Automatically Annotating Spectral Trees Using a Generated List of Fragment Structures

To familiarize yourself with fragment annotation in the Mass Frontier™ 8.0 application, follow the topics in this tutorial to detect components in a chromatogram, generate a list of fragment structures for one of the identified components, and annotate the component's spectral tree with the fragment structures.

Contents

Prerequisite

To follow the procedures in this tutorial, your processing computer must have installations of the Mass Frontier 8 and Server Manager 3 applications.

Demo Data Files

This tutorial uses the following file that resides in the Demo Data folder on the application computer.

File	Description
sulfamethoxazolehybrid-pos.raw	Xcalibur RAW file from a direct infusion experiment for a sulfamethoxazole standard acquired on a Thermo Scientific Orbitrap Elite™ MS
sulfamethoxazole.mol file	A two-dimensional structure file for the target analyte

To substitute the demo files with your own data files, you must have the following files:

- An Xcalibur™ RAW file
- Two-dimensional structure files (MOL format) for the analytes in your experimental data

Opening a Raw Data File and Detecting Components

In this tutorial, you use the Chromatogram Processor module to open a raw data file and detect components.

Note You can also run library searches and mzLogic analyses from the Chromatogram Processor module.

Follow these topics in the order listed:

1. Opening a Raw Data File for Processing
2. Detecting Components in a Direct Infusion Chromatogram

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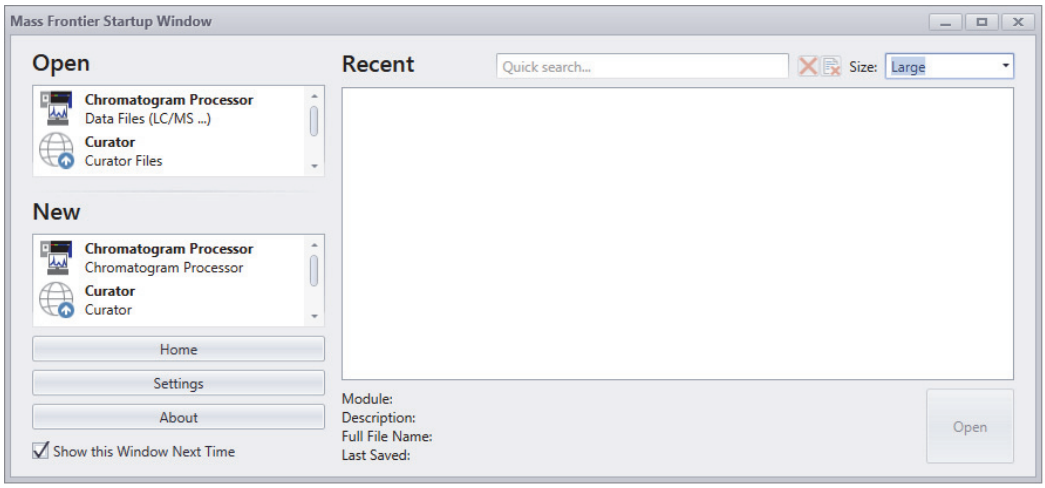
Opening a Raw Data File for Processing

❖ To open the example raw data file

1. Do one of the following:

   - From the Mass Frontier Startup Window, under Open, click **Chromatogram Processor**.

     Figure 1. Mass Frontier Startup Window

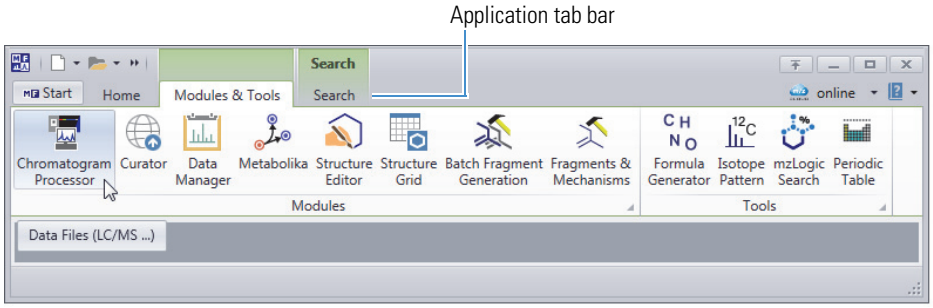
     

     Note If you clear the Show this Window Next Time check box, the next time you open the application, it opens with the Modules & Tools toolbar displayed.

   –or–

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

     Figure 2. Modules & Tools toolbar

2. In the Open Chromatogram dialog box, browse to the following folder, select **sulfamethoxazolehybrid-pos.raw**, and click **Open**.

   drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Chromatograms

   A new instance of the Chromatogram Processor module opens as a tabbed document with the following views (Figure 3):

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

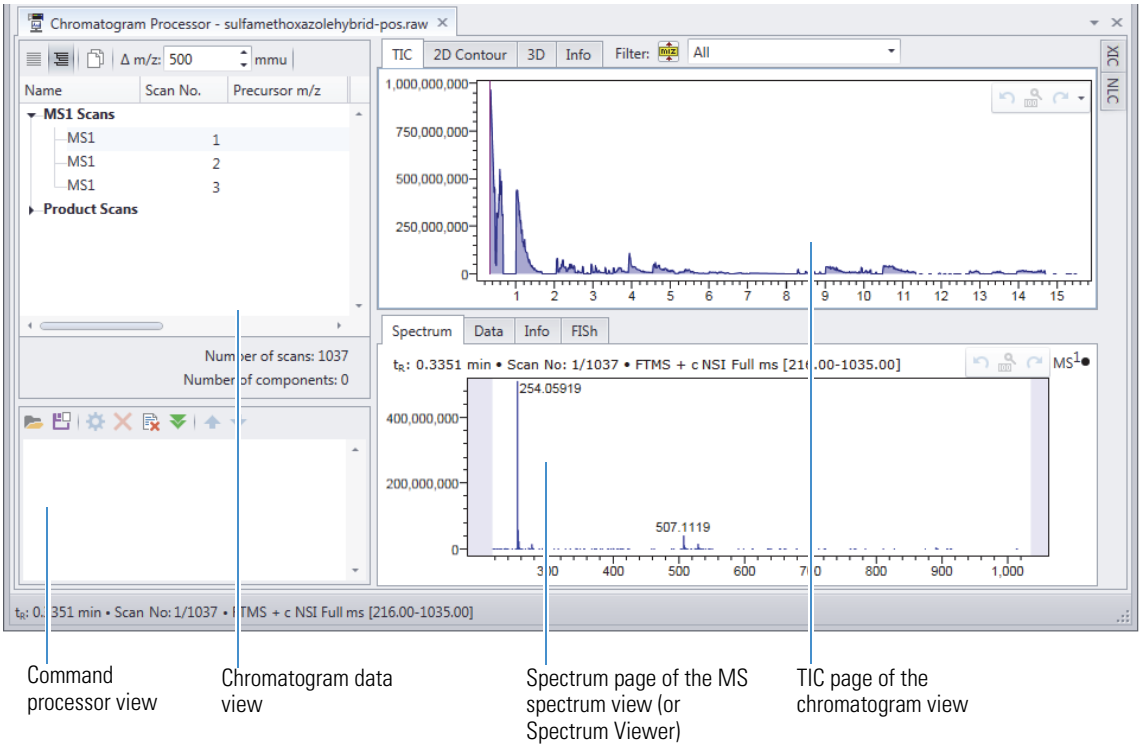
     Note Applying a component detection algorithm to the chromatogram adds a list of detected components to this view.

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

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

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

Figure 3. TIC chromatogram and scan number 1 for the selected raw data file



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

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

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

You cannot hide the chromatogram view.

Note For details about the Chromatogram Processor views, refer to the *Chromatogram Processor Module* chapter in the *Mass Frontier 8.0 User Guide*.

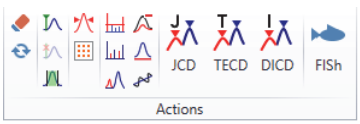
Detecting Components in a Direct Infusion Chromatogram

Use the direct infusion components detection (DICD) algorithm to detect the components in the example data file from a direct infusion experiment.

Note For the example data, use the DICD algorithm. For LC/MS data, use the JCD algorithm. For more information about component detection, refer to the *Chromatogram Processor Module* chapter in the *Mass Frontier 8.0 User Guide*.

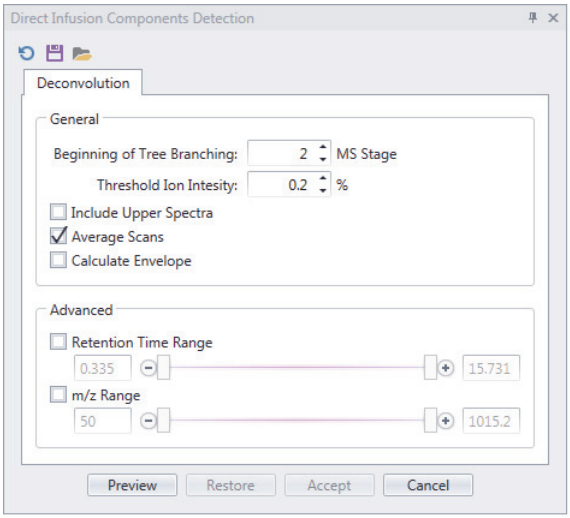
❖ To detect the components in a chromatogram from a direct infusion experiment

1. In the Actions group of the Chromatogram Processor toolbar, click **DICD**.



The Direct Infusion Components Detection view opens on the right.

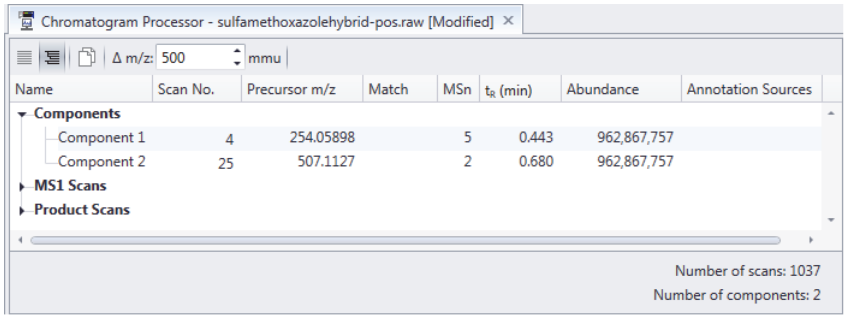
Figure 4. Direct Infusion Components Detection view with the default settings



2. To make sure that the default settings are selected, click the **Reset Parameters to the Default State** icon, .
3. To detect components and create a spectral tree, click **Preview**.

   After the processing finishes, the following items appear:

   - In the chromatogram data view, the Components list appears above the MS1 Scans list. The components are numbered in ascending order by retention time [t_R (min)]. The number of detected components appears at the bottom of the view.



   - On the TIC page of the chromatogram view, blue triangles appear above the apexes of the chromatographic peaks for the detected components.
   - On the left of the MS spectrum view, a spectral tree depicts the selected component as a hierarchically arranged set of spectra beginning with the MS1 stage at the top and increasing MS^n stages at each lower level. Each node contains spectra for the same precursor pathway, but with different ion activations and collision energies. The spectra in each node are color-coded by ion activation type (Table 1).

     Figure 5. Spectrum tree view with the default color-coding for the ion activation types

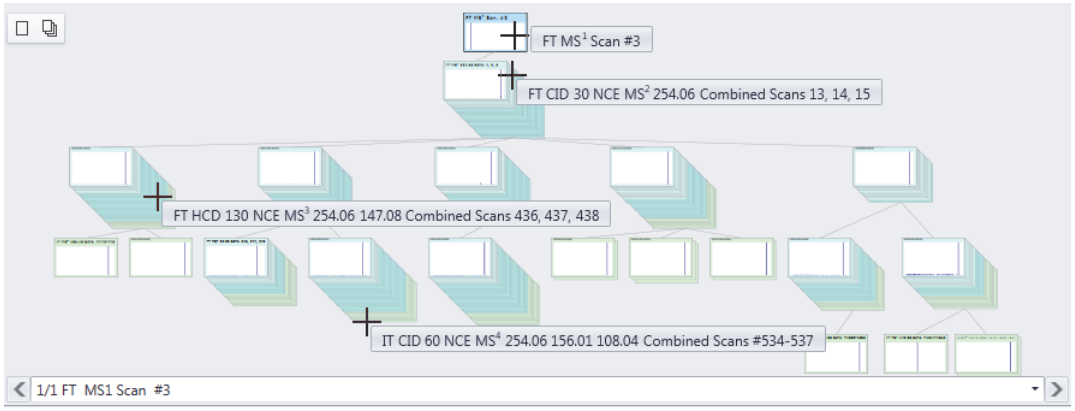


Table 1 describes the default color-coding for the spectrum borders. When you select a spectrum, its border color changes to light blue with a black outline (Figure 5 and Figure 6).

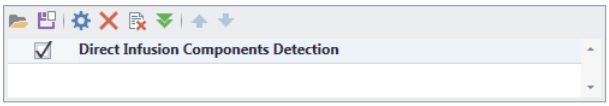
Table 1. Color-coding for spectrum borders by ion activation type

Ion activation type	Default border color
FT CID	
FT HCD	
IT CID	
IT HCD	

Tip You can change the color-coding for the ion activation types in the MS Tree view of the Options dialog box.

4. Click **Accept**.

   The DICD view closes and the applied action appears in the command processor view.



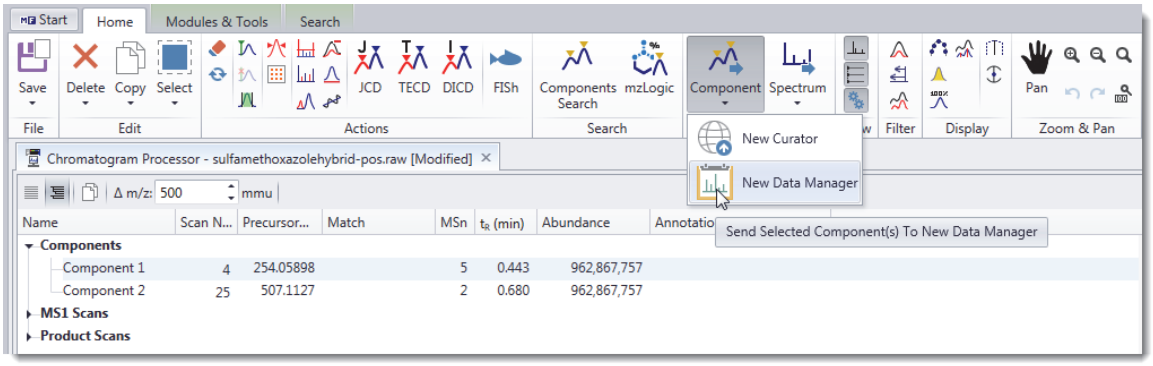
Go to the next topic, "Sending a Component to the Data Manager Module."

Sending a Component to the Data Manager Module

After you apply a component detection algorithm to the raw data, you can use the Data Manager module to select the structure for a specific component.

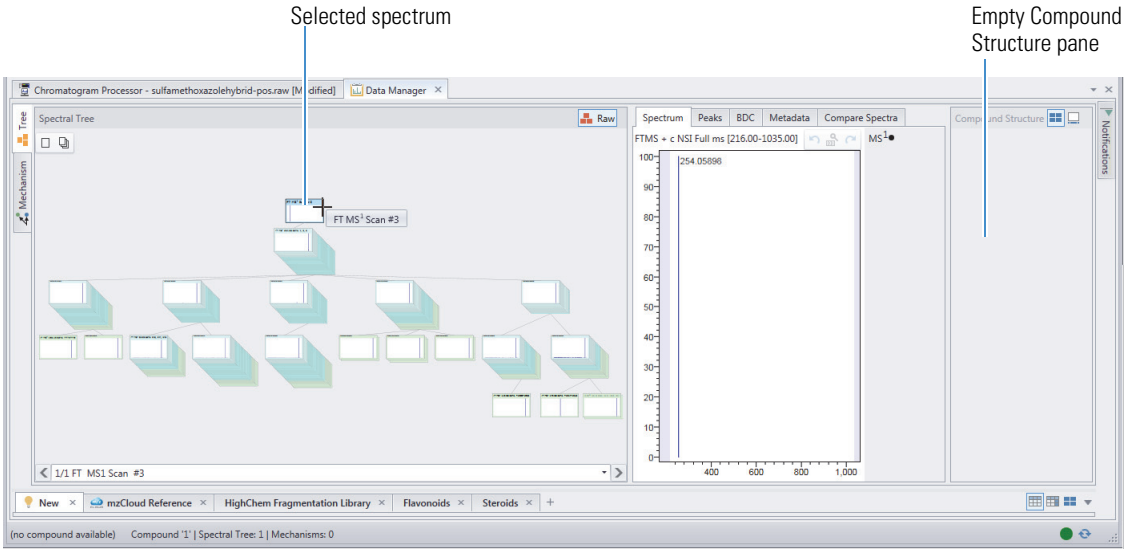
❖ To send a component to the Data Manager module

1. In the chromatogram data view, select **Component 1** (*m/z* 254. 05898 at 0.443 minutes).
2. In the Send To group of the Chromatogram Processor toolbar, click **Component** and select **New Data Manager**.



The Data Manager page displays the spectrum tree on the left with the MS1 level spectrum selected, the spectrum pane in the middle, and an empty Compound Structure pane on the right. Because you sent an unannotated component, the Compound Structure pane is empty (Figure 6).

Figure 6. Data Manager page showing the spectrum tree for component 1 and the spectrum from scan #3



Go to the next topic, "Assigning a Structure to the Component."

Assigning a Structure to the Component

You can assign a structure to a component in the Data Manager module.

❖ To assign a structure to the component

1. After you send a component to the Data Manager module, select the MS1 (top) level node in the Spectral Tree pane, and then double-click the empty **Compound Structure** pane on the right (Figure 6).

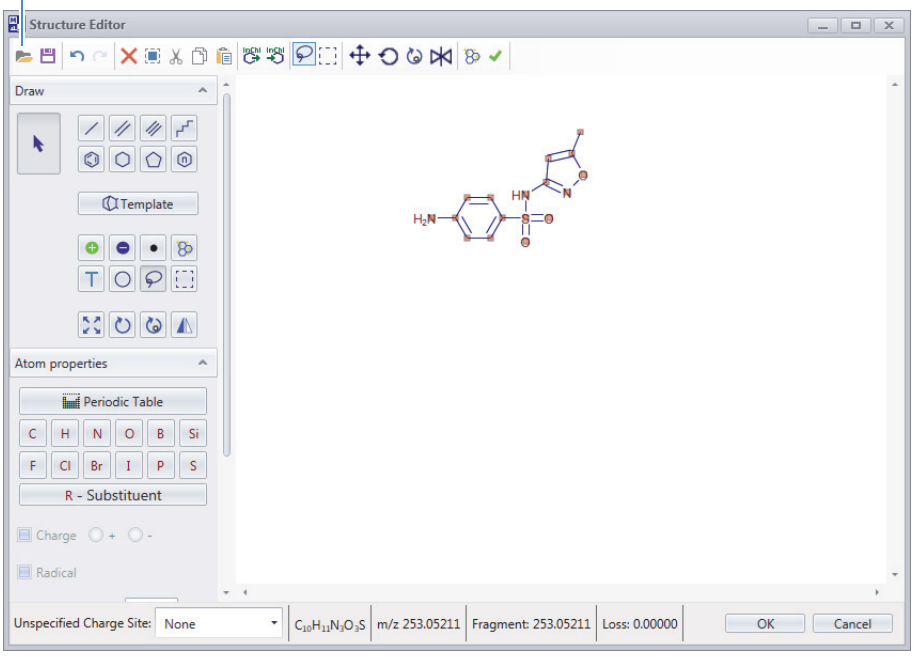
   The Structure Editor opens.

2. In the Structure Editor toolbar, click the **Open** icon, . Then, browse to the following folder, select the **sulfamethoxazole.mol** file, and click **Open**.

   drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Structures

   The selected structure appears in the editor's drawing area.

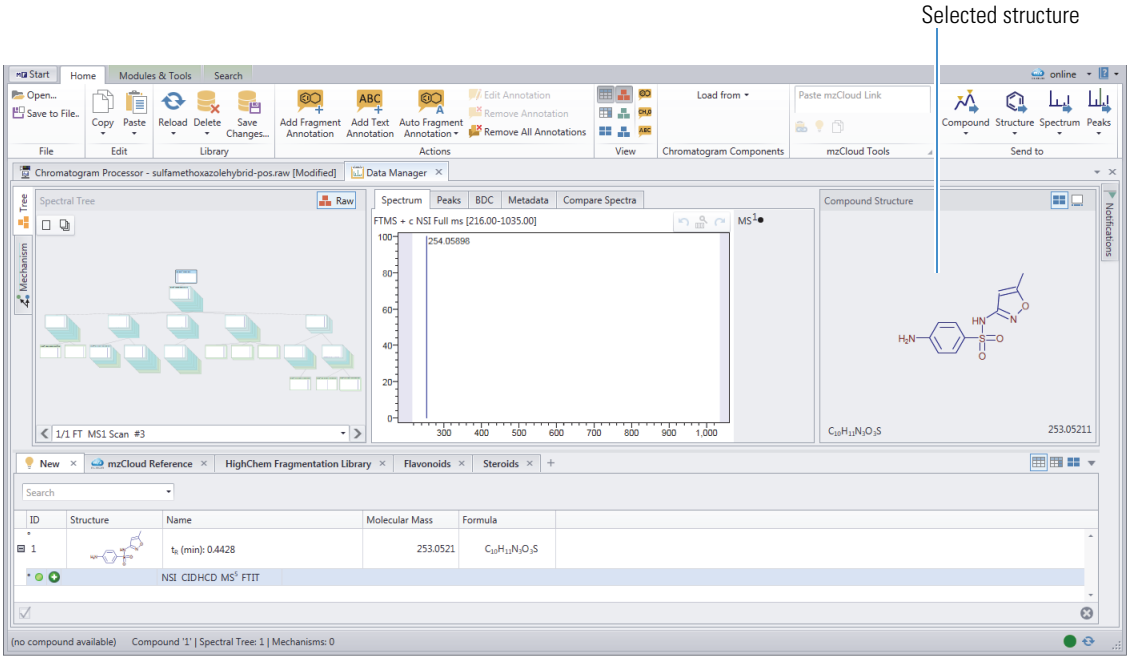
   Opens the Open Structure dialog box where you can select a structure file.



3. Click **OK**.

The selected structure appears in the Compound Structure pane on the Data Manager page.

Figure 7. Data Manager page for component 1 with a populated Compound Structure pane



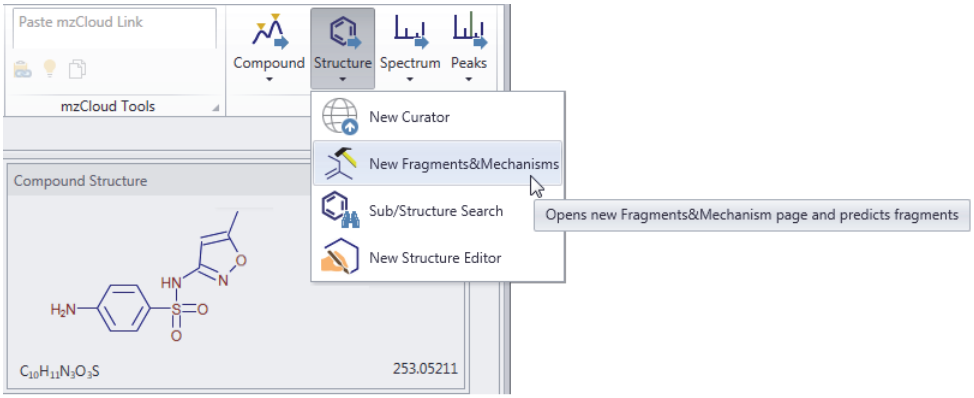
Go to the next topic, "Initiating the Fragmentation Process in Data Manager."

Initiating the Fragmentation Process in Data Manager

To create a list of fragment structures for a component from the Data Manager module, send a component's structure to the Fragments & Mechanisms module.

❖ To initiate the fragmentation process

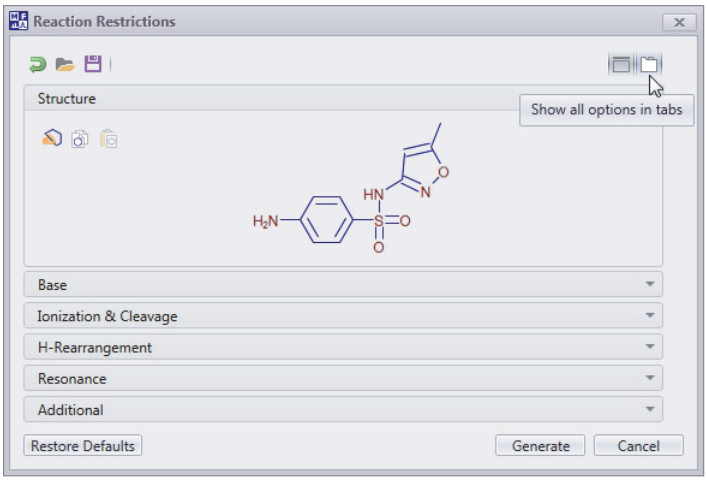
1. In the Send To group of the Data Manager toolbar, click **Structure**, and then select **New Fragments & Mechanisms**.



The Reaction Restrictions dialog box opens with all the sections on one page (Figure 8).

2. To change the layout to tabbed pages, click the **Show All Options in Tabs** icon, .

Figure 8. Reaction Restrictions dialog box with the single page layout



3. On the Base page of the Reaction Restrictions dialog box, do the following.
   a. By default the General Fragmentation Rules check box is selected. Keep this selection.

      The general fragmentation rules include the mechanisms listed in the Reaction Mechanisms Overview dialog box (Figure 18).

   b. Select the **Use HighChem Fragmentation Library** check box.

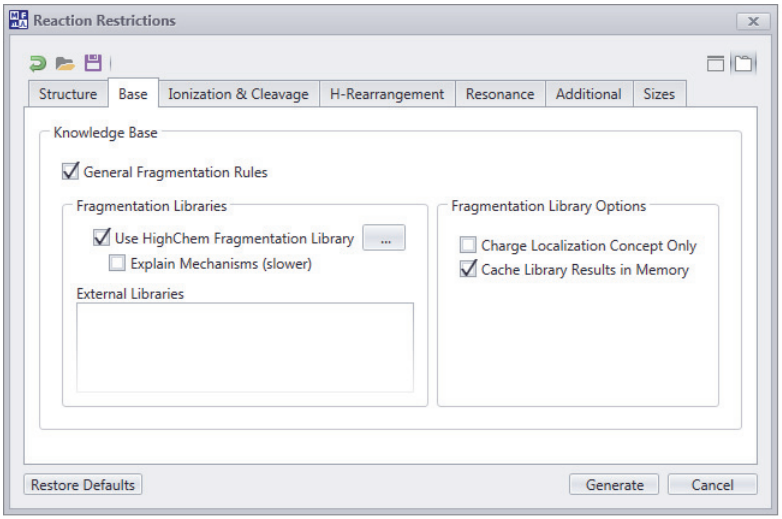
      The HighChem Fragmentation library includes >52 000 fragmentation schemes and ~220 000 individual reactions.

> **IMPORTANT** Depending on the structure's size, fragmentation prediction using the HighChem Fragmentation Library can take significantly longer than using only the general rules. Enabling caching improves the fragmentation speed, but significantly increases the RAM memory usage.
>
> Clear the Cache Library Results in Memory check box if your processing computer does not meet the recommended requirements for the Mass Frontier application or if you are using the Batch Fragment Generation wizard to process multiple structures.

   c. Select the **Cache Library Results in Memory** check box.

Figure 9 shows the selections for this tutorial.

Figure 9. Base page selections for this tutorial

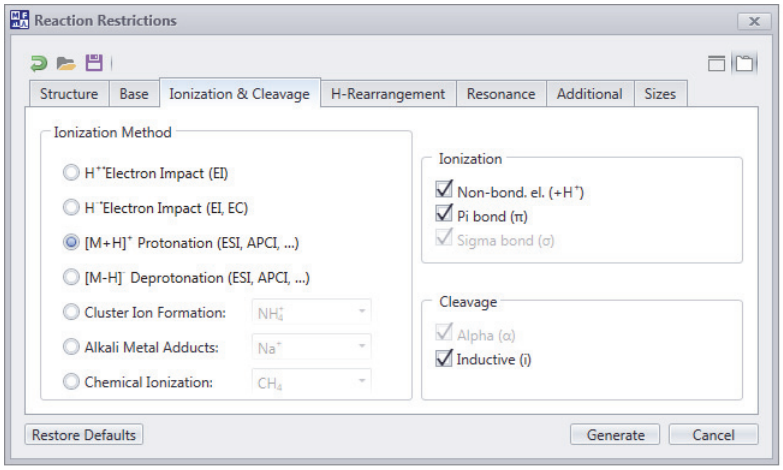


4. For the remaining pages of the Reactions Restrictions dialog box, keep the default settings for the example data.
   a. On the Ionization & Cleavage page, select the ionization method for the experimental data.

      For the example data file, keep the default setting of **[M+H]+ Protonation (ESI, APCI, ...)**.

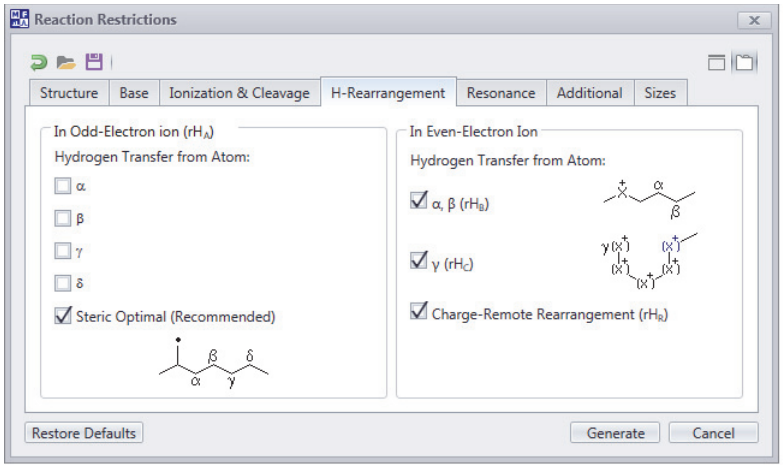
> **IMPORTANT** Selecting an incorrect ionization method might result in poor mapping of the generated fragments to the spectral peaks.

Figure 10. Default settings for the Ionization & Cleavage page



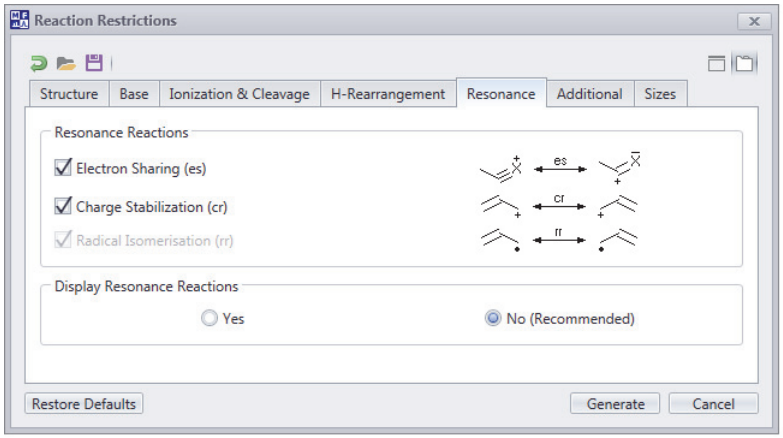
   b. On the H-Rearrangement page, keep the default settings.

Figure 11. Default settings for the H-Rearrangement page



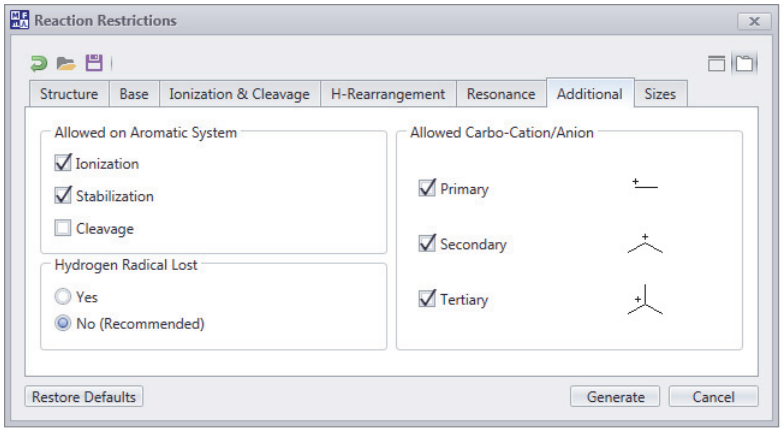
   c. On the Resonance page, keep the default settings.

Figure 12. Default settings for the Resonance page



d. On the Additional page, keep the default settings.

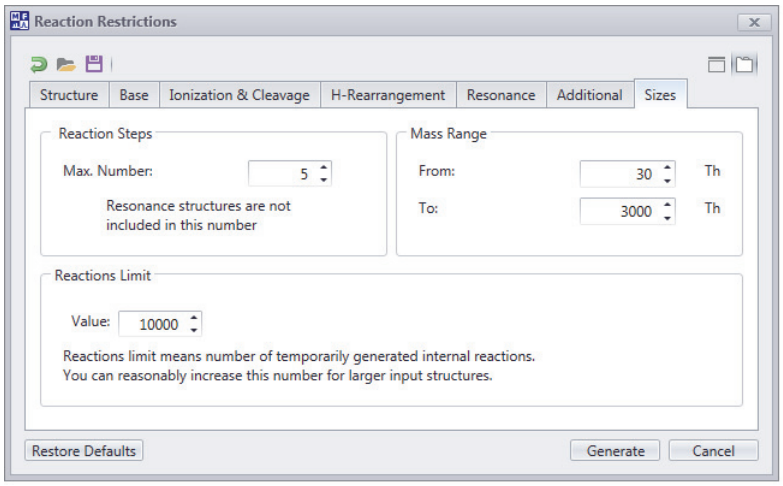
Figure 13. Default settings for the Additional page



e. On the Sizes page, select the reaction steps, mass range, and reaction limits for your experimental data. For this tutorial, keep the default settings (Figure 14).

IMPORTANT The Reactions Limit parameter refers to the number of temporarily generated internal reactions. Thermo Fisher Scientific recommends that you increase the setting for large structures even though increasing this value increases the fragment prediction time.

Figure 14. Default settings for the Sizes page



5. Click **Generate**.

   The Fragment Generation dialog box that displays the progress of the analysis opens.

6. Wait for processing to finish.

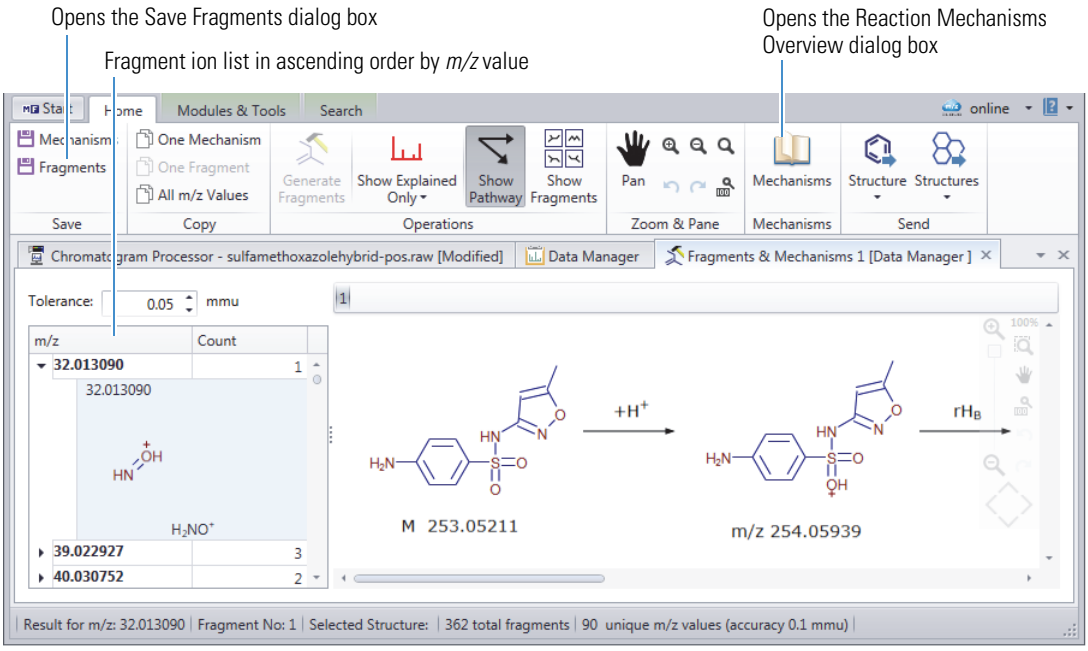
Note For details about the parameters in the Reaction Restrictions dialog box, refer to the *Fragments & Mechanisms Module* chapter in the *Mass Frontier 8.0 User Guide*.

Tip The fragmentation procedure can become extremely CPU intensive depending on the depth of the fragmentation pathway prediction.

When the fragmentation process is complete, the Fragmentation Generation dialog box closes and the Fragments & Mechanisms module opens as a tabbed page with a list of predicted fragments.

A list of the predicted fragments grouped by their *m/z* values within the tolerance value appears in the left pane, and the reaction pathway for the selected fragment appears in the right pane (Figure 15).

Figure 15. Fragments & Mechanisms page showing the fragmentation pathway for the first fragment in the fragments list

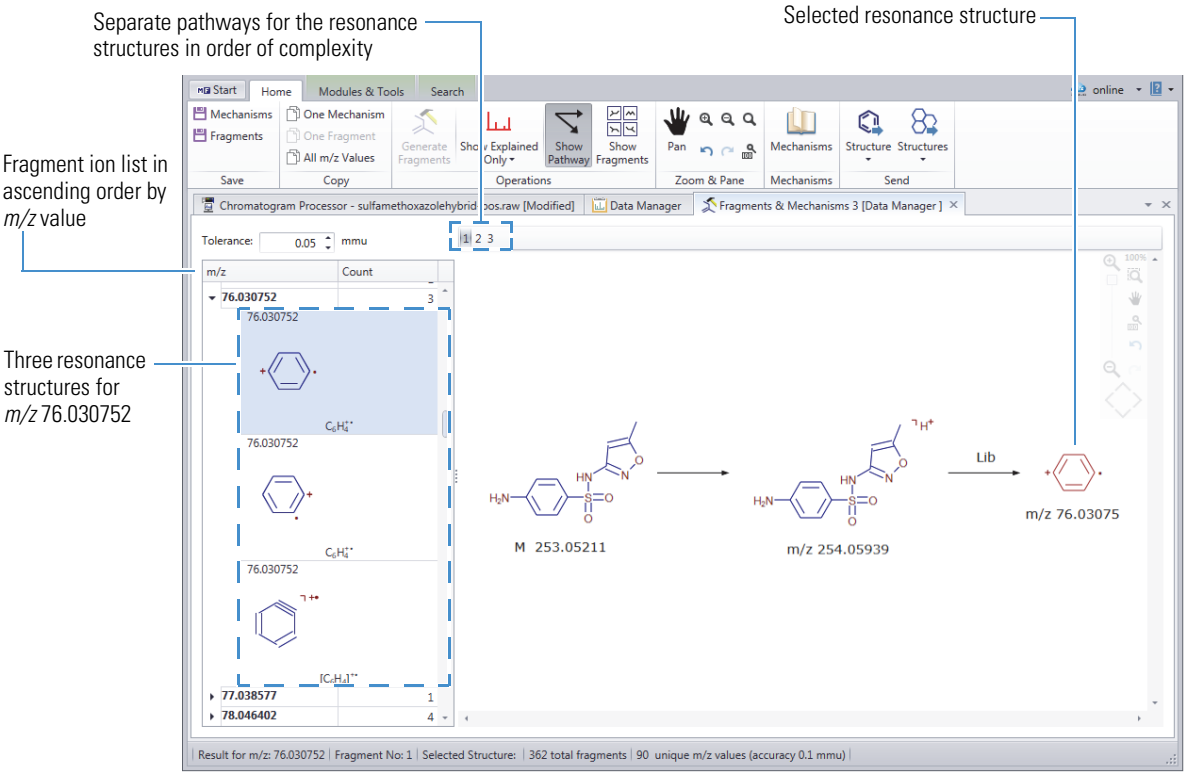


Note A fragment can have multiple resonance structures with identical *m/z* values. The resonance structures within the *m/z* group are listed in order of the complexity of the fragmentation pathway, starting with the fragmentation reaction that involves fewer steps.

7. On the Fragments & Mechanisms page, do the following:
   - To view the fragmentation pathway for a specific fragment, select the fragment's *m/z* value in the m/z column, and then click **Show Pathway** in the Operations group of the Fragments & Mechanisms toolbar.

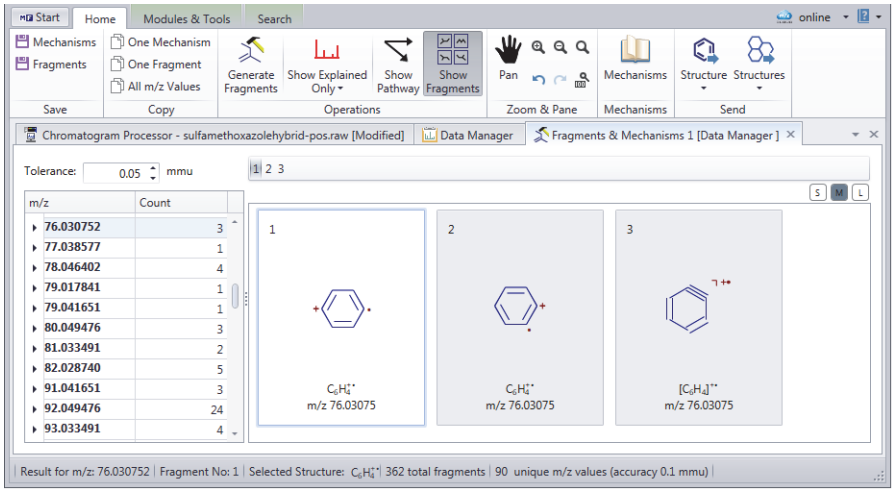
     The selected resonance structure for the final fragment ion is displayed in red.

Figure 16. Show Pathway view with the fragmentation pathway for a selected resonance structure for *m/z* 76.030752



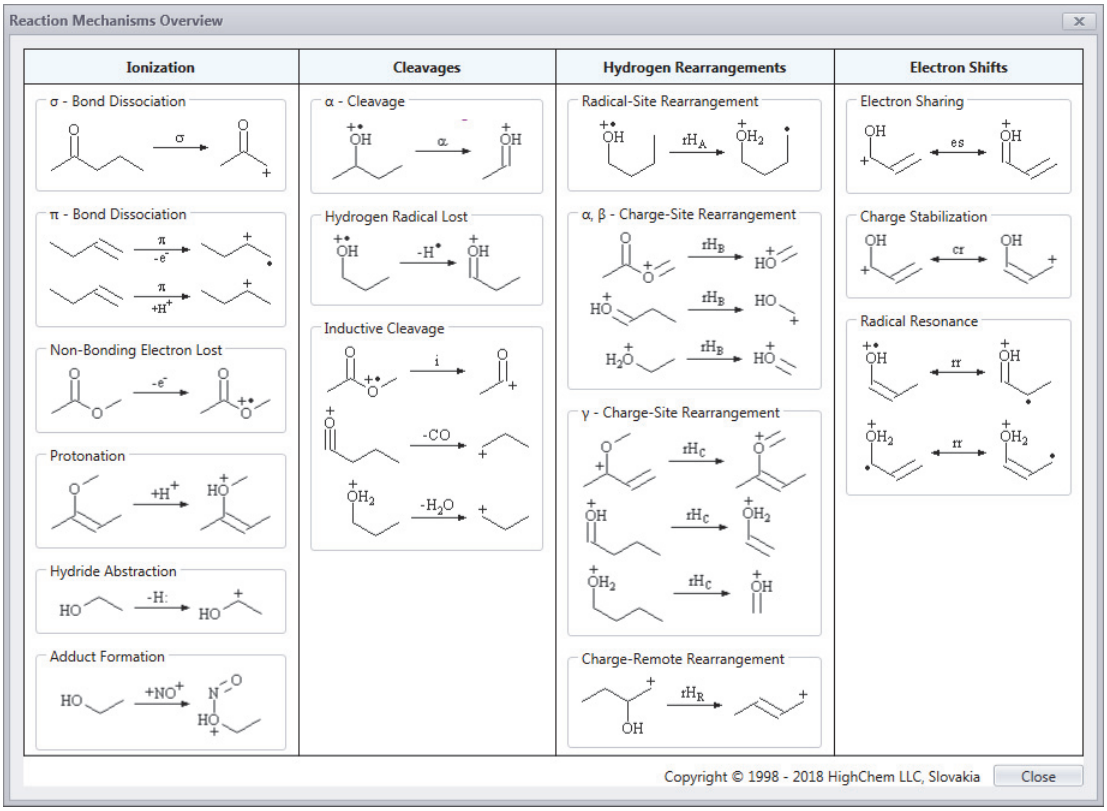
- To view the resonance structures for a fragment as cards, click **Show Fragments** in the toolbar.

Figure 17. Show Fragments view with resonance structures for the selected fragment shows as cards



8. (Optional) To open the Reaction Mechanisms Overview dialog box, click **Mechanisms** in the Fragments & Mechanisms [Data Manager] toolbar (Figure 15). To continue the tutorial, close the dialog box.

Figure 18. Reaction Mechanisms Overview dialog box



Tip Since the fragmentation process is time consuming, saving the fragments to a file for later use can save time. To save the fragments list to an SDF file, in the Save group of the Fragments & Mechanisms toolbar, click **Fragments**. Then, browse to an appropriate folder, name the file, and click **Save**.

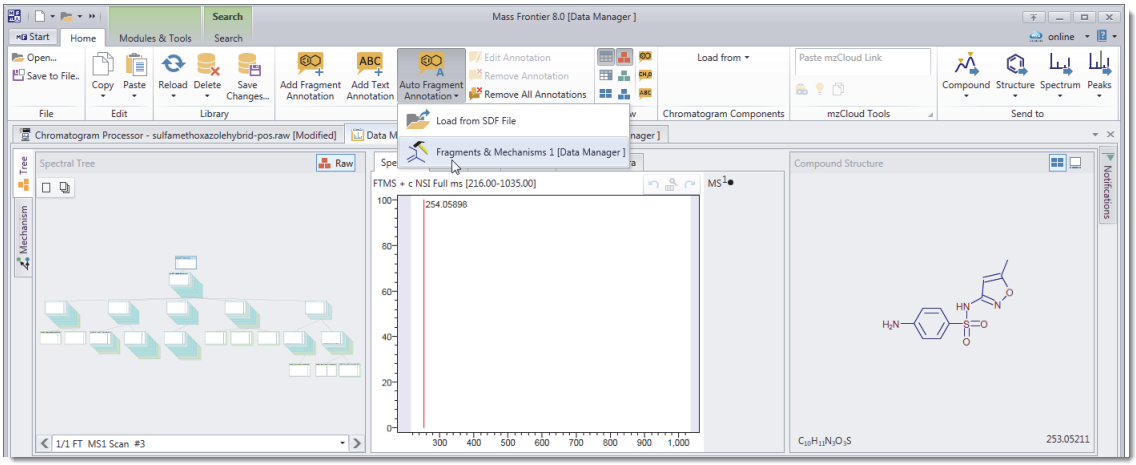
Go to the next topic, "Automatically Annotating Spectra with Fragment Structures."

Automatically Annotating Spectra with Fragment Structures

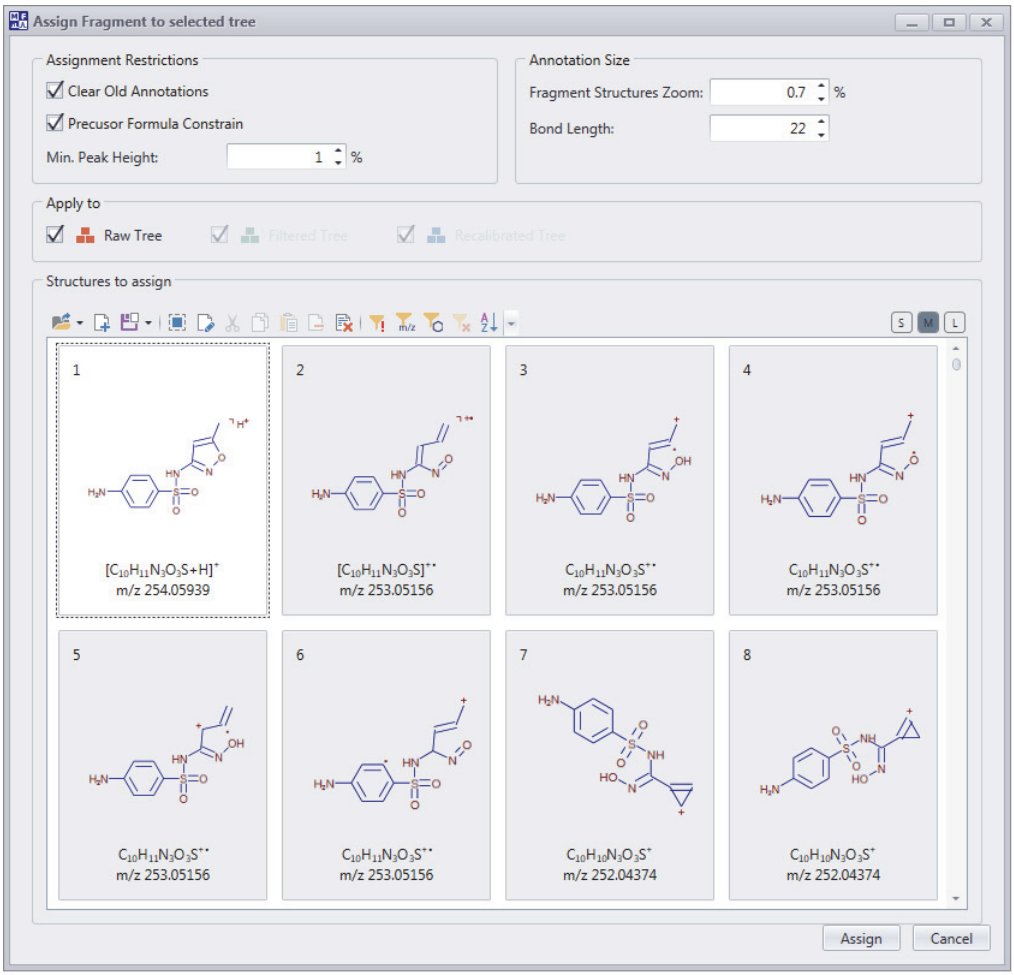
Use the Auto Fragment Annotation feature on the Data Manager page to automatically annotate a component's spectral tree with the fragment structures from the Fragments & Mechanisms page.

❖ To automatically annotate the spectrum tree for a component with fragment structures

1. If you have not already done so, follow the previous topics in this tutorial to set up a list of fragment structures for a specific component.
2. Click the **Data Manager** tab.
3. In the Actions group of the Data Manager toolbar, click **Auto Fragment Annotation**, and then select **Fragments & Mechanisms [Data Manager]**.



The Assign Fragment to Selected Tree dialog box opens.



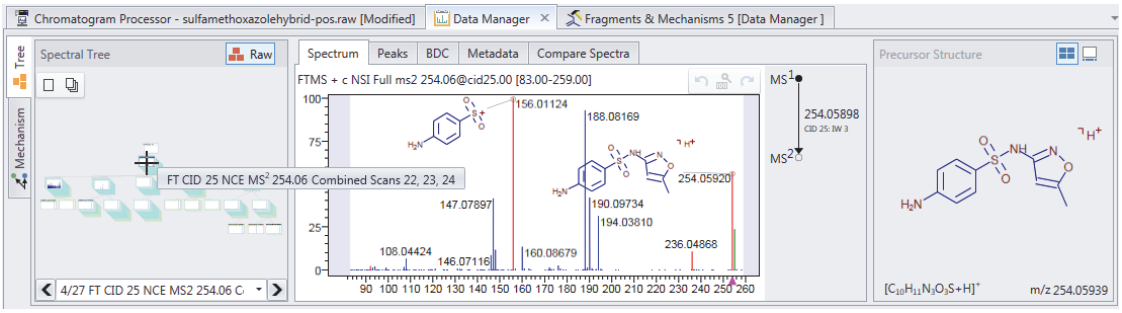
4. For this tutorial, click **Assign** to assign all the structures.

> **Tip** To assign only a selected subset of the structures, select their cards, and then click **Assign**.

> **Note** When the Precursor Formula Constrain check box is selected, the application assigns only structure annotations for the given spectrum that are valid subformulas of any of its precursor structure annotations.

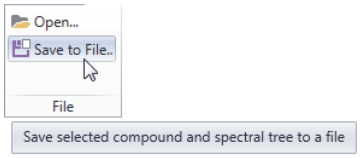
5. At the number of fragments prompt, click **OK** to accept the assigned structures.
6. Browse to individual spectra from the tree.

   The annotated spectral peaks are displayed in red.



7. Do one or both of the following:

   - To save the spectral record in the Mass Frontier 8.0 Data Manager format (.mfcdc), in the File group of the Data Manager toolbar, click **Save to File**. Then, browse to an appropriate folder, name the file, and click **Save**.



   - To save the spectral record to a user library, in the Library group of the Data Manager toolbar, click **Save Changes**, select the user library, and click **Save**.

   The record is added to the selected library with a new library ID.

> **Note** If the added record is not displayed, click **Reload > Reload Compound** in the Library group of the Data Manager toolbar.

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