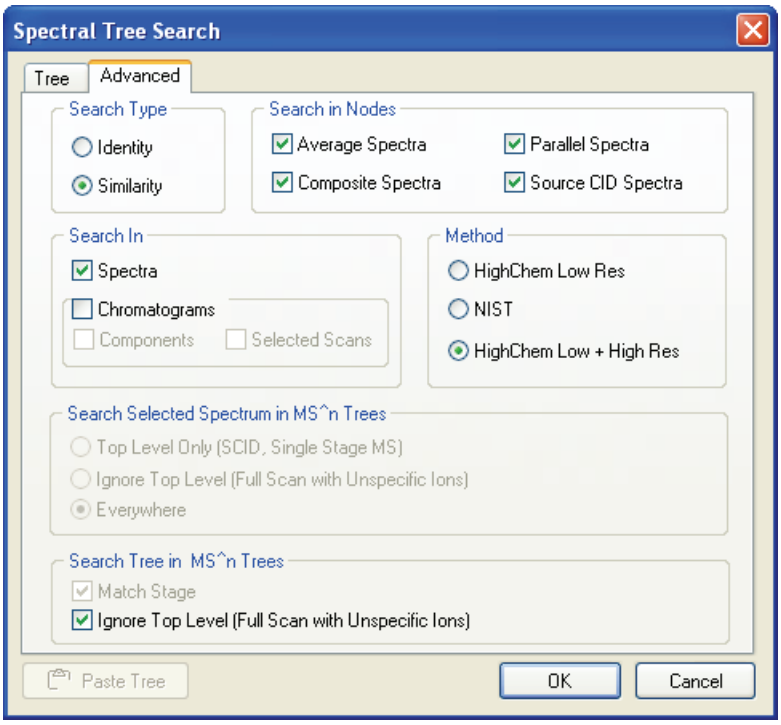


Table 4. Advanced parameters (Sheet 1 of 2)

Parameter	Description
Search Type	
Identity	Locates a library spectrum that closely matches an unknown. Provides an exact match of query and library structure.
Similarity	Retrieves spectra library entries of similar compounds when the unknown is not in the library or its spectrum is distorted so badly that a reliable match is not possible.
Search in Nodes	
Average Spectra	Searches the average spectra in the spectral tree nodes.
Composite Spectra	Searches the composite spectra in the spectral tree nodes.
Parallel Spectra	Searches the parallel spectra in the spectral tree nodes.
Source CID Spectra	Searches source collision-induced dissociation spectra in the spectral tree nodes.
Search In	
Spectra	Searches spectra.
Chromatograms	Searches chromatograms: components, selected scans, or both.

Table 4. Advanced parameters (Sheet 2 of 2)

Parameter	Description
Method	
HighChem Low Res	Specifies a HighChem Low Res tree comparison method.
NIST	Specifies a NIST tree comparison method.
HighChem Low + High Res	Specifies a HighChem Low + High Res tree comparison method.
Search Selected Spectrum in MS^n Trees	
Top Level Only (SCID, Single Stage MS)	Searches only the top-level tree (full scan, source CID).
Ignore Top Level (Full Scan with Unspecific Ions)	Excludes the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.
Search Tree in MS^n Trees	
Match Stage	Uses an identity search with the MS^n stage of the tree spectra.
Ignore Top Level (Full Scan with Unspecific Ions)	Excludes the top-level tree (full scan, source CID).
Paste Tree	Available when you have a copied spectral tree on the Clipboard. Lets you search for a spectral tree other than the currently selected spectral tree in the Database Manager window.

Spectrum Search

The Mass Frontier application uses search algorithms developed by HighChem and the National Institute of Standards and Technology (NIST). The HighChem Low Res and NIST algorithms are based on the optimized dot-product function, and an additional term that is based on ratios of peak intensities. The HighChem Low + High Res algorithm is based on weight distance between spectra. The application includes peak accuracies when it calculates the match factor.

The query spectrum can originate from the Database Manager, Components Editor, or Chromatogram Processor modules by selecting a spectral scan in a chromatogram. In addition, you can paste the query spectrum from the Clipboard into the search dialog box. In this case, you can copy the spectrum into the Mass Frontier application only.

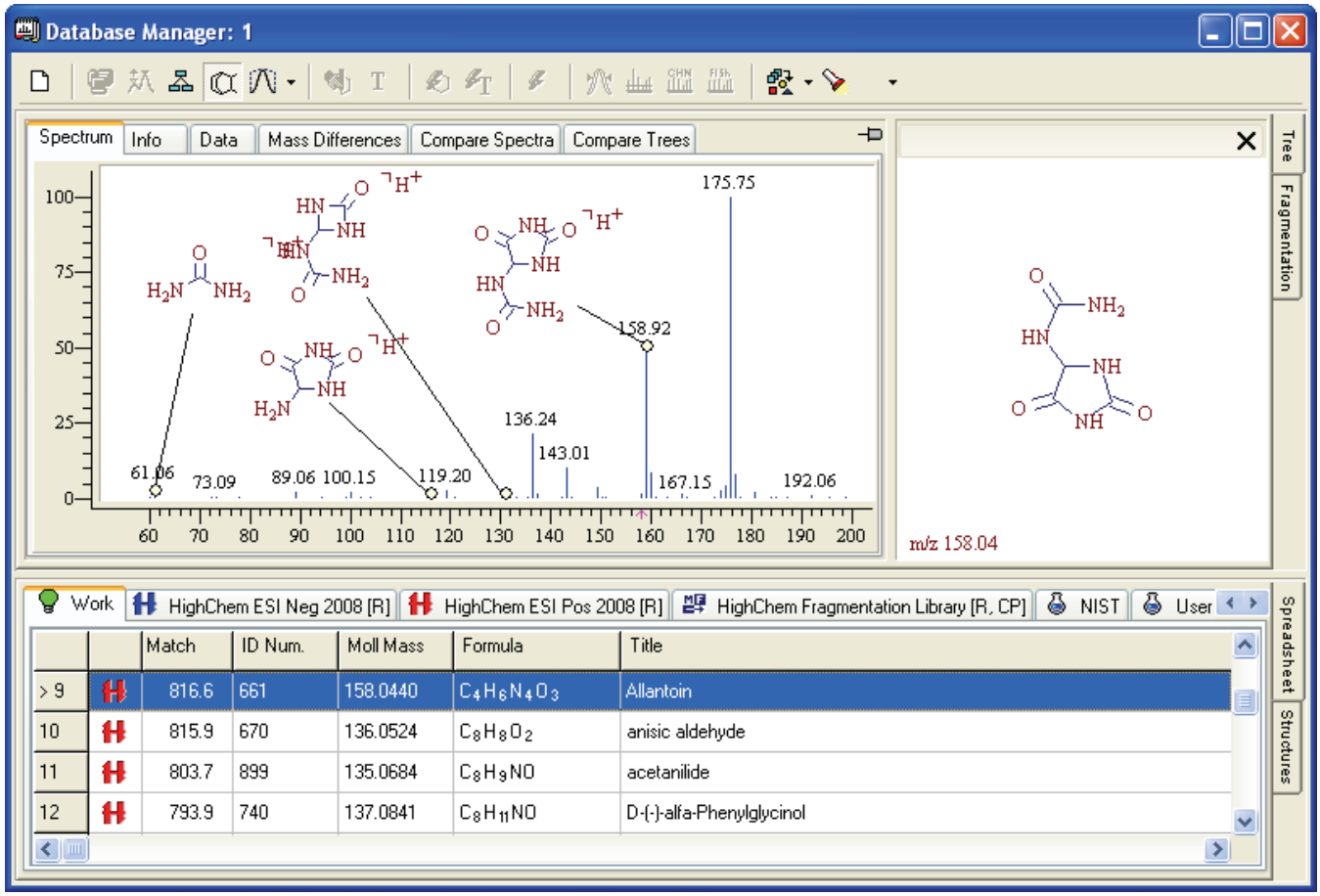
With the spectrum search option you can choose between Identity or Similarity searches. Identity searching is designed to locate a library spectrum that closely matches an unknown. You use similarity searching to retrieve spectra library entries of similar compounds when the unknown is not in the library or its spectrum is distorted so badly that a reliable match is not possible.

After doing a spectrum search, the application stores the search results in the Work library in the Spreadsheet view. After the search is complete, the application adds a match factor column (Match) to the Spreadsheet view. See "Spectrum search results" on page 80. The match factor is a number from 1 to 999 that specifies the measure of similarity between the query spectrum and the library reference spectrum. A match factor of 999 means a perfect match. To draw your attention to a match greater than 930, a "lightning" icon, , is displayed in the Match column of the Spreadsheet view.

The Spectrum Search dialog box includes the following pages:

- Spectrum page in the Spectrum Search dialog box
- Advanced page in the Spectrum Search dialog box

Figure 32. Spectrum search results



If the Identity search does not provide an acceptable match—that is, the unknown has not been positively identified—you might use a Similarity search. In this case, the algorithm does not use the high mass peak index, but uses wider abundance ranges instead. The search results from a Similarity search can be of value in deducing structure, especially in establishing a structural proposal for an unknown spectrum.

To establish a structural proposal for an unknown, you can switch to the Structures view. From the search results of similar compounds you might recognize some common structural features, which are displayed in the structures grid. You can copy the structures to the Structure Editor and put the pieces of the structural "puzzle" together to create an initial structure. You can then paste this structure back into a Database Manager window to the record that holds the unknown spectrum. After this, comparing the peaks in the spectrum with generated fragments can provide valuable information about the consistency of the proposed structure and unknown spectrum. If the *m/z* values of the fragments do not match the spectrum, you can modify the structure and repeat this procedure. However, if the structures in the search results are highly diverse and dissimilar, you must combine this approach with other methods.

Figure 33. Spectrum page in the Spectrum Search dialog box

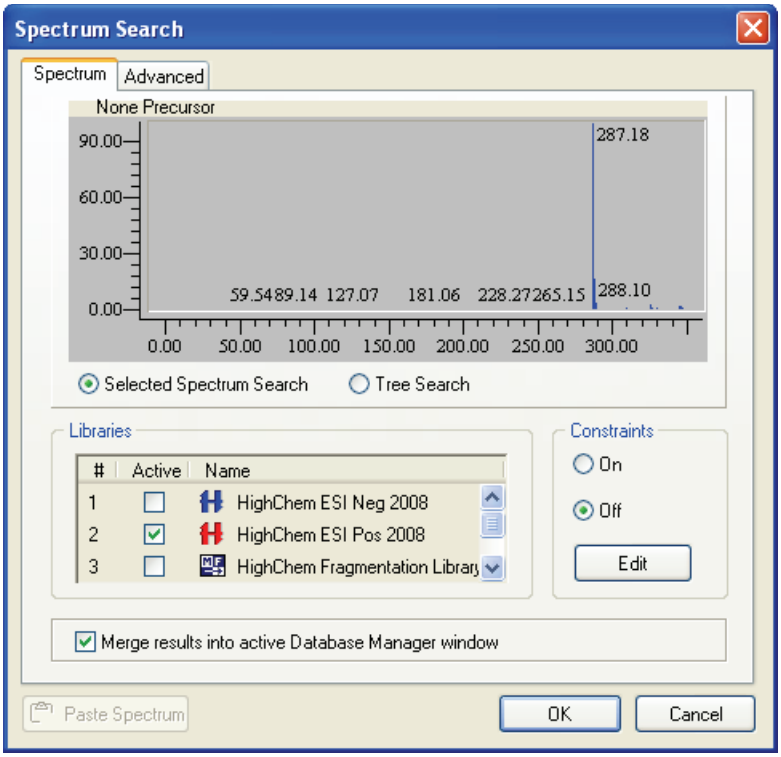


Table 5. Spectrum parameters

Parameter	Description
Selected Spectrum Search	Default.
Tree Search	Changes this search to a Tree search. See "Spectral Tree Search" on page 74.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.
Paste Tree	Available when you have a copied spectral tree on the Clipboard. Lets you search for a spectral tree other than the currently selected spectral tree in the Database Manager window.

Figure 34. Advanced page in the Spectrum Search dialog box

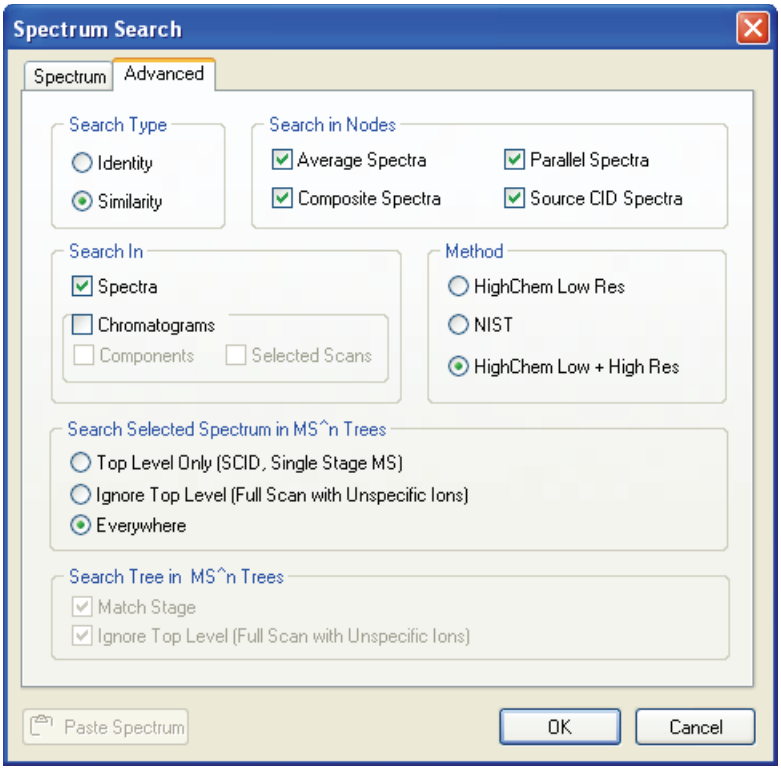


Table 6. Advanced parameters (Sheet 1 of 2)

Parameter	Description
Search Type	
Identity	Locates a library spectrum that closely matches an unknown. Provides an exact match of query and library structure.
Similarity	Retrieves spectra library entries of similar compounds when the unknown is not in the library or its spectrum is distorted so badly that a reliable match is not possible.
Search in Nodes	
Average Spectra	Searches the average spectra in the spectral tree nodes.
Composite Spectra	Searches the composite spectra in the spectral tree nodes.
Parallel Spectra	Searches the parallel spectra in the spectral tree nodes.
Source CID Spectra	Searches source collision-induced dissociation spectra in the spectral tree nodes.
Search In	
Spectra	Searches spectra.
Chromatograms	Searches chromatograms: components, selected scans, or both.

Table 6. Advanced parameters (Sheet 2 of 2)

Parameter	Description
Method	
HighChem Low Res	Specifies a HighChem Low Res spectrum comparison method.
NIST	Specifies a NIST spectrum comparison method.
HighChem Low + High Res	Specifies a HighChem Low + High Res spectrum comparison method.
Search Selected Spectrum in MS^n Trees	
Top Level Only (SCID, Single Stage MS)	Searches only the top-level tree (full scan, source CID).
Ignore Top Level (Full Scan with Unspecific Ions)	Excludes the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.
Search Tree in MS^n Trees	
Match Stage	Uses an identity search with the MS^n stage of the tree spectra.
Ignore Top Level (Full Scan with Unspecific Ions)	Excludes the top-level tree (full scan, source CID).
Paste Spectrum	Available when you have a copied spectrum on the Clipboard. Lets you search for a spectrum other than the currently selected spectrum in the Database Manager window.

(Sub)Structure Search

You can make a structure or substructure query from the Structure Editor window, the Database Manager window, or a fragment copied from the Fragments & Mechanism window.

Structure and substructure searches are important features for retrieving library entries. The structure search is the most straightforward method for finding compounds in a library. 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. Drawing or importing a structure query is easier than typing a complicated name or CAS number.

While a structure search (Identity search) provides an exact match of query and library structure, 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. See "(Sub)Structure Search Rules" on page 89.

A substructure search offers two additional search options: Substructure Best Match and Substructure Match Ring Bonds. A substructure can sometimes fit at several locations of a larger structure. When the Substructure Best Match option representing the closest match is found, it appears in red on the Structure page. See "(Sub)Structure search results" on page 85. Using this option lengthens calculation times. 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 also lengthens calculation times.

Use a (sub)structure search for multiple purposes:

- To study mass spectra of structurally related molecules.
- To positively identify an unknown or to interpret a mass spectrum. Compounds retrieved through a substructure search can provide analogies to the fragmentation processes in the spectrum under consideration.

When searching structures with an unspecified charge site or substituents, be aware of the search rules that apply. You can perform a structure similarity search using substituents.

Note A substructure search ignores the number and positions of hydrogen atoms.

The (Sub)Structure Search dialog box includes the following pages:

- Data page in the (Sub)Structure Search dialog box
- Advanced page in the (Sub)Structure Search dialog box

Figure 35. (Sub)Structure search results

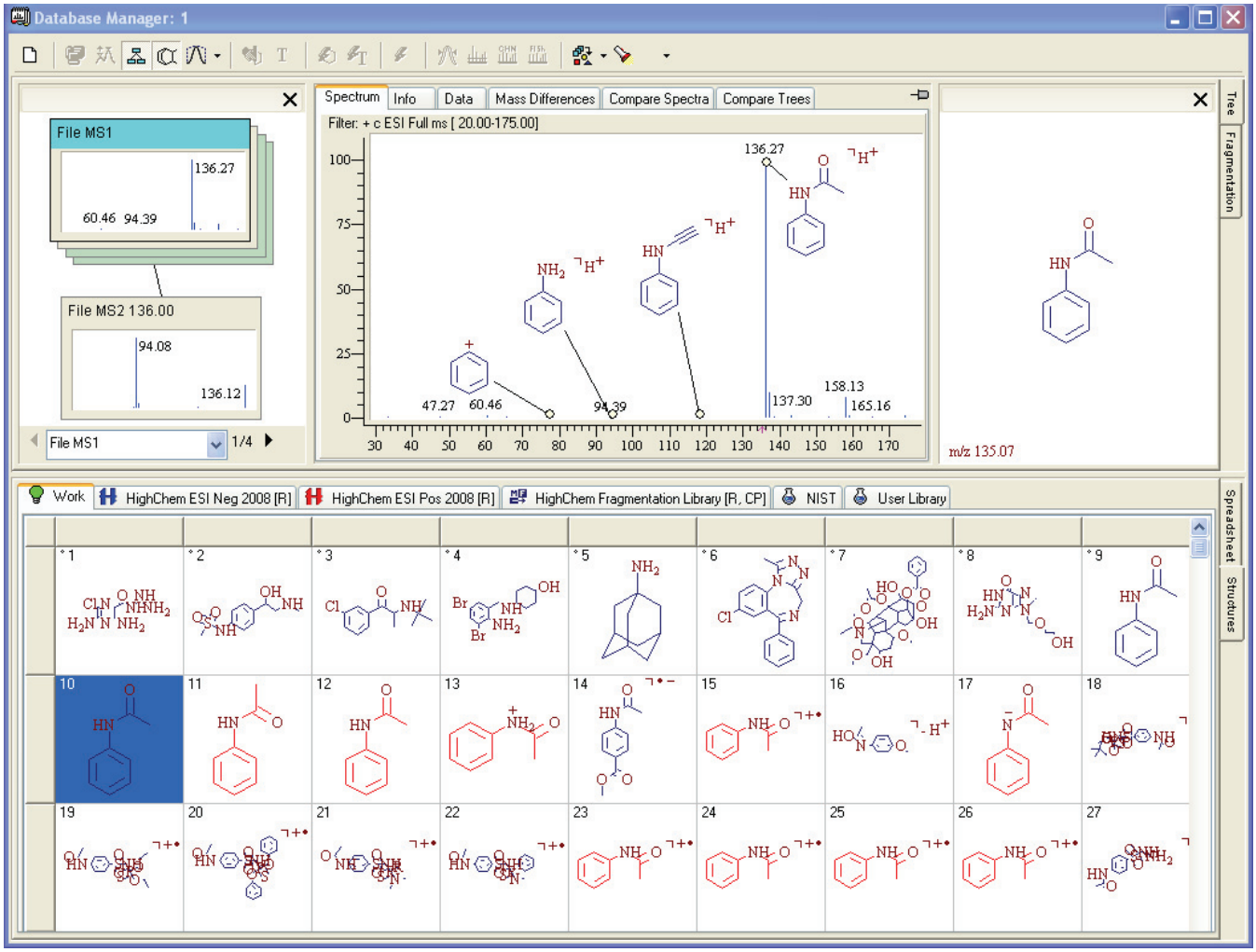


Figure 36. Data page in the (Sub)Structure Search dialog box

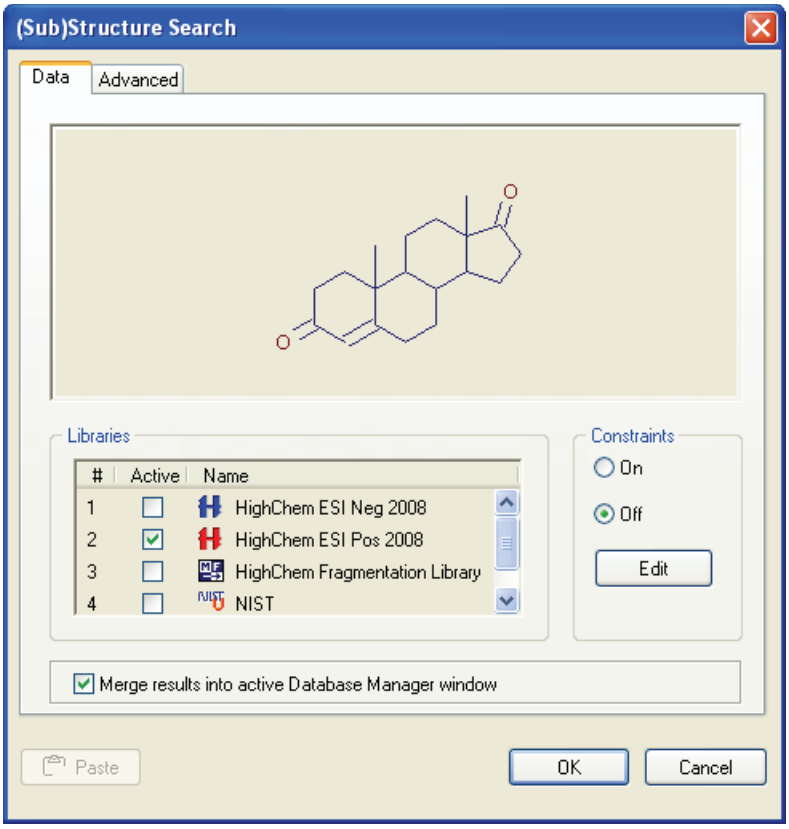


Table 7. Data parameters

Parameter	Description
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.
Paste SubStructure	Available when you have a copied substructure on the Clipboard. Lets you search for a substructure other than the currently selected substructure in the Database Manager window.

Figure 37. Advanced page in the (Sub)Structure Search dialog box

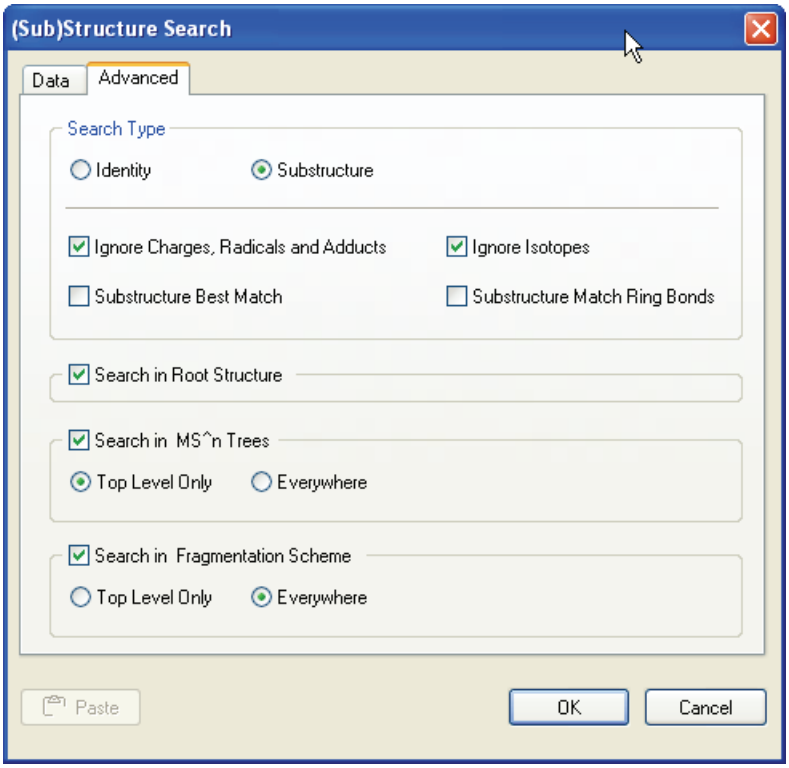


Table 8. Advanced parameters (Sheet 1 of 2)

Parameter	Description
Search Type	
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*.
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. When the Substructure Best Match option representing the closest match is found, it appears in red on the Structure page. Using this option lengthens calculation times.

Table 8. Advanced parameters (Sheet 2 of 2)

Parameter	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 calculation times.
Search in Root Structure	Searches for the specified molecular mass in the root structure.
Search in MS^n Trees	
Top Level Only	Searches only the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.
Search in Fragmentation Scheme	
Top Level Only	Searches only the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.
Paste SubStructure	Available when you have a copied substructure on the Clipboard. Lets you search for a substructure other than the currently selected substructure in the Database Manager window.

(Sub)Structure Search Rules

When searching structures with an unspecified charge site or substituents (see "Editing Atom Properties" on page 141), the following search rules apply.

Table 9. SubStructure search rules (Sheet 1 of 2)

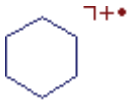
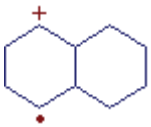
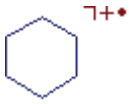
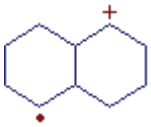
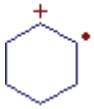
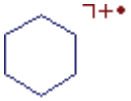
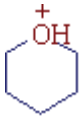
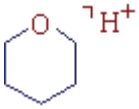
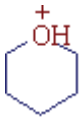
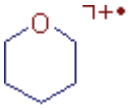
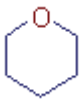
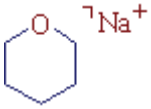
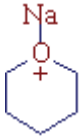
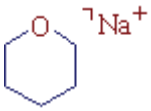
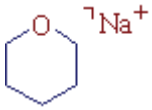
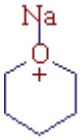
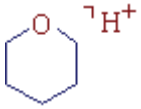
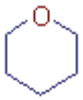
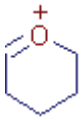
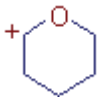
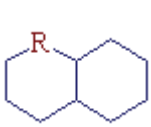
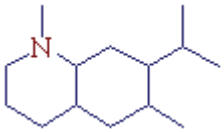
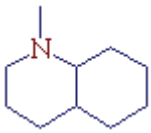
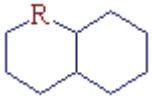
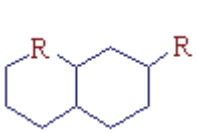
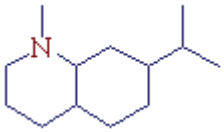
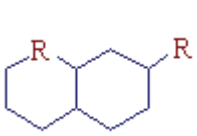
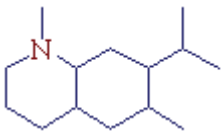
Search type	Query	Library	Search result
S			True
S			False
I			True
I			True
I			False
S			True
S			True

Table 9. SubStructure search rules (Sheet 2 of 2)

Search type	Query	Library	Search result
I			True
S			False
I			True
S			True
S			False
I			True
I			False

S = Substructure search
I = Identity search

Name Search

The name search option provides incremental search capabilities, or a type-ahead feature. As you start typing a portion of a name, the application displays a list of compounds that begin with the letters typed. The chemical structure is displayed for the highlighted name. You can use the up and down arrows to browse the displayed names.

The name search covers all three name types—IUPAC, Synonyms\References, and Commercial Product—as defined on the Info page.

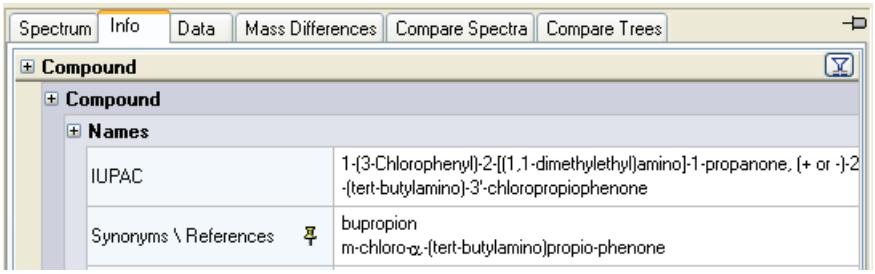


Figure 38. Name Search dialog box

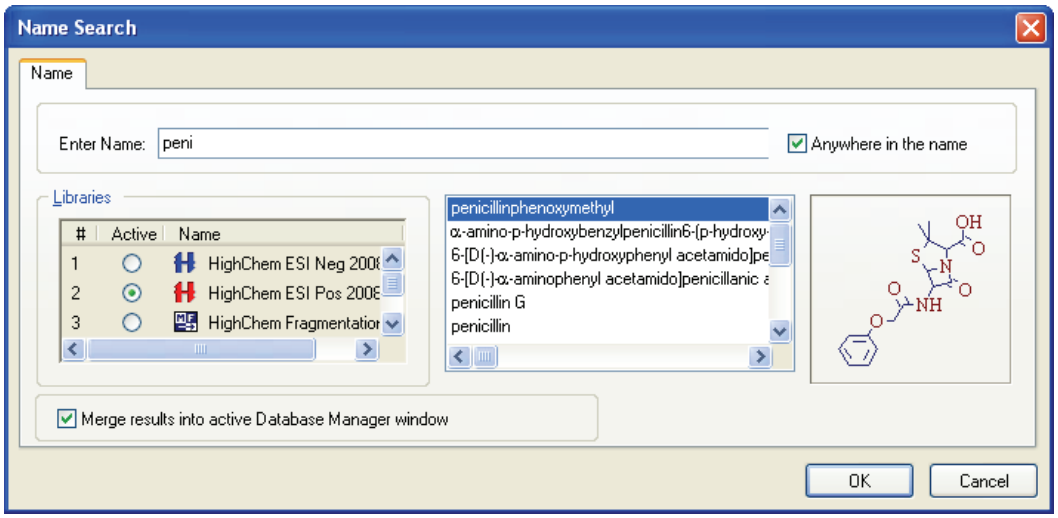


Table 10. Name Search parameters

Parameter	Description
Enter Name	Text that the search process matches in the selected libraries.
Anywhere in the Name	Matches the entered text anywhere it appears in the name.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

Formula Search

The formula search option searches for all compounds with a specific molecular formula. You can use lowercase letters to enter the formula, unless it leads to an ambiguous query (for example, “si” could be interpreted as Si or SI). Use the correct case to avoid misinterpretation.

The Formula Search dialog box includes the following pages:

- Formula page in the Formula Search dialog box
- Advanced page in the Formula Search dialog box

Figure 39. Formula page in the Formula Search dialog box

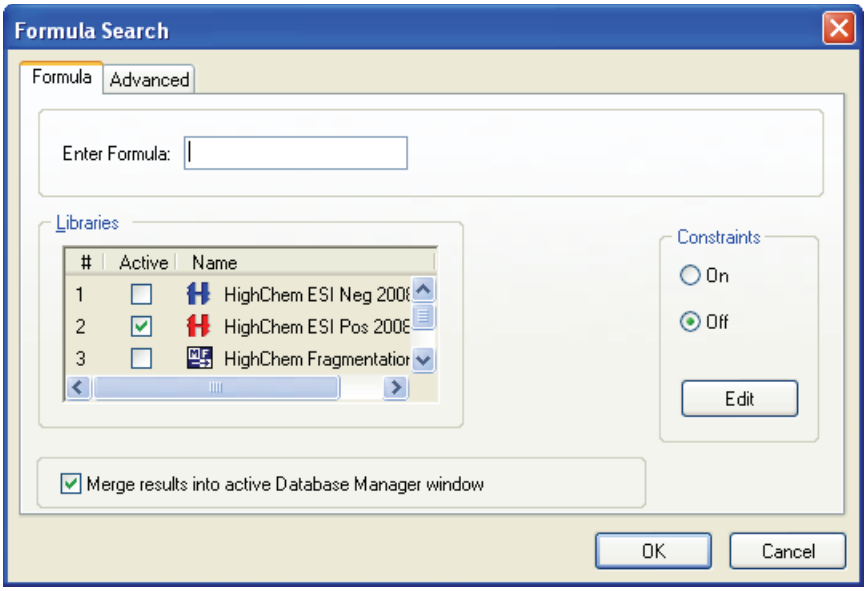


Table 11. Formula parameters

Parameter	Description
Enter Formula	Specifies the formula to search for.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See “Search Constraints” on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

Figure 40. Advanced page in the Formula Search dialog box

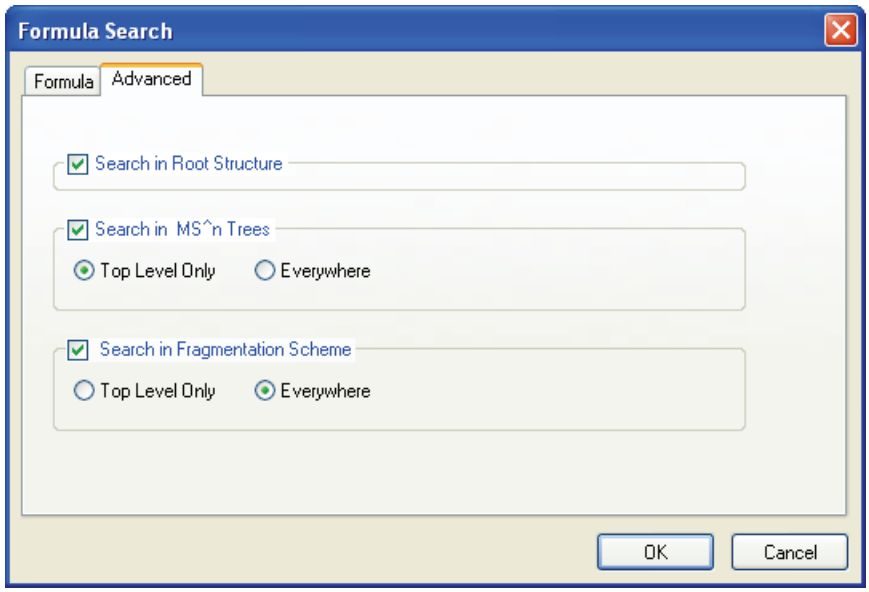


Table 12. Advanced parameters

Parameter	Description
Search in Root Structure	Searches for the specified molecular mass in the root structure.
Search in MS^n Trees	
Top Level Only	Searches only the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.
Search in Fragmentation Scheme	
Top Level Only	Searches only the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.

Molecular Mass Search

The molecular mass search option retrieves all compounds matching the queried molecular mass with a mass tolerance of 0.5 mass units. The results are ranked according to their closeness to the queried molecular mass.

The Molecular Mass Search dialog box includes the following pages:

- Molecular Mass page in the Molecular Mass Search dialog box
- Advanced page in the Molecular Mass Search dialog box

Figure 41. Molecular Mass page in the Molecular Mass Search dialog box

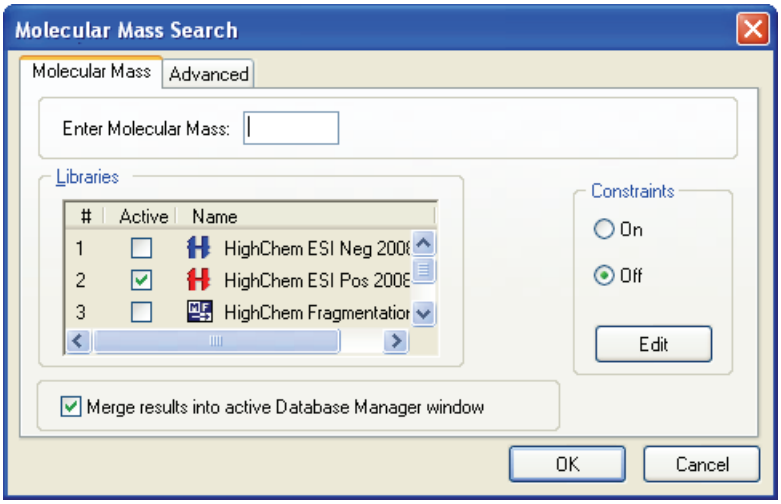


Table 13. Molecular Mass parameters

Parameter	Description
Enter Molecular Mass	Specifies the molecular mass of the compounds. The search results include all compounds that match the specified whole number with a tolerance of 0.5 mass units.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

Figure 42. Advanced page in the Molecular Mass Search dialog box

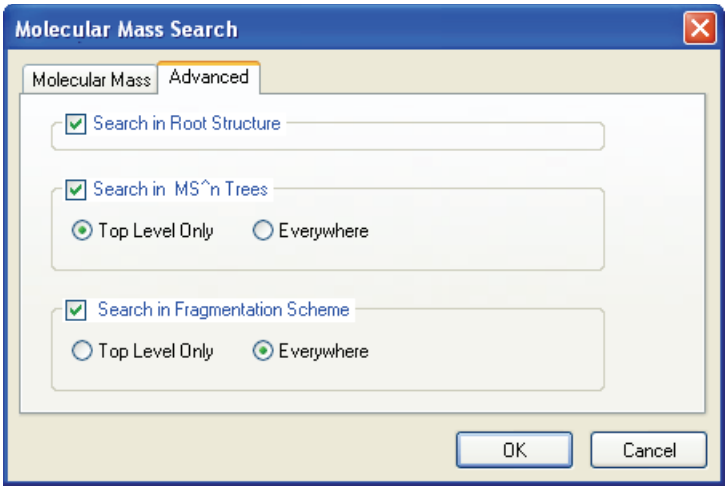


Table 14. Advanced parameters

Parameter	Description
Search in Root Structure	Searches for the specified molecular mass in the root structure.
Search in MS^n Trees	
Top Level Only	Searches only the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.
Search in Fragmentation Scheme	
Top Level Only	Searches only the top-level tree (full scan, source CID).
Everywhere	Searches the entire tree.

ID Number Search

Each library entry is individually numbered. The ID number option searches for a single ID number or for a range of ID numbers. The ID search dialog box contains two edit boxes to input the ID range: **From** and **To** boxes. To retrieve a single ID number, leave the To box blank. The Max. ID box displays the maximum ID number that can be found.

Note When you delete a record from a library, the Spreadsheet view decrements the ID numbers of records with a higher ID than the deleted record. This means that deleting one or more records leaves no ID number gaps in the library.

Figure 43. ID Number Search dialog box

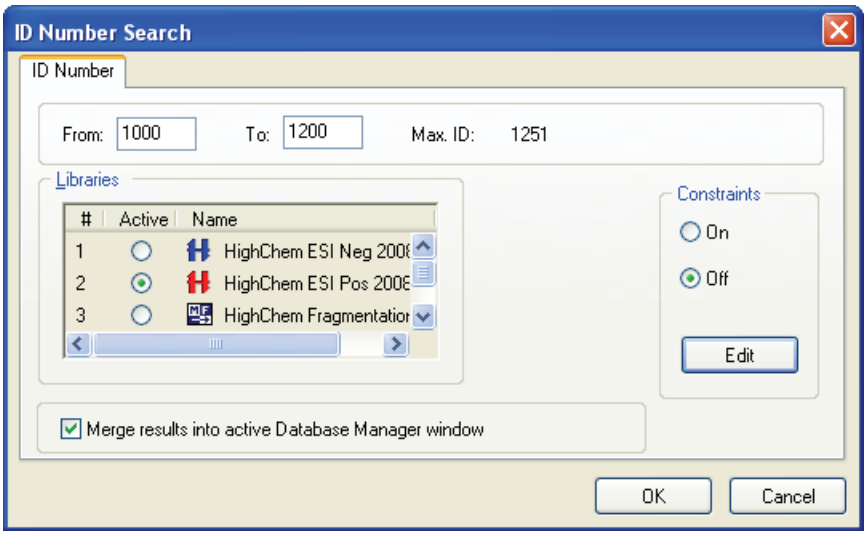


Table 15. ID Number parameters

Parameter	Description
From/To	Specifies a range of record numbers from the first column of the library Spreadsheet view.
Max ID	Specifies the highest record number in the Spreadsheet view.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

CAS Number Search

The Chemical Abstract Service (CAS) registry number search option is available for all libraries. Do not enter dashes when entering CAS numbers; the dashes automatically appear.

Figure 44. CAS Number Search dialog box

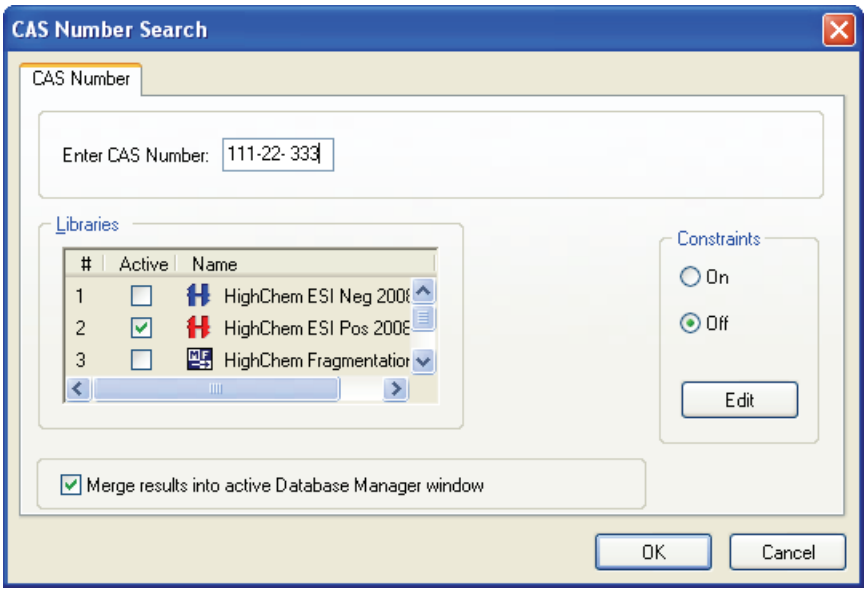


Table 16. CAS Number parameters

Parameter	Description
Enter CAS Number	Specifies the 8-digit CAS number.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

Fragmentation Data Search

The fragmentation data search option searches for a compound on the Info page. See "Using the Info Page" on page 38.

The Fragmentation Data Search dialog box includes the following pages:

- Data page in the Fragmentation Data Search dialog box
- Advanced page in the Fragmentation Data Search dialog box

Figure 45. Data page in the Fragmentation Data Search dialog box

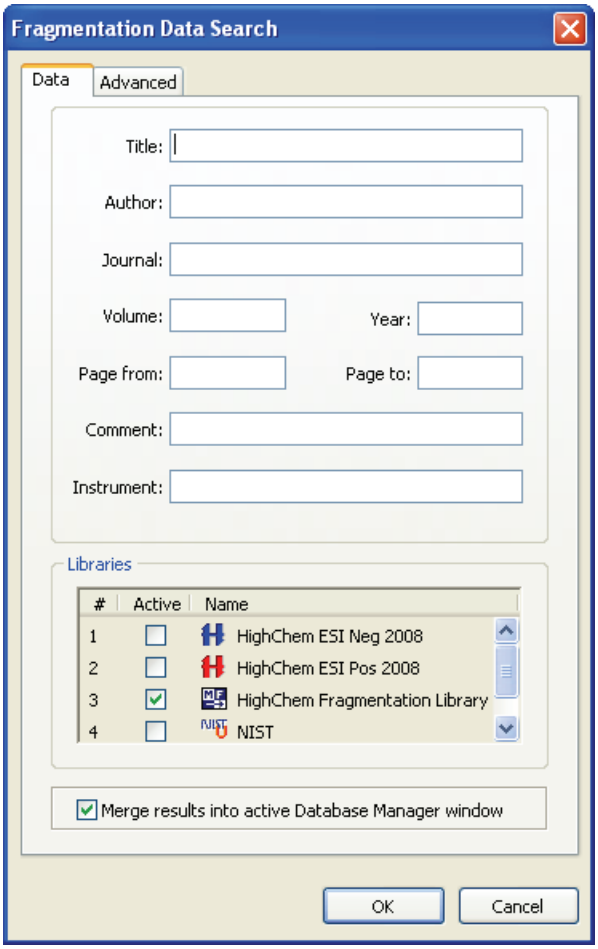


Table 17. Data parameters (Sheet 1 of 2)

Parameter	Description
Title	Specifies the keywords to search for.
Author	Name of any of the authors as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.

Table 17. Data parameters (Sheet 2 of 2)

Parameter	Description
Journal	Name of the journal as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Volume	Volume number as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Year	Year of the publication as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Page from / Page to	Page range as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Comment	Comment as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Instrument	Name of the instrument as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

Figure 46. Advanced page in the Fragmentation Data Search dialog box

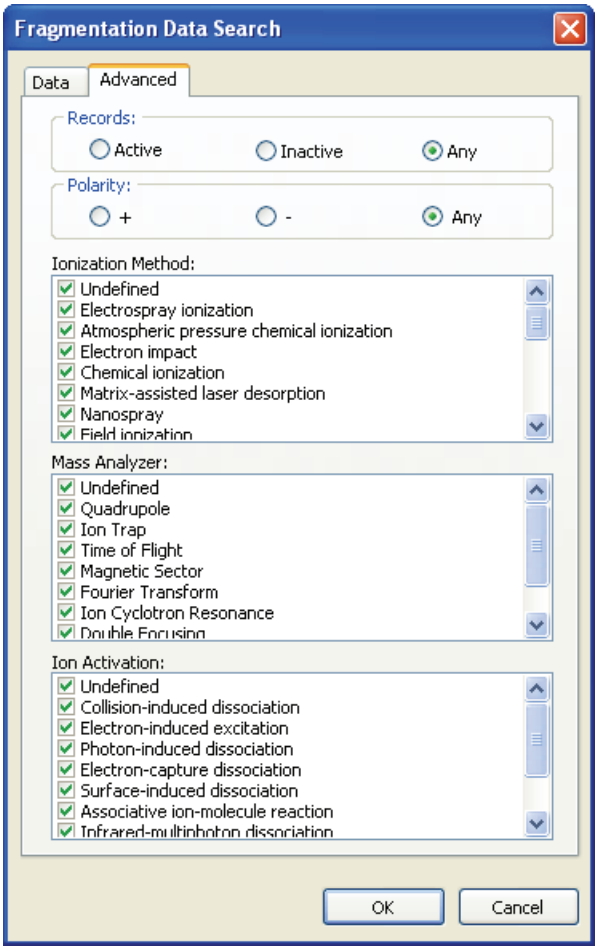


Table 18. Advanced parameters

Parameter	Description
Records	
Active/Inactive/Any	Active status of records as specified in the Active column of the Spreadsheet view for the library.
Polarity	
+/–/Any	Polarity as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Ionization Method	Method of ionization as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Mass Analyzer	Mass analyzer as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.
Ion Activation	Method of ion activation as specified in the Fragmentation Scheme data on the Info page. See "Using the Info Page" on page 38.

Retention Time Search

Each spectrum of a tree or stand-alone spectrum of a record can include a retention time value (Spectrum Info/Scan Info/RT item on the Info page). Using the retention time search, you can retrieve spectra whose retention time values fit into a range of retention values.

Figure 47. Retention Time Search dialog box

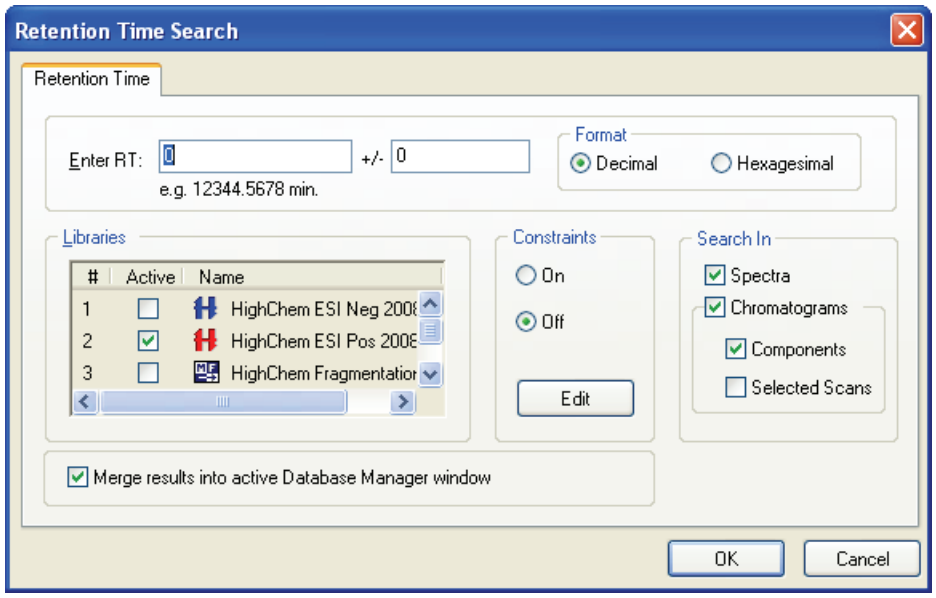


Table 19. Retention Time parameters (Sheet 1 of 2)

Parameter	Description
Enter RT / +/–	Retention time range to search.
Format	
Decimal	Specifies the retention time in base 10.
Hexagesimal	Specifies the retention time in base 60.
Libraries	Specifies the libraries to search. You can search both read-only and user libraries.
Constraints	The **On** option applies the search constraints defined in the Search Constraints dialog box. See "Search Constraints" on page 103. The **Off** option ignores the search constraints defined in the Search Constraints dialog box.

Table 19. Retention Time parameters (Sheet 2 of 2)

Parameter	Description
Search In	
Spectra	Searches spectra.
Chromatograms	Searches chromatograms: components, selected scans, or both.
Merge Results…	• When selected, the application displays the search results in the current Database Manager window and adds the search results to the Work spreadsheet. • When cleared, the application displays the search results in a new Database Manager window.

Search Constraints

You can restrict all search types, except the name search, by a set of constraints. The dialog boxes for each of these searches contain an option for activating constraints and a button for editing search constraints. When you click Edit, the Search Constraints dialog box opens for setting specific criteria to search selected libraries for matches. See "Search Constraints dialog box with default settings" on page 104.

Four constraint types are available: Molecular Mass range, Number of Atoms range, Allowed Elements, and Good-Bad List. Searches conducted with activated constraints can be time consuming because each library entry is examined to find those that match the criteria you have set. The search constraints are especially useful when you have large libraries and want to retrieve search results that are of interest for your specific problem.

Use the Good List (required substructures) when you want to focus your search results on the particular compound classes you are most interested in. The Bad List (forbidden substructures) eliminates from a result list all structures containing unwanted functional groups. For example, you can use the Good-Bad List in a search of acids with a specific molecular formula, or you can search for spectra similar to an unknown, with the requirement that ketones may not appear in the result list.

The following example shows a Search Constraints dialog box with C, N, and O as allowed elements. The Good-Bad list is set for esters as a required (good) functional group and for naphthalene as a forbidden (bad) functional group.

Figure 48. Search Constraints example

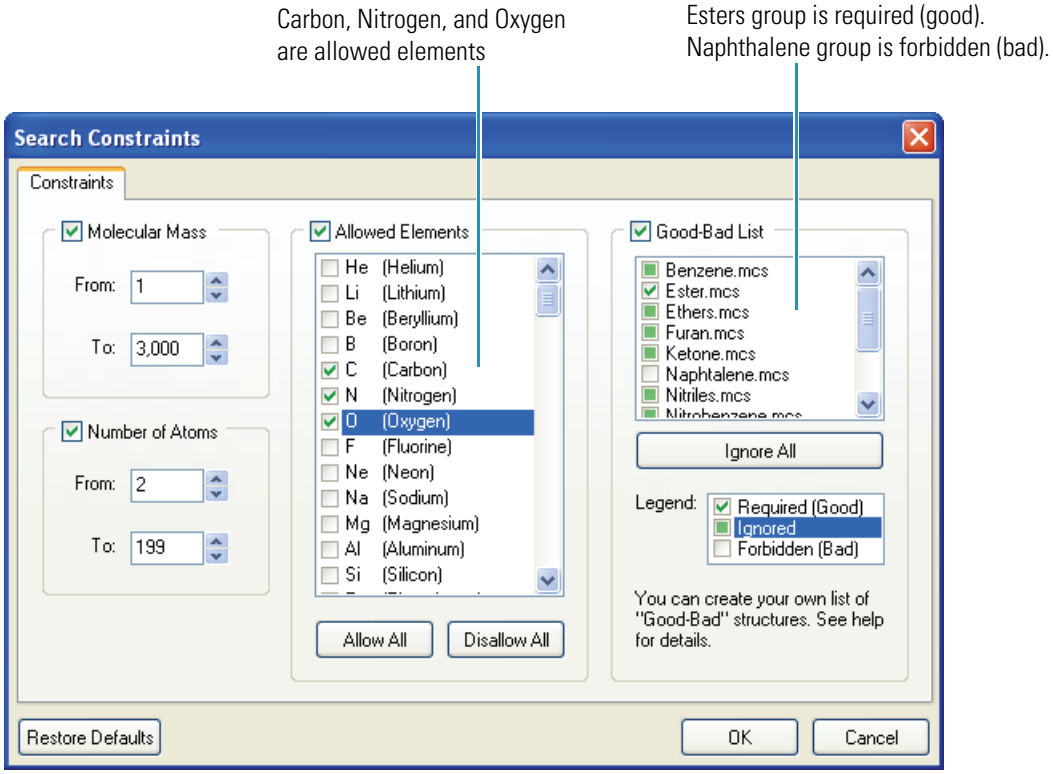


Figure 49. Search Constraints dialog box with default settings

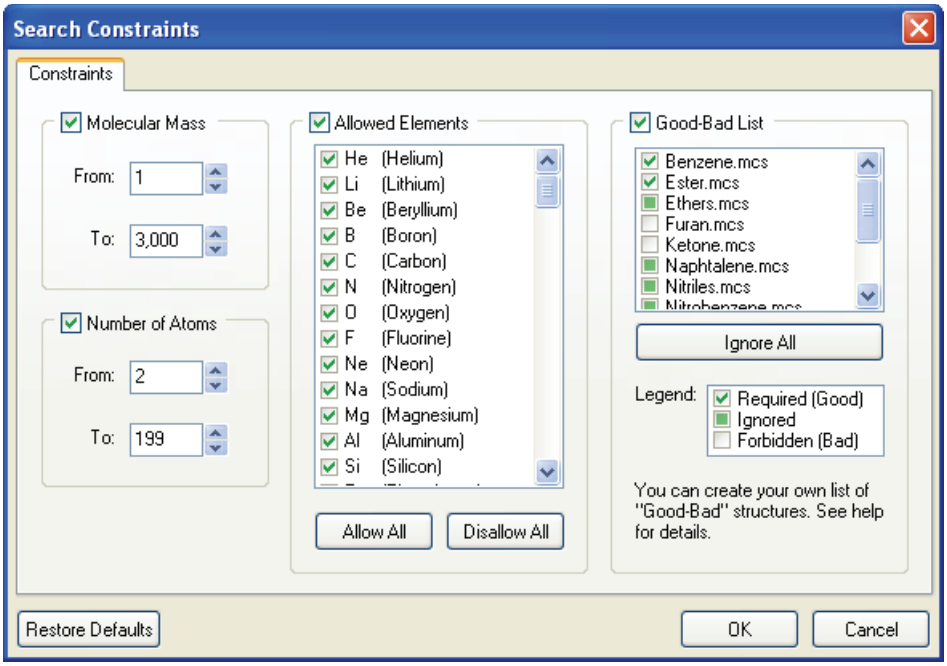


Table 20. Search Constraints parameters

Parameter	Description
Molecular Mass From/To	Searches for molecular masses in the specified range.
Number of Atoms From/To	Searches for compounds with the number of atoms in the specified range.
Allowed Elements	Specifies the elements you want to include in your search.
Allow All	Selects the check boxes for all elements.
Disallow All	Clears the check boxes for all elements.
Good-Bad List	Specifies the required (good) and forbidden (bad) substructures to used to refine your search. Each check box can be in one of three states: Required, Ignored, or Forbidden.
Ignore All	Clears all substructures in the Good-Bad list.
Legend	Displays the check box symbols used to indicate Required, Ignored, or Forbidden substructures in the Good-Bad list.
Restore Defaults	Resets the default values for all areas on the page.

Spectral Tree Module

The Mass Frontier application uses spectral tree representation for MS^n spectra. The tree structure best reflects the hierarchical spectra dependencies in tandem experiments. The graphical user interface provides for the management and processing of spectral trees. You can reconstruct spectral trees from data files, automatically extract them from data-dependent chromatographic components, or manually create them. For additional information, see "Using Components Detection and Spectra Deconvolution" on page 299.

All the spectral modules support trees except the Spectra Classifier, which uses total composite spectra generated from trees. The Mass Frontier application uses a newly developed algorithm for comparing spectral trees, which is integrated in the database search procedures.

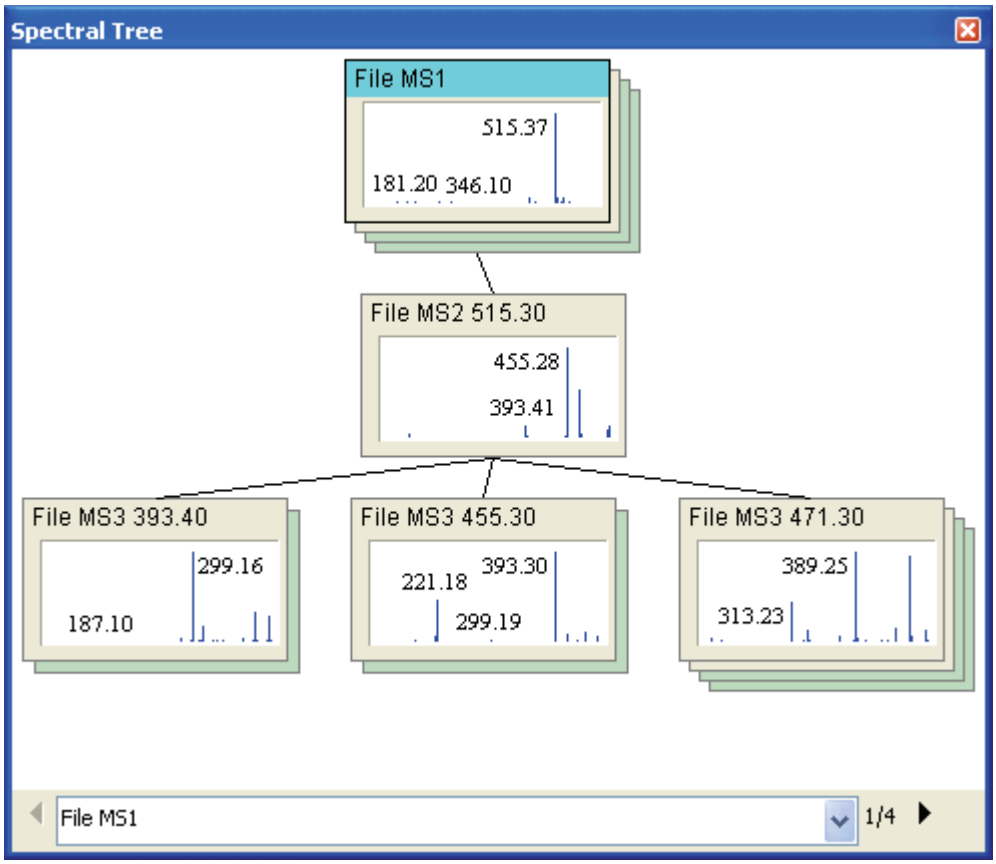
Contents

With spectral tree components, you can do any of these tasks:

- Store and search trees in libraries.
- Annotate every node spectrum.
- Create chromatographic libraries.
- Exchange trees between modules using copy-and-paste commands.
- Export and import spectral trees from or to an Excel spreadsheet in text format.

Note Most management and processing actions distinguish between a single spectrum and a tree. When handling data that contains trees, be very clear whether you want to apply a particular action to an entire tree or a selected single spectrum. The displayed information is also associated with the tree, the spectrum, or both depending on the information type. For additional information, see "Using Records in the Database Manager Window" on page 37.

Spectral Tree Window



Spectral Tree Arrangement

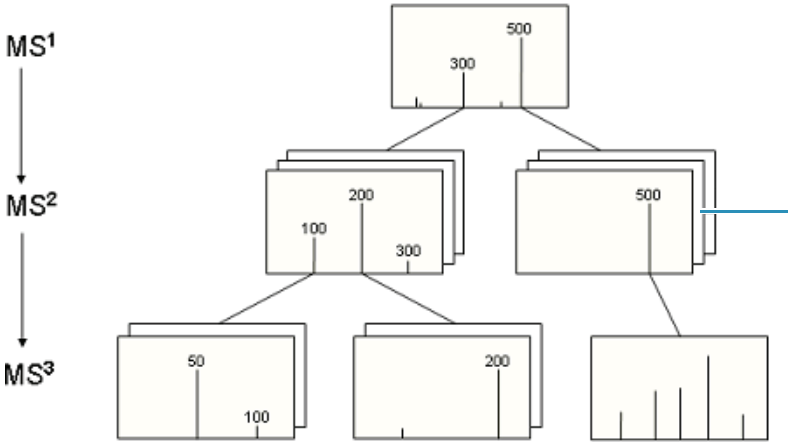
The Mass Frontier application uses spectral tree representation for MS^n spectra. A spectral tree consists of the following components.

Table 21. Spectral Tree components

Components	Description
Levels	Symbolize MS^n stages starting at n=1.
Nodes	Holders of node items.
Node connectors	Graphical symbols of precursor *m/z* values, or precursor *m/z* ranges if the isolation width is included.
Node items	Stand for the product or calculated spectra of identical precursor *m/z* values or *m/z* ranges or for chromatograms.

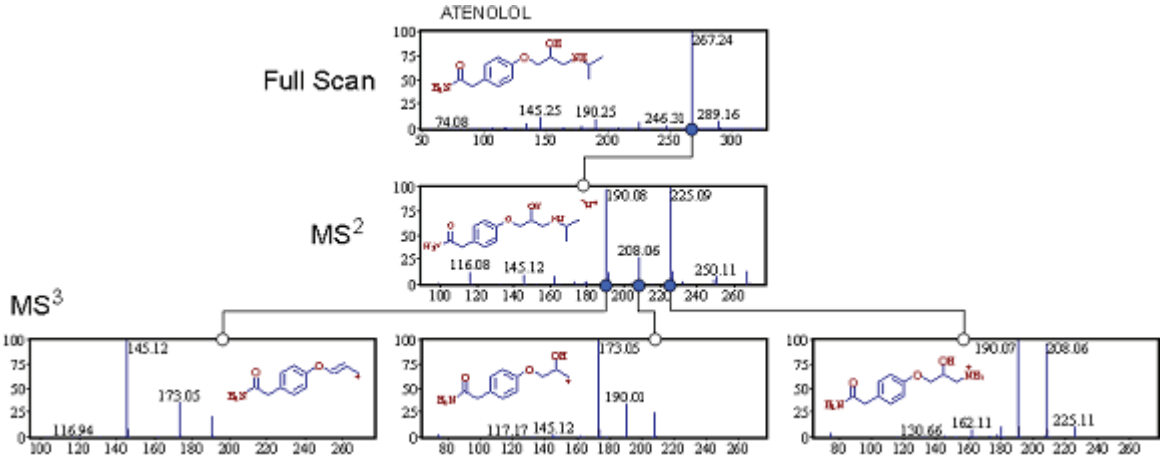
Node product spectra, called parallel spectra, represent spectra acquired at various collision energies and isolation widths or that use wideband activation. They can also be zoom spectra, source CID spectra, or any other spectra that enhance reproducibility in compound identification. If a node contains more than two parallel spectra, the average and composite spectra are automatically calculated. In addition to spectra, each node can contain a chromatogram (see "Spectral Tree Chromatograms" on page 123).

Figure 50. Parallel spectra



Note Chemical structures are associated with nodes and not with entire trees. This means you can assign fragments for product spectra. The top-level node MS^1 should hold the structure of the neutral compound.

Figure 51. Nodes



You can store complete trees in a library and update them at any time. For additional information, see Chapter 5, "Library Utilities." Any complementary information associated with a single stage spectrum or a chromatogram can be associated with a node spectrum or node chromatogram (see "Using the Info Page" on page 38).

Spectral Tree Layout

A spectral tree consists of levels, nodes, node connectors, and node items. Node items are divided into five groups that are differentiated by the displayed color: Single, Average, Composite, Source CID spectrum, and Chromatogram.

Because display and editing actions affect the selected node item, its color helps distinguish item selection. For additional information, see "Manually Create and Edit a Spectral Tree" on page 114.

❖ To change color settings

1. Choose **Options > Settings** from the Mass Frontier main menu.

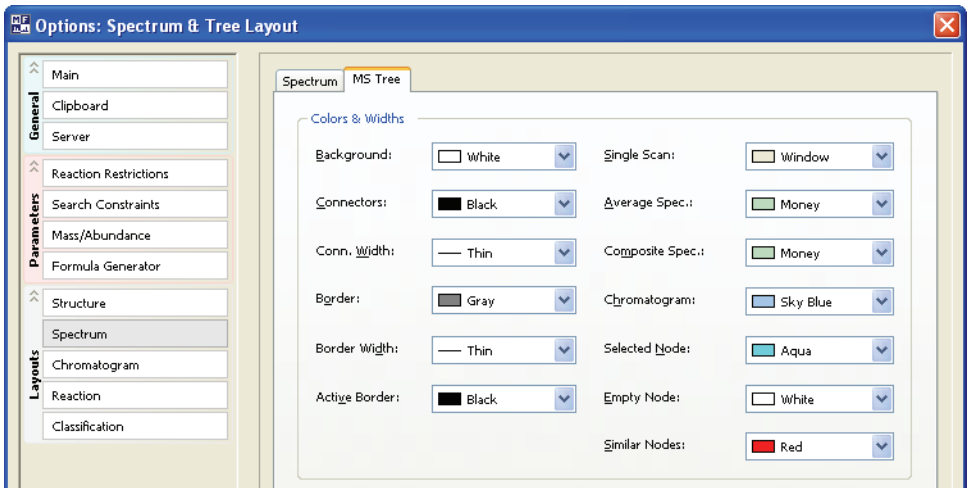
   The Options dialog box opens.

2. Click **Spectrum** in the Layouts section.

   The Spectrum page of the Options: Spectrum & Tree Layout dialog box opens.

3. Click the **MS Tree** tab.

   The MS Tree page opens.



4. Make your color selections and click **OK**.

Generating a Spectral Tree

You can use any of these methods to create a spectral tree:

- Reconstruct a Tree from Multiple Raw Data Files
- Reconstruct a Tree from a Single Raw Data File
- Generate Trees from Chromatographic Components
- Manually Create and Edit a Spectral Tree

The tree reconstruction automatically assigns node spectra to specific node utilities in addition to creating levels, nodes, and node connections.

Reconstruct a Tree from Multiple Raw Data Files

The spectral tree reconstruction feature reads Xcalibur raw data files and automatically creates a tree according to the precursor *m/z* and isolation width values. All files (spectra) in a directory should be from an identical chemical entity (compound or chromatographic component)—one directory per tree per compound. This feature works on Xcalibur raw data files acquired using direct injection and a single MS^n stage. Each file in a directory can be separately acquired at a specific collision energy and can be a wideband activation spectrum or a zoom or source CID spectrum. The sample preparation can also be diverse (pH, concentration, buffer, and so on). Example files are available in the following directory on a Windows XP system:

C:\Documents and Settings\All Users\Documents\ HighChem\
Mass Frontier 7.0\Chromatograms

Note The directory location on the Windows Vista operating system varies depending on the version.

❖ To import trees using tree reconstruction

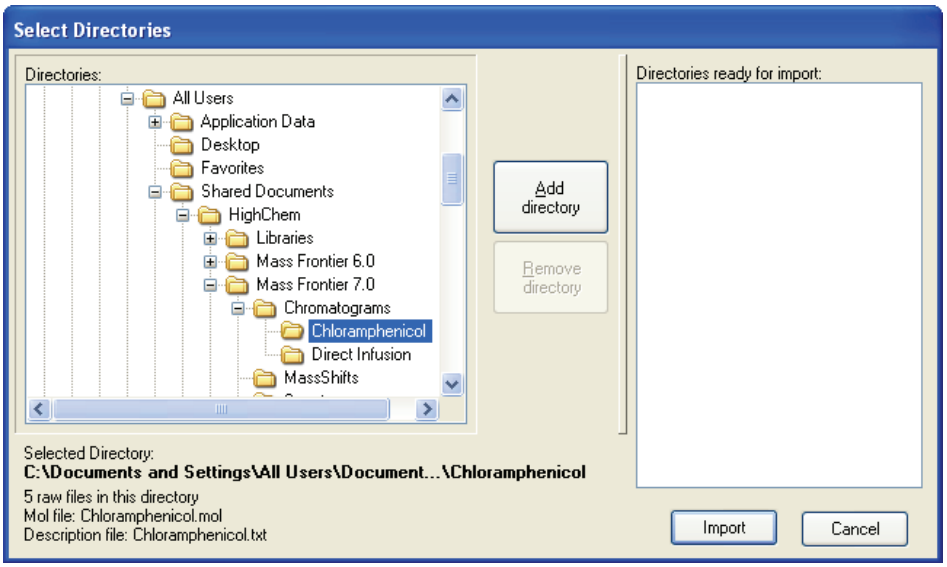
1. Do one of the following:

   Right-click the tree pane in Database Manager and choose **Import > MS Trees** from the shortcut menu.

   –or –

   Choose **File > Import > MS Trees** from the Mass Frontier main menu.

   The Select Directories dialog box opens.



2. In the Directories pane, select a directory to import.

   You can view the contents of the selected directory in the Directory Content pane.

3. Click **Add Directory**.

   The selected directory appears in the Directories Ready for Import pane.

4. Repeat steps 2 and 3 for as many directories as you want.

5. Click **Import**.

   The imported directories create MS^n libraries.

Reconstruct a Tree from a Single Raw Data File

You can create a spectral tree for a reference compound by reading various MS^n spectra acquired through direct infusion in one run and stored in a single raw data file.

❖ To create a spectral tree from a single run using tree reconstruction

1. Do one of the following:

   Choose **Tools > Chromatogram Processor** from the Mass Frontier main menu.

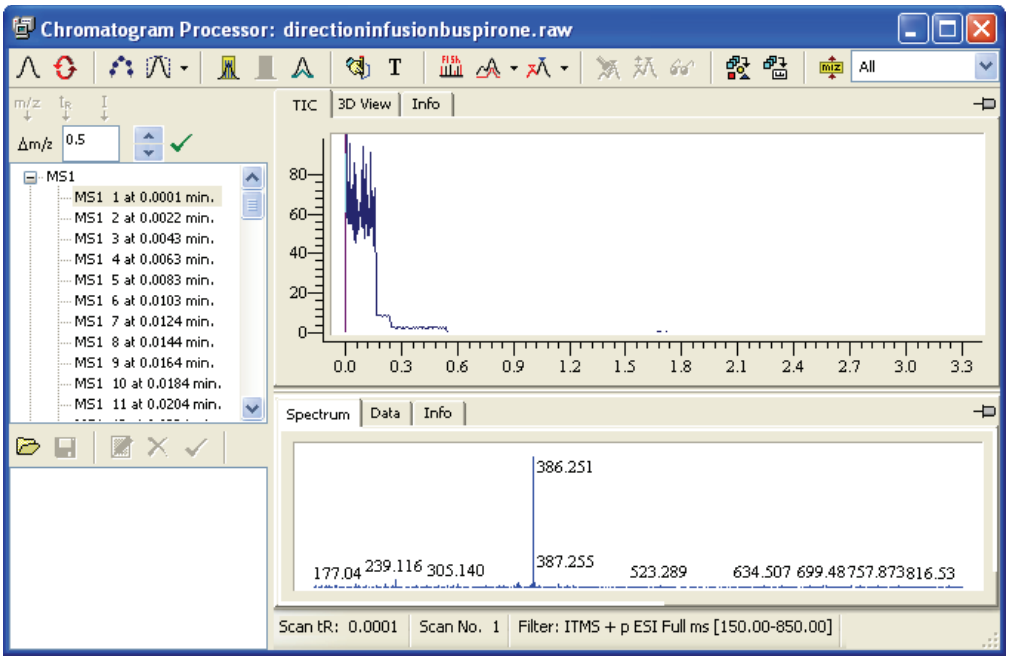
   –or–

   Click the **Chromatogram Processor** icon,  , in the Mass Frontier toolbar.

   The Open Chromatogram dialog box opens.

2. Select a direct infusion file and click **Open**.

   The file opens in the Chromatogram Processor window.



3. Do one of the following:

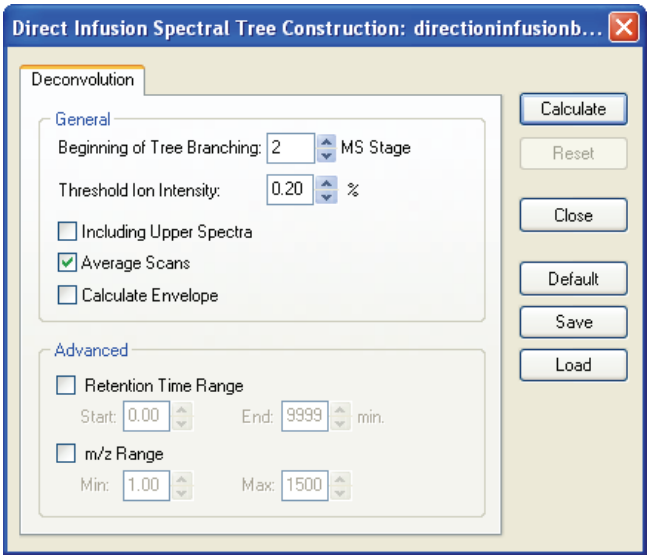
   Click the **Components Detection & Spectra Deconvolution** icon and choose **Direct Infusion** from the menu.

   –or–

   Right-click and choose **Component Detection > Direct Infusion** from the shortcut menu.

   The Direct Infusion Spectral Tree Construction dialog box opens. For additional information, see "Direct Infusion Algorithm" on page 315.

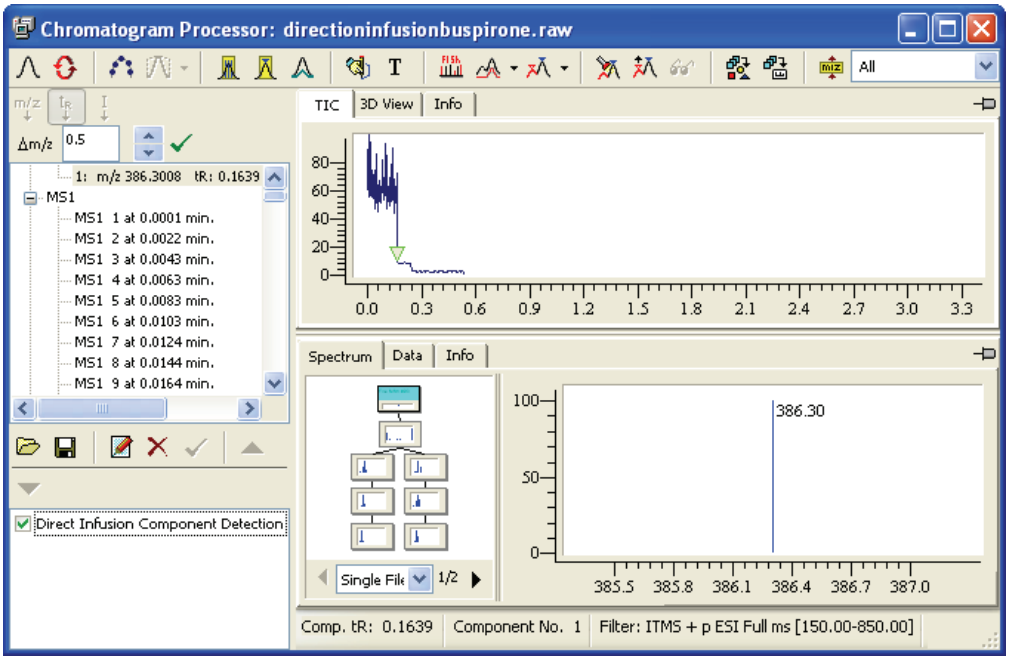
Figure 52. Direct Infusion Spectral Tree Construction dialog box



4. Specify your parameters and click **Calculate**.
5. When the process finishes, click **Close**.
6. In the Chromatogram Processor window, click a component triangle in the TIC pane.

   The reconstructed spectral tree is displayed in the lower pane.

Figure 53. Chromatogram Processor window



Generate Trees from Chromatographic Components

You can generate spectral trees from chromatographic components. To create a tree from a chromatogram, use the Component Detection & Spectra Deconvolution feature in the Chromatogram Processor module. For additional information, see "Using Components Detection and Spectra Deconvolution" on page 299.

Manually Create and Edit a Spectral Tree

You can manually create and edit spectral trees in the Spectral Tree pane in the Database Manager window. You can construct a tree by creating an empty tree framework, adding new nodes, and then successively pasting or importing spectra into the nodes.

Follow these procedures:

- To open a Spectral Tree window from the toolbar
- To add a new node
- To define the precursor m/z value
- To edit the node caption of a parallel spectrum or chromatogram

❖ To open a Spectral Tree window from the toolbar

Click the Spectral Tree icon,  , in the Database Manager toolbar.

❖ To add a new node

1. Right-click the parent node and choose **Add > Add Node** from the shortcut menu.

   This command is available only for editable libraries.

2. Fill an empty node with a spectrum from the Clipboard or import one from a file.

   a. Right-click the node and choose **Paste > Paste Parallel Spectrum**.

      This command is available only when the Clipboard contains a spectrum.

   b. To add another empty node product spectrum (parallel spectrum), right-click the node and choose **Add > Add Parallel Spectrum**.

      A node can contain only one empty parallel spectrum. If you add more than one parallel spectrum to the node, the application automatically generates the average and composite parallel spectra, which are differentiated by color.

If you import a raw data file into the node, the application calculates the average spectrum from all scans.

You cannot import a chromatogram raw data file into a spectrum node. To create a node with a chromatogram, paste a data-reduced chromatogram from the Chromatogram Processor module. For additional information, see "Spectral Tree Chromatograms" on page 123 and "Searching Chromatographic Libraries" on page 182.

❖ To define the precursor *m/z* value

Do one of the following:

Type the value in the edit box in the Structure pane.

–or–

a. Paste or draw the ionic product fragment into the Structure pane.

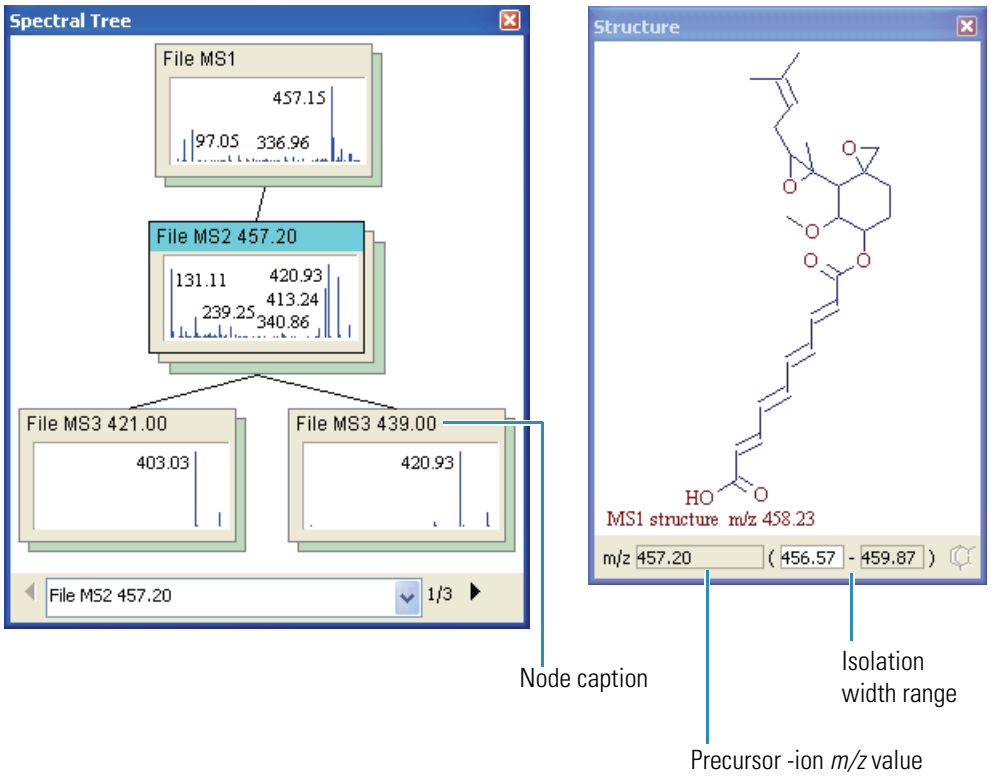
b. Click the **Restore Precursor *m/z*** icon, , in the lower right corner of the Structure pane.

For additional information, see "To paste a structure to a record" on page 54.

A tree must have precursor *m/z* values and the isolation width of the product nodes. The Mass Frontier application automatically determines the default value of the isolation width, but you can manually change it.

❖ To edit the node caption of a parallel spectrum or chromatogram

Double-click the caption label and type the new formatted text into the Annotation dialog box.



Copying and Pasting a Spectral Tree

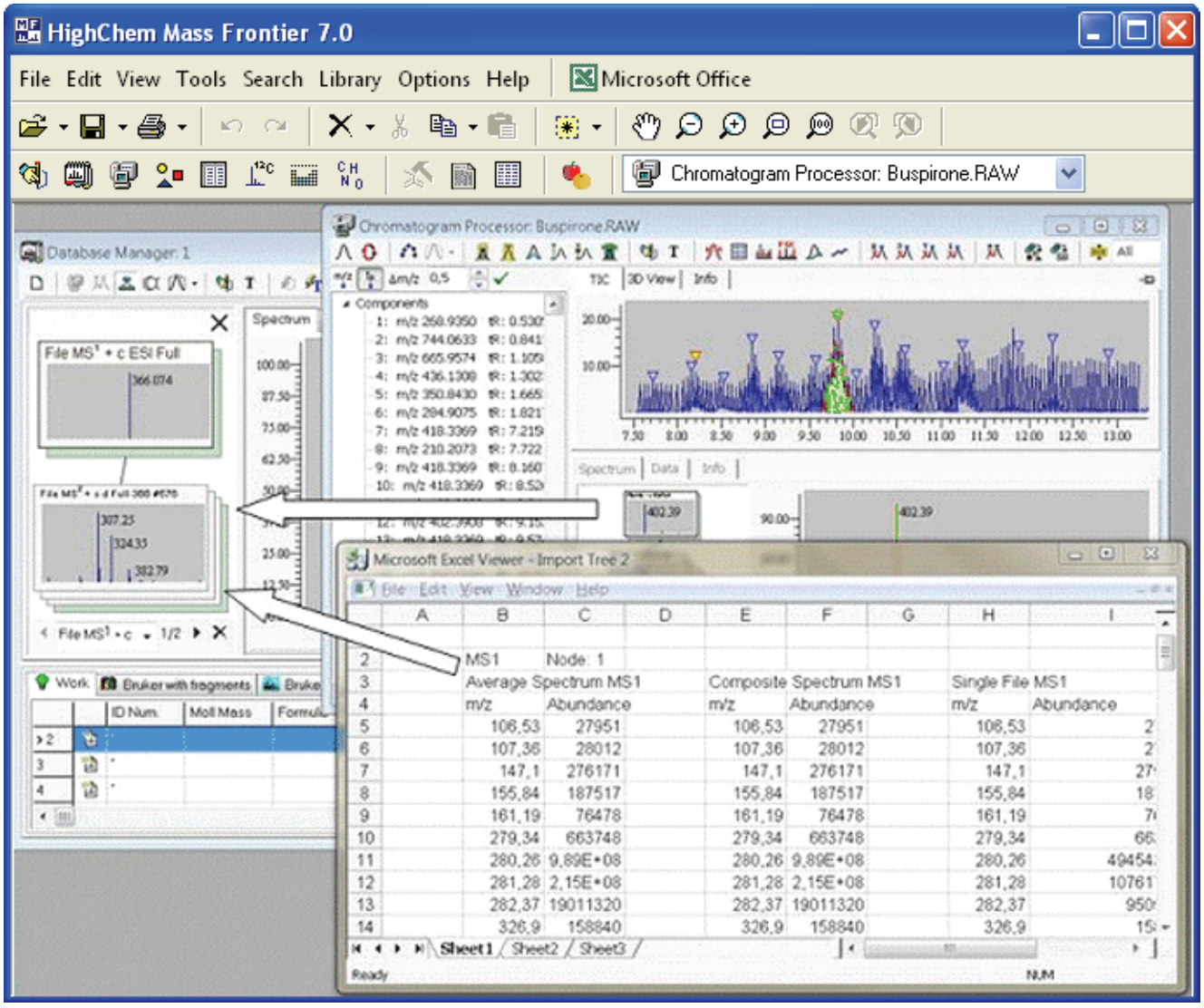
You can exchange trees among windows, records, chromatographic components, or applications (for example, Excel).

For additional information, see "Using Records in the Database Manager Window" on page 37 or "Processing Data-Reduced Chromatograms" on page 69.

Follow these procedures:

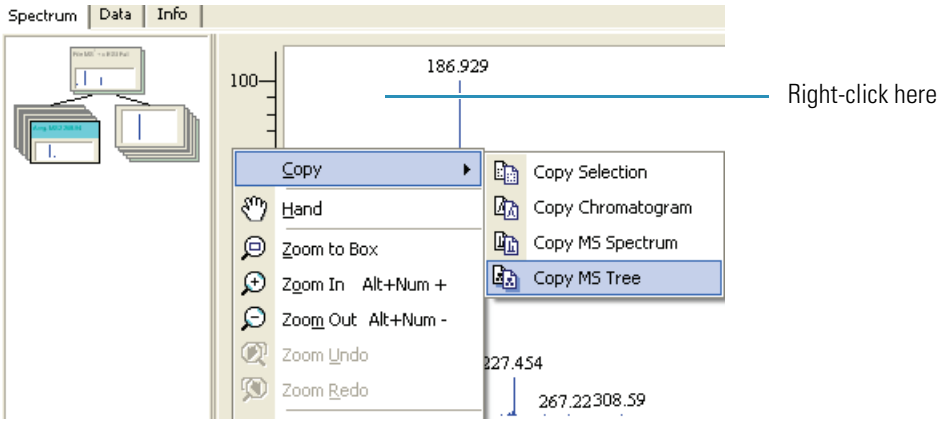
- To copy a spectral tree from a Chromatogram Processor to a Database Manager
- To copy a spectral tree from a Database Manager to an Excel spreadsheet
- To copy a spectral tree from a Chromatogram Processor to an Excel spreadsheet

Figure 54. Copying data in a Mass Frontier window



❖ To copy a spectral tree from a Chromatogram Processor to a Database Manager

1. Right-click the **Chromatogram Processor** spectrum pane and choose **Copy > Copy MS Tree** from the shortcut menu.



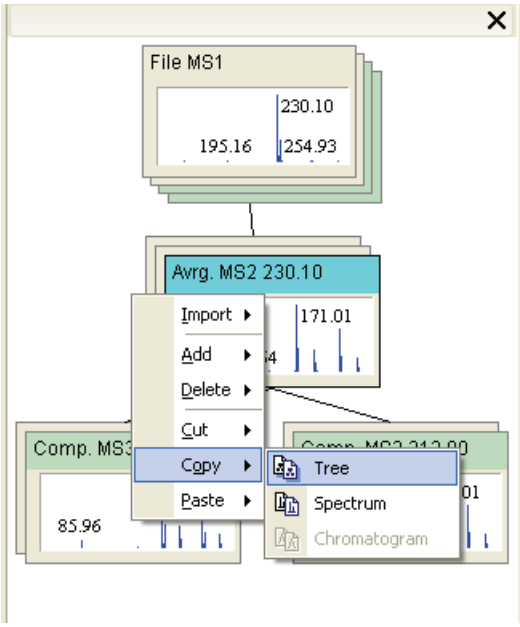
Note There is no shortcut menu in the tree pane itself; you must right-click the spectrum pane.

2. In the Database Manager tree pane, right-click and chose **Paste > Tree** from the shortcut menu.

   The application pastes your spectral tree and the associated spectrum in the Database Manager tree pane.

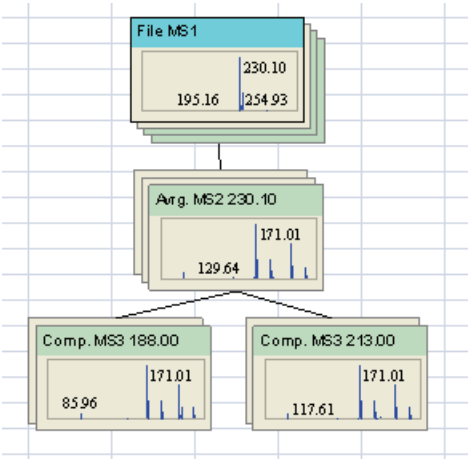
❖ To copy a spectral tree from a Database Manager to an Excel spreadsheet

1. Right-click the Database Manager tree pane and choose **Copy > Tree** from the shortcut menu.



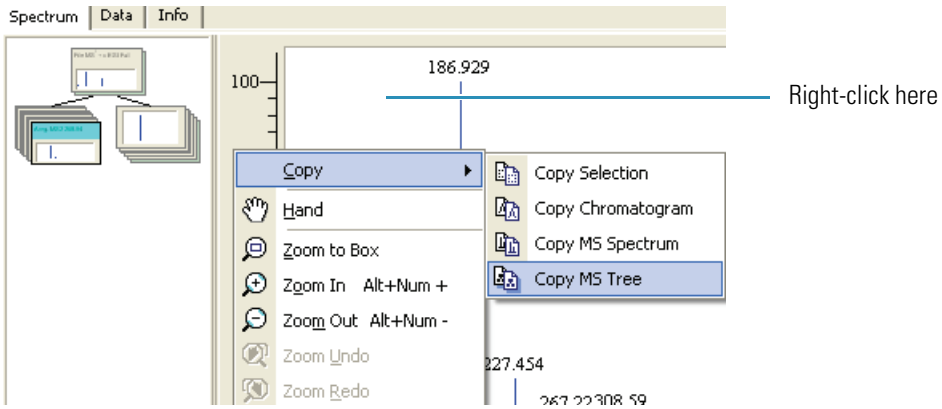
2. In the Excel spreadsheet, right-click and chose **Paste** from the shortcut menu.

   The application pastes your spectral tree graphic in the spreadsheet.



❖ To copy a spectral tree from a Chromatogram Processor to an Excel spreadsheet

1. Right-click the **Chromatogram Processor** spectrum pane and choose **Copy > Copy MS Tree** from the shortcut menu.



Note There is no shortcut menu in the tree pane itself; you must right-click the spectrum pane.

2. In the Excel spreadsheet, right-click and chose **Paste** from the shortcut menu.

   The application pastes your spectral tree graphic in the spreadsheet.

Spectral Tree Node Items

Some mass spectrometry 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 Mass Frontier application uses spectral trees that can contain nodes with several 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), or for a chromatogram.

Node product spectra represent spectra that were acquired at various collision energies and isolation widths or that use wideband activation. They can also be zoom spectra, source CID spectra, or any other spectra that enhance reproducibility in compound identification. If a node contains more than two parallel spectra, the application automatically calculates the average and composite spectra. In addition to spectra, each node can contain a chromatogram.

You might use node spectra for any of these reasons:

- They can significantly contribute to correct compound identification in a tree library search.
- They allow the study of fragmentation processes by changing the experimental conditions.
- They permit the efficient organization of product spectra.

The spectral node strategy strengthens the robustness of all the mathematical processing methods and, compared to simple spectra averaging, does not distort the highly nonlinear peak ratio progress.

You can easily access node spectra from a library and create or edit them using the graphical interface. Every tree item has an editable annotation caption.

You can select node items by clicking the edge of the spectral or chromatographic node item, or by browsing in the box displayed below the tree.

Searching Spectral Trees

You can use the Mass Frontier application for processing MS^n spectra in hierarchically consistent spectral trees that can be searched in spectral or chromatographic libraries. Select from various options on the Advanced page in the Spectra or Tree Search dialog box. Each node in spectral trees can consist of four types of spectra (average, composite, parallel, and source CID), and you can specify the type of spectra that is searched. For additional information, see "Processing Data-Reduced Chromatograms" on page 69 or "Spectral Tree Arrangement" on page 106.

❖ To perform a spectral tree search

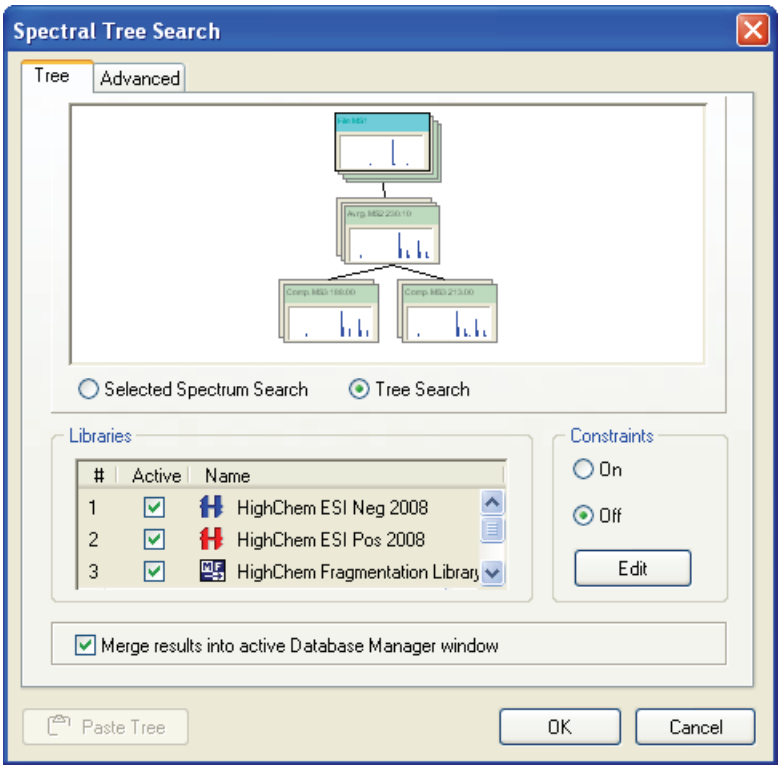
1. Do one of the following:

   Click the **Search** button,  , in the Database Manager window and choose **Tree** from the menu.

   –or–

   Choose **Search > Tree** from the Mass Frontier main menu.

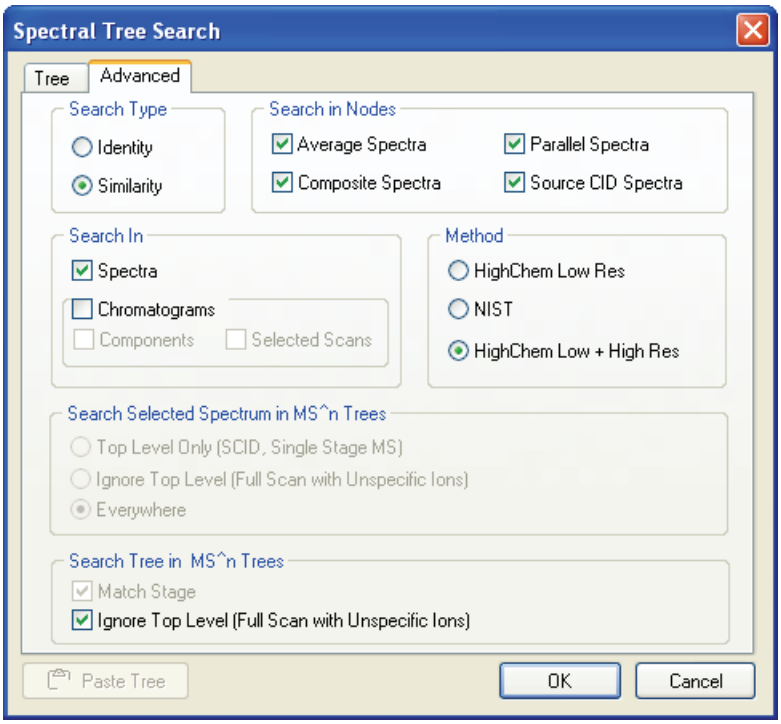
   The Tree page of the Spectral Tree Search dialog box opens.



2. Select either the **Selected Spectrum Search** option or the **Tree Search** option.
3. In the Libraries area, select the **Active** check box for the libraries you want to search.

4. Click the **Advanced** tab.

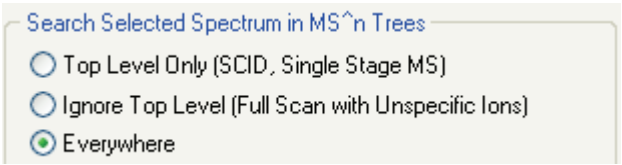
   The Advanced page of the Spectral Tree Search dialog box opens.



5. In the Search in Nodes area, select the types of nodes you want to search.

   If you have a library that consists exclusively of source CID spectra and your unknown spectrum is also a source CID, select only the **Source CID Spectra** check box. If no such library is available, you can search source CID spectra in product CID spectra, but use care regarding the search results. Source CID spectra might contain fragmentation products from all the ions present in the source, including adduct or cluster ions, while product CID spectra are preferably generated from protonated or deprotonated ions.

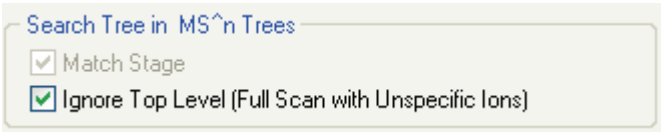
6. If you selected the **Selected Spectrum Search** option on the Tree page, select one of the following search options.



   The application compares the spectrum to every spectrum in the tree hierarchy and individually calculates the math factor.

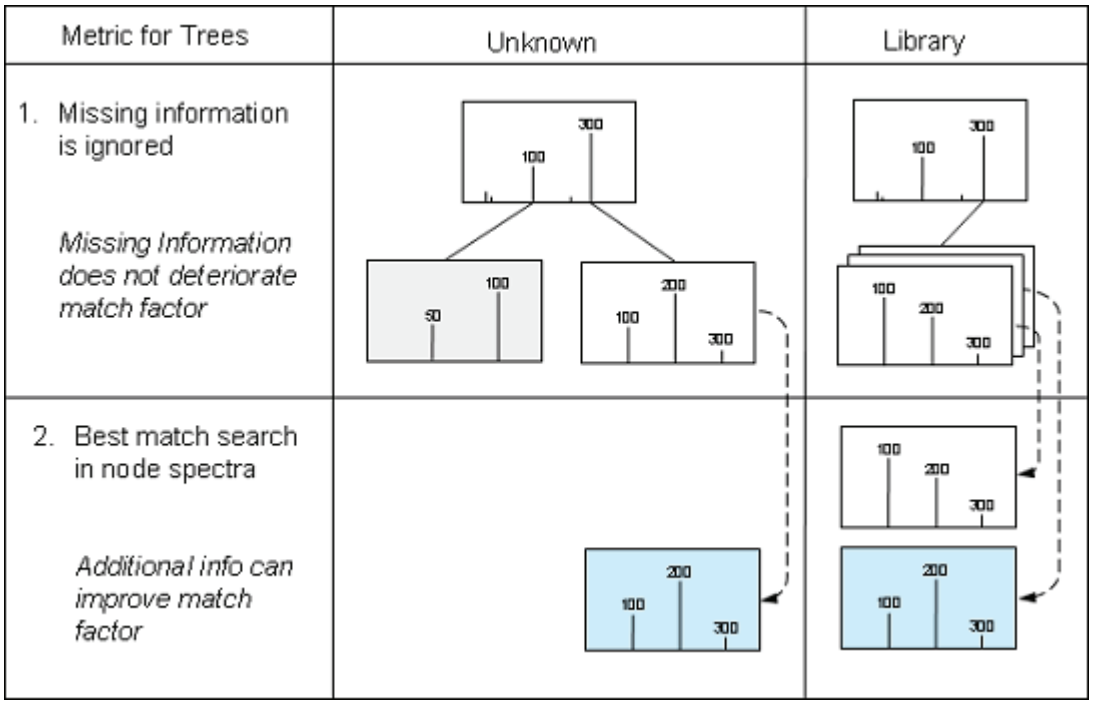
   - Top Level Only—Searches only the top-level tree (full scan, source CID).
   - Ignore Top Level—Excludes the top-level tree (full scan, source CID).
   - Everywhere—Searches the entire tree.

7. If you selected the **Tree Search** option on the Tree page, select one of the following search options.



   - Ignore Top Level—Excludes the top-level tree (full scan, source CID).
   - Match Stage—Uses an identity search with the MS^n stage of the tree spectra.

When you search a tree (unknown) against spectral trees in a library, the application compares the spectrum according to a special logic. It compares the corresponding spectra on an identical level (MS^n stage) with a common precursor *m/z* by using an algorithm based on the optimized dot-product. The application ignores a spectrum that appears on one side only, which does not adversely affect the search result. If there are several corresponding spectra (single node with average, composite, parallel, or other spectra), the Mass Frontier application accepts the best match (optimistic approach). The total match factor is calculated from all the non-zero match factors.



Spectral Tree Chromatograms

The Mass Frontier application supports spectral trees that can contain nodes with data-reduced chromatograms. You can assign chromatograms to every node, which lets you store product ion chromatograms for a particular precursor *m/z* value.

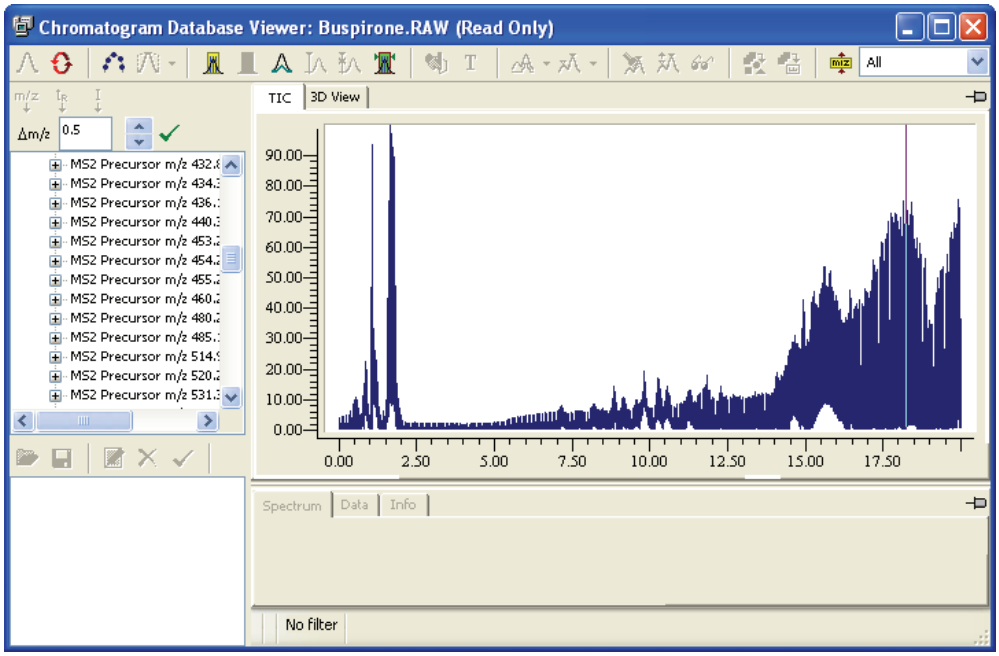
For additional information, see "Searching Chromatographic Libraries" on page 182, "Copying and Pasting a Spectral Tree" on page 116 or "Manually Create and Edit a Spectral Tree" on page 114.

In contrast to spectra, the application limits you to only one chromatogram per node. Tree chromatograms with components or selected scans are fully searchable. You can review or reload a node chromatogram from the original data file in the Chromatogram Database Viewer. For additional information, see "Processing Data-Reduced Chromatograms" on page 69.

❖ To open the Chromatogram Database Viewer

Click the **Show Complete Chromatogram** icon,  , in the Database Manager toolbar.

The Chromatogram Database Viewer window opens.



You can edit node chromatogram components or selected scans in the Components Editor window. For additional information, see Chapter 9, "Components Editor Module."

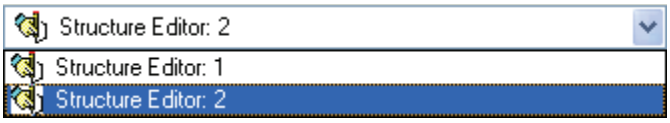
Structure Editor Module

Structural information is essential for interpreting and investigating structure-spectra relationships because mass spectra reflect the structural features of molecules. With the Structure Editor drawing tool, you can interactively handle all kinds of structural information. Use the Structure Editor to edit, import, export, and check chemical structures.

Contents

Working with the Structure Editor

You can open multiple Structure Editor modules in the Mass Frontier application. Use the list in the Mass Frontier main window to switch between them.



The Structure Editor supports two kinds of structure formats:

- Molecular Design Ltd. MOL or SDF files with the .mol or .sdf extension
- HighChem, Maximal Compressed Structure format with the .mcs extension

These formats are also supported in the Database Manager module. Templates are stored in MCS format, using the .tml extension. For additional information, see "Using Templates" on page 137.

The Mass Frontier application restricts searches using a set of structural constraints or the "Good-Bad" list. For example, you can instruct the system to conduct a library search comparing an unknown spectrum with only the spectra of ketones. This feature provides many possibilities for targeting your search results. For additional information, see "Search Constraints" on page 103.

The "Good-Bad" structures are stored in the …\Constraints directory, and the structures are saved in MCS format (.mcs). The Mass Frontier application automatically retrieves all MCS structures from the Constraints directory and puts them in a "Good-Bad" list in the Constraints dialog box.

Follow these procedures:

- To open the Structure Editor window
- To begin drawing a chemical structure
- To change the size of the Structure Editor window
- To restore the default state of the Structure Editor
- To open a structure
- To save a structure

❖ **To open the Structure Editor window**

Do one of the following:

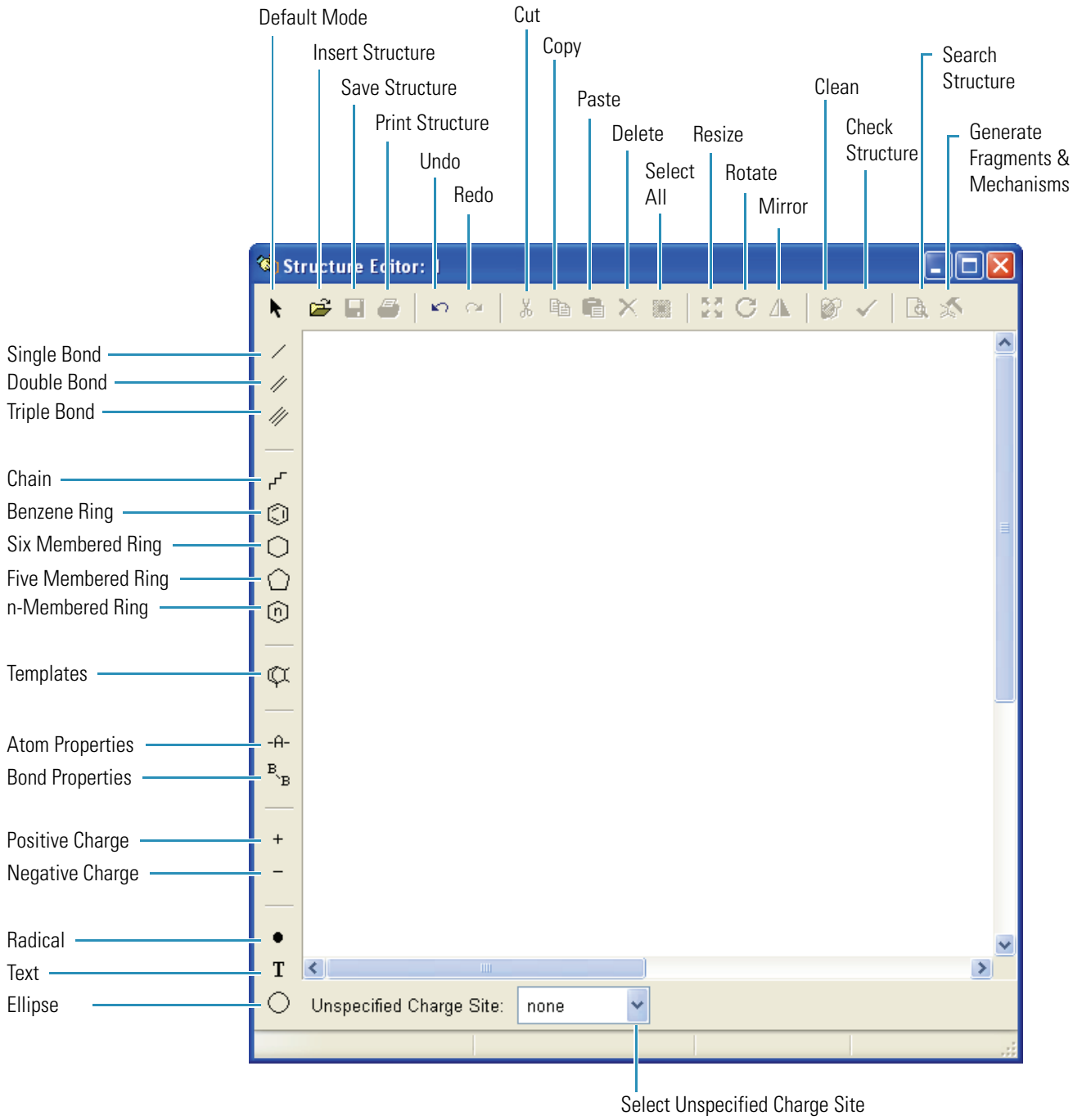
Click the **Structure Editor** button,  , in the Mass Frontier toolbar.

–or–

Choose **Tools > Structure Editor** from the Mass Frontier main menu.

The Structure Editor window opens.

Figure 55. Structure Editor window



❖ To begin drawing a chemical structure

1. Click any of the buttons in the vertical toolbar.

   The cursor changes shape to represent the current drawing mode.

2. Do one of the following:

   Click the drawing area to place an element.

   –or–

   Drag the cursor to create elements such as single/double/triple bonds or a chain.

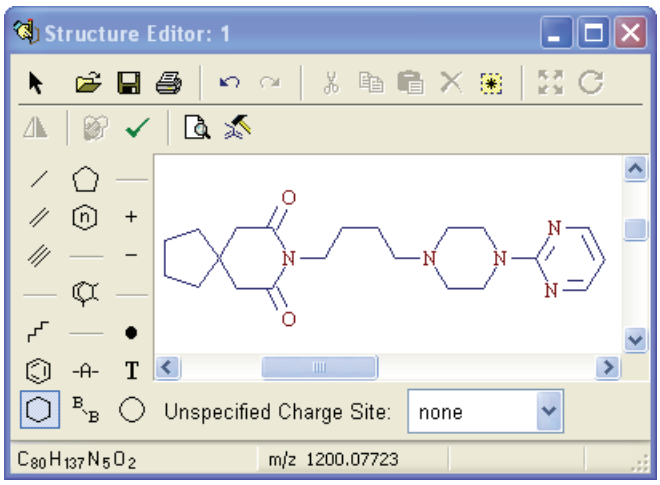
All elements are recursive—that is, the application draws a new element each time you click.

Note Holding your cursor over each drawing button displays a ToolTip that describes the type of structure each element creates.

❖ To change the size of the Structure Editor window

Drag the borders of the Structure Editor to change the width or height.

With a smaller window, you can have several open modules on your application window; a larger window gives you more drawing area.



❖ To restore the default state of the Structure Editor

Do one of the following:

Click the **Default Mode** button, , in the upper left corner of the window.

–or–

Right-click the drawing area and choose **Default Mode** from the shortcut menu.

By default, no drawing element is selected. The plain arrow cursor indicates that the Structure Editor is in the default state.

❖ To open a structure

Click the **Open Structure** button,  , select a structure, and click **Open**.

When you open a file that contains more than one structure (.sdf file), only the first structure in the file is loaded into the Structure Editor.

❖ To save a structure

1. Do one of the following:

   Click the **Save Structure** button,  .

   –or–

   Choose **File > Save > Structure** from the Mass Frontier main menu.

2. Specify a structure name in the .mol format.

Because Mass Frontier is a 32-bit application, you can save structures under their actual names, regardless of length (for example, 1-Amino-2-hydroxyindane.mol).

Customizing the Structure Layout

You can change the way structures and other objects are displayed in the Structure Editor drawing area. The various layout items help tailor the graphics to your individual report or publication needs.

The structure layout settings apply to all structures in the application. Changing a structure layout item affects all structures in the Structure Editor, Database Manager, Fragments and Mechanisms, and Fragments Comparator modules.

Follow these procedures:

- To open the Structure Layout dialog box
- To return all options to their defaults
- To display carbon symbols
- To display hydrogen symbols
- To display the symbols for hydrogen atoms attached to carbon atoms
- To edit the font for atoms
- To change the appearance of bonds
- To change the color of the background and selected atoms
- To enter a text note
- To change the text notes

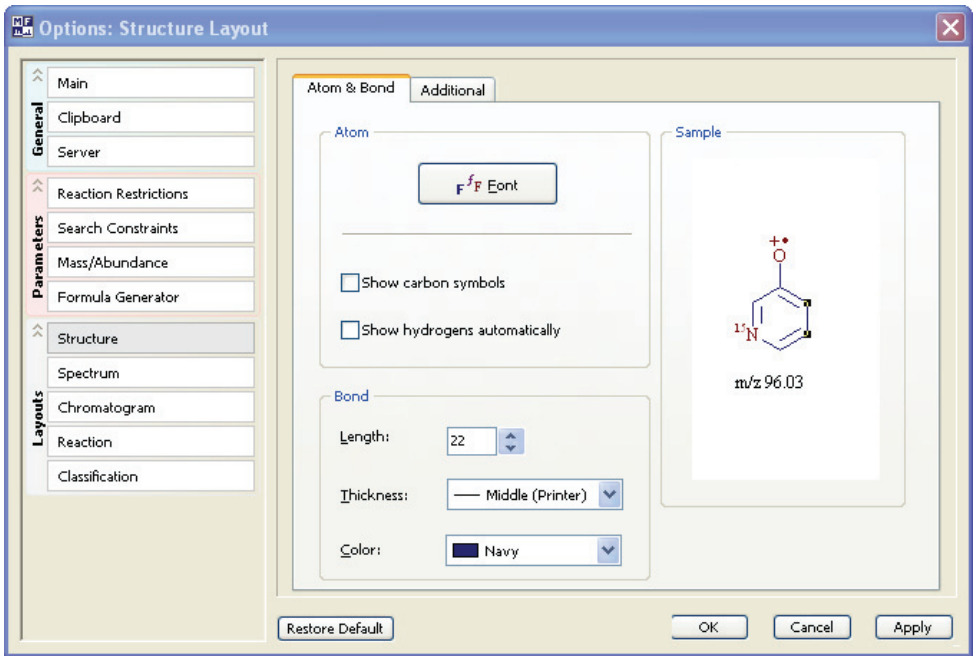
❖ To open the Structure Layout dialog box

1. Choose **Options > Settings** from the Mass Frontier main menu.

   The Options dialog box opens.

2. Click **Structure** in the Layouts section.

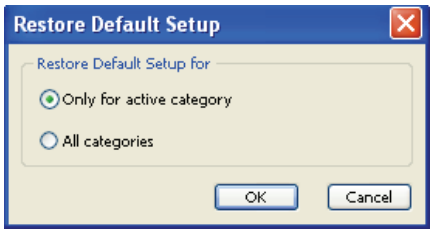
   The Atom & Bond page of the Structure Layout dialog box opens.



❖ To return all options to their defaults

1. Click **Restore Default**.

   The Restore Default Setup dialog box opens.



2. Do one of the following:

   To reset the default values for only the options in the selected category (in this case, the Structure pages), select the **Only for Active Category** option.

   –or–

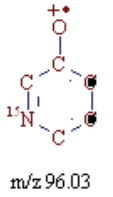
   To reset all the settings in the Options dialog box to their defaults, select the **All Categories** option.

3. Click **OK**.

❖ To display carbon symbols

In the Atom area, select the **Show Carbon Symbols** check box.

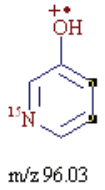
Carbon symbols are displayed in the Sample area.



❖ To display hydrogen symbols

In the Atom area, select the **Show Hydrogens Automatically** check box.

Hydrogen symbols are displayed in the Sample area.

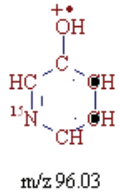


By default, the Structure Editor displays hydrogen symbols for heteroatoms only. It does not display the symbols for hydrogen atoms attached to carbon atoms.

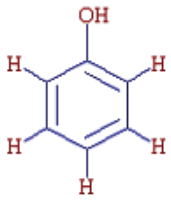
❖ To display the symbols for hydrogen atoms attached to carbon atoms

In the Atom area, select both the **Show Carbon Symbols** and the **Show Hydrogens Automatically** check boxes.

Hydrogen symbols and hydrogen atoms attached to carbon atoms are displayed in the Sample area.



When you draw non-isotopic explicit hydrogen atoms, the application removes them in the Fragments and Mechanisms module because they can make the mechanism network unclear, especially for complex hydrogen rearrangement steps. A substructure search ignores the number and positions of hydrogen atoms.

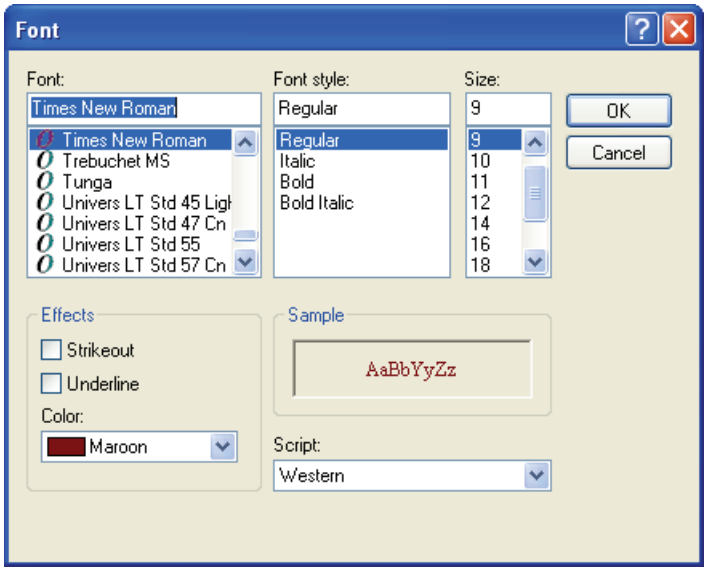


❖ To edit the font for atoms

1. In the Atom area, click the **Font** button, 

.

   The Font dialog box opens.



2. Specify the way you want your font to appear and click **OK**.

❖ To change the appearance of bonds

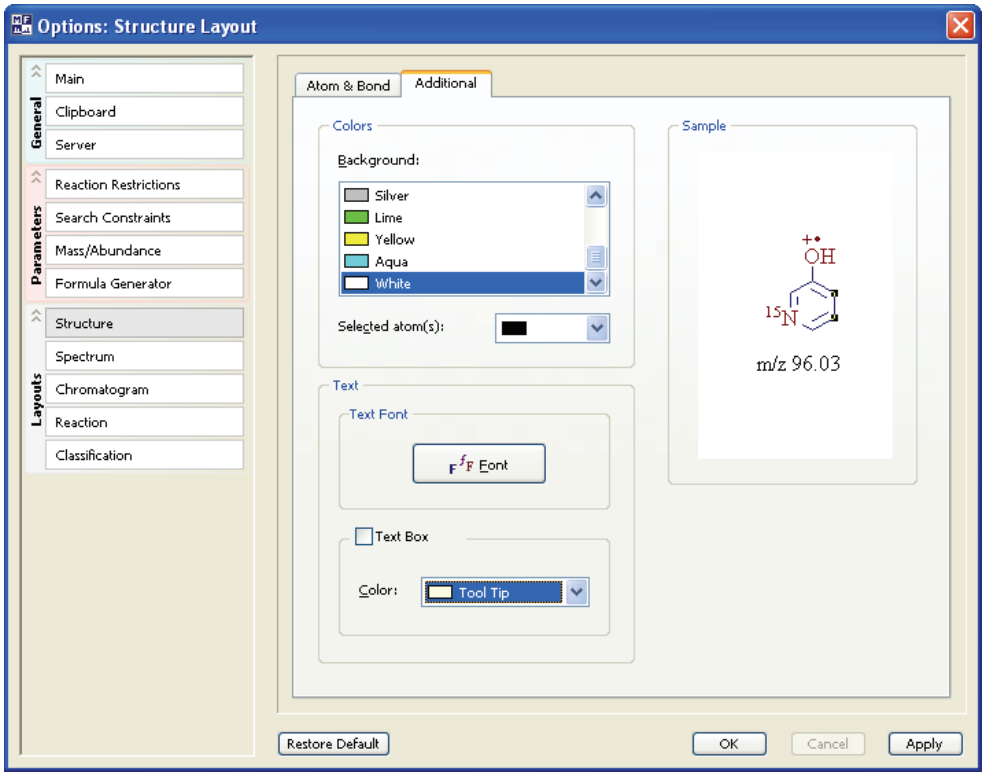
In the Bond area, select the Length, Thickness, or Color for your symbols.

Tip (For black-and-white print only) If you have set bright colors for bonds or atoms, the lines and fonts might appear indistinct. To avoid this problem, specify darker colors for all structural items, including spectra, chromatograms, and mechanisms.

❖ To change the color of the background and selected atoms

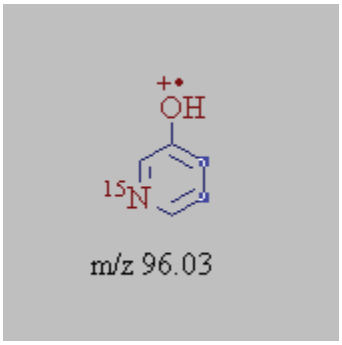
1. Click the **Additional** tab.

   The Additional page of the Structure Layout dialog box opens.



2. In the Colors area, do the following:

   a. Select a color for the background of the drawing area in the Structure Editor, as in the following example.



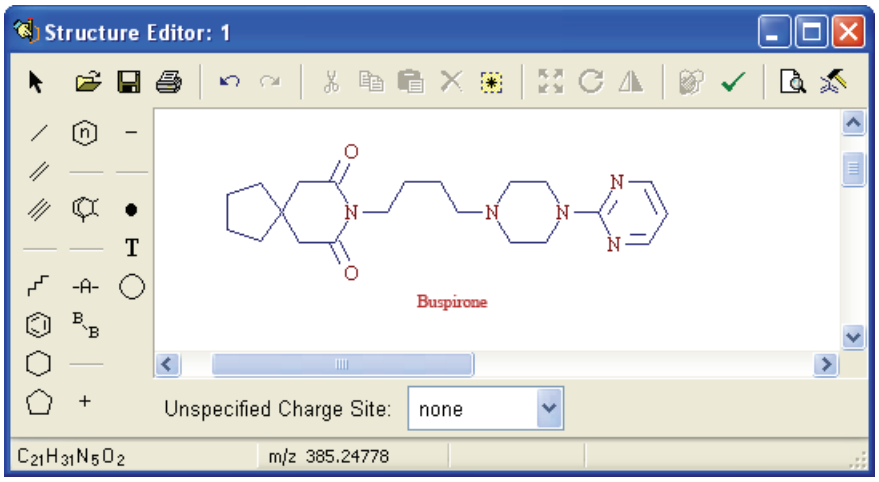
   When you copy the structures to the Clipboard or print the structures, the background color remains white.

   b. Select a color for the selected atoms in the drawing area.

   Use the Sample area to identify an appropriate combination of colors for the background and selected atoms.

❖ To enter a text note

1. Click the **Text** button, T .
2. Click the drawing area to place the text.
3. Type the text.



4. Confirm the text by clicking outside the text area or clicking any button in the Structure Editor window.

You can label a structure or display a text note on the screen or on the printout. You can create up to 127 separate text notes. To change the font, color, size, or background of the text notes, see "To change the text notes" on page 136.

Note Text notes are not associated with structures. The Open, Save, Copy, and Paste commands apply only to structures. When you use these commands, the Structure Editor ignores the text notes even when you select a structure together with a text. Additionally, you can perform structure-handling routines, such as resizing, rotating, or mirroring only on structures.

❖ To change the text notes

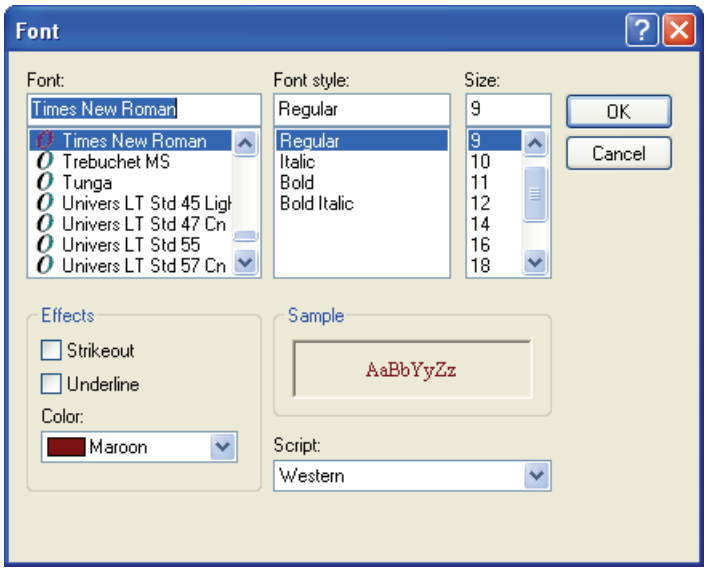
1. Click the **Additional** tab.

   The Additional page of the Structure Layout dialog box opens.

2. In the Text area, click the **Font** button, 

.

   The Font dialog box opens.



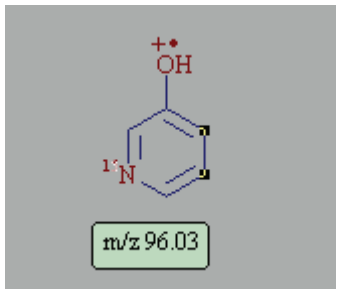
3. Specify the way you want your font to appear and click **OK**.

4. To add a box around the text, do the following:

   a. Select the **Text Box** check box.

   b. From the Colors list, select a fill color for the text box.

   Example filled text box:



Using Templates

The Mass Frontier application comes with more than 200 predefined templates.

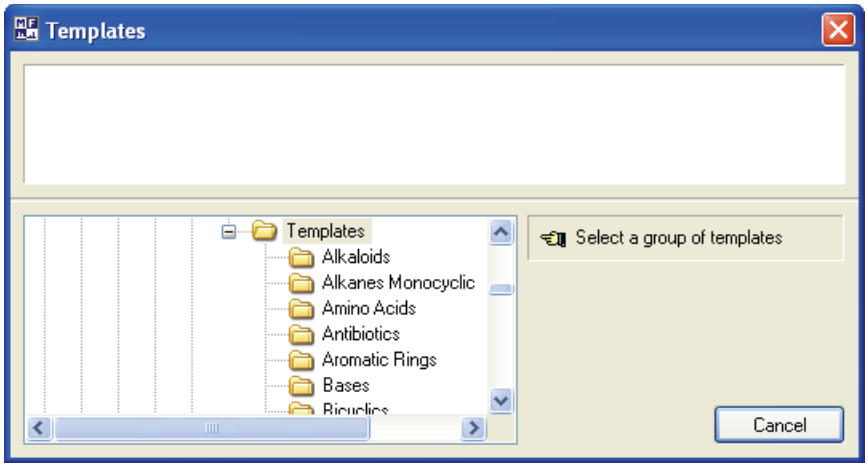
Follow these procedures:

- To open the Templates dialog box
- To insert a template structure into the Structure Editor
- To build your own templates

❖ To open the Templates dialog box

Click the **Templates** button,  .

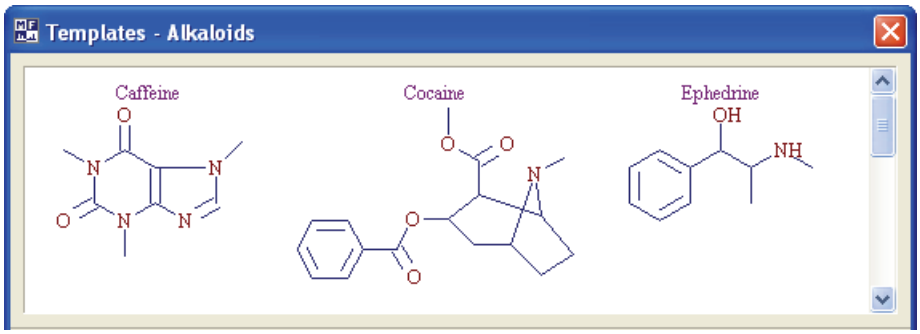
The Templates dialog box opens.



❖ To insert a template structure into the Structure Editor

1. Select a group of templates in the directory list.

   The upper pane displays the structures of the selected group.



2. Click an atom or bond of a structure, depending on whether you want to attach the template to an atom or a bond of a structure in the Structure Editor.

   The Templates dialog box closes.

3. Click the drawing area of the Structure Editor to place or attach the selected template structure.
4. Right-click and choose **Default Mode** from the shortcut menu to stop placing the selected structure.

   The application continues to place the selected template structure each time you click until you return to default mode or select another drawing tool.

❖ To build your own templates

1. Draw a template structure in the Structure Editor.
2. Click the **Save Structure** button,  , or choose **File > Save** from the Mass Frontier main menu.

   The Save Structure dialog box opens.
3. Select the **Template (*.tml)** format in the Save as Type list of the Save Structure dialog box.

   The application stores each group of templates in a separate subdirectory of the template root directory. Subdirectories are named after compound groups (for example, Steroids). The files within each subdirectory are named after actual structures using the .tml extension (for example, Cholesterol.tml).
4. Navigate to the following directory:

   Program Files\HighChem\Mass Frontier 7.0\Templates
5. To save your template to an existing templates folder, do the following:
   a. Select the folder.
   b. In the File Name box, name the template.
   c. Click **Save**.
6. To save your template to a new templates folder, do the following:
   a. Right-click the Save Structure dialog box.
   b. Choose **New > Folder** from the shortcut menu.
   c. Name and select the new folder.
   d. In the File Name box, name the template.
   e. Click **Save**.

Modifying Atoms and Bonds

Any modification that you make to a structure applies only to the selected atoms or bonds. When you initiate a (Sub)Structure search, the application automatically searches for the selected atoms or bonds. For additional information, see "(Sub)Structure Search" on page 84.

This section includes the following topics:

Selecting Atoms and Bonds

The Structure Editor uses function buttons and menus to manipulate the atom and bond structures.

Follow these procedures:

❖ To select a group of atoms

Do one of the following:

To select a group of atoms that are adjacent to each other, drag the cursor to form a rectangle around the atoms.

–or–

To select a group of atoms that are not adjacent to each other, hold down the SHIFT key and select the individual atoms.

❖ To select all of the atoms and bonds in the structure

Do one of the following:

Click the **Select All** button,  .

–or–

Double-click anywhere in the drawing area of the Structure Editor, except on atoms or bonds.

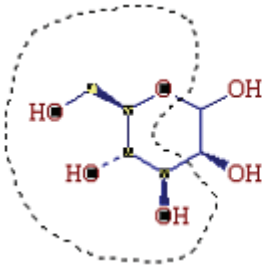
❖ To choose a selection mode

Do one of the following:

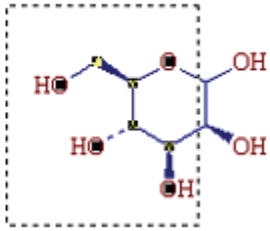
Right-click the **Default Mode** button, , and choose the appropriate selection mode from the shortcut menu.

–or–

Right-click anywhere in the drawing area of the Structure Editor, and choose **Lasso Selection** or **Rectangle Selection** from the shortcut menu.



Lasso Selection



Rectangle Selection

❖ To move a structure

1. Select the atoms or bonds you want to move.
2. Drag the selected structures to the new location.

Editing Atom Properties

Use the Atom Properties dialog box to change the charge state or isotope of an atom or to change the entire element.

Follow these procedures:

- To edit only the element of a single atom
- To edit the element, charge, radical, or nucleon number of a single atom
- To edit only the element of multiple atoms
- To replace an element with a substituent

❖ To edit only the element of a single atom

1. Select the atom you want to change.
2. Do one of the following:

   To change an element that has a single-character symbol—C, H, N, O, B, F, K, P, S, I, V, W, Y, U, and R— press the appropriate key on the keyboard.

   –or–

   To change to chlorine (Cl) or bromine (Br), use SHIFT+C or SHIFT+B, respectively.

   The application applies the selected element symbol to the selected atom.

❖ To edit the element, charge, radical, or nucleon number of a single atom

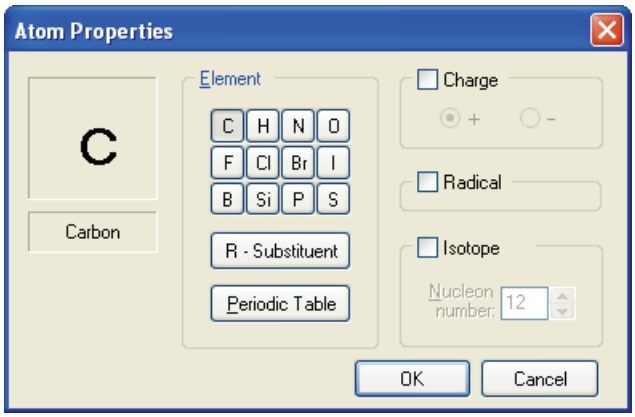
1. Do one of the following:

   Click the **Atom Properties** button, -A-, and then click the atom you want to change.

   –or–

   Right-click and choose **Default Mode** from the shortcut menu, and then double-click the atom you want to change.

   The Atom Properties dialog box opens.



2. Make changes by clicking the appropriate Element button, selecting the Charge and Radical check boxes, or entering a value in the Nucleon Number box.
3. To change the atom to an element not listed in the Element area, do the following:
   a. Click **Periodic Table**.

      The Periodic Table opens. For additional information, see "Report Creator" on page 19.
   b. Select an element on the periodic table.
   c. Click **OK**.
4. In the Atom Properties dialog box, click **OK**.

   Changes you make in the Atom Properties dialog box affect only the selected atom.

❖ To edit only the element of multiple atoms

1. Hold down the SHIFT key and click each atom you want to change.

   You can change only the element of multiple atoms. You cannot change the other parameters available on the Atom Properties dialog box.
2. Do one of the following:

   To change an element that has a single-character symbol—C, H, N, O, B, F, K, P, S, I, V, W, Y, U, and R— press the appropriate key on the keyboard.

   –or–

   To change to chlorine (Cl) or bromine (Br), use SHIFT+C or SHIFT+B, respectively.

   The element symbol is applied to all selected atoms.

❖ To replace an element with a substituent

1. Do one of the following:

   Click the **Atom Properties** button, -A- , and then click the atom you want to change.

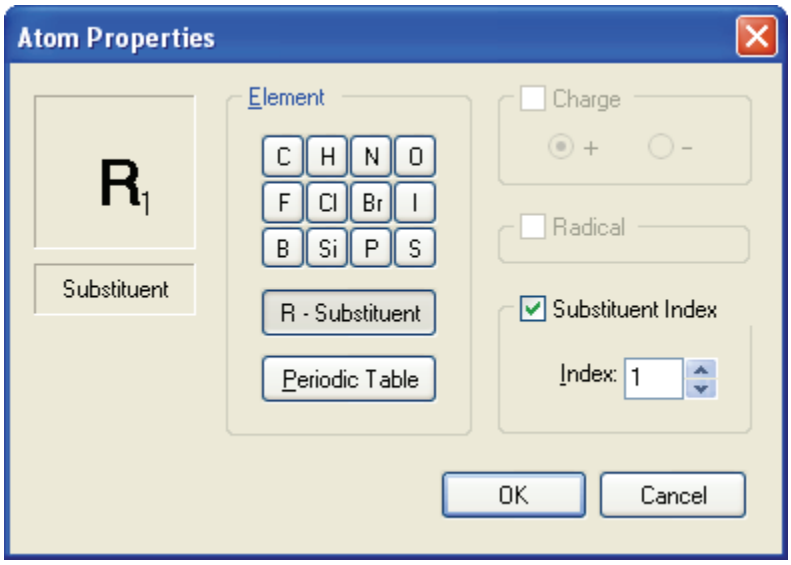
   –or–

   Right-click and choose **Default Mode** from the shortcut menu, and then double-click the atom you want to change.

   The Atom Properties dialog box opens.

2. Click **R-Substituent**.

   The Substituent Index area appears in the dialog box.



3. Select a value for the substituent index, or clear the **Substituent Index** check box.
4. Click **OK**.

The symbol "R" represents a substituent. Substructure search and fragments generation algorithms consider substituents with identical indexes as equal and substituents with different indexes as not equal. For additional information, see Chapter 6, "Fragments and Mechanisms Module."

When searching structures with substituents, specific search rules apply. For additional information, see "(Sub)Structure Search Rules" on page 89.

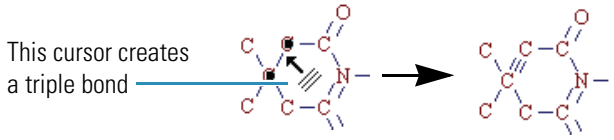
Editing Bond Properties

Use the Bond Properties dialog box to change bond multiplicity, bond style, and bond color.

❖ To change the multiplicity of a bond

Click , or , or and then click the bond you want to change.

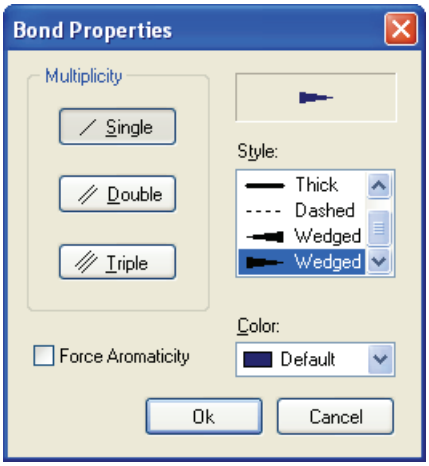
These commands are recursive; the application changes the selected bond each time you click. The cursor indicates the type of bond you are using.



❖ To change the multiplicity, color, line style, or aromaticity of a bond

1. Click the **Bond Properties** button, , and then click the bond you want to change.

   The Bond Properties dialog box opens.



2. To change the multiplicity of the selected bond, select **Single**, **Double**, or **Triple** in the Multiplicity area.
3. To change the line style used to display the bond, select a style in the Style list.

   Only single bonds use Wedged line styles.
4. To change the color used to display the bond, select a color in the Color list.
5. To force aromaticity to the selected bond, select the **Force Aromaticity** check box.

   The Mass Frontier application automatically recognizes aromatic bonds in an appropriate six-membered ring or in polyaromatic structures. However, if unusual semiaromatic or aromatic resonance structures are required, the aromatic bond can be forced to a selected bond or bonds.
6. Click **OK**.

Understanding Unspecified Bond Locations

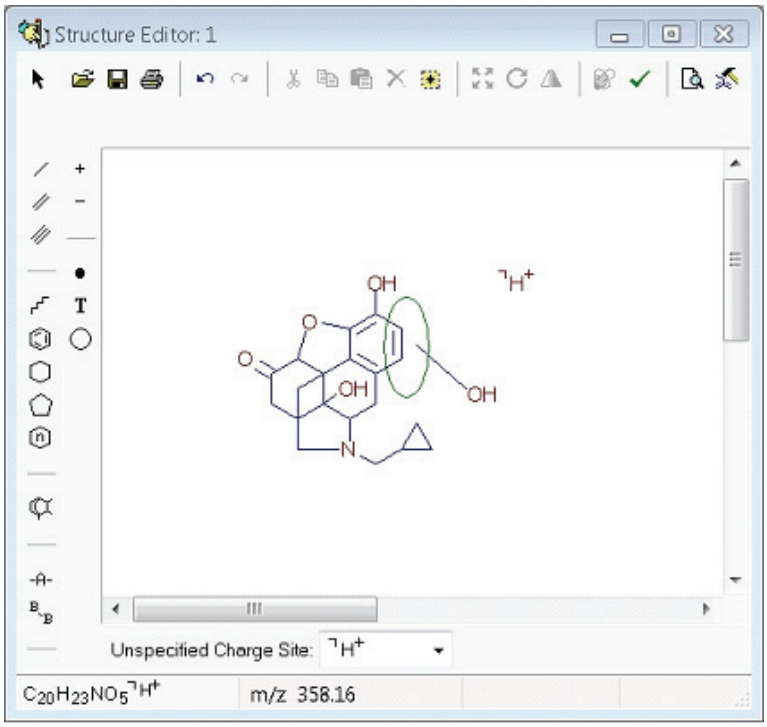
Mass spectrometry cannot always provide information about the exact position of a functional group on a chain or ring portion of a structure. In several application areas, however, even incompletely characterized molecules can be sufficient to study a particular phenomenon. A typical example is metabolite characterization in an early drug discovery process where knowing the precise location of the site of biotransformation action is of little importance.

For displaying and calculating the monoisotopic mass or the isotopic pattern of a fragment or molecule with an unspecified bond location (see "Isotope Pattern" on page 15), use the Ellipse tool,  . The ellipse visually defines the region on a structure where you can potentially attach a functional group. To correctly interpret an unspecified bond location, the ellipse must enclose one or more atoms of the core structure and a single atom of a functional group that is not attached to the core structure.

Note The Ellipse tool is available only in the Structure Editor module. You cannot use a structure with an unspecified bond location in other Mass Frontier modules. The Mass Frontier application does not support the generation of fragments and mechanisms from such structures.

Understanding Unspecified Charge Sites

Use the Mass Frontier application for processing structures where the charge site is not specified. This kind of molecule representation is important when using ionic structures because the favored ionization site or the explicit charge site during fragmentation reactions is often unclear. You can choose from several ion types in the system.



❖ To create an ionic structure with an unspecified charge site

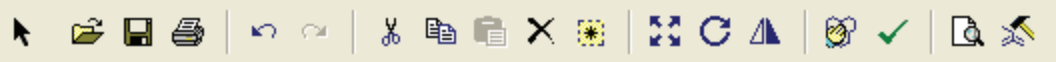
Select one of the ion types from the list at the bottom of the Structure Editor.

Every structure with an unspecified charge site has this symbol, ⌝ , on the upper left part of the structure.

Be aware of potential complications when you use 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 with 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. For additional information, see "Base Page" on page 201.

Using Toolbar Functions

You can manipulate a structure using several buttons in the Structure Editor toolbar.



Follow these procedures:

- To resize a structure
- To copy a structure or part of a structure to the Clipboard
- To paste a structure to the Structure Editor
- To rotate a structure in the Structure Editor
- To mirror a structure
- To clean a structure
- To check a structure

❖ To resize a structure

1. Select the structure or part of the structure you want to resize.
2. Click the **Resize** button,  .
3. Drag one of the small rectangles on the structure edge and release the mouse button.

   Dragging one of the diagonal rectangles keeps the aspect ratio constant during structure resizing.

❖ To copy a structure or part of a structure to the Clipboard

1. Select the structure or part of the structure you want to copy.
2. Click the **Copy** button,  , or choose **Edit > Copy** from the Mass Frontier main menu.

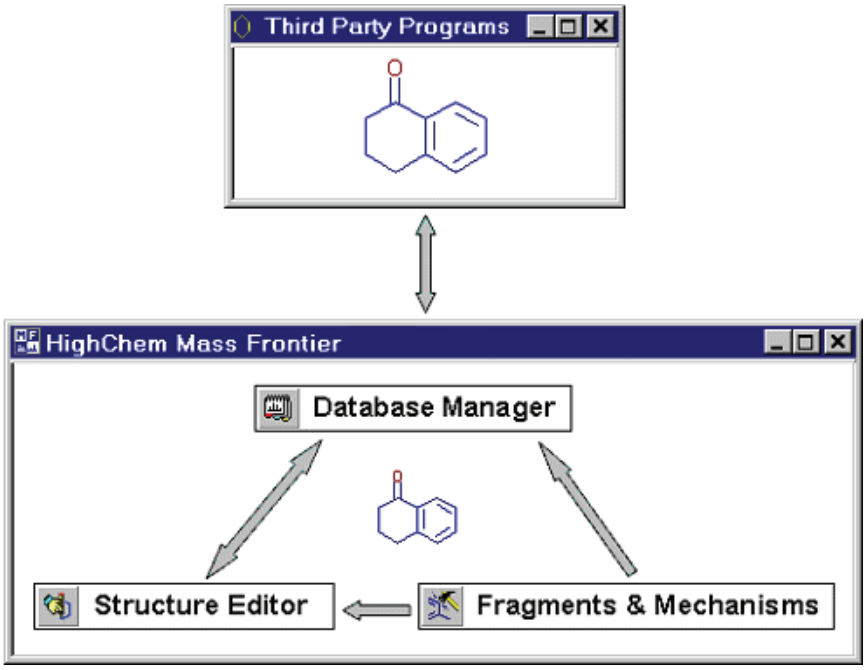
Note The application copies only the selected atoms and their associated bonds.

The Mass Frontier application supports use of the Windows Clipboard for the exchange of structural information between modules. In addition, you can use copy-and-paste functions inside the Structure Editor. To efficiently draw larger structures, use the copy-and-paste functions.

In addition to structure exchange between modules, you can use the Mass Frontier application for structure export to other applications that deal with structural information. When you copy a structure, the application automatically copies two different formats to the Clipboard: structural information in .mol format and graphics in Windows Metafile Format (.wmf).

When you paste a structure into the structure editing software, the application automatically uses the .mol format. When you paste the structure into a text editor, spreadsheet, or other application that works with graphics, the application automatically uses the graphical information.

Figure 56. Structure Exchange using copy-and-paste commands



❖ To paste a structure to the Structure Editor

Click the **Paste** button, , or choose **Edit > Paste** from the Mass Frontier main menu.

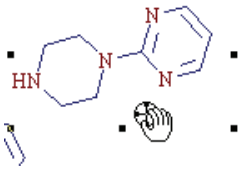
When you copy a structure or fragment anywhere in the application or in a third-party structure drawing tool, you can paste it to the Structure Editor. If necessary, you can change or correct the structure, and then return it to its original location. This is especially useful for structural elucidation. For example, you can copy a structure from the Database Manager window, paste it to the Structure Editor, make appropriate changes, and then move it back to the Database Manager. If the spectrum and the structural proposal are not consistent, you can repeat the process.

When you copy a structure or fragment in a program other than the Mass Frontier application, you can paste this structure as long as the external structure drawing software supports .mol format and this format is activated. The majority of drawing tools for structures support .mol format with this format set as the default. If you paste a structure from an external source, it might appear larger in the Mass Frontier application than in the original software. If this occurs, make the structure smaller using the Resize tool.

❖ To rotate a structure in the Structure Editor

1. Select the structure or part of the structure you want to rotate.
2. Click the **Rotate** button, .

   The center of rotation is indicated by a small circle with a cross in the middle, .
3. Move the center of rotation by dragging the circle with a cross.



4. Drag any of the small rectangles on the structure edge to rotate the selected structure around the center.

❖ To mirror a structure

1. Select the structure or part of the structure you want to mirror.
2. Click the **Mirror** button, .
3. Click one of the small rectangles on the structure edge.
   - The top and bottom rectangles flip the selected structure along a horizontal axis.
   - The left and right rectangles flip the selected structure along a vertical axis.

❖ To clean a structure

1. Do one of the following:

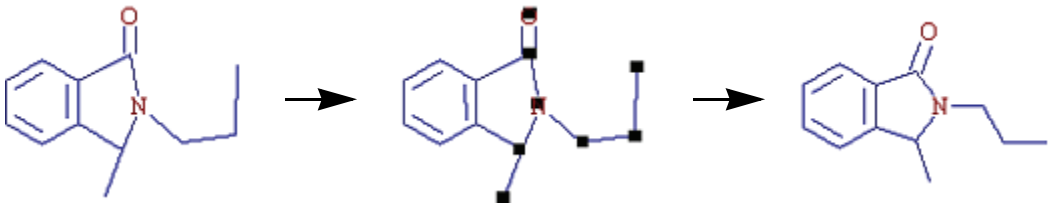
   Select an entire structure.

   –or–

   Select only the atoms you want to clean.

   The selected atoms must be connected.
2. Click the **Clean** button, .

   The Clean function helps you create a professional look for your structures.



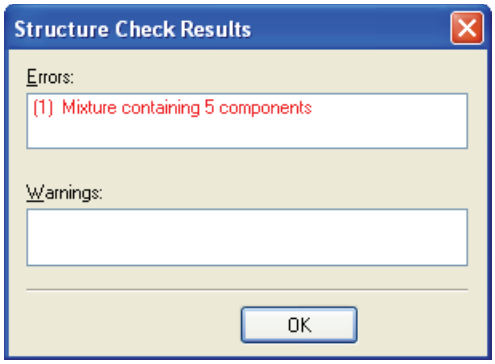
Note In some complicated cases, the Clean function can lead to structures that you might not find satisfactory. If this occurs, choose **Edit > Undo** from the Mass Frontier main menu.

After finishing a structure drawing, always check for errors before proceeding with any other procedure. When you generate the fragments and mechanisms, the application automatically checks the structure for errors and, if any error is discovered, the generation automatically discontinues. Before you run Fragments and Mechanisms, the Fragmentation Library window automatically performs a structure check.

❖ To check a structure

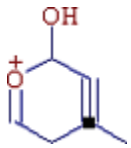
1. Click the **Check Structure** button,  .

   The Structure check tool searches for formal errors and unusual structural features. If a structure is formally incorrect or if the Structure check tool finds its validity questionable, the Structure Check Results message box lists the errors and warnings.



2. Click **OK** to close this dialog box.

   The Mass Frontier application automatically selects the atoms and bonds that are considered incorrect. The system considers structures that are not connected as mixtures and reports them as errors, but it does not select the mixtures.



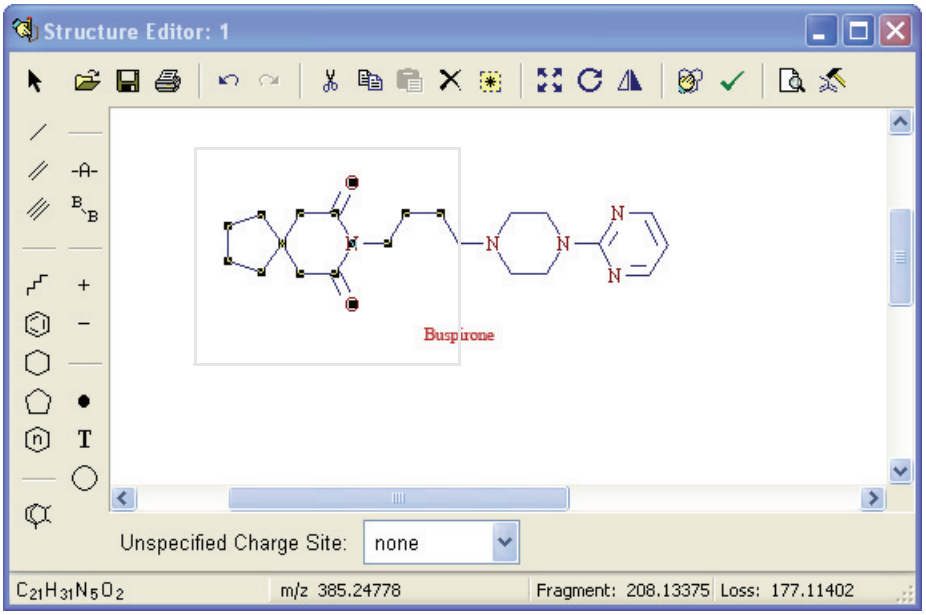
Note This option does not perform quantum mechanical or thermodynamical calculations that address possible structural stability.

Displaying the Monoisotopic Molecular Mass

When you select part of a structure, the Structure Editor displays the molecular formula and monoisotopic molecular mass of the selected atoms and the corresponding loss in the status bar of the Structure Editor. For additional information, see "Monoisotopic Mass" on page 381.

Information about the molecular formula and monoisotopic molecular mass is useful for simple consistency checking of a mass spectrum and chemical structure.

Figure 57. Molecular formula and monoisotopic molecular mass



Library Utilities

The Mass Frontier application provides several methods for creating and maintaining mass spectral, chromatographic, and fragmentation libraries. To help you visually distinguish between libraries, each library has its own icon. You can select an icon for the user libraries you create, but the application automatically assigns icons to the HighChem MS^n libraries. You can install up to 255 libraries at once.

The Mass Frontier Server Manager is a stand-alone application for managing and administering libraries on the Microsoft SQL Server (SQL Server).

The Mass Frontier application supports both low- and high-resolution mass spectra. You can store mass spectra as single spectra or as MS^n trees with multiple node spectra. You can also store and search data-reduced chromatograms with selected scans or components. You can update any record in a library except the HighChem MS^n libraries, which are read-only.

The Mass Frontier application unifies spectral and fragmentation libraries into a single library format.

Contents

Using the Server Manager

Not only can you access libraries over a computer network by using the Mass Frontier Server Manager, but you can access a library on the SQL Server from multiple instances of the Mass Frontier application. Use the Server Manager to manage spectral and fragmentation libraries for all instances of the Mass Frontier application. You can also use the Server Manager to find and manage servers over computer networks.

This section includes the following topics:

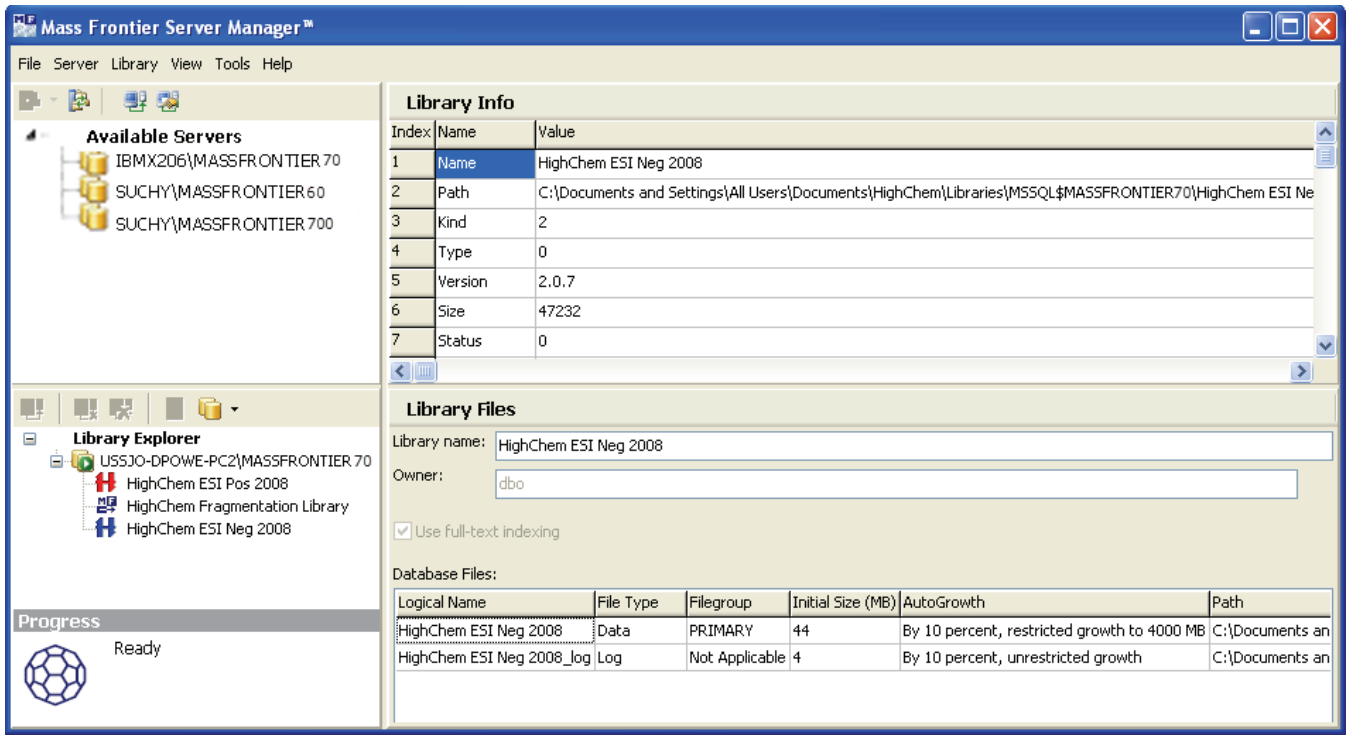
- Using the Server Manager
- Managing Servers
- Managing Libraries
- Importing the NIST/EPA/NIH Mass Spectral Database
- Error Logs

❖ To open the Server Manager

Choose **Library > Server Manager** from the Mass Frontier main menu.

This menu item is accessible only if the Server Manager is installed on the same computer as the Mass Frontier software. If the Server Manager is installed on a network computer, start the Server Manager from this location.

Figure 58. Mass Frontier Server Manager window



Setting Up the Server Manager

To access the SQL Server through the Server Manager, you must configure both the firewall protecting the SQL Server and the SQL Server browser so that they can receive incoming connections.

At startup, the Server Manager attempts to find and connect to the server located on the local computer. If it cannot find this server or if you want to work with servers on other computers, you can do one of the following:

Choose **Server > Add Server Manually** to add a server (link) in the Server Manager window.

–or–

Choose **Server > Enumerate Available Servers** to search all available servers on the network.

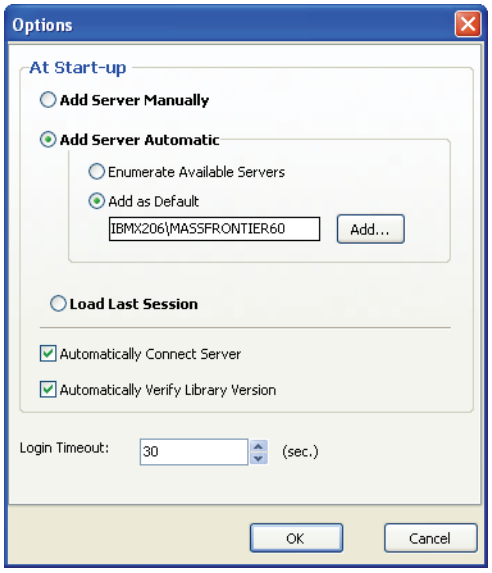
Note Enumerating servers on a computer network can be time-consuming, depending on the network size. Some network configurations do not allow automated identification of servers using enumeration. In this case, you must manually connect the server. For instructions, see "To manually connect to a server" on page 156.

When you regularly or exclusively work with a specific server, you can set it as the default.

❖ To specify a default server

1. Choose **Tools > Options** from the Server Manager menu.

   The Options dialog box opens.



2. Select the **Add Server Automatic** option.
3. Select the **Add as Default** option.

4. Type the name of the default server or click **Add** and locate the server.
5. Click **OK**.

Note To connect to a server that was installed with the Mass Frontier 4.0, 5.0, or 6.0 applications, you must reconfigure the server for network functionality (see "To reconfigure a previous version of the Mass Frontier SQL Server" on page 170). Server configuration has no effect on the Mass Frontier application and its libraries.

Managing Servers

You can manage your server connections from the Server Manager window.

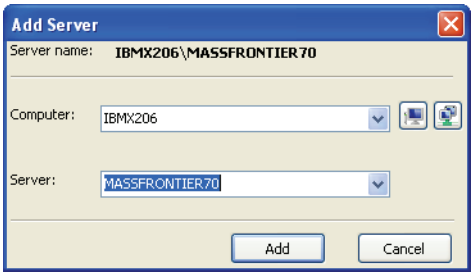
Follow these procedures:

- To manually connect to a server
- To connect to a preferred server from the Available Servers list
- To interrupt a server connection
- To remove a server from the Library Explorer list

❖ To manually connect to a server

1. Choose **Server > Add Server Manually**.

   The Add Server dialog box opens.



2. To specify the computer, do one of the following:

   Click the **Select Computer** icon, , and browse to a computer.

   –or–

   Type the computer name.

   The available servers for the selected computer are listed in the Server list.

3. To specify the server, do one of the following:

   Select from the available servers in the Server list.

   –or–

   Type the server name (for example, MASSFRONTIER70).

4. (Optional) To automatically enumerate computers on the network, click the **Enumerate Network Computers** icon,  .
5. Click **Add**.

   The server name appears in the Available Servers pane in the Server Manager window.

❖ To connect to a preferred server from the Available Servers list

1. Select the server in the Available Servers pane.

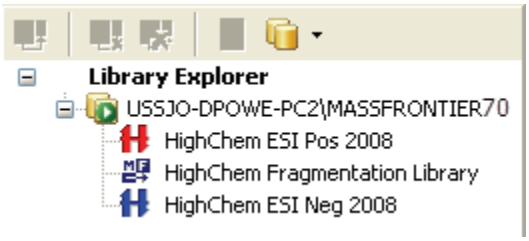
2. Do one of the following:

   Click the **Connect Server** icon,  .

   –or–

   Right-click and choose **Connect Server** from the shortcut menu.

   The application connects to the selected server and displays the server and the list of available libraries installed on the server in the Library Explorer pane.



Note You can manage libraries (install, remove, create, or delete) only when a server is connected.

❖ To interrupt a server connection

1. Select the server in the Library Explorer pane or the Available Servers pane.
2. Do one of the following:

   Click the **Disconnect Selected Server** icon,  , in the Library Explorer toolbar.

   –or–

   Choose **Server > Disconnect Server** from the Server Manager main menu.

   You can reconnect to the server at any time. For additional information, see "To manually connect to a server" on page 156.

❖ To remove a server from the Library Explorer list

1. Select the server in the Library Explorer pane.
2. Do one of the following:

   Click the **Remove Server from Library Explorer** icon,  , in the Library Explorer toolbar.

   –or–

   Choose **Server > Remove Server** from the Server Manager main menu.

   The Server Manager removes the server only from the Library Explorer list; it is not removed from your computer. To return the server to the Library Explorer list, see "To manually connect to a server" on page 156.

Managing Libraries

You can store spectral data and fragmentation mechanisms in user libraries. Because creating a user library is easy, you can organize your mass spectral, chromatographic, fragmentation, and structural data in separate user libraries.

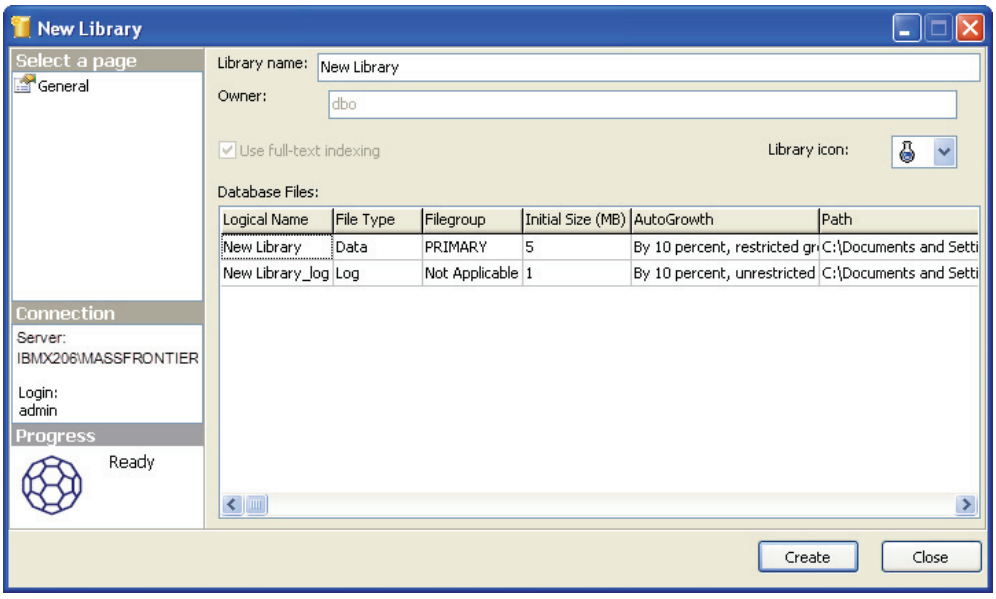
Follow these procedures:

- To create a new user library
- To install a library
- To remove a library
- To permanently remove a library
- To back up a library
- To restore a library from backup files
- To reindex a library
- To shrink a library
- To convert a library to Mass Frontier 7.0 format
- To refresh a library list
- To reconfigure a previous version of the Mass Frontier SQL Server

❖ To create a new user library

1. Choose **Library > Create Library** from the Server Manager main menu.

   The New Library window opens.



2. Type the library name.

   You can use only the characters a–z, A–Z, 0–9, and SPACE.

3. Select a library icon.

   By default, new libraries use a blue beaker, but there are many icons from which to choose.

4. If you must change the default directory (not recommended), do the following:

   a. Click the Path column in the Database Files table.

      A browse button appears on the far right.

   b. Click the button and navigate to a new directory.

5. To create the new library, click **Create**.

   When the library is successfully created, the icon and name are displayed in the Library Explorer.

You can create an unlimited number of user libraries, but the SQL Server can hold a maximum of 255 libraries at one time. If you require more libraries and have reached the maximum, remove a library that you do not need and then create or install a new library.

Note You can create a library only on the same computer where the SQL Server is located. For example, if your SQL Server is located on a network computer, you cannot create a library on your local computer.

❖ To install a library

1. Do one of the following:

   Choose **Library > Install Library** from the Server Manager main menu.

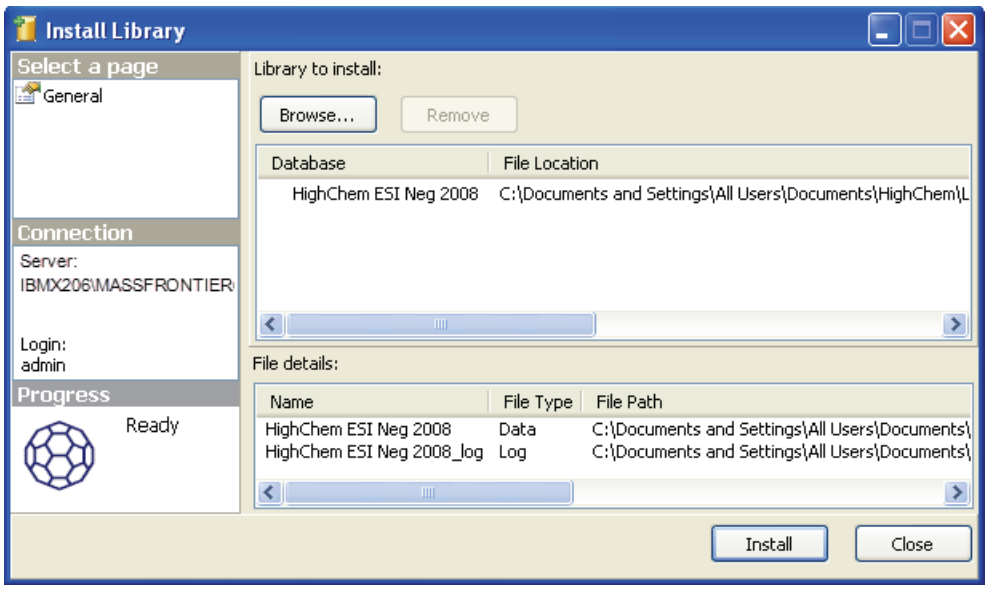
   –or–

   Select the server name in the Library Explorer pane, right-click and choose **Install Library** from the shortcut menu.

   The Locate Library File browser opens.

2. Select a library file with the .mdf extension and click **OK**.

   The Install Library dialog box opens.



3. To install another library, click **Browse** and select a library file with the .mdf extension.

   Repeat this step for as many libraries as you want to install.

4. Click **Install**.

   When the library is successfully installed, a new library item appears in the Library Explorer pane.

   If the application encounters a problem installing a library, an error icon, , appears next to the library item in the list of libraries. To see the details of the problem, double-click the library item.

5. To remove a library from the list of previously chosen libraries, select the library item and click **Remove**.

Note You can install a library only on the same computer where the SQL Server is located. For example, if your SQL Server is located on a network computer, you cannot install a library on your local computer. If you have a library file on a local computer, you must copy it to the computer where the SQL Server is located.

Tip You can install a library only when the data is in the Mass Frontier library format (version 4.0 or later). For libraries in NIST format (NIST Library and user libraries from the Mass Frontier 3.0 and earlier versions), use the Import NIST Library command. For additional information, see "Importing the NIST/EPA/NIH Mass Spectral Database" on page 171.

❖ To remove a library

1. Select the library in the Library Explorer pane.

   Notes

   - Too many installed libraries can slow the performance of the system; removing unused libraries can improve system performance.
   - You cannot copy or delete database files (.mdf) when a library is installed.

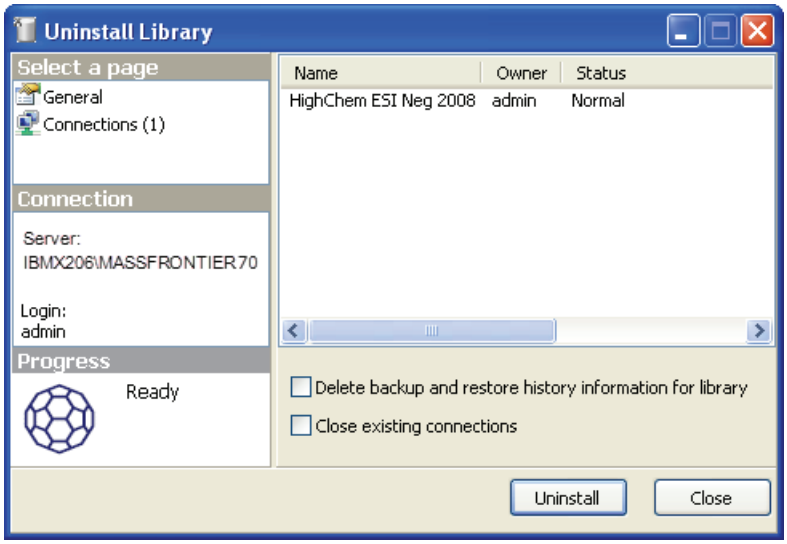
2. Do one of the following:

   Choose **Library > Uninstall Library** from the Server Manager main menu.

   –or–

   Right-click and choose **Uninstall Library** from the shortcut menu.

   The Uninstall Library window opens.



   You cannot remove a library with an already established connection to the application.

3. (Optional) To find out if another user is connected to the library, click **Connections** in the Select a Page pane.
4. (Optional) To remove a library that has an established connection, select the **Close Existing Connections** check box.

   Applications that are connected to a removed library do not work properly. You can use the Close Existing Connections feature if an application remains connected after an unexpected termination.
5. (Optional) To remove backup information from the system database, select the **Delete Backup and Restore History Information for Library** check box.

   To optimize processing speed, keep this option cleared.
6. Click **Uninstall**.

Removing a library does not delete it. The application removes the library reference without the loss of spectral, structural, and fragmentation information. At any time, you can reinstall a library that has been removed. For additional information, see To install a library.

❖ To permanently remove a library

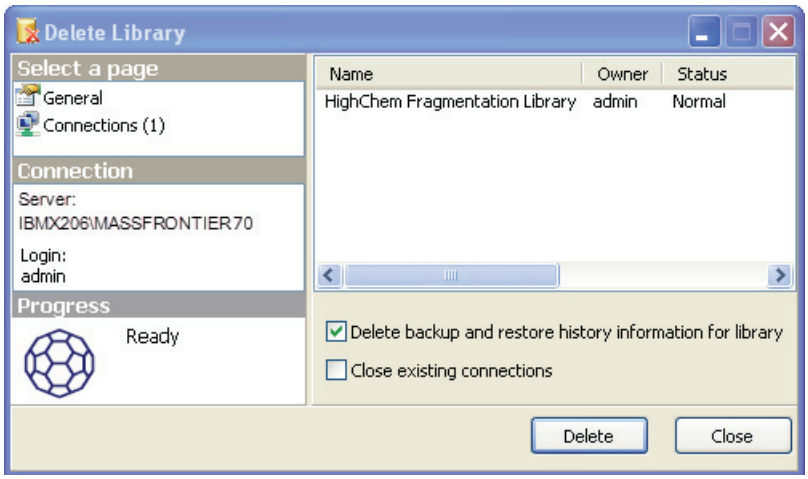
1. Select the library in the Library Explorer pane.
2. Do one of the following:

   Choose **Library > Delete Library** from the Server Manager main menu.

   –or–

   Right-click and choose **Delete Library** from the shortcut menu.

   The Delete Library window opens.



   You cannot remove a library with an already established connection to the application.
3. (Optional) To find out if another user is connected to the library, click **Connections** in the Select a Page pane.

4. (Optional) To remove a library that has an established connection, select the **Close Existing Connections** check box.

   Applications that are connected to a removed library do not work properly. You can use the Close Existing Connections feature if an application remains connected after an unexpected termination.

5. (Optional) To remove backup information from the system database, select the **Delete Backup and Restore History Information for Library** check box.

   To optimize processing speed, keep this option cleared.

6. Click **Delete**.

The Delete Library option irreversibly removes the library and all associated files from the hard disk. No recovery action is possible unless you back up the library before deleting it. For additional information, see To back up a library.

❖ To back up a library

1. Select the library in the Library Explorer pane.

   IMPORTANT Do not back up or restore libraries by using a copy-and-paste operation; use only the Server Manager's backup and restore procedures.

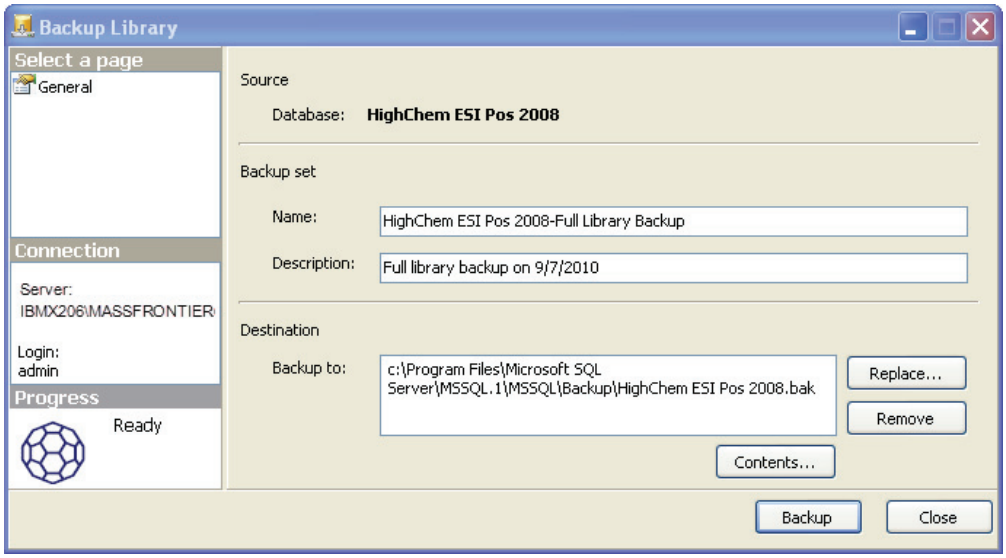
2. Do one of the following:

   Choose **Library > BackUp Library** from the Server Manager main menu.

   –or–

   Right-click and choose **BackUp Library** from the shortcut menu.

   The Backup Library window opens.



3. (Optional) Edit the text in the Name and Description boxes.

   The text in these fields helps identify the specific backup when you restore a library. For additional information, see To restore a library from backup files.

4. (Optional) To change the default destination of the backup file, click **Replace** and specify the directory and file name.

5. (Optional) To see the size and time stamp of the backup file, click **Contents**.

6. Click **Backup**.

Use this backup procedure to continuously store changes from previous backups into the same file. The application automatically creates a time stamp for each backup.

❖ To restore a library from backup files

1. Select the source library in the Library Explorer pane.

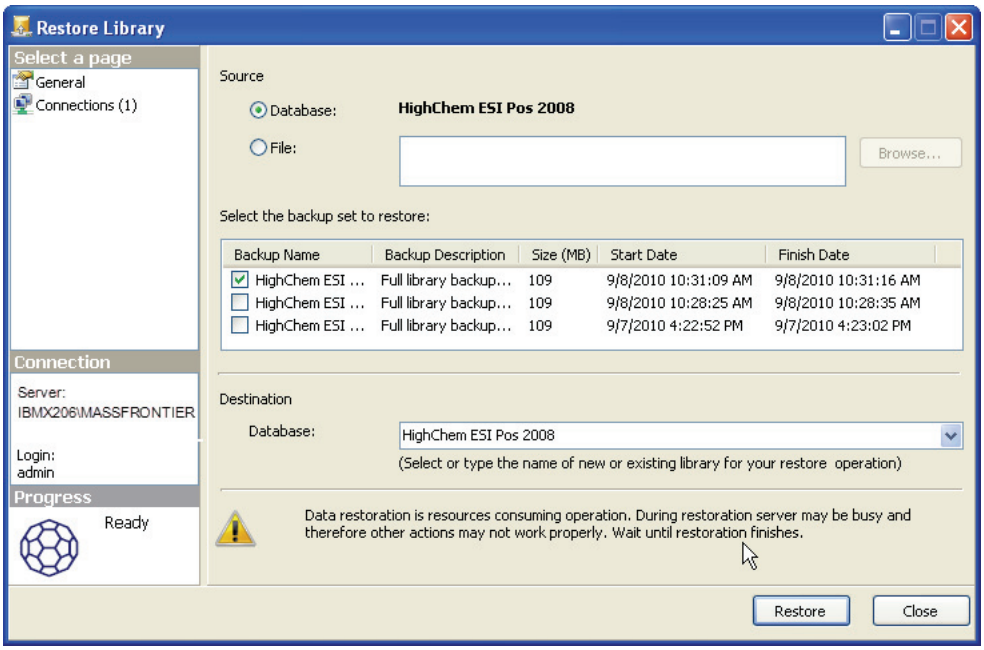
2. Do one of the following:

   Choose **Library > Restore Library** from the Server Manager main menu.

   –or–

   Right-click and choose **Restore Library** from the shortcut menu.

   The Restore Library window opens.



3. In the Select the Backup Set to Restore list, select the check box of the data set you want to restore.

   Use the time stamp to identify the correct data set. You can restore library data from previous backups into a new library or into an existing library.

Tip If you restore library data into an existing library, you will irreversibly overwrite its data. Instead, restore the backup library to a new destination library with a unique name.

4. Type a new destination library name in the Database list.

   You can select a library name from the list, but this action irreversibly overwrites the data in that library.

5. Click **Restore**.

   You cannot restore a library with an already established connection to the application.

6. (Optional) To find out if another user is connected to the library, click **Connections** in the Select a Page pane.

7. (Optional) To remove a library that has an established connection, select the **Close Existing Connections** check box.

   Applications that are connected to this library do not work properly. You can use the Close Existing Connections feature if an application remains connected after an unexpected termination.

Note Data restoration is a resource-consuming operation. During restoration, the server might be busy so that other actions in the Server Manager might not work properly. Wait until restoration finishes to proceed.

❖ To reindex a library

1. Select the library in the Library Explorer pane.

   IMPORTANT Make backup copies of libraries before reindexing them. For additional information, see "To back up a library" on page 164.

2. Do one of the following:

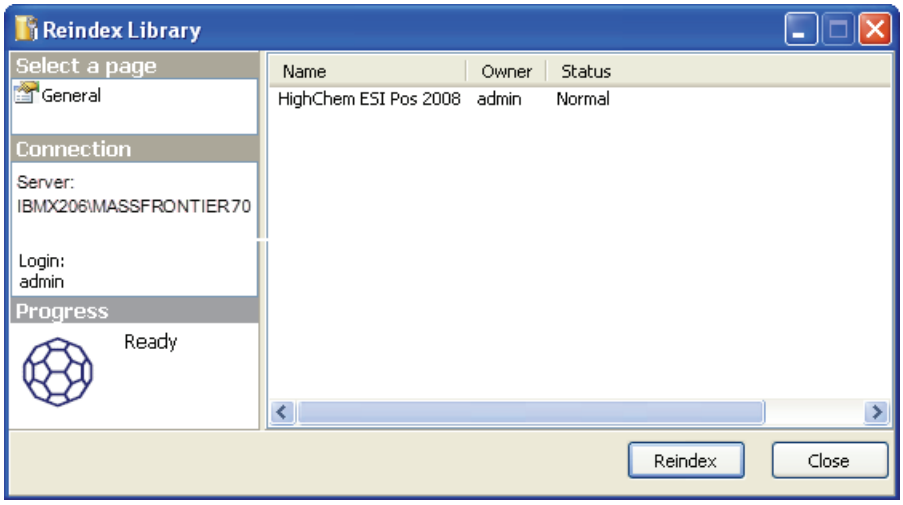
   Choose **Library > Reindex Library** from the Server Manager main menu.

   –or–

   Right-click and choose **Reindex Library** from the shortcut menu.

   The Reindex Library window opens.

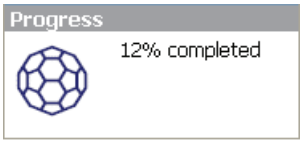
   Figure 59. Reindex Library window

   This feature defragments the library index and can increase performance.

3. Click **ReIndex**.

   Depending on the amount of stored data, reindexing requires from several minutes to several hours to perform. The Progress pane reports the completion status.

❖ To shrink a library

1. Select the library in the Library Explorer pane.

   Note Make backup copies of libraries before you shrink them. For additional information, see To back up a library.

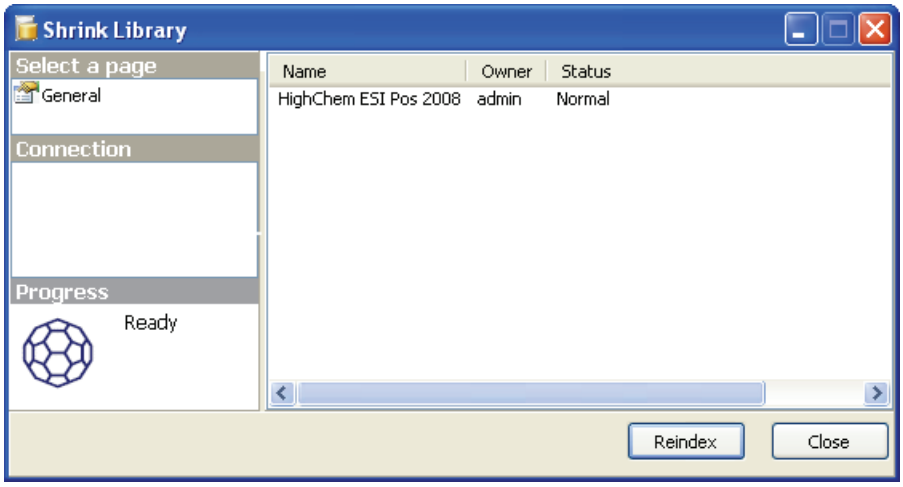
2. Do one of the following:

   Choose **Library > Shrink Library** from the Server Manager main menu.

   –or–

   Right-click and choose **Shrink Library** from the shortcut menu.

   The Shrink Library window opens.



Some operations, such as Conversion or Import Library, cause inflation of library files that might require reducing the size of the library. For additional information, see "To convert a library to Mass Frontier 7.0 format" on page 169 or "Importing the NIST/EPA/NIH Mass Spectral Database" on page 171.

The Mass Frontier 7.0 application uses library formatting that is not compatible with previous versions. To use libraries created in older versions of the application, you must convert them to the new format. Converting a library requires connecting to its corresponding SQL Server.

Because previous versions of the SQL Server were not configured for network operations, you must reconfigure your previous version of the SQL Server to enable network operations. For additional information, see "To reconfigure a previous version of the Mass Frontier SQL Server" on page 170.

❖ To convert a library to Mass Frontier 7.0 format

1. Select the library in the Library Explorer pane.
2. Do one of the following:

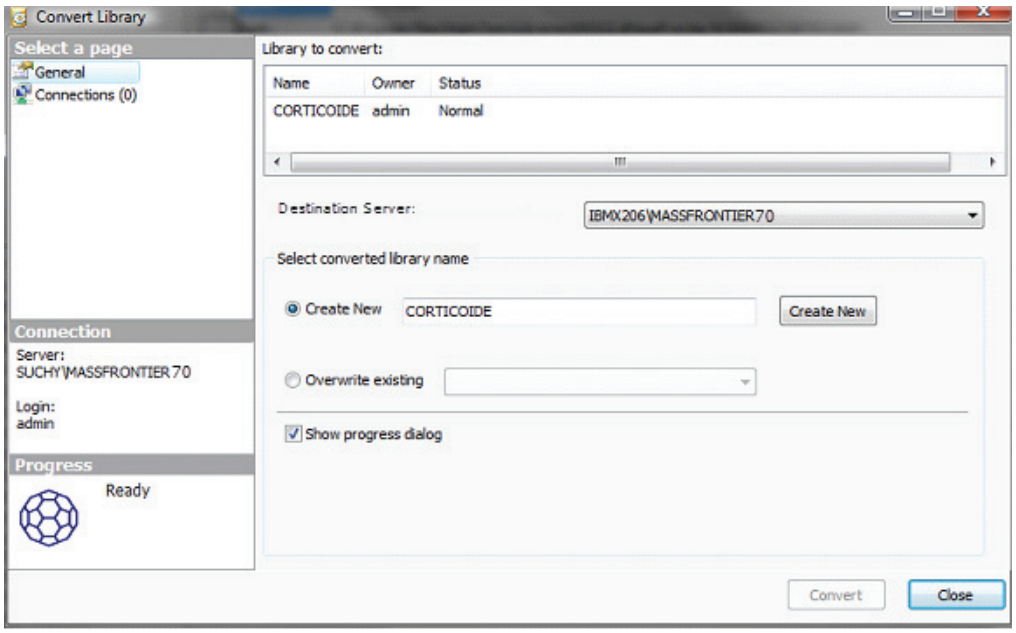
   Choose **Library > Convert Library** from the Server Manager main menu.

   –or–

   Right-click and choose **Convert Library** from the shortcut menu.

   Note If the selected library is already in Mass Frontier 7.0 format, the Convert Library commands are not available.

   The Convert Library window opens.



3. From the Destination Server list, select a server.
4. To select a destination for the imported library, do one of the following:
   a. Select the **Create New** option to create a new library for the converted library.
   b. Type the name of the new library and click **Create New**.

      The New Library dialog box opens.

      –or–

   a. Select the **Overwrite Existing** option to place data into an existing library on the selected server.
   b. Select the destination library from the list.
5. Click **Convert**.

❖ To refresh a library list

1. Select the library folder in the Library Explorer pane.

   The application does not automatically update the list of available libraries. To see the current list of libraries, you must manually refresh the list.

   The Refresh Library List icon is enabled only when a library folder is selected.

2. Click the **Refresh Library List** icon,  .

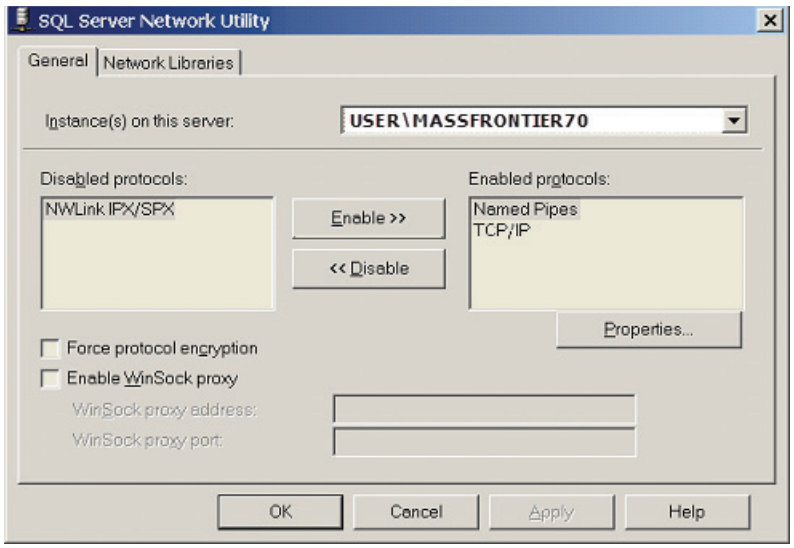
❖ To reconfigure a previous version of the Mass Frontier SQL Server

> **Note** Because previous versions of the SQL Server were not configured for network operations, you must reconfigure your previous version of the SQL Server to enable network operations. For additional information, see To reconfigure a previous version of the Mass Frontier SQL Server.

1. From the Windows taskbar, choose **Start** > **Run**.
2. In the Open box, type **svrnetcn** and click **OK**.

   The SQL Server Network Utility dialog box opens.

   Figure 60. SQL Server Network Utility dialog box



3. In the Instances on This Server list, select an instance of SQL Server to configure.
4. In the Disabled Protocols list, select **Named Pipes** and **TCP/IP**.
5. Click **Enable >>**.

   The application moves the selected protocols to the Enabled Protocols list.

6. Click **OK**.
7. Restart the SQL Server Service or restart your computer.

Importing the NIST/EPA/NIH Mass Spectral Database

The Mass Frontier application supports NIST/EPA/NIH Mass Spectral Database 2005 (NIST 2005 Library). You can either install the NIST 2005 Library—if the library is in the Mass Frontier format—or import it in NIST (.mdf) file format. While the NIST library is importing, the data is converted to the Mass Frontier database format (SQL Server). This process can take up to several hours.

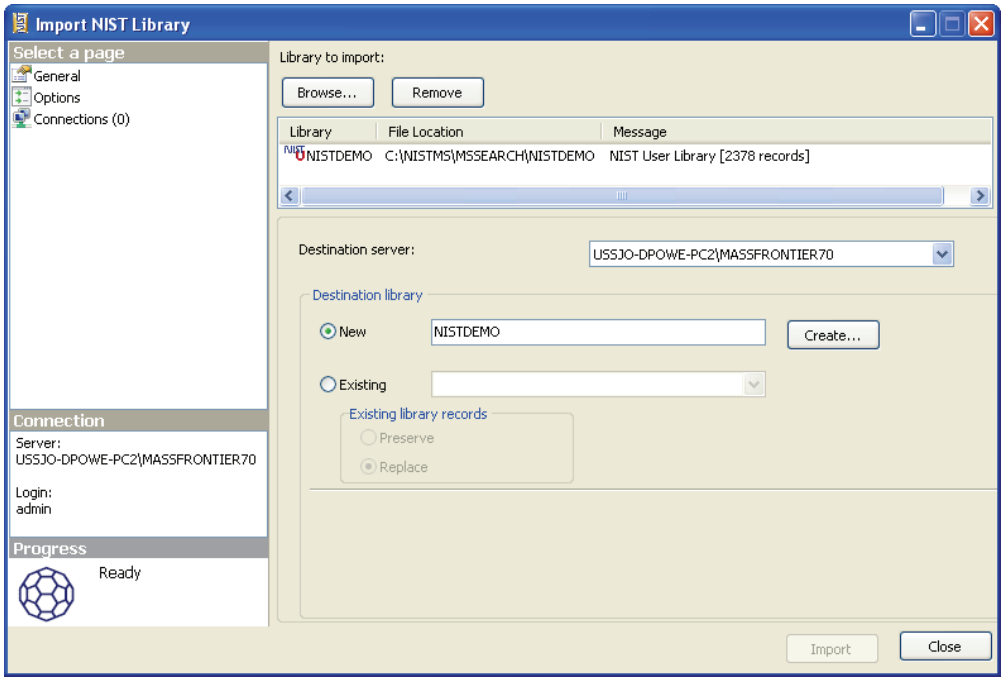
Follow these procedures:

- To import the NIST Library or any user library in NIST format
- To specify mass spectra, ionization mode, or precursor-ion data from a NIST spectra

❖ To import the NIST Library or any user library in NIST format

1. Choose **Library > Import NIST Library** from the Server Manager main menu.

   The Import NIST Library dialog box opens.



2. In the Library to Import area, click **Browse** and select the NIST library.
3. In the Destination Server list, select a server.

4. To select a destination for the imported library, do one of the following:
   a. Select the **New** option to create a new library for the imported library.
   b. Type the name of the new library and click **Create**.

      The New Library dialog box opens. For additional information, see To create a new user library.

      –or–

   c. Select the **Existing** option to place data into an existing library on the selected server.
   d. Select the destination library from the list.
5. Click **Import**.

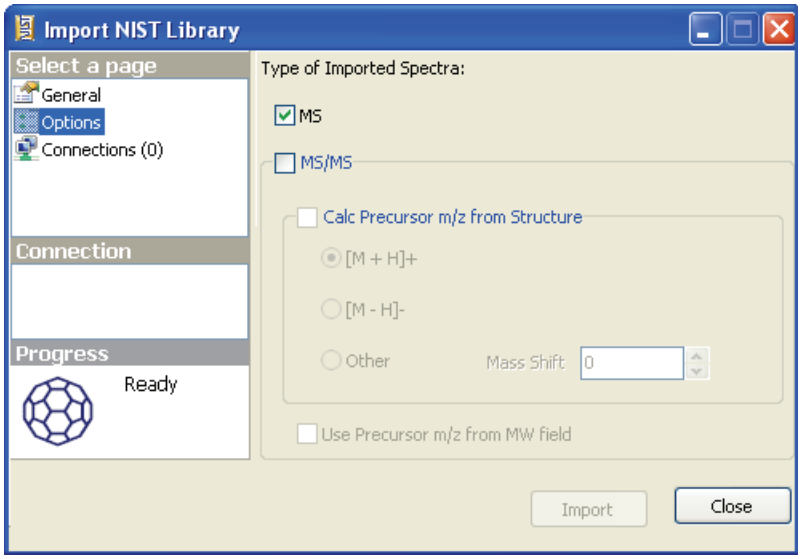
The Mass Frontier application does not recognize the mass spectra type, ionization mode, or precursor-ion information from a NIST spectra.

❖ To specify mass spectra, ionization mode, or precursor-ion data from a NIST spectra

1. In the Select a Page pane, select **Options**.

   The Options page opens.

   Figure 61. Options page of the Import NIST Library dialog box



2. Specify the options for MS or MS/MS imported spectra.
3. Click **Import**.

   When the import job is completed, the Import NIST Library dialog box closes.

Error Logs

When the Mass Frontier application encounters problems with libraries or the Server Manager, it saves the errors to two error log files: the native SQL Server error log and the Server Manager error log.

Follow these procedures:

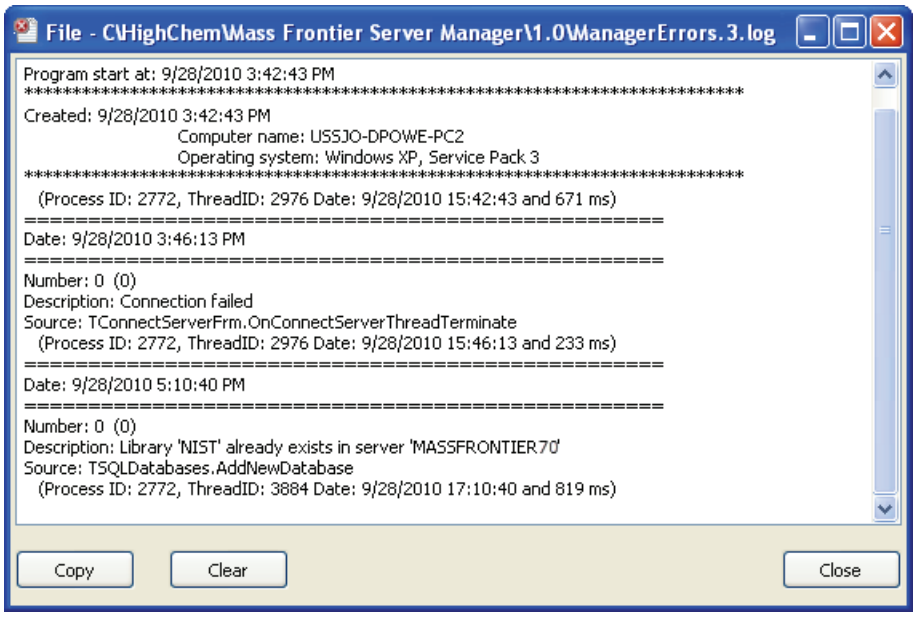
- To open the Server Manager error log
- To open the SQL Server error log

❖ To open the Server Manager error log

1. Choose **File > Program Error Log** from the Server Manager main menu.

   The Manager Errors log file opens.

   Figure 62. Manager Errors log file



2. To copy the log text to the Clipboard, click **Copy**.

   The Copy command copies all text in the log; you cannot select only a portion of the text to copy.

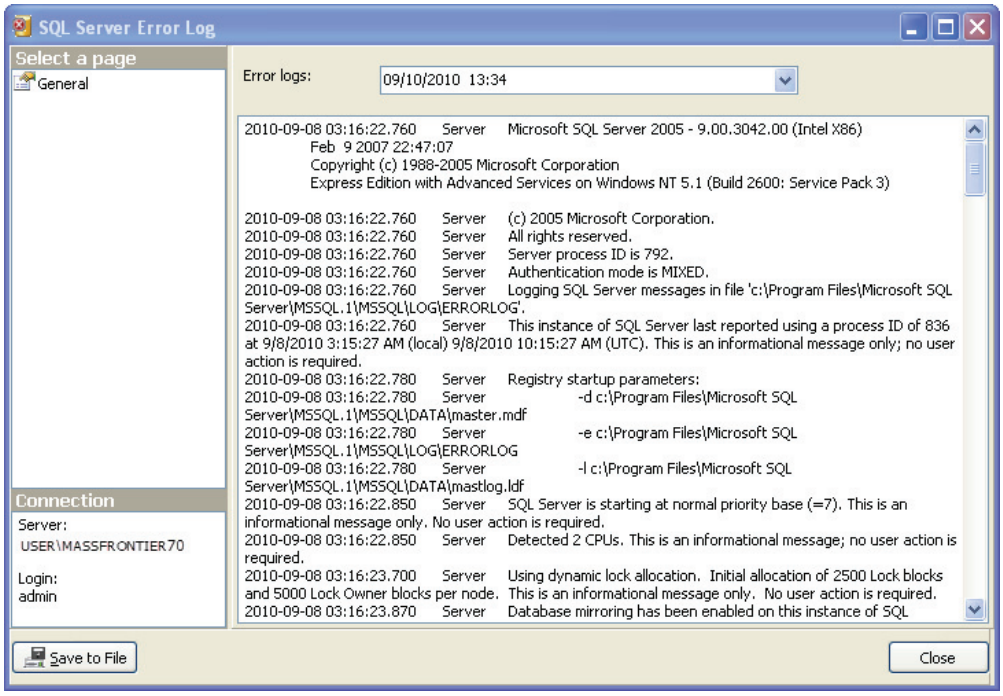
3. To delete all entries in the log, click **Clear**.

   You cannot undo this command.

❖ To open the SQL Server error log

1. Choose **Server > SQL Server Error Log** from the Server Manager main menu.

   The SQL Server Error Log window opens.



2. Select a specific log file from the Error Logs list.

   The SQL Server maintains six error logs. Each time you start a new instance of the SQL Server, a new log file begins.

3. To save the current log file to a text file, click **Save to File**.

   The Save to File command saves all text in the log; you cannot select only a portion of the text to save.

Using the Microsoft SQL Server 2005 Express Edition

The Mass Frontier library utilities are based on the Microsoft SQL Server 2005 Express Edition (SQL Express). SQL Express is a database engine based on core SQL Server technology. It is a reliable storage engine and query processor for desktop applications. SQL Express is a royalty-free, distributable database engine that is fully compatible with SQL Server. SQL Express is included in the Mass Frontier installation.

SQL Express is the background application for the Mass Frontier application. Library utilities are integrated in the graphical interface; you do not directly interact with the database engine.

The SQL Express database engine creates databases with a maximum file size of 4 GB. Any libraries created in the application cannot exceed this file size. If you try to store data above this limit, the Mass Frontier application informs you that this action cannot be completed and you should store the extra data in a new library.

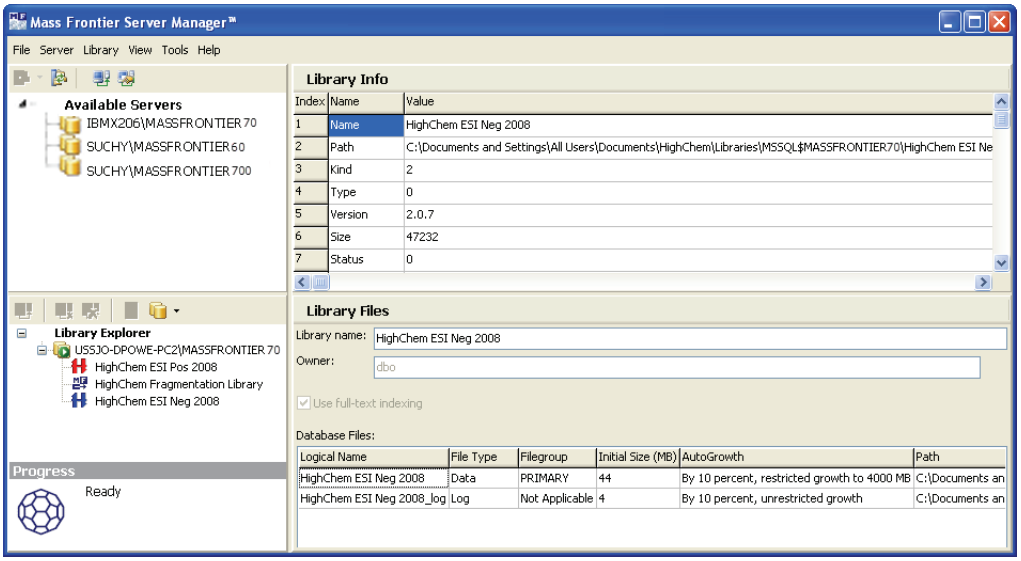
This file size issue occurs more often with chromatographic libraries than with spectral libraries. For example, the NIST 2002 database with 175000 spectra takes up 800 MB in the SQL Express format. However, if you must create large spectral or chromatographic databases, you can upgrade SQL Express to the higher SQL Server editions (for example, Enterprise, Standard, or Personal) that have terra byte capacity. The Mass Frontier libraries are fully compatible with other editions of the SQL Server. A database expert must individually set up later SQL Server editions. For additional information on how to upgrade SQL Express, contact Microsoft or the HighChem, Ltd. database group.

SQL Express is managed by the Mass Frontier Server Manager.

❖ To start the Server Manager

Choose **Library > Server Manager** from the Mass Frontier main menu.

Figure 63. Server Manager window



Using the HighChem Spectral Tree Library

The HighChem spectral trees (MS^n spectra) are available in the Mass Frontier application. This collection[1] contains high quality ion trap spectra of human and veterinary pharmaceuticals, endogenous metabolites, drugs of abuse, and doping agents. The library contains spectral trees acquired by electrospray ionization (ESI) in both the positive and negative ion modes. Each spectral tree contains a name, structure, and CAS number. The HighChem Spectral Tree Library contains more than 13 000 individual spectra of 1251 positive and 524 negative ESI spectral trees. In addition, 702 positive and 263 negative ESI spectral trees are associated with fragmentation mechanisms[2].

IMPORTANT HighChem owns all the intellectual property rights to spectral, structural, and fragmentation data in the HighChem Spectral Tree Library. You may not copy, translate, distribute, publish, or use this data in connection with other applications without the written approval of HighChem, Ltd., Slovak Republic.

[1] Compiled by Dr. Ernst Pittenauer, Vienna University of Technology, Getreidemarkt 9/164, A-1060 Vienna, Austria and Dr. Petr Simek, Academy of Sciences of the Czech Republic, Branisovska 3, CZ-37005 Ceske Budejovice, Czech Republic

[2] Dr. Ernst Pittenauer, Vienna University of Technology, Getreidemarkt 9/164, A-1060 Vienna, Austria

Managing Library Records in the Database Manager

Use the Mass Frontier Database Manager to add new records (spectra, trees, chromatograms, structures, compound identification information, and fragmentation mechanisms) to a user library. A user library is any library you created in the Server Manager module. For instructions, see To create a new user library.

You can change and save any record information (spectra, trees, chromatograms, structures, compound identification information, and fragmentation mechanisms) in a library. To indicate a change in a record, the application displays a small circle in the ID number column of the Database Manager Spreadsheet view. Changes in records are not automatically updated in a database; you must manually save them. For additional information, see Chapter 2, "Database Manager Module."

Follow these procedures:

- To open the Database Manager window
- To add records to a user library
- To save changes in a library
- To delete library records

❖ To open the Database Manager window

1. Do one of the following from the Mass Frontier application window:

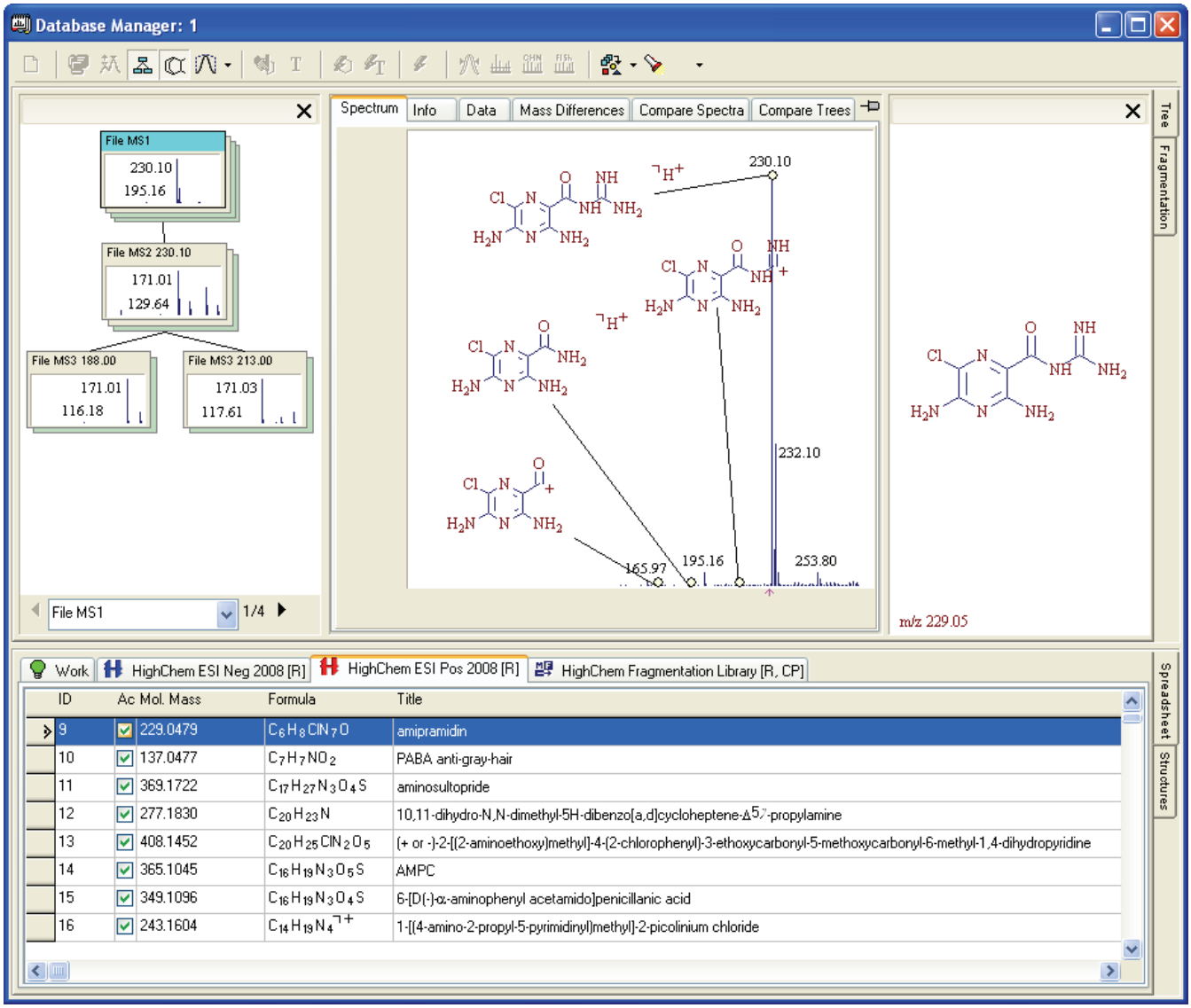
   Choose **Tools > Database Manager** from the Mass Frontier main menu.

   –or–

   Click the Database Manager icon,  , in the Mass Frontier toolbar.

   The Database Manager window opens.

Figure 64. Database Manager window

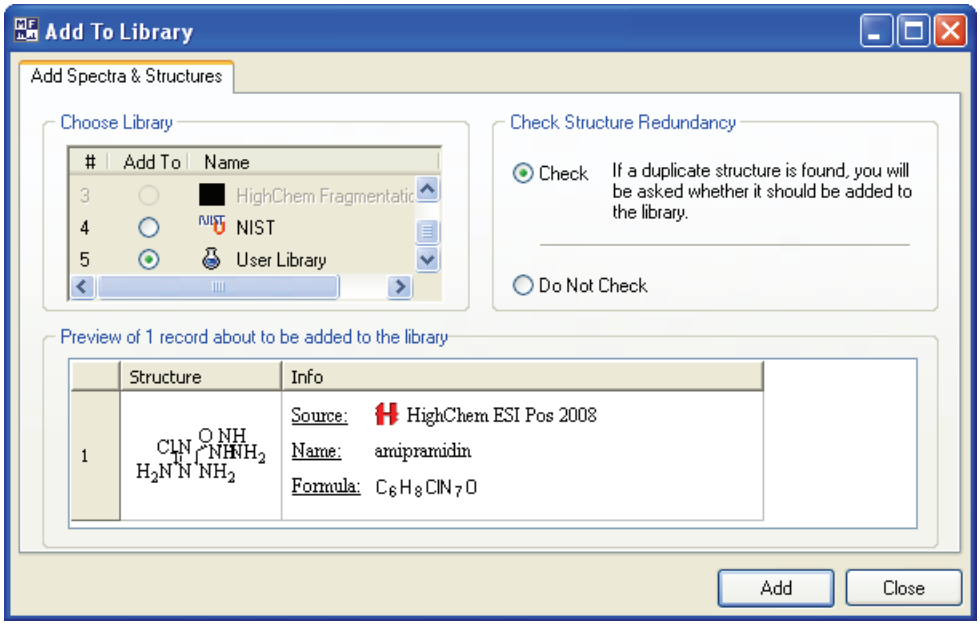


You can add a new record in the Database Manager in the Work tab only. You can update an existing record in any library that is not read-only.

❖ To add records to a user library

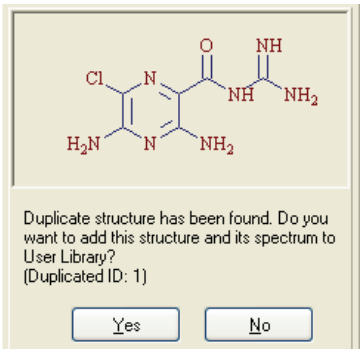
1. In the Spreadsheet of any library in the Database Manager, select the records you want to add to the user library.
2. Choose **Library > Add To Library** from the Mass Frontier main menu.

   The Add To Library window opens.



3. In the Choose Library area, select the destination library.
4. (Optional) Turn off redundancy checking by selecting the **Do Not Check** option.

   By default, the application checks whether the structure associated with the spectrum you are adding to the user library is already present in the library. The application compares each structure to be added to the user library with structures already in the library. If the structure already exists in the library, the following message appears:



5. Click **Add**.

Note A single structure accompanying a spectrum in a user library cannot contain more than 199 non-hydrogen atoms.

❖ To save changes in a library

1. In the Database Manager, select the a library you want to save.
2. Choose **Library > Save Changes In Library** from the Mass Frontier main menu.

When you save a newly created record, the application redirects you to the Add To Library dialog box. For additional information, see To add records to a user library.

Note If you close a Database Manager window or the Mass Frontier application with unsaved changes, the application prompts you to save them.

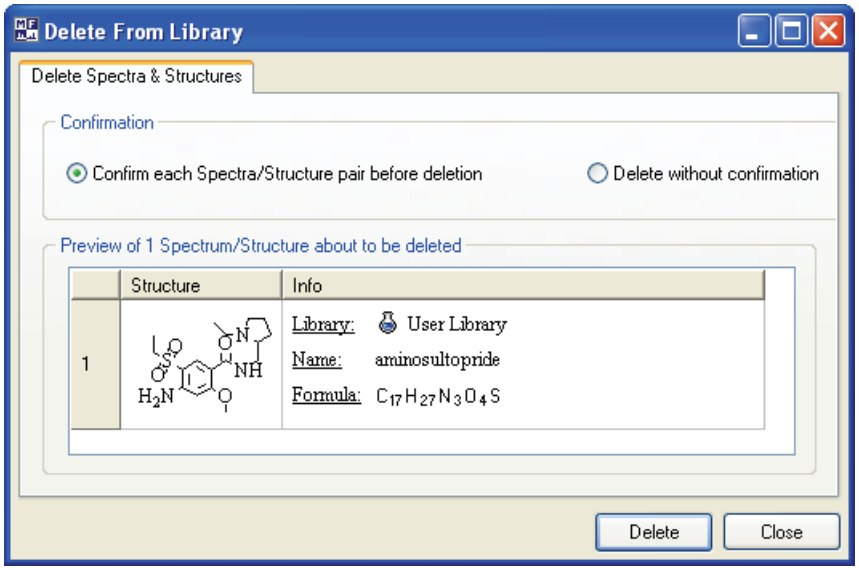
❖ To delete library records

1. Use any search method to retrieve the record or records you want to delete.

   IMPORTANT When you delete a library record, the information is irreversibly lost. You cannot undo the delete library record function. You cannot delete library records from read-only libraries.

2. Select the records you want to delete in the Database Manager Spreadsheet.
3. Choose **Library > Delete from Library** from the Mass Frontier main menu.

   The Delete From Library window opens.



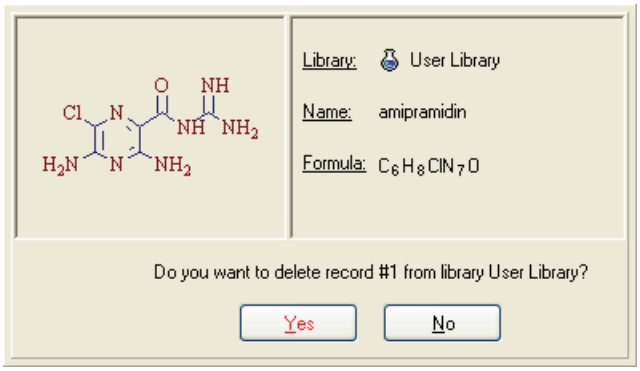
4. Carefully review the spectra and structures.

5. Do one of the following:

   a. To delete all displayed records at once, select the **Delete Without Confirmation** option.

   b. To delete record-by-record, select the **Confirm Each Spectra/Structure Pair Before Deletion** option.

      The application prompts you to confirm the deletion of each selected record in the library, even if you selected only one. For example:



6. Click **Delete**.

   When you delete a record from a library, the application automatically decrements the ID numbers of records with a higher ID than the deleted record so that there are no ID number gaps in the library.

Searching Chromatographic Libraries

The Mass Frontier application can create searchable libraries of data-reduced chromatographic data. You can export retention times, TIC profiles, selected scans, or detected component spectra or trees to user libraries and search them using various criteria. Use this feature to search a particular spectrum in a series of GC/LC/MS^n library records (target analysis) or to perform cross matching of all scans or components in a chromatogram against spectral data of other chromatographic records (dereplication). Chromatograms are organized in a similar manner to spectral libraries, the difference being that a single library entry represents a data-reduced chromatogram rather than a simple spectrum or tree.

For additional information, see "Exporting Scans, Components, and Reduced Chromatograms" on page 325 or "Searching Components" on page 334.

Fragments and Mechanisms Module

The Mass Frontier application can automatically generate possible fragments, including complete fragmentation and rearrangement mechanisms starting from your chemical structure. The Fragments & Mechanisms module provides information about basic fragmentation and rearrangement processes that might occur in a mass spectrometer.

Contents

Generated fragments and corresponding mechanisms are particularly useful for the following:

- Checking consistency between a chemical structure and its mass spectrum
- Confirming library search identifications
- Recognizing the structural differences between spectra of closely related compounds
- Interpreting the spectra of isotopically-labeled compounds
- Illustrating the structure-spectra relationship for educational purposes

Features

The Fragments & Mechanisms module uses a mathematical approach to simulating unimolecular ion-decomposition reactions. For additional information, see "Previewing Unimolecular Reactions" on page 193.

The system, which generates possible fragmentation and rearrangement pathways, is based on these assumptions:

- General fragmentation and rearrangement rules

  The system optionally predicts reaction pathways that are based on general fragmentation and rearrangement rules. This feature does not include compound-specific mechanisms that cannot be applied generally. At first, this might seem to be a disadvantage; however, you can use this feature in combination with a substructure search for identifying specific compound classes. For additional information, see "Unexplained Peaks" on page 219.

- Fragmentation Library mechanisms

  The system optionally accesses an intelligent fragmentation mechanism knowledge base for predicting unimolecular decomposition reactions. HighChem Fragmentation Library currently contains approximately 19000 individual mechanisms. You can also include your own mechanisms in fragmentation prediction. For additional information, see "Base Page" on page 201, Chapter 7, "Fragmentation Library," , or "Using Library Reactions in Fragmentation Prediction" on page 237.

- 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. For additional information, see "Resonance Page" on page 209.

  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.

- Unimolecular linear reaction mechanisms

  The Mass Frontier 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.

- Even-Electron rule

  The application generates reaction mechanisms in accordance with the Even-Electron rule. The Even-Electron rule says that the homolytic bond cleavages of an even electron ion are energetically unfavorable. This means that the application never generates radical cations from an even-electron ion.

- Bond cleavages only

  Using the General fragmentation and rearrangement rules option, the application can only generate fragments from bond cleavages. It does not support bond creation, except creation of an H-*X* bond (*X* = any element) in hydrogen rearrangements. For this option, the system does not include ring contractions, cyclizations, or skeletal and non-hydrogen rearrangements. The Fragmentation Library option supports bond creation with all rearrangements and ring transformations. For additional information, see "H-Rearrangements Page" on page 207 or Chapter 7, "Fragmentation Library."

- Ionization methods

  The application supports electron impact, protonation, deprotonation, cluster ion formation, alkali metal adducts, and chemical ionization methods.

- Formally possible solutions

  The mechanisms generated by the Mass Frontier 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, multistep hydrogen rearrangements.

Generating 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 the Structure Editor, the Database Manager, or a Fragments & Mechanisms window. Before the Mass Frontier application generates the program, it checks the input structure for errors. If it finds any errors, a message box displays the errors and cancels the generation.

- When you start a generation from the Structure Editor, the application assumes that the complete structure is intended as input for the generation.
- When you start a generation from the Database Manager window, the application automatically links the generated fragments in the Fragments & Mechanisms window with the corresponding spectrum in the Database Manager window. The application highlights peaks that have the same *m/z* value as the generated fragments. Selecting a highlighted peak reveals all the pathways leading to it. By default, peaks are highlighted in red.

❖ To start a generation of possible fragmentation and rearrangement pathways

1. Do one of the following:

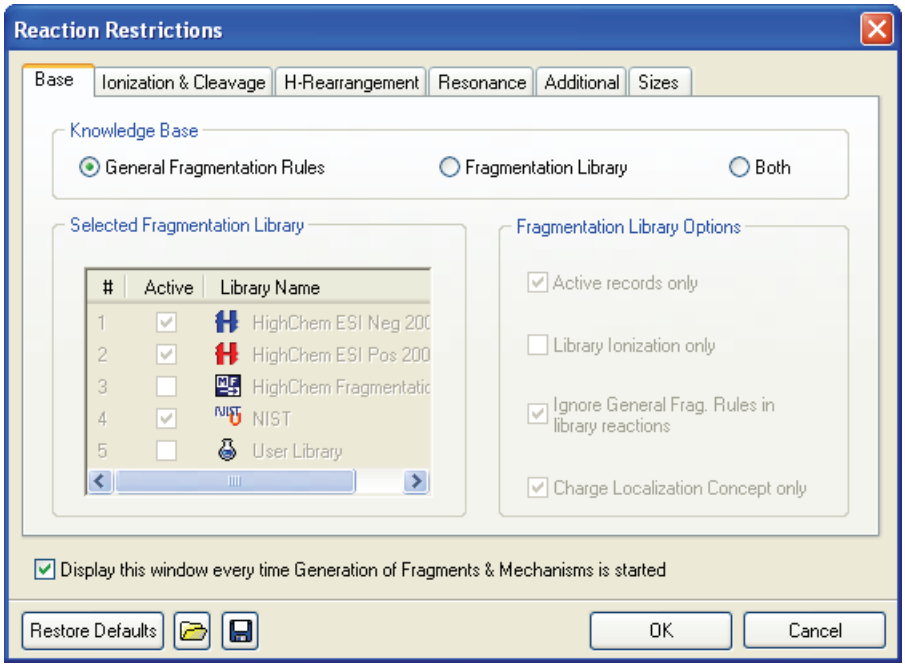
   Click the **Fragments and Mechanism** button,  , in the Mass Frontier toolbar.

   –or–

   Choose **Tools > Fragments and Mechanisms** from the Mass Frontier main menu.

   The Reaction Restrictions dialog box opens. For detailed information about the Reaction Restrictions dialog box, see "Specifying Reaction Restrictions" on page 198.

Figure 65. Reaction Restrictions dialog box



2. Specify your parameters in the Reaction Restrictions dialog box and click **OK**.

   The Generation of Fragments & Mechanisms dialog box displays the progress as the application checks for structure errors. See "Generation of Fragments & Mechanisms dialog box" on page 187.

   When the checks are complete, the Fragments & Mechanisms window opens. See "Using the Fragments & Mechanisms Window" on page 189.

Figure 66. Generation of Fragments & Mechanisms dialog box

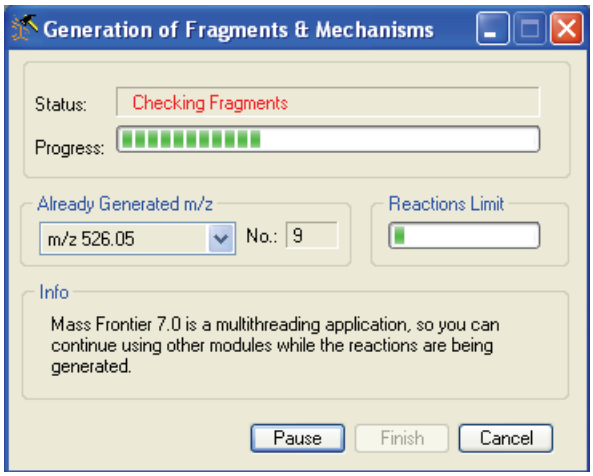


Table 22. Generation of Fragments & Mechanisms parameters (Sheet 1 of 2)

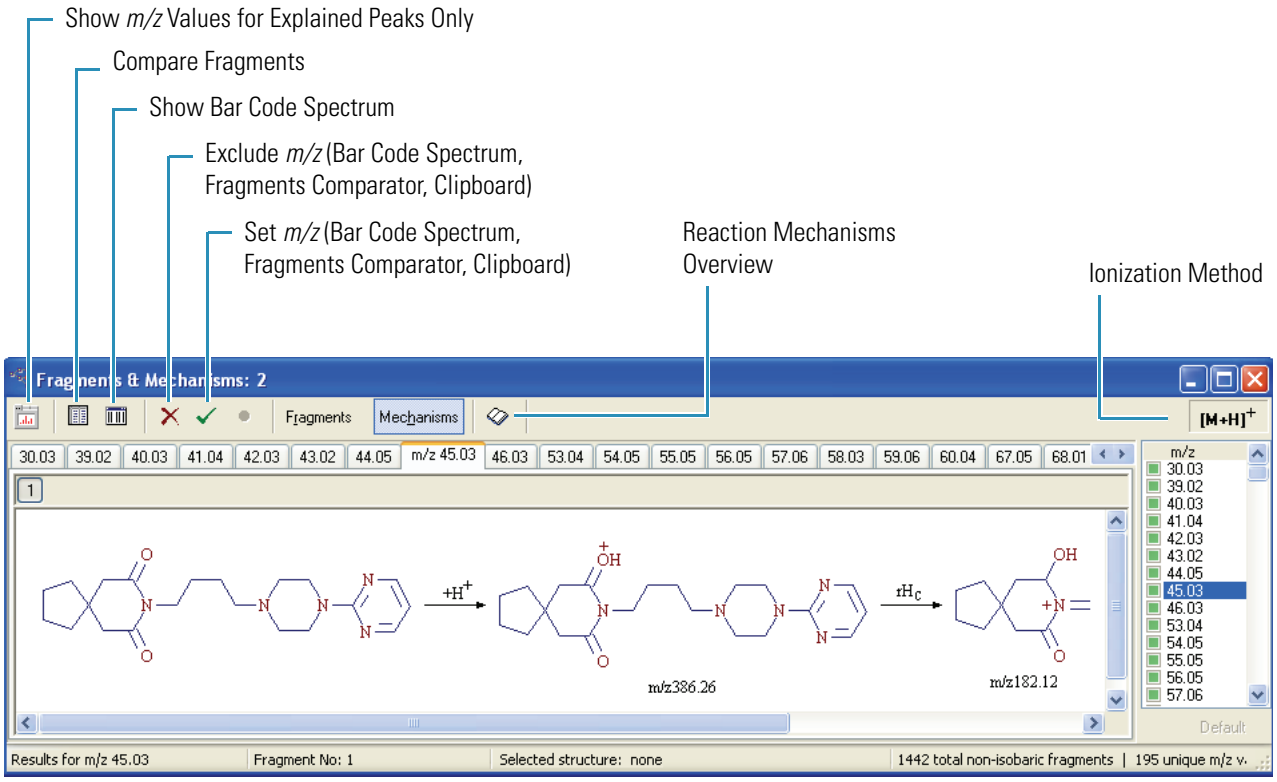
Parameter	Description
Status	Reports the current check.
Progress	Status bar reports the relative completion state of the current check.
Already Generated *m/z*	List of *m/z* values of the ions that have already been generated and the total number of ions that have already been generated.

Table 22. Generation of Fragments & Mechanisms parameters (Sheet 2 of 2)

Parameter	Description
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 an error 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. However, if you are missing an important fragment because you assume the generation was interrupted, you can increase the reactions limit. For additional information, see "Sizes Page" on page 213.
Info	With this multithreading strategy, you can simultaneously perform multiple tasks. For example, while the application is generating reactions, you can search libraries or analyze your spectra. You can also run two or more generations of fragments and mechanisms at the same time.
Pause	Temporarily interrupts generation to redirect processing power 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.

Using the Fragments & Mechanisms Window

When the application finishes a reaction-generating process, a Fragments & Mechanisms window opens. You cannot directly open this window from any menu or toolbar. A Fragments & Mechanisms window displays either the complete mechanisms of ion-decomposition reactions or only the resulting fragments.



Follow these procedures:

- To customize the m/z tab display
- To display a mechanism or fragment for an m/z value
- To copy fragments or mechanisms
- To display the isobaric fragments for a particular m/z value

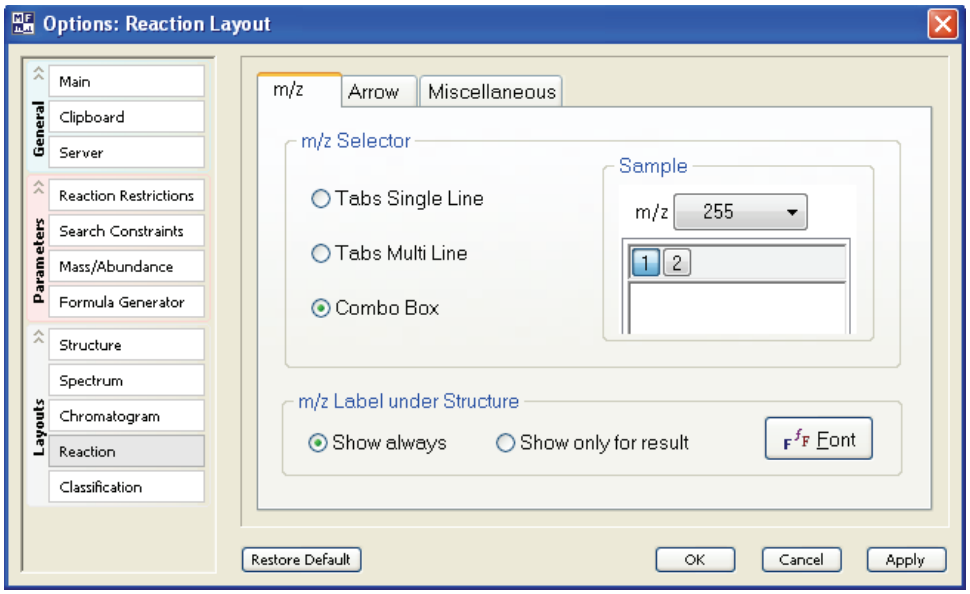
❖ To customize the *m/z* tab display

1. Choose **Options > Settings** from the Mass Frontier main menu.

   The Options dialog box opens.

2. Click **Reaction** in the Layouts section.

   The *m/z* page of the Options: Reaction Layout dialog box opens.



3. Specify the display style for your *m/z* tabs.

   The Sample area shows how each display style will look.

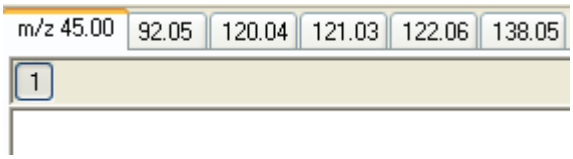
4. Click **OK**.

   The next time you generate fragments and mechanisms, the application uses the specified display changes. Current Fragments & Mechanisms windows are not affected.

❖ To display a mechanism or fragment for an *m/z* value

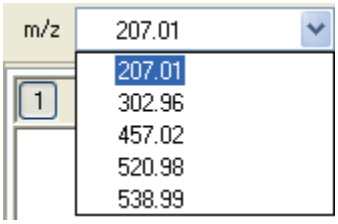
Do one of the following:

Click the appropriate *m/z* tab.



–or –

Choose the *m/z* value from the *m/z* list.



The display style depends on the settings you specified in the Options dialog box.

❖ To copy fragments or mechanisms

1. Click the **Copy** button on the Mass Frontier main menu and choose one of the following:

   - Mechanism

     This command copies mechanisms in both graphic format (Enhanced Windows Metafile) and data format.

   - Fragment

     This command is available only when you have a selected fragment in the Fragments & Mechanisms window.

   - List of Fragments

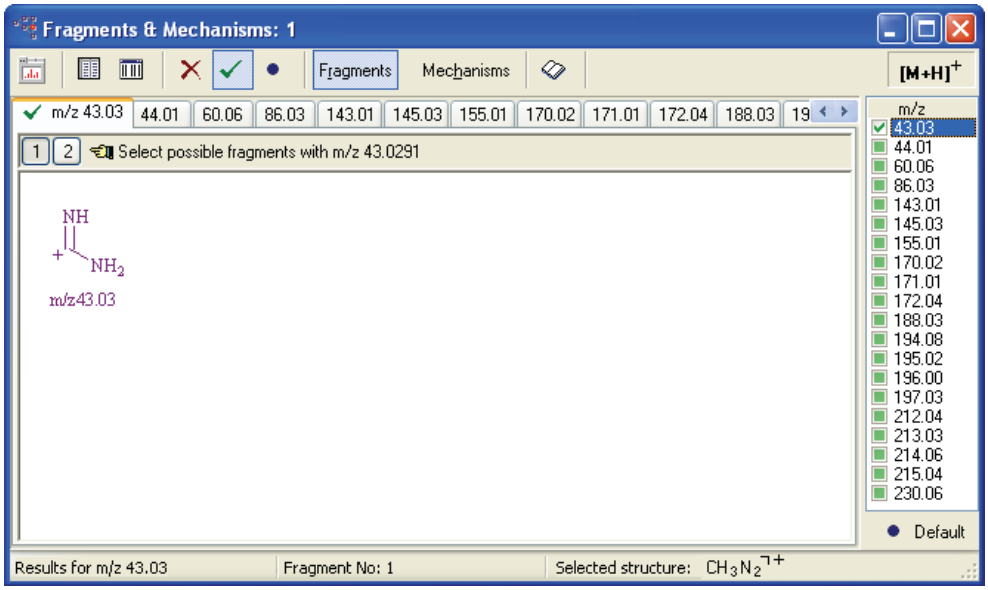
     This command copies all fragments listed in the Fragments & Mechanisms window.

2. Paste the copied element into a Structure Editor window, a Database Manager window, or any Windows application.

❖ To display the isobaric fragments for a particular *m/z* value

You can generate several possible isobaric fragments for a particular *m/z* value. When more than one fragment is generated for an *m/z* value, the application displays each numbered fragment separately.

To display the isobaric fragments with their corresponding mechanisms, click the numbered button next to the hand pointer.



The fragments are sorted according to the simplicity of their production mechanism, so fragment No.1 is produced by the simplest (shortest) mechanism. The isobaric fragments are usually isomers of the same fragments with a different charge, radical, or p-bond location.

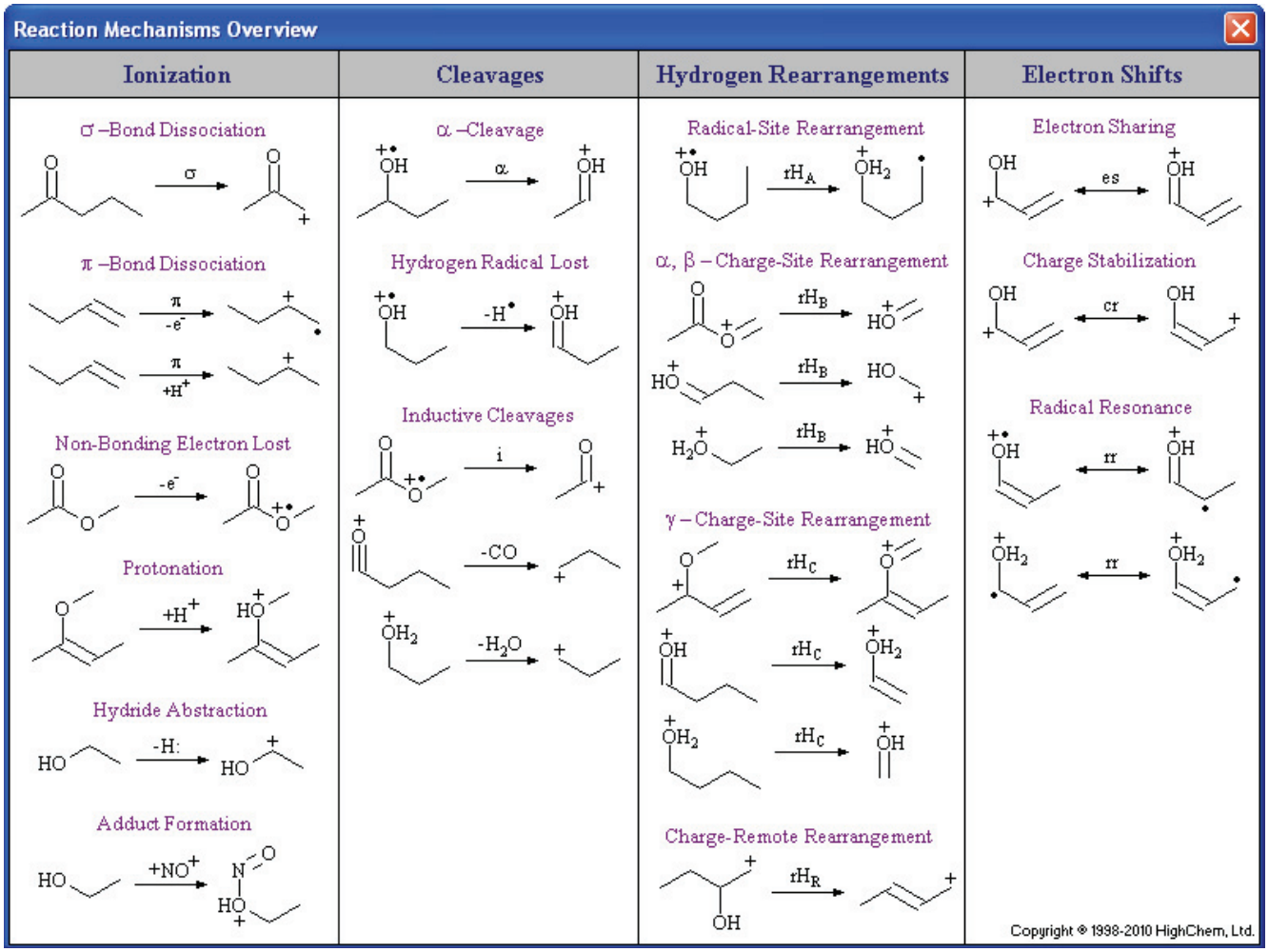
Previewing Unimolecular Reactions

The Mass Frontier application uses general unimolecular reactions for prediction of fragmentation, rearrangement, and resonance mechanisms.

❖ To preview unimolecular reactions

Click the **Reaction Mechanisms Overview** button,  , in a Fragments & Mechanisms window.

The Reaction Mechanisms Overview window opens.



Reaction Formalism

The reaction formalism used in the application follows these conventions:

α	Alpha cleavage
π	Pi electron ionization
σ	Sigma electron ionization
cr	Charge stabilization
-e⁻	Non-bonding electron ionization
es	Electron sharing
i	Inductive cleavage
$+H^+$	Protonation
$-H^\bullet$	Hydrogen radical loss
-H:	Hydride abstraction
rH_A	Radical site rearrangement
rH_B	Charge site rearrangement (α, β)
rH_C	Charge site rearrangement (γ)
rH_R	Charge-remote rearrangement
$rH_{1,2}$	Hydrogen shift to adjacent position
rr	Radical resonance
Lib	Fragmentation Library Mechanism

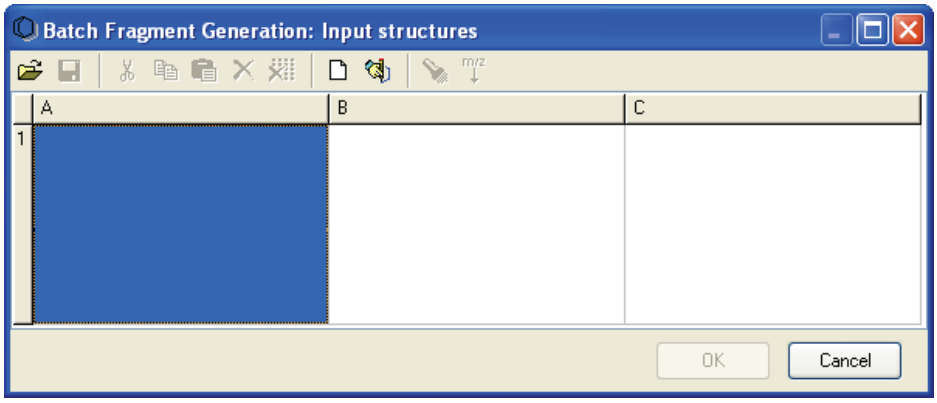
Generating Fragments for Multiple Structures

The Mass Frontier application supports batch processing for fragment prediction. You can generate fragments without mechanisms for multiple input structures in a single batch.

❖ To generate fragments for multiple input structures

1. Choose **Tools > Batch Fragment Generation** from the Mass Frontier main menu.

   The Batch Fragment Generation: Input Structures dialog box opens.



2. To add structures, do any of the following:

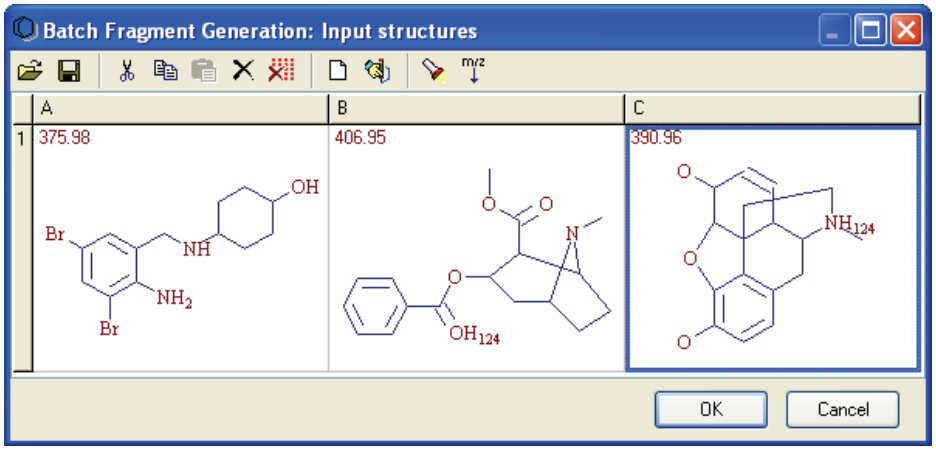
   Click the **Open** button,  , and import structures from .sdf or .mol files.

   –or–

   Paste structures from the Clipboard.

   –or–

   Create structures in the Structure Editor window, and then copy and paste them.

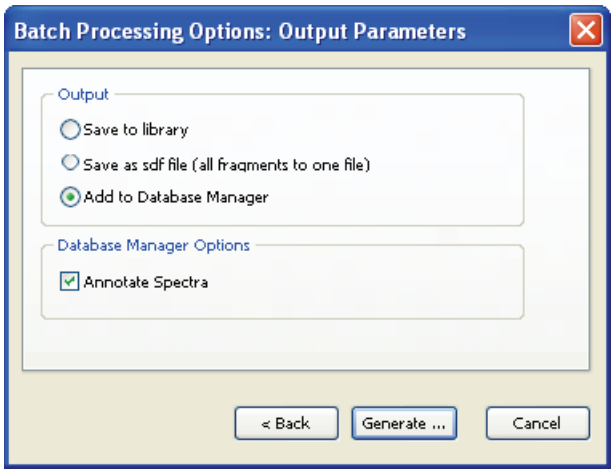


3. Click **OK**.

   The Batch Processing Options: Reaction Restriction dialog box opens. The parameters in this dialog box are almost identical to the Reaction Restrictions dialog box. See "Specifying Reaction Restrictions" on page 198.

4. Specify your parameters in the Batch Processing Options: Reaction Restriction dialog box and click **Next**.

   The Batch Processing Options: Output Parameters dialog box opens.



5. Select one of the following output options:

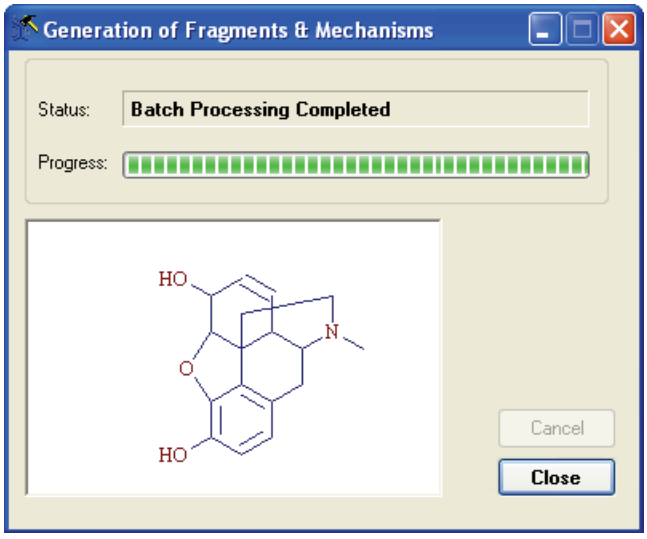
   - Save to a library.
   - Save in .sdf file format.

     This file format, defined by Molecular Design Ltd. (MDL), is an ASCII file format that can contain multiple compounds in .sdf file format.
   - Add to a new Database Manager window.

6. To annotate the spectra, select the **Annotate Spectra** check box.

7. Click **Generate**.

   The Generation of Fragments & Mechanisms dialog box reports the progress.



8. When the batch is completed, click **Close**.

   For each input structure, the application generates a separate set of fragments and saves them in the specified output format.

   Fragment generation is executed in its own thread, which means you can use other Mass Frontier functions during the generation.

Specifying Reaction Restrictions

Use the features in the Reaction Restrictions dialog box to change the default settings of the ionization method and the basic fragmentation and rearrangement mechanisms.

The Reaction Restrictions dialog box automatically opens when you begin generating fragments. You can also open the Options dialog box from the Mass Frontier main menu or toolbar and access identical Reaction Restrictions parameters.

This section includes the following topics:

- Managing Reaction Restrictions Files
- Using the Reaction Restrictions Dialog Box
- Specifying Precision and Isobaric Differentiation

❖ To open the Reaction Restrictions pages of the Options dialog box

1. Choose **Options > Settings** from the Mass Frontier main menu.
2. Click **Reaction Restrictions** in the Parameters section.

   The Reaction Restrictions pages of the Options dialog box open. The parameters are exactly the same as the parameters in the Reaction Restrictions dialog box that automatically opens when you generate fragments and mechanisms. See "Reaction Restrictions dialog box" on page 186.

Managing Reaction Restrictions Files

Use the Reaction Restrictions dialog box to specify many parameters for a fragment. You can save parameter values to a file and then import that file to apply the same parameter values to other fragments.

❖ To save reaction restrictions to a file

1. Specify the restrictions for all pages of the Reaction Restrictions dialog box.
2. Click the **Save** button,  .

   The Save Reaction Restrictions dialog box opens.
3. Type a file name for the file.

   The file type defaults to .rrs.
4. Click **Save**.

You can import these restriction settings when you generate fragments for similar compounds.

Note There is no Save option when you use the Reaction Restrictions pages of the Options dialog box.

❖ To import reaction restrictions from a file

1. Click the **Open** button,  .

   The Open Reaction Restrictions dialog box opens.

2. Select an .rrs file to import.

3. Click **Open**.

   The application overwrites all parameters in the Reaction Restrictions dialog box with the saved parameters in the file.

4. Click **OK** to make these restrictions active.

   All changes in the Reaction Restrictions dialog box take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.

Note There is no Open option when you use the Reaction Restrictions pages of the Options dialog box.

Using the Reaction Restrictions Dialog Box

The Reaction Restrictions dialog box automatically opens when you begin generating fragments. You can manually open identical pages of parameters from the Options menu.

This section includes the following topics:

❖ To restore default settings

Click **Restore Defaults**.

The application restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.

❖ To hide the Reaction Restrictions dialog box each time you start fragment generation

Clear the **Display This Window Every Time Generation of Fragments & Mechanisms Is Started** check box.

The application will not display the Reaction Restrictions dialog box when you start fragment generation.

If you want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the next procedure.

❖ To restore the Reaction Restrictions dialog box each time you start fragment generation

1. Choose **Options > Settings** from the Mass Frontier main menu.
2. Click **Reaction Restrictions** in the Parameters section.
3. Select the **Display This Window Every Time Generation of Fragments & Mechanisms Is Started** check box, and click **OK**.

   The Reaction Restrictions dialog box opens each time you start fragment generation.

Base Page

Use the Base page to specify the type of knowledge base the system uses for predicting fragmentation pathways. See "Base page of the Reaction Restrictions dialog box" on page 202.

Follow these procedures:

- To minimize the generation of useless fragments
- To disable automatic simulation
- To speed up fragment generation
- To exclude mechanisms with unspecified charge sites

❖ To minimize the generation of useless fragments

Some mechanisms of small fragments can fit virtually every structure and can generate an immense number of useless fragments.

1. In the Spreadsheet view for the library, clear the **Active** check box for these structures in the Fragmentation Library.

   For additional information about active records, see "Spreadsheet View" on page 67.

2. In the Reaction Restrictions dialog box, select the **Active Records Only** check box.

   The fragment generation ignores the inactive records in the Fragmentation Library.

❖ To disable automatic simulation

Select the **Library Ionization Only** check box and add an ionization mechanism to the library.

> **Note** When a neutral molecule is provided, the application automatically simulates the ionization process according to the selected ionization mode on the Ionization & Cleavage page. You must first disable this automatic simulation feature to use a different ionization site. For additional information, see "Ionization and Cleavage Page" on page 204.

❖ To speed up fragment generation

1. Select the **General Fragmentation Rules** option in the Knowledge Base area.

   The fragmentation library mechanisms must follow general fragmentation rules.

2. Clear the **Ignore General Frag. Rules in Library Reactions** check box in the Fragmentation Library Options area.

❖ To exclude mechanisms with unspecified charge sites

Select the **Charge Localization Concept Only** check box in the Fragmentation Library Options area.

This option excludes mechanisms in the fragmentation library that contain unspecified charge sites from the generation process.

Note Structures with an unspecified charge site in the fragmentation library or in the starting structure slow the generation process. When you use an unspecified charge site, the system must consider a large number of combinations for each step. When possible, avoid using unspecified charge sites in the fragmentation library.

Figure 67. Base page of the Reaction Restrictions dialog box

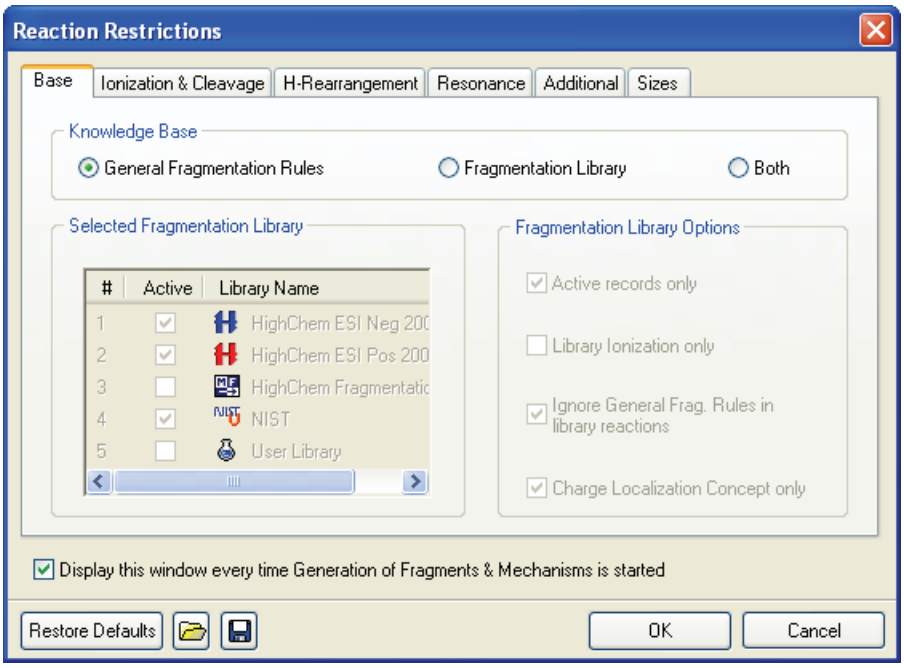


Table 23. Base parameters (Sheet 1 of 2)

Feature	Description
Knowledge Base	
General Fragmentation Rules	Specifies general rules for generating fragments and mechanisms.
Fragmentation Library	Activates the Selected Fragmentation Library and the Fragmentation Library Options areas where you can choose the libraries and options to use for generating fragments and mechanisms. The HighChem Fragmentation Library contains approximately 19000 mechanisms, so calculation times are significantly longer when you choose this library.

Table 23. Base parameters (Sheet 2 of 2)

Feature	Description
Both	Uses both the general rules and the selected libraries to generate fragments and mechanisms.
Selected Fragmentation Library	Specifies the fragmentation libraries to use for generating fragments and mechanisms.
Fragmentation Library Options	
Active Records Only	Ignores records in the Fragmentation Library that are not marked as Active. When this option is not selected, the fragmentation generation function considers all mechanisms from the selected libraries.
Library Ionization Only	Disables the automatic simulation of the ionization process.
Ignore General Frag. Rules in Library Reactions	Skips the checking of general fragmentation rules to speed up the generation.
Charge Localization Concept Only	Excludes mechanisms in the fragmentation library that contain unspecified charge sites from the generation process.
Display This Window Every Time Generation of Fragments & Mechanisms Is Started	Opens the Reaction Restrictions dialog box each time you start fragment generation. Clear this check box if you want to use the same parameter settings for subsequent fragment generations and do not want to see this dialog box each time. If you clear this option and want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the instructions "To restore the Reaction Restrictions dialog box each time you start fragment generation" on page 200.
Restore Defaults	Restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.
Open	Imports the restriction parameter values from an .rrs file.
Save	Saves the current restriction parameter values to an .rrs file.

Ionization and Cleavage Page

Use the Ionization & Cleavage page to specify the ionization method.

When comparing generated fragments and mechanisms with a mass spectrum, carefully choose the correct ionization method. The application warns you if the reaction restrictions are set for protonation techniques or chemical ionization and you are attempting to compare generated fragments with a spectrum from the NIST library, which contains only EI spectra. However, if the spectrum is from a file, the application does not check mass spectrum type and ionization techniques for consistency. For additional information, see "Importing the NIST/EPA/NIH Mass Spectral Database" on page 171.

Figure 68. Ionization & Cleavage page of the Reaction Restrictions dialog box

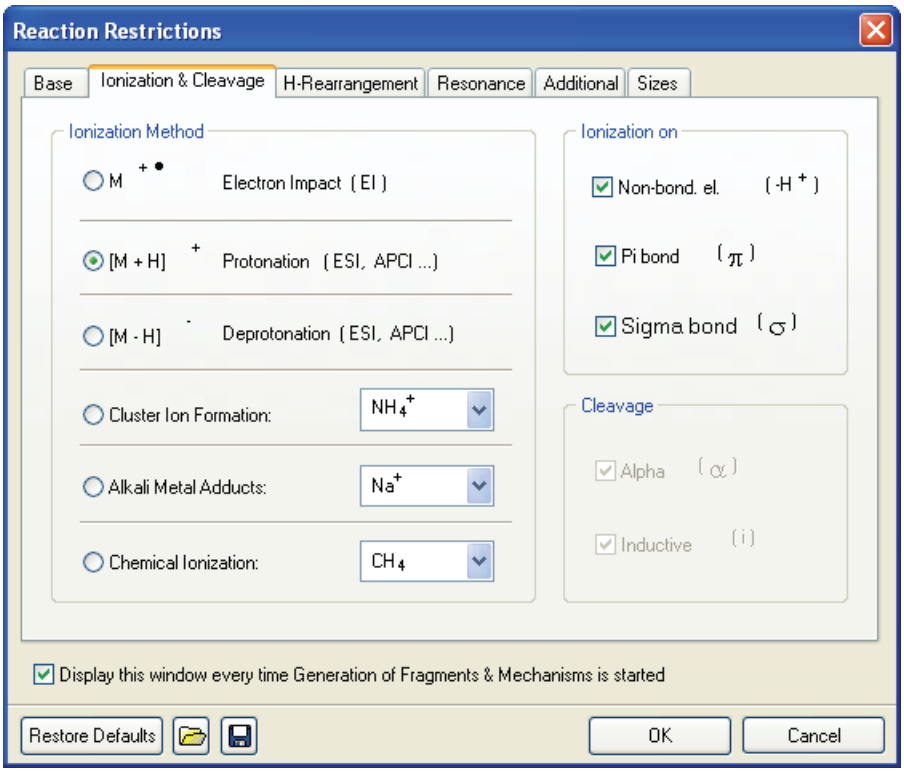


Table 24. Ionization & Cleavage parameters (Sheet 1 of 3)

Feature	Description
Ionization Method	
Electron Impact	Electron Impact (EI) mode that produces $M^{+\bullet}$ ions.
Protonation	Positive ionization $[M+H]^+$. 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 24. Ionization & Cleavage parameters (Sheet 2 of 3)

Feature	Description
Deprotonation	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. The deprotonation option does not support General Fragmentation Rules in the knowledge base. For additional information, see "Base Page" on page 201. You can only use deprotonation in connection with the Fragmentation Library. For additional information, see Chapter 7, "Fragmentation Library."
Cluster Ion Formation	$[M+NH_4]^+$ or $[M+H_3O]^+$
Alkali Metal Adducts	$[M+Li]^+$, $[M+Na]^+$, $[M+K]^+$
Chemical Ionization	The chemical ionization option offers three basic ionization reactions: protonation, hydride abstraction (see "Reaction Formalism" on page 194), and adduct formation. In addition, you can select one of six most common reaction gases: methane (CH_4), hydrogen (H_2), isobutane ($i–C_4H_{10}$), ammonia (NH_3), water (H_2O), and nitrogen monoxide (NO).
Ionization On	
Non-Bond El.	Performs ionization on non bonded electrons.
Pi Bond [π]	Performs ionization on Pi bonds. Available only when Electron Impact or Protonation ionization methods are selected.
Sigma Bond [σ]	Performs ionization on Sigma bonds. Available only when Electron Impact ionization method is selected.
Cleavage	
Alpha [α]	Use alpha cleavage when generating fragments and mechanisms. Available only when Electron Impact ionization method is selected.
Inductive [i]	Use inductive cleavage when generating fragments and mechanisms.

Table 24. Ionization & Cleavage parameters (Sheet 3 of 3)

Feature	Description
Display This Window Every Time Generation of Fragments & Mechanisms Is Started	Opens the Reaction Restrictions dialog box each time you start fragment generation. Clear this check box if you want to use the same parameter settings for subsequent fragment generations and do not want to see this dialog box each time. If you clear this option and want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the instructions "To restore the Reaction Restrictions dialog box each time you start fragment generation" on page 200.
Restore Defaults	Restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.
Open	Imports the restriction parameter values from an .rrs file.
Save	Saves the current restriction parameter values to an .rrs file.

H-Rearrangements Page

Use the H-Rearrangement page in the Reaction Restriction dialog box to specify the controls for setting four basic hydrogen rearrangements:

rH_A	Radical site rearrangement
rH_B	Charge site rearrangement (α, β)
rH_C	Charge site rearrangement (γ)
rH_R	Charge-remote rearrangement

The hydrogen rearrangements that involve radical (odd-electron ions) rH_A are set by default for hydrogen transfer from a steric optimal atom, usually from a γ-atom (McLafferty rearrangement). A hydrogen shift to an adjacent position (rH1,2) is activated by default and cannot be deactivated. For additional information, see "Reaction Formalism" on page 194.

There are two reasons for changing the default setup of rearrangements:

- You are missing an important peak and you suspect an unusual rearrangement. You can compel hydrogen transfer from an α, β, γ, or δ atom.
- You might want to simplify a mechanism by deactivating rearrangements that cause redundant reaction steps.

Figure 69. H-Rearrangement page of the Reaction Restrictions dialog box

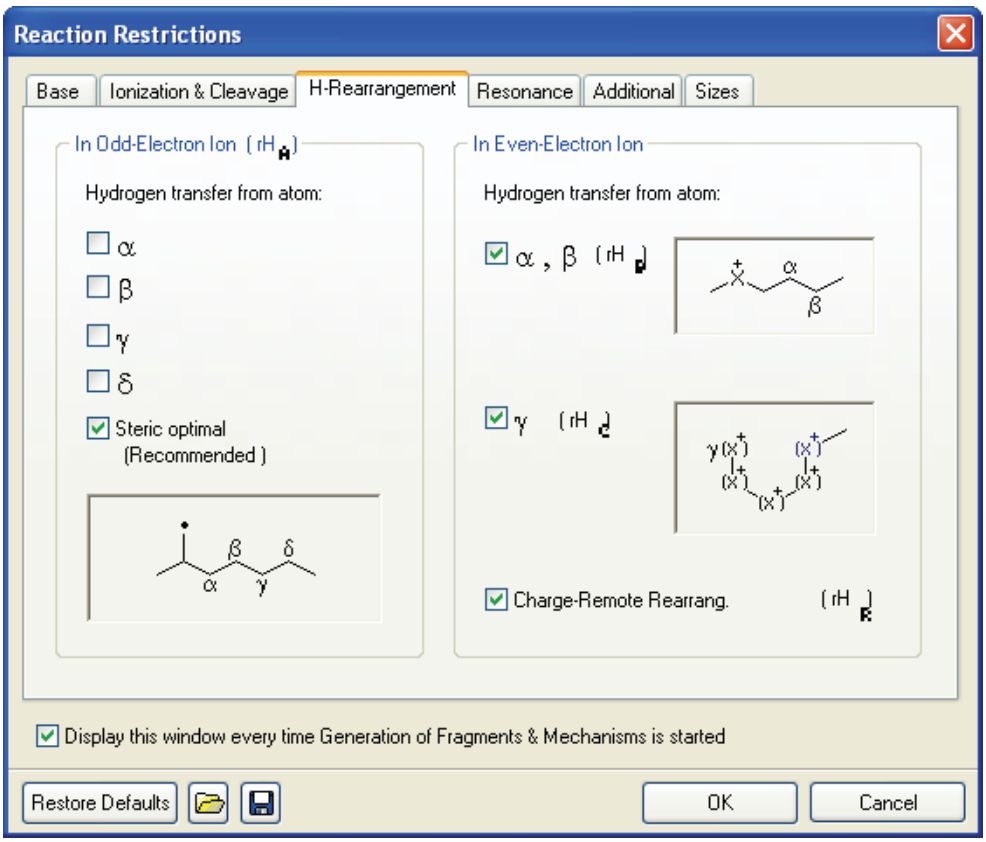


Table 25. H-Rearrangement parameters

Feature	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]
Display This Window Every Time Generation of Fragments & Mechanisms Is Started	Opens the Reaction Restrictions dialog box each time you start fragment generation. Clear this check box if you want to use the same parameter settings for subsequent fragment generations and do not want to see this dialog box each time. If you clear this option and want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the instructions "To restore the Reaction Restrictions dialog box each time you start fragment generation" on page 200.
Restore Defaults	Restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.
Open	Imports the restriction parameter values from an .rrs file.
Save	Saves the current restriction parameter values to an .rrs file.

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. If you have difficulty understanding such reduced mechanisms, you can display all resonance reactions.

Figure 70. Resonance page of the Reaction Restrictions dialog box

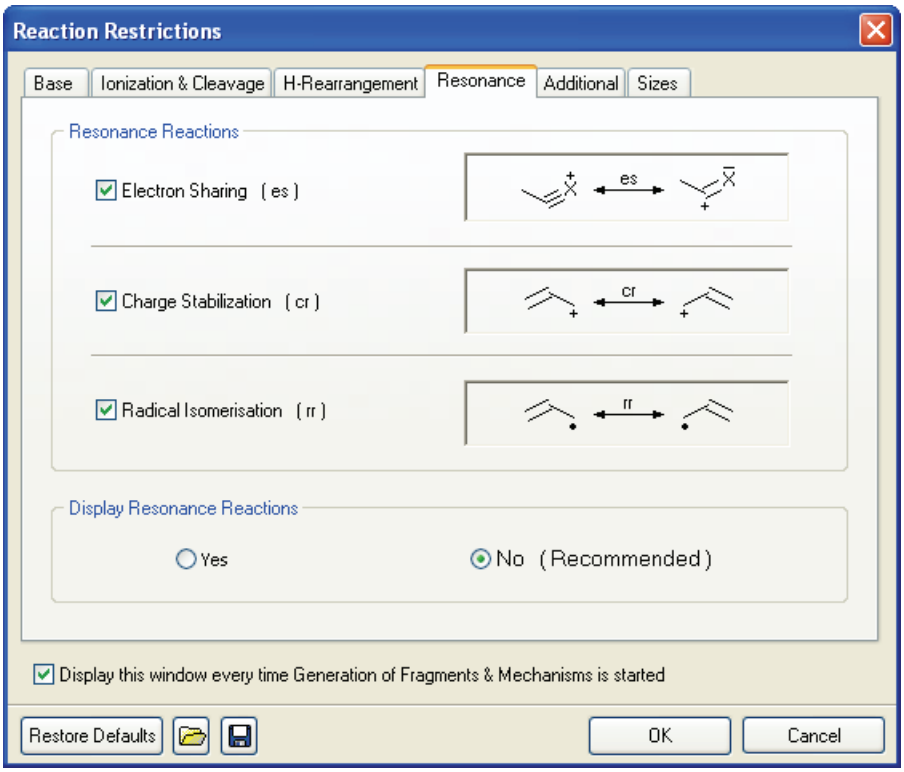


Table 26. Resonance parameters (Sheet 1 of 2)

Feature	Description
Resonance Reactions	Specifies any of the following reactions: • Electron Sharing [es] • Charge Stabilization [er] • Radical Isomerisation [rr]
Display Resonance Reactions	
Yes	Displays all resonance reactions.
No (Recommended)	Does not display resonance reactions.

Table 26. Resonance parameters (Sheet 2 of 2)

Feature	Description
Display This Window Every Time Generation of Fragments & Mechanisms Is Started	Opens the Reaction Restrictions dialog box each time you start fragment generation. Clear this check box if you want to use the same parameter settings for subsequent fragment generations and do not want to see this dialog box each time. If you clear this option and want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the instructions "To restore the Reaction Restrictions dialog box each time you start fragment generation" on page 200.
Restore Defaults	Restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.
Open	Imports the restriction parameter values from an .rrs file.
Save	Saves the current restriction parameter values to an .rrs file.

Additional Page

By default, the application does not activate cleavage on an aromatic ring. However, you might activate bond cleavage on aromatic rings when working with small aromatic compounds. For example, when you generate fragments and mechanisms of phenol, you can generate the important fragment corresponding to the peak *m/z* 66 only after activating cleavage on aromatic systems.

By default, cleavage on aromatic systems is deactivated because of the quantity of fragments that can be generated from large aromatic compounds and because the aromatic bond is a very strong bond.

Figure 71. Additional page of the Reaction Restrictions dialog box

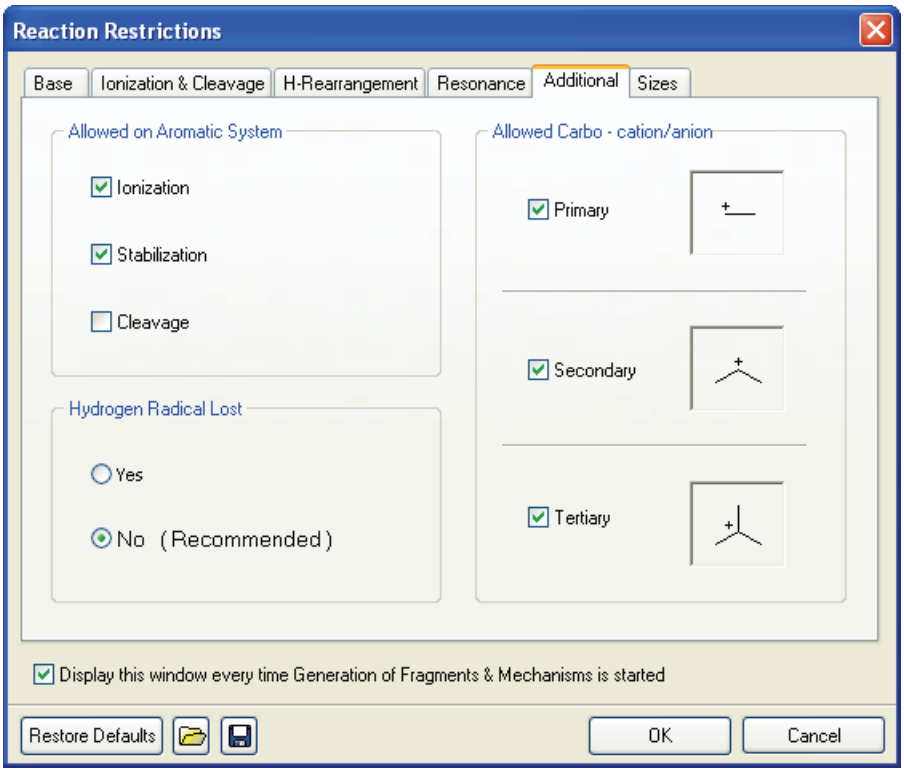


Table 27. Additional parameters

Feature	Description
Allowed on Aromatic System	Specifies any of the following bonds on aromatic rings: • Ionization • Stabilization • Cleavage
Hydrogen Radical Lost	
Yes	
No (Recommended)	
Allowed **Carbo - Cation/Anion**	Specifies any of the following: • Primary • Secondary • Tertiary
Display This Window Every Time Generation of Fragments & Mechanisms Is Started	Opens the Reaction Restrictions dialog box each time you start fragment generation. Clear this check box if you want to use the same parameter settings for subsequent fragment generations and do not want to see this dialog box each time. If you clear this option and want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the instructions "To restore the Reaction Restrictions dialog box each time you start fragment generation" on page 200.
Restore Defaults	Restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.
Open	Imports the restriction parameter values from an .rrs file.
Save	Saves the current restriction parameter values to an .rrs file.

Sizes Page

On the Sizes page in the Reaction Restrictions dialog box, you can limit the size and complexity of a reaction pathway generation.

Increasing the maximum number of reaction steps can exponentially increase the number of fragments produced for a specified reaction path. 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 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 an error 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. However, if you are missing an important fragment because you assume the generation was interrupted, you can increase the reactions limit.

Figure 72. Sizes page of the Reaction Restrictions dialog box

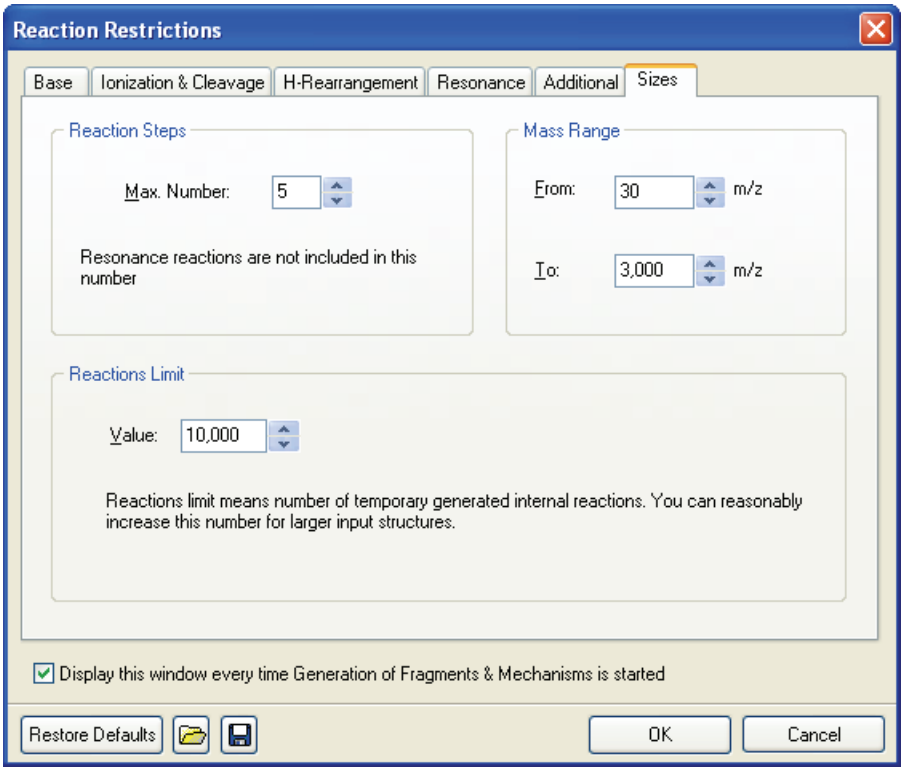


Table 28. Sizes parameters

Feature	Description
Reaction Steps	
Max Number	Specifies the maximum number of cascaded fragment reactions.
Reactions Limit	
Value	Specifies the number of temporary internal reactions.
Mass Range	
From	Specifies the lower end of the *m/z* range.
To	Specifies the upper end of the *m/z* range.
Display This Window Every Time Generation of Fragments & Mechanisms Is Started	Opens the Reaction Restrictions dialog box each time you start fragment generation. Clear this check box if you want to use the same parameter settings for subsequent fragment generations and do not want to see this dialog box each time. If you clear this option and want to return to displaying the Reaction Restrictions dialog box each time you start fragment generation, follow the instructions "To restore the Reaction Restrictions dialog box each time you start fragment generation" on page 200.
Restore Defaults	Restores all default values in the Reaction Restrictions dialog box. These values take effect after the regeneration of fragments and mechanisms and affect all subsequent generations. The current Fragments & Mechanisms windows are not affected.
Open	Imports the restriction parameter values from an .rrs file.
Save	Saves the current restriction parameter values to an .rrs file.

Working with Generated Fragments

The Fragments & Mechanisms module automatically generates fragments and detailed fragmentation and rearrangement mechanisms from your chemical structure.

This section includes the following topics:

Linking Generated Fragments with a Spectrum

Use the Mass Frontier application to link generated fragments with a mass spectrum, which helps explain peaks in a spectrum. When you start a generation of fragments and mechanisms from the Database Manager window, the generated fragments are automatically linked with peaks in a spectrum according to their *m/z* values. After a generation, highlighted (explained) peaks are displayed in a different color—by default, red—in the original mass spectrum. Selecting a highlighted peak reveals all the mechanisms leading to it. In addition, you can automatically assign generated fragments.

❖ To automatically assign generated fragments

In the Database Manager window, right-click the spectrum pane and choose **Auto Annotation > Generated Fragments** from the shortcut menu.

The corresponding Fragments & Mechanisms window must be open.

For additional information, see "Assigning Fragments to Spectral Peaks" on page 61.

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, which can produce this isotopic profile.

You cannot predict energies and barriers in ionized molecules, which prevents the prediction of all the peaks in a mass spectrum. In addition, the fragment predictability range is usually between 50 and 90 percent. However, a prominent unexplained peak can be of special value for interpreting or identifying an unknown. An unexplained peak can indicate a compound-specific mechanism that occurs in molecules with similar structural features or with a common substructure. Several mechanisms that have been observed only in a specific group of compounds cannot be applied generally when proposing fragmentation and rearrangement pathways.

If you suspect a compound-specific mechanism of fragment formation, verify your assumption by conducting a substructure search. Then you can compare the explained and unexplained peaks in the spectra retrieved by the substructure search.

Eliminating Generated Fragments Not Present in a Spectrum

The Fragments & Mechanisms module shows all *m/z* values that have been generated with the specified Reaction Restrictions settings. When the generated fragments are linked with a spectrum—that is, the fragments were generated from a Database 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, showing only those fragments that can be linked with a spectrum (explained peaks) is useful.

For additional information, see "Specifying Reaction Restrictions" on page 198 or "Linking Generated Fragments with a Spectrum" on page 216.

❖ **To eliminate *m/z* values that cannot be linked with peaks in a spectrum**

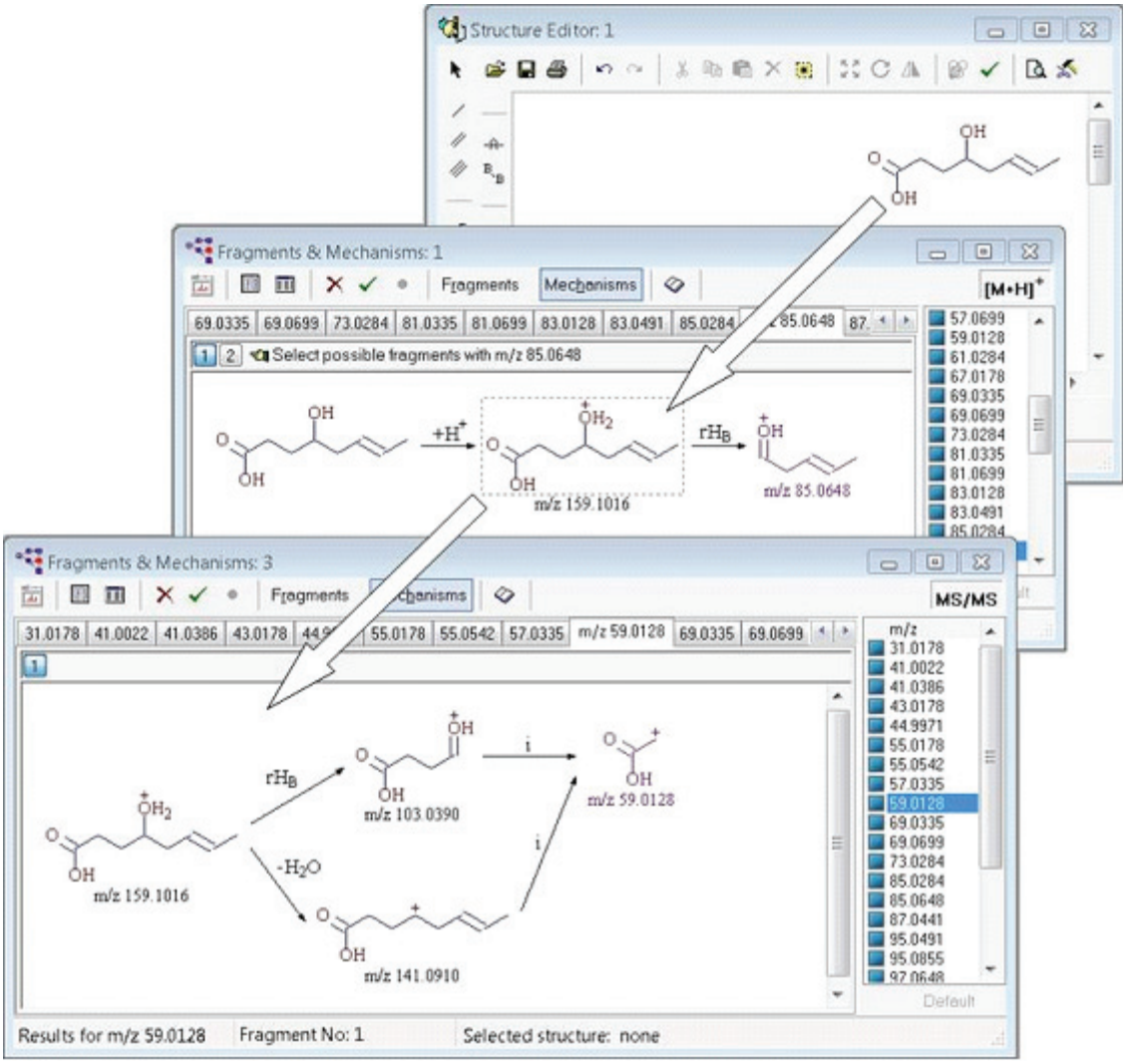
Click the **Show *m/z* Values For Explained Peaks Only** button,  , in the Fragments & Mechanisms window.

When you eliminate *m/z* values that do not correspond to a spectrum, these values are not permanently deleted. To restore the original display, click the **Show *m/z* Values For Explained Peaks Only** button again.

This **Show *m/z* Values For Explained Peaks Only** button switches the display between all generated *m/z* values and *m/z* values for explained peaks only.

Simulating Fragmentation Processes in MS/MS Experiments

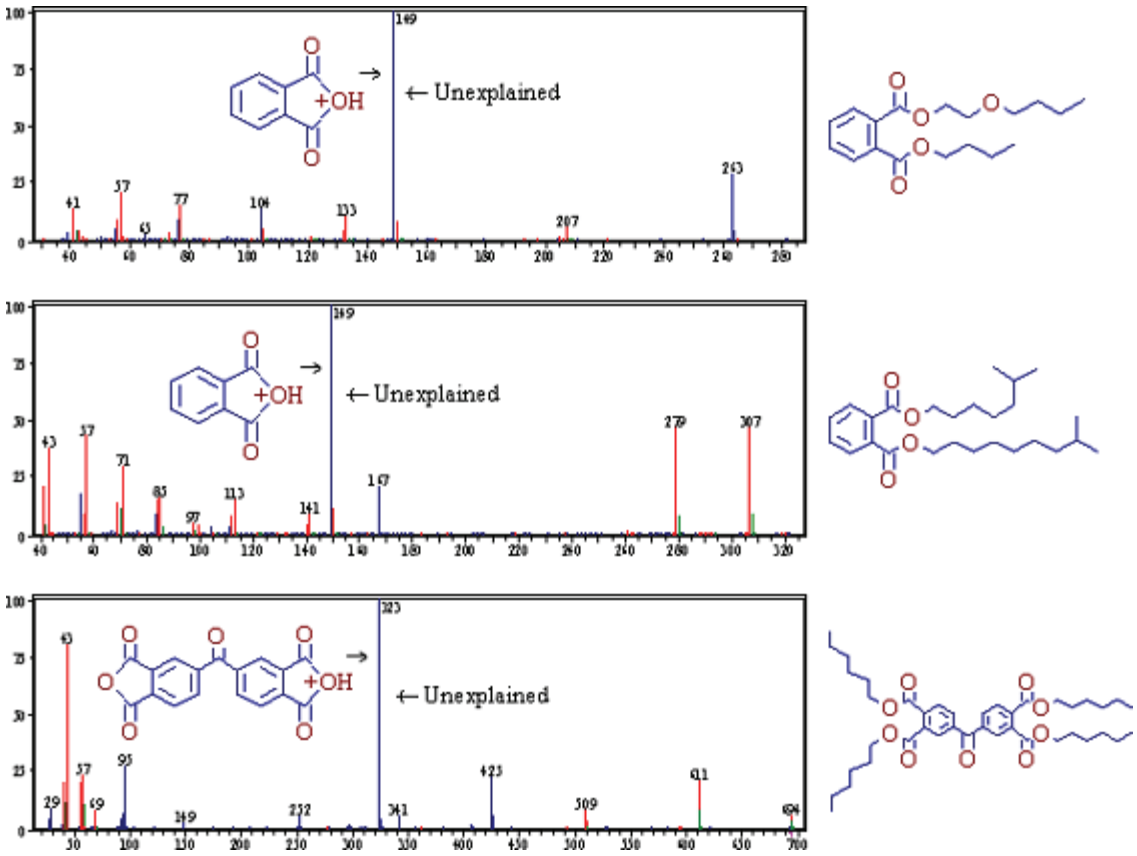
To simulate fragmentation processes in MS^n experiments, you can generate the fragments and mechanisms from neutral compounds or from ions or you can select a product fragment (parent ion) in a Fragments & Mechanism window and start the generation from there. You can simulate an unlimited number of consecutive secondary ion decomposition reactions.



Unexplained Peaks

In some cases, the fact that the application cannot explain a peak (because the corresponding fragment was probably formed by a compound-specific mechanism) can help in identifying characteristic structural groupings that give rise to the peak. For example, the phthalates produce a characteristic ion with *m/z* 149, which is formed by a highly specific mechanism. You can easily recognize the peak at *m/z* 149 as a contaminant from elasticized polymers. The application is not able to explain this peak because its corresponding fragment is formed by an unusual hydrogen rearrangement and cyclization, which are not supported. To distinguish between a randomly unexplained peak and a compound-specific peak, you must find examples in the library. Using a substructure search, you can retrieve compounds that contain a phthalate group as a common substructure. For additional information, see "(Sub)Structure Search" on page 84.

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, a phthalate with a functional group at position 3, 4, 5, or 6 will have its prominent peak shifted to higher masses by the mass of this functional group. Such an unexplained, prominent peak, present in the spectra of structurally similar compounds, can be a strong indicator of a compound-specific fragmentation process. This information can serve as evidence toward identifying a substructure under investigation.



Bar Code Spectra

The Mass Frontier application can automatically predict possible fragments from a chemical structure using general rules and fragmentation libraries, along with initial determination of the structural plausibility of generated ions. Predicting mass spectra is hindered by the difficulty of predicting thermochemical data, thermodynamic stability of product ions, and reaction rates. However, you can use generated fragments and their *m/z* values for creating bar code spectra. A bar code spectrum contains peaks at predicted *m/z* values with identical (maximal) intensity.

For additional information, see "Previewing Unimolecular Reactions" on page 193 or "Using Library Reactions in Fragmentation Prediction" on page 237.

Follow these procedures:

- To create a bar code spectrum
- To compare multiple bar codes

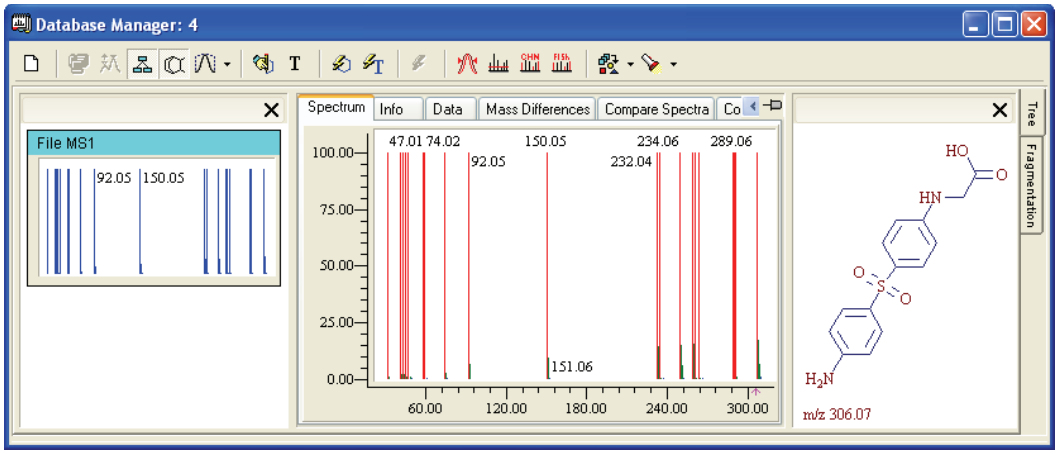
❖ To create a bar code spectrum

1. Click the **Bar Code Spectrum** button,  , in the Fragments & Mechanisms window.

   The Select Target dialog box opens.

2. Select one of the open Database Manager windows, or click **New Database Manager** to open a new window.

   The created bar code spectrum is displayed in the selected Database Manager window.



Bar code spectra are automatically linked with their original Fragments & Mechanisms windows. When you select any bar code peak in the Database Manager window, the Fragments & Mechanisms window displays the corresponding fragments and their formation mechanisms. This link remains in place as long as the corresponding Fragments & Mechanisms window is open. For additional information, see "Linking Generated Fragments with a Spectrum" on page 216.

You can use bar code spectra in several strategies for investigating spectra-structure relationships. The primary purpose of generating bar code spectra is that they make identifying spectral differences possible in structurally similar compound classes for which mass spectra are not available. To study fragmentation dissimilarities between structurally-related compounds, comparing multiple bar codes in the Database Manager window is easier than manually comparing fragments and their *m/z* ratios between multiple Fragments & Mechanisms windows. For additional information, see "Using the Compare Spectra Page" on page 43.

❖ To compare multiple bar codes

In the following example, you are interpreting an unknown spectrum and you have established two structural proposals. To find out which structure belongs to the unknown spectrum, do the following:

1. Draw both structures in separate Structure Editor windows.
2. Generate fragments for both structures.
3. Create bar code spectra and save them to the same Database Manager window.
4. Compare the bar code spectra on the Compare Spectra page in the Database Manager window.

   In the Difference Spectrum pane, you should see the specific peaks that this pair of spectra do not have in common.
5. Compare these specific peaks with the unknown spectrum and take a closer look at the fragmentation mechanisms of these peaks.

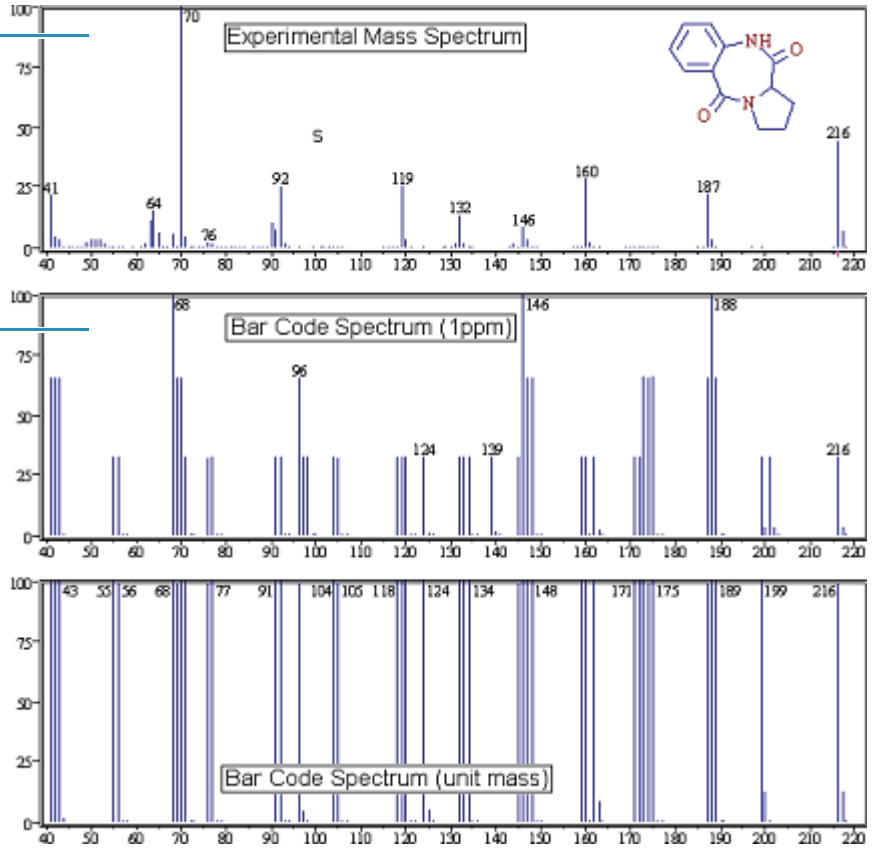
When a specific peak is present in the unknown spectrum and the mechanism of formation seems to be simple and plausible, you can select the most likely structure. This approach, using bar codes, is far superior to simply comparing explained peaks, because you can simultaneously apply it to a large number of structural proposals.

In the following example, the abundance is proportional to the number of generated *m/z* values that fit into a single integer *m/z* value.

Figure 73. Comparing multiple bar codes

Generated using 1ppm mass precision (monoisotopic mass settings)

Generated using unit mass precision



Experimental and Bar Code spectra of
Pyrrolidine[2,1-c]-2H,5H-1,4-benzodiazepin-2,5-dione, 1,3-dihydro- (Electron impact mode)

Marking Fragments

You can use the generated fragments for fragment comparison of different structures or for export to an Excel spreadsheet. The automated generator can predict a large number of theoretically possible fragments. To extract only selected fragments for use during further processing, mark fragments (*m/z* values) that should be either considered or ignored.

The fragment marks apply to bar code spectra, the Fragments Comparator window, and copying to an Excel spreadsheet.

For additional information, see "Bar Code Spectra" on page 220 or "Comparing Fragments" on page 241.

❖ To mark an *m/z* value using the toolbar buttons

Select the tab for the *m/z* value and click one of the following buttons:

Mark	Description
Set	The Set mark has the highest priority. When any *m/z* values are marked *Set*, only these values are considered.
Exclude	When you mark fragments Exclude, the marked fragments are not considered.
Default	When no fragments are marked (by default), then all the fragments are considered.

❖ To mark an *m/z* value using the check boxes in the *m/z* list

1. Click the check box for an *m/z* value in the *m/z* list until it displays the correct state.

   The check box can have one of three states:

   - **Set**
   - **Exclude**
   - **Default**

2. To mark all the *m/z* values as Default, click the **Default** button, Default , at the bottom of the list.

Note Changes you make to marks are instantly reflected in the Bar Code spectrum. After you send or copy a list of fragments with specific marks to the Fragments Comparator, the application ignores all subsequent changes. The same applies for a fragment list copied to an Excel spreadsheet. For additional information, see "Comparing Fragments" on page 241.

Specifying Precision and Isobaric Differentiation

Use the features of the Mass/Abundance pages to specify *m/z* precision and isobaric differentiation of calculated fragments.

❖ To set the *m/z* precision of calculated fragments

1. Choose **Options > Settings** from the Mass Frontier main menu.
2. Click **Mass/Abundance** in the Parameters section.
3. Click the **Mass Precision** tab.

   The Mass Precision page opens.

   For detailed information about setting the parameters on the Mass Precision page, see "Precision" on page 384.

❖ To set the isobaric differentiation of calculated fragments

1. Choose **Options > Settings** from the Mass Frontier main menu.
2. Click **Mass/Abundance** in the Parameters section.
3. Click the **Mass Accuracy** tab.

   The Mass Accuracy page opens.

   For detailed information about setting the parameters on the Mass Accuracy page, see "Accuracy" on page 381.

Fragmentation Library

Use the Fragmentation view in the Database Manager window to display fragmentation reactions in all your libraries and to create and manage fragmentation mechanism databases in your editable libraries.

Contents

The Fragmentation view contains a graphical editor for entering fragmentation reactions, which you can store in the library database with complementary information for the reaction. You can search all fields of the database, such as authors, ionization method, mass analyzer, or structures.

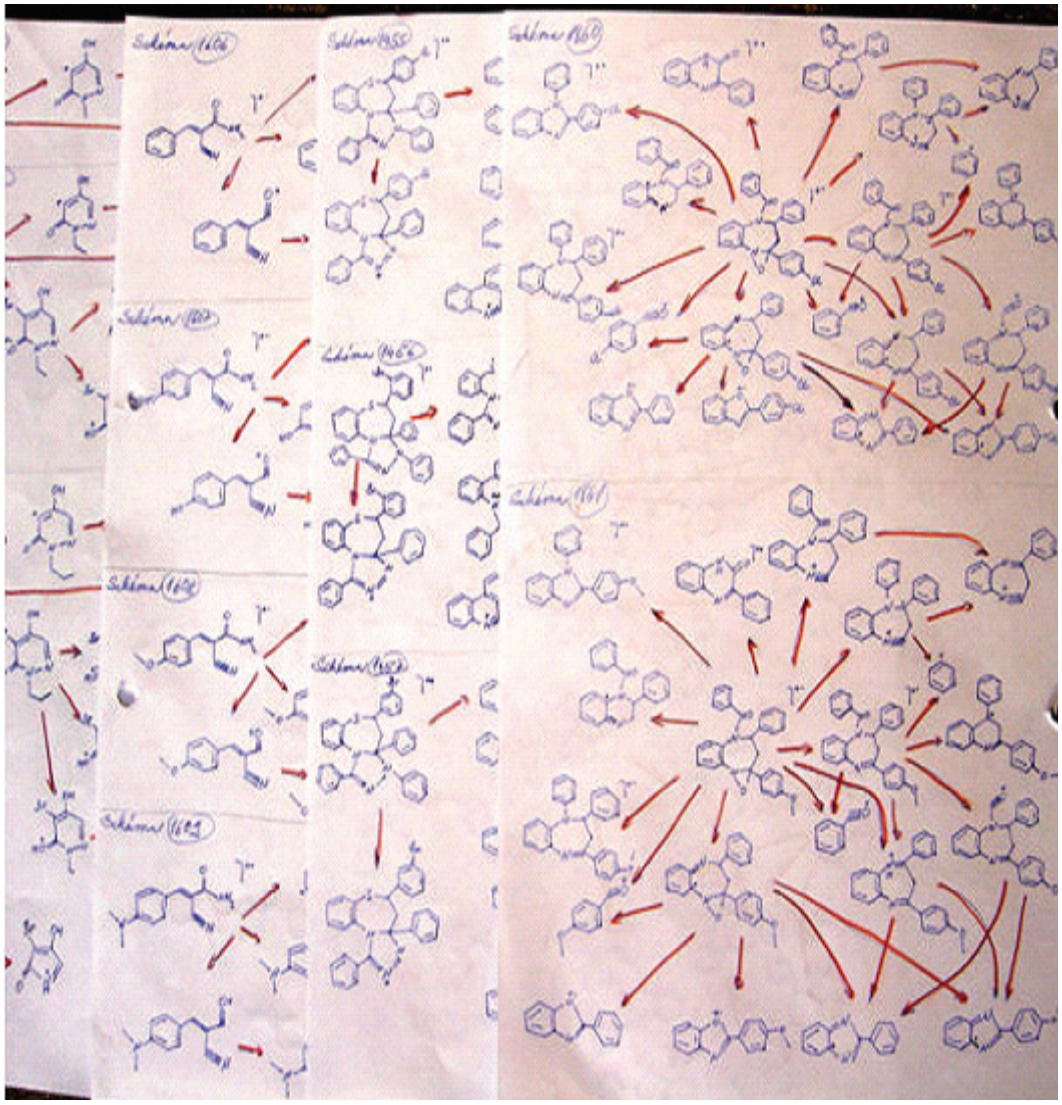
The fragmentation library uses an expert system to automatically extract a decomposition mechanism for each fragmentation reaction in the database and determine the compound class range to which you can apply the mechanism. This system applies database mechanisms to your structures and automatically predicts the fragmentation reactions for a specified compound.

HighChem collated and entered fragmentation mechanisms published in all the available printed media dedicated to mass spectrometry. Each reaction, along with related chemical structures, was manually drawn in Reaction Editor and saved in the HighChem Fragmentation Library, which currently contains approximately 130 000 individual reactions. See "Fragmentation mechanisms from the library" on page 227. Fragmentation pathways are accompanied with complementary information such as the title, the authors, and the information source. This library collection serves as a knowledge base for predicting fragmentation pathways from your structures.

Total number of	Stand as of 5/2009
Fragmentation Schemes	30.936
Individual Reactions	129.229
Chemical Structures	151.762

To ensure the data is of the highest quality, the software rigorously evaluates fragmentation mechanisms 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 has revealed formally 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.

Figure 74. Fragmentation mechanisms from the library



Note The HighChem Fragmentation Library is a read-only library. You cannot copy, paste, or edit anything in the library.

Drawing Fragmentation Reactions

Use the Fragmentation view to enter and edit fragmentation reactions.

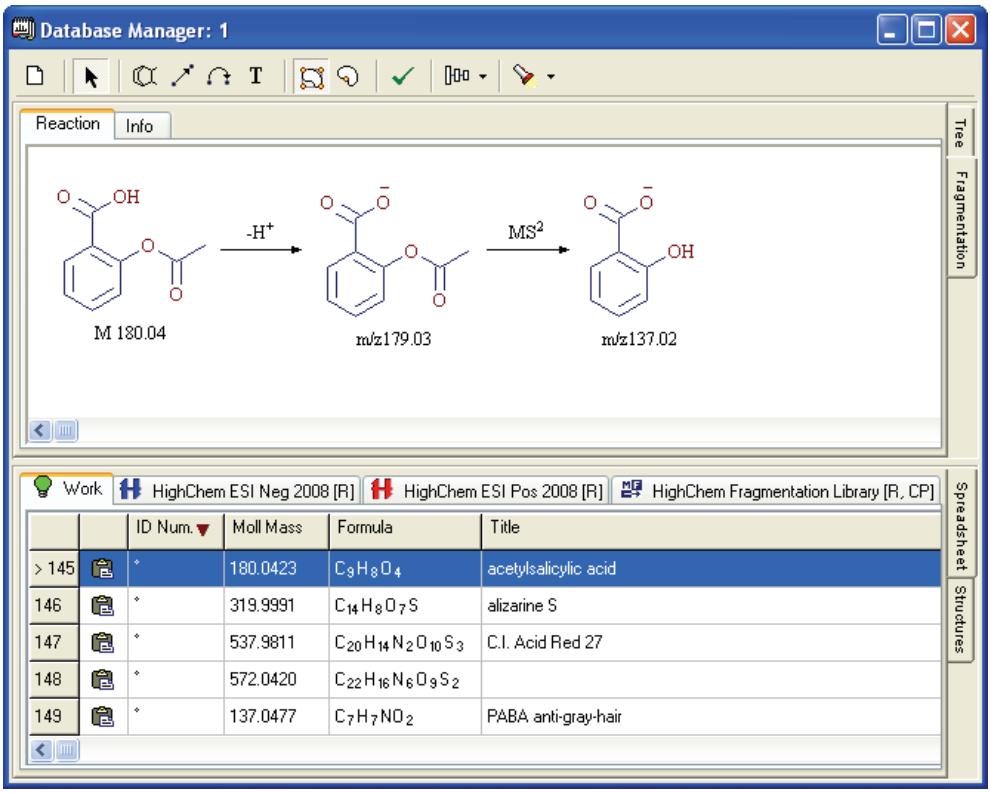
Follow these procedures:

- To open the Fragmentation view for the current compound
- To create a new structure
- To edit an existing structure
- To add a reaction arrow
- To edit the arrow label

❖ To open the Fragmentation view for the current compound

Click the **Fragmentation** tab on the right side of the Database Manager window.

The Fragmentation view for the selected compound opens.



❖ To create a new structure

1. Do one of the following to open the Structure Editor dialog box:

   Double-click the Reaction page drawing area.

   –or–

   Click the **Add/Edit Structure** button in the toolbar.

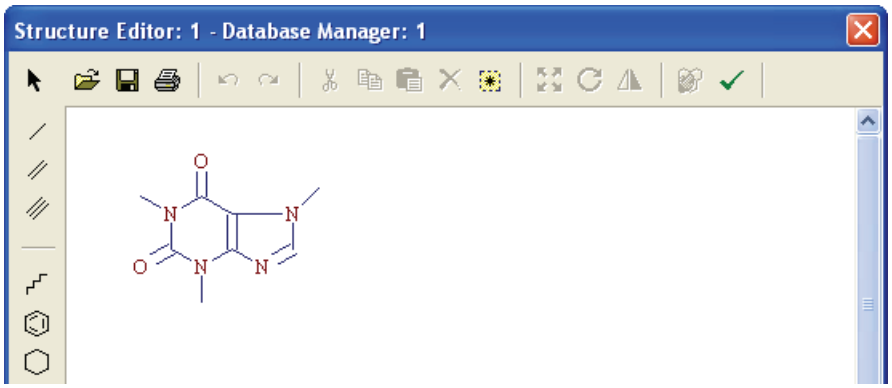
   –or–

   Right-click a blank drawing area and choose **New Structure** from the shortcut menu.

   The Structure Editor dialog box opens. For detailed instructions about using the Structure Editor, see Chapter 4, "Structure Editor Module."

2. Create your structure in the Structure Editor.

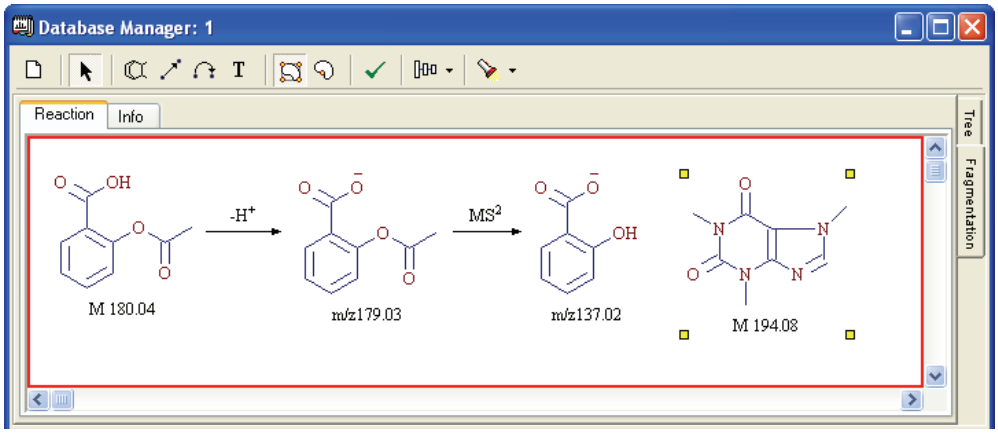
   Figure 75. Structure Editor dialog box

   You cannot open another window until you close the Structure Editor dialog box.

3. Click **OK**.

   The application adds your fragment to the Reaction page drawing area.

   The new, unconnected structure is indicated with yellow handles, ▫. If a scheme is formally incorrect, the software places a red border around it.

4. Click the **Check** button,  , to identify critical errors in the scheme.

   You cannot save schemes with one or more critical errors (such as an unconnected fragment or the presence of more than one scheme) to the database.

5. Drag the structure to any location on the drawing area.

❖ To edit an existing structure

1. Do one of the following to open the structure in the Structure Editor dialog box:

   Double-click the fragment.

   –or–

   Select the structure and click the **Add/Edit Structure** button,  , in the Reaction page toolbar.

2. Use the tools in the Structure Editor dialog box to edit the structure.

   For detailed instructions, see Chapter 4, "Structure Editor Module."

3. To confirm your changes, click **OK**.

   The application updates the modified structure in the Reaction page drawing area.

❖ To add a reaction arrow

1. Do one of the following:

   a. Click the **Draw Straight Arrow** button,  , in the Reaction page toolbar.

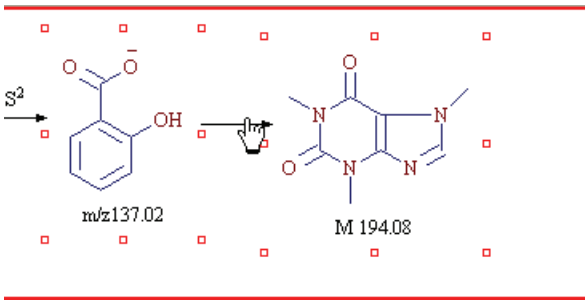
   b. Position the cursor where you want the arrow on the drawing area, and click again.

   –or–

   a. Right-click a structure and choose **New Arrow**.

   b. Click the arrow and drag it to the correct location on the drawing area.

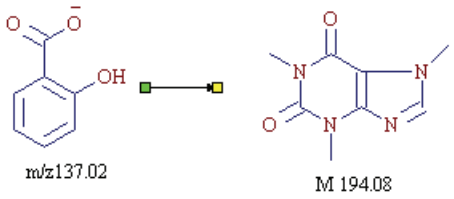
2. To be certain that the arrow connects the correct structures, look for the red selection squares.



   As you move a connection arrow, red selection squares appear around the structure or arrow when the objects are close enough to connect.

For the application to correctly extract mechanisms, you must properly connect the structures in a reaction scheme with arrows (see "Extracting Mechanisms" on page 235). The application considers stand-alone structures and disconnected arrows as errors and ignores them.

When a structure is connected with at least one arrow, the Reaction page displays the selection squares in green ■. When a structure is not connected, the Reaction page displays the selection squares in yellow ■. The same color-coding applies to arrows. When an arrow is connected with a structure, this arrow end appears in green. When the arrow end is not connected, it appears in yellow.



❖ To edit the arrow label

1. Double-click the arrow.

   The Arrow Caption dialog box opens.

2. Type or paste the text into the dialog box.
3. Use the formatting tool in the Arrow Caption dialog box to format your text.
4. Click **OK**.

Editing a Fragmentation Scheme

In addition to a fragmentation reaction, you can also maintain text data in a database record.

❖ To open the Fragmentation Scheme data for the current compound

1. Click the **Fragmentation** tab on the right side of the Database Manager window.

   The Fragmentation view for the selected compound opens.

2. Click the **Info** tab on the top of the Fragmentation view.

   The Info page for the selected compound opens. By default, all records on the Info page are expanded.

   Note To hide all empty records, right-click and choose **Hide Empty Values** from the shortcut menu.

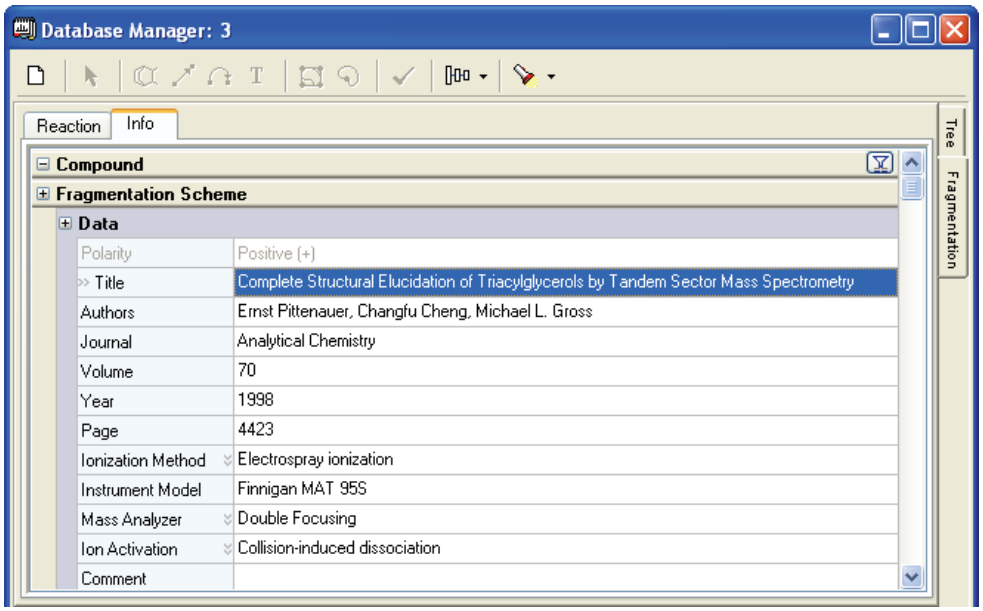
3. To display the Fragmentation Scheme data, do one of the following:

   Click the plus sign next to Compound (to collapse the compound records).

–or–

Scroll down to the Fragmentation Scheme section.

Figure 76. Fragmentation Scheme data on the Info page



Working with Records

The Mass Frontier application applies the mechanisms of fragmentation reactions to your structure and automatically predicts fragmentation reactions for the specified compound. However, the application might apply some mechanisms that are erroneous or too general. Some mechanisms of small fragments can fit to virtually every structure and can generate a large number of useless fragments.

Excluding specific records can prevent the generation of a large number of fragments, which dramatically slows the generation process and makes reviewing the predicted fragments difficult. You can change record activity in the Active column of the Spreadsheet view.

Follow these procedures:

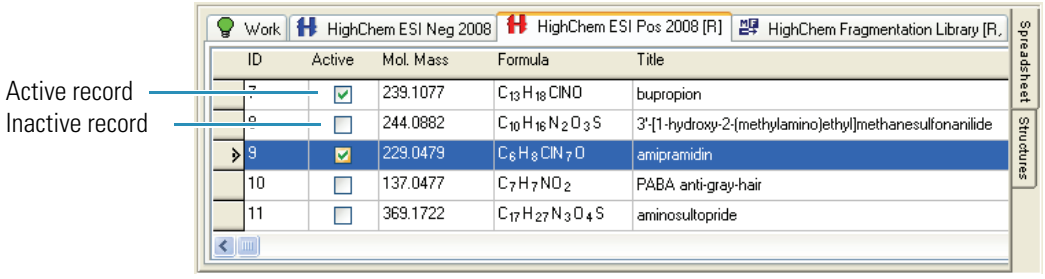
- To exclude a record from the generation of fragments
- To include or exclude multiple records from the generation of fragments
- To sort records in the Spreadsheet view
- To save changes to the reaction and fragmentation scheme

❖ To exclude a record from the generation of fragments

1. Select the record in the Spreadsheet view for the library.
2. Clear the **Active** check box.

> **Note** Changes to an Active check box are immediately updated in the database and do not need to be saved.

Figure 77. Active check box in Spreadsheet view



❖ To include or exclude multiple records from the generation of fragments

1. Select the records you want to activate.
2. Right-click the records and choose **Activate All Selected Records** or **Deactivate All Selected Records** from the shortcut menu.

❖ To sort records in the Spreadsheet view

Click the column header.

You can sort any of the following criteria in ascending or descending order:

- Active and inactive records
- ID numbers (sorted 1–*n*)
- Molecular mass (sorted from smallest to largest)
- Formula (sorted from fewest to most Carbons, followed by fewest to most Hydrogens, and so forth)
- Title (sorted alphabetically)

❖ To save changes to the reaction and fragmentation scheme

Choose **Library** > **Save Changes in Library** from the Mass Frontier main menu.

The Mass Frontier application performs three sequential actions:

- Checks the fragmentation scheme on the Reaction page for formal correctness. If the software detects an error, you are prompted to either save the scheme as it is or return to the record to correct the problem.
- Extracts a fragmentation mechanism for every single unimolecular reaction in the fragmentation scheme, using advanced algorithms. This process can take several seconds for complex reactions. For additional information about extracting fragmentation mechanisms, see "Extracting Mechanisms" on page 235.
- Stores the reaction scheme, additional text, numerical data, and extracted mechanisms in the database.

When you save a scheme with just a single error, the application does not extract the reaction mechanisms and ignores the entire record when predicting fragments and mechanisms. At any time, you can return to an erroneous record to fix the problem so that the software can perform fragmentation prediction for the record.

Reaction symbols and reaction abbreviations appear only when you save a scheme. For additional information, see "Reaction Symbols" on page 236 or "Reaction Formalism" on page 194.

Extracting Mechanisms

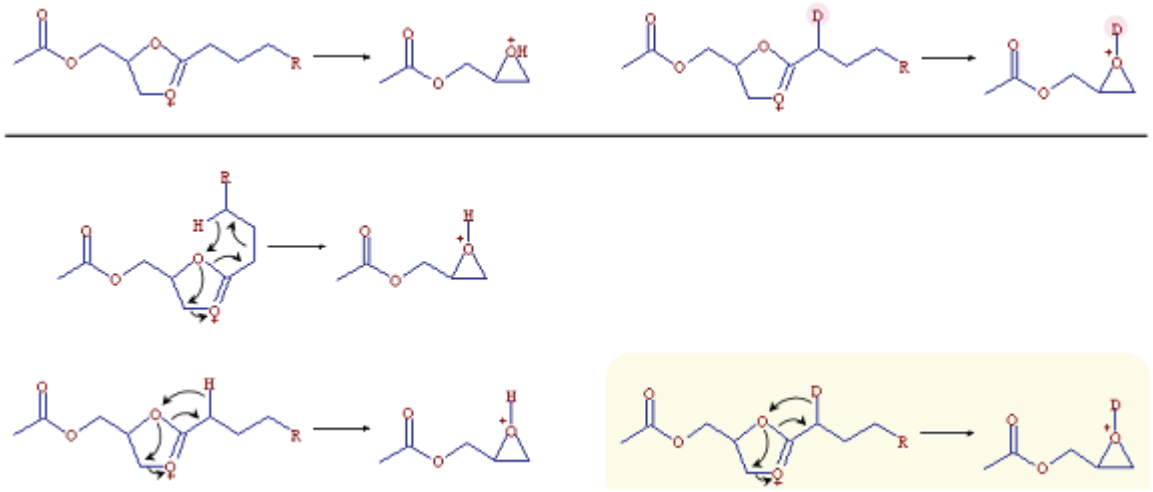
The automated prediction of fragmentation and rearrangement pathways extracts unimolecular decomposition mechanisms from reactions you provide. The application decodes the underlying principle of fragmentation mechanisms from reaction drawings and builds a knowledge base of fragmentation events, replacing the need for the manual input of atom-atom correspondence in precursor and product ion pairs. For additional information, see "Drawing Fragmentation Reactions" on page 228 or "Base Page" on page 201.

The application processes specific fragmentation details (similar to labeled or generic structures) for use in experimental mechanistic studies to direct the dissociation route. Using deuteria or substituents participation, the decoding algorithm unambiguously extracts the underlying mechanism.

Figure 78. Extracted mechanisms

Two mechanistic possibilities can be extracted from the depicted fragmentation reaction.

Isotope labeling directs the decoding algorithm to the required rearrangement.



Because many reactions stored in a fragmentation library follow general fragmentation rules that can be predicted using preprogrammed unimolecular reactions, the library distinguishes between class-specific mechanisms and general fragmentation reactions. After you save a reaction to the database, the application attempts to identify a general fragmentation rule and assigns the relevant reaction symbol above the arrow. You can edit the reaction symbol of every reaction on the Reaction page by double-clicking the arrow and entering the new formatted text into the annotation dialog box.

Note Reaction symbols do not appear immediately after the reaction is drawn, but only after you save the record in a library. For a description of reaction symbols, see "Reaction Symbols" on page 236.

With respect to fragmentation prediction, determining the preferred ionization site is as important as detailed knowledge of the fragmentation mechanism. The application supports the virtual generation of charged molecules based on library ionization reactions using the

exact location of the positive, negative, or unspecified charge location symbol that you can assign to a structure drawing. For additional information about modifying the charge location symbols, see "Modifying Atoms and Bonds" on page 139.

Reaction Symbols

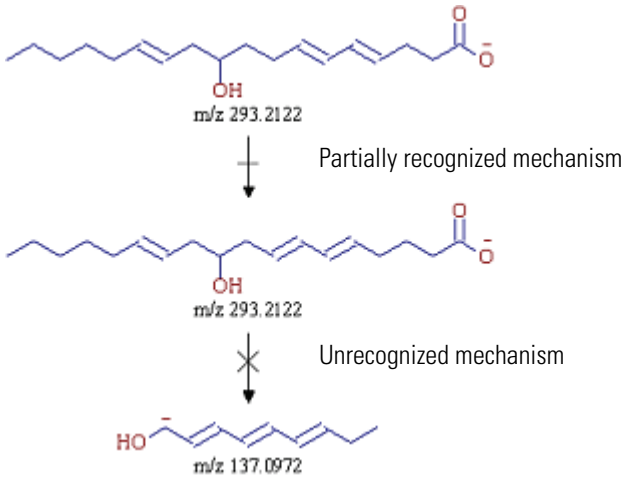
The Mass Frontier application automatically extracts fragmentation mechanisms from a reaction drawing after you save a scheme. When a reaction follows one of the preprogrammed general fragmentation rules, the arrow is labeled with the particular rule abbreviation. For descriptions of these abbreviations, see "Reaction Formalism" on page 194.

Even when a reaction is formally correct, deriving a reaction mechanism from your drawing might not be possible because you entered an unfeasible fragmentation mechanism or the unimolecular reaction is incomprehensible to the application. When a mechanism cannot be extracted, the application puts a cross through the reaction arrow ( ). In this case, the mechanism is reduced to the exact precursor and product structures and only the identical neutral or ionic precursor is matched with your structure in the fragmentation prediction process.

The application can sometimes decode a mechanism from a drawn reaction, but the atom-matching procedure will not be able to find the corresponding atom counterparts on both sides of the reaction leading to partially recognized mechanisms. When this occurs, the reaction arrow appears with a small line through it at right angles. This kind of reaction can be used only for fragment prediction for some input structures according to the decision of the fragmentation algorithm. Even when your input fragment looks similar to the precursor in the library reaction, a partially recognized reaction mechanism might not be selected for fragments prediction.

To overcome such a problem with unrecognized or partially recognized mechanisms, try to decompose complex one-step mechanisms into several simple reaction steps and then save these in a fragmentation library.

Figure 79. Graphical indicators of unrecognized mechanisms



Using Library Reactions in Fragmentation Prediction

Library reactions create a database of fragmentation and rearrangement mechanisms that you can apply to your structures to predict the fragmentation pathways occurring in a mass spectrometer. The library mechanisms are extensions to the general fragmentation rules, which might not cover all the complex processes for a broad spectrum of ionization and ion activation techniques. For additional information, see "Previewing Unimolecular Reactions" on page 193.

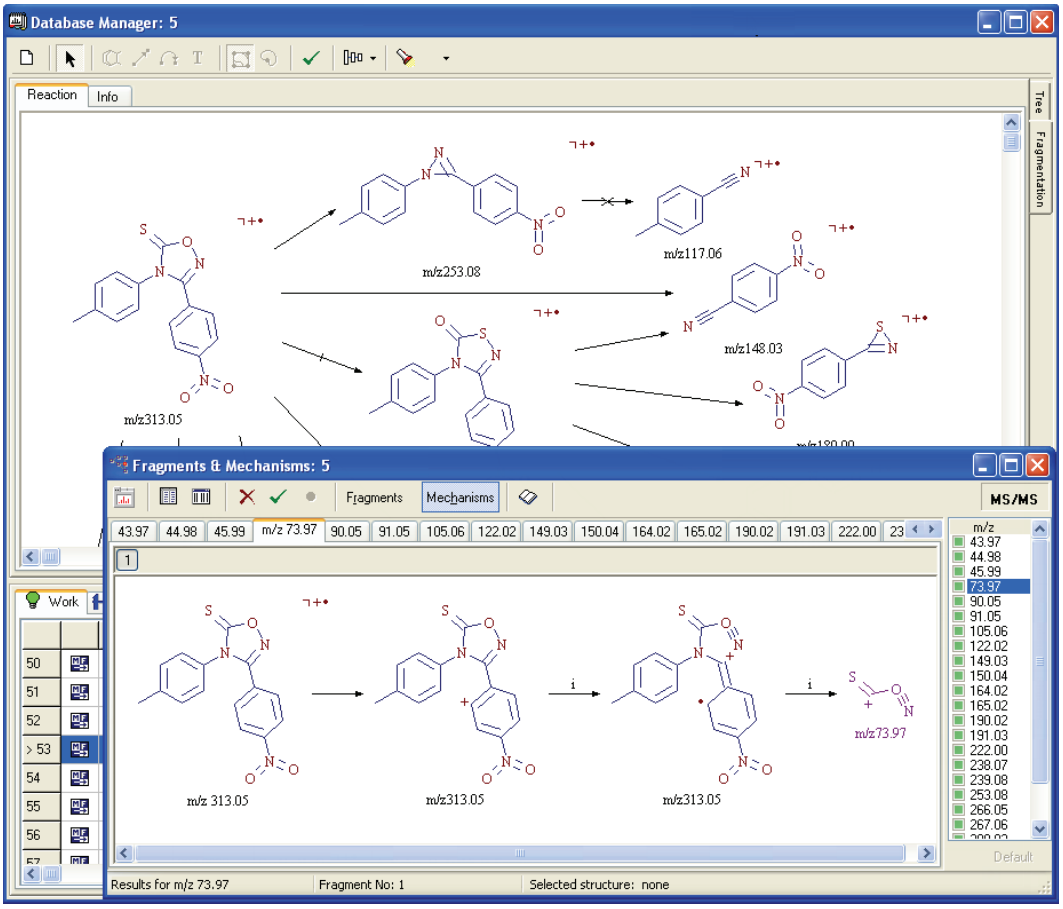
Library mechanisms provide flexibility in altering predicted fragmentation pathways and entering highly specific mechanisms that apply to a limited class of compounds. Because the Mass Frontier application contains approximately 130000 reactions, the fragmentation predictability is much higher than if only general rules were applied. This is particularly true for low energetic experiments such as ESI or APCI that often yield complicated skeletal and hydrogen rearrangements, unusual ring closures, or complex fragmentations.

Any recognized and active mechanism that has been saved into a library serves as a knowledge base for predicting fragmentation pathways. After drawing and saving a reaction into any fragmentation library, you can use the mechanism template for predicting fragmentation pathways for any structure to which the derived mechanism can be applied. The applicability range depends on many factors, but structurally similar compounds with a common ring scaffold usually exhibit identical fragmentation mechanisms.

Fragment stability and general ion energetics depend on many thermodynamical parameters, and even a slight structural dissimilarity between two molecules can result in large differences in the course of fragmentation pathways. For example, two identical structures with a simple hydroxy group difference can occasionally exhibit completely different spectra and the derived fragmentation analogy based on library reactions might not always reflect the real fragmentation events for structural analogs.

When a fragmentation reaction is predicted using a library reaction, you can double-click the Lib arrow label to see the corresponding mechanism. Both template and generated fragmentation mechanisms are displayed in red in the Fragmentation view and in the Fragments & Mechanisms window. See "Generated fragmentation mechanisms" on page 238.

Figure 80. Generated fragmentation mechanisms



Searching for Fragmentation Criteria

Fragmentation libraries are fully searchable using various combinations of search criteria.

Because fragmentation libraries largely contain ionic structures that can undergo resonance reactions, the Mass Frontier application can perform a resonance substructure search. This search feature ensures the correct retrieval of all resonance structures, even when the query structure is in a different resonance form than the library structure. This feature is fully automatic, so you need not worry about the particular resonance state of the ionic structures. However, be aware of this functionality when reviewing search results, as the query and library structures in positive search results might appear different if the resonance reactions are possible. In this case, do not consider this difference as an error, but rather be aware that there might be complex resonance variations. For additional information about resonance reactions, see "Resonance Page" on page 209.

You can ignore charges and radicals in (sub)structure searches if their location is ambiguous. When searching structures with an unspecified charge location or substituents, review the applicable search rules. For descriptions of these search rules, see "(Sub)Structure Search Rules" on page 89.

Follow these procedures:

- To search Fragmentation data
- To search (Sub)Structure data

❖ To search Fragmentation data

1. Choose **Search > Fragmentation Data** from the Mass Frontier main menu.

   The Fragmentation Data Search dialog box opens.

2. Select the libraries you want to search.

3. Type the search criteria on the Data page or select criteria on the Advanced page.

   For a detailed description of the Fragmentation Data Search dialog box, see "Fragmentation Data Search" on page 98.

4. Click **OK**.

   Depending on the merge option you select, the search results appear in the Work library pane of the Database Manager window or in a new Database Manager window.

❖ To search (Sub)Structure data

1. Choose **Search > (Sub)Structure** from the Mass Frontier main menu.

   The (Sub)Structure Search dialog box opens.

2. Select the libraries you want to search.

3. Select search criteria on the Data page or the Advanced page.

   For a detailed description of the (Sub)Structure Search dialog box, see "(Sub)Structure Search" on page 84.

4. Click **OK**.

   The search finds all the reactions in the selected libraries with reaction precursor or product structures that match your queried (sub)structure.

   Depending on the merge option you select, the search results appear in the Work library pane of the Database Manager window or in a new Database Manager window. The search results display the query substructure in red in the library structure.

Comparing Fragments

Use the Fragments Comparator module to process and compare fragments generated in the Fragments & Mechanisms window. The fragments can originate from different structures. This module supports fragments generated using any ionization method. Using fragment marks in the Fragments & Mechanisms window, you can export a selected set of fragments into the Fragments Comparator module. The fragments are organized in columns. Each column represents a set of fragments provided by an associated Fragments & Mechanisms window. The Fragments Comparator module can hold a large number of fragment sets, limited only by system resources. For detailed instructions for marking fragments, see "Marking Fragments" on page 223.

The Fragments Comparator was designed as an integral part of the Fragments & Mechanisms module. When you double-click any fragment in the Fragments Comparator module, the associated mechanisms appear in the Fragments & Mechanisms window. The Fragments Comparator can recall only mechanisms that are present in an open Fragments & Mechanisms window. When you close the associated Fragments & Mechanisms window, the link is lost.

The comparison feature is especially useful when analyzing the fragmentation products of structurally related compounds. Common fragments point toward a common substructure in terms of fragmentation. Fragment differences can indicate fragments along with corresponding peaks in a spectrum that are characterized by distinct structural details. Predicted *m/z* values of fragments that are different for structurally related compounds are displayed on the Compare Spectra page in the Database Manager window. For a detailed description of the Compare Spectra page, see "Using the Compare Spectra Page" on page 43.

Fragments Comparator Window

The Fragments Comparator window consists of Table and Structures pages. The Table page lists the numerical *m/z* values of fragments, and the Structures page displays structural drawings of possible fragments. Because the Fragments & Mechanisms module can generate several isobaric isomers for a single *m/z* value, the Structures page imports only the first fragment for each generated *m/z* value. The Mass Frontier application simultaneously imports fragments into these two pages but manages the information independently. Moving or deleting a column on one page does not affect the other page.

The Fragments Comparator window can display structures of fragments only as long as the associated Fragments & Mechanisms window is open. When you close the associated Fragments & Mechanisms window, the corresponding column of fragments on the Structures page is removed.

On the Table page, you can select a column or a part of a column and copy the data. You can then paste the *m/z* values into an Excel spreadsheet or a similar application.

You can resize the cell on the Structure page with the Cell Size track bar, move columns in both pages, and delete the columns for imported fragments (the leftmost columns).

Figure 81. Table page in the Fragments Comparator window

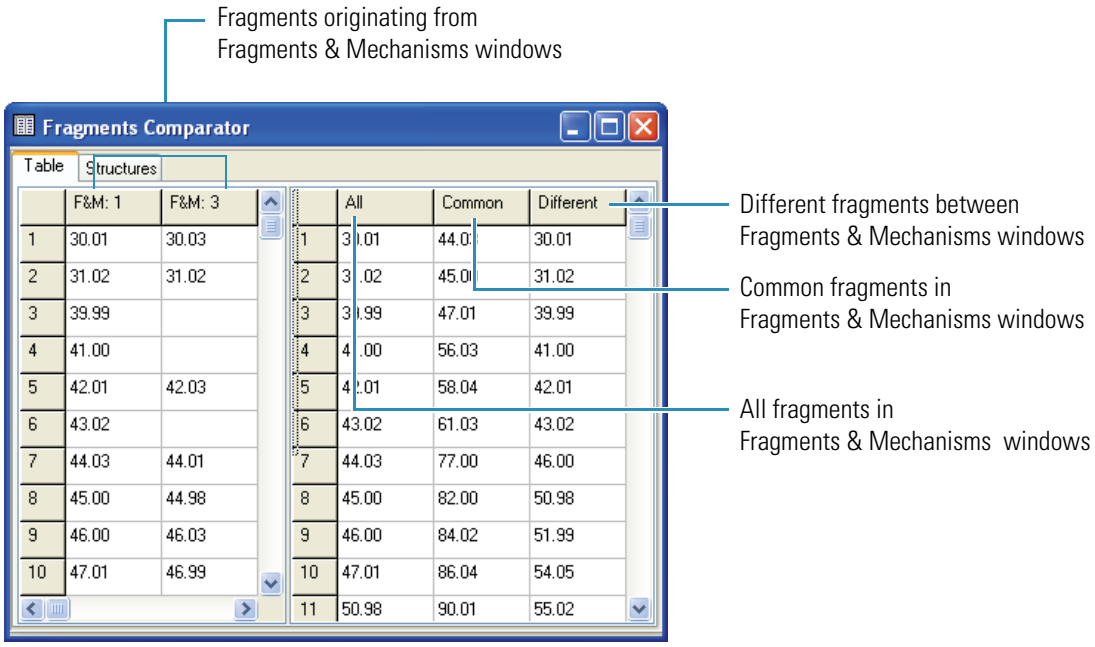
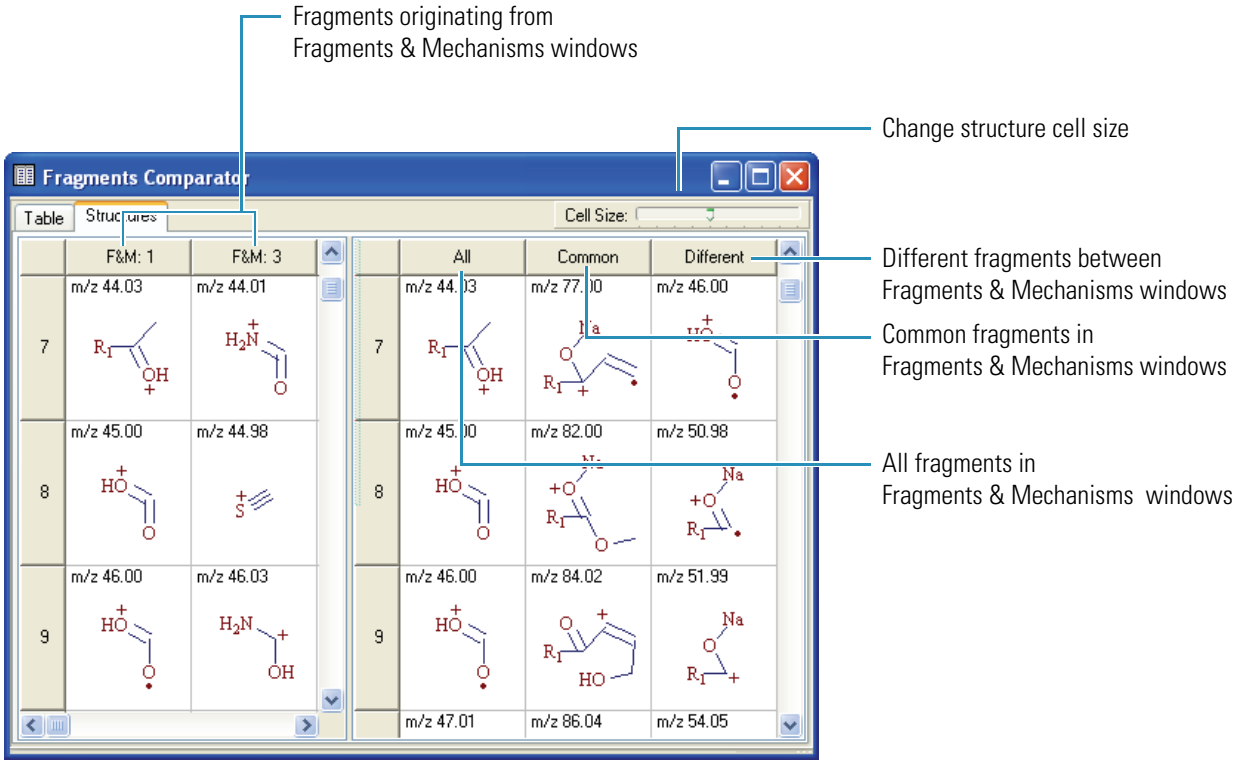


Figure 82. Structures page in the Fragments Comparator window



Comparison Results

Both the Table and Structures pages in the Fragments Comparator window are divided into two parts. The left side displays columns of imported fragments for each Fragments & Mechanisms window. The right side displays three columns that show three types of comparison results. The first result column shows all available fragments (logical OR), the second column shows all common fragments (logical AND), and the third column shows different fragments (logical NAND).

Note The Mass Frontier application compares all fragments by their *m/z* values using Resolution Settings (see "Accuracy" on page 381). The fragments are usually predicted in several isomeric forms, making a structural comparison unreasonable. Because the application compares the fragments by *m/z* values, the calculated precision as defined in the Resolution Settings significantly influences the comparison results.

Chromatogram Processor Module

Use the Chromatogram Processor module for the extraction and processing of mass spectral scans from GC/MS or LC/MS files or from MS^n data. Use this module to perform component detection, spectra deconvolution, spectral averaging, and background subtraction.

You can display three types of chromatograms: Total Ion Chromatograms (TIC), MS and MS/MS chromatograms, and Extracted Ion Chromatograms (XIC). With the XIC feature, you can display individual mass chromatograms of ions that are characteristic for a specific compound.

Mass spectral scans, deconvoluted components, and various types of MS^n data can be classified using these methods: PCA, SOM, or Fuzzy Clustering. You can search spectra in a library for positive compound identification or copy the spectra to a Database Manager window for further processing and archiving. You can also simultaneously open multiple chromatograms. Fully customizable chromatogram and mass spectrum layouts are available.

In the Chromatogram Processor window, you can copy chromatograms with extracted spectra for importing into reports, spreadsheets, or other Windows programs. MS^n data is displayed in tree structures so that you can view the dependencies. This module does not include target analysis or automatic quantitation of ions.

Contents

Supported GC/MS and LC/MS Data File Formats

The Mass Frontier application supports various data file formats for importing GC/MS and LC/MS files:

- Thermo Xcalibur raw data files (MS and MS^n)
- Thermo Scientific LCQ™, GCQ™, ITS40, and Magnum
- Varian Saturn™ (Agilent)
- Agilent™ ChemStation™
- JACAMP (DOS, Windows, and UNIX™)

You can import these files to the Chromatogram Processor, and you can save single scans in JACAMP, MSP format, or as a HighChem Chromatogram file. The HCC format saves all actions applied to chromatogram.

This application supports “centroid” type data and displays centroid mass spectra in a bar graph. This application does not support “profile” type data for mass spectra.

Because of various netCDF standards for implementation, the application might not be able to read some .cdf files.

Note The Mass Frontier application supports features connected with chromatographic spectral trees for Xcalibur raw data (.raw) files only. For additional information, see “Processing MSn Data” on page 323.

Working in a Chromatogram Processor Window

In the Chromatogram Processor window, you can copy chromatograms with extracted spectra for importing into reports, spreadsheets, or other Windows programs. MS^n data is displayed in tree structures so that you can view the dependencies. Using text boxes, you can annotate chromatographic scans or components. This module does not include target analysis, structures, or automatic quantitation of ions.

Follow these procedures:

- To make the chromatogram files editable
- To open a Chromatogram Processor window
- To export scans or components to a Database Manager window
- To copy data to a Database Manager window
- To copy components to a Spectra Classifier window

❖ To make the chromatogram files editable

1. In Windows Explorer, select all the files whose attributes you want to change.

   The Mass Frontier software comes with GC/MS and Xcalibur MS^n demonstration files, located in the C:\Documents and Settings\All Users\Documents\HighChem\Mass Frontier 7.0\Chromatograms directory.

2. Right-click the files and choose **Properties** from the shortcut menu.
3. Clear the **Read-only** check box in the Properties dialog box and click **OK**.

> **Note** You cannot open GC/LC/MS files from a CD-ROM. If you have files on a CD-ROM, copy these files to the hard drive and disable the read-only file attribute to make them accessible for importing.

❖ To open a Chromatogram Processor window

1. Do one of the following:

   Click the **Chromatogram Processor** button,  , in the Mass Frontier toolbar.

   –or–

   Choose **File > Open > GC/LC/MS** from the Mass Frontier main menu.

   –or–

   Double-click a HighChem chromatogram (.hcc) in Windows Explorer.

   The Open Chromatogram dialog box opens.

2. Select the raw data file and click **Open**.

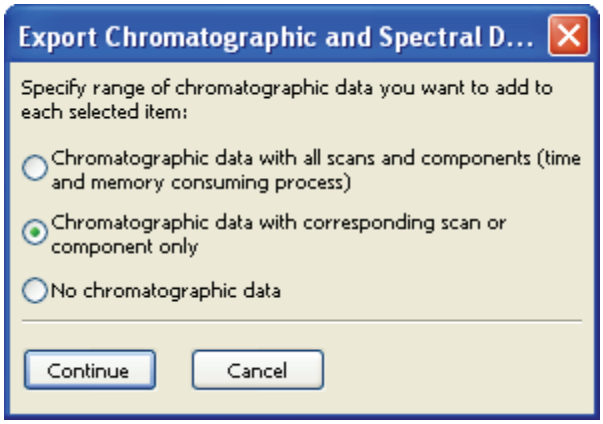
   The Chromatogram Processor window opens. See "Chromatogram Processor window" on page 250.

❖ To export scans or components to a Database Manager window

1. Select the scans or components.
2. Click the **Add Selected Scans/Components to Database Manager** button,  .

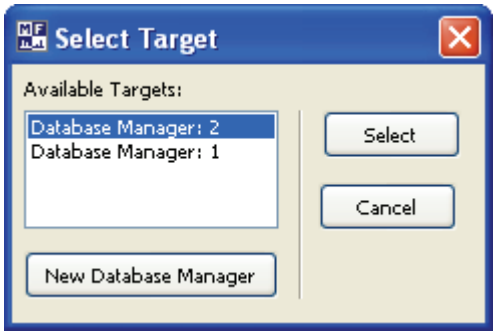
   Because copying components can take several minutes, use this method to monitor the processing and, if needed, interrupt the operation.

   The Export Chromatographic and Spectral Data dialog box opens.



3. Select the option for the type of data you want to export.
4. Click **Continue**.

   The Select Target dialog box opens.



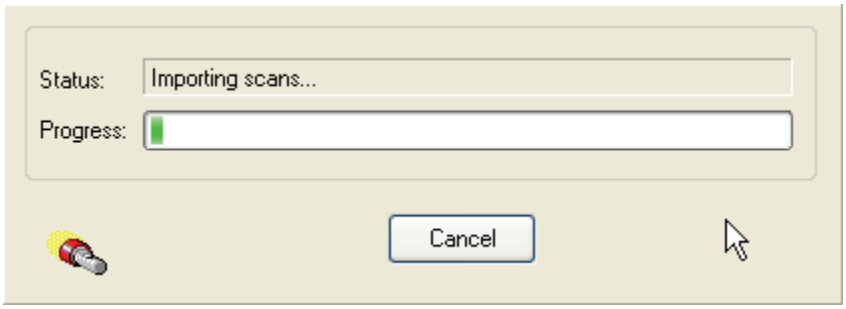
5. Do one of the following:

   Select the Database Manager window for the exported data, and then click **Select**.

   –or–

   Click **New Database Manager**.

   The export process begins.



6. To stop the export process, click **Cancel**.

❖ To copy data to a Database Manager window

1. Select the components.
2. Right-click and choose **Copy > Copy *option*** from the shortcut menu.

   You can copy a selection, chromatogram, MS spectrum, or an MS tree.

3. In a Database Manager window, right-click and choose **Paste > *option*** from the shortcut menu.

For more information about exporting and importing chromatograms, see "Exporting Scans, Components, and Reduced Chromatograms" on page 325.

❖ To copy components to a Spectra Classifier window

1. Select the scans or components.
2. Click the **Add Selected Scans or Components to Spectra Classifier** button,  .

   The Spectra Classifier window opens, and the selected components are displayed in the Available Groups of Spectra list.

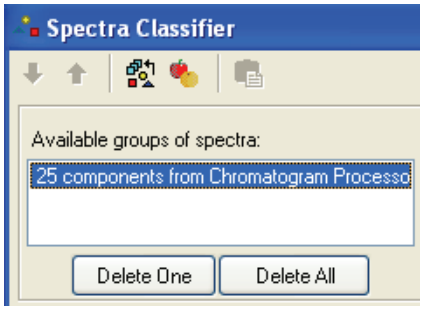
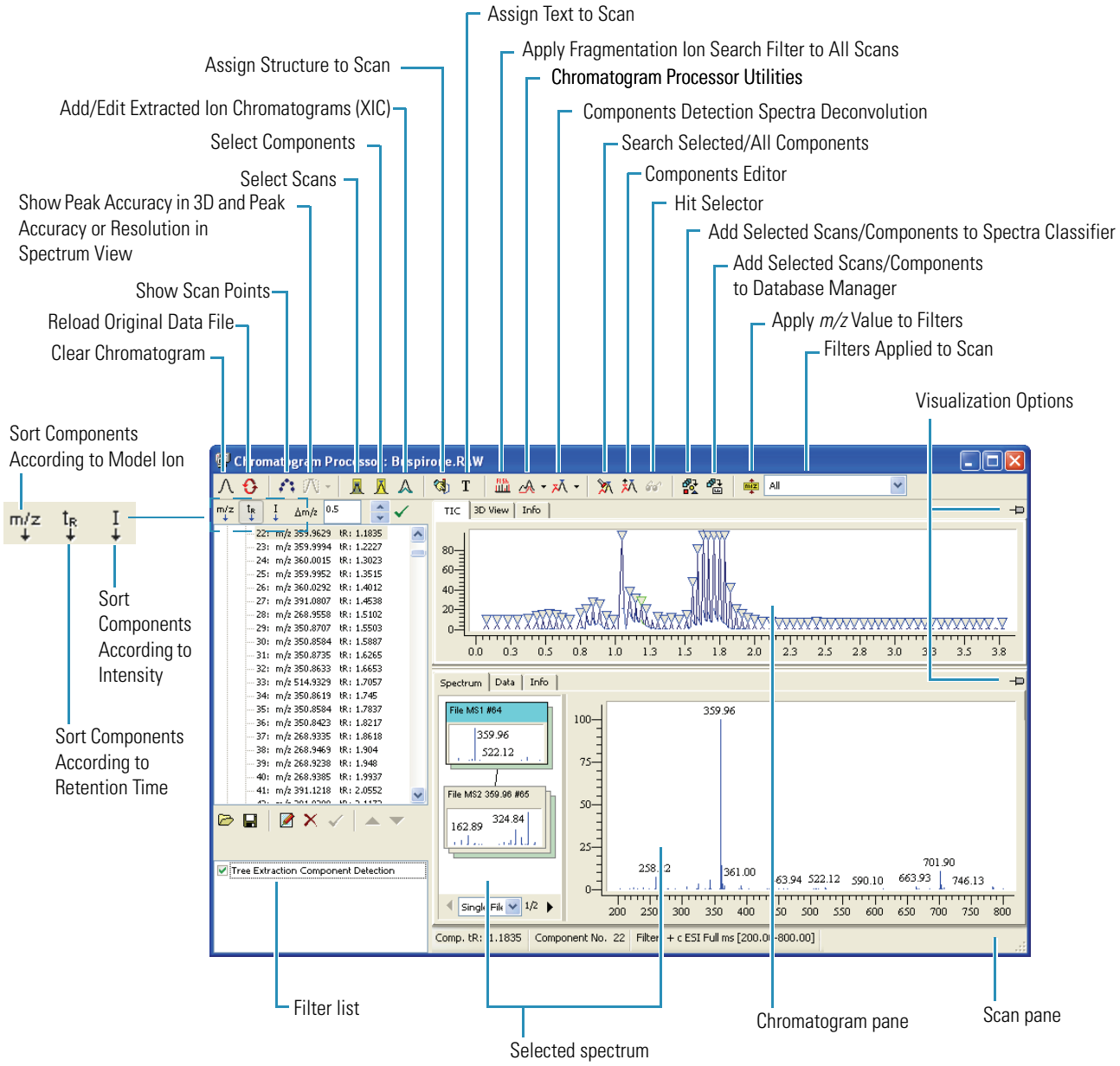


Figure 83. Chromatogram Processor window



Using the Chromatogram Pane

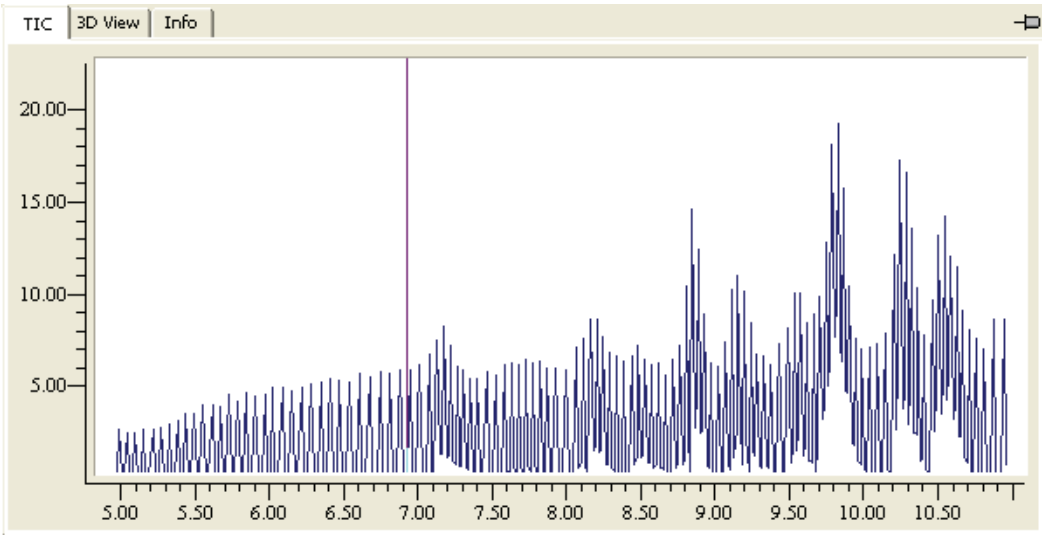
The Chromatogram pane includes the following pages:

- TIC Page
- 3D View Page
- Info Page

TIC Page

The Chromatogram Processor window displays Total Ion Current (TIC) chromatograms on the TIC page.

Figure 84. TIC page in the Chromatogram pane



Follow these procedures:

- To zoom in on the retention time or the intensity axis
- To use the toolbar zoom and pan features
- To select a scan from the TIC display
- To use the chromatogram pane visualization options
- To use the spectrum pane visualization options

❖ To zoom in on the retention time or the intensity axis

1. Drag the cursor in the horizontal (retention time) or vertical (intensity) scale to form a rectangle around the region you want to enlarge.

Figure 85. Enlarging the retention time scale

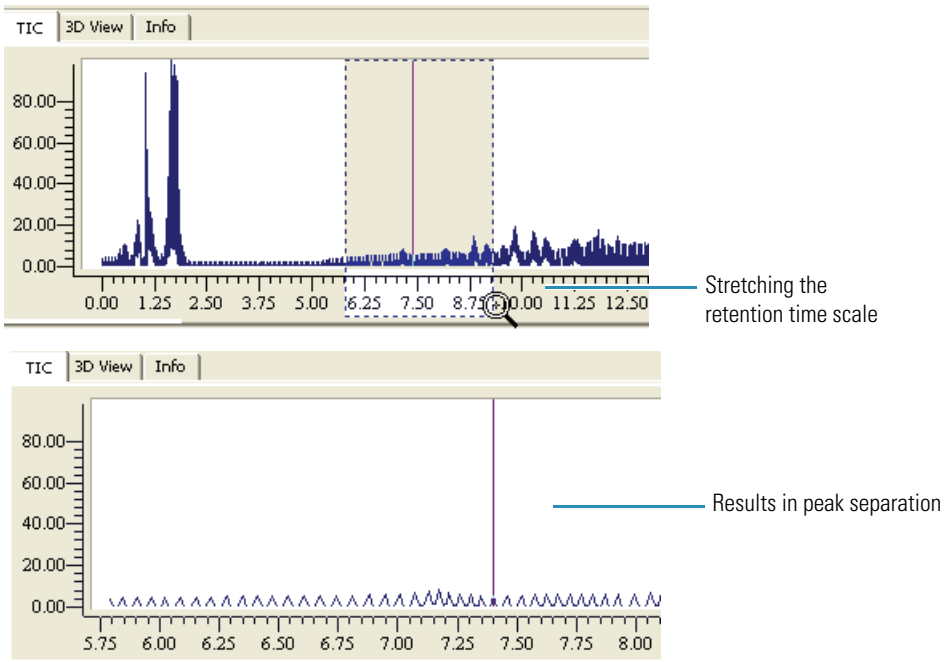
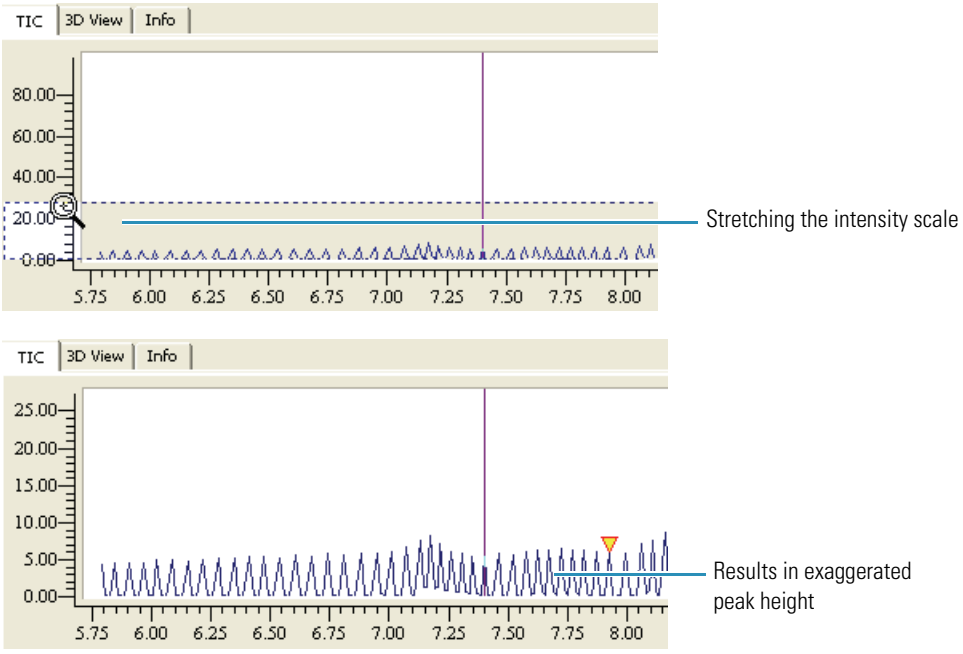


Figure 86. Enlarging the intensity scale



2. To return to the original scale, right-click and choose **Zoom > Zoom Reset** from the shortcut menu.

❖ To use the toolbar zoom and pan features

- Click the **Hand** button, , in the Mass Frontier toolbar and drag the hand to pan horizontally or vertically.
  Similarly, hold down the H keyboard key or the Spacebar to change the cursor to the hand.
- Click the **Zoom Out** button, , in the Mass Frontier toolbar.
- Click the **Zoom In** button, , in the Mass Frontier toolbar.
- Click the **Zoom to Box** button, , in the Mass Frontier toolbar and describe a rectangle.
  Similarly, hold down the Z keyboard key and describe a rectangle.
- Click the **Zoom Reset** button, , in the Mass Frontier toolbar to return to full scale.
- Click the **Zoom Undo** button, , in the Mass Frontier toolbar to undo the last zoom operation.
- Click the **Zoom Redo** button, , in the Mass Frontier toolbar to restore the last zoom operation.
- Use the scroll wheel on the mouse to zoom the entire display.

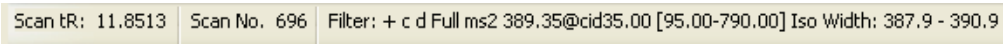
❖ To select a scan from the TIC display

1. Click the chromatogram pane to select a retention time.

   The scan corresponding to the selected retention time is displayed on the Spectrum page. A vertical line in the chromatogram pane indicates the selected scan.

2. To move the scan point to the next or previous scan, click the keyboard arrow keys.

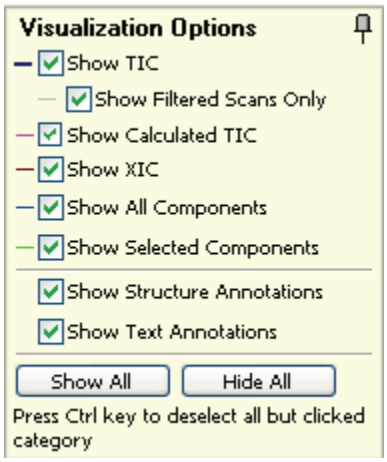
   Retention times (tR), scan numbers, and scan filters of active scans are displayed in the status bar.



❖ To use the chromatogram pane visualization options

1. In the Chromatogram pane, click the **Visualization Options** button, .

   The **Visualization Options** pane opens.



2. Select the options for the chromatogram display.

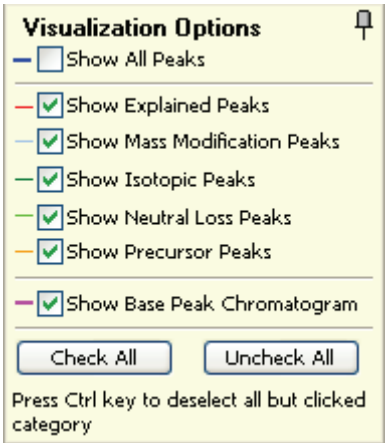
   The effect is immediate; the options pane remains open while you experiment with combinations of display options.

3. When you are satisfied with the display options, click the **Visualizations Options** button again to close the pane.

❖ To use the spectrum pane visualization options

1. In the Spectrum pane, click the **Visualization Options** button, .

   The **Visualization Options** pane opens.



2. Select the options for the spectrum display.

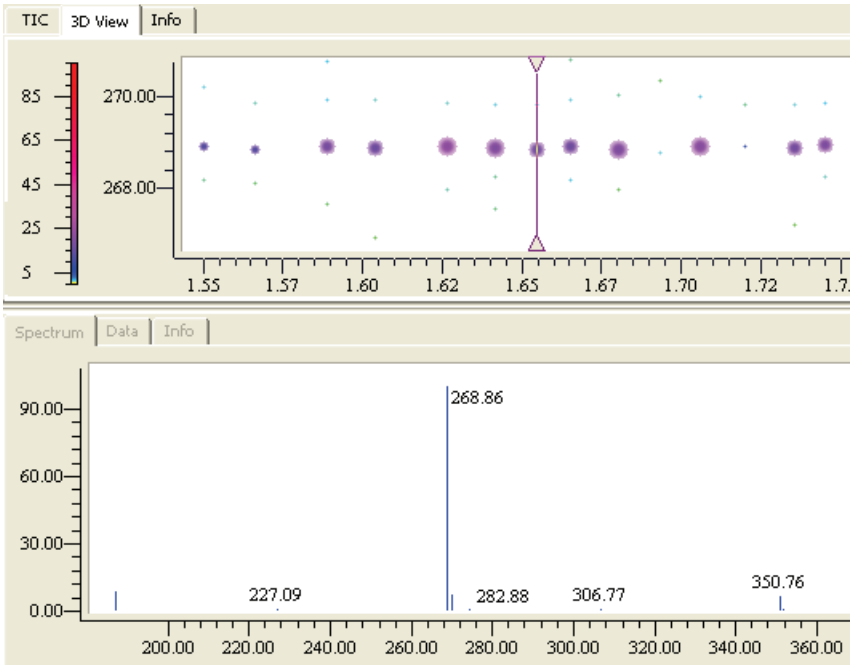
   The effect is immediate; the options pane remains open while you experiment with combinations of display options.

3. When you are satisfied with the display options, click the **Visualizations Options** button again to close the pane.

3D View Page

The 3D View page in the Chromatogram Processor window shows GC/MS or LC/MS data in a two-dimensional plot (retention time versus *m/z* value) where intensity as a third dimension is color-coded.

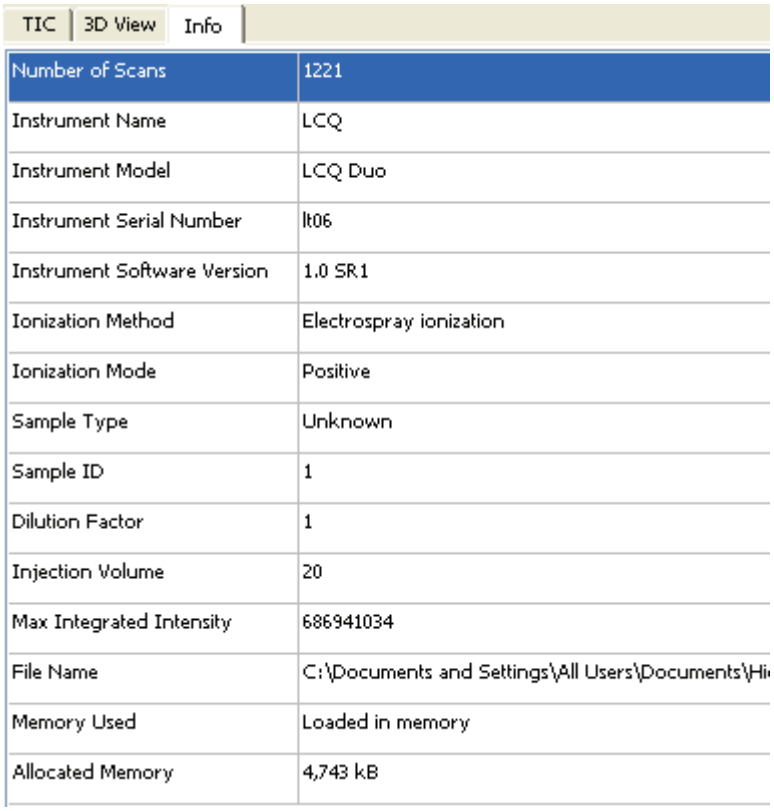
Figure 87. 3D View page in the Chromatogram Processor window



Info Page

The Info page in the Chromatogram Processor window shows additional information saved in the data file. Each GC/MS or LC/MS file format contains a different list of items that describe sample, instrument, experimental condition, and other information. The application extracts information from the file and displays it on the Info page.

Figure 88. Info page in the Chromatogram pane

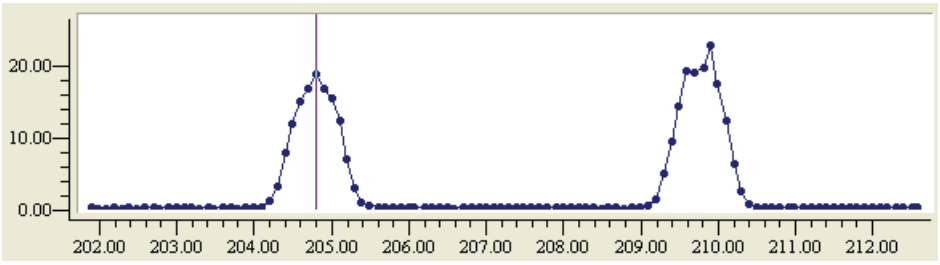


Averaging Scans

The application can extract the average mass spectrum from several scans.

❖ To extract the average mass spectrum from several scans

1. Click the **Show Scan Points** button, , in the Chromatogram Processor toolbar.

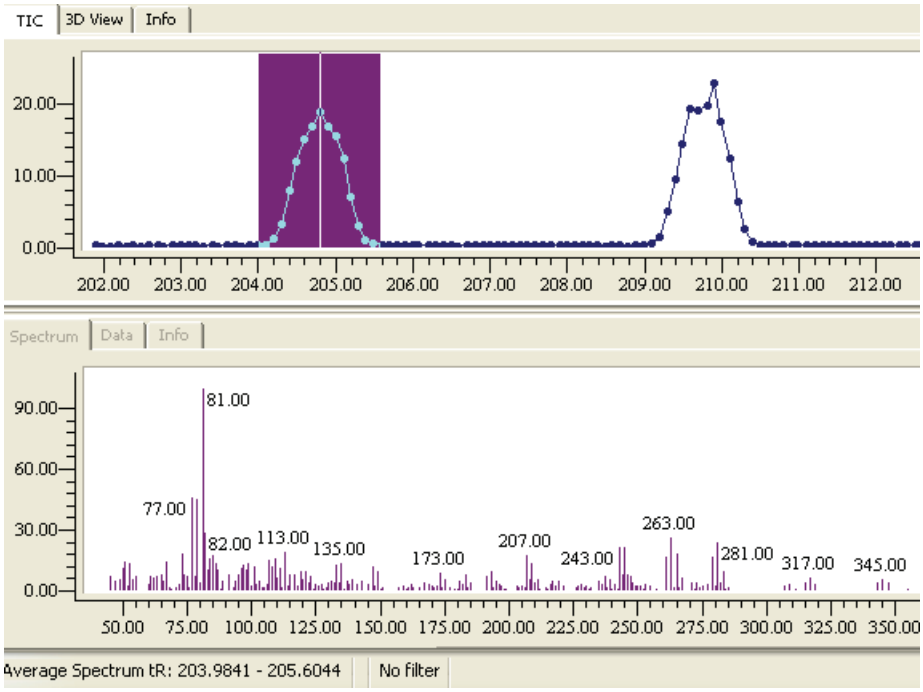


2. Click the **Chromatogram Processing Utilities** button, , and choose **Scans Average**.

3. Drag the cursor in the Chromatogram pane to indicate the region of the scans to be averaged.

   The marked region is displayed in the same color as the active scan line (by default purple). The average spectrum is displayed on the Spectrum page.

Figure 89. Averaged scans



4. To cancel the spectra average mode and restore the single scan mode, click anywhere in the chromatogram pane.

Processing the Data

The interfacing of gas and liquid chromatography with mass spectrometry often produces a signal that is not associated with the information you want. The Mass Frontier application provides tools for GC/MS and LC/MS data processing to improve the useful signal.

This section includes the following topics:

- Fragment Ion Search (FISh)
- Force Mass Tolerance
- Matrix Conversion
- Thresholding
- Baseline Correction and Noise Elimination
- Smoothing
- Base Peak Chromatogram
- Background Subtraction

Chromatographic data processing prepares valid data for automated detection and deconvolution of chromatographic components. There are two principally different ways of combining data processing and component detection:

- Preprocess data in advance by using a variety of methods for every processing type, separate with several customizable options, and then begin component detection.
- Perform data processing at the same time as component detection, using predefined methods and options (optimized for common chromatograms) in the same window that is used for component detection. See "Using Components Detection and Spectra Deconvolution" on page 299.

Fragment Ion Search (FISh)

Fragment Ion Search (FISh) is a data processing method used for intelligent extraction of useful information from chromatograms. The FISh feature removes spectral peaks that are not present in the predefined template. A list of fragments or *m/z* values serves as the input template (Fragment Source). When you apply the FISh algorithm, all spectral peaks except for the template peaks are removed from the chromatogram. You can use this feature to extract structurally related spectral peaks, for example, peaks related to the parent drug in metabolite ID, or to eliminate the noise or impurity peaks.

Follow these procedures:

- To apply fragment ion search to your data
- To drag actions from one Chromatogram Processor window to another
- To view an explanation of FISh results

❖ To apply fragment ion search to your data

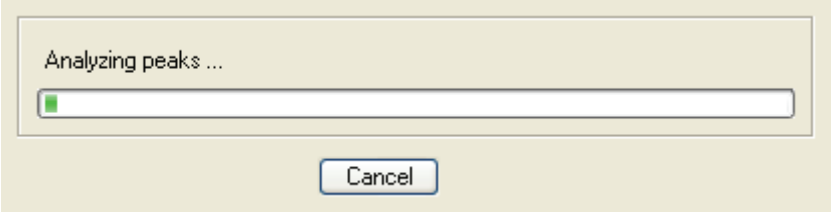
1. Click the **Apply Fragmentation Ion Search** button,  , in the Chromatogram Processor toolbar.

   The FISh Filter dialog box opens. See "Model page in the FISh Filter dialog box" on page 265.

2. Specify your fragment ion search parameters.

3. Click **Calculate**.

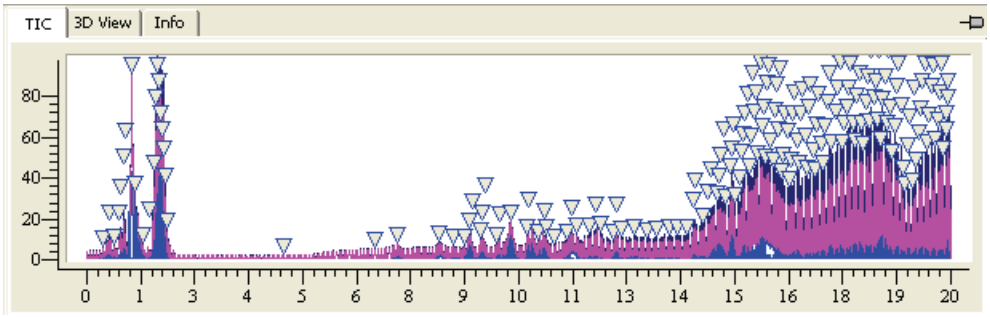
   The application displays the status of the FISh processing.



Note Do not click Cancel in this status box unless you intend to stop the FISh process.

4. When the fragment ion search is complete, click **Close**.

   The Chromatogram Processor window displays the detected components.



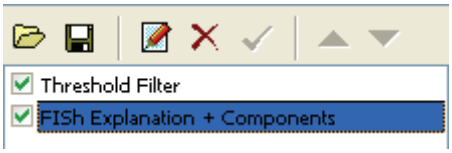
❖ To drag actions from one Chromatogram Processor window to another

1. Open multiple Chromatogram Processor windows.

2. Select, define, and calculate the filters and detection methods in the source Chromatogram Processor window.

   The application adds these calculated filters and methods to the Actions pane Chromatogram Processor window.

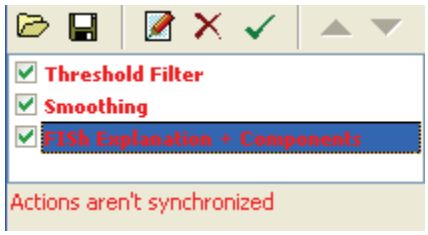
3. Select an action in the Actions pane of the source Chromatogram Processor window.



4. Drag the action to the Actions pane of the target Chromatogram Processor window.
5. Drag as many actions as you want from the any open source window to the target window.

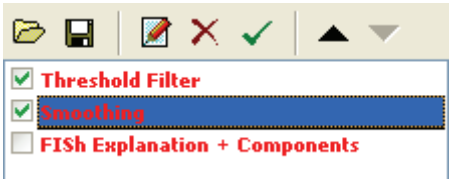
   The application copies each action and its associated parameters from the source Chromatogram Processor window to the target Chromatogram Processor window.

   The Actions pane reports that the actions are not synchronized.

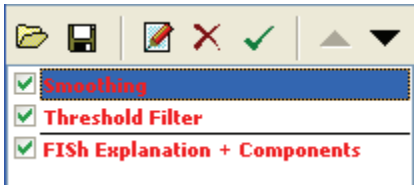


   The detection method must always be the final action, but you can change the order of other filters in the Action pane.

6. Select the action you want to move and click the up or down arrow in the Actions pane toolbar.



   The application moves the selected action.



7. To apply these actions to the current raw data file, do the following:
   a. Select the check box for each of the actions you want to process.
   b. Click the **Apply Actions to Chromatogram** button,  , in the Action pane toolbar.

   The Mass Frontier application processes each of the selected actions for the current raw data file.

❖ To view an explanation of FISh results

1. After FISh processing, right-click a spectrum in the Spectrum pane and choose **FISh Explanation** from the shortcut menu.

   The application adds a FISh page and displays the results.

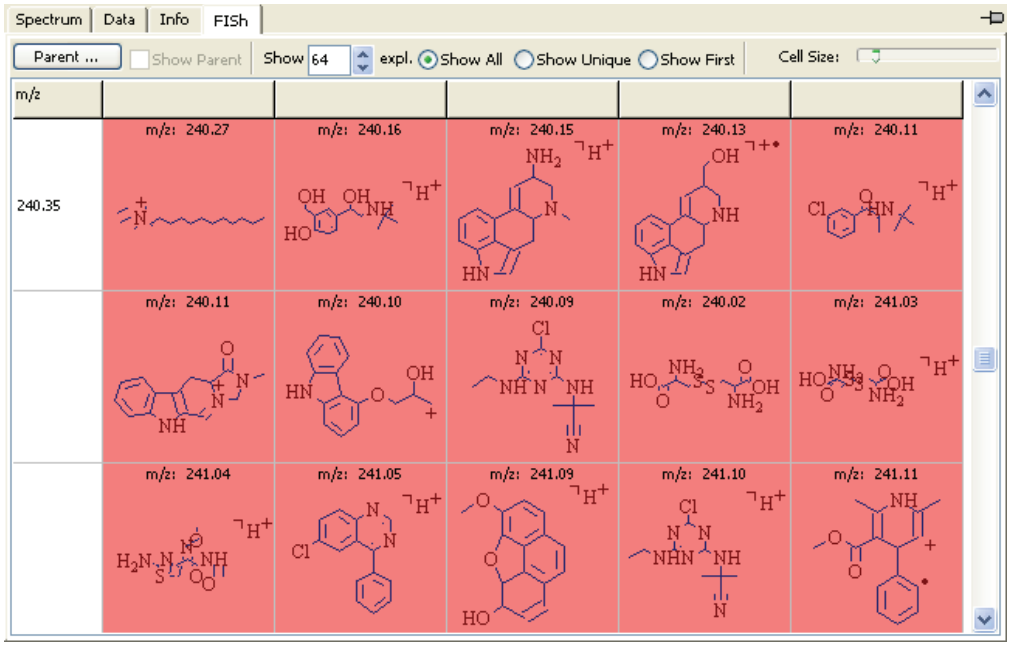


Table 29. FISh parameters

Parameter	Description
Parent	Opens the Structure Editor where you can define the parent.
Show Parent	Highlights in red all fragments common to the parent.
Show	Lets you select the maximum number of fragments to display in this page.
Show All	Displays all fragments.
Show Unique	Displays only unique fragments.
Show First	Displays only the first fragments in each *m/z* group.
Cell Size	Lets you resize the cells to fit more or fewer fragments in the display.

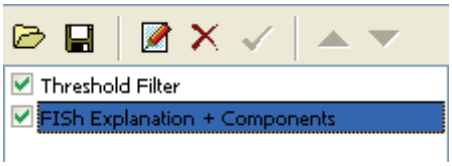
Saving FISh Parameters to a File

Follow these procedures:

- To save all FISh processing parameters to a file
- To edit FISh parameters before saving them to a file
- To save FISh model parameters to a file
- To save FISh options parameters to a file

❖ To save all FISh processing parameters to a file

1. When all processing is complete, click the **Save Actions to a File** button, , in the Actions pane of the Chromatogram Processor window.



   The Save Chromatogram Actions dialog box opens.

2. Type a name for the Mass Frontier Chromatogram Actions (.hcca) file and click **Save**.

   The applications saves the model and options parameters to the specified file.

❖ To edit FISh parameters before saving them to a file

1. Click the **Change Actions Parameters** button, , in the lower left pane of the Chromatogram Processor window.

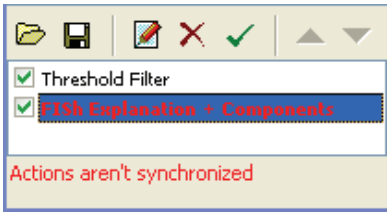
   The Fish Filter dialog box opens. This dialog box does not have a Calculate button. You can edit the FISh parameters on this dialog box, but you cannot begin FISh processing.

   The Fish Filter dialog box includes the following pages:

   - "Model page in the FISh Filter dialog box" on page 265
   - "Options page in the FISh Filter dialog box" on page 267

2. When you have finished changing the parameters, click **OK**.

   A message in the Actions pane reports that, because of your edits, the FISh parameters are no longer synchronized with the previous filters. This is acceptable only for saving the edited parameters to a file.



❖ To save FISh model parameters to a file

1. On the Model page of the FISh Filter dialog box, select the **Save FISh Model** check box.

   The Save FISh Flat File dialog box opens.

2. Type the name for the FISh Flat (.fish) file and click **Save**.

   When you calculate the FISh filter, the application writes the model parameters to a .fish file.

❖ To save FISh options parameters to a file

1. On the Options page of the FISh Filter dialog box, click **Save**.

   The Save FISh Parameters File dialog box opens.

2. Type the name for the FISh Parameters (.fish.par) file and click **Save**.

   When you calculate the FISh filter, the application writes the options parameters to a .fish.par file.

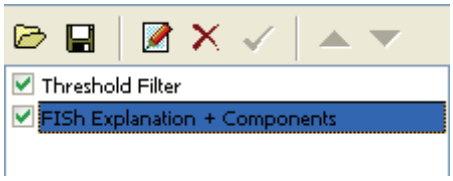
Loading FISh Parameters from a File

Follow these procedures:

- To load all FISh processing parameters from a file
- To load FISh model parameters from a file
- To load FISh options parameters from a file

❖ To load all FISh processing parameters from a file

1. Click the **Load Actions from a File** button,  , in the Actions pane of the Chromatogram Processor window.

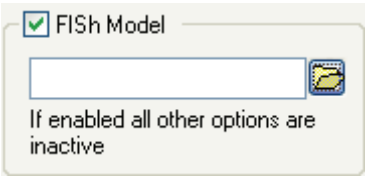
   The Load Chromatogram Actions dialog box opens.

2. Select a Mass Frontier Chromatogram Actions (.hcca) file and click **Open**.

   When you calculate the FISh filter, the application uses the model and options parameters from the specified .hcca file.

❖ To load FISh model parameters from a file

1. On the Model page of the FISh Filter dialog box, select the **FISh Model** check box.



2. Click the Open button,  .

   The Open FISh Flat File dialog box opens.

3. Select a FISh Flat (.fish) file and click **Open**.

   When you calculate the FISh filter, the application uses the model parameters in the specified .fish file.

❖ To load FISh options parameters from a file

1. On the Options page of the FISh Filter dialog box, click **Load**.

   The Open FISh Parameters File dialog box opens. Select a FISh Parameters (.fish.par) file and click **Open**.

   When you calculate the FISh filter, the application uses the options parameters in the specified .fish.par file.

Figure 90. Model page in the FISh Filter dialog box

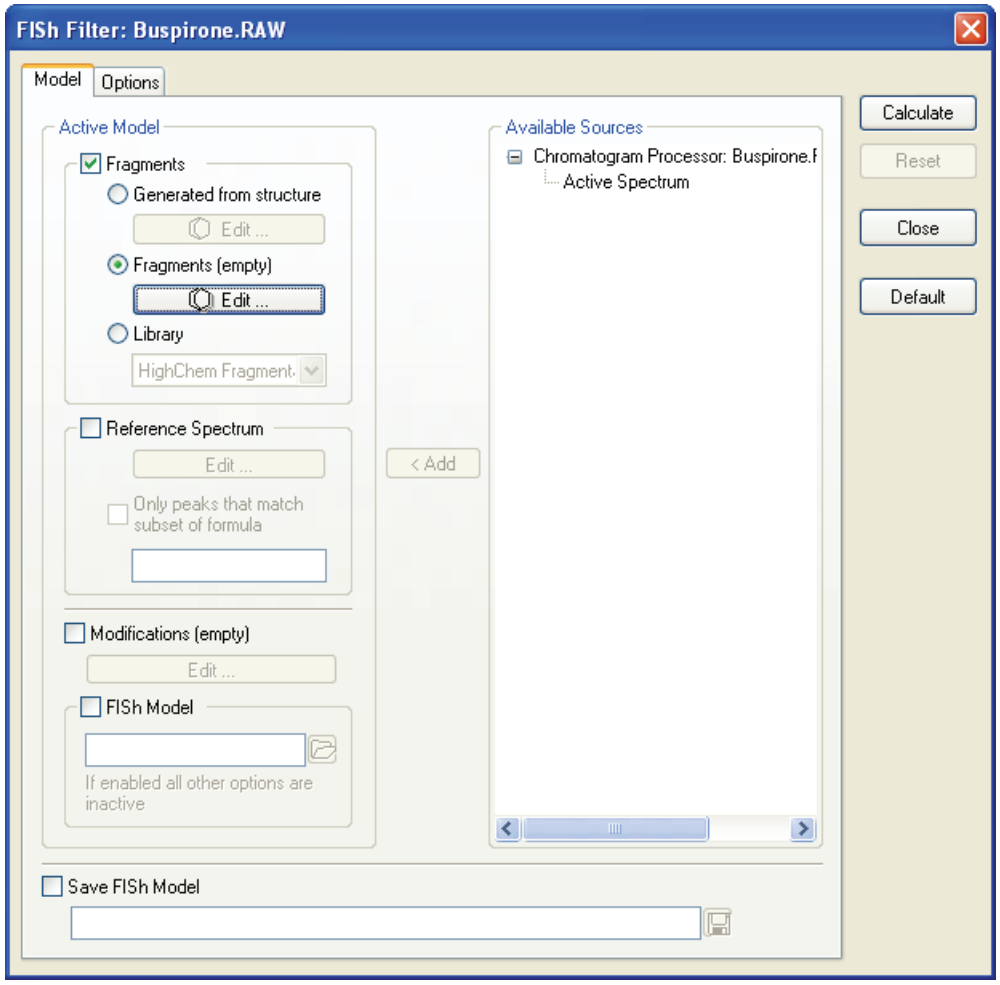


Table 30. Model parameters (Sheet 1 of 2)

Parameter	Description
Fragments	Defines the template list of fragments or *m/z* values that are not removed from a chromatogram.
Generated from Structure	Uses a list of fragments that you create in the Structure Editor module.
Fragments	Uses a list of fragments that you can load from an SDF file.
Library	Uses all fragments from the selected library.
Reference Spectrum	Defines the *m/z* values to use as a reference spectrum.
Edit	Opens the Input *m/z* List dialog box where you can create a list of *m/z* values. These values do not have implicit tolerance values, so enter these *m/z* values with the proper number of decimal digits.

Table 30. Model parameters (Sheet 2 of 2)

Parameter	Description
Only Peaks that Match Subset of Formula	Enter a reference formula to limit the number of fragments.
Modifications	Specifies a modifications list.
Edit	Opens the Modifications dialog box where you can create new modifications or select predefined modifications.
FISh Model	Use parameters from a saved FISh model (.fish.par) file. When you choose to use a saved file, all other parameter options are unavailable.
Save FISh Model	Saves the active fragment source to the specified (.fish.par) file.
Buttons	
Calculate	Begins the fragment ion search process.
Reset	Resets the chromatogram to its original (before fragment ion search) state. Uses the determined Mass Merge Power value in Baseline Correction and Noise Elimination, Smoothing, and the JCD Component Detection Algorithm as the starting value.
Close	Closes the fragment ion search dialog box.
Default	Returns the Mass Merge Power parameter to its default value.

Figure 91. Options page in the FISh Filter dialog box

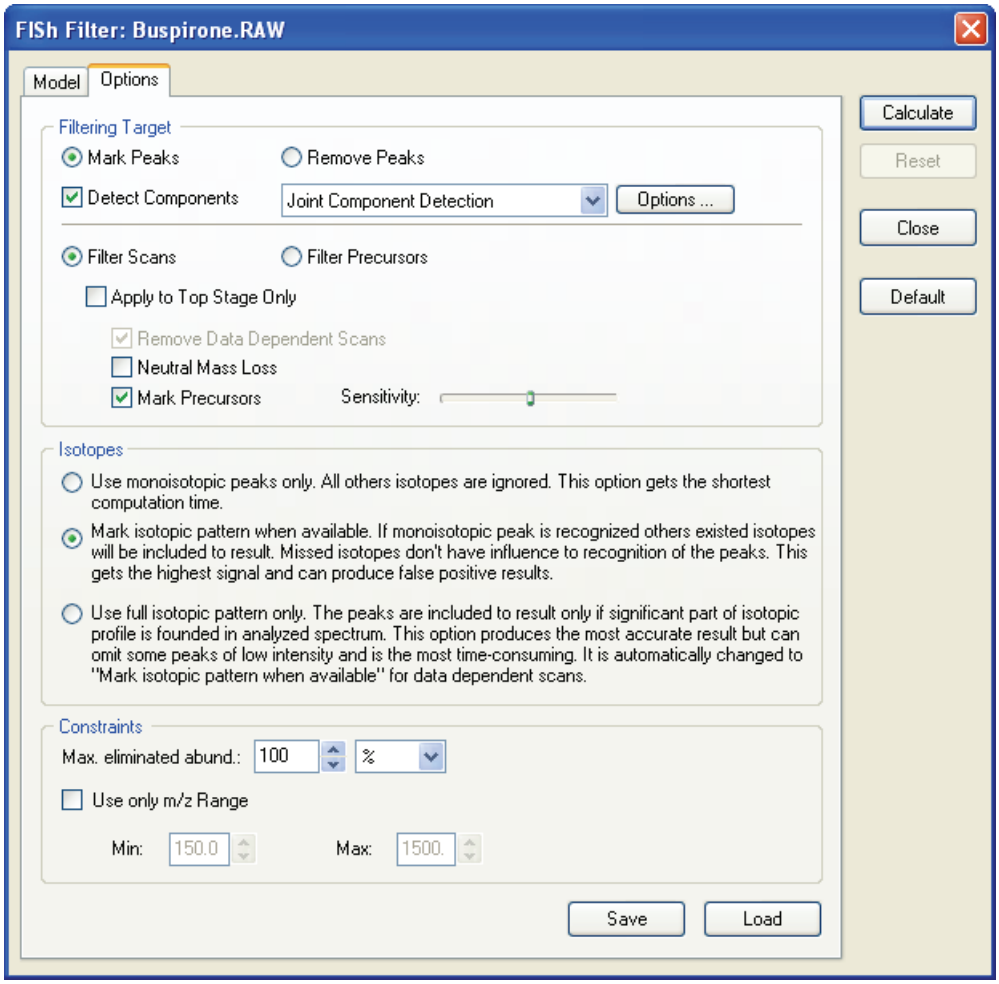


Table 31. Options parameters (Sheet 1 of 3)

Parameter	Description
Filtering Target	
Mark Peaks	Marks the threshold peaks in red when they are large enough to be above threshold value (the point where Mass Frontier preserves peaks). Specifies that the predefined peaks are marked in pink and corresponding TIC are drawn in green. No peaks are removed.
Remove Peaks	Specifies that peaks that are not predefined in the template are removed.
Detect Components	Specifies JCD or TECD detection options.

Table 31. Options parameters (Sheet 2 of 3)

Parameter	Description
Filter Scans	Specifies that only peaks are removed from scans, in contrast to the Filter Precursors option where entire scans are removed. The Mass Frontier application displays these options when you have a data-dependent chromatogram. • Apply to Top Stage Only: When selected, the filter is applied only to top stage scan (usually MS^1 scans). Data-dependent scans are not affected in this case. • Remove Data Dependent Scans: This option is active only if Apply to Top Stage Only is selected. When selected, the algorithm removes whole data-dependent scans for which the precursor peak was removed. When cleared, the filter is applied to a data-dependent scan in the same manner as a top stage scan (individually to each peak).
Filter Precursors	Specifies that all spectra with a precursor ion not found in the predefined list are removed from the chromatogram and precursor ions are removed from parent spectra. This option is enabled for data-dependent chromatograms only. • Filter Top Stage Scans: Modifies the top stage scans (usually MS^1 scans).
Isotopes	
Use Monoisotopic Peaks Only	All other isotopes are ignored. This option uses the shortest computation time.
Mark Isotopic Pattern when Available	If a monoisotopic peak is recognized, the application includes other existing isotopes in the result. Missed isotopes do not influence the recognition of the peaks. This option produces the highest signal and can produce false positive results.
Use Full Isotopic Pattern Only	The peaks 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 can omit some peaks of low intensity and consumes the most time. The Mass Frontier application changes the option to Mark Isotopic Pattern when Available for data-dependent scans.
Constraints	
Maximum Eliminated Abundance	Peaks with intensity higher than this value are not removed.
Use only *m/z* Range	Min/Max

Table 31. Options parameters (Sheet 3 of 3)

Parameter	Description
Buttons	
Save	Opens the Save FISh Parameters File dialog box where you can save your current parameters to a FISh parameters file (.fish.par).
Load	Opens the Open FISh Parameters File dialog box where you can choose a FISh parameters file (.fish.par) to load.

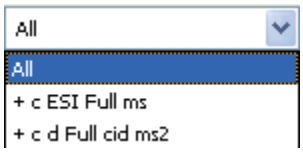
Force Mass Tolerance

Use the Force Mass Tolerance feature to change mass tolerance values for scans that you select. When processing spectra with different mass tolerance settings of different origins, you might want to force tolerance values to create comparable data. The Mass Frontier application uses tolerance values when comparing two *m/z* values.

Note Use the Force Mass Tolerance feature to check the correctness of search and calculation procedures by creating unit resolution from high accuracy/resolution data. You cannot improve accuracy using this feature.

❖ To run the Force Mass Tolerance process

1. Select the type of scans you want to process from the filter list.



   If you select Force Tolerance Only to Current Filter in the Force Tolerance dialog box, the software processes only visible scans.

2. Click the **Chromatogram Processing Utilities** button,  , and choose **Force Mass Tolerance**.

   The Force Tolerance dialog box opens. See "Force Tolerance dialog box" on page 271.

3. Specify your tolerance parameters.

   If you select Force Tolerance Only to Current Filter, the software processes only visible scans.

4. Click **Calculate**.

5. When the process is complete, click **Close**.

Figure 92. Force Tolerance dialog box

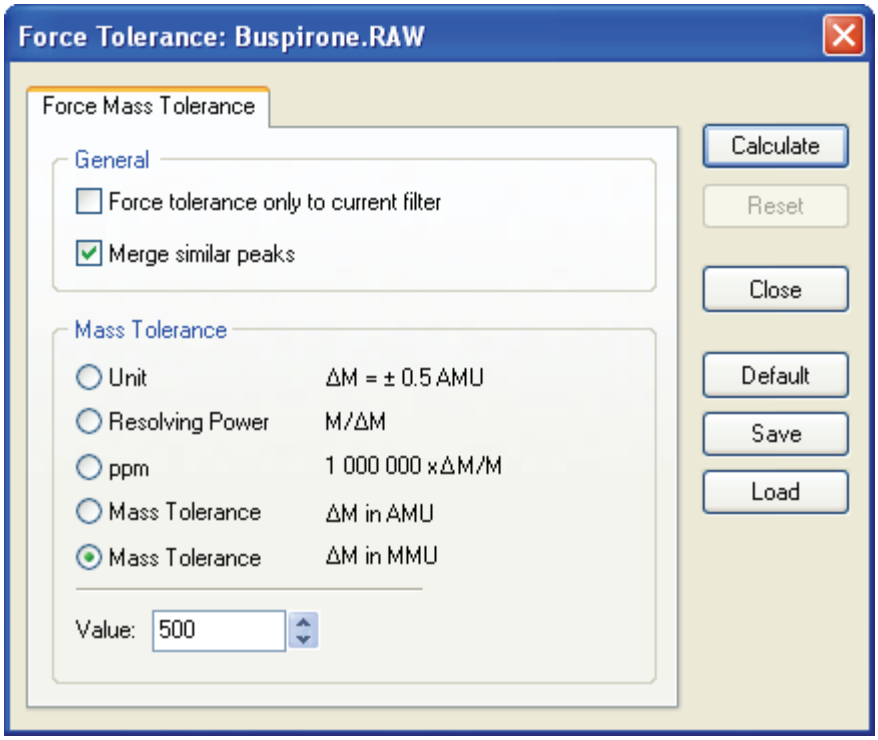


Table 32. Force Tolerance parameters (Sheet 1 of 2)

Parameter	Description
General	
Force Tolerance Only to Current Filter	Entered mass tolerance is forced only to scans visible in Chromatogram Processor.
Merge Similar Peaks	The peaks that overlap are merged after forcing the tolerance.
Mass Tolerance	
	The application supports these tolerance types: • **Unit** • **Resolving power** M/ΔM • **ppm** 1 000 000 x ΔM/M • **Mass Accuracy** (ΔM in AMU or ΔM in MMU) where: M = *m/z* (mass-to-charge ratio) $\Delta M = M_2 - M_1$ (M_1, M_2 are two adjacent peaks) Peaks (*m/z* values) that fall into the ΔM band are merged into a single peak (*m/z* value).
Value	Value of mass tolerance.

Table 32. Force Tolerance parameters (Sheet 2 of 2)

Parameter	Description
Buttons	
Calculate	Begins the forced mass tolerance process.
Reset	Resets the chromatogram to its original (before forced mass tolerance) state.
Close	Closes the mass tolerance dialog box.
Default	Returns all mass tolerance parameters to their default values.
Save	Opens the Save Force Tolerance Parameters File dialog box where you can save your current parameters to a Force Tolerance parameters file (.tolerance.par).
Load	Opens the Open Force Tolerance Parameters File dialog box where you can choose a Force Tolerance parameters file (.tolerance.par) to load.

Matrix Conversion

Some processing utilities, such as Baseline Correction and Noise Elimination, Smoothing, and the JCD Component Detection Algorithm, use internal matrix calculations. For high resolution/accuracy data, how the matrix is created can have a significant influence on the outcome of the processing utilities.

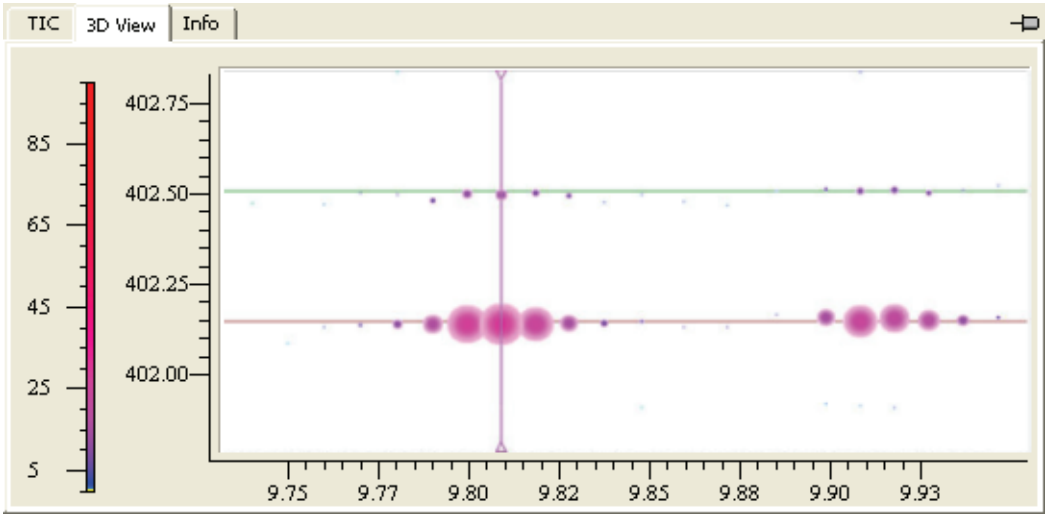
Note Use this feature mainly for visual determination of Mass Merge Power.

For additional information, see "Baseline Correction and Noise Elimination" on page 285, "Smoothing" on page 290, or "JCD Algorithm" on page 301.

The Mass Frontier application automatically converts a chromatogram to a matrix, where the first dimension is the scan number and the second dimension is an *m/z* value. Values of matrix cells are abundances. However, the oscillating *m/z* values of an identical ion might overlap with a different oscillating isobaric ion. In this case, it is very difficult to decide which *m/z* value should be taken as an independent dimension. Using the Matrix Conversion feature, you can control the alignment of oscillating *m/z* values to a specific value and you can visually control this process.

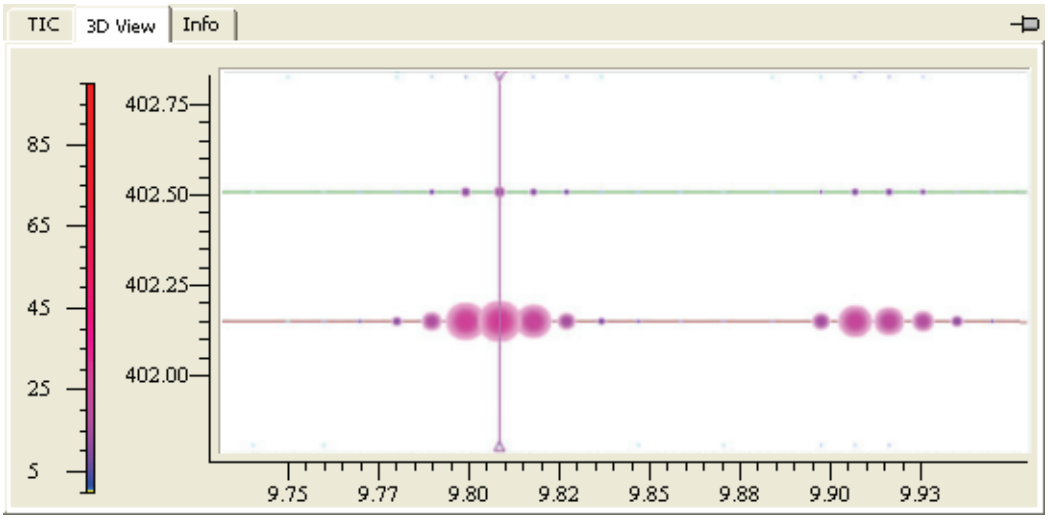
In the following example, the peaks are oscillating around *m/z* 402.15 and *m/z* 402.5 before matrix conversion.

Figure 93. Oscillating peaks on the 3D View page



In the following example, the peaks are aligned to discrete *m/z* 402.15 and *m/z* 402.5 after matrix conversion.

Figure 94. Aligned peaks on the 3D View page



Matrix conversion uses the Mass Merge Power parameter to determine the strength of the alignment process. The software uses this parameter also in Baseline Correction and Noise Elimination, Smoothing, and the JCD Component Detection Algorithm; however, when you calculate matrix alignment, you can watch the immediate effect on the chromatogram.

❖ To apply matrix conversion to your data

1. Click the **Chromatogram Processing Utilities** button, , and choose **Matrix**.

   The Matrix Conversion dialog box opens. See "Matrix Conversion dialog box" on page 275.

2. Specify the mass merge power percentage.
3. Click **Calculate**.
4. To undo a conversion, click **Reset**.

   The application returns to the Mass Merge Power value specified in Baseline Correction and Noise Elimination, Smoothing, and the JCD Component Detection Algorithm as a starting value.

5. When the conversion process is complete, click **Close**.

Figure 95. Matrix Conversion dialog box

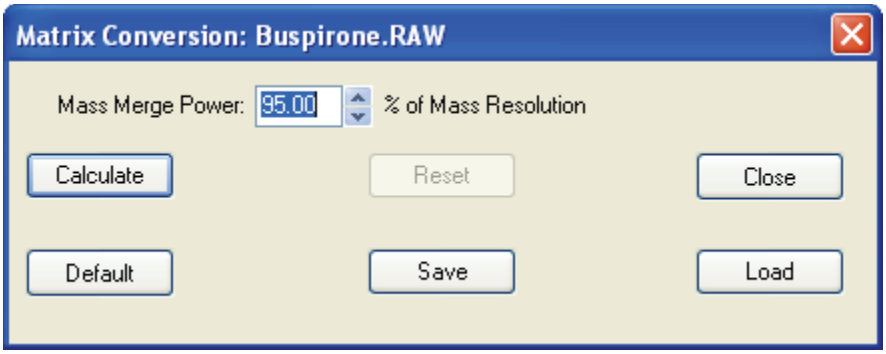


Table 33. Matrix Conversion parameters

Parameter	Description
Mass Merge Power	Specifies the percentage of mass resolution calculated from all scans in a chromatogram. It specifies the mass difference interval within the algorithm and merges spectral peaks into one *m/z* value. A low value can result in more individual *m/z* dimensions and a high value can result in merging more ions into one.
Buttons	
Calculate	Begins the matrix conversion process.
Reset	Resets the chromatogram to its original (before matrix conversion) state. Uses the determined Mass Merge Power value in Baseline Correction and Noise Elimination, Smoothing, and the JCD Component Detection Algorithm as the starting value.
Close	Closes the matrix conversion dialog box.
Default	Returns the Mass Merge Power parameter to its default value.
Save	Opens the Save To Matrix Conversion Parameters File dialog box where you can save your current parameters to a Matrix Conversion parameters file (.matrix.par).
Load	Opens the Open Matrix Conversion Parameters File dialog box where you can choose a Matrix Conversion parameters file (.matrix.par) to load.

Thresholding

Thresholding is a data processing method that analyzes every scan to reduce ion intensities or delete spectral peaks if algorithmic criteria have been met. The main purpose of thresholding is to eliminate noise or peaks originating from minor components that are not of interest. Thresholding requires fulfilling the condition that the intensity of noise peaks is smaller than the intensity of a signal and that the noise peaks count is significantly higher than the signal peaks count.

Note In contrast to Baseline Correction and Noise Elimination (see "Baseline Correction and Noise Elimination" on page 285), which operates in a chromatographic time domain, the thresholding feature processes each scan as a stand-alone, independent object that ignores the previous or following scans. Setting thresholds can significantly shorten JCD computation time for large chromatograms.

This section includes the following topics:

❖ To apply thresholding to your data

1. Click the **Chromatogram Processing Utilities** button,  , and choose **Thresholding**.

   The Threshold Filter dialog box opens. See "Threshold Filter dialog box" on page 277.

2. Specify your thresholding parameters.
3. Click **Calculate**.
4. When the thresholding process is complete, click **Close**.

Figure 96. Threshold Filter dialog box

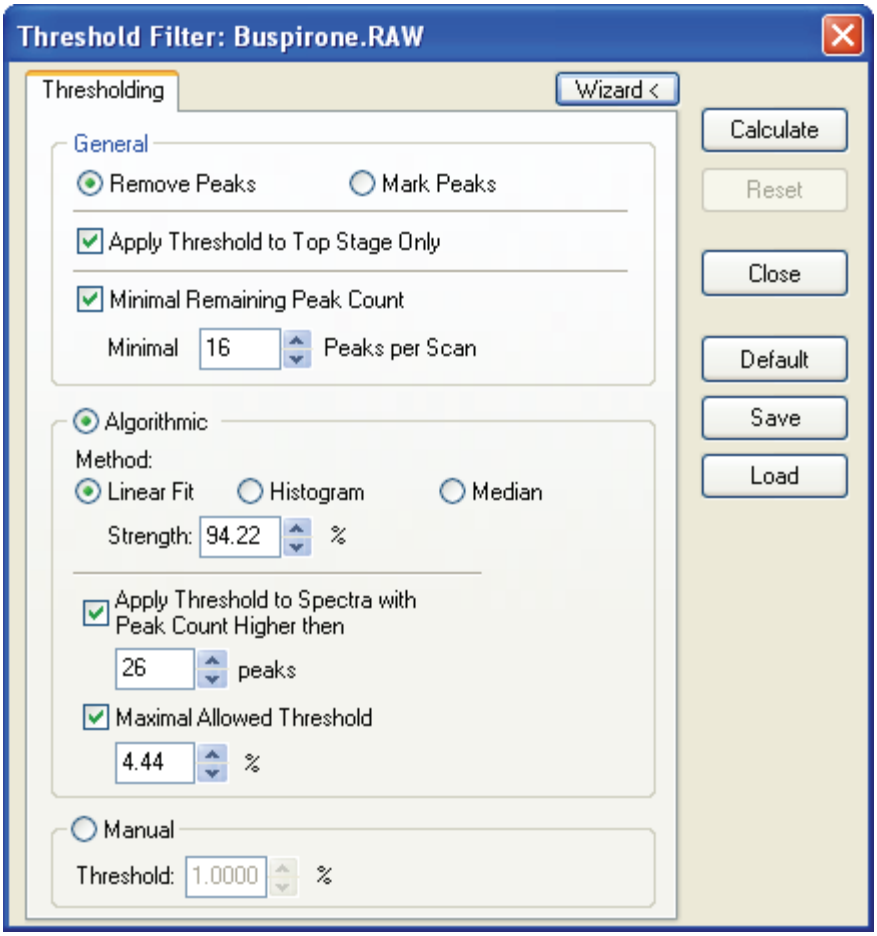


Table 34. Threshold Filter parameters (Sheet 1 of 2)

Parameter	Description
General	
Remove Peaks	Removes the threshold peaks.
Mark Peaks	Marks the threshold peaks in red when they are large enough to be above threshold value (the point where the Mass Frontier application preserves peaks). No peaks are removed. The same logic is applied for FISh filtering.
Apply Threshold to Top Stage Only	Specifies that only MS^1 (full scan) scans are processed.
Minimal Remaining Peak Count Minimal Peaks per Scan	Specifies that the number of the most intense peaks specified in the Minimal box is not affected by thresholding.

Table 34. Threshold Filter parameters (Sheet 2 of 2)

Parameter	Description
Algorithmic	Enables algorithmic thresholding parameters.
Method	Specifies that one of the following algorithms is used: Linear Fit, Histogram, or Median. See "Specifying Methods" on page 279.
Strength	In the Linear Fit or Histogram method, the calculated threshold value is multiplied by this value. In the Median method, this value is used for calculation of filter length.
Apply Threshold to Spectra with Peak Count Higher Than	Specifies that algorithmic thresholding is applied to spectra with several peaks higher than the specified value.
Maximal Allowed Threshold	Specifies that the algorithmic threshold is applied to spectral peaks with an intensity lower than the specified value.
Manual	Enables manual thresholding parameters.
Threshold	Specifies that all peaks that exhibit an intensity lower than the specified value are deleted.
Buttons	
Wizard	Displays a simplified version of the Threshold filter parameters. See "Using the Threshold Filter Wizard" on page 283.
Calculate	Begins the thresholding process.
Reset	Resets the chromatogram to its original (before thresholding) state.
Close	Closes the Threshold Filter dialog box.
Default	Returns all thresholding parameters to their default values.
Save	Opens the Save Threshold Parameters File dialog box where you save your current parameters to a threshold parameters file (.threshold.par).
Load	Opens the Open Threshold Parameters File dialog box where you can choose a threshold parameters file (.threshold.par) to load.

Specifying Methods

The threshold filter uses one of three options for methods:

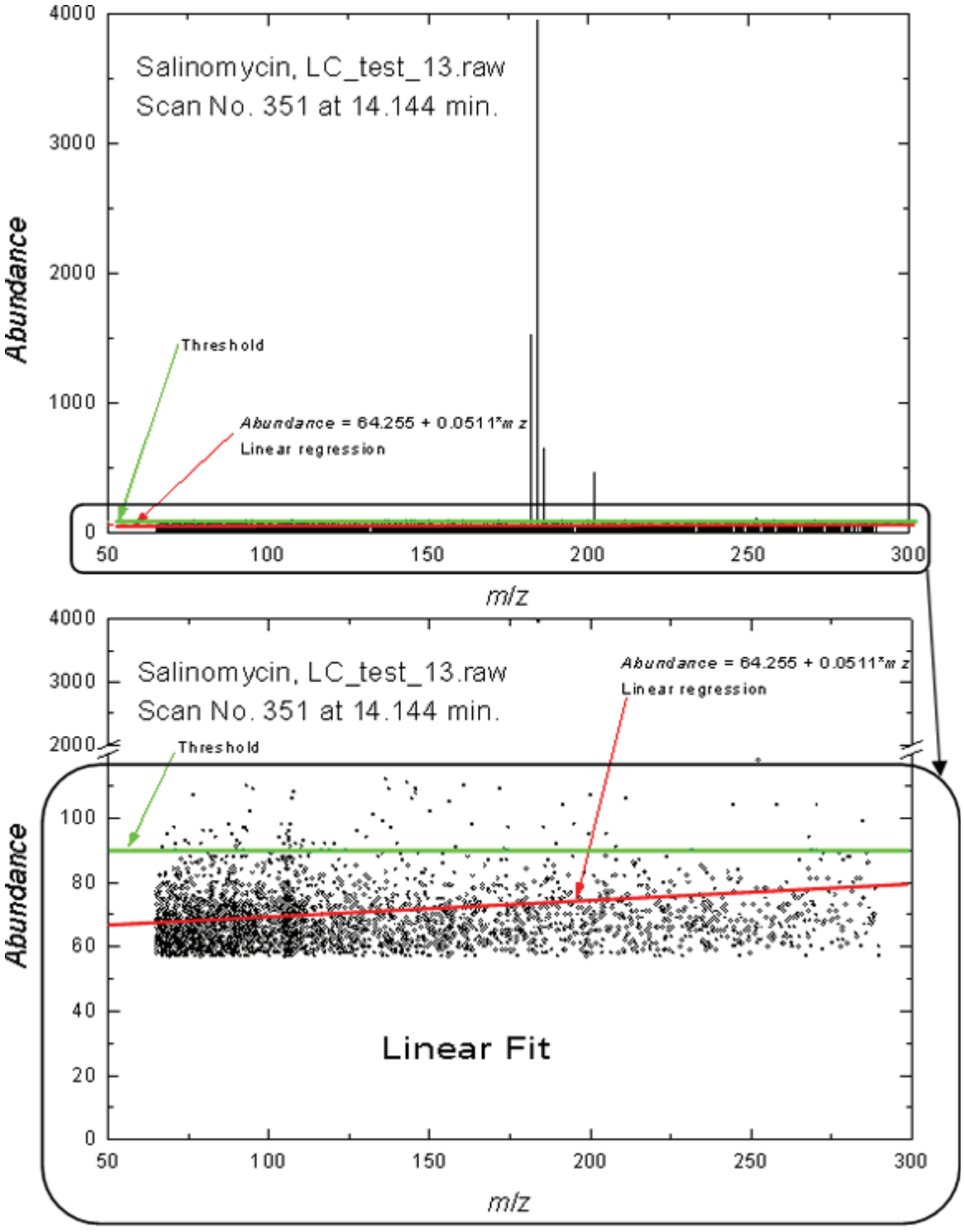
- Linear Fit
- Histogram
- Median

Linear Fit

The process of determining a linear fit occurs as follows:

- The Mass Frontier application 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.
- The Mass Frontier application considers outlier peaks as signals and excludes them from noise.
- In iterative form, the Mass Frontier application applies linear regression and exclusion of outlier peaks (signal) to each considered peak while finding no new outlier points.
- The Mass Frontier application determines a threshold value as the intensity of the most intensive noise peak (the highest peak that is not excluded from noise).

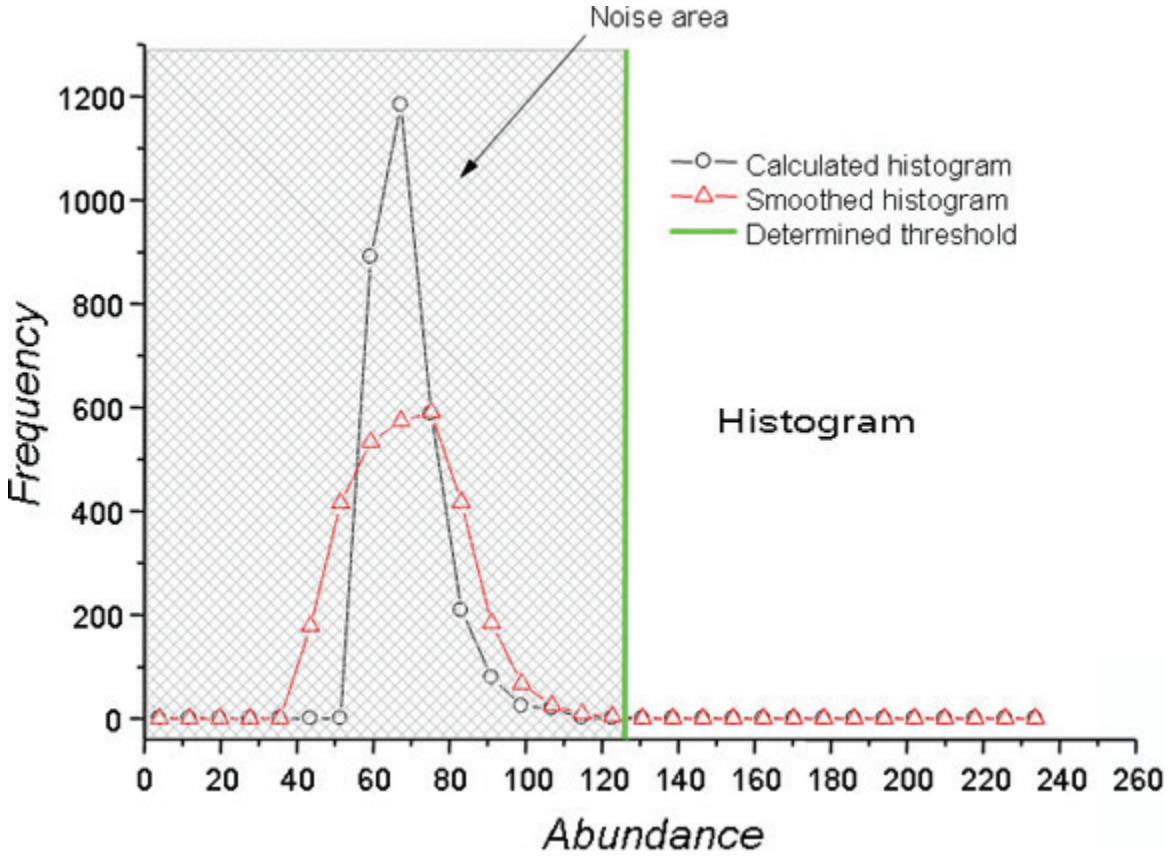
Figure 97. Linear Fit method



Histogram

A histogram is created from abundances of all peaks in analyzed scans (black circles on the figure), and then each histogram is smoothed (red triangles) and the end of the peak or the minimum between two peaks is considered as the threshold value (green line).

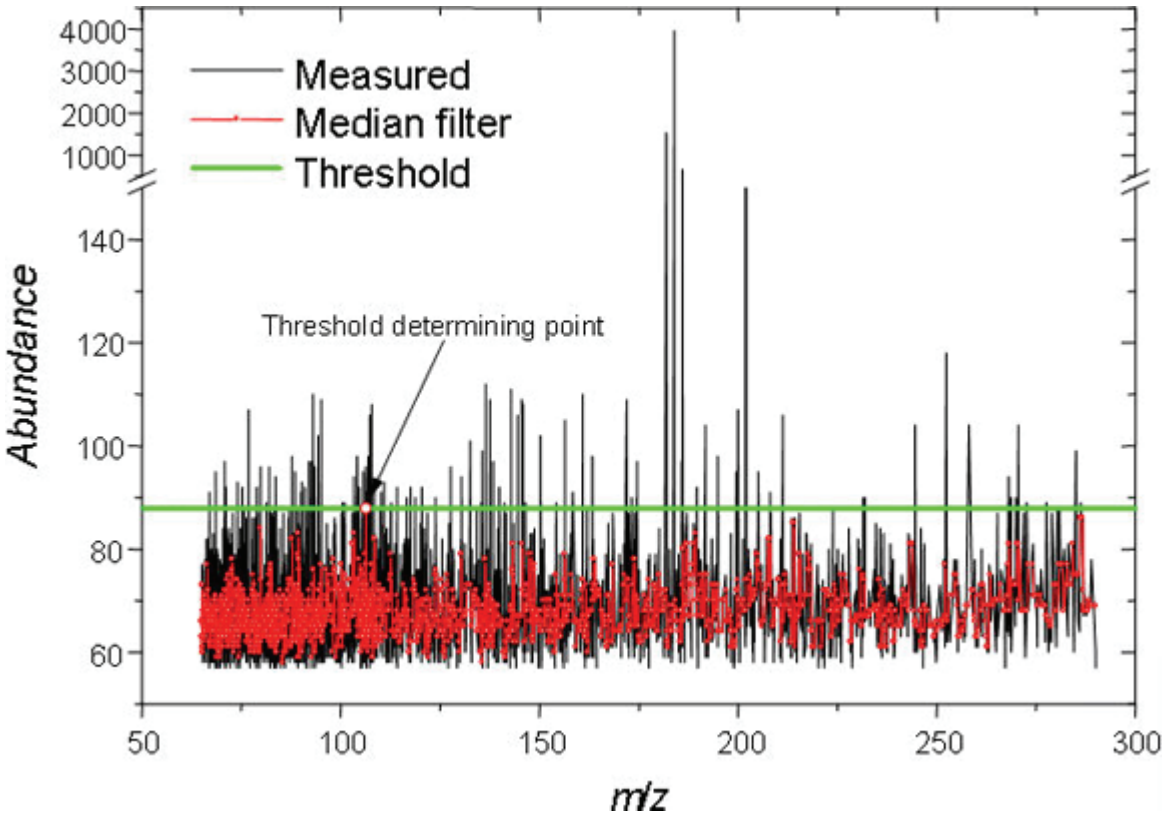
Figure 98. Histogram method



Median

The median filter is applied to an analyzed scan (red line). The maximal filtered abundance is used as the threshold value.

Figure 99. Median method



Using the Threshold Filter Wizard

The Threshold Filter wizard is a simplified version of the Threshold Filter parameters.

❖ To open the Threshold Filter wizard

1. In the Threshold Filter dialog box, click the **Wizard** button, Wizard <.

   The Threshold Filter dialog box minimizes to a simplified version.

2. To return to the complete version of the Threshold Filter dialog box, click **Details**.

Figure 100. Threshold Filter Wizard dialog box

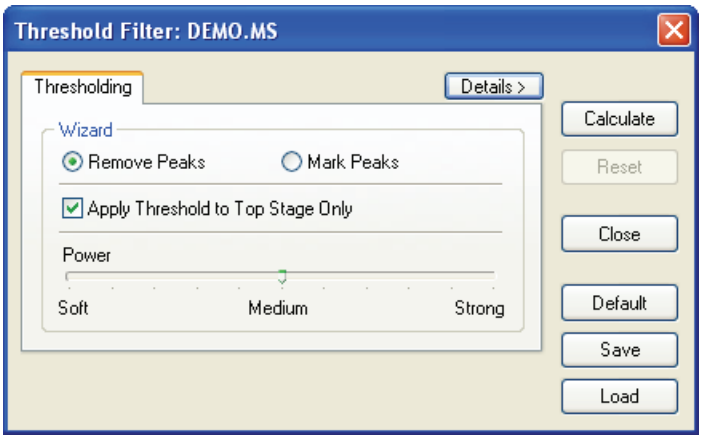


Table 35. Threshold Filter Wizard parameters (Sheet 1 of 2)

Parameter	Description
Wizard	
Remove Peaks	Directly removes the threshold peaks.
Mark Peaks	Mark the threshold peaks in red only with corresponding TIC showing in red as well. No peaks are removed.
Apply Threshold to Top Stage Only	Specifies that only MS^1 (full scan) scans are processed.
Power	Specifies the percentage of mass resolution calculated from all scans in a chromatogram on a linear scale. This value specifies the mass difference interval within the algorithm and merges spectral peaks into one *m/z* value. A Soft value can result in more individual *m/z* dimensions and a Strong value can result in merging more ions into one.

Table 35. Threshold Filter Wizard parameters (Sheet 2 of 2)

Parameter	Description
Buttons	
Details	Displays the full version of the Thresholding Filter parameters. See "Threshold Filter dialog box" on page 277.
Calculate	Begins the thresholding process.
Reset	Resets the chromatogram to its original (before thresholding) state.
Close	Closes the Threshold Filter dialog box.
Default	Returns all thresholding parameters to their default values.
Save	Opens the Save Threshold Parameters File dialog box where you save your current parameters to a threshold parameters file (.threshold.par).
Load	Opens the Open Threshold Parameters File dialog box where you can choose a threshold parameters file (.threshold.par) to load.

Baseline Correction and Noise Elimination

Baseline correction and noise elimination algorithms analyze ion profiles (ion chromatograms) of all ions appearing in spectra over the entire region of a chromatogram. In contrast to thresholding, where the individual scans and their spectral peaks are independently analyzed and modified, baseline correction and noise elimination analyze and modify spectral peaks in a specified retention time range.

Note In contrast to thresholding, which processes each scan independently while ignoring the previous or following scans, the Baseline Correction and Noise Elimination feature processes data in a chromatographic time domain, following trends over a specified retention time range.

❖ To apply baseline correction and noise elimination to your data

1. Click the **Chromatogram Processing Utilities** button,  , and choose **Baseline**.

   The Baseline & Noise dialog box opens. See "Baseline & Noise dialog box" on page 286.

2. Specify your baseline correction and noise elimination parameters.
3. Click **Calculate**.
4. When the baseline and noise process is complete, click **Close**.

Figure 101. Baseline & Noise dialog box

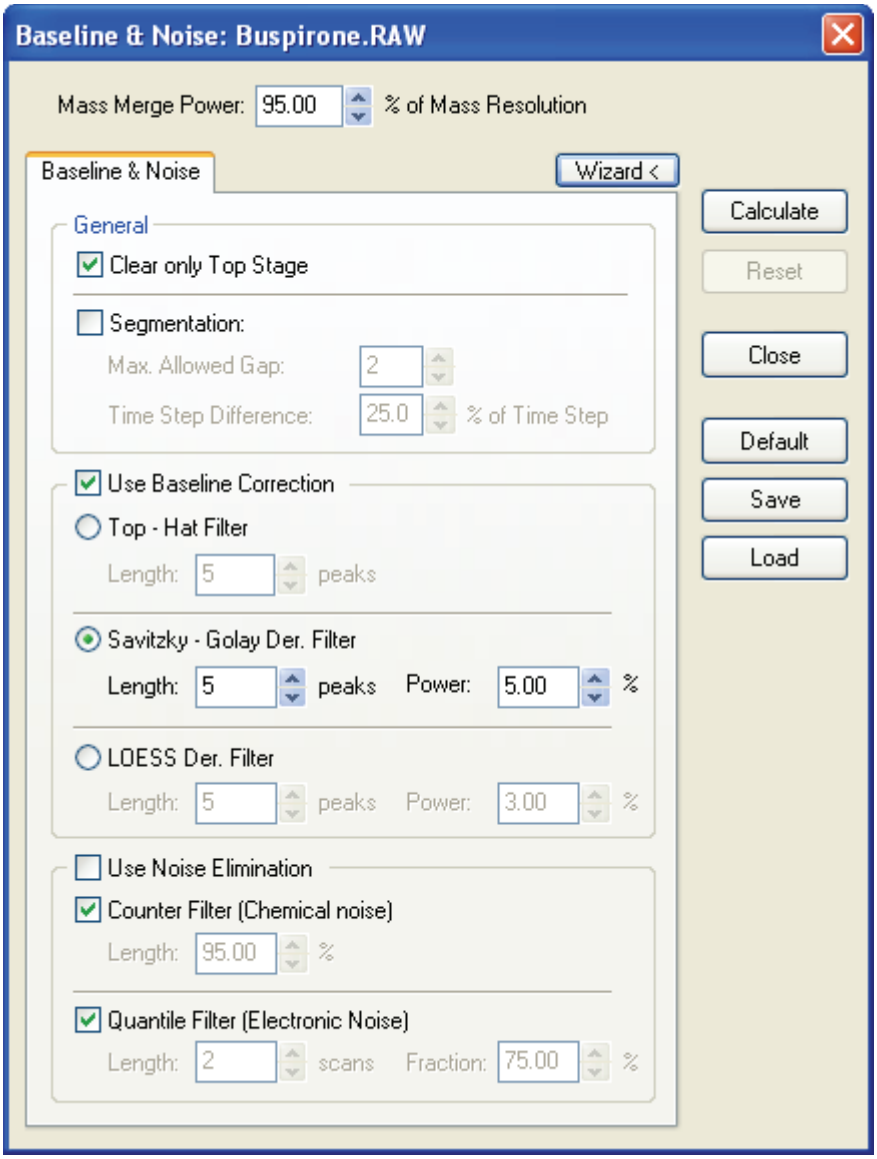


Table 36. Baseline & Noise parameters (Sheet 1 of 3)

Parameter	Description
Mass Merge Power	Specifies the mass difference within which the algorithm merges spectral peaks into one *m/z* value. A low value might result in more components (oscillating ions) and a high value can result in fewer components (merging ions).
General	
Clear Only Top Stage	Processes only spectra in MS^1 stage (full scan).

Table 36. Baseline & Noise parameters (Sheet 2 of 3)

Parameter	Description
Segmentation	Divides the chromatogram into discrete parts to which the algorithms are applied separately. This option can provide better results if a chromatogram exhibits diverse shape, peak density, and baseline characteristics over the retention time scale. Avoid segmentation if you use a LOESS derivative filter or if results obtained without segmentation are acceptable.
Use Baseline Correction	Applies one of the following methods to your chromatogram: Top-Hat filter, Savitzky-Golay derivative filter, or LOESS derivative filter.
Top-Hat Filter	A morphological two-step filter. First, the algorithm searches the average value (opening) of signal on an area around the analyzed peak and then it subtracts this value from the original analyzed peak.
Savitzky-Golay Derivative Filter	Baseline is defined by the point where the first derivation of a smoothed ion profile (values from Savitzky-Golay filter) is smaller than the value represented by the parameter Power. This baseline is then subtracted from the original ion profile. Time domain is determined by the Length parameter.
LOESS Derivative Filter	Principally identical with the Savitzky-Golay derivative filter but it does not require equidistant sampling.
Use Noise Elimination	Applies the following methods individually or simultaneously: Counter filter reduces chemical noise and Quantile filter reduces electronic noise.
Counter Filter	Counts the occurrence of non-zero peaks in ion profile. If the percentage of signal occurrence is higher than the Length parameter, the ion is considered as chemical noise and the whole ion profile is removed.
Quantile Filter	Counts the occurrence of non-zero peaks in neighboring space on both sides (2×Length + 1 peaks). If the percentage of signal occurrence is smaller than the Fraction parameter, the analyzed peak is removed. This filter removes narrow peaks.
Buttons	
Wizard	Displays a simplified version of the Baseline & Noise filter parameters. See "Baseline & Noise wizard dialog box" on page 288.
Calculate	Begins the baseline correction and noise elimination process.
Reset	Resets the chromatogram to its original (before baseline correction and noise elimination) state.

Table 36. Baseline & Noise parameters (Sheet 3 of 3)

Parameter	Description
Close	Closes the Baseline & Noise dialog box.
Default	Returns all baseline correction and noise elimination parameters to their default values.
Save	Opens the Save Baseline & Noise Parameters File dialog box where you save your current parameters to a threshold parameters file (.baseline.par).
Load	Opens the Open Baseline & Noise Parameters File dialog box where you can choose a threshold parameters file (.baseline.par) to load.

Figure 102. Baseline & Noise wizard dialog box

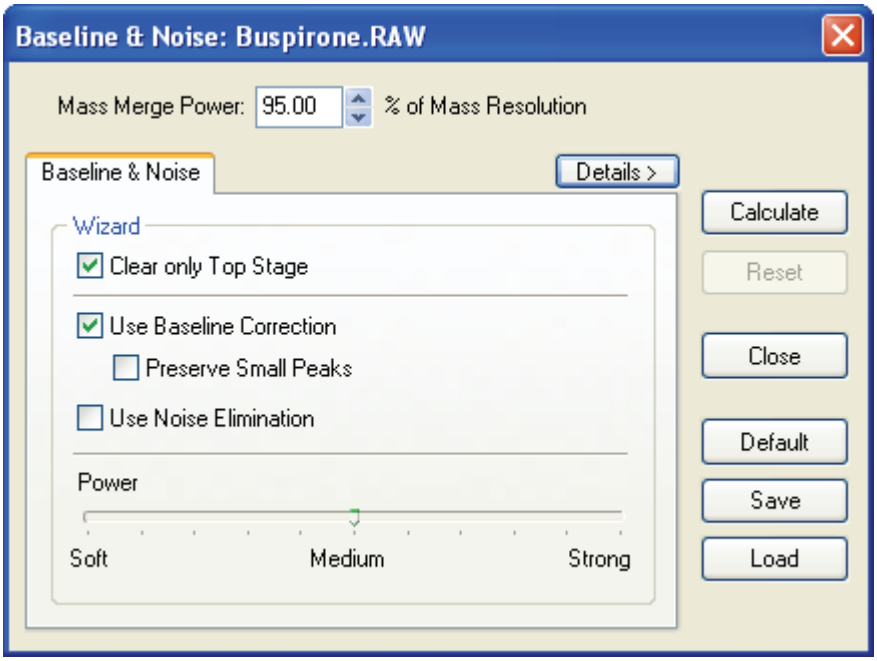


Table 37. Baseline & Noise parameters (Sheet 1 of 2)

Parameter	Description
Mass Merge 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).
Clear Only Top Stage	Processes only spectra in MS^1 stage (full scan).
Use Baseline Correction	Applies the correction method specified in the detailed Baseline & Noise dialog box.

Table 37. Baseline & Noise parameters (Sheet 2 of 2)

Parameter	Description
Preserve Small Peaks	Preserves small peaks when baseline correction is used.
Use Noise Elimination	Applies the methods specified in the detailed Baseline & Noise dialog box: Counter filter reduces chemical noise and Quantile filter reduces electronic noise.
Buttons	
Details	Displays a detailed version of the Baseline & Noise filter parameters. See "Baseline & Noise dialog box" on page 286.
Calculate	Begins the baseline correction and noise elimination process.
Reset	Resets the chromatogram to its original (before baseline correction and noise elimination) state.
Close	Closes the Baseline & Noise Filter dialog box.
Default	Returns all baseline correction and noise elimination parameters to their default values.
Save	Opens the Save Baseline & Noise Parameters File dialog box where you save your current parameters to a threshold parameters file (.baseline.par).
Load	Opens the Open Baseline & Noise Parameters File dialog box where you can choose a threshold parameters file (.baseline.par) to load.

Smoothing

The Mass Frontier 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 increase correct component detection and eliminate spikes, which cause false positive results. For additional information, see "Using Components Detection and Spectra Deconvolution" on page 299.

❖ To apply smoothing to your data

1. Click the **Chromatogram Processing Utilities** button, , and choose **Smoothing**.

   The Smoothing dialog box opens.

2. Specify your smoothing parameters.
3. Click **Calculate**.
4. When the smoothing process is complete, click **Close**.

Figure 103. Smoothing dialog box

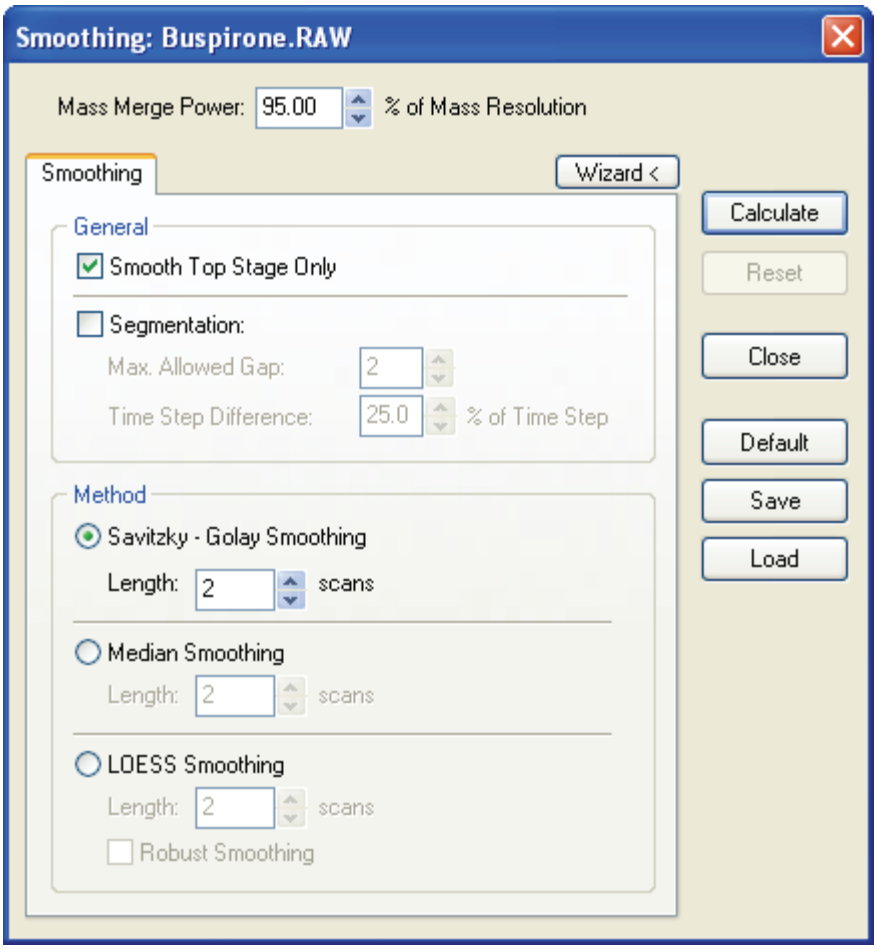


Table 38. Smoothing parameters (Sheet 1 of 2)

Parameter	Description
Mass Merge Power	Specifies the mass difference within which the algorithm merges spectral peaks into one *m/z* value. A low value might result in more components (oscillating ions) and a high value can result in fewer components (merging ions).
General	
Smooth Top Stage Only	Applies smoothing only to MS^1 stage (full scan).
Segmentation	Divides the chromatogram into discrete parts so that the software can apply separate algorithms to each part. This option can provide better results if the chromatogram exhibits diverse shape, peak density, and baseline characteristics in the retention time scale. Avoid segmentation if using a LOESS or Median filter or if results obtained without segmentation are acceptable.
Method	This list includes the following smoothing methods: Savitzky-Golay, Median, and LOESS. In contrast to Median and LOESS methods, the Savitzky-Golay method requires time-equidistant scans.
Method	
Median Smoothing	Smooths each ion profile in the chromatogram using the Median filter. The Length parameter determines the filter length.
Savitzky-Golay Smoothing	Smooths each ion profile in the chromatogram using the Savitzky-Golay filter. The Length parameter determines the filter length. This smoothing requires equidistant time steps between scans. If smoothed results are not acceptable, use another method or check segmentation.
LOESS Smoothing	Smooths each ion profile in the chromatogram using the LOESS filter. The Length parameter determines the filter length. This filter works in a similar way as the Savitzky-Golay filter but is slower and does not require equidistant time steps between scans. When Robust Smoothing is on, then the smoothing is slower but more robust to outlier data in ion profiles.

Table 38. Smoothing parameters (Sheet 2 of 2)

Parameter	Description
Buttons	
Wizard	Displays a simplified version of the Smoothing filter parameters. See "Smoothing wizard dialog box" on page 293.
Calculate	Begins the smoothing process.
Reset	Resets the chromatogram to its original (before smoothing) state.
Close	Closes the Smoothing dialog box.
Default	Returns all smoothing parameters to their default values.
Save	Opens the Save Smoothing Parameters File dialog box where you save your current parameters to a smoothing parameters file (.smooth.par).
Load	Opens the Open Smoothing Parameters File dialog box where you can choose a smoothing parameters file (.smooth.par) to load.

Figure 104. Smoothing wizard dialog box

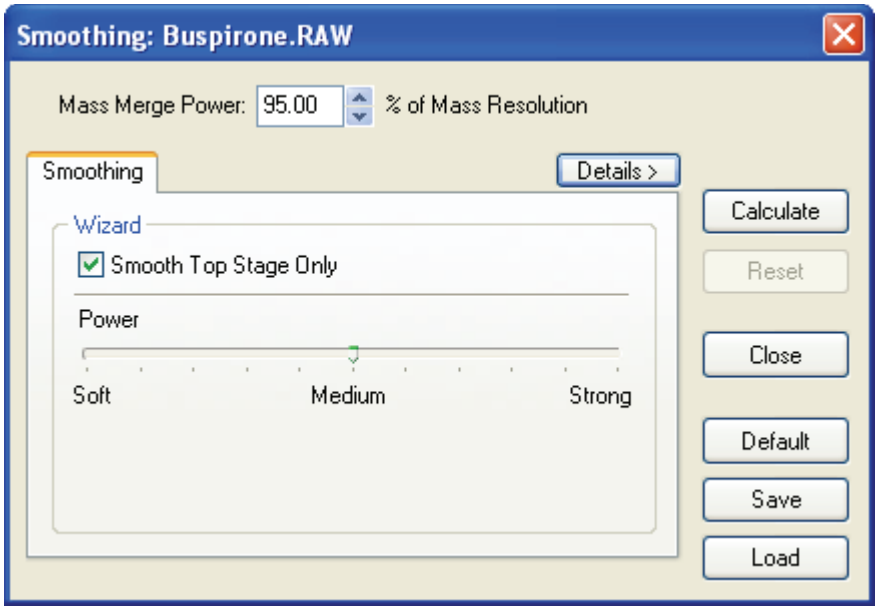


Table 39. Smoothing wizard parameters (Sheet 1 of 2)

Parameter	Description
Mass Merge Power	Specifies the mass difference within which the algorithm merges spectral peaks into one *m/z* value. A low value might result in more components (oscillating ions) and a high value can result in fewer components (merging ions).
General	
Smooth Top Stage Only	Applies smoothing only to MS^1 stage (full scan).
Segmentation	Divides the chromatogram into discrete parts so that the software can apply separate algorithms to each part. This option can provide better results if the chromatogram exhibits diverse shape, peak density, and baseline characteristics in the retention time scale. Avoid segmentation if using a LOESS or Median filter or if results obtained without segmentation are acceptable.
Method	This list includes the following smoothing methods: Savitzky-Golay, Median, and LOESS. In contrast to Median and LOESS methods, the Savitzky-Golay method requires time-equidistant scans.
Method	
Median Smoothing	Smooths each ion profile in the chromatogram using the Median filter. The Length parameter determines the filter length.

Table 39. Smoothing wizard parameters (Sheet 2 of 2)

Parameter	Description
Savitzky-Golay Smoothing	Smooths each ion profile in the chromatogram using the Savitzky-Golay filter. The Length parameter determines the filter length. This smoothing requires equidistant time steps between scans. If smoothed results are not acceptable, use another method or check segmentation.
LOESS Smoothing	Smooths each ion profile in the chromatogram using the LOESS filter. The Length parameter determines the filter length. This filter works in a similar way as the Savitzky-Golay filter but is slower and does not require equidistant time steps between scans. When Robust Smoothing is on, then the smoothing is slower but more robust to outlier data in ion profiles.
Buttons	
Details	Displays a detailed version of the Smoothing filter parameters. See "Smoothing dialog box" on page 290.
Calculate	Begins the smoothing process.
Reset	Resets the chromatogram to its original (before smoothing) state.
Close	Closes the Smoothing dialog box.
Default	Returns all smoothing parameters to their default values.
Save	Opens the Save Smoothing Parameters File dialog box where you save your current parameters to a smoothing parameters file (.smooth.par).
Load	Opens the Open Smoothing Parameters File dialog box where you can choose a smoothing parameters file (.smooth.par) to load.

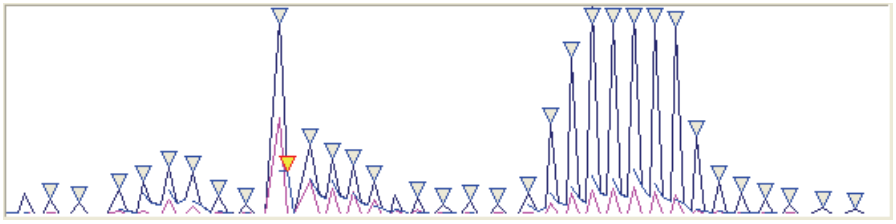
Base Peak Chromatogram

The Base Peak Chromatogram process calculates a TIC profile based on the most intense spectral peak in each scan. This option is a display feature only; no data is modified.

❖ To apply the Base Peak Chromatogram to your data

Click the **Chromatogram Processing Utilities** button, , and choose **Base Peak Chromatogram**.

The application marks the most intense peak for each scan in the Chromatogram pane.



Background Subtraction

To eliminate the background signal from an active scan or from the average of spectral scans, you can perform a manual background subtraction.

❖ To set the location of two representative background scans

1. Click the **Chromatogram Processing Utilities** button,  , and choose **Background Subtraction**.

   The cursor changes appearance,  , to indicate this functionality.

2. Click a scan point in the chromatogram pane.

   A green vertical line indicates the background scans. The Scan pane displays the resulting spectrum.

3. To cancel a background subtraction, click the **Chromatogram Processing Utilities** button,  , and choose **Delete Background Subtraction**.

   The Extracted Ion Chromatogram feature can help you choose the two most representative background scans. You can use these background scans in the Manual background subtraction option in Automated Components Detection and Spectra Deconvolution procedures.

For additional information, see "Using the Extracted Ion Chromatogram Features" on page 297 and "Using Components Detection and Spectra Deconvolution" on page 299.

❖ To see the effects of the background subtraction on a scan

1. Copy the extracted and the original spectra to a Database Manager window.

   See "Working in a Chromatogram Processor Window" on page 247.

2. Compare them using the spectra comparison routine on the Compare Spectra page.

Using the Extracted Ion Chromatogram Features

Use the Mass Frontier application to display a chromatogram for an extracted ion in a different color. The extracted ion chromatogram (XIC) is sometimes called an individual, or single ion chromatogram, ion profile. The program can display up to 16 extracted ion chromatograms per window.

You can use background subtraction in conjunction with the Extracted Ion Chromatogram feature. In this case, the XIC profiles are extracted from background-subtracted scans.

❖ To display an XIC for a particular mass-to-charge ratio

1. Do one of the following:

   Click a spectral peak in the Scan pane.

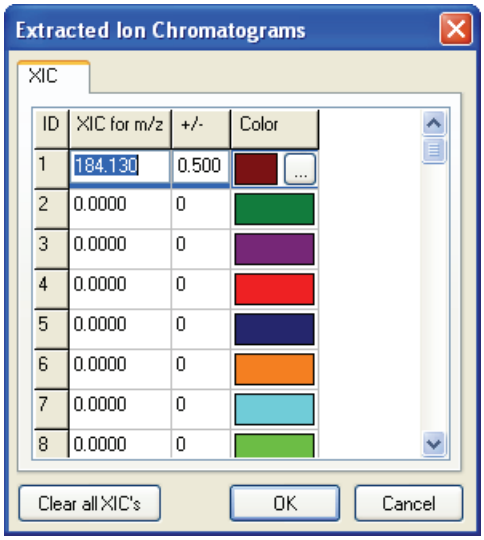
   –or–

   Click the **Data** tab and double-click the *m/z* value in the mass-to-charge ratio table.

   –or–

   Click the **Add/Edit Extracted Ion Chromatograms (XIC)** button,  .

   The Extracted Ion Chromatograms dialog box opens.



2. Change or add the mass-to-charge ratio.
3. Define XIC tolerance.

   Set a value of zero (the default value) to compare the XIC for *m/z* value with peaks using peak accuracy only.

   Set a non-zero value of tolerance (± *m/z* value) to compare the peak *m/z* value/accuracy with "XIC for *m/z*" ± its tolerance.

4. Select a color for a particular *m/z* value.

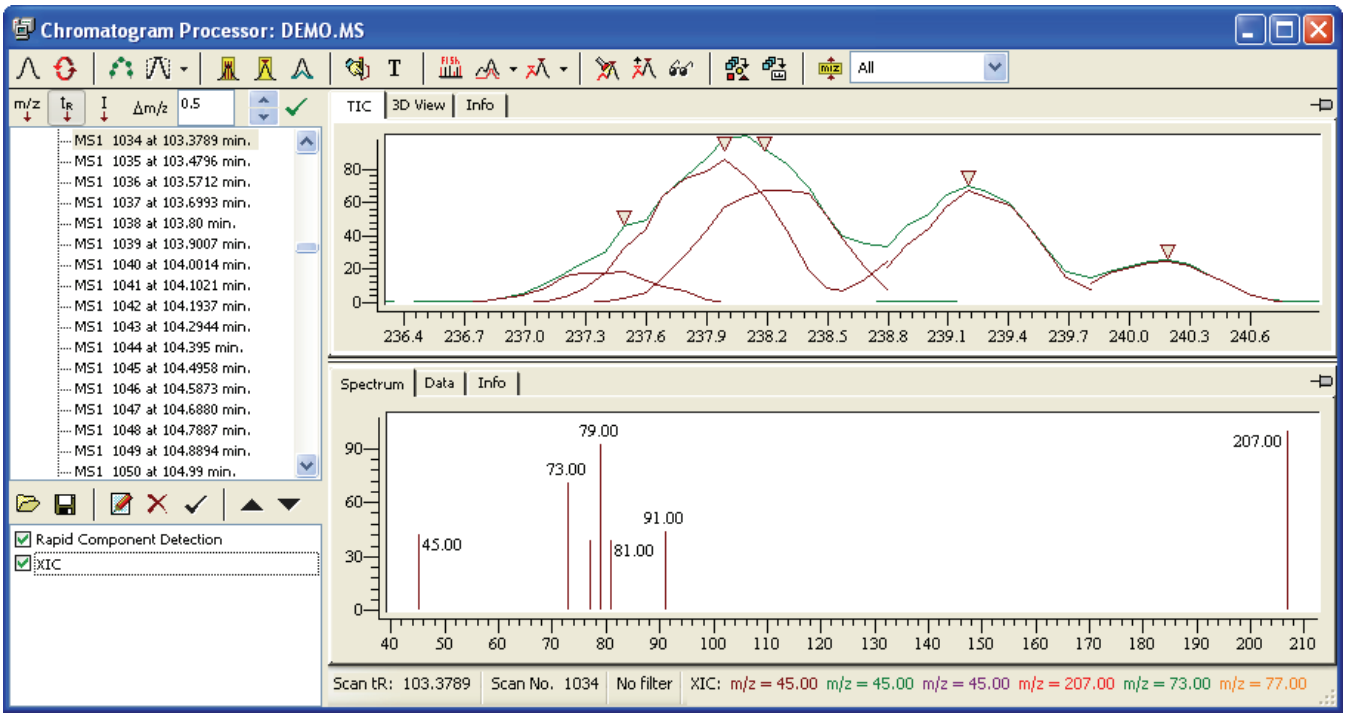
5. Click **OK**.

   The application extracts the XIC from the original file, a process that can be time-consuming for chromatograms with a large number of scans. However, because the application is multi-threading, you can still use other windows during the XIC extraction.

An extracted ion chromatogram is useful for verifying automated component detection and spectra deconvolution results. An XIC helps you to recognize mixture components in a peak region. Examine an XIC of model peaks for any component you might want to use in further analysis. Some structural (alkanes) or optical isomers produce almost identical mass spectra, and even if you can clearly see two or more maxima in a peak region, an XIC might not reveal a multi-component profile.

An XIC can help you determine whether the composition of the background changes over the course of a run. To view the background profile, extract the XIC of a base peak or a prominent peak from a scan that is clearly in a non-peak region. If the XIC of a background peak has a variable profile around the peak you are focusing on, choose two scans with different XIC profiles for background subtraction.

Figure 105. Extracted ion chromatogram ion profiles



Using Components Detection and Spectra Deconvolution

The Mass Frontier application incorporates an automated system for detecting chromatographic components in complex GC/MS or LC/MS runs and extracting mass spectral signals from closely coeluting components (deconvolution). You can search individual mass spectra or spectral trees obtained after deconvolution in libraries or you can classify them using Principal Component Analysis or Neural Networks. For additional information about PCA or Neural Networks, see "Principal Component Analysis (PCA)" on page 341 or "Self-Organizing Maps" on page 343.

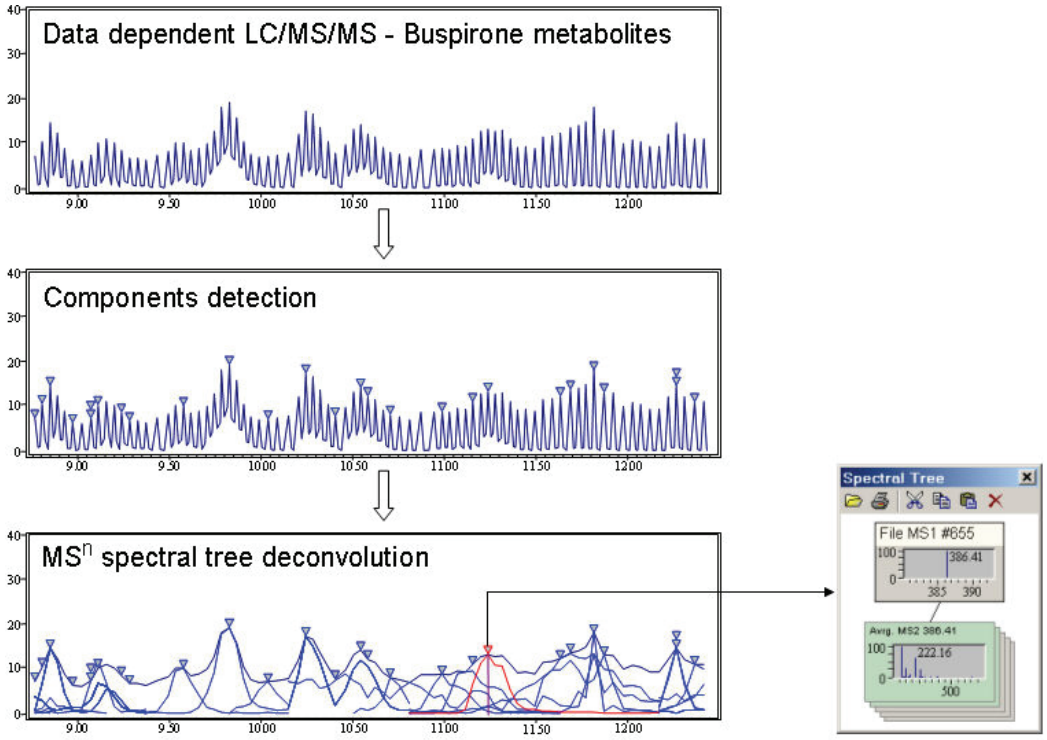
This Components Detection and Spectra Deconvolution system involves the combined use of the following procedures:

- Noise examination, signal filtering, and smoothing

  See "Processing the Data" on page 258.
- Baseline definition and demarcation of chromatographic peaks
- Background scan determination and background subtraction
- Component candidate detection and model ion selection (*m/z*)
- Correlation of model ion profiles and component confirmation or rejection
- Spikes elimination
- Calculation of exact component retention time
- Spectra deconvolution using linear algebra

This system is designed for broad types of chromatographic runs, for both GC/MS, LC/MS, and GC/LC/MS^n analyses, for clean and noisy signals, and for simple or complex chromatograms (see "Processing MSn Data" on page 323). However, you might need to change some parameters to optimize the system for specific applications. This automated procedure is designed for small- and medium-sized organic compounds. Do not use this procedure for the processing of proteins, peptides, oligonucleotides, or other biomolecules.

Figure 106. Components detection and spectra deconvolution example



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

The application identifies components using the following algorithms:

- JCD Algorithm
- RCD Algorithm
- TECD Algorithm
- Direct Infusion Algorithm

JCD Algorithm

Joint Components Detection (JCD) is based on the statistical analysis of all ion profile maxima. Ion profiles (ion chromatograms) 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, but be aware that it requires significant computer processing resources.

To significantly reduce computation time for large files, apply a threshold filter to the chromatogram before performing JCD. See "Thresholding" on page 276 for more information about applying thresholds.

❖ To run the Joint Components Detection algorithm

1. Click the **Components Detection & Spectra Deconvolution** button,  , and choose **JCD**.

   The Joint Detection Algorithm dialog box opens.

2. Specify your JCD parameters.

   Note These parameters are interdependent, so changing one parameter can affect algorithms linked with other parameters.

3. Click **Calculate**.

4. When the detection process is complete, click **Close**.

Figure 107. Deconvolution page of the Joint Detection Algorithm dialog box

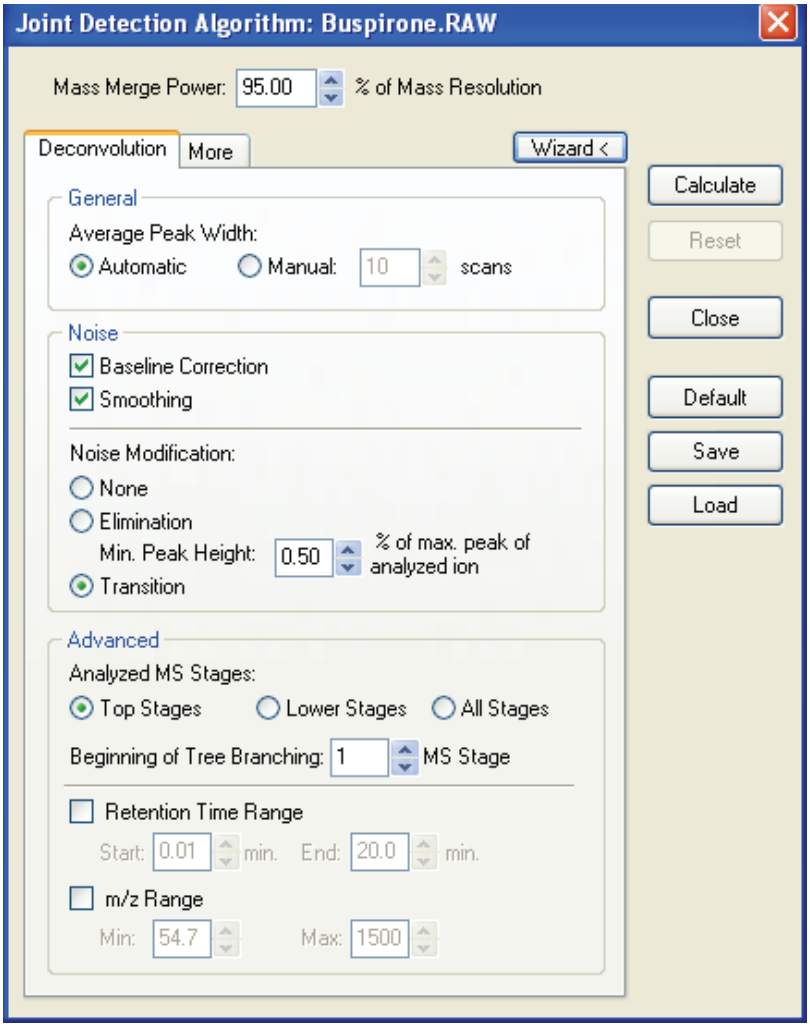


Table 40. Deconvolution parameters (Sheet 1 of 3)

Parameter	Description
Mass Merge Power	Specifies the mass difference within which the algorithm merges spectral peaks into one *m/z* value. A low value might result in more components (oscillating ions) and a high value can result in fewer components (merging ions).
Average Peak Width	Specifies the chromatographic peak width in scans that the algorithm uses to identify a potential component. If a value is too high, the result might be the loss of narrow peaks. If a value is too low, it might split a real component into two different components. Average Peak Width is applied only for scans that are specified in Analyze MS Stages parameter. For example, if Analyze MS Stages is set to Top Stages, then Average Peak width is applied to MS^1 scans only.

Table 40. Deconvolution parameters (Sheet 2 of 3)

Parameter	Description
Baseline Correction	Applies an automated baseline correction for each ion profile using the Top-Hat algorithm. Use this option only for chromatograms with an elevated baseline.
Smoothing	Performs the smoothing of each ion profile using the Average Peak Width value and the LOESS algorithm. If the Average Peak Width parameter is too large or peaks are shorter than four to five scans, then the smoothing can remove small peaks.
Noise Modification	Determines how to adjust the intensity values of spectral peaks with comparable abundances to noise level. You can choose from None, Elimination, or Transition.
None	Spectral peak intensities are not altered.
Elimination	Spectral peaks with intensities lower than the specified value are eliminated. Use this method when low abundant peaks are not of interest. Min. Peak Height: The threshold value applied to each abundance in an ion profile. This value is determined as a percentage of the maximum of the analyzed ion profile. All abundances smaller than this threshold are removed. This option speeds up calculations.
Transition	Default. Artificial noise is added to replace random spikes with constant noise for better detection of low abundant components. Abundances with zero value are replaced by artificial noise.
Analyzed MS Stages	Determines which MS stages are considered in the analysis of ion profiles—that is, which MS stages are used for detecting components. The remaining stages are only used for spectral tree reconstruction. Select one of the following options: • Top Stages level: Recommended in most cases. • Lower Stages level: Useful if Data Dependent experiment is set so that ions are isolated according to a predefined list of *m/z* values and top-stage spectra are noisy. • All Stages level: Useful for the preliminary analysis of complex data.
Top Stages (default)	Analyzes only the top stage ions present in the data, where "top" means MS^1, or MS/MS if MS^1 is not present. The algorithm builds the component tree by joining the corresponding lower stage spectra that meet the following criteria: they occur within the component envelope and the software detected the precursor *m/z* in the top stage as a component ion (if the deconvoluted MS^1 spectrum contains peaks that have been further isolated, the corresponding MS/MS spectra are assigned to the spectral tree). Recommended option for most cases.

Table 40. Deconvolution parameters (Sheet 3 of 3)

Parameter	Description
Lower Stages	Analyzes all ions except those from the top MS stages. The algorithm calculates the spectrum of the top stage from the total ion abundance of the top stage. The resulting spectral trees do not have as much depth as those from the Top Stages option. This option can be useful if the full-scan chromatogram is not well resolved and the lower stages contain enough scans with the same precursor value.
All Stages	Analyzes all ions regardless of the MS stage. The resulting spectral trees do not have as much depth as those from the Top Stages option. This option is useful in some cases when the FISh algorithm is applied in metabolite ID (see "Base Peak Chromatogram" on page 295).
Beginning of Tree Branching	Determines MS stage where a spectral tree is divided into two or more independent components based on the precursor ion.
Retention Time Range	Specifies only a part of a chromatogram to be analyzed (time range), which can speed up your work. In this case, the algorithm ignores regions outside of the specified range.
m/z Range	Determines the *m/z* value range to be analyzed. Ignores other spectral regions. This feature is useful when processing large chromatograms.
Buttons	
Wizard	Displays a simplified version of the component detection parameters. See "Joint Detection Algorithm wizard dialog box" on page 307.
Calculate	Begins the component detection process.
Reset	Resets the chromatogram to its original (before component detection) state.
Close	Closes the component detection dialog box.
Default	Returns all component detection parameters to their default values.
Save	Opens the Save JCD Parameters File dialog box where you can save your current parameters to a JCD parameters file (.jcd.par).
Load	Opens the Open JCD Parameters File dialog box where you can choose a JCD parameters file (.jcd.par) to load.

Figure 108. More page of the Joint Detection Algorithm dialog box

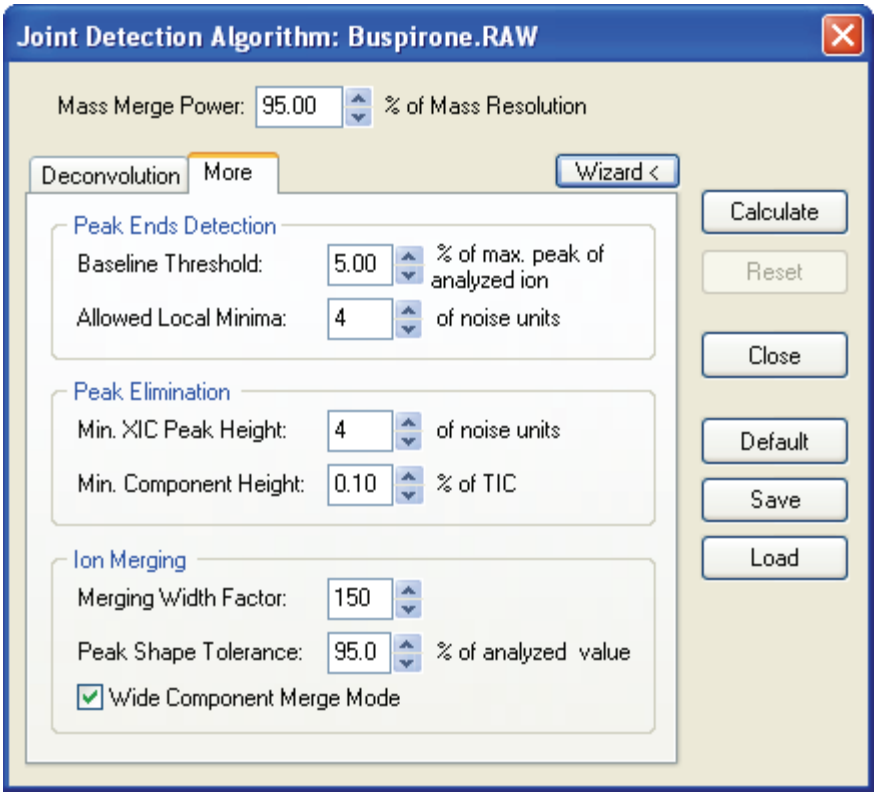


Table 41. More parameters (Sheet 1 of 2)

Parameter	Description
Baseline Threshold	Specifies the peak baseline as a percentage of base peak height.
Allowed Local Maxima	Specifies the sensitivity to local minima of ion profiles. Peak ends are detected if the local minimum of analyzed ion profile is greater than this value. Value is determined as a multiple of estimated noise.
Min. XIC Peak Height	Specifies the minimal ion profile peak height to be considered as a potential component. Value is determined as a multiple of the estimated noise.
Min. Component Height	Specifies the minimal intensity of component peaks. This value is determined as a percentage of intensity of the total ion chromatogram at the position of the component maxima.

Table 41. More parameters (Sheet 2 of 2)

Parameter	Description
Merging Width Factor	Specifies a limit for the time difference of the ion profiles maxima. Too high a value can cause the merging of randomly coeluting components. Too low a value can split a component into more false-positive components. The software merges ion profile peaks within this range into a single component. For additional information, see "Wide Component Merge Mode" on page 314.
Peak Shape Tolerance	Specifies the degree (%) of similarity of ion profile shapes. If two ion shapes meet the specified percentage for the sharpness tolerance, as well as other parameters, the algorithm merges the ions into a single component.
Wide Component Merge Mode	Specifies whether to use a limit for the time difference of the ion profile maxima in a combination of the following parameters: Average Peak Width and Merging Factor. Select this check box (default) to avoid splitting a component into ion peaks that are detected as redundant components in chromatograms with wide peaks. Clear this check box if an incorrect component merge occurs. Recommended for most LC/MS experiments.

Figure 109. Joint Detection Algorithm wizard dialog box

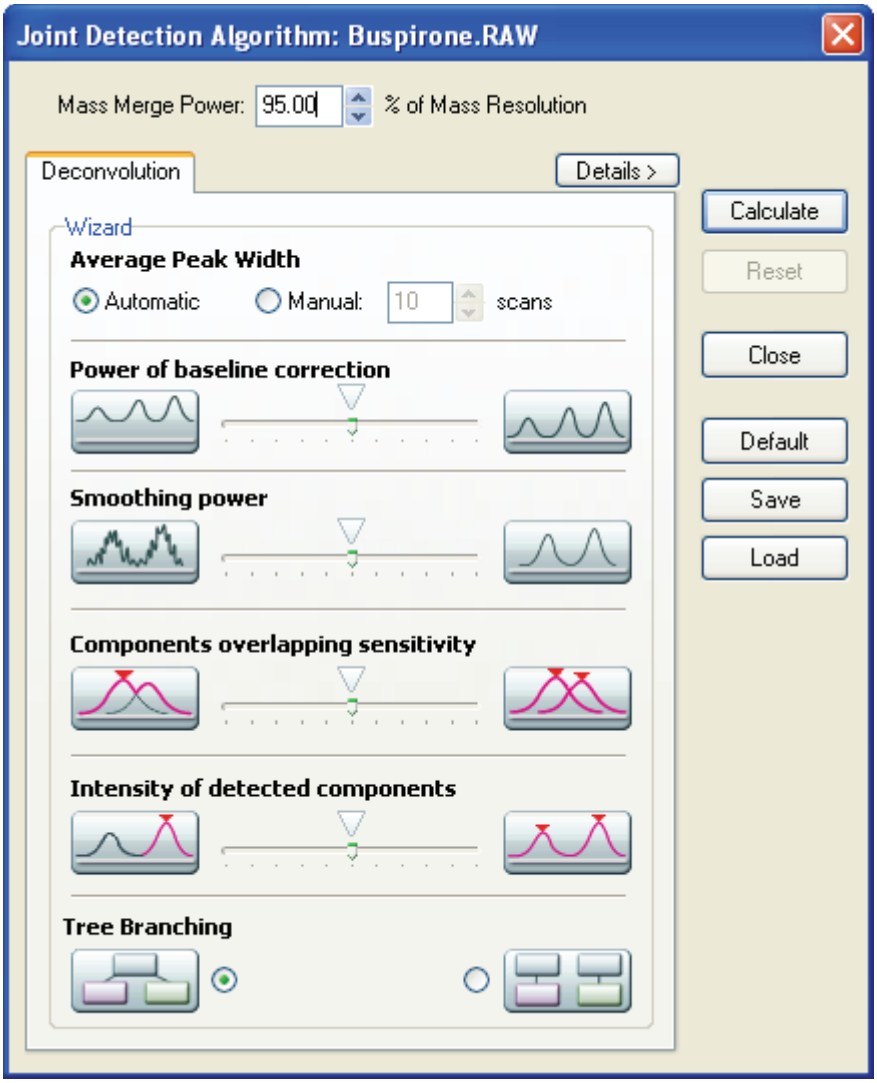


Table 42. Joint Detection Algorithm wizard parameters (Sheet 1 of 2)

Parameter	Description
Average Peak Width	Specifies an automatically calculated average peak width, or lets you specify the average 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 overlap sensitivity within a low-to-high range.

Table 42. Joint Detection Algorithm wizard parameters (Sheet 2 of 2)

Parameter	Description
Intensity of Detected Components	Specifies the intensity of detected components within a low-to-high range.
Tree Branching	Specifies one of the following:  Parent ions are merged into one component.  Each parent ion produces an individual tree.

RCD Algorithm

Rapid Components Detection (RCD) is based on a model ion that represents a characteristic ion (*m/z* value in MS^1 spectra) for every detected component. The algorithm starts with component candidate detection and model ion selection and continues with the correlation of model ion profiles to confirm or reject a candidate.

❖ To run the Rapid Components Detection algorithm

1. Click the **Components Detection & Spectra Deconvolution** button,  , and choose **RCD**.

   The R-Component Detection Algorithm dialog box opens. This dialog box includes the following pages:

   - "Deconvolution page of the R-Component Detection Algorithm dialog box" on page 310
   - "More page of the R-Component Detection Algorithm dialog box" on page 312

2. Specify your RCD parameters.

   Note These parameters are interdependent, so changing one parameter can affect algorithms linked with other parameters.

3. Click **Calculate**.

4. When the detection process is complete, click **Close**.

Figure 110. Deconvolution page of the R-Component Detection Algorithm dialog box

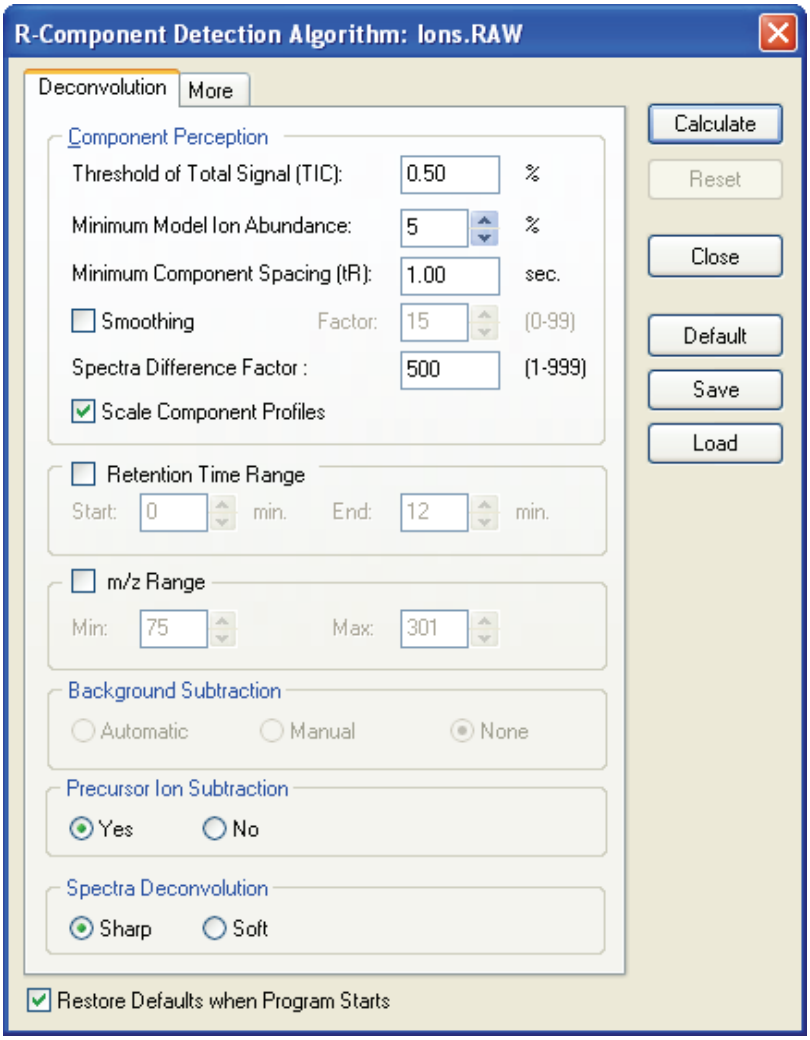


Table 43. Deconvolution parameters (Sheet 1 of 3)

Parameter	Description
Threshold of Total Signal	The program uses a different threshold level from the one specified in the data file. In especially noisy chromatograms, setting the threshold higher can reduce the number of false positive results. However, if you feel the algorithm is too restrictive and is missing some chromatographic peaks, you can lower the default value.
Minimum Model Ion Abundance	The algorithms search for spectral peaks (model ions) that have the most rapid rise and fall of a signal in a peak region. To eliminate spikes and random fluctuations, a model ion should exhibit a minimum abundance value.
Minimum Component Spacing	Eliminates the components within a specified retention time interval.

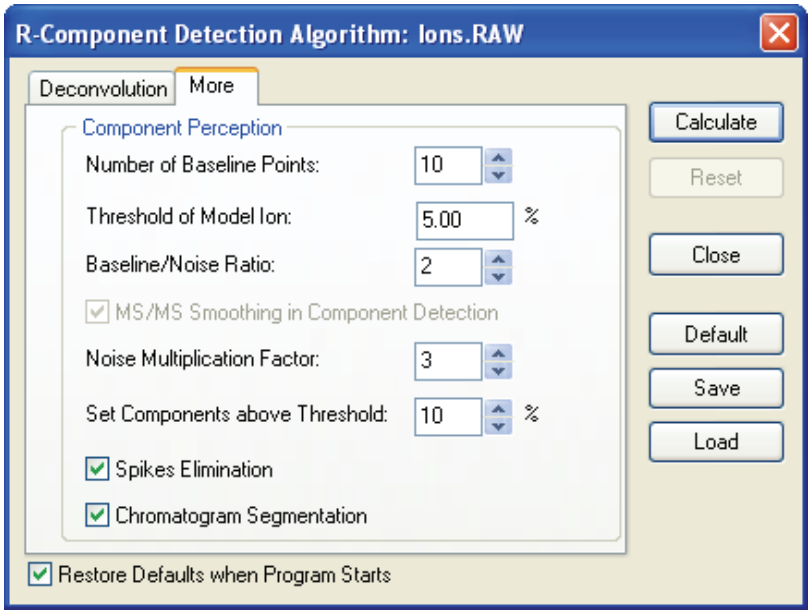
Table 43. Deconvolution parameters (Sheet 2 of 3)

Parameter	Description
Smoothing	Use this option when you must analyze noisy data. The program automatically determines the smoothing factor according to the signal-to-noise ratio. You can change this value or switch off the smoothing. The system uses an exponential filter similar to the analog RC filter.
Spectra Difference Factor	To eliminate false positive component detection, the adjacent components must show some degree of spectral dissimilarity that is represented as the match factor used in library searching. The spectra Difference Factor value is the minimal match factor between spectra that detected components should exhibit.
Background Subtraction	When you select the Automatic option, the program attempts to find a region of relatively constant signal intensity before and after every peak and sets two background scans there. To use the Manual option, set the background scans by clicking the Chromatogram Processor Utilities button and choosing Background Subtraction before starting the detection and deconvolution process (see "Background Subtraction" on page 296). You can set two manual background scans anywhere in the chromatogram. When you select the None option, background subtraction is not performed.
Precursor Ion Subtraction	When a product ion chromatogram is being deconvoluted, you can subtract the selected precursor ion from all scans to improve component detection. However, if a component does not fragment and you can only observe a precursor ion, you should not apply the subtraction because you can overlook this component. Note that this application does not support scan events that are often used in connection with product ion scanning.
Scale Component Profile	Scale the component profile (envelope) to fit the intensity of a full scan.
Retention Time Range	Where you specify only a part of a chromatogram to be analyzed (time range), which can speed up your work. In this case, the algorithm ignores regions outside of the specified range.
m/z Range	Determines the *m/z* value range to be analyzed and ignores other spectral regions. This feature is useful when processing large chromatograms.

Table 43. Deconvolution parameters (Sheet 3 of 3)

Parameter	Description
Spectra Deconvolution	You can apply two extraction algorithms, the choice depending on the intended use for the component spectra. When components are intended for a library search (see "Spectrum Search" on page 79), use Sharp spectra deconvolution. When the purpose of component detection is target analysis, use Soft deconvolution. Generally, Sharp deconvolution subtracts peaks from coeluting components with a higher multiplication factor, and so it produces spectra with fewer peaks and lower intensities of isobaric peaks than Soft deconvolution.
Restore Defaults when Program Starts	Resets these parameters to the default values each time you start the Mass Frontier application.
Buttons	
Calculate	Begins the component detection process.
Reset	Resets the chromatogram to its original (before component detection) state.
Close	Closes the component detection dialog box.
Default	Returns all parameters to their default values.
Save	Opens the Save RCD Parameters File dialog box where you can save your current parameters to a RCD parameters file (.rcd.par).
Load	Opens the Open RCD Parameters File dialog box where you can choose a RCD parameters file (.rcd.par) to load.

Figure 111. More page of the R-Component Detection Algorithm dialog box



TECD Algorithm

The TECD (Total Extraction Components Detection) algorithm creates spectral trees for every section of a chromatogram that starts with an MS^1 scan. Each generated tree is then divided into subtrees based on the value of the Minimal Tree Depth parameter. Generated subtrees are merged into components based on the precursor ion *m/z* value identity and the spectral tree match factor similarity.

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

❖ To run the Total Extraction Components Detection algorithm

1. Click the **Components Detection & Spectra Deconvolution** button,  , and choose **TECD**.

   The Total Extraction Component Detection Algorithm dialog box opens.

2. Specify your TECD parameters.

   Note These parameters are interdependent, so changing one parameter can affect algorithms linked with other parameters.

3. Click **Calculate**.

4. When the detection process is complete, click **Close**.

Figure 112. Total Extraction Component Detection Algorithm dialog box

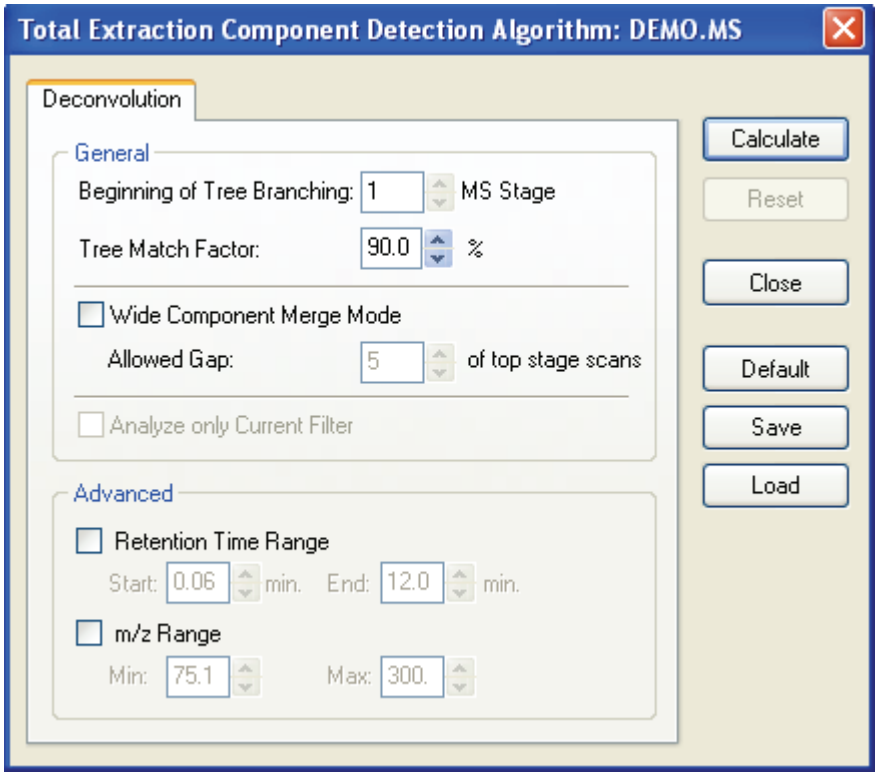


Table 44. Total Extraction Component Detection Algorithm parameters (Sheet 1 of 2)

Parameter	Description
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 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 as the same component. Matching spectral trees is defined as having identical precursors up to the level specified by the Minimal Tree Depth value and a Tree Match Factor value that exceeds the specified value.
Wide Component Merge Mode	Enables a comparison of the spectral trees for potential matching and merging, not only in adjacent sections, but also in sections up to the distance specified by the Allowed Gap value.
Allowed Gap	When comparing the spectral trees for potential merging, specifies the maximum distance between nonadjacent tree sections.
Analyze only Current Filter	Processes only visible scans (scans chosen by scan filter) using the TECD algorithm.

Table 44. Total Extraction Component Detection Algorithm parameters (Sheet 2 of 2)

Parameter	Description
Retention Time Range	Specifies only a part of a chromatogram to be analyzed (time range), which can speed up your work. In this case, the algorithm ignores regions outside of the specified range.
m/z Range	Determines the *m/z* value range that is analyzed. Ignores other spectral regions. This feature is useful when processing large chromatograms.
Buttons	
Calculate	Begins the component detection process.
Reset	Resets the chromatogram to its original (before component detection) state.
Close	Closes the component detection dialog box.
Default	Returns all component detection parameters to their default values.
Save	Opens the Save TECD Parameters File dialog box where you can save your current parameters to a TECD parameters file (.tecd.par).
Load	Opens the Open TECD Parameters File dialog box where you can choose a TECD parameters file (.tecd.par) to load.

Direct Infusion Algorithm

The Direct Infusion 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 MS^n scans acquired in one run and constructing a tree according to their MS stage and precursor ion *m/z* values.

❖ To run the Direct Infusion algorithm

1. Click the **Components Detection & Spectra Deconvolution** button,  , and choose **Direct Infusion**.

   The Direct Infusion Spectral Tree Construction dialog box opens.

2. Specify your Direct Infusion parameters.

   Note These parameters are interdependent, so changing one parameter can affect algorithms linked with other parameters.

3. Click **Calculate**.

4. When the construction process is complete, click **Close**.

Figure 113. Direct Infusion Spectral Tree Construction dialog box

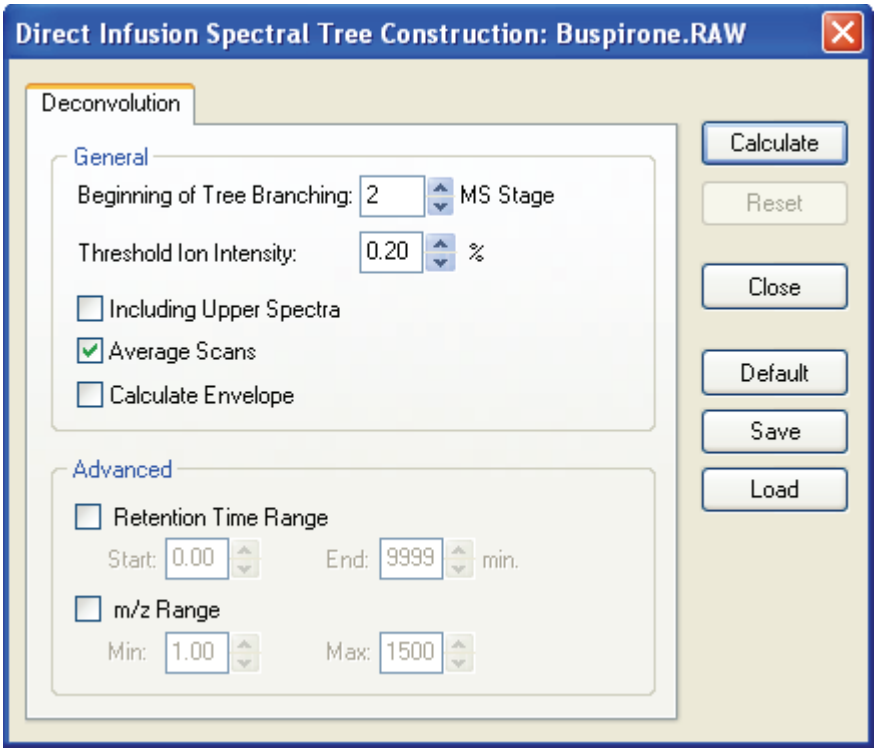


Table 45. Direct Infusion Spectral Tree Construction parameters (Sheet 1 of 2)

Parameter	Description
Beginning of Tree Branching	Specifies the MS stage that should only have a single precursor. The value determines the MS stage for a tree branch division. When you use the default value of 1, the algorithm creates a single tree from all the scans in a run (file). When you use a value of 2, and the analyzed run contains MS/MS spectra with different precursor *m/z* values, the algorithm creates separate trees for each MS/MS scan with a unique precursor ion *m/z* value.
Threshold Ion Intensity	Removes spectral peaks of lower intensity than specified from each analyzed scan.
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.
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. You can then preview scans that belong to a particular tree.

Table 45. Direct Infusion Spectral Tree Construction parameters (Sheet 2 of 2)

Parameter	Description
Retention Time Range	Specifies only a part of a chromatogram to be analyzed (time range), which can speed up your work. In this case, the algorithm ignores regions outside of the specified range.
m/z Range	Determines *m/z* value range for analysis. Ignores other spectral regions. This feature is useful when processing large chromatograms.
Buttons	
Calculate	Begins the spectral tree construction process.
Reset	Resets the chromatogram to its original (before spectral tree construction) state.
Close	Closes the spectral tree construction dialog box.
Default	Returns all spectral tree construction parameters to their default values.
Save	Opens the Save Direct Infusion Parameters File dialog box where you can save your current parameters to a Direct Infusion parameters file (.infusion.par).
Load	Opens the Open Direct Infusion Parameters File dialog box where you can choose a Direct Infusion parameters file (.infusion.par) to load.

Processing Extracted Spectra

Use the Database Manager or Spectra Classifier windows to further process a spectrum obtained from the average of scans, from scans with a subtracted background, or from component deconvolution. You can also directly search for the spectrum in a Chromatogram Processor library.

You can classify mass spectra obtained in the Chromatogram Processor using Principal Component Analysis, Neural Networks, or Fuzzy Clustering methods.

For additional information, see "Organizing Spectra" on page 345, "Principal Component Analysis (PCA)" on page 341, "Fuzzy Clustering" on page 344, or Chapter 12, "Neural Networks Module."

Follow these procedures:

- To choose scans or deconvoluted spectra for classification
- To search the extracted spectra
- To transfer the extracted spectrum or tree to a Database Manager window
- To transfer the extracted spectrum or tree to a Spectra Classifier window
- To copy a spectrum to a Windows application

❖ To choose scans or deconvoluted spectra for classification

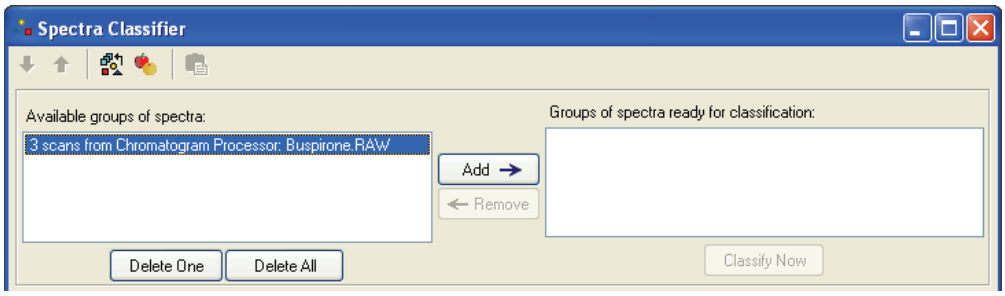
1. Select the scans or components that you want to classify.

   You can select scans from the chromatogram pane or components from the filter list.

2. Click the **Add Selected Scans or Components to Spectra Classifier** button,  .

   The Spectra Classifier window opens.

   Figure 114. Spectra Classifier window with spectra from a Chromatogram Processor



❖ To search the extracted spectra

1. Click the **Search** button,  .

   The Component Search dialog box opens.

2. To perform a tree search, see "Spectral Tree Search" on page 74.

3. To perform a spectrum search, see "Spectrum Search" on page 79.

❖ To transfer the extracted spectrum or tree to a Database Manager window

1. Select the components.

2. Right-click and choose **Copy > Copy *option*** from the shortcut menu.

   You can copy an MS spectrum or an MS tree.

3. In a Database Manager window, right-click and choose **Paste > *option*** from the shortcut menu.

4. To process the components, see Chapter 2, "Database Manager Module."

❖ To transfer the extracted spectrum or tree to a Spectra Classifier window

1. Select the components.

2. Click the **Add Selected Scans or Components to Spectra Classifier** button,  .

   The Spectra Classifier window opens.

3. To process the components, see Chapter 10, "Spectra Classifier Module."

❖ To copy a spectrum to a Windows application

1. Select the spectrum.

2. Right-click and choose **Copy > Copy MS Spectrum** from the shortcut menu.

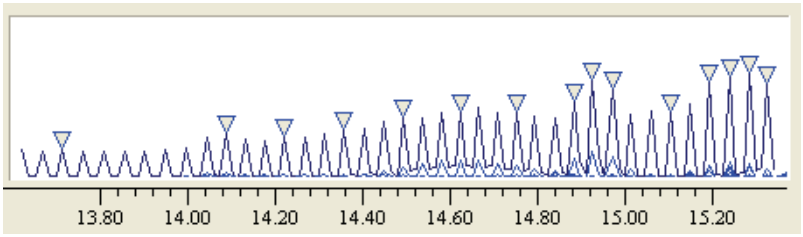
   The application copies the chromatogram graphic and the spectrum from the Scan page to the Clipboard.

3. Open any Windows application and paste the contents of the Clipboard.

> **Note** Use the **Paste Special** command in the Windows applications to paste these graphics.

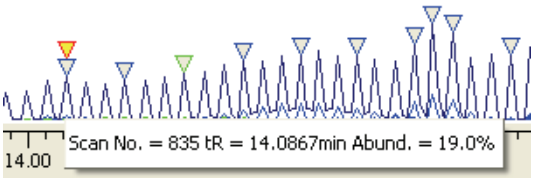
Working with Detected Components

Detected components are marked with a triangle in the original chromatogram (see "Using Components Detection and Spectra Deconvolution" on page 299). Because the program calculates the precise retention time (tR) of each component, the component triangle can appear between scan points.



Note To display a deconvoluted spectrum of a component rather than of a scan, click the triangle that marks the detected component in the chromatogram pane. To display an original scan at the position of a detected component, click above the triangle.

When you pause the cursor over a component triangle, a ToolTip displays the component number, precise retention time, and model ion *m/z* value that were used in the automated detection and deconvolution processes.



To edit or search chromatographic components in the Components Editor window, click the **Components Editor** button,  .

To process detected components in the Database Manager or in the Spectra Classifier modules, you must select them, copy them, and paste them into the Database Manager or Spectra Classifier windows.

For details about processing detected components, see "Processing Data-Reduced Chromatograms" on page 69 or Chapter 10, "Spectra Classifier Module." .

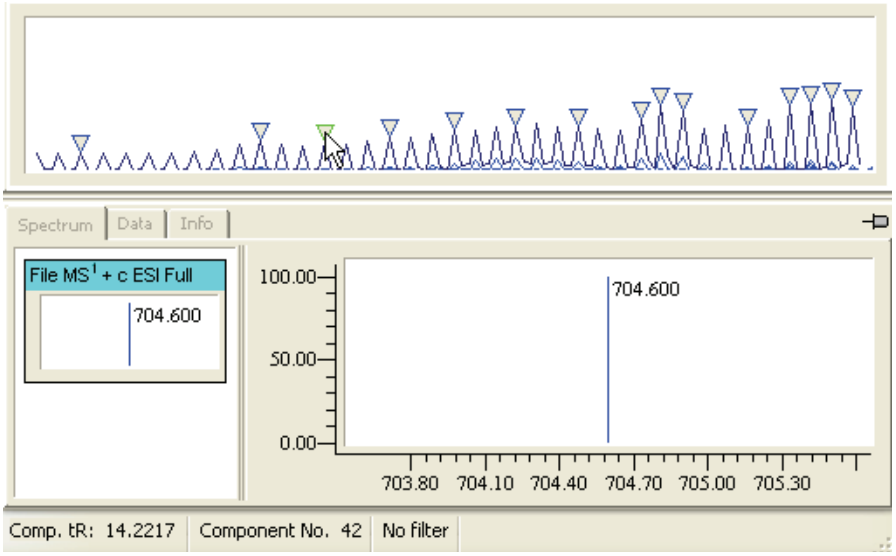
Follow these procedures:

- To select a component
- To select a range of components
- To select all detected components in a run
- To copy the selected components to a Database Manager window
- To copy the selected components to a Spectra Classifier window

❖ To select a component

Click the triangle on the peak.

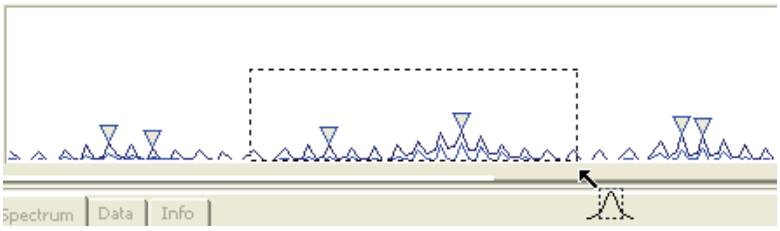
A deconvoluted spectrum appears in the scan pane.



You can process this spectrum the same way you would any spectra, for example, search for it in a library or copy it to a Database Manager window.

❖ To select a range of components

1. Click the **Select Components** button,  .
2. Draw a rectangle around the components.



The application makes a strict distinction between original scans and detected components and does not mix them. When selecting a chromatographic region, the application prefers components over scans. When you select a chromatographic region that contains both scans and components, the software selects only the components. When you select a region that does not contain any components, the software selects all scans in that region.

❖ To select all detected components in a run

Right-click the chromatogram pane and choose **Select > Select All Components** from the shortcut menu.

❖ To copy the selected components to a Database Manager window

1. Select the components.
2. Right-click and choose **Copy > Copy *option*** from the shortcut menu.

   You can copy a selection, chromatogram, MS spectrum, or an MS tree.
3. In a Database Manager window, right-click and choose **Paste > *option*** from the shortcut menu.
4. To process data-reduced chromatograms, see Chapter 2, "Database Manager Module."

❖ To copy the selected components to a Spectra Classifier window

1. Select the components.
2. Click the **Add Selected Scans or Components to Spectra Classifier** button,  .

   The Spectra Classifier window opens.
3. To process the components, see Chapter 10, "Spectra Classifier Module."

Processing MS^n Data

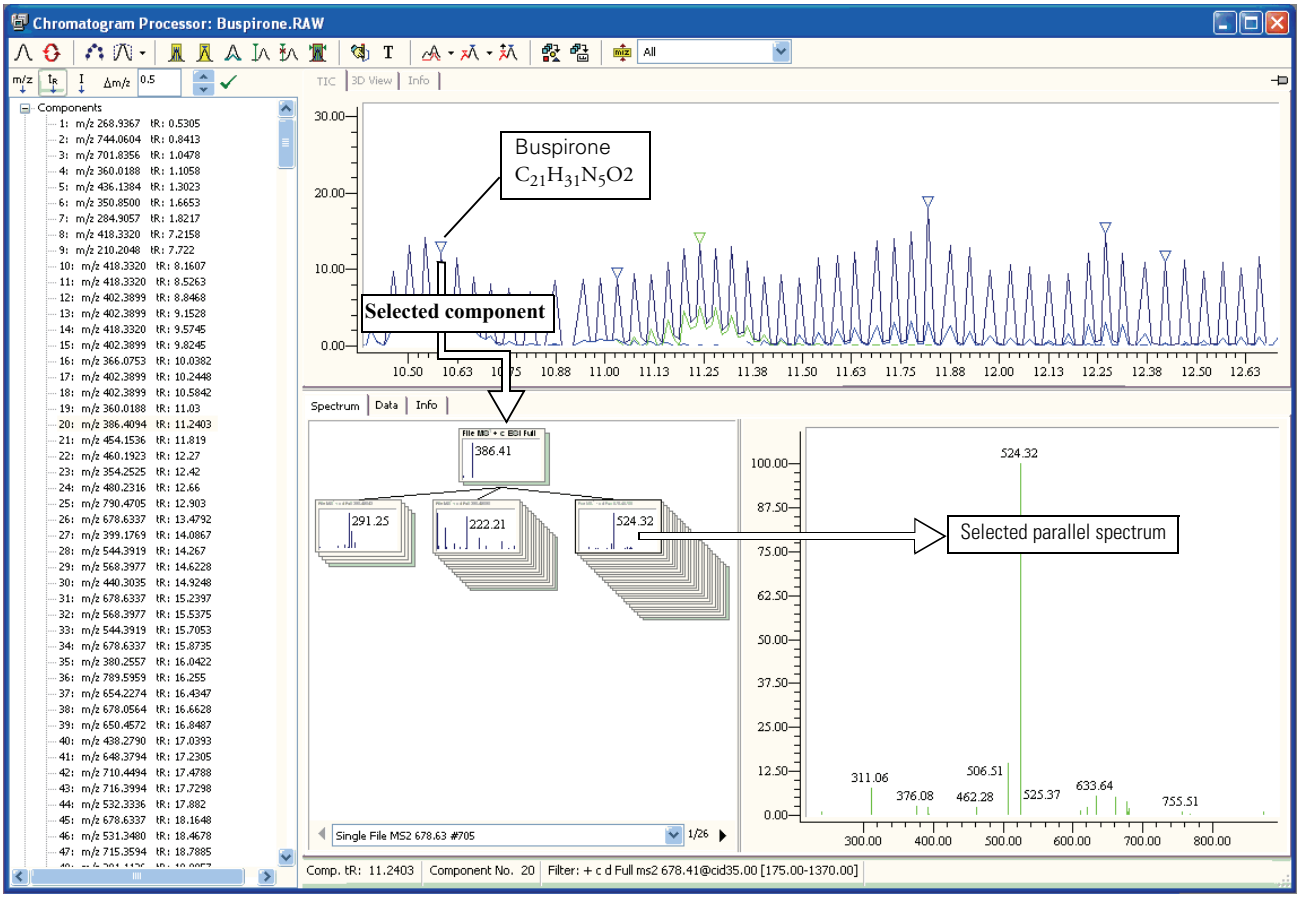
In the Chromatogram Processor window, you can view and process Xcalibur data-dependent experiments and product ion scanning raw data files and extract spectral trees (MS^n spectra) from chromatograms. When you open Xcalibur data in a Chromatogram Processor window, the window displays a tree view control. Use this tree view control to select one or more product scans at any MS^n stage. Deconvoluted components that are present are listed at the bottom of the tree control.

For additional information, see "Using Components Detection and Spectra Deconvolution" on page 299, "Spectral Tree Arrangement" on page 106, or "Working with Detected Components" on page 320.

When you apply component detection and spectra deconvolution procedures to data-dependent chromatograms, the application generates components as spectral trees. You can process the tree components in the same way as regular spectra. You can edit them in the Components Editor window, search them in spectral libraries, or classify them using the Spectra Classifier module. You can only view spectral trees in the Chromatogram Processor; to edit them, paste them into a Database Manager window to access all editing utilities.

For additional information, see Chapter 9, "Components Editor Module," or Chapter 10, "Spectra Classifier Module."

Figure 115. Xcalibur data in a Chromatogram Processor window



Exporting Scans, Components, and Reduced Chromatograms

You can export scans, components, and chromatograms from the Chromatogram Processor window to a Database Manager or Spectra Classifier window for additional processing.

Follow these procedures:

- To export selected scans and components to a Database Manager window
- To export a data-reduced chromatogram to a Database Manager window

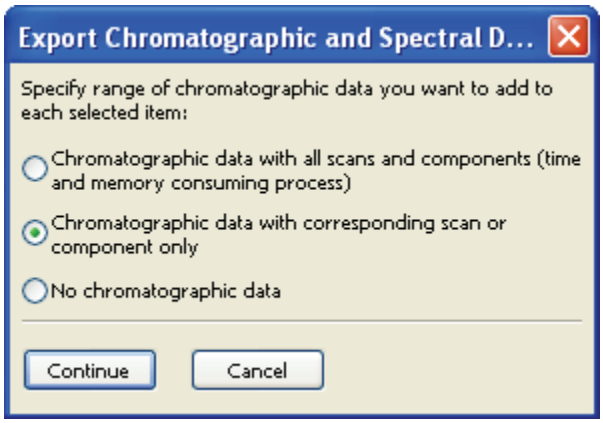
❖ To export selected scans and components to a Database Manager window

1. Select the scans or components.

   For details about selecting scans or components, see "Working with Detected Components" on page 320.

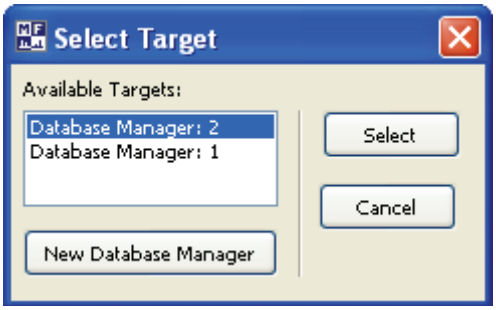
2. Click the **Add Selected Scans/Components to Database Manager** button,  .

   The Export Chromatographic and Spectral Data dialog box opens.



3. Select one of the export options.
4. Click **Continue**.

   The Select Target dialog box opens.



5. Do one of the following:

   Select one of your open Database Manager windows and click **Select**.

   –or–

   Click **New Database Manager**.

   The application imports the selected data to the Database Manager window.

❖ To export a data-reduced chromatogram to a Database Manager window

1. Select the scans or components.

   For details about selecting scans or components, see "Working with Detected Components" on page 320.

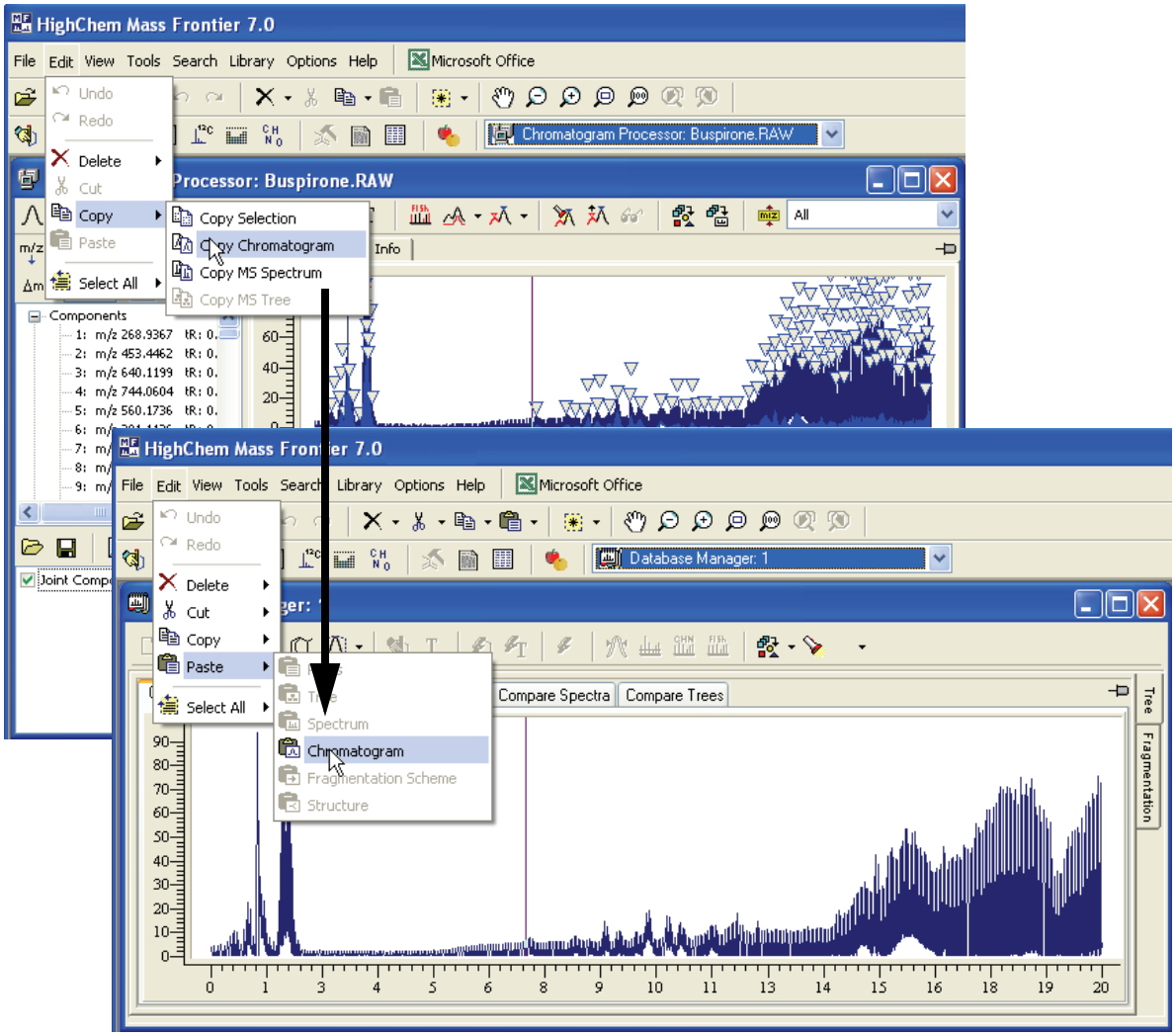
2. Right-click and choose **Copy > Copy Chromatogram** from the shortcut menu.

3. In a Database Manager window, right-click and choose **Paste > Chromatogram** from the shortcut menu.

   You can save data-reduced chromatograms in a Database Manager window to any library using the same procedure that you would use to save spectra or trees.

   Chromatographic scans or trees are fully searchable. For additional information, see "Searching Components" on page 334 or "Processing Data-Reduced Chromatograms" on page 69.

Figure 116. Copying a chromatogram from Chromatogram Processor to Database Manager



Annotating Chromatographic Peaks

You can add chemical structures and formatted text to any chromatographic peak in the Chromatogram Processor window.

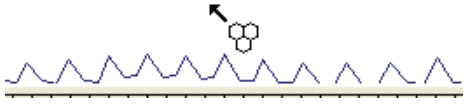
Note You cannot search structures assigned to chromatographic peaks.

Follow these procedures:

- To add a chemical structure to a chromatographic peak
- To add formatted text to a chromatographic peak

❖ To add a chemical structure to a chromatographic peak

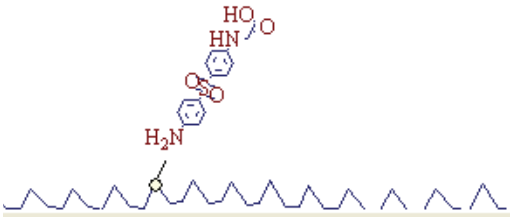
1. Click the **Assign Structure to Scan** button, .
2. Click the scan where you want to place a structure.



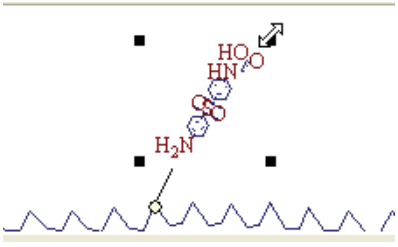
A new Structure Editor window opens where you can draw a fragment. For information about drawing fragments in the Structure Editor window, see Chapter 4, "Structure Editor Module."

3. After you finish drawing, click **OK**.

   The application connects your structure with the selected scan.

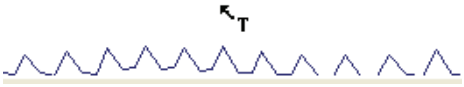


4. To connect the added structure to a different scan, select the structure and drag the connecting circle to the required scan.
5. To resize the structure fragment, select the structure and drag one of the corner rectangles.

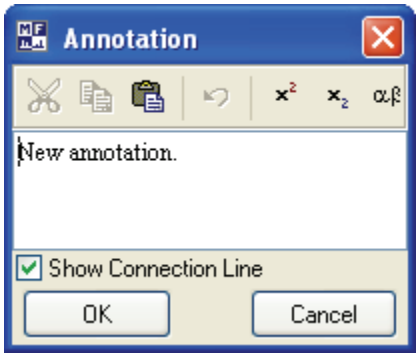


❖ To add formatted text to a chromatographic peak

1. Click the **Assign Text to Scan** button, T .
2. Click the scan where you want to place the text.

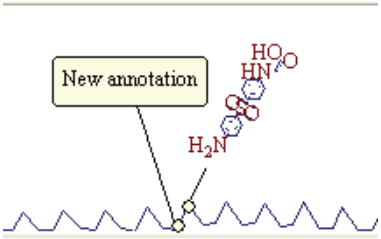


The Annotation dialog box opens.



3. Type your text.
4. To draw a line connecting the peak to the annotation, select the **Show Connection Line** check box.
5. Click **OK**.

   The application connects your text to the selected scan.



6. To connect the added text to a different scan, select the text and drag the connecting circle to the required scan.

Components Editor Module

The Components Editor module consists of a complete set of management tools to delete unrelated components, add chemical structures, edit extensive data fields, process spectral trees, annotate spectral peaks, and sort search hit lists for every component in a processed chromatogram.

Contents

Using the Components Editor Window

Use the Components Editor window to edit, search, and organize chromatographic components stored in a library or generated by the Components Detection and Spectra Deconvolution feature.

You cannot open the Components Editor module directly from the Mass Frontier application window. You must generate this module from your data in the Chromatogram Processor window.

❖ To open a Components Editor window

1. From a Chromatogram Processor window, click the **Components Detection & Spectra Deconvolution** button,  , and choose one of the component detection algorithms.

   See "Using Components Detection and Spectra Deconvolution" on page 299.

2. Identify the detection parameters and click **Calculate**.

   For detailed descriptions of the detection algorithm parameters, see the following:

   - "JCD Algorithm" on page 301
   - "RCD Algorithm" on page 309
   - "TECD Algorithm" on page 313
   - "Direct Infusion Algorithm" on page 315

3. When the detection and deconvolution process finishes, click **Close**.

   The TIC page identifies the detected components with a triangle at each detected peak.

4. In the Chromatogram Processor window, click the **Components Editor** button,  .

   The Components Editor window opens. See "Components Editor window" on page 333.

   The Components Editor window closely resembles the Database Manager window; however, the processing item is a chromatographic component rather than a database record. To distinguish between the modules, the Components Editor window has a light blue bar on the left side. Both modules behave almost identically. For a detailed explanation of the panes and their functionality, see Chapter 2, "Database Manager Module."

   Each chromatographic component is represented by a single row. The columns contain supplementary component information. One of the columns lists Model Ion values that have been used for component detection. These values help you orient yourself and find components of interest. In most cases, the model ion is the base peak in the full-scan spectrum; however, if closely coeluting components have isobaric base peaks, the algorithms select different model ions to distinguish these components.

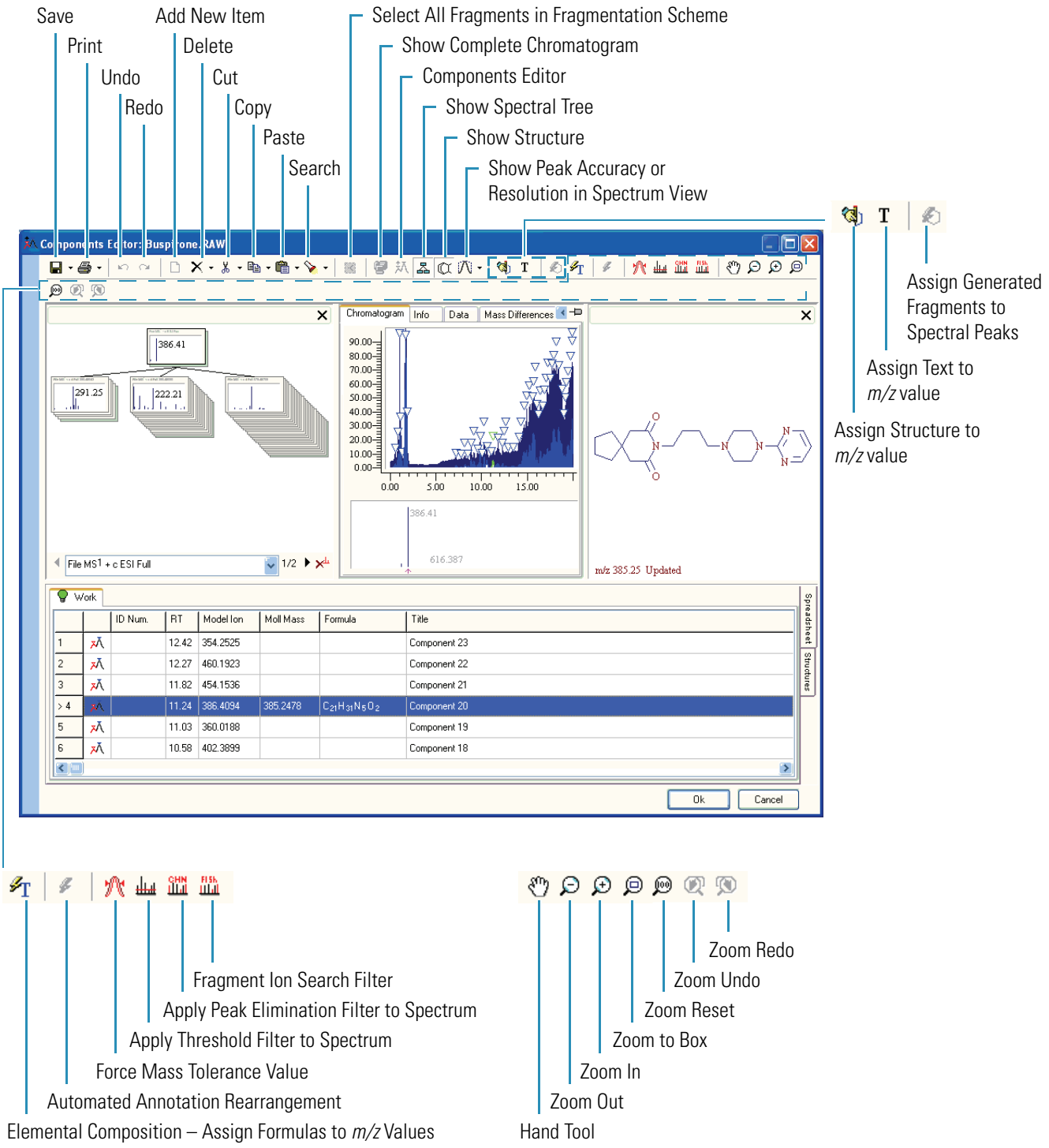
5. Make your changes to the chromatogram.

6. To save your changes and close this module, click **OK**.

   The application keeps changes with the chromatogram. To save the chromatogram to a chromatographic library, see "Processing Data-Reduced Chromatograms" on page 69.

Note You can also edit associated components of a library chromatogram (see "Searching Chromatographic Libraries" on page 182).

Figure 117. Components Editor window



Searching Components

You can use the Components Editor window to search one selected subset or all the chromatographic components in the spectral or chromatographic libraries. For additional information, see "Searching Chromatographic Libraries" on page 182.

Follow these procedures:

- To search for a component from the processed chromatogram
- To search all the components from the processed chromatogram
- To remove a component from the hit list
- To process the hit list of a component

❖ To search for a component from the processed chromatogram

1. Select the components in the Spreadsheet view.
2. Click the **Search** button, , and choose **Search Selected Components**.

   The Spectral Tree Search dialog box opens.
3. Enter your search criteria and click **OK**.

   For details about the Spectral Tree Search parameters, see "Searching Spectral Trees" on page 120.

   When the search is successful, the application stores the results in the Spreadsheet view in the Components Editor window.

   The highest match factor for each component search appears in the Match column of the Spreadsheet view. If the search did not find any plausible hits, the match factor is not displayed.

   The match factor is a number from 1 to 999 that specifies the measure of similarity between the query spectrum and the library reference spectrum. A match factor of 999 means a perfect match. Matches greater than 930 display a lightning bolt, , in the Match column.

	Match	ID Num.	RT	Model Ion	Moll Mass	Formula	Title
	934.2+		15.28	458.2671	457.1584	$C_{20}H_{27}NO_{11}$	Amygdalin

❖ To search all the components from the processed chromatogram

1. Click the **Search** button, , and choose **Search All Components**.

   Note Searching a large number of components can take a long time, depending on the library size.

   The Spectral Tree Search dialog box opens.

2. Enter your search criteria and click **OK**.

   For details about the Spectral Tree Search parameters, see "Searching Spectral Trees" on page 120.

   When the search is successful, the application stores the results in the Spreadsheet view in the Components Editor window. The spreadsheet displays the title for all hits in red.

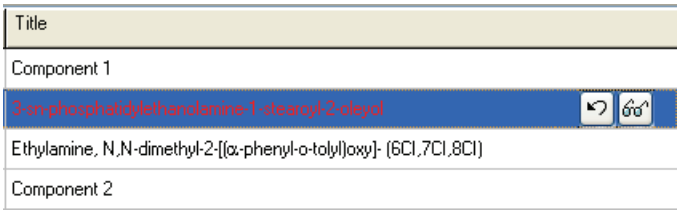
   The highest match factor for each component search appears in the Match column of the Spreadsheet view. If the search did not find any plausible hits, the match factor is not displayed.

   The match factor is a number from 1 to 999 that specifies the measure of similarity between the query spectrum and the library reference spectrum. A match factor of 999 means a perfect match. Matches greater than 930 display a lightning bolt, ⚡, in the Match column.

	Match ▼	ID Num.	RT	Model Ion	Moll Mass	Formula	Title
	⚡ 934.2+		15.28	458.2671	457.1584	$C_{20}H_{27}NO_{11}$	Amygdalin

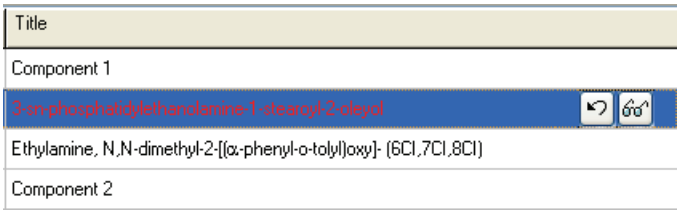
❖ To remove a component from the hit list

1. Select the component row.
2. Click the **Reject Library Hit** button, ↩.



❖ To process the hit list of a component

1. Select the component row.
2. Click the **Hit Selector** button, 🔍.

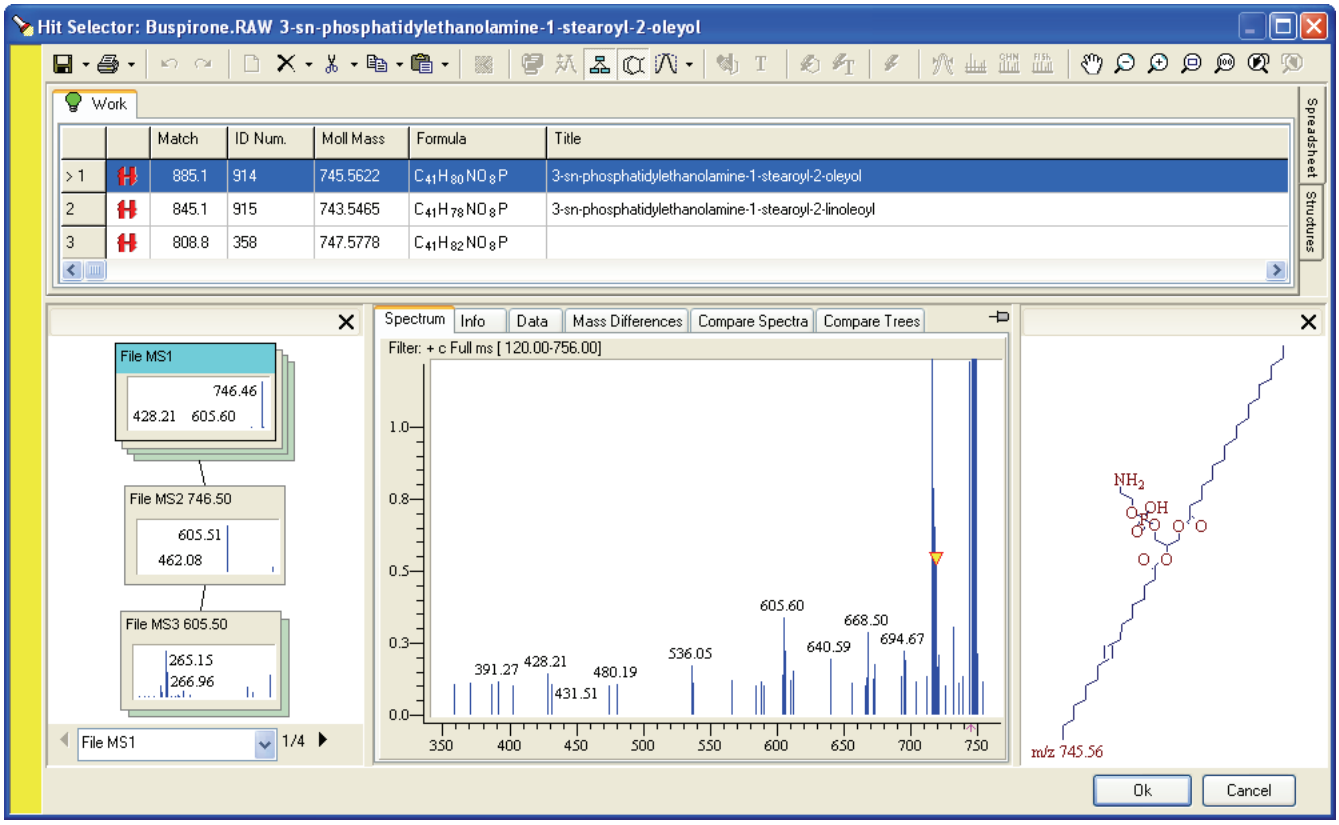


   The Hit Selector window opens.

   To distinguish the Hit Selector window from the Components Editor and Database Manager windows, the Hit Selector window has a yellow bar on the left side.

   The Hit Selector window lists the best matches found during the library search. The match factor describes the similarity of the match to your component.

Figure 118. Hit Selector window



3. In the Hit Selector window, review the hit list and do one of the following:

   - To accept a library record that corresponds to the component, select the component in the spreadsheet and click **OK**.

     When you accept a library record for a component, all relevant information (structure, name, molecular mass, ion types, and so on) are adopted and entered in the component fields.

   - To reject all components in the hit list, click **Cancel**.

Spectra Classifier Module

Use the Spectra Classifier module to retrieve and organize spectra, which you can then submit to Principal Component Analysis (PCA), Neural Networks Self-Organizing Maps (SOM), or Fuzzy Clustering.

Contents

Classification Methods Background

Classification greatly enhances library search and fragmentation prediction methods. The Mass Frontier application uses classification methods that close the triangle of computer-oriented methods for interpreting mass spectral data. The computer-oriented methods available in this application complement each other but are based on different principles. These methods provide the means to create alternative strategies and enable comprehensive data interpretation.

Advantages of Classification Methods in the Mass Frontier System

In contrast to statistical software packages, the Mass Frontier system applies classification methods directly to mass spectral data. All preprocessing procedures, which are essential to using classification methods, are automatic. This built-in process ensures that you use classification methods correctly, even if you have minimal knowledge of the multivariate statistic.

The primary goal of spectral classification is to find a correlation between the properties of compounds and their mass spectra. Because physical and chemical properties and biological activities of chemical compounds are to a large extent a function of molecular structure, the results of classification analysis reflect structural features that are determined by fragmentation ions appearing in a mass spectrum. The important advantage of classification methods is that you do not need a detailed knowledge of the complex spectra-structure relationship to get satisfactory results. Classification strategy in this application is based on graphical presentation of the results, which you can easily view on the screen (see "Working in the Spectra Projector Window" on page 361).

Spectra Classification Methods

Computer methods of analyzing mass spectral data center on three fundamental methodologies: library search techniques, expert system procedures, and classification methods.

For additional information, see "Using the Search Utilities" on page 72, "Previewing Unimolecular Reactions" on page 193, or "Example Workflow" on page 357.

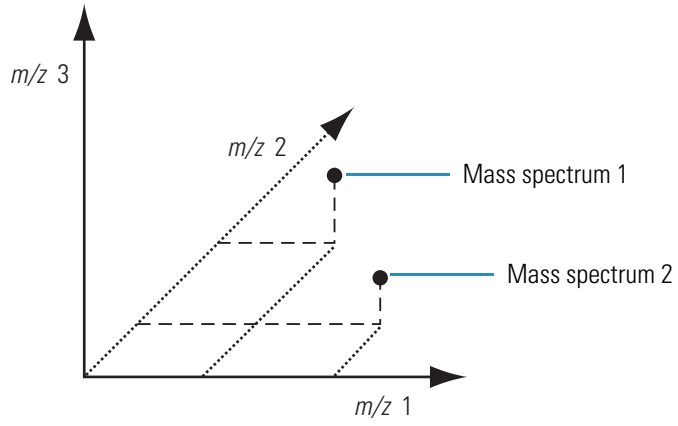
This application contains three classification methods:

- Principal Component Analysis (PCA)
- Fuzzy Clustering
- Self-Organizing Maps

Self-Organizing Maps (SOM) is a special class of neural networks. Use these methods, based on different principles, to explore complex data from various perspectives. Principal Component Analysis (PCA) uses multivariate statistics, fuzzy clustering assigns data to clusters, and SOM is based on competitive learning.

In the multivariate statistic, you can consider each spectrum as a single point in an *n*-dimensional space, with the intensities being the coordinates of this point. A dimension (axis) of that space represents a mass-to-charge ratio, *m/z*, of the considered peak. This means that the dimensionality is determined by the *m/z* value of the last peak in the spectrum. For example, the EI spectrum of hydrogen exhibits two peaks at *m/z* =1 (intensity 2%) and *m/z* = 2 (100%). You can view this spectrum as a point in a two-dimensional space with the coordinates [2, 100].

Figure 119. Multivariate statistic example



In reality, spectra have a far higher dimensionality than two. When the dimensionality is too high, or several coordinates are equal to zero (usually a mass spectrum does not have peaks at every *m/z* value), the classification methods might not provide the required results. As a result, a reduction of dimensionality is carried out either before a spectrum is placed in *n*-dimensional space, or during the classification process.

The basic hypothesis of multivariate statistical methods assumes that the distance between points (spectra) in an *n*-dimensional space is related to a relevant property of the compounds that represent these points. When the points are close enough to form a cluster or a separated region, you can assume that the compounds that correspond to these points exhibit common or similar properties. To ensure the results of the classification methods have statistical significance, place a large number of spectra (usually one or more groups, each with 10–1000 spectra) in the same *n*-dimensional space. Then apply multivariate statistical methods, with various parameters, to evaluate these points (spectra). The objective of a classification process is to separate these points (spectra) into two or more classes according to the desired structural or other properties.

Principal Component Analysis (PCA)

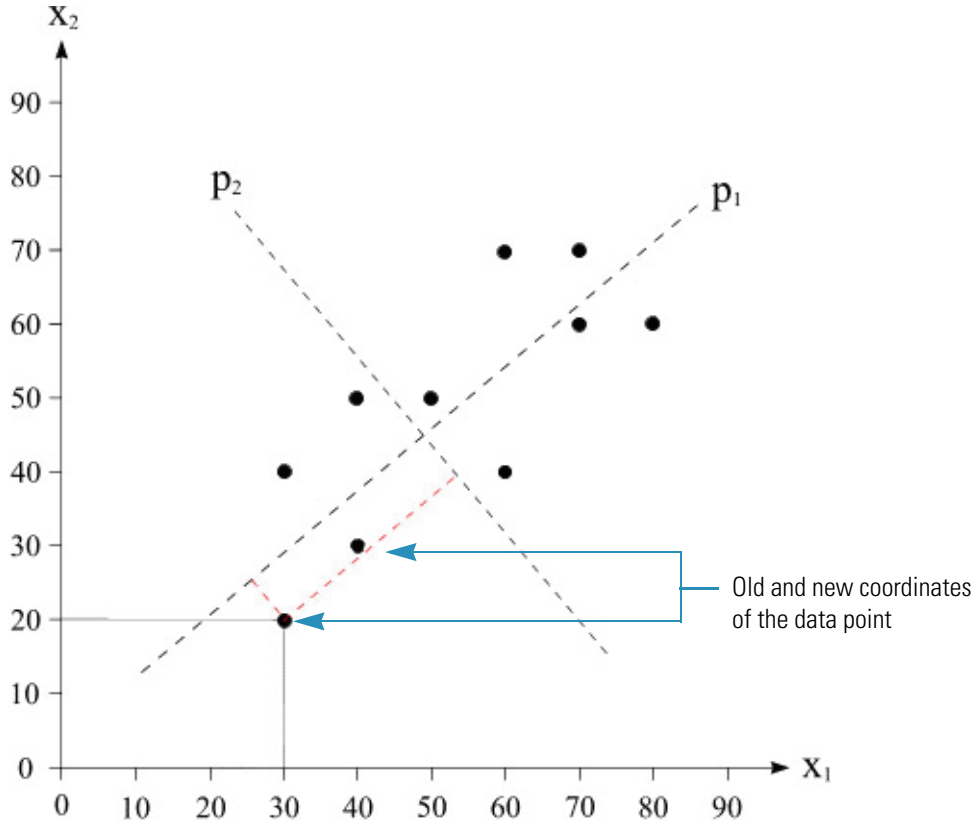
Principal Component Analysis reduces the dimensionality of a data set where a large number of interrelated—that is, correlated—variables exist, while retaining as much as possible the variation present in the data set. In mass spectrometry, the data set consists of the mass spectra of different compounds. The mass spectra are expressed as the intensities of individual *m/z* ratios, or variables.

PCA attempts to find a new coordinate system that can be expressed as the linear combination of the original variables (*m/z*) so that the major trends in the data are described. Mathematically, PCA relies on eigenvalue/eigenvector decomposition of the covariance or the correlation matrix of the original variables. PCA decomposes the data matrix *X* as the multiplication of two matrices *P* (the matrix of new coordinates of data points) and *T´* (transposition of the coefficients matrix of the linear combination of the original variables):

$$X = P.T'$$

Generally, the data can be adequately described using far fewer coordinates, also called principal components, than original variables. PCA also serves as a data-reduction method and a visualization tool. When the data points are plotted in the new coordinate system, the relationships and clusters are often more apparent than when the data points are plotted with the original coordinates.

Figure 120. Geometrical interpretation of PCA



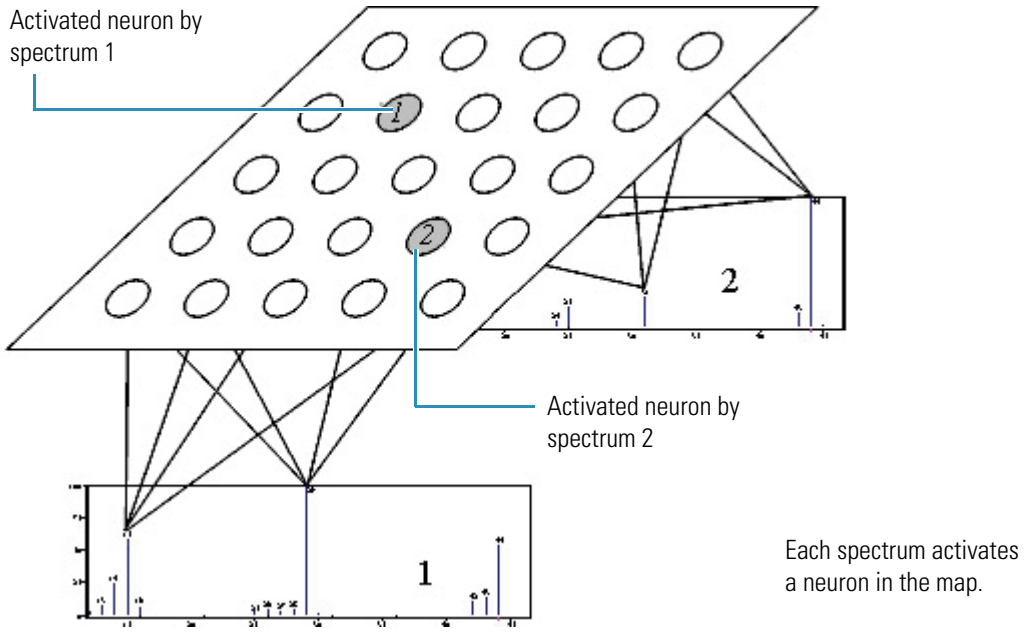
Geometrical interpretation of PCA: The axes of the new coordinate system—principal components p1 and p2—are created as the linear combinations of the original axes. New coordinates (principal components (PC)) are orthogonal (perpendicular) to each other. There is greater variation in the direction of p1 than in either of the original variables, but very little variation in the direction of p2. For data sets with more than two variables, the first PC describes the direction of the greatest variation in the data set, the second PC describes the direction of the second greatest variation, and so on.

Self-Organizing Maps

Self-Organizing Maps, sometimes called Kohonen networks, are a special class of neural networks. A self-organizing map consists of neurons placed at the nodes of a two-dimensional lattice. The neurons become selectively activated to various input mass spectra, or classes of spectra, in the course of a competitive learning process. The neurons compete among themselves to be activated or excluded. You can view SOM as a nonlinear generalization of PCA.

The principal goal of self-organizing maps is to transform a set of *n*-dimensional input spectra into a discrete two-dimensional map and to display this transformation. Each input spectrum presented to the network activates a neuron according to a complex set of interrelationships between spectra. In SOM, each mass spectrum must always activate a neuron and this spectrum is shown on the particular neuron. Spectra that activate the same neuron belong, in terms of classification, to the same pattern. To ensure that the self-organizing process has a chance to develop properly, the networks should be exposed to a certain number of different spectra. As a result, this application requires a minimum of 10 spectra in a self-organizing process.

Figure 121. SOM architecture as used in the Mass Frontier application

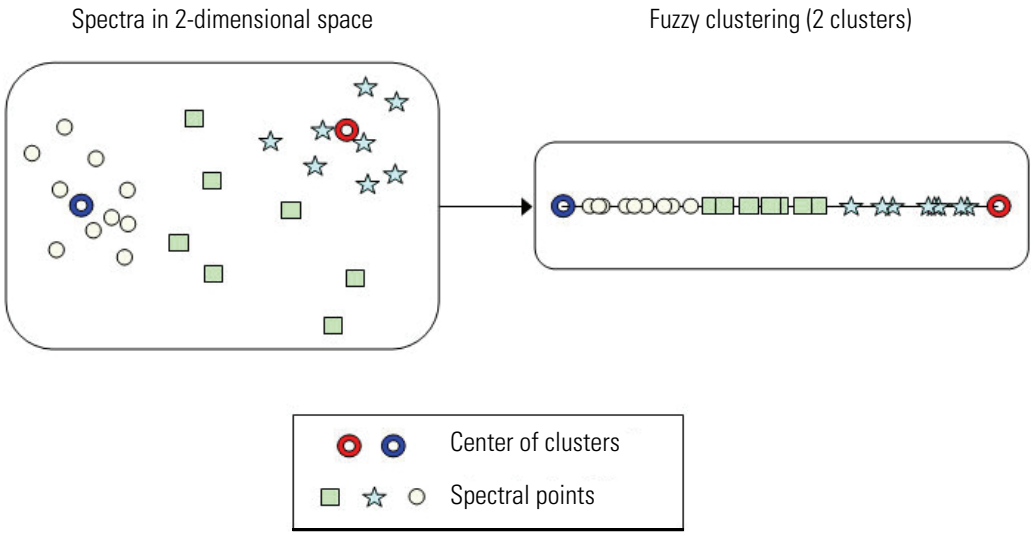


Note The SOM classification method exhibits one atypical feature to be aware of: an identical data set produces different results if the input data is processed in a different order. This ordering is an inherent feature of neural networks and is not a result of faulty algorithms.

Fuzzy Clustering

Cluster analysis is a technique for grouping data into clusters to find common structural features in spectral data. Membership degrees between zero and one are used in fuzzy clustering, instead of crisp assignments of the data to clusters. Fuzzy clustering is based on the dot-product distance between the center of clusters and experimental spectral points. The dot-product is calculated from mass spectral *n*-dimensional space with the intensities being the coordinates and *m/z* values dimensions. The number of peaks whose intensities are above the predefined threshold determines the dimensionality of spectral space. The number of clusters is usually pre-defined. For additional information, see Chapter 11, "Spectra Projector Module."

Figure 122. Fuzzy clustering example



Organizing Spectra

Spectra are organized into groups that can have their own names and graphic representations. When spectra are organized into groups, you can distinguish between the classes of spectra that should arise from the submitted groups of spectra after the classification process.

You can use the Mass Frontier application for the classification of spectra according to physical or chemical properties (point of origin, toxicity, aromaticity, and so on). For spectra that cannot be found in a library, classification can involve identification of substructure types or compound classes (structure elucidation) to establish and confirm structural proposals. Classification can also be useful in cases when you must retrieve only structurally related compounds from a complex chromatogram (metabolite research).

In classification analysis, distinguishing between two terms, *groups* and *classes,* is important. You can assign classes only after the classification method (Principal Component Analysis, Fuzzy Clustering, or Neural Networks (SOM)) clearly shows the occurrence of clusters or regions that consist of spectra with the desired properties. Before classification, you can only allocate spectra to groups according to preselected criteria. Often the objective of the classification process is to obtain classes that closely resemble groups of spectra submitted to classification.

You can save and open records with the .ref file extension, which are present in the Database Manager spreadsheet. This feature is especially useful for maintaining groups of spectra that are sent from the Database Manager to the Spectra Classifier window.

When you have retrieved spectra for classification, you might want to save these records, rather than save the spectra as ASCII files, such as JCAMP-DX (.jdx) or NIST MSP (.msp) files. In contrast to .jdx or .msp files, a reference file stores the locations where all the relevant information about each spectrum is saved (spectrum, structure, experimental conditions, and so on). These files are easy to manipulate; you can add or remove one or more records to them. You cannot save deconvoluted spectra from the Chromatogram Processor window as records (see "Working with Detected Components" on page 320).

Working in the Spectra Classifier Module

The Spectra Classifier window is the gateway to PCA, Neural Networks (SOM), and Fuzzy Clustering classification. When you send or paste any spectra to the Spectra Classifier window, the spectrum or spectra are automatically assigned to a group. The application automatically gives a name to a new group, which you can rename. You can add up to 255 groups of spectra to Spectra Classifier, and each group can consist of an unlimited number of spectra. Conversely, it is also possible for a group to only contain a single spectrum.

This section includes the following topics:

Opening the Spectra Classifier Window

You can open an empty Spectra Classifier window or select spectra from the Database Manager or Chromatogram Processor window and populate those spectra to a Spectra Classifier window.

Follow these procedures:

❖ To open a Spectra Classifier window

Do one of the following:

Click the **Spectra Classifier** button,  , in the Mass Frontier toolbar.

–or –

Choose **Tools > Spectra Classifier** from the Mass Frontier main menu.

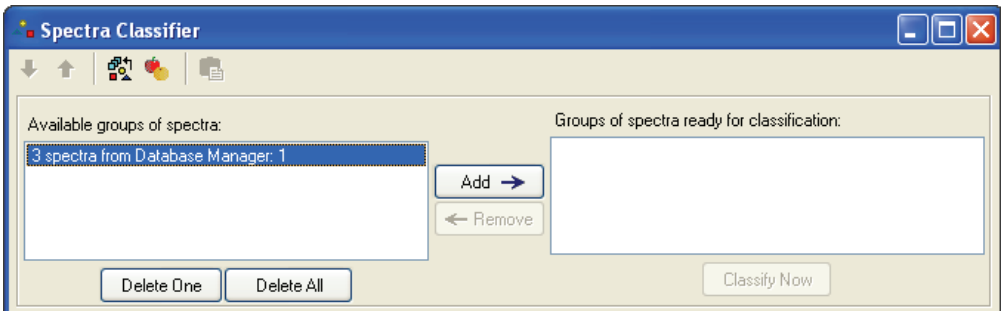
An empty Spectra Classifier window opens. See "Spectra Classifier Window" on page 355. You can open only one Spectra Classifier window at a time in the system. If a Spectra Classifier window is already open, it becomes active (and might already contain spectra).

❖ To select spectra for classification from the Database Manager

1. Select the records in the Database Manager window.
2. Click the **Add Selected Records to Spectra Classifier** button,  , and choose **Spectra Classifier**.

   The Spectra Classifier window opens.

   Figure 123. Spectra Classifier window with spectra from a Database Manager



❖ To select spectra for classification from the Chromatogram Processor

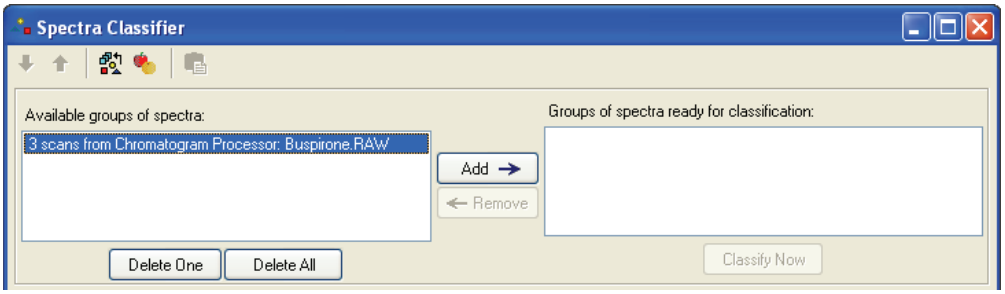
1. Select scans or components in the Chromatogram Processor window.

   You can select scans from the chromatogram pane or components from the filter list.

2. Click the **Add Selected Scans or Components to Spectra Classifier** button,  .

   The Spectra Classifier window opens.

   Figure 124. Spectra Classifier window with spectra from a Chromatogram Processor



Managing Groups of Spectra

In the Spectra Classifier window, you can select the spectra you are ready to classify, remove spectra groups from the Spectra Classifier window, or assign names to the spectra groups.

Follow these procedures:

- To copy and paste spectra for classification
- To add an available spectra group to the ready-for-classification list
- To remove a spectra group from the ready-for-classification list
- To delete a group of spectra from the Spectra Classifier window
- To assign an appropriate name to each group

❖ To copy and paste spectra for classification

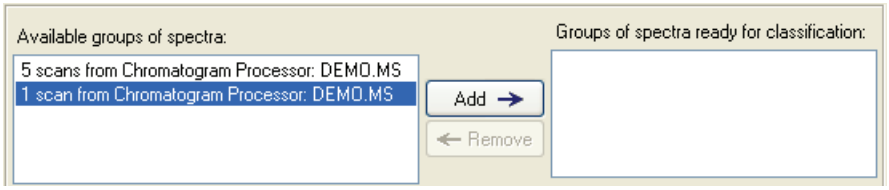
1. In a Database Manager window or a Chromatogram Processor window, select the spectra you want to classify.
2. In the Spectra Classifier window, click the **Paste** button,  .

   The application adds the selected spectra to the Available Groups of Spectra list.

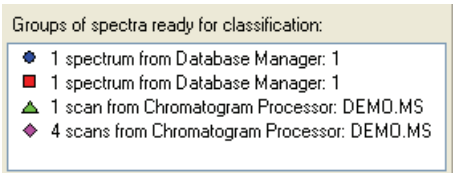
❖ To add an available spectra group to the ready-for-classification list

1. Select the group of scans in the Available Groups of Spectra list.
2. Click **Add**.

   The application moves the selected group to the Groups of Spectra Ready for Classification list.



   The application automatically assigns a graphic to each group in the Groups of Spectra Ready for Classification list.



   To change the group's graphic, choose **Options > Settings** from the Mass Frontier main menu, and then choose **Classification** in the Layouts area. The colors and types of graphics are used only to distinguish the groups in the plots and have no influence on the results of the analysis.

❖ To remove a spectra group from the ready-for-classification list

1. Select the group of scans in the Groups of Spectra Ready for Classification list.
2. Click **Remove**.

   The application moves the selected group to the Available Groups of Spectra list.

❖ To delete a group of spectra from the Spectra Classifier window

1. Select the group of scans in the Available Groups of Spectra list.
2. Do one of the following:

   Click **Delete One** to remove only the selected scan.

   –or–

   Click **Delete All** to remove all scans in the Available Groups of Spectra list.

   The application deletes the specified scans from the Spectra Classifier window.

> **Note** You can delete scans only from the Available Groups of Spectra list. You cannot delete scans from the Groups of Spectra Ready for Classification list.

❖ To assign an appropriate name to each group

1. In either list of spectra groups, select a group.
2. In the Selected Group of Spectra box, change the name of the group of spectra.

> **Note** You can use the copy (CTRL+C) and paste (CTRL+V) commands.

Viewing Spectra Data

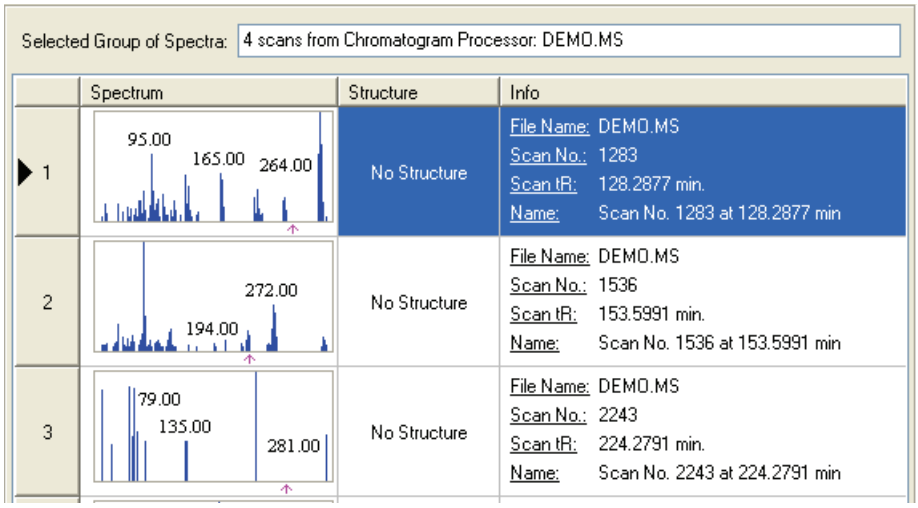
In the Spectra Classifier window, you can view the spectra and structures for a spectra group or open the source (Database Manager or Chromatogram Processor) module for a spectra group.

❖ To preview spectra records

In either list of spectra groups, select a group.

The table displays spectra, structures, and additional information.

Figure 125. Spectra records



❖ To open the source for a spectra group

Double-click a spectra group in either of the two lists.

The Database Manager window or Chromatogram Processor where the corresponding spectra originated opens with these records selected.

IMPORTANT The Spectra Classifier can hold only spectra that are still present in the original Database Manager or Chromatogram Processor window. After you create a group of spectra in the Spectra Classifier, do not move the spectra from the original Database Manager window or clear the component spectra from a chromatogram in the Chromatogram Processor. If you violate either of these conditions, the target group of spectra is removed from the Spectra Classifier.

Classifying Spectra

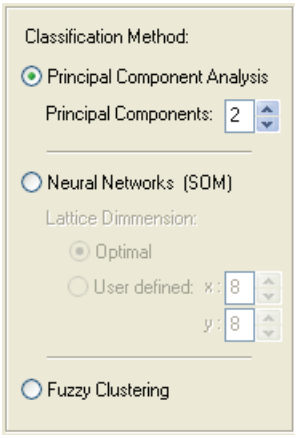
After you have selected all the groups you want to classify and chosen the classification method, you can classify the spectra groups. While the application is running the classification, you can continue to work in other Mass Frontier windows. Depending on the type of classification method you choose, the application displays the results in a Spectra Projector or a Neural Networks window.

Follow these procedures:

- To begin Principal Component Analysis classification
- To begin Neural Networks (SOM) classification
- To begin Fuzzy Clustering classification

❖ To begin Principal Component Analysis classification

1. In the Classification Method area, select the **Principal Component Analysis** option.



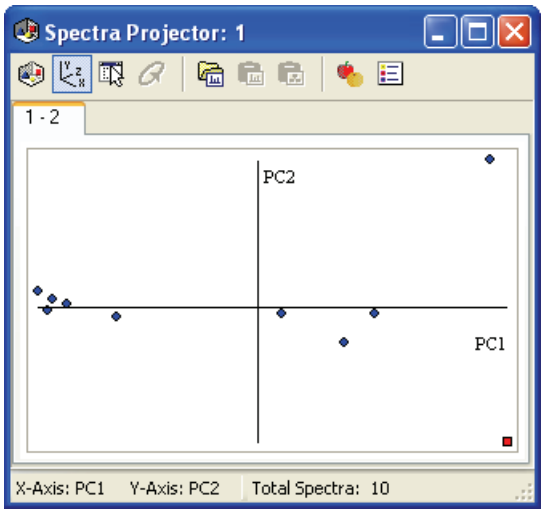
2. Specify the number of principal components to be calculated.
3. Click **Classify Now.**

   The application classifies all groups of spectra in the Groups of Spectra Ready for Classification list according to the selected classification method.

> **Note** You can continue to work in other Mass Frontier windows while the application processes a classification.

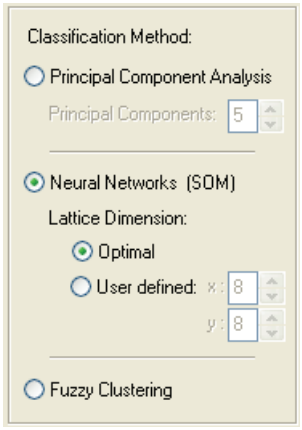
The application displays the results of a Principal Component Analysis classification in a Spectra Projector window. For a complete description, see Chapter 11, "Spectra Projector Module."

Figure 126. Spectra Projector window resulting from PCA classification



❖ To begin Neural Networks (SOM) classification

1. In the Classification Method area, select the **Neural Networks** option.



2. Select the **Optimal** option (recommended), or select the **User Defined** option and specify an **x** and **y** value.

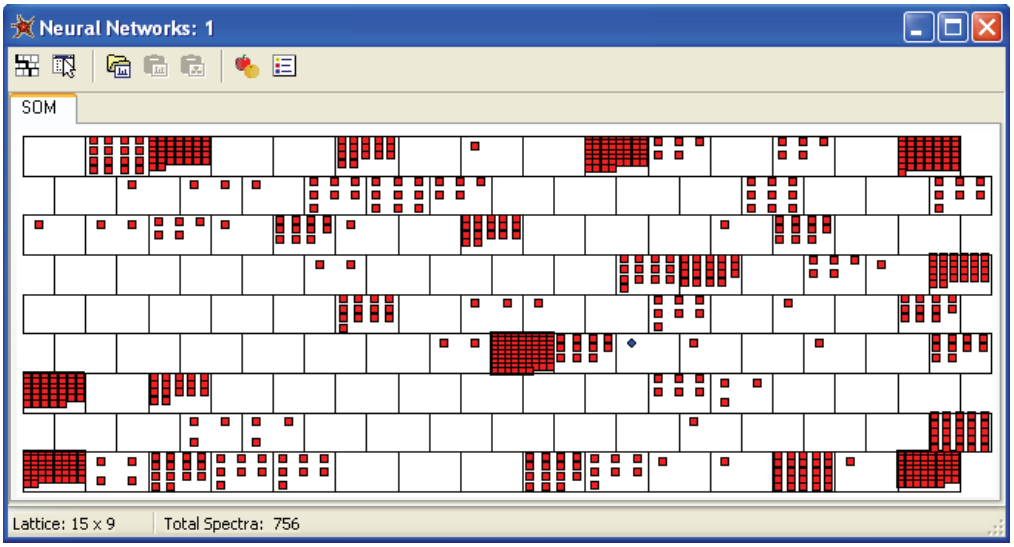
3. Click **Classify Now.**

   The application classifies all groups of spectra in the Groups of Spectra Ready for Classification list according to the selected classification method.

> **Note** You can continue to work in other Mass Frontier windows while the application processes a classification.

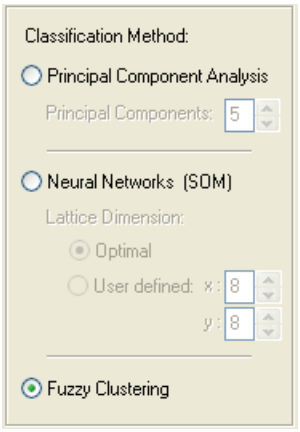
The application displays the results of a Neural Networks classification in a Neural Networks window. For a complete description, see Chapter 12, "Neural Networks Module."

Figure 127. Neural Networks window resulting from spectra classification



❖ To begin Fuzzy Clustering classification

1. In the Classification Method area, select the **Fuzzy Clustering** option.



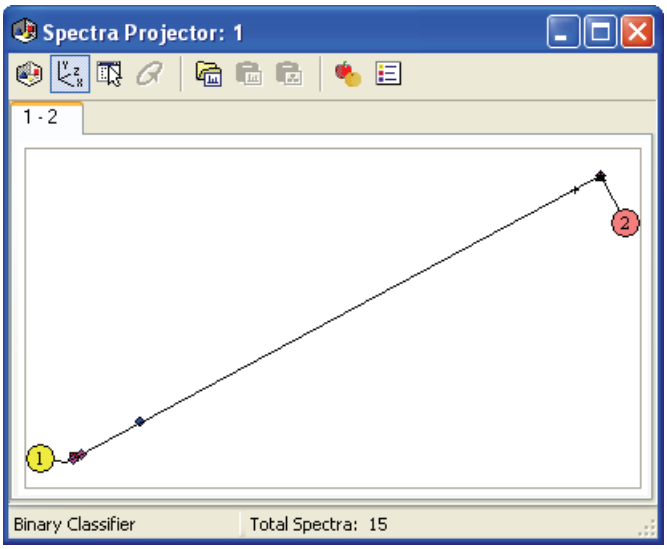
2. Click **Classify Now.**

The application classifies all groups of spectra in the Groups of Spectra Ready for Classification list according to the selected classification method.

Note You can continue to work in other Mass Frontier windows while the application processes a classification.

The application displays the results of a Fuzzy Clustering classification in a Spectra Projector window. For a complete description, see Chapter 11, "Spectra Projector Module."

Figure 128. Spectra Projector window resulting from Fuzzy Clustering classification



Spectra Classifier Window

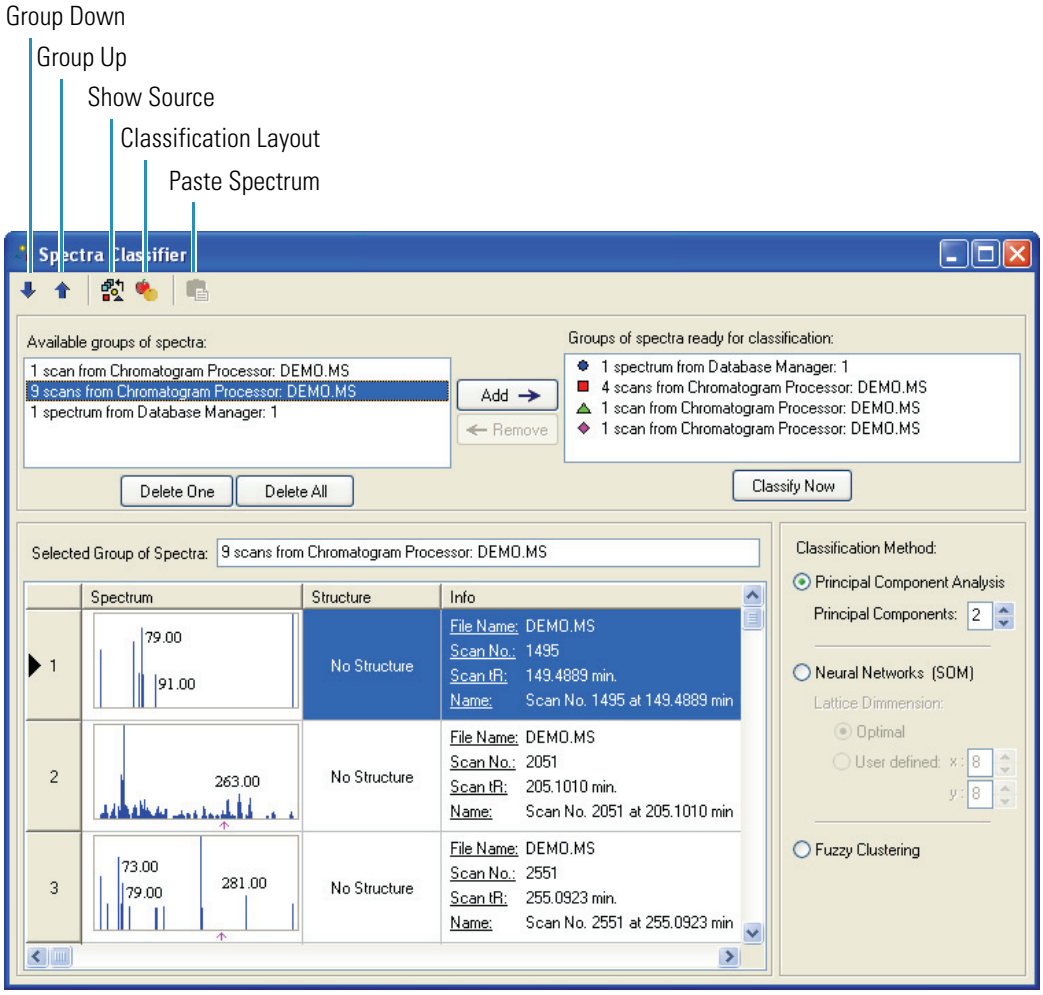


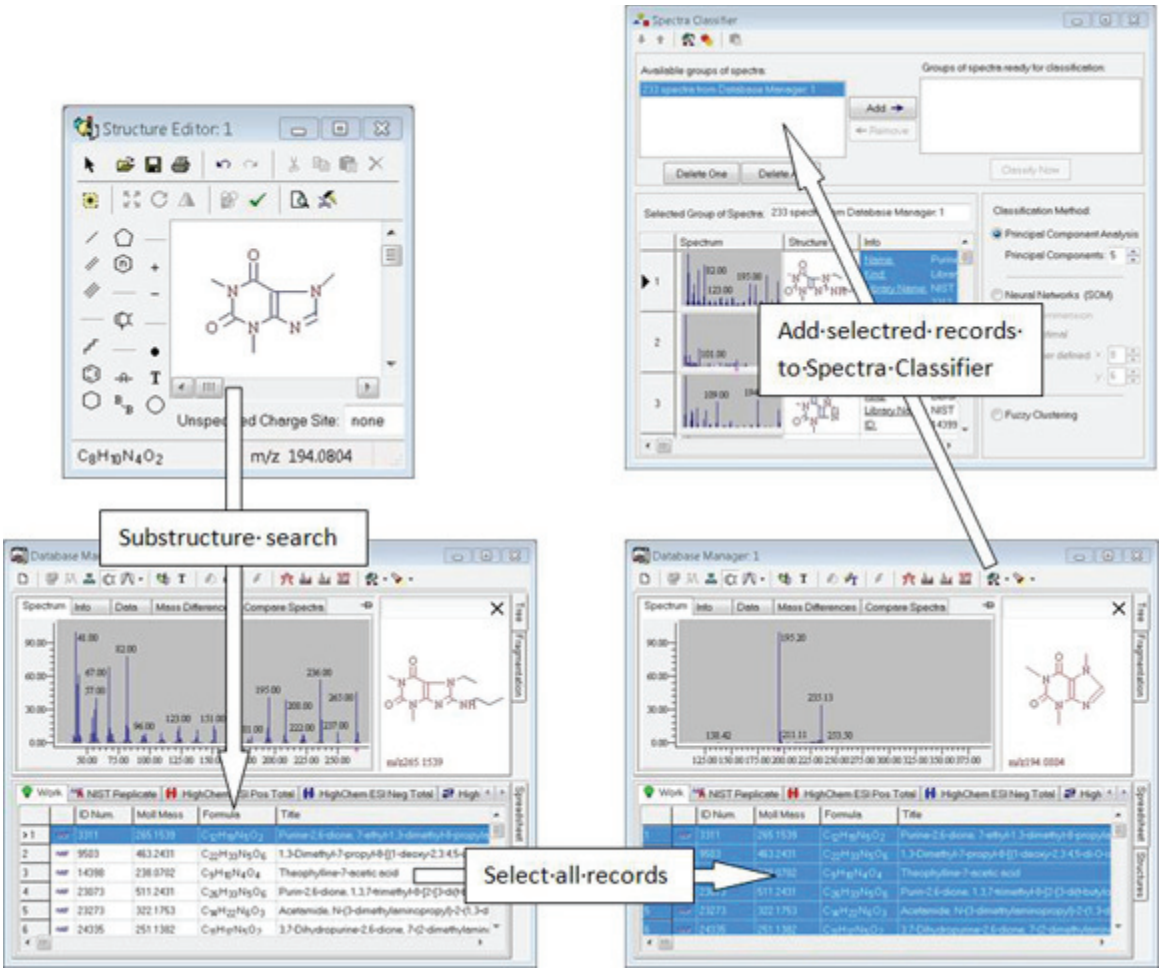
Table 46. Spectra Classifier parameters (Sheet 1 of 2)

Parameter	Description
Available Groups of Spectra	Lists groups of spectra that have been sent (or copied) from a Database Manager or Chromatogram Processor window.
Groups of Spectra Ready for Classification	Lists the groups of spectra to classify when you click **Classify Now**.
Add →	Moves the selected group to the Groups of Spectra Ready for Classification list.
← Remove	Moves the selected group to the Available Groups of Spectra list.
Delete One	Removes only the selected scan from the Spectra Classifier window.
Delete All	Removes all scans in the Available Groups of Spectra list.

Table 46. Spectra Classifier parameters (Sheet 2 of 2)

Parameter	Description
Classify Now	Classifies all groups of spectra in the Groups of Spectra Ready for Classification list according to the selected classification method.
Selected Group of Spectra	Editable name of the currently selected spectra group.
Classification Method	
Principal Component Analysis / Principal Components	Specifies the PCA classification method and the number of coordinates used to describe the data. For a detailed description, see "Principal Component Analysis (PCA)" on page 341.
Neural Networks (SOM)	Specifies the SOM classification method, which is a self-organizing map that consists of neurons placed at the nodes of a two-dimensional lattice. For a detailed description, see "Self-Organizing Maps" on page 343.
Optimal	(Recommended) Specifies an optimized two-dimensional lattice.
User Defined	Specifies the **x** and **y** dimensions of the lattice.
Fuzzy Clustering	Specifies the fuzzy clustering classification method that groups data into clusters to find common structural features in the spectral data. For a detailed description, see "Fuzzy Clustering" on page 344.

Example Workflow



This example workflow demonstrates how to classify the derivatives of nicotine and caffeine.

You have an EI spectrum of an unknown compound that you suspect is a metabolite of either nicotine or caffeine, and you must find out which of these two compound classes it belongs to.

1. Retrieve a sufficient number of spectra for each group (for example, nicotines and caffeines).
2. To form clusters of each group, submit to a principal component analysis.
   a. Perform a substructure search, where the search query is the structure of nicotine and caffeine.
   b. When a sufficient number of spectra for each group is found (more than 10 spectra), select these records and add them, separately for each group, to the Spectra Classifier.
3. Assign an appropriate name to each group using the Selected Group of Spectra text box.

   You can use the copy (CTRL+C) and paste (CTRL+V) commands.

4. Select a line in the left pane of Spectra Classifier and click **Add**, and then repeat this for the next group.

   Note Spectral trees are supported by all the spectral modules except the Spectra Classifier, which uses total composite spectra generated from trees.

5. When all the groups of spectra that you want to classify are in the Groups of Spectra Ready for Classification list, launch the classification process by clicking **Classify Now**.

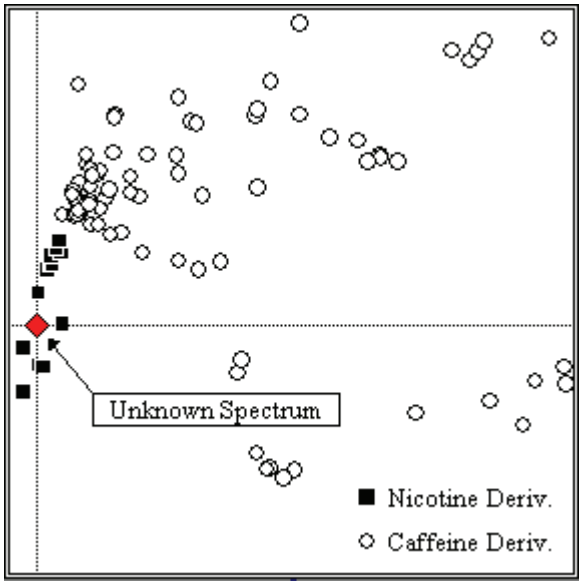
   After the generation is finished, a new Spectra Projector window opens.

   You have retrieved two groups of spectra: nicotine and caffeine derivatives. You now want to examine an unknown metabolite that likely originated from nicotine or caffeine.

6. After preparing the two groups of spectra in the Spectra Classifier window, generate a Spectra Projector window by using the **PCA** option.

   The following figure shows that the two groups are separated into clusters.

   Figure 129. Nicotine and caffeine separated into clusters



7. Add the unknown metabolite to the projection plane by pasting or opening its spectrum in Spectra Projector.

   The projection of this external spectrum clearly shows that it belongs to the nicotine class (squares).

8. Confirm or reject this result using the fragmentation pattern of nicotine.

Spectra Projector Module

The Spectra Projector module displays the results of a Principal Component Analysis (PCA) or Fuzzy Clustering method. In a Spectra Projector window, mass spectra are projected as points on a two-dimensional (2-D) plane or a three-dimensional (3-D) twistable space, according to principal components or fuzzy cluster combinations. The objective of classification analysis is to find classes of spectra on the projection that exhibit common or similar properties. You can use the Spectra Projector window to place an external spectrum onto a projection to examine its class membership.

You can apply classification to spectra that cannot be previously distributed into groups by classifying only one group of spectra and attempting to find points on the projection that represent compounds with the expected properties. In the Spectra Projector window, you can select these points to examine their spectra and structures.

For additional information, see "Principal Component Analysis (PCA)" on page 341 or "Fuzzy Clustering" on page 344.

Contents

Generating a Spectra Projector Window

You cannot directly open a Spectra Projector window from the Mass Frontier application. You must generate it from the Spectra Classifier module by specifying the Principal Component Analysis or the Fuzzy Clustering option for classification. You can open an unlimited number of Spectra Projector windows in the Mass Frontier application. For additional information, see Chapter 10, "Spectra Classifier Module."

You cannot alter classification results after they have been generated. To remove a spectrum (point) from a projection plane, remove this spectrum from the input data and then launch a new classification process.

Figure 130. Spectra Projector generated with the PCA classification method

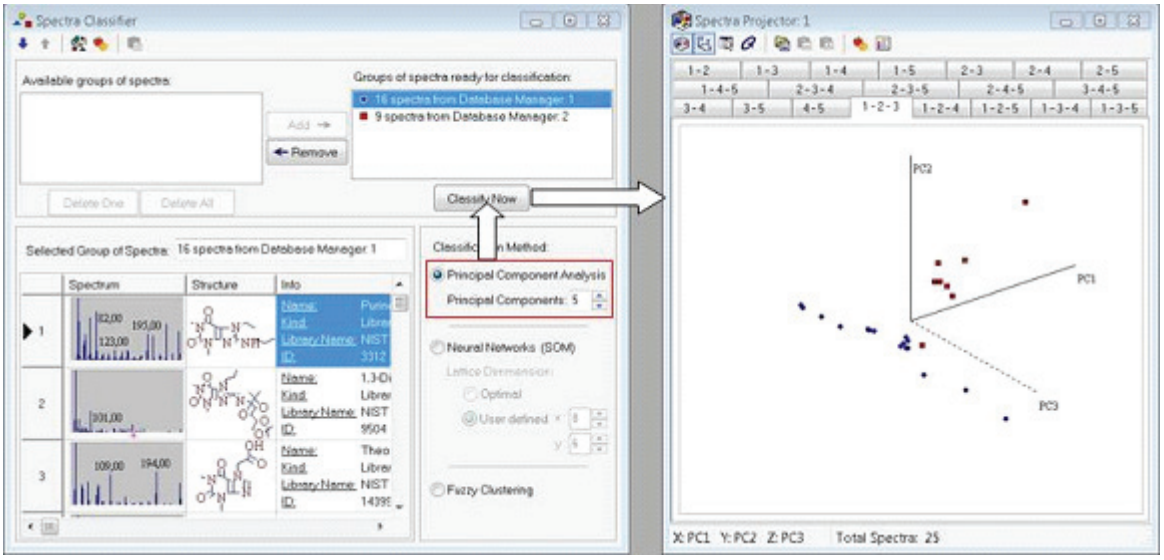
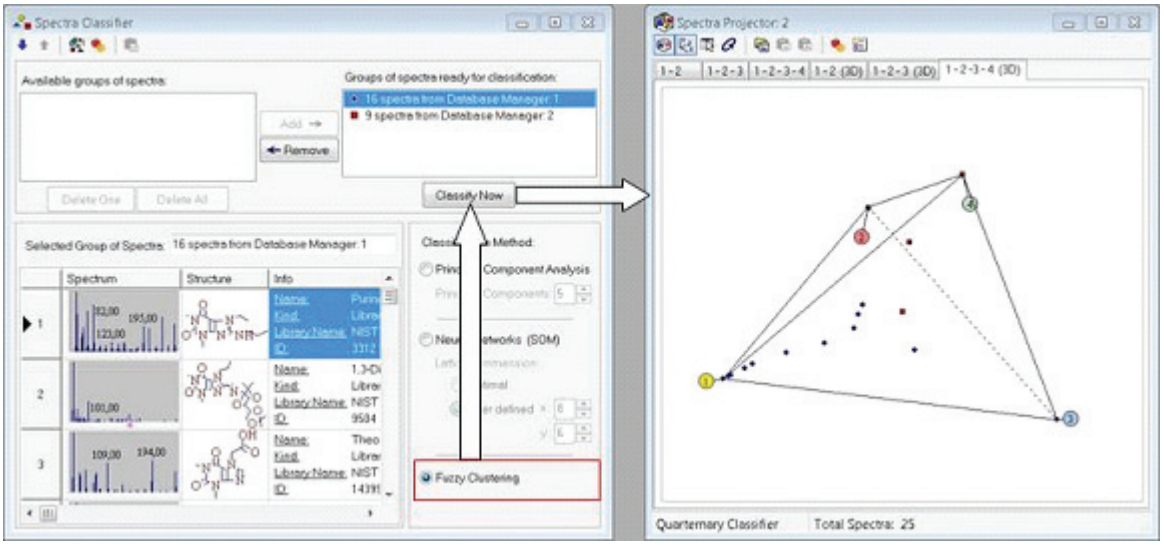


Figure 131. Spectra Projector generated with the Fuzzy Clustering classification method

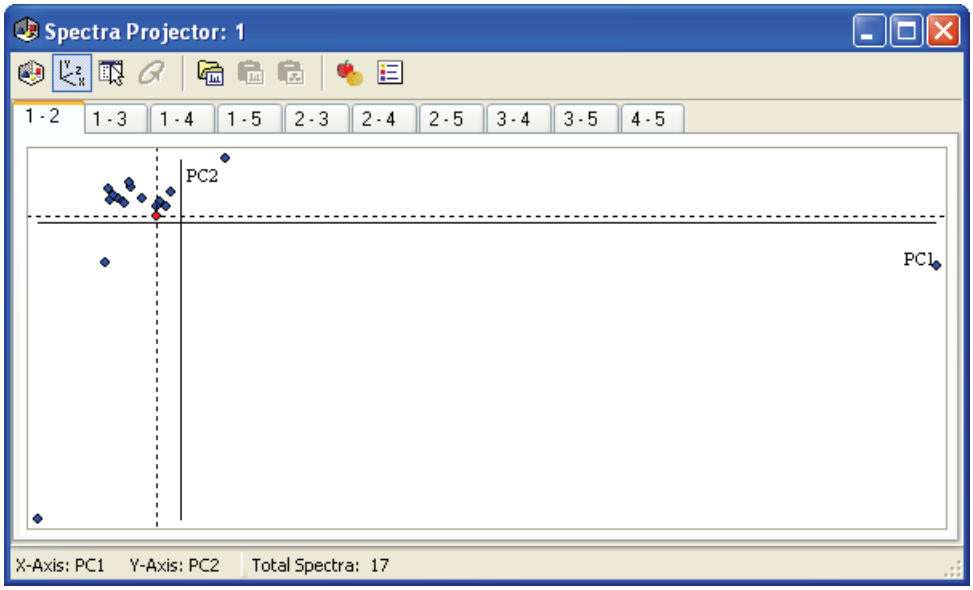


Working in the Spectra Projector Window

The Spectra Projector window displays spectra as points on a 2-D plane or in a 3-D space. You can select a combination of two principal components, which make up the 2-D projection plane. When you are viewing PCA results using the 3-D projection mode, you can select a combination of the three associated principal components.

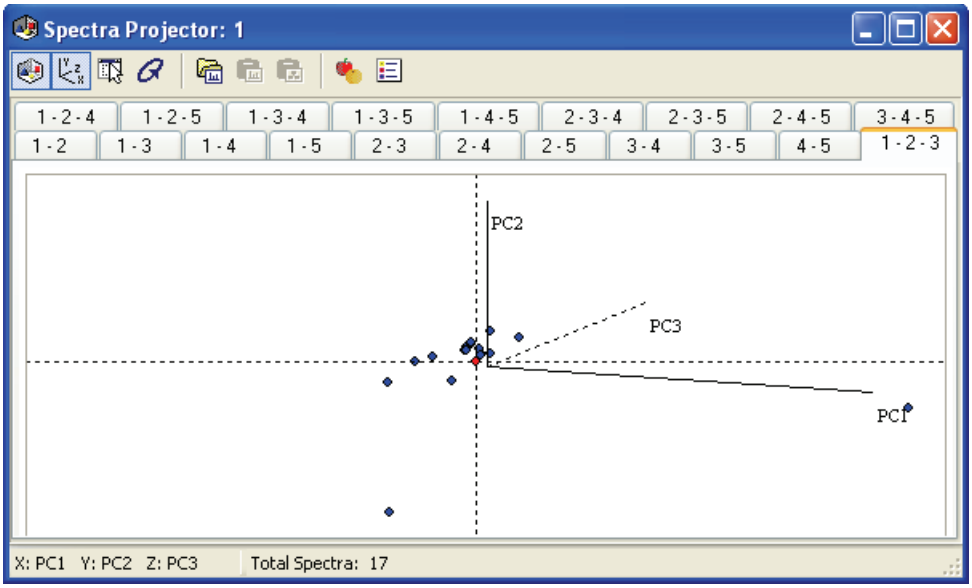
For example, when you click a 2-D projection tab labeled "2-5," the spectra are projected onto the plane of the 2nd and 5th principal components. The number of tabs is determined by the number of principal components that have been selected in the Spectra Classifier module. The Spectra Projector window displays a tab for every possible combination of the selected principal components. For example, when you generate the Spectra Projector module with three principal components, three tabs are available (1-2, 2-3, and 1-3). When you use the five default principal components, 10 tabs are available (1-2, 1-3, 1-4, 1-5, 2-3, 2-4, 2-5, 3-4, 3-5, and 4-5).

Figure 132. 2-D projection plane



In the 3-D mode, the number of combinations of principal components for more than five components is significantly higher in comparison to the 2-D projection plane. As a result, when you want to analyze your spectra in the 3-D mode, use no more than five principal components. Set the number of principal components in the Spectra Classifier window before you begin the PCA calculation. For additional information, see Chapter 10, "Spectra Classifier Module."

Figure 133. 3-D space



The status bar in a Spectra Projector window displays the number of selected principal components and the number of spectra that have been classified (the total number of spectra from all groups).

Follow these procedures:

- To scale the projection plane
- To show a legend of spectra
- To zoom and pan in the graphical display

❖ To scale the projection plane

1. Select the tab for the projection you want to view.

   You can view each projection at a different scale.

2. Right-click the Spectra Projector window and choose **Zoom** > ***zoom option*** from the shortcut menu.

   - Use **Zoom to Box** to define a rectangle that enlarges to fill the window.
   - Use **Zoom In** to display less of the projection plane at a larger scale.
   - Use **Zoom Out** to display more of the projection plane at a smaller scale.

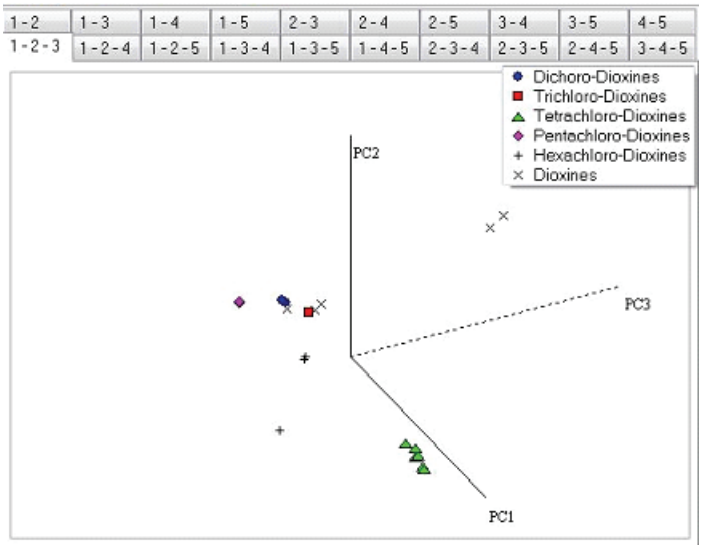
3. To return to the original scale, right-click the Spectra Projector window and choose **Zoom** > **Zoom Reset** from the shortcut menu.

❖ To show a legend of spectra

Right-click the Spectra Projector window and choose **Zoom > Show Legend** from the shortcut menu.

The application displays a list of spectra with their symbols.

Figure 134. 3-D plot with a legend



❖ To zoom and pan in the graphical display

- Click the **Hand** button, , in the Mass Frontier toolbar and drag the hand to pan horizontally or vertically.
  Similarly, hold down the H keyboard key or the Spacebar to change the cursor to the hand.
- Click the **Zoom Out** button, , in the Mass Frontier toolbar.
- Click the **Zoom In** button, , in the Mass Frontier toolbar.
- Click the **Zoom to Box** button, , in the Mass Frontier toolbar and describe a rectangle.
  Similarly, hold down the Z keyboard key and describe a rectangle.
- Click the **Zoom Reset** button, , in the Mass Frontier toolbar to return to full scale.
- Click the **Zoom Undo** button, , in the Mass Frontier toolbar to undo the last zoom operation.
- Click the **Zoom Redo** button, , in the Mass Frontier toolbar to restore the last zoom operation.
- Use the scroll wheel on the mouse to zoom the entire display.

Using 3-D Projection Mode

The principle of PCA classification and Fuzzy Clustering is to reduce the dimensionality of a spectral space to a level comprehensible to the human eye. Because a paper or screen plot is inherently 2-D, the method of choice is usually 2-D PCA or Fuzzy Clustering. However, if a 3-D projection on a 2-D computer screen is accompanied with motion, you can better perceive this simulation as a 3-D space. In the Spectra Projector window, you can rotate the plot to help you perceive this simulation in 3-D mode.

Spectra classification of complex data set in a 3-D space can be more effective and reliable than a simple 2-D plot. When analyzing PCA or Fuzzy Clustering results, do not rely entirely on 2-D plots. Two or more clusters that overlap in a 2-D plot might be separated in 3-D space, or two seemingly separate clusters discernible in a 2-D plot might appear in 3-D mode to be too close together to be considered as two different objects.

❖ To view and rotate PCA or Fuzzy Clustering results in 3-D mode

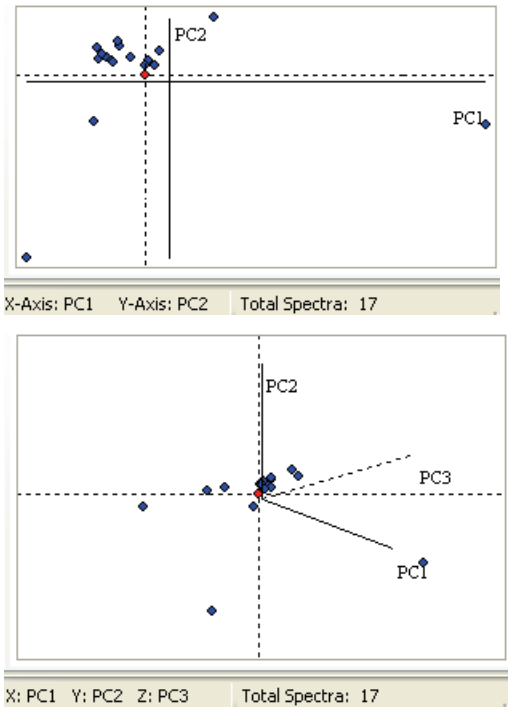
1. Click the **3D Projection** button, .

   The application displays the classification plot in 3-D space.

2. Click the **Show Axis** button, .

   The application overlays the *x*, *y*, and z axes on the display.

   Figure 135. 3-D projection with axes



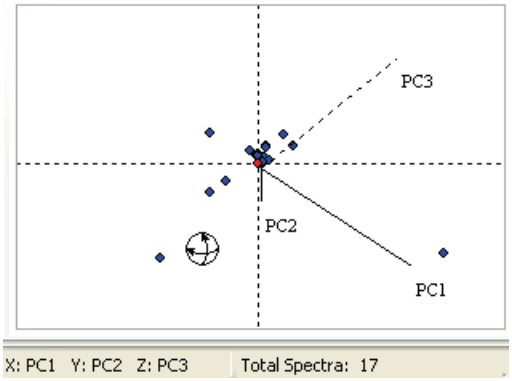
2-D projection

3-D projection with axes

3. Click the **Rotate** button, .

   The cursor changes to a rotation sphere, .

4. Click the classification plot and drag the cursor to rotate the plot.



Opening and Saving Classification Results

In the Spectra Projector window, you can save or open projection planes that have been generated with particular initial settings (number of principal components).

Follow these procedures:

- To open spectra projection planes
- To save spectra projection planes

❖ To open spectra projection planes

1. Do one of the following:

   Click the **Open** button,  , in the Mass Frontier main toolbar and choose **PCA Projections**.

   –or–

   Choose **File > Open > PCA Projections** from the Mass Frontier main menu.

   The Open Spectra Projections dialog box opens.

2. Select a Projection File (.pca2) file and click **Open**.

   The selected file opens in a new Spectra Projector window.

❖ To save spectra projection planes

1. Do one of the following:

   Click the **Save** button,  , in the Mass Frontier main toolbar.

   –or–

   Choose **File > Save** from the Mass Frontier main menu.

   The Save Spectra Projections dialog box opens.

2. Type a name for the saved file and click **Save**.

   The application saves projection planes as graphics, without the possibility of recalling the original spectra, which are displayed as points. To access the spectra with structures from projection planes, save the records in the Database Manager, as described in the Spectra Classifier chapter. In this case, you must regenerate the classification from the original data set that was saved as records; you can, however, add an external spectrum.

   For additional information about saving records, see "Using Records in the Database Manager Window" on page 37 or "Organizing Spectra" on page 345.

Accessing Spectra from a Spectra Projector Window

An important part of classification analysis is knowing which spectrum is represented by a point on a projection plane. As the application links corresponding modules, it recalls spectra with structures or chromatographic components from any projection plane.

Note The Spectra Projector module is able to recall only records that are present in an open Database Manager window. When you close the Database Manager window that is the source of the input data for classification, the link between a point and its spectrum is interrupted. The application automatically warns you if you try to close a Database Manager window that is linked with one or more Spectra Projector windows. To keep the links between points and spectra intact, do not move records in or between Database Manager windows using cut-and-paste commands.

When the data originates from Chromatogram Processor, keep the window open (see "Processing Extracted Spectra" on page 318).

❖ To access a single spectrum, scan, or deconvoluted component

Double-click a point on the projection.

The linked module opens on top of all other windows, and the application selects the corresponding record or component and displays the spectrum.

For additional information about saving spectra to records, see "Using Records in the Database Manager Window" on page 37 or "Working with Detected Components" on page 320.

❖ To access several spectra in a region

Do one of the following:

a. Right-click and choose **Select** from the shortcut menu.

b. Draw a region around the spectra.

–or–

a. Click the **Select Spectra and Show Their Origin** button,  .

b. Draw a region around the spectra.

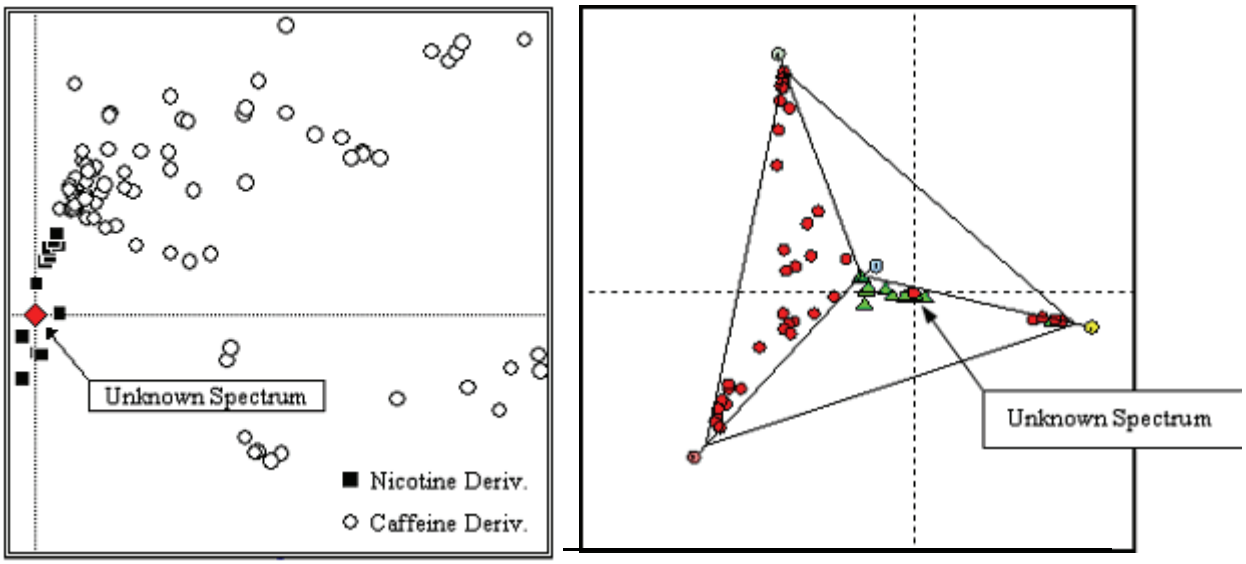
- When the selected spectra originate from a Database Manager window, you are prompted to copy the records with spectra and structures to either the last active Database Manager window or to a new one (see "Example Workflow" on page 357).
- When your spectra originate from a chromatogram in a Chromatogram Processor window, the corresponding scans or components appear in the same color as they appear in the PCA or Fuzzy Clustering plot.

Adding External Spectrum to a Projection

The objective of principal component projections of mass spectra is to find classes of compounds with common or similar properties. You can then use sufficiently separated classes to investigate the class membership of an unknown spectrum (see "Example Workflow" on page 357). Use the Spectra Projector window to add an external spectrum, which was not used for classification, to the projection plane. When the added spectrum is clearly projected into a particular class region, you can assume that this compound has similar, usually structural, properties.

The following figure shows that two groups are separated into clusters. You can add the unknown spectrum to the projection plane by pasting or opening its spectrum in a Spectra Projector window. The projection of this external spectrum shows that it belongs to the nicotine class (squares, PCA; or triangles, Fuzzy Clustering). You can confirm or reject this result using the fragmentation pattern of nicotine.

Figure 136. Nicotine and caffeine separated into clusters



Neural Networks Module

The Neural Networks module displays classification results from Self-Organizing Maps (SOM), which are a special class of neural networks.

Contents

A self-organizing map is a network of neurons arranged in the form of a two-dimensional (2-D) lattice. You can define the size of a lattice or let the application automatically calculate it. During a classification, neurons become selectively activated to various input spectra as a result of a competitive learning process.

The objective of classification analysis using SOM is to find classes of spectra on the map that exhibit common or similar properties. When one or more spectra activate the same neuron, you can assume the spectra belong to a common class. In this case, the spectra should exhibit certain similarities. You can consider spectra that activate neighboring neurons and those neurons that have low Euclidian distance between each other (shown by borderline thickness), to be related.

In neural networks, each mass spectrum must always activate a neuron and this spectrum is shown on the particular neuron. Neurons are displayed as rectangles. Spectra are represented as symbols or numbers, and are placed onto neurons. Because the spectra are located in discrete objects, the interpretation of SOM is relatively easy in contrast to PCA and Fuzzy Clustering where you do not have diffuse clusters. However, when a single spectra activates a larger numbers of neurons, this advantage is lost.

Spectra that activate the same neuron belong to the same classification pattern. All spectra drawn inside a neuron box are equal for classification purposes and their graphical positions within a neuron are irrelevant.

Note The SOM classification method exhibits one atypical feature to be aware of: Different results are produced for an identical data set if the input data is processed in a different order. This impact of order on results is an inherent feature of neural networks and not a result of faulty algorithms.

For additional information, see "Self-Organizing Maps" on page 343 or "Fuzzy Clustering" on page 344.

Generating a Neural Networks Module

Use the Spectra Classifier module to generate a Neural Networks module. You cannot directly open a Neural Networks window from the Mass Frontier main menu (similar to the Spectra Projector). After you generate the network, you cannot alter the classification results. To remove a spectrum (symbol) from a network, you must remove this spectrum from the input data and then launch a new classification process. You can open an unlimited number of Neural Networks windows at any time in the application.

❖ To generate a Neural Networks module

1. Select spectra from the Database Manager or Chromatogram Processor window and populate those spectra to a Spectra Classifier window.

   For detailed instructions, see "Opening the Spectra Classifier Window" on page 346.

2. In the Spectra Classifier window, select the data you want to classify.

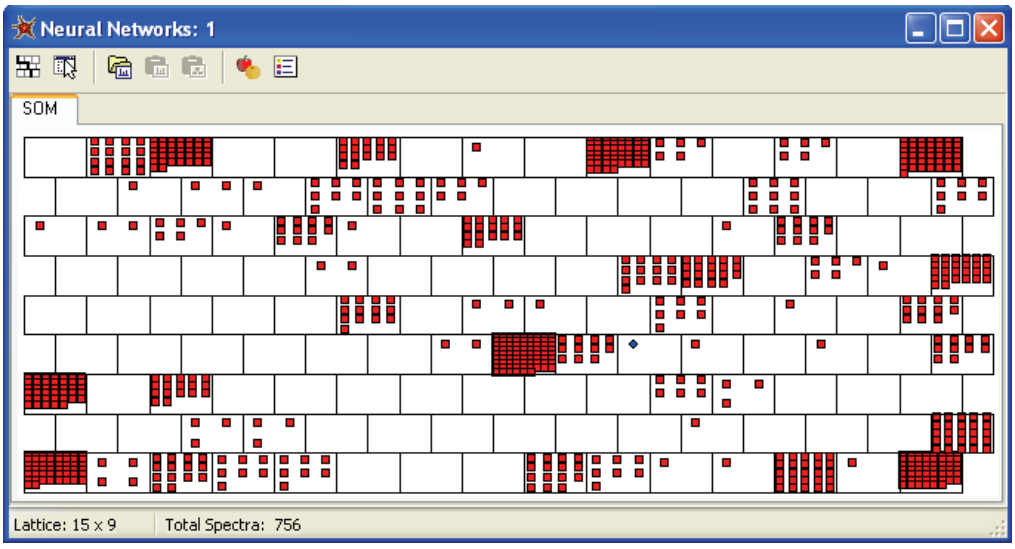
   For detailed instructions, see "Managing Groups of Spectra" on page 348.

3. To begin the Neural Networks classification process, select the **Neural Networks** option and click **Classify Now**.

   For detailed instructions, see "Classifying Spectra" on page 351.

   The application displays the results of a Neural Networks classification in a Neural Networks window.

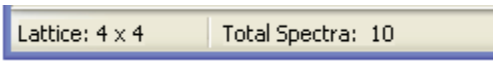
Figure 137. Neural Networks window resulting from spectra classification



Using the Neural Networks Window

A Neural Networks window displays a topological map of neurons, represented as rectangles. Every neuron has a minimum of two and a maximum of six neighboring neurons. Spectra are displayed as symbols or numbers within neurons.

The status bar in a Neural Network window displays the lattice dimension and how many spectra have been classified (the total number of spectra from all groups).



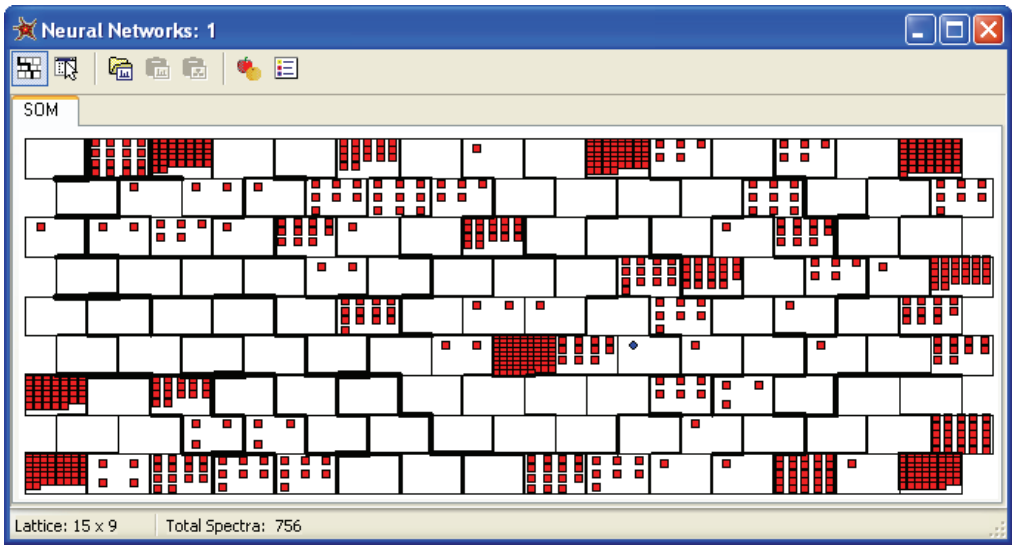
Follow these procedures:

- To show distances between neurons
- To scale the lattice
- To use the zoom and pan features
- To open a neural networks file
- To save neural networks to a file

❖ To show distances between neurons

Click the **Show Distances Between Neurons** button,  .

To show the approximate distances between neighboring neurons, the application applies different thicknesses to their borders.



You can use information about neuron distances when classifying an unknown spectrum. The distances show how a neuron that has been activated by an unknown correlates with neighboring neurons. The distances can also suggest that spectra from a common neuron might have been activated by multiple, close neurons.

Although neurons are displayed in a regular 2-D lattice, the actual Euclidian distances between neurons vary. The line thickness is proportional to the distance between immediate neighbors. The thinner the line, the closer together are the neurons.

Note Spectra that activate the same neuron belong to the same classification pattern. This means that all spectra drawn inside a neuron box are equal for classification purposes and their graphical positions within a neuron are irrelevant.

❖ To scale the lattice

1. Right-click the Neural Networks window and choose **Zoom > *zoom option*** from the shortcut menu.
   - Click **Zoom to Box** to describe a rectangle that enlarges to fill the window.
   - Click **Zoom In** to display less of the neural networks lattice at a larger scale.
   - Click **Zoom Out** to display more of the neural networks lattice at a smaller scale.
2. To return to the original scale, right-click the Neural Networks window and choose **Zoom > Zoom Reset** from the shortcut menu.

❖ To use the zoom and pan features

- Click the **Hand** button,  , in the Mass Frontier toolbar and drag the hand to pan horizontally or vertically.
  Similarly, hold down the H keyboard key or the Spacebar to change the cursor to the hand.
- Click the **Zoom Out** button,  , in the Mass Frontier toolbar.
- Click the **Zoom In** button,  , in the Mass Frontier toolbar.
- Click the **Zoom to Box** button,  , in the Mass Frontier toolbar and describe a rectangle.
  Similarly, hold down the Z keyboard key and describe a rectangle.
- Click the **Zoom Reset** button,  , in the Mass Frontier toolbar to return to full scale.
- Click the **Zoom Undo** button,  , in the Mass Frontier toolbar to undo the last zoom operation.
- Click the **Zoom Redo** button,  , in the Mass Frontier toolbar to restore the last zoom operation.
- Use the scroll wheel on the mouse to zoom the entire display.

❖ To open a neural networks file

1. Do one of the following:

   Click the **Open** button,  , in the Mass Frontier main toolbar and choose **Neural Networks**.

   –or–

   Choose **File > Open > Neural Networks** from the Mass Frontier main menu.

   The Open Neural Networks dialog box opens.

2. Select a Neural Networks (.nn2) file and click **Open**.

   The lattice for the selected file opens in a new Neural Networks window.

❖ To save neural networks to a file

1. Do one of the following:

   Click the **Save** button,  , in the Mass Frontier main toolbar.

   –or–

   Choose **File > Save** from the Mass Frontier main menu.

   The Save Neural Networks dialog box opens.

2. Type a name for the saved file and click **Save**.

The Mass Frontier application saves neural networks as graphics, without the possibility of recalling the original spectra, displayed as symbols. To access the spectra from a lattice, save the spectra to records in the Database Manager window as described in the Spectra Classifier chapter. In this case, regenerate the classification from the original data set that was saved.

For additional information about saving spectra to records, see "Using Records in the Database Manager Window" on page 37 or "Organizing Spectra" on page 345.

Accessing Spectra from a Neural Networks Window

An important part of classification analysis is knowing which spectrum is represented by a symbol on the lattice. As the Mass Frontier application links corresponding modules, it recalls spectra with structures or chromatographic components from any neural network.

Note The Neural Networks module is able to recall only records that are present in an open Database Manager window. When you close the Database Manager window that is the source of the input data for classification, the link between a symbol and its spectrum is interrupted. The application automatically warns you if you try to close a Database Manager window that is linked with one or more Neural Networks windows. To keep the links between symbols and spectra intact, do not move records in or between Database Manager windows.

When the data originates from a Chromatogram Processor window, keep the window open (see "Processing Extracted Spectra" on page 318).

❖ To access a single spectrum, scan, or deconvoluted component

Double-click a symbol on the lattice.

The linked module opens on top of all other windows, and the application selects the corresponding record or component and displays the spectrum.

❖ To access several spectra in a region

Do one of the following:

a. Right-click and chose **Select** from the shortcut menu.

b. Draw a region around the spectra.

–or–

a. Click the **Select Spectra and Show Their Origin** button,  .

b. Draw a region around the spectra.

- When the selected spectra originate from a Database Manager window, the application copies the original data to the Database Manager window. For additional information, see "Example Workflow" on page 357.
- When your spectra originate from a chromatogram in a Chromatogram Processor window, the corresponding scans or components are selected in the original Chromatogram Processor window and appear in the same color as they appear in the Neural Networks window.

Formula Generator Module

Use the Formula Generator module to calculate a list of theoretical molecular formulas that best 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, elements used, and the maximum allowed number of atoms for each isotope, always carefully evaluate these parameters before starting a generation.

Contents

You can either manually enter the *m/z* value or have the software automatically retrieve the value from any spectral peak in the Database Manager window or the Chromatogram Processor window. In the Formula Generator module, you can also calculate the isotopic pattern for any generated formula.

Generating Formulas from Peaks

Use the Formula Generator module to calculate the possible elemental composition for any spectral peak by selecting the peak in a Database Manager or Chromatogram Processor window.

Follow these procedures:

- To open a Formula Generator window
- To specify molecular formula options
- To automatically calculate the m/z tolerance value
- To generate possible formulas for a peak

❖ To open a Formula Generator window

1. Do one of the following:

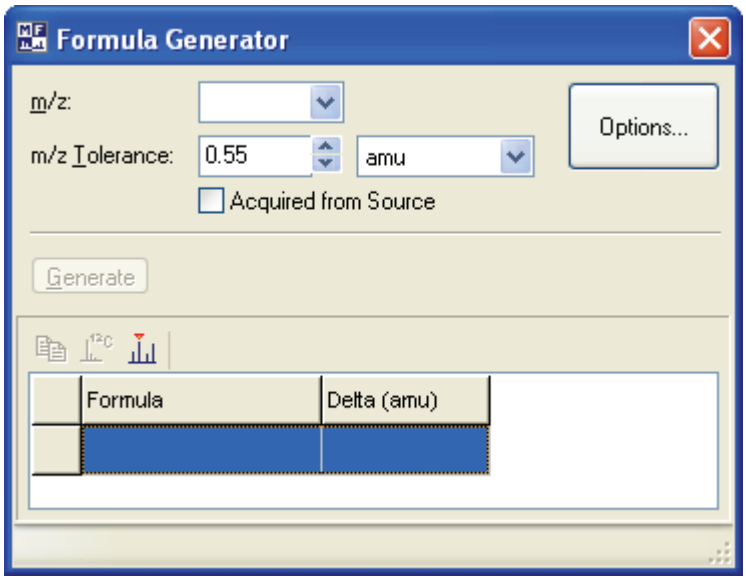
   Click the **Formula Generator** button,  , in the Mass Frontier main toolbar.

   –or–

   Choose **Tools > Formula Generator** from the Mass Frontier main menu.

   The Formula Generator window opens.

   Figure 138. Formula Generator window



❖ To specify molecular formula options

1. In the Formula Generator window, click **Options**.

   The Molecular Formula Settings dialog box opens to the Peaks page.

2. Review the default settings on the Peaks page.

   See "Peaks page" on page 377.

3. Click the **Limits** tab to see the Limits page. Verify that the Charge setting matches the polarity of the MS mode you used for spectral acquisition.

   See "Limits page" on page 378.

4. Click the **Elements** tab to see the Elements in Use page. Verify the maximum number of elements used for calculation.

   See "Elements in Use page" on page 379.

5. When you have verified the options, click **OK**.

   Use the options in the Molecular Formula Settings dialog box to speed up calculations, restrict the number of possible formulas, or set the charge state and polarity.

Figure 139. Peaks page

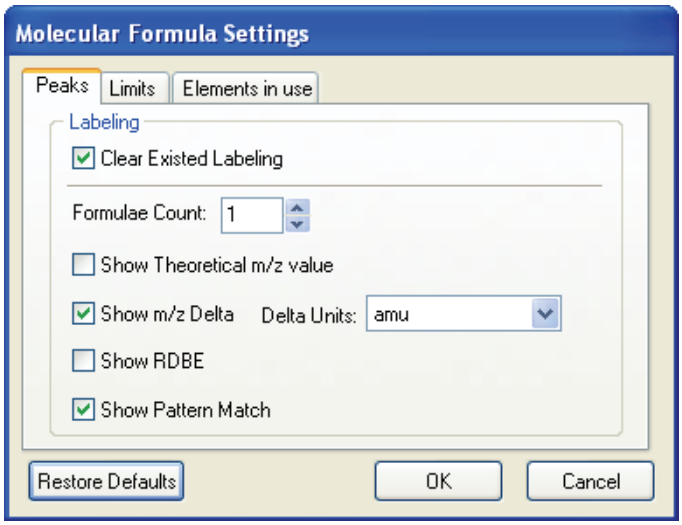


Table 47. Peaks parameters (Sheet 1 of 2)

Parameter	Description
Clear Existing Labeling	Removes all columns from the formula list except the Formula column. This option is meaningful only in the Auto Annotation > Generate Formulas feature in the Database Manager window.
Formula Count	Limits the number of formulas displayed in the formula list.

Table 47. Peaks parameters (Sheet 2 of 2)

Parameter	Description
Show Theoretical m/z value	Displays the Theoretical *m/z* column in the formula list.
Show m/z Delta Delta Units	Displays the Delta (*units*) column in the formula list.
Show RDBE	Displays the Ring Double Bond Equivalents (RDBE) column in the formula list.
Show Pattern Match	Displays the Pattern Match (%) column in the formula list.

Figure 140. Limits page

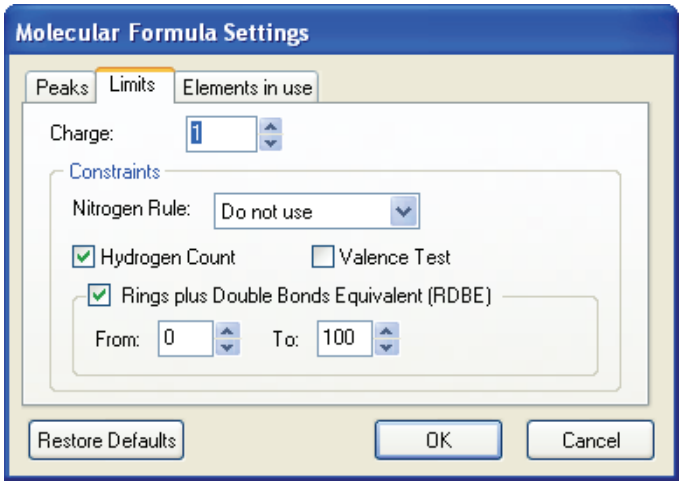


Table 48. Limits parameters

Parameter	Description
Charge	Charge state.
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)
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 be connected in any way using valences common in organic chemistry.
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.

Figure 141. Elements in Use page

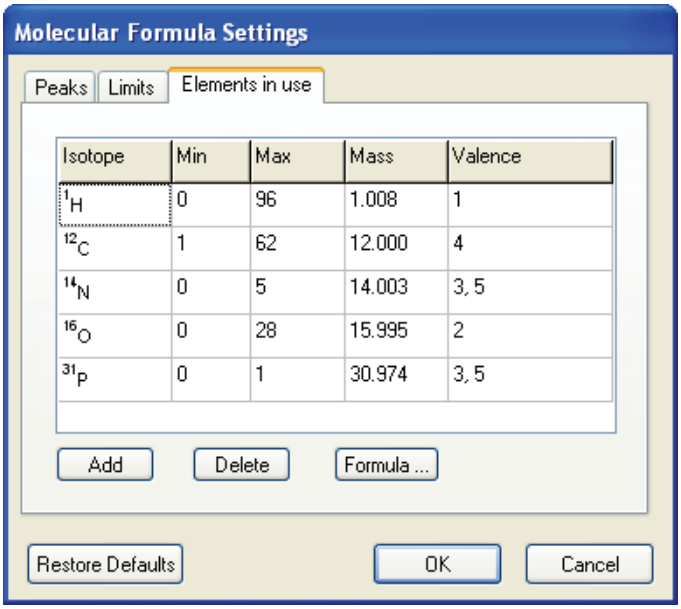


Table 49. Elements in Use parameters (Sheet 1 of 2)

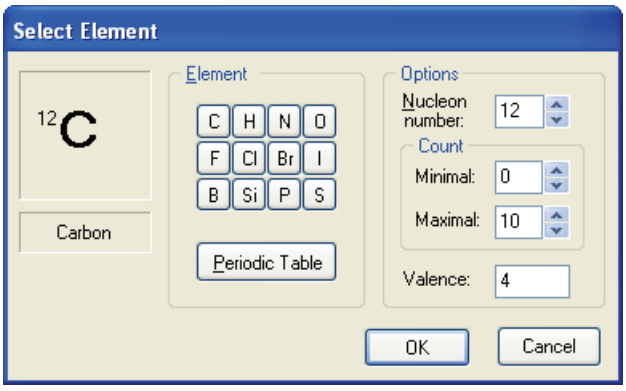
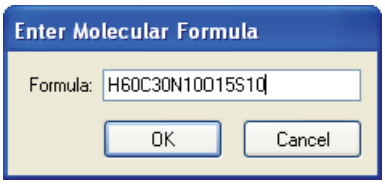
Parameter	Description
Elements in Use	Specifies whether Mass Frontier will consider isotopes of particular elements with a maximum number when calculating formulas. For information about isotope patterns, see "Displaying Isotope Patterns" on page 58.
Add	Opens the Select Element dialog box where you can specify a new element. The application uses the new element when calculating formulas. 

Table 49. Elements in Use parameters (Sheet 2 of 2)

Parameter	Description
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 software uses the new formula to derive upper limits for individual elements (see "Elements in Use page" on page 379). These limits are considered when the software calculates formulas. 

❖ To automatically calculate the *m/z* tolerance value

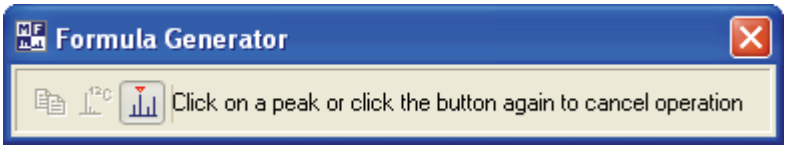
Select the **Acquired from Source** check box.

When you generate possible formulas for a peak, the application automatically calculates the *m/z* tolerance value from the source spectra.

❖ To generate possible formulas for a peak

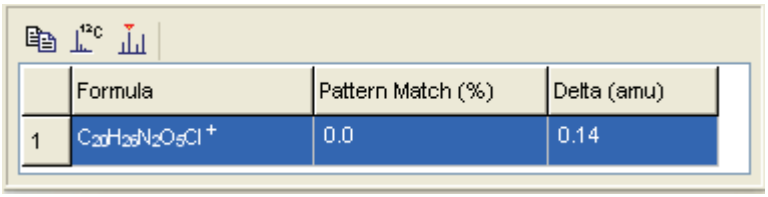
1. In the Formula Generator window, click the **Pick a Peak** button,  .

   The Formula Generator window minimizes to a bar.

2. Click a spectral peak in the Database Manager or Chromatogram Processor window.

   The Formula Generator window lists all possible formulas. All the decimal places are accurately transferred to the Formula Generator. To choose which columns of information are displayed in this list, see "Peaks page" on page 377.

Specifying Mass/Abundance Options

This section includes the following topics:

Monoisotopic Mass

All the calculated masses displayed in the Formula Generator window are monoisotopic masses. The monoisotopic mass of an ion is the mass of the isotopic peak, which is composed of the most abundant isotopes of its elements. Because the system calculations support only single-charged ions (z=1), the calculated exact masses (not measured) in structure-based modules are equal to their *m/z* value.

Accuracy

The Mass Frontier application uses two parameters associated with *m/z* value: Accuracy and Resolution. Resolution is the smallest difference in m/z of two centroid peaks that a detector can distinguish Accuracy is the estimated difference between the observed and the real *m/z* value of a peak.

The Mass Frontier application determines these values from the raw data file. If you select the Use User Defined option in the mass accuracy page, the software sets both values to the same user defined value.

Use accuracy settings for the differentiation of adjacent peaks (spectra) and *m/z* values (calculations). Because the application processes only centroid spectra, the differentiation is defined as the spacing between resolved peaks (spectra) or as the smallest distinguishable difference in *m/z* values (calculations). No additional parameters, such as peak width, are taken into account.

Note Changing the accuracy in the Tolerance Settings dialog box does not affect spectra that you open in the Chromatogram Processor or fragments that the application has already calculated in the Fragments & Mechanisms and Fragments Comparator modules. When you require spectra or fragments with a new setting, you must reload the spectra or regenerate the fragments.

❖ To specify accuracy settings

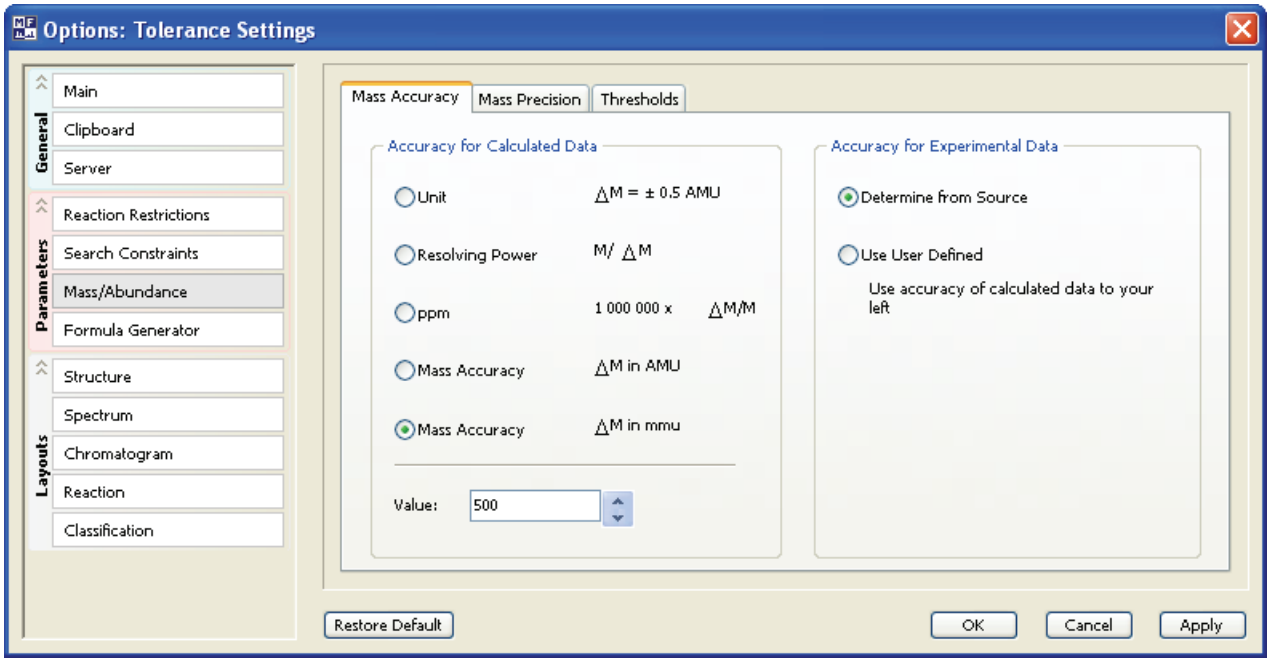
1. Choose **Option > Settings** from the Mass Frontier main menu.

   The Options dialog box opens.

2. Click **Mass/Abundance** in the Parameters section.

The Mass Accuracy page in the Tolerance Settings dialog box displays options for specifying accuracy settings.

Figure 142. Mass Accuracy page



3. Define the appropriate type, value, and unit in the Accuracy for Calculated Data area.

   The application supports these tolerance types:

   - Unit
   - Resolving power M/ΔM
   - ppm 1 000 000 x ΔM/M
   - Mass Accuracy (ΔM in AMU or ΔM in mmu)

   where:

   M = *m/z* (mass-to-charge ratio)

   $\Delta M = M_2 - M_1$ (M_1, M_2 are two adjacent peaks)

   Peaks (*m/z* values) that fall into the ΔM band are considered to be identical (*m/z* value).

4. To set a tolerance for imported spectra or GC/LC/MS chromatograms from a processed file, do one of the following in the Accuracy for Experimental Data area:

   Select the **Determine from Source** option to adopt the tolerance settings saved in a file.

   –or–

   Select the **Use User Defined** option to use the same tolerance settings specified for calculations in the Accuracy for Calculated Data area.

You cannot improve the resolution of experimental data by choosing a user-defined type. Avoid setting tolerance values that are better than the resolution of the mass spectrometer that you use for data acquisition. The Use User Defined option can artificially reduce the resolution of experimental data when working with low- and high-resolution spectra in the same data set: spectra search, target analysis, or classification.

For additional information, see "Using the Search Utilities" on page 72 for spectra search, "Searching Chromatographic Libraries" on page 182 for target analysis, Chapter 10, "Spectra Classifier Module." for classification, or "Supported GC/MS and LC/MS Data File Formats" on page 246.

Precision

Precision refers to *m/z* values and is defined as the position of the last digit relative to the decimal point that is displayed. Precision settings are used only for the display of *m/z* values and peak positions on the *m/z* scale. In contrast to tolerance, the Mass Frontier application ignores precision settings in all calculations, always using the highest possible precision. To assure the correct display of *m/z* values, you must set the precision to the same or a higher order than the tolerance.

Note When working with experimental spectra, some of the digits of the displayed *m/z* values might not be significant.

❖ To specify precision settings

1. Choose **Option > Settings** from the Mass Frontier main menu.

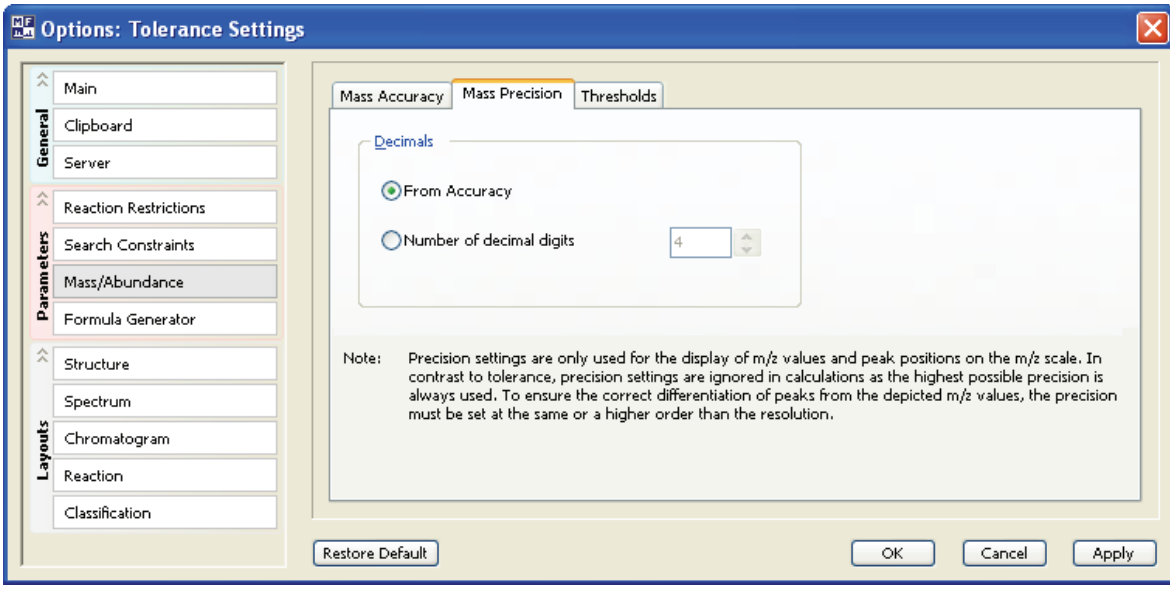
   The Options dialog box opens.

2. Click **Mass/Abundance** in the Parameters section.

3. Click the **Mass Precision** tab.

   The Mass Precision page in the Tolerance Settings dialog box displays options for specifying precision settings.

   Figure 143. Mass Precision page



4. Do one of the following:

   Select the **From Accuracy** option to use settings specified on the Mass Accuracy page.

   –or–

   Select the **Number of Decimal Digits** option and, in the adjacent box, specify the number of decimal places to use.

Microsoft Office

The Mass Frontier application supports the Object Linking and Embedding (OLE) features of the Microsoft Excel spreadsheet and the Microsoft Word applications —that is, you can open these applications and their native data files inside the Mass Frontier main window. This feature is useful for data exchange between the Mass Frontier modules and Excel worksheets.

Note The Mass Frontier application does not support the direct import or export of native Excel files (.xls) to the Database Manager module.

Note The Mass Frontier installation package does not include the Microsoft Office application. To use the Object Linking and Embedding features of Excel and Word, you must purchase the Microsoft Office application separately. Install Microsoft Office before you install the Mass Frontier software (see "Installing the Mass Frontier Application" on page 21).

Contents

Exchanging Data

You can use copy-and-paste commands to exchange text tables, graphics, mass spectra, and trees in table format between the Microsoft Excel and the Mass Frontier applications.

❖ To use a Microsoft Word document

1. Choose **Microsoft Office > Open Microsoft Word Document** from the Mass Frontier main menu.

   The Open Microsoft Word Document dialog box opens.

2. Select a document and click **Open**.

   A Word document opens in the Mass Frontier window. The Word menu and toolbars are displayed below the Mass Frontier toolbars. The Word menu and toolbars do not include the Open or Save commands.

3. To save a Word document, choose **Microsoft Office > Save Microsoft Word Document** from the Mass Frontier main menu.

4. Use copy-and-paste commands to exchange data between the Mass Frontier modules and the Word document.

❖ To use an Excel document

1. Choose **Microsoft Office > Open Microsoft Excel Document** from the Mass Frontier main menu.

   The Open Microsoft Excel Document dialog box opens.

2. Select a document and click **Open**.

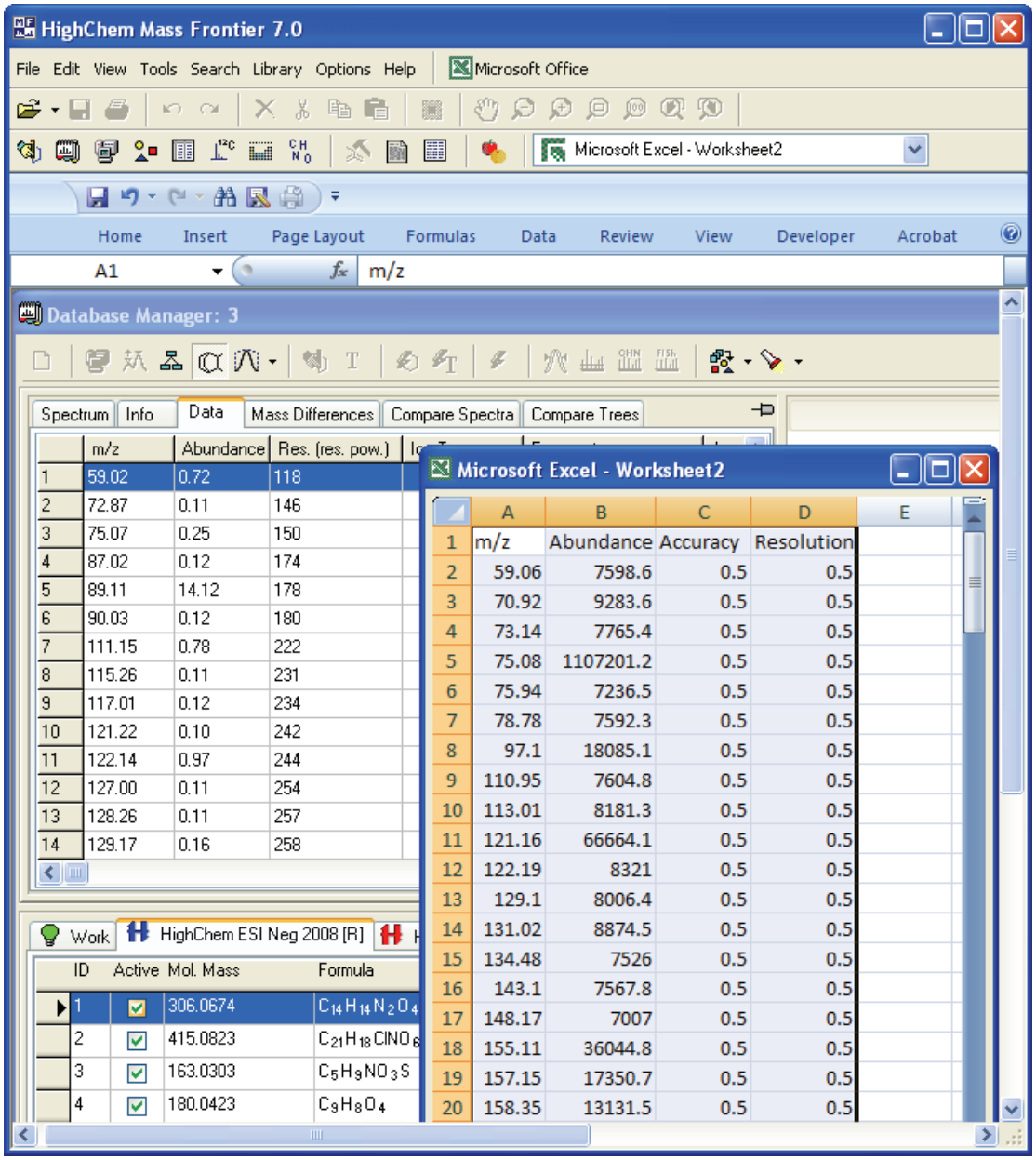
   A Microsoft Excel spreadsheet opens in the Mass Frontier window. See "Embedded Excel spreadsheet" on page 387.

   The Excel menu and toolbars are displayed below the Mass Frontier toolbars. The Excel menu and toolbars do not include the Open or Save commands.

3. To save an Excel document, choose **Microsoft Office > Save Microsoft Excel Document** from the Mass Frontier main menu.

4. Use copy-and-paste commands to exchange data between the Mass Frontier modules and the Excel spreadsheet.

Figure 144. Embedded Excel spreadsheet



Exporting Data to an Excel Spreadsheet

You can export text tables, graphics, mass spectra in numerical table format, and spectral trees from the Database Manager to an Excel spreadsheet. Because you can access all these types at the same time, you must specify the type of data you want to copy to the Excel spreadsheet. For instructions for copying spectral tree data from the Mass Frontier application, see "Copying and Pasting a Spectral Tree" on page 116.

Follow these procedures:

- To export data records
- To export mass spectrum or mass differences graphics
- To export a mass spectrum in numerical table format
- To use an Excel spreadsheet as an editor

❖ To export data records

1. Select one or more records on the Spreadsheet page in the Database Manager window.
2. Do one of the following:

   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Selected Rows** from the menu.

   –or–

   Choose **Edit > Copy > Selected Rows** from the Mass Frontier main menu.
3. Click the Excel spreadsheet to make it active.
4. Right-click the Excel spreadsheet, and choose **Paste** from the shortcut menu.

   The application pastes the records into the spreadsheet.

	A	B	C	D	E
1					
2		*	229.0479	C6H8ClN7O	amipramidin
3		*	244.0882	C10H16N2O3S	3'-[1-hydroxy-2-(methylam
4		*	239.1077	C13H18ClNO	bupropion
5					

❖ To export mass spectrum or mass differences graphics

1. Choose **Microsoft Office > New Microsoft Excel Worksheet** from the Mass Frontier main menu.
2. Do one of the following in the Database Manager window:

   Click the **Spectrum** tab.

   –or–

   Click the **Mass Differences** tab.

   The mass spectrum or mass differences spectrum must be visible before you can copy it. For detailed information about the content of the spectrum and mass differences pages, see Chapter 2, "Database Manager Module."
3. Do one of the following:

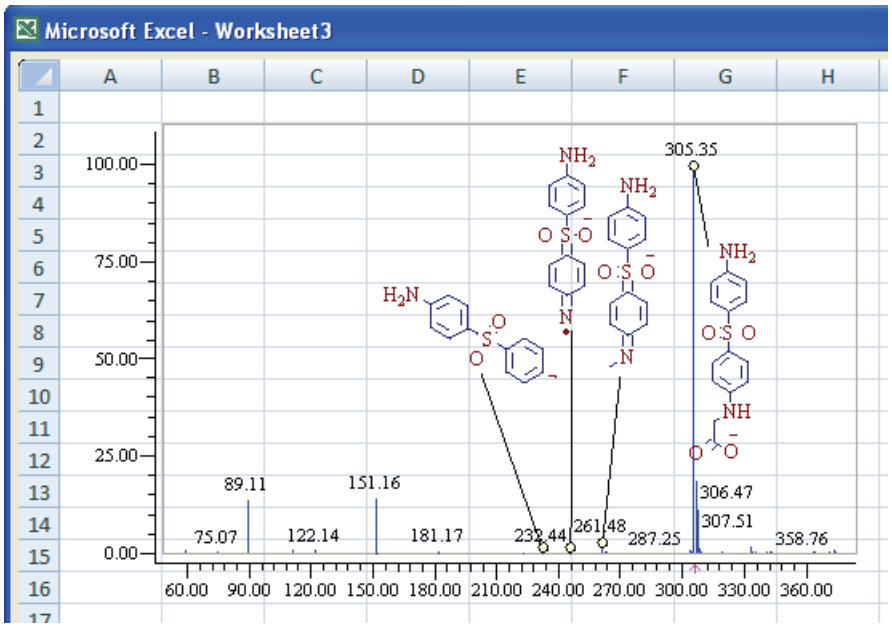
   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Spectrum** from the menu.

   –or–

   Choose **Edit > Copy > Spectrum** from the Mass Frontier main menu.
4. Click the Excel spreadsheet to make it active.
5. Right-click the Excel spreadsheet, and choose **Paste Special** from the shortcut menu.

   The Paste Special dialog box opens.
6. Select **Picture (Enhanced Metafile)** and click **OK**.

   The application pastes the spectra graphics into the spreadsheet.



❖ To export a mass spectrum in numerical table format

1. In a Database Manager window, click the **Data** tab to make the *m/z* and abundance table visible.
2. Do one of the following:

   Click the **Copy** button,  , in the Mass Frontier toolbar and choose **Spectrum** from the menu.

   –or–

   Choose **Edit > Copy > Spectrum** from the Mass Frontier main menu.
3. Click the Excel spreadsheet to make it active.
4. Right-click the Excel spreadsheet, and choose **Paste** from the shortcut menu.

   The application pastes the record data into the spreadsheet.

Microsoft Excel - Worksheet1

	A	B	C	D
1	m/z	Abundance	Accuracy	Resolution
2	33.09	35493672	0.5	0.5
3	39.11	15956602	0.5	0.5
4	47.27	37226953	0.5	0.5
5	60.46	141307891	0.5	0.5
6	61.29	16480493	0.5	0.5
7	65.42	84593373	0.5	0.5
8	94.39	23626432	0.5	0.5
9	136.27	13775699456	0.5	0.5
10	137.3	1340118840	0.5	0.5
11	138.34	88444076	0.5	0.5
12	143.17	144339023	0.5	0.5
13	149.28	13902487	0.5	0.5
14	153.22	52275005	0.5	0.5
15	158.13	1096194728	0.5	0.5
16	159.12	109676827	0.5	0.5

❖ To use an Excel spreadsheet as an editor

Export a spectrum or tree to an Excel spreadsheet, edit it, and then import the edited spectrum or tree into the Database Manager window.

Using the Excel application's editing features is useful when, for example, you have an experimental spectrum with several noise peaks at high *m/z* values that you want to delete or you want to extract part of a spectrum that is important to your report or presentation. For obvious reasons, do not add, delete, or alter prominent peaks.

Importing Spectra from an Excel Spreadsheet

You can import mass spectra or spectral trees stored in an Excel spreadsheet to the Database Manager window (see "Copying and Pasting a Spectral Tree" on page 116). You can organize spectral tables horizontally or vertically.

Note The Mass Frontier application supports standard tables with separated numbers, so you can also import spectra from other applications.

❖ To import spectra from an Excel spreadsheet

1. Make sure your table contains mass spectra.
2. Select the table you want to export into the Mass Frontier application.
3. Right-click the Excel spreadsheet, and choose **Copy** from the shortcut menu.
4. Choose **Edit > Paste > Spectrum** in the Mass Frontier main menu.

❖ To correctly interpret *m/z* values and abundance

Follow one of these conventions:

- When the spectral table is vertical, the first column must be the *m/z* value and the second must be abundance.
- When the spectra are vertically oriented, the first column is abundance, and the second column is the *m/z* value, then label the first row of the first column "Abundance" and the first row of the second column "*m/z*".
- When the spectral table is horizontally oriented, then the first row must be the *m/z* value and the second row must be abundance.
- When the spectra are oriented horizontally, the first row is abundance, and the second row is the *m/z* value, then label the first column of the first row "Abundance" and the first column of the second row "*m/z*".
- When you import more than one spectrum at a time, then the first column or row (depending on the orientation) must be the *m/z* values and all the other columns (or rows) must be abundance.

Report Creator Module

To create customizable reports, use the Report Creator module to extract text and graphics from other modules in the Mass Frontier application. You can also specify the order in which objects from the module appear in the report. You can print or export reports to PDF files and save the report layout.

You can extract text and graphics from the following modules:

- Database Manager
- Structure Editor
- Chromatogram Processor
- Spectra Projector
- Neural Networks
- Fragments and Mechanisms

You can create reports only from open windows. You cannot report data that is simply stored in the Mass Frontier application; it must appear on the screen before you can add it to a report in the Report Creator window.

This section includes the following topics:

❖ To open the Report Creator window

1. Open the modules with the data you want to use in a report.
2. Do one of the following:

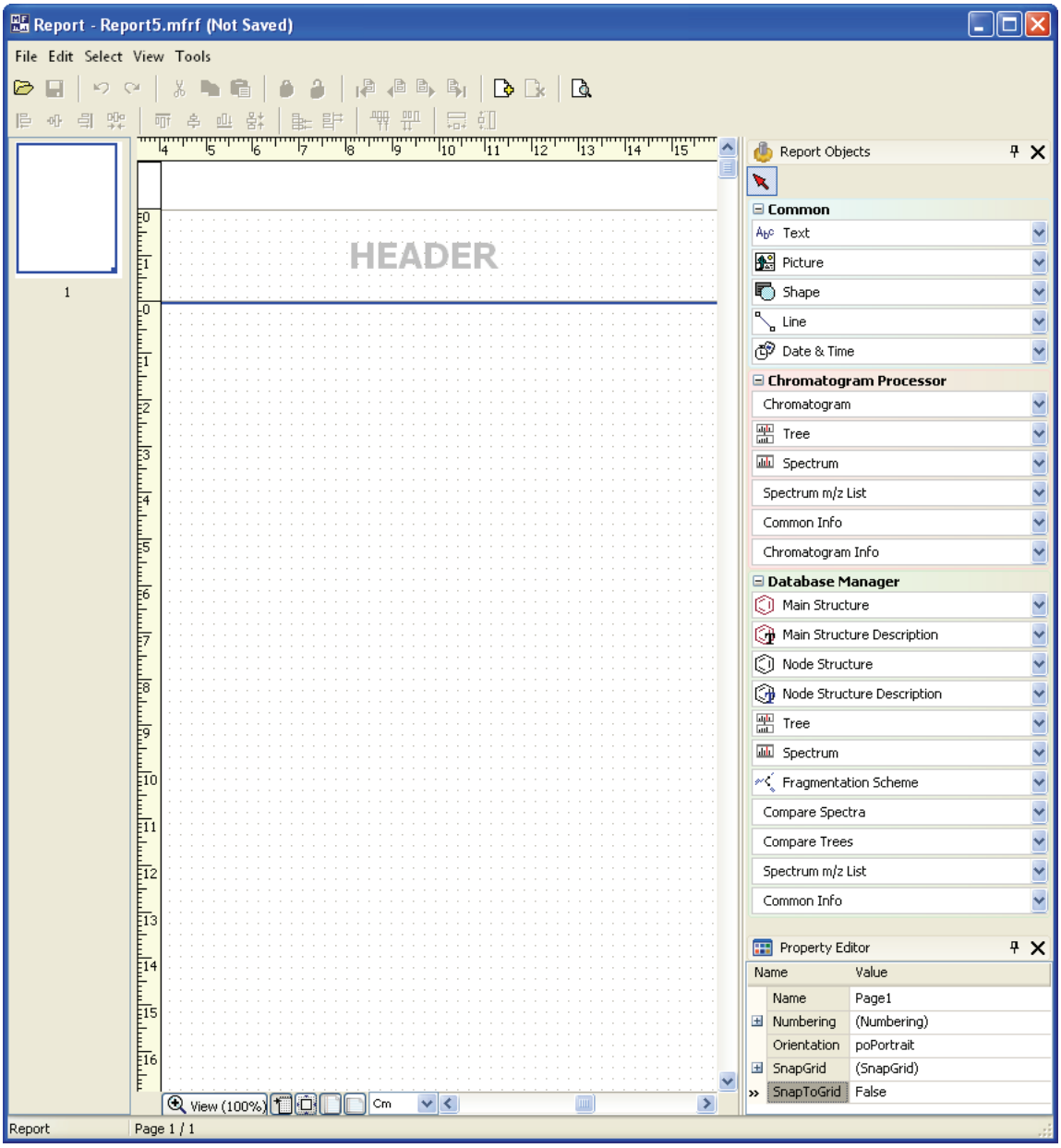
   Click the **Report Creator** button,  , in the Mass Frontier main toolbar.

   –or–

   Choose **Tools > Report Creator** from the Mass Frontier main menu.

   The Report Creator window opens.

Figure 145. Report Creator window

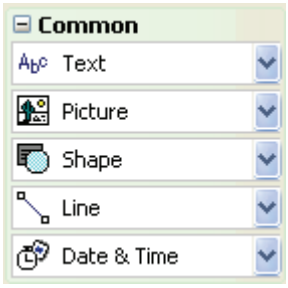


The Report Creator window is divided into three panes:

- The leftmost pane displays a thumbnail of each of the pages of your report.
- The center pane displays the contents of your report.
- The rightmost pane displays the commands you can use to add and modify elements in your report.

Adding Graphic Elements to a Report

Use Report Creator features to add common graphical elements to the report.



Follow these procedures:

- To add a page to a report
- To add text
- To load a graphic
- To draw a line
- To add a data/time stamp

❖ To add a page to a report

1. Click the **Add Page** button,  .

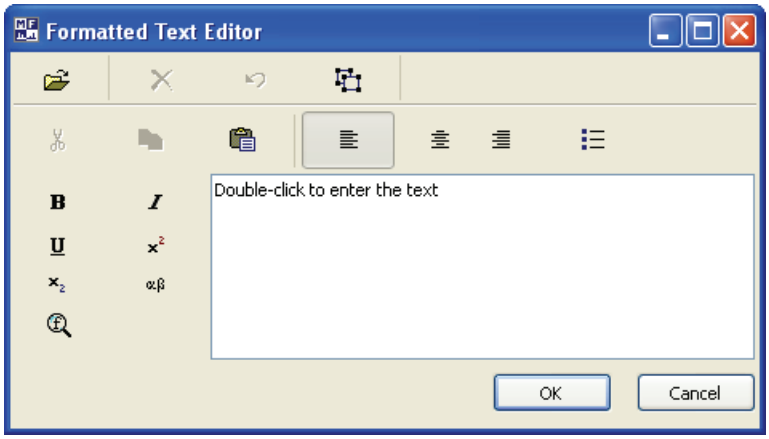
   The application adds a blank page to the end of the report.

2. Use the thumbnail views in the leftmost pane to move between pages.

❖ To add text

1. Click **Text** and click on the report where you want the text.
2. Double-click the rectangle.

   The Formatted Text Editor dialog box opens.



3. Select the default text and type your text.
4. Use the text editing features to format your text.
5. Click **OK**.
6. To verify that your text looks as you expect, choose **File > Preview** from the Report menu.

   The Preview page shows a print view of your report, without a background grid or positioning rectangles.
7. To close the Preview page, click the **Close** button,  , or press ESC.

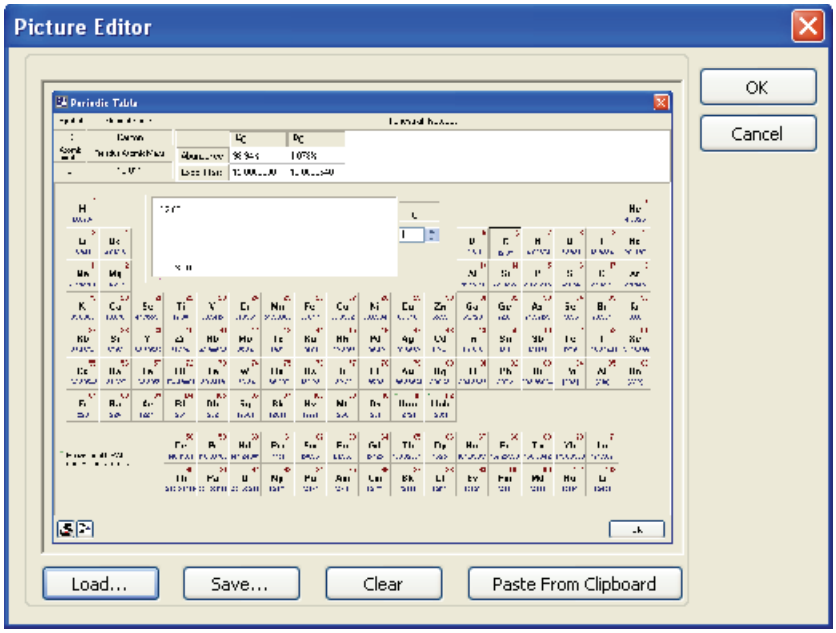
❖ To load a graphic

1. In the Report Creator window, click **Picture** and click on the report where you want the graphic.
2. Double-click the rectangle.

   The Picture Editor dialog box opens.
3. Click **Load**.
4. The Load Picture dialog box opens.
5. Locate a graphic and click **Open**.

   The application supports these graphic formats: .gif, .jpg, .jpeg, .bmp, .ico, .emf, and .wmf.

   The application displays the graphic in the Picture Editor dialog box.



6. Click **OK**.

If the aspect ratio of the graphic is not similar to the rectangle in the Report Creator window, the graphic might be misshaped.

7. Stretch the graphic rectangle to fit the aspect ratio of the graphic.
8. To resize the graphic rectangle, right-click and choose **Position > Scale** or **Position > Size** from the shortcut menu.
9. To verify that your graphic looks as you expect, choose **File > Preview** from the Report menu.

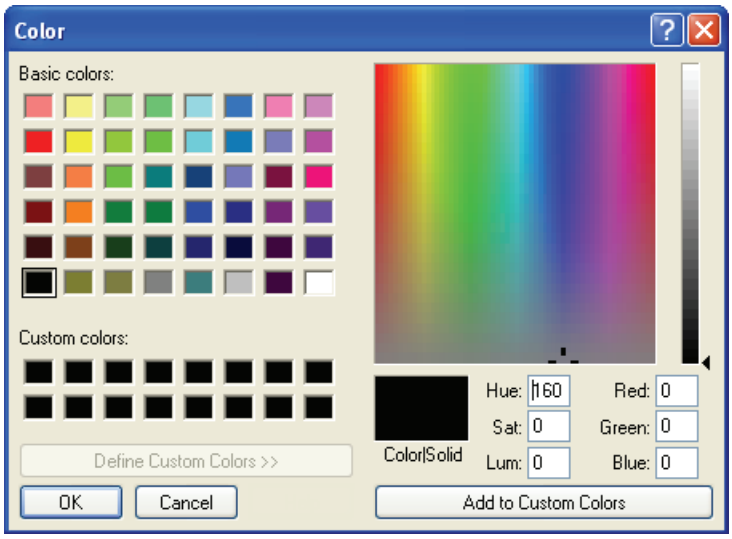
   The Preview page shows a print view of your report, without a background grid or positioning rectangles.
10. To close the Preview page, click the **Close** button, , or press ESC.

❖ To draw a line

1. Click **Line** and click on the report where you want the line.

   The application places the line at the bottom edge of the rectangle. You cannot draw a vertical line.
2. To change the color of the line, double-click the rectangle.

   The Color dialog box opens.



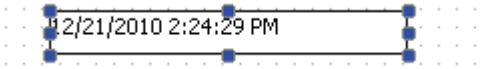
3. Select a color for your line and click **OK**.
4. To resize the graphic rectangle, right-click and choose **Position > Scale** or **Position > Size** from the shortcut menu.

   These commands do not change the thickness of the line.

❖ To add a data/time stamp

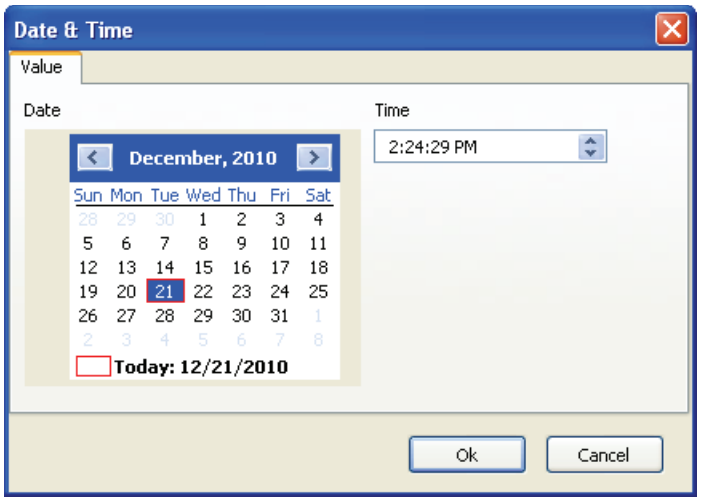
1. Click **Date & Time** and click on the report where you want the time stamp.

   The application inserts the current date and time in a rectangle.



2. To change the date and time, double-click the rectangle.

   The Date & Time dialog box opens.

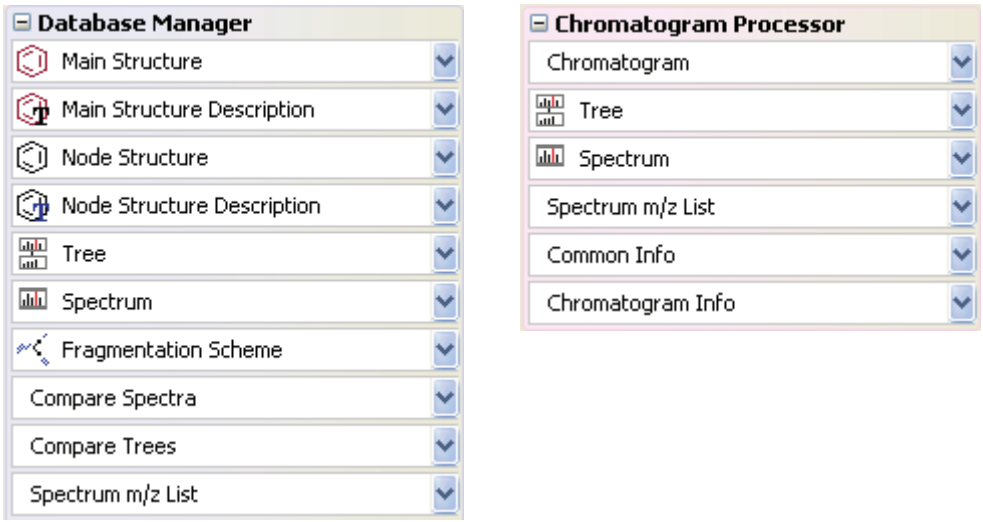


   You do not have to use the current date and time on the report.

3. To change the date, click a new date on the calendar.
4. To change the time, click the up or down arrows in the Time box.
5. Click **OK**.

Adding Data Elements to a Report

Use Report Creator features to add data elements from a Database Manager or Chromatogram Processor window to a report.



Follow these procedures:

- To copy a graphic from the Database Manager or Chromatogram Processor window
- To add a structure
- To add a tree
- To add a spectrum
- To add a fragmentation scheme
- To add Compare Spectra graphics
- To add Compare Trees graphics

❖ To copy a graphic from the Database Manager or Chromatogram Processor window

1. Copy a tree or structure in a Database Manager or Chromatogram Processor window.

   The application saves this graphic to the Clipboard.

2. In the Report Creator window, click **Picture** and click on the report where you want the graphic.

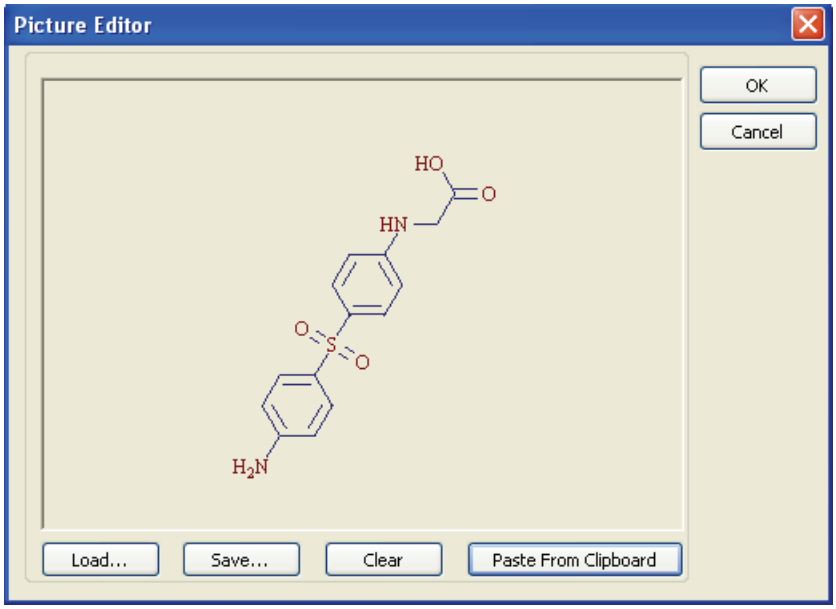
3. Double-click the rectangle.

   The Picture Editor dialog box opens.

4. Click **Paste From Clipboard**.

Note If the Paste From Clipboard button is not enabled, there is nothing on the Clipboard to paste. You cannot copy a graphic to the Clipboard while this dialog box is open. You must cancel this dialog box, copy a graphic to the Clipboard, and return to this dialog box.

The application displays the graphic in the Picture Editor dialog box.



5. Click **OK**.

   If the aspect ratio of the graphic is not similar to the rectangle in the Report Creator window, the graphic might be misshaped.

6. Stretch the graphic rectangle to fit the graphic.

7. To resize the graphic rectangle, right-click and choose **Position > Scale** or **Position > Size** from the shortcut menu.

8. To verify that your graphic looks as you expect, choose **File > Preview** from the Report menu.

   The Preview page shows a print view of your report, without a background grid or positioning rectangles.

9. To close the Preview page, click the **Close** button, , or press ESC.

❖ To add a structure

1. Click **Main Structure** and click on the report where you want the structure.

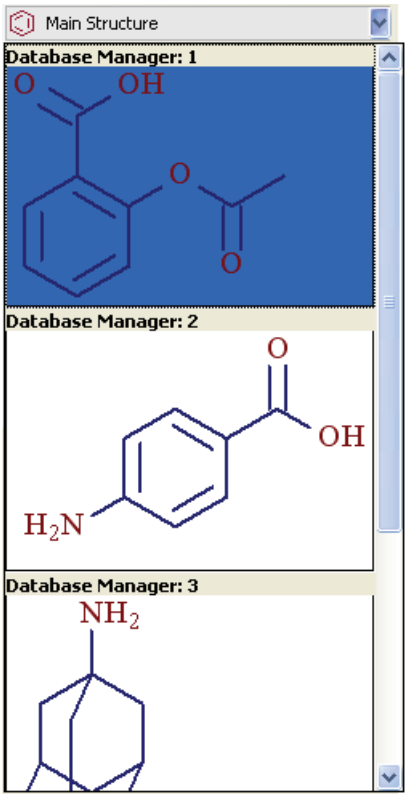
   The application pastes the current structure in a rectangle.

2. Double-click the rectangle until the structure you want is displayed.

   As you double-click, the application cycles through all available structures.

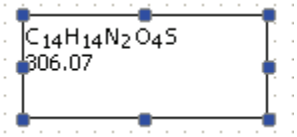
   Note When the original structure is smaller than the required structure, stretch the rectangle to an appropriate size.

3. To view all the available structures, click the Main Structure down arrow.



4. To add the chemical formula and molecular mass, click **Main Structure Description** and click on the report where you want the description.

   The application adds the chemical formula and molecular mass for the structure.



❖ To add a tree

1. Click **Tree** and click on the report where you want the tree.

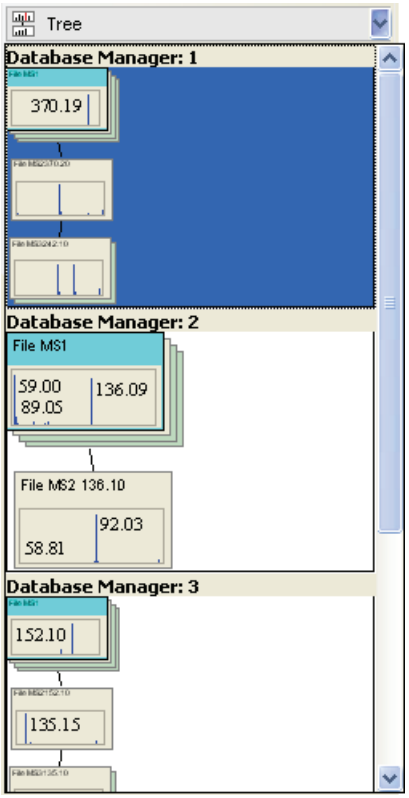
   The application pastes the current tree in a rectangle.

2. Double-click the rectangle until the tree you want is displayed.

   As you double-click, the application cycles through all available trees.

   Note When the original tree is smaller than the required tree, stretch the rectangle to an appropriate size.

3. To view all the available trees (one from each open Database Manager or Chromatogram Processor window), click the Tree down arrow.



❖ To add a spectrum

1. Click **Spectrum** and click on the report where you want the spectrum.

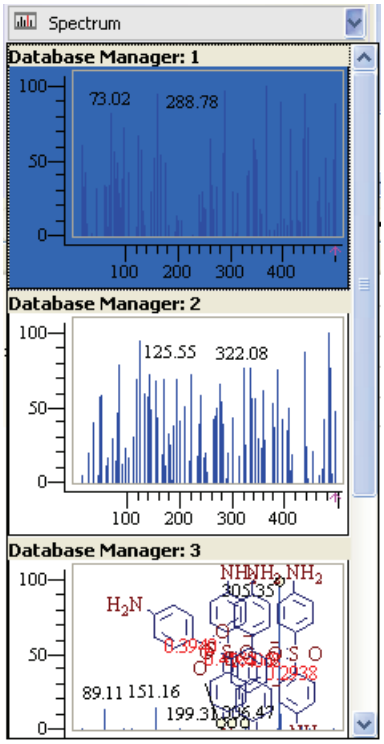
   The application pastes the current spectrum in a rectangle.

2. Double-click the rectangle until the spectrum you want is displayed.

   As you double-click, the application cycles through all available spectrum.

   Note When the original spectrum is smaller than the required spectrum, stretch the rectangle to an appropriate size.

3. To view all the available spectra (one from each open Database Manager or Chromatogram Processor window), click the Spectrum down arrow.



❖ To add a fragmentation scheme

1. Click **Fragmentation Scheme** and click on the report where you want the fragment.

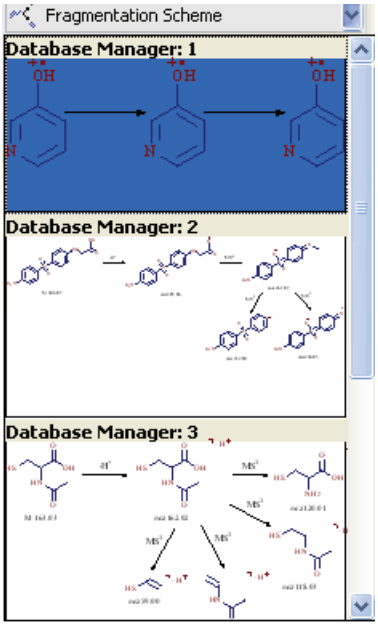
   The application pastes the current fragment in a rectangle.

2. Double-click the rectangle until the fragment you want is displayed.

   As you double-click, the application cycles through all available fragmentation schemes.

   Note When the original fragment is smaller than the required fragment, stretch the rectangle to an appropriate size.

3. To view all the available spectra (one from each open Database Manager window), click the Spectrum down arrow.



❖ To add Compare Spectra graphics

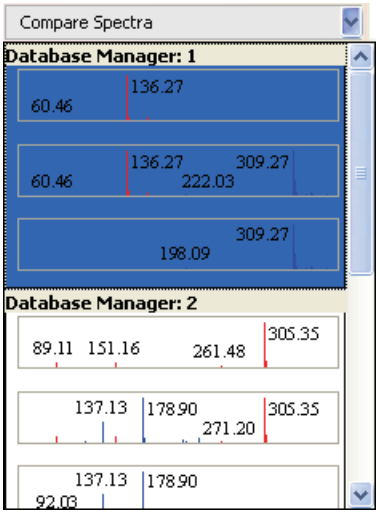
1. Click **Compare Spectra** and click on the report where you want the compared spectra.

   The application pastes the current compare spectra data in a rectangle.

2. Double-click the rectangle until the correct compare spectra data is displayed.

   As you double-click, the application cycles through all available compare spectra data.

3. To view all the available compare spectra data (one from each open Database Manager window), click the Compare Spectra down arrow.



❖ To add Compare Trees graphics

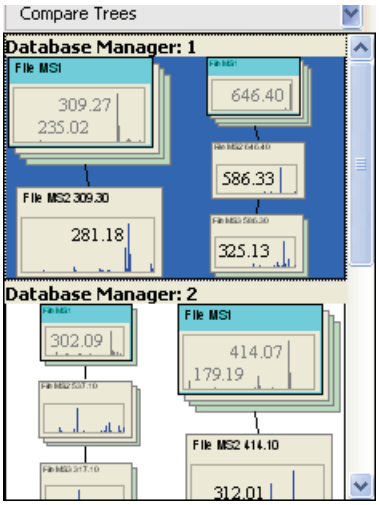
1. Click **Compare Trees** and click on the report where you want the compared trees.

   The application pastes the current compare trees data in a rectangle.

2. Double-click the rectangle until the correct compare trees data is displayed.

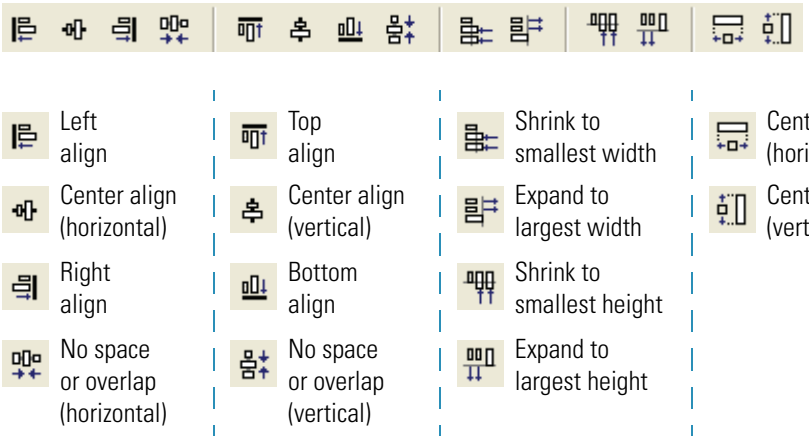
   As you double-click, the application cycles through all available compare trees data.

3. To view all the available compare trees (one from each open Database Manager window), click the Compare Trees down arrow.



Aligning Elements on a Report

The alignment toolbar includes commands to align all selected elements in your report.



❖ To align elements

1. Hold down the SHIFT key and select all elements you want to align.

   The alignment toolbar buttons are available only when elements are selected.

2. Click the appropriate alignment button in the toolbar.
3. If the effect is not what you want, click the **Undo** button, .

Saving, Printing, or Exporting a Report

Use the Report Creator to save a report as a Mass Frontier format file (.mfrf), or as a Mass Frontier Snapshot file (.mfrs), or as a template to use as a base for creating other reports.

You can also export a report as a PDF or TIFF file.

Follow these procedures:

- To save a report
- To save a report as a template
- To preview or print a report
- To export a report as a PDF file
- To export a report as a TIFF file

❖ To save a report

1. Click the **Save** button,  .

   The Save As dialog box opens.

   Note If you have previously saved this report, the application immediately saves your changes and does not open the Save As dialog box.

2. Type a name for the Mass Frontier Report File (.mfrf), and click **Save**.

   The application saves the report in the …\HighChem\Mass Frontier 7.0 \Reports folder.

❖ To save a report as a template

1. Choose **File > Save As** from the Report Creator menu.

   The Save As dialog box opens.

2. Open the Templates folder.

3. Type a name for the Mass Frontier Report File (.mfrf), and click **Save**.

   The application saves the report in the …\HighChem\Mass Frontier 7.0\ Reports\Templates folder.

You can use this report as a base for making another report by choosing File > Open and navigating to the Templates folder.

❖ To preview or print a report

1. Click the **Print Preview** button,  .

   The Preview window opens.

2. Click the **Print** button,  , to print the report.

You can save the report as a print preview (.ppv) file by clicking the Save As button,  .

3. Click the **Close** button,  , to close the Preview window.

❖ To export a report as a PDF file

1. Choose **File > Export As PDF** from the Report Creator menu.

   The Save As dialog box opens.

2. Navigate to a location for the PDF file.
3. Type a name for the PDF file.
4. Click **Save**.

   The report opens in an Adobe Acrobat window.

❖ To export a report as a TIFF file

1. Choose **File > Export As TIFF** from the Report Creator menu.

   The Save As dialog box opens.

2. Navigate to a location for the TIFF file.
3. Type a name for the TIFF file.
4. Click **Save**.

   The report opens in a Windows Picture and Fax Viewer window.

Example Reports

The following example reports show added graphics, structures, spectra, spectral trees, data, and text.

Example Metabolite ID Database Report

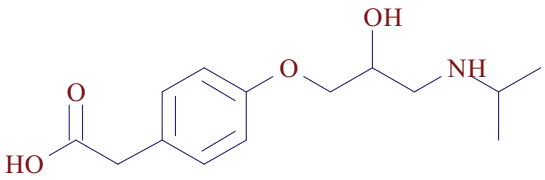
Metabolite ID Database Report 1/18/2011 8:43:04 AM

Thermo SCIENTIFIC

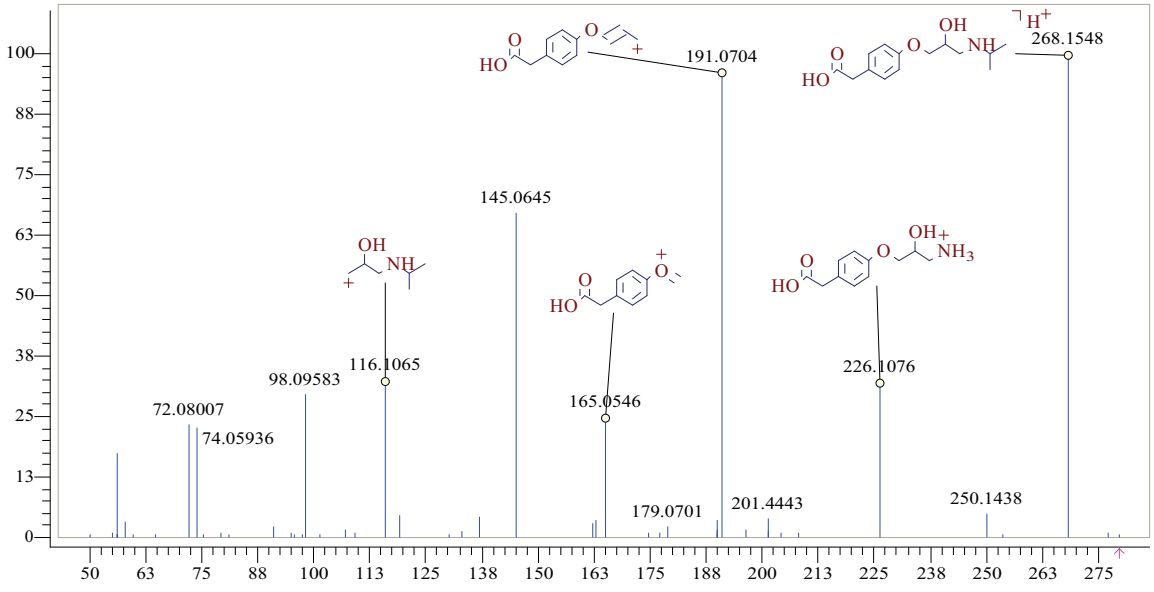
Component Information:

Compound	
Compound	
Names	
Synonyms \ References	Metabolite #1 - Amide Cleavage

Component Structure:



Fragmentation Spectra:



Example Metabolite ID FISh Chromatogram Report

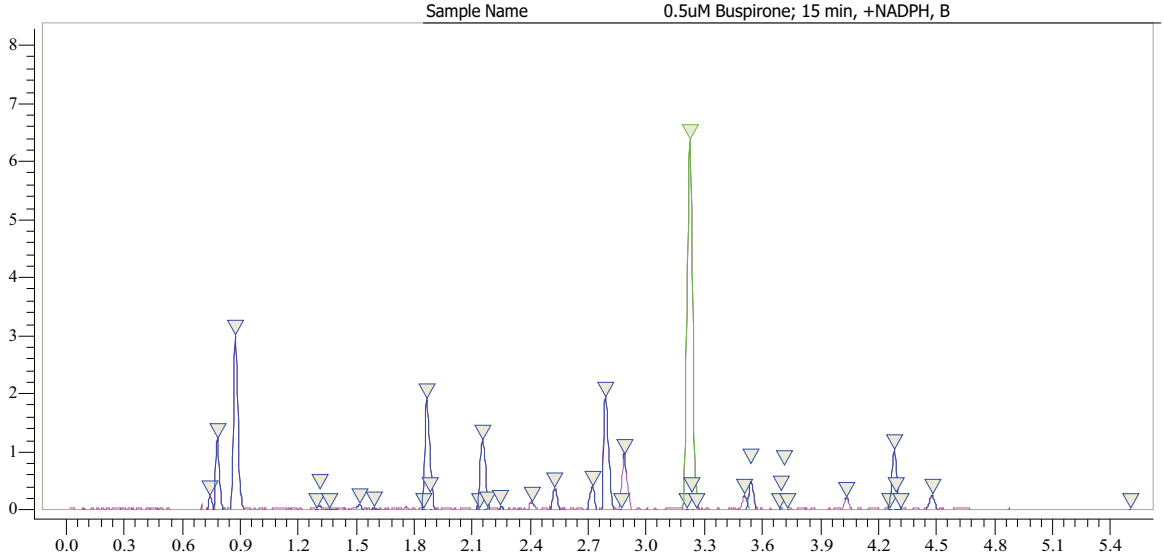
Metabolite ID FISh Chromatogram Report 1/18/2011 9:12:33 AM

Thermo SCIENTIFIC

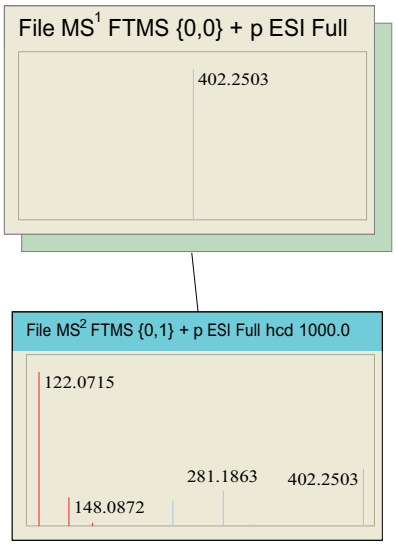
FISh Chromatogram with Components

Selected component in Green

Number of Scans	3374
Comments	2.1x150 1.7u Waters Acquity, H2O/ACN, 0.1%HCOOH gradient
Instrument Model	LTQ Orbitrap
Instrument Serial Number	0001
Instrument Hardware Version	Level1
Ionization Method	Electrospray ionization
Ionization Mode	Positive
Sample Type	Unknown
Sample Name	0.5uM Buspirone; 15 min, +NADPH, B

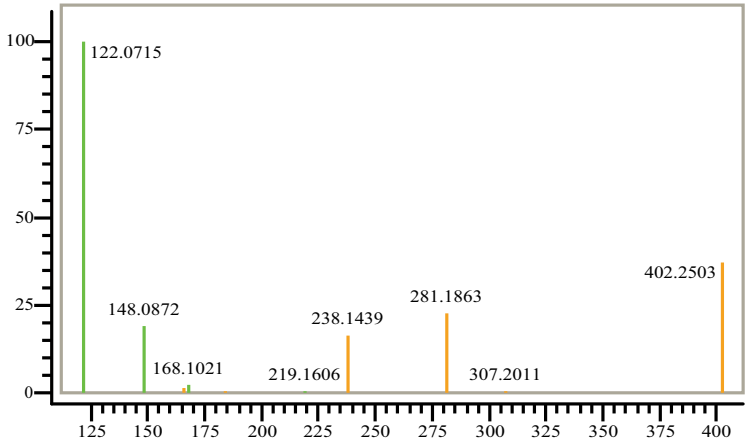


Component Spectral Tree



Component MS2 Scan

Spectrum	
Scan Info	
Caption	File MS2 FTMS {0,1} + p ESI Full hcd 1000.0



Index

Numerics

A

B

C

D

E

F

G

H

I

J

K

L

M

T

U

W

X