Mass Frontier 8.1 tutorial to Detect Structurally-Related Compounds with the FISh Algorithm

This tutorial describes how to detect structurally-related compounds by running a Fragment Ion Search™ (FISh) analysis. Applying a FISh filter to a chromatogram removes all the spectral peaks except for the defined FISh model peaks. You can use this feature to extract spectral peaks for structurally-related features, for example, compounds related to the parent drug in metabolite ID, API impurity ID, or compounds that share a common list of fragments.

Contents

Demo data files

This tutorial uses the following files that reside in the Demo Data folder on the application computer.

File	Description
OMP-Sigma-70K. raw	A raw data file acquired with an LC-ESI/MS/MS experiment using an Orbitrap™ QExactive™ Plus MS. The sample solution was prepared from an omeprazole standard. Omeprazole is a therapeutic drug for acid reflux.
Omeprazole FISh Fragments.sdf	A structure file that contains twelve fragment structures for the parent compound—omeprazole
OMP-Sigma-70K.chpro.jcd	A component detection file that contains the component detection settings for an LC/MS experiment
Omeprazole.mol	A structure file that contains the structure for omeprazole—the analyte of interest in this tutorial

Check the global settings

Before you begin this tutorial, check the global settings for the mass accuracy and color-coding of the spectrum peaks.

❖ To restore the default settings for mass accuracy and spectrum peak color-coding

1. Open the Mass Frontier application by double-clicking its desktop icon,  , or by choosing **Thermo Mass Frontier 8.1 > Mass Frontier 8.1** from the Windows™ Start menu.

   The application opens to the Mass Frontier startup window or the Modules & Tools toolbar.

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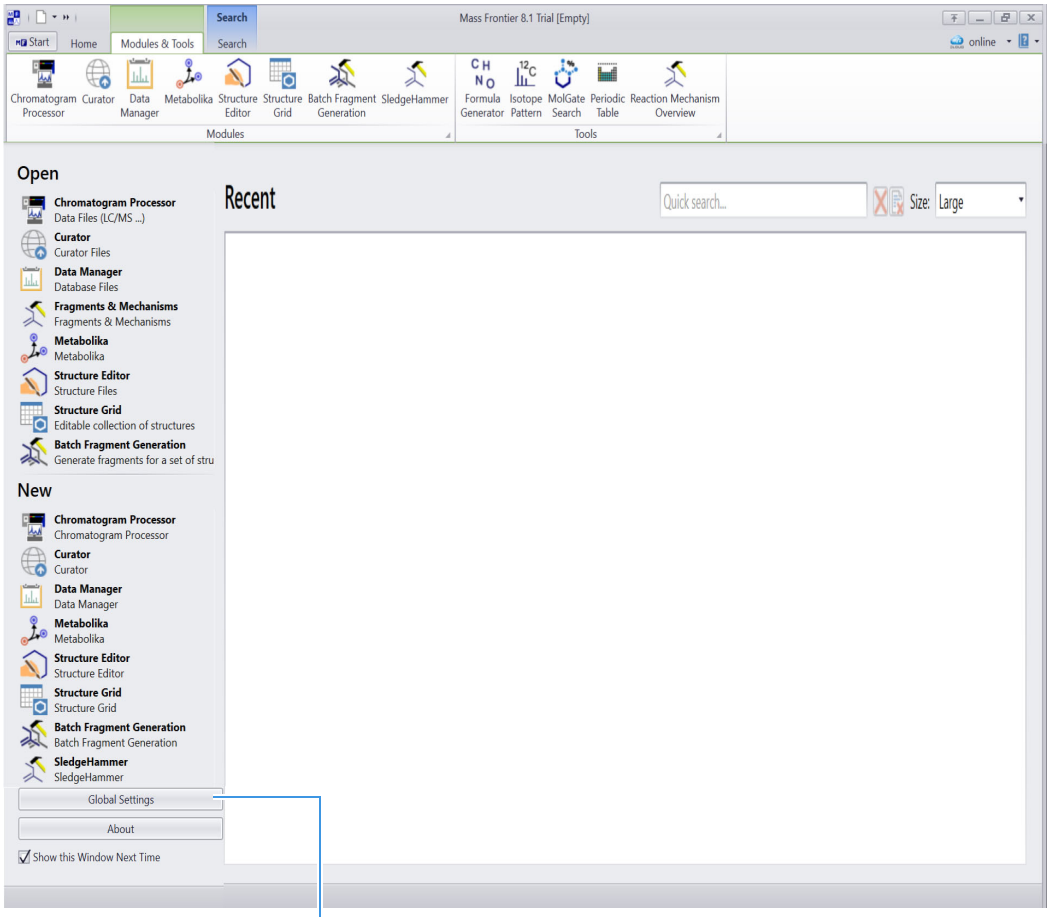
2. Do one of the following:

   - From the Mass Frontier startup window, click **Global Settings**.

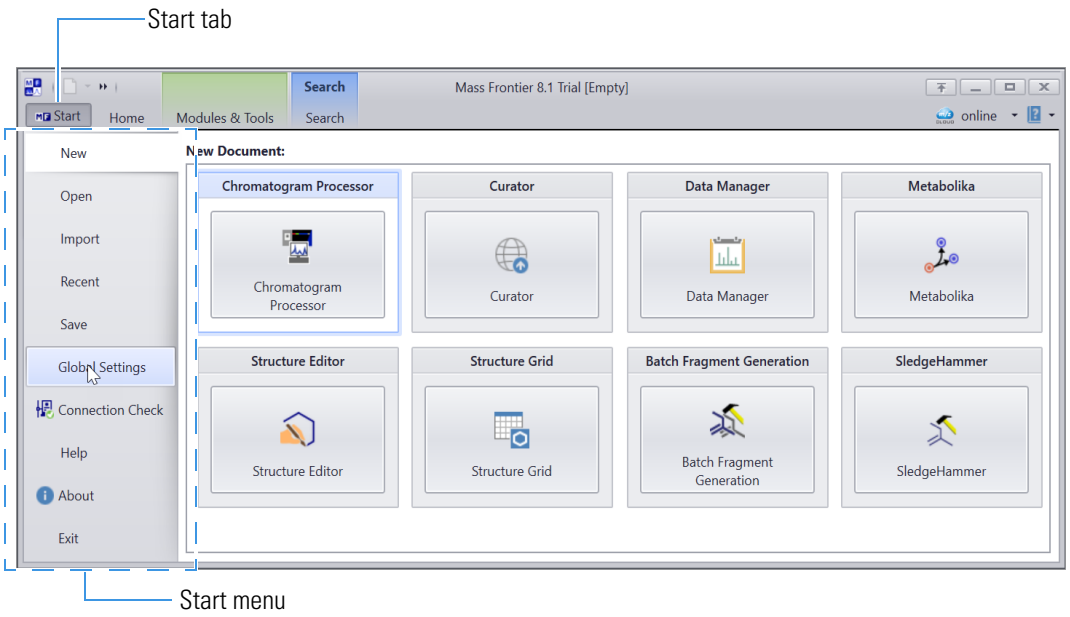
     Figure 1. Mass Frontier startup window



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

–or–

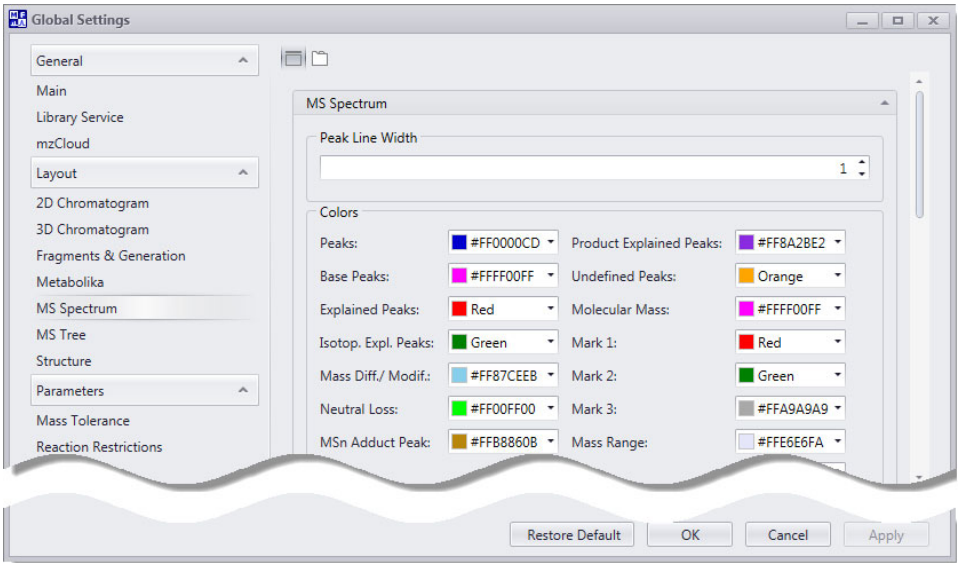
   - From the application tab bar, click the **Start** tab to display the Start menu, and then choose **Global Settings**.



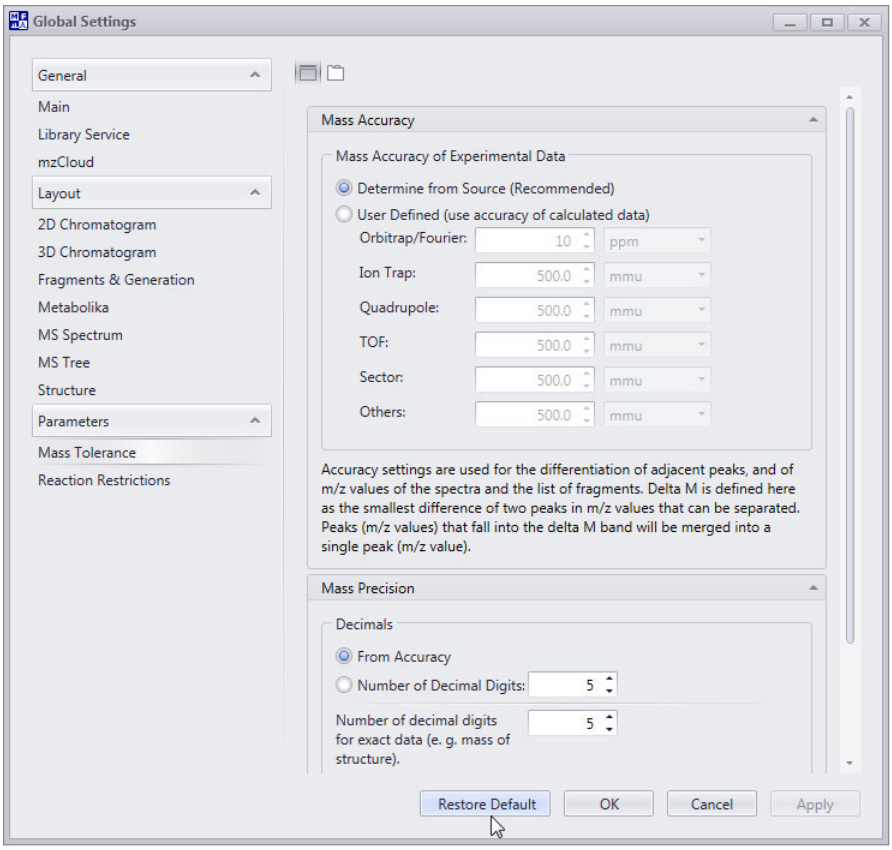
The Global Settings dialog box opens (Figure 2).

3. In the left pane, under Layout, click **MS Spectrum**, and then click **Restore Default**.

Figure 2. Global Settings dialog box – MS Spectrum view with the default settings



4. In the left pane under Parameters, click **Mass Tolerance**, and then click **Restore Default**.



IMPORTANT Make sure that the Determine from Source option (default) is selected for the mass accuracy of the experimental data; a user-defined setting for mass accuracy can change the number of detected components.

Run and Review a FISh analysis

To run a FISh analysis and review the results, follow these topics in order:

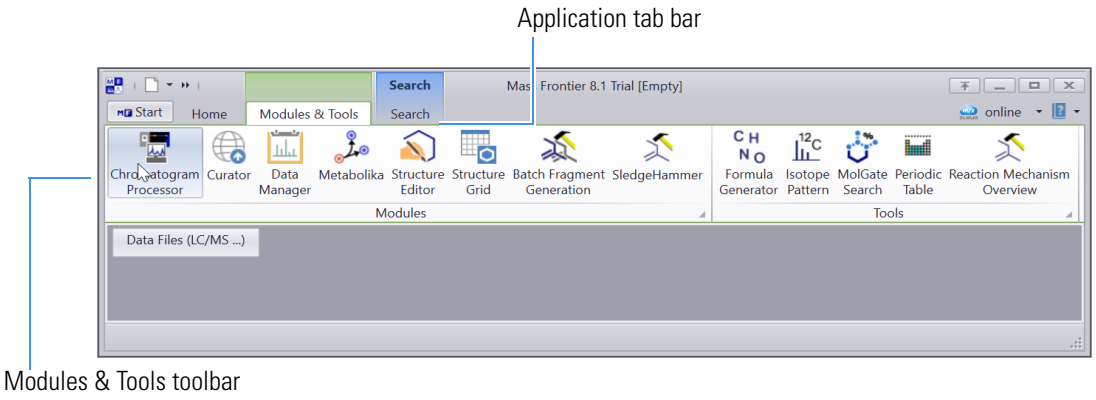
1. Open a raw data file for processing
2. Apply a fragment ion search filter
3. View the FISh trace
4. Review the detected components

Open a raw data file for processing

❖ **To open the example raw data file**

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

Figure 3. Modules & Tools toolbar



2. In the Open Chromatogram dialog box, browse to the following folder, select **OMP-Sigma-70K.raw**, and click **Open**.

   drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.1\Demo Data\Chromatograms

   A new instance of the Chromatogram Processor module opens as a tabbed document with the following views (Figure 4):

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

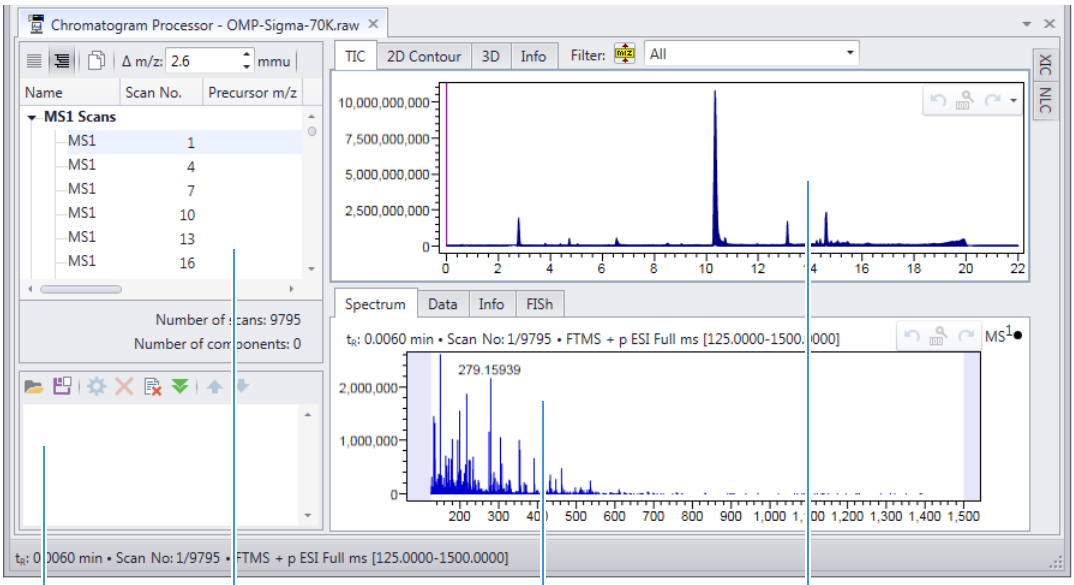
     Note Applying a component detection algorithm to the chromatogram adds a list of detected components to this pane.

   - The chromatogram view 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 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 4. 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, .

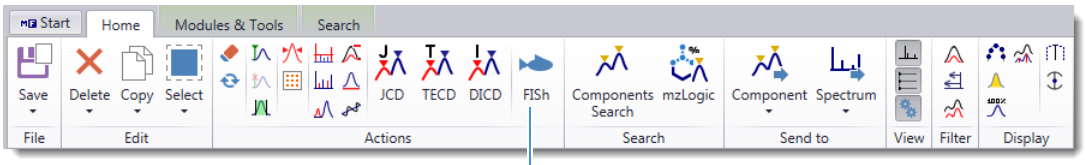
You cannot hide the chromatogram view.

Apply a fragment ion search filter

❖ To apply a fragment ion search filter to the TIC chromatogram

Note The fragment ion search filter that you specify for FISh detection is called the FISh model.

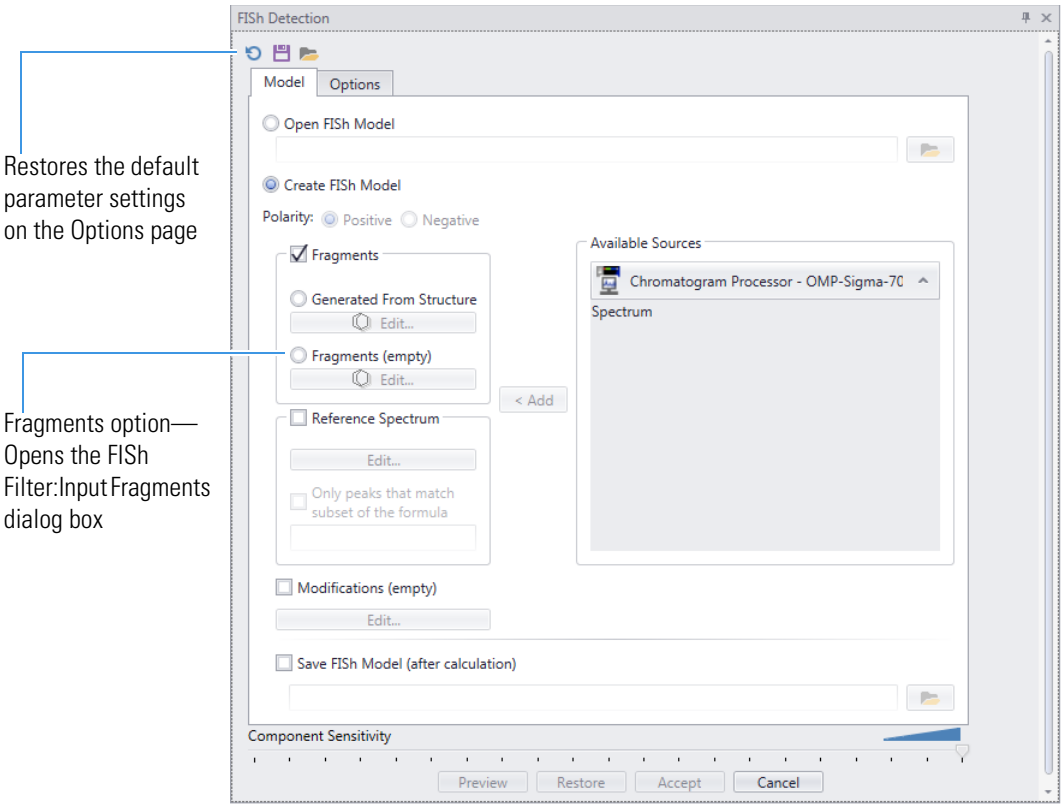
1. In the Actions group of the Chromatogram Processor toolbar, click **FISh**.



The Model page of the FISh Detection view opens to the right of the chromatogram and spectrum views (Figure 5).

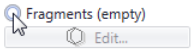
Note You use the Model page to set up the FISh model for the fragment ion search.

Figure 5. Model page of the FISh Detection view with the default settings



2. In the Fragments area, set up the fragments list as follows:

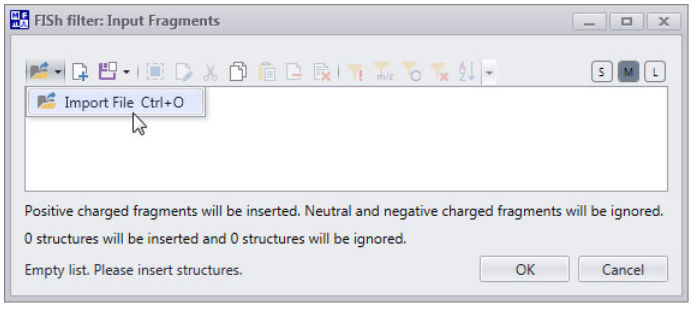
   a. Select the **Fragments** option.

   The FISh Filter: Input Fragments dialog box opens.

   Tip To edit the fragment options after closing the FISh Filter: Input Fragments dialog box, click **Edit**.

   b. Click the **Import** icon, , and then click **Import File**.

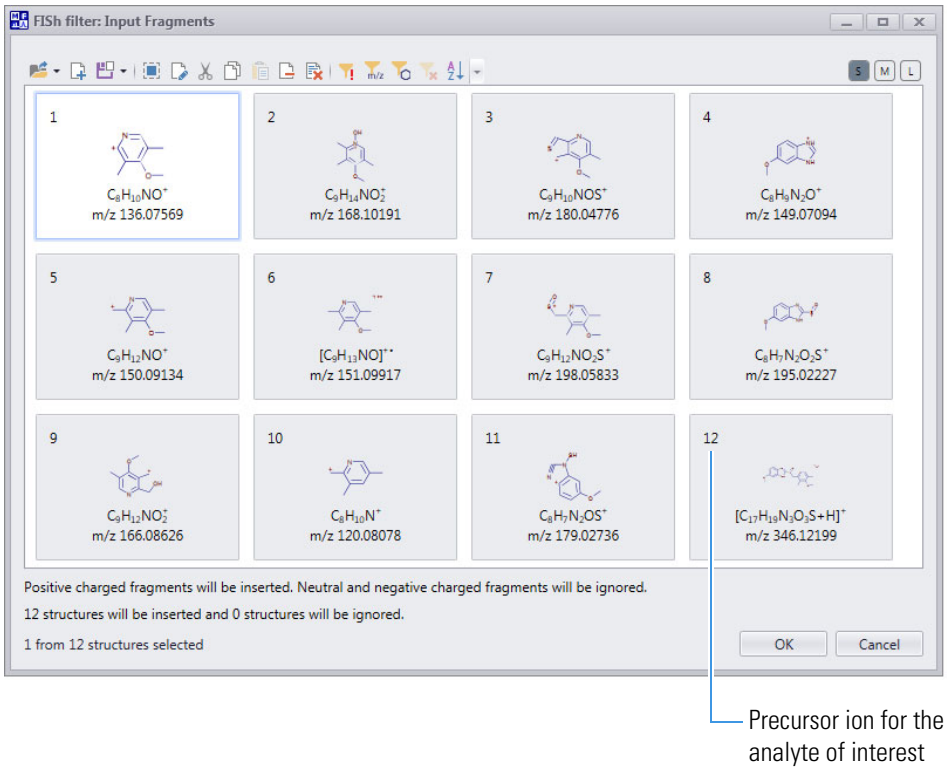
   c. Browse to the following folder, select **Omeprazole FISh Fragments.sdf**, and click **Open**.

   drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.1\Demo Data\Structures

   Twelve structures appear in the dialog box (Figure 6). The structure with the highest *m/z* value (*m/z* 346.12199) is the protonated ion of omeprazole.

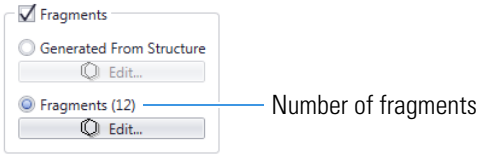
Note For a FISh analysis, make sure that the fragments list includes the analyte's precursor ion.

Figure 6. Fragment structures in the Omeprazole FISh Fragments.sdf file



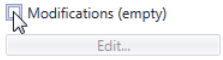
d. Click **OK**.

The number of fragments to use appears to the right of the Fragments option.



3. Add one modification to the FISh model as follows:

a. On the Model page of the FISh Detection view, select the **Modifications** check box.

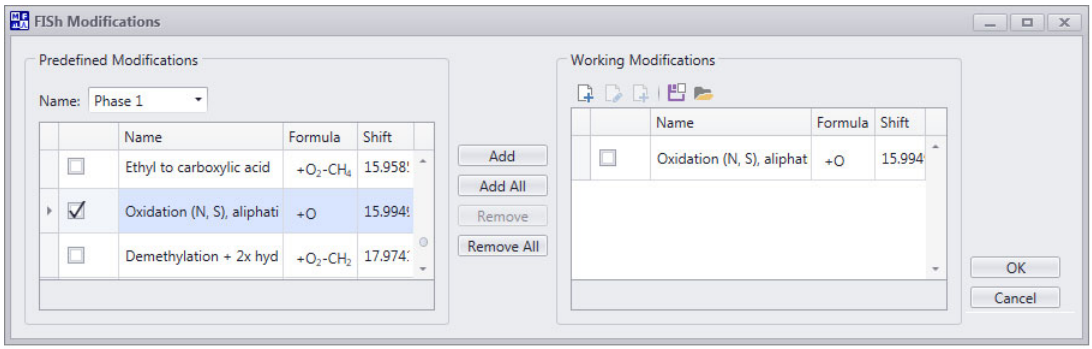


The FISh Modifications dialog box opens.

Tip To edit the FISh modifications after closing the FISh Modifications dialog box, click **Edit**.

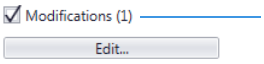
b. In the Predefined Modifications area, select the **Oxidation** (+O) check box, and then click **Add** (Figure 7).

Figure 7. FISh Modifications dialog box with one modification



c. Click **OK**.

The number of modifications to consider appears to the right of the Modifications check box.

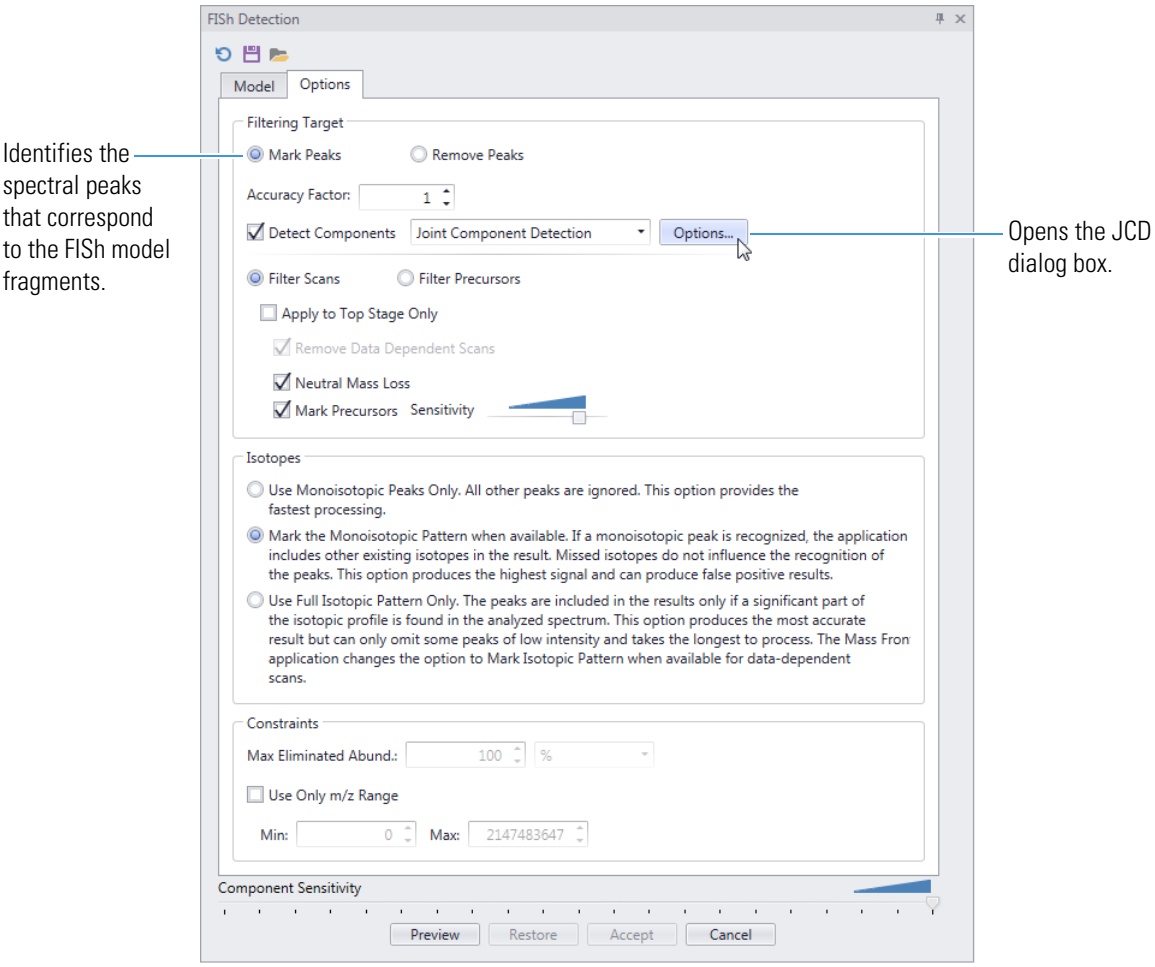


4. To reset the parameter settings on the Options page, click the **Reset** icon, .

Note You use the Options page to specify the component detection algorithm and which spectral peaks you want the application to use to build the FISh chromatogram.

5. Click the **Options** tab to open the Options page (Figure 8). Keep the default settings for selecting which spectral peaks contribute to the ion current at each time point in the FISh chromatogram.

Figure 8. Options page of the FISh Detection view



6. Set up component detection as follows:

   a. On the Options page of the FISh Detection view, click **Options**.

      The JCD dialog box opens (Figure 9).

      By default, all the sliders in the Deconvolution pane of the JCD view are set to the middle.

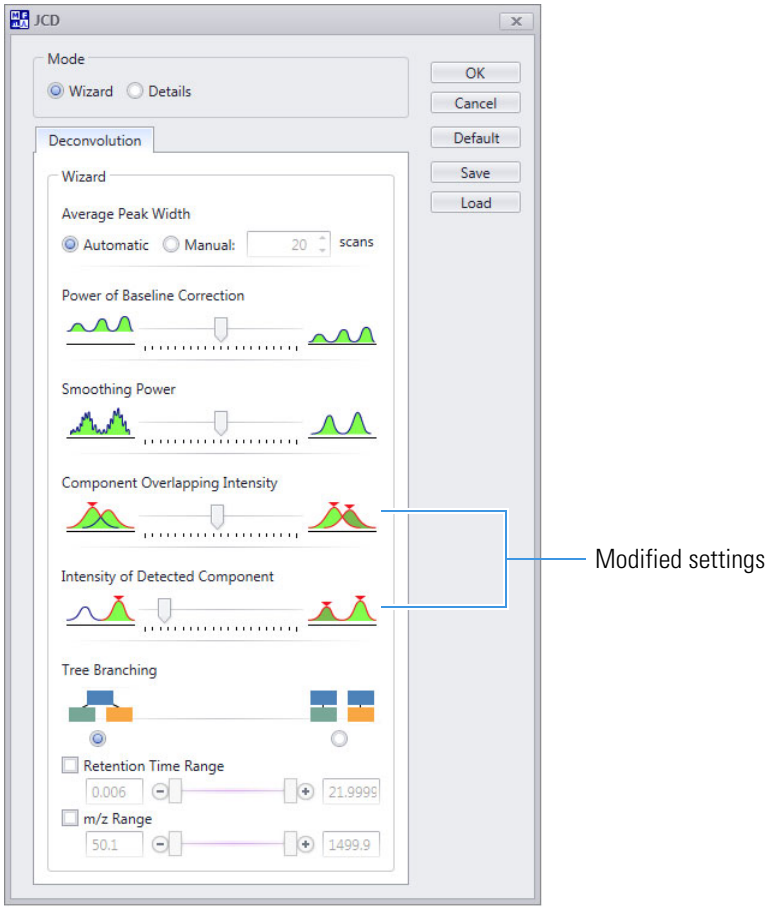
   b. In the JCD dialog box, click **Load**.

   c. Browse to the following folder, select the **OMP-Sigma-70K.chpro.jcd** file, and click **Open**.

      drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.1\Demo Data\Chromatograms

      The selected .chpro.jcd file moves the Intensity of Detected Component slider to the left, which increases the minimum intensity threshold for a component and typically decreases the number of detected components. It also slightly changes the Component Overlapping Intensity setting.

      Figure 9. Component detection settings (from selected file)



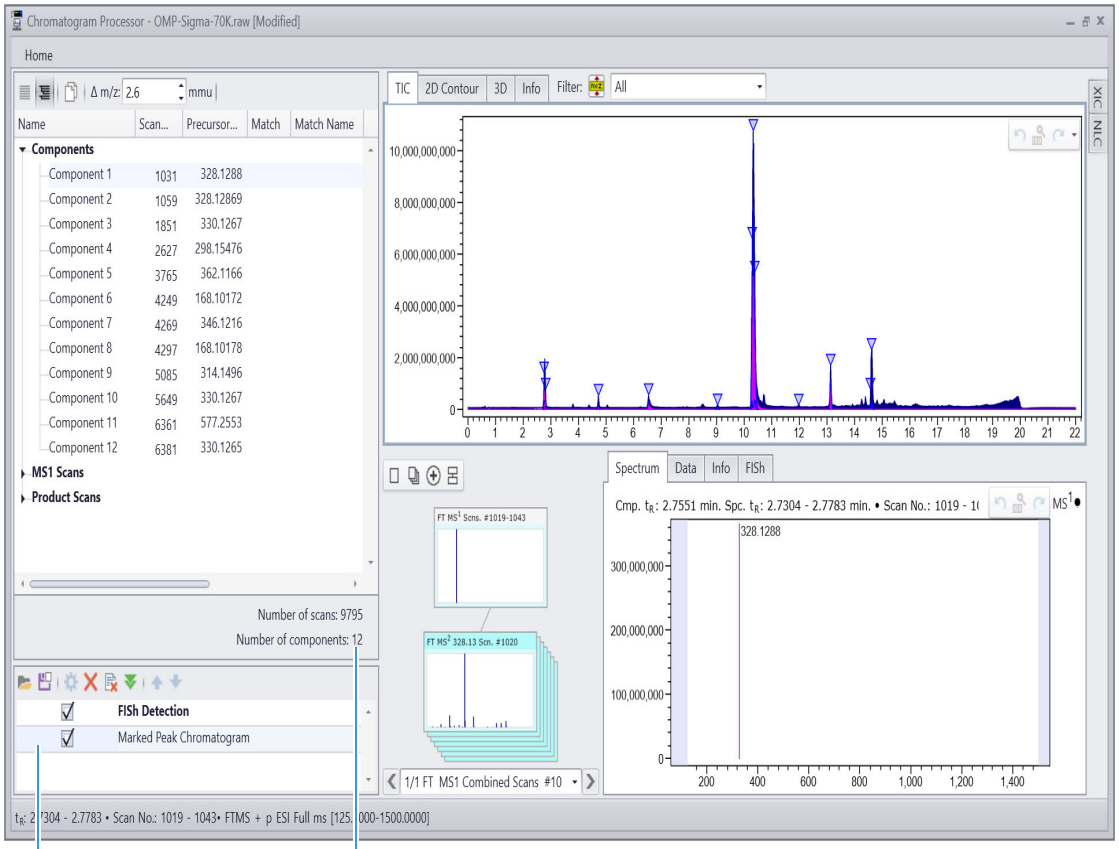
   d. Click **OK**.

7. In the FISh Detection view, click **Preview**.

8. After the analysis finishes, click **Accept**.

The command processor view lists the FISh Detection filter as an applied action. A list of detected components appears in the chromatogram data view, and the blue triangles indicate the detected components.

Figure 10. Accepted FISh analysis



Applied actions in the command processor view

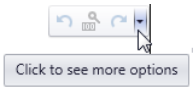
Number of detected components in the chromatogram data view—12

View the FISh trace

Use the options menu to display selected chromatograms.

❖ To view the FISh chromatogram

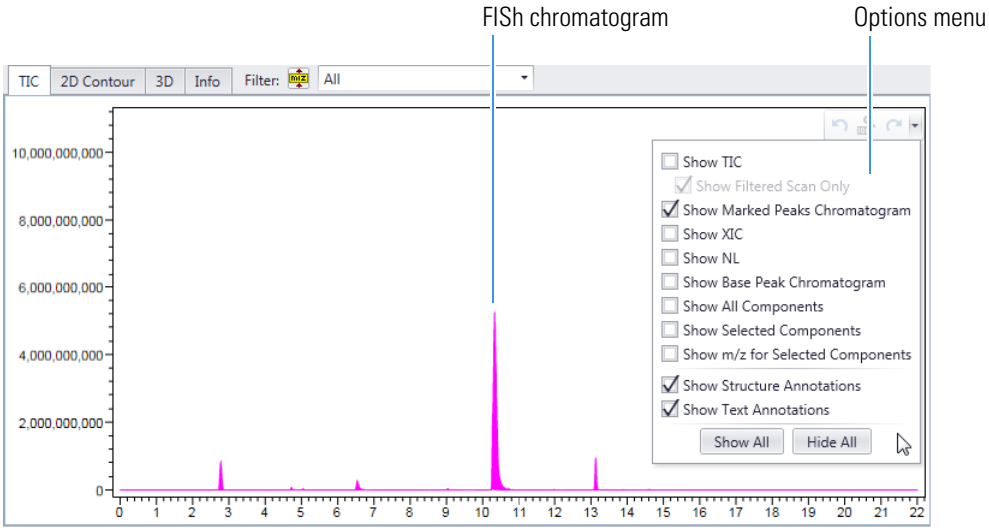
Open the options menu and clear the **Show TIC**, **Show All Components**, and **Show Selected Components** check boxes.



A FISh chromatogram is a TIC chromatogram that is calculated from the identified (marked) *m/z* peaks for the specified FISh model. By default, the application displays calculated TIC chromatograms in pink (Figure 11).

Tip You can change the color settings for chromatogram in the 2D Chromatogram view of the Global Settings dialog box.

Figure 11. FISh chromatogram (marked peaks chromatogram)

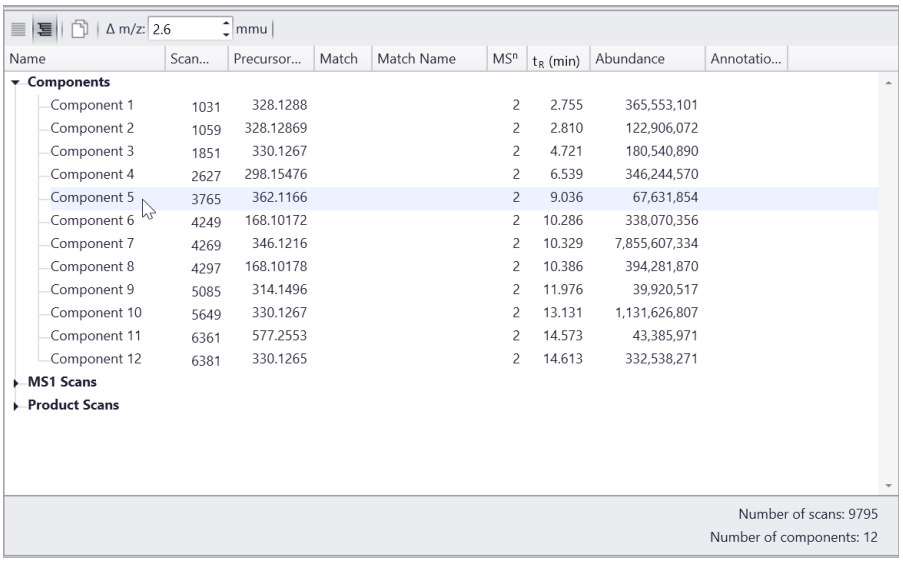


Review the detected components

❖ To inspect the spectra for a detected component

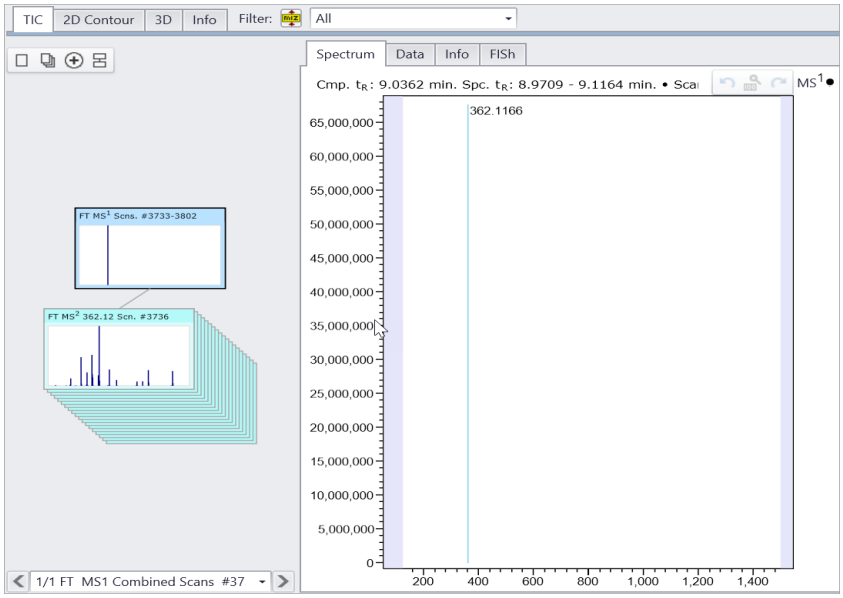
1. In the Components list, select **Component 5** (*m/z* 362.1166 at 9.036 min).

Figure 12. Chromatogram data view with a list of 12 components



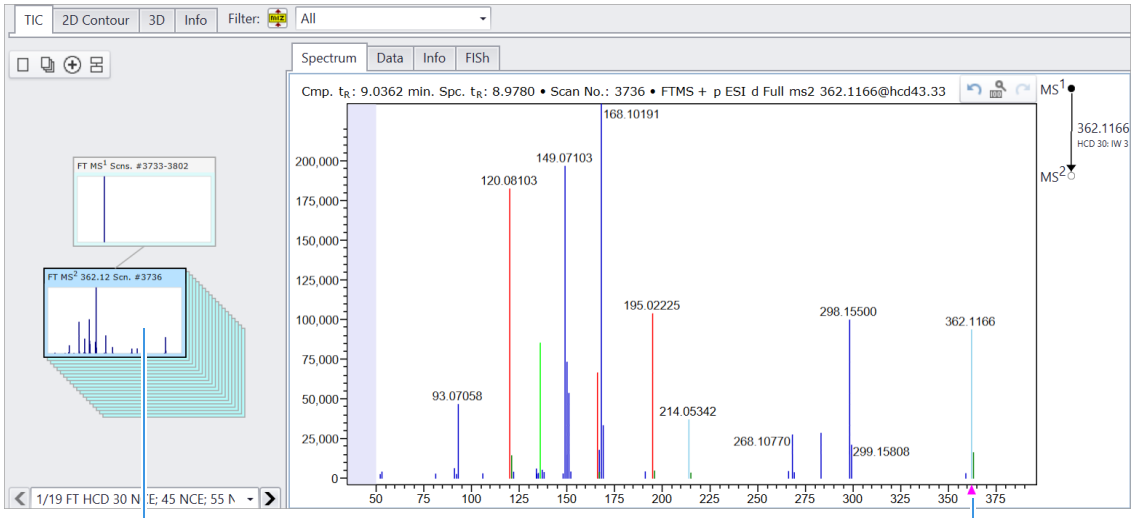
A combined scan for the MS1 scan stage appears on the Spectrum page of the MS spectrum view (Figure 13).

Figure 13. MS spectrum view open to the Spectrum page



2. To display the first MS^2 scan for the selected component, click the MS^2 node of the spectrum tree.

Figure 14. Spectrum page showing scan no. 3736 at full scale



On the Spectrum page, each FISh peak is color-coded:

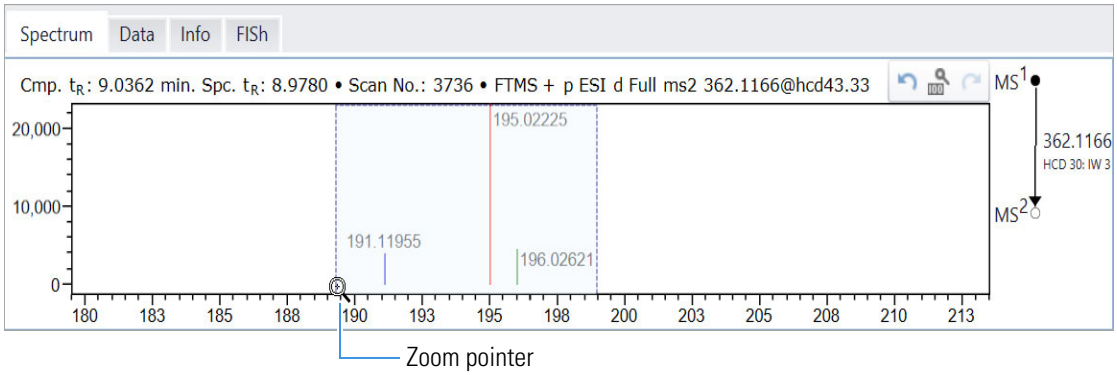
- ( ) Explained — Red indicates that the peak matches a specified fragment.
- ( ) Mass Diff./Modified — Light blue indicates that the peak matches a shifted fragment for a specified modification.
- ( )Neutral Loss — Lime green indicates that the peak matches a specified fragment from a neutral loss.
- ( ) Isotope explained peak — Green indicates that the peak matches an isotopic ion for a specified fragment.

The unexplained peaks are a royal blue .

3. To zoom in on a specific peak, drag the pointer from left to right across a small range of *m/z* values on the *x* axis.

   This figure shows scan no. 3736 with the selection of the *m/z* 191–196 range on the *x* axis.

Figure 15. Scan no. 3736 showing the selection of the *m/z* 191–196 range on the *x* axis



4. To show the explanation for a FISh peak, point to the peak until the triangular marker appears (Figure 16). Then, right-click and choose **Show FISh Explanation *m/z Value*** (Figure 17).

   Figure 16 shows the spectrum peak for *m/z* 195.0219 ( ). The peak is red, which indicates that its *m/z* value matches one of the fragments in the user-specified fragments list (see card 8 in Figure 6).

Figure 16. Triangular marker that appears when you point to a peak

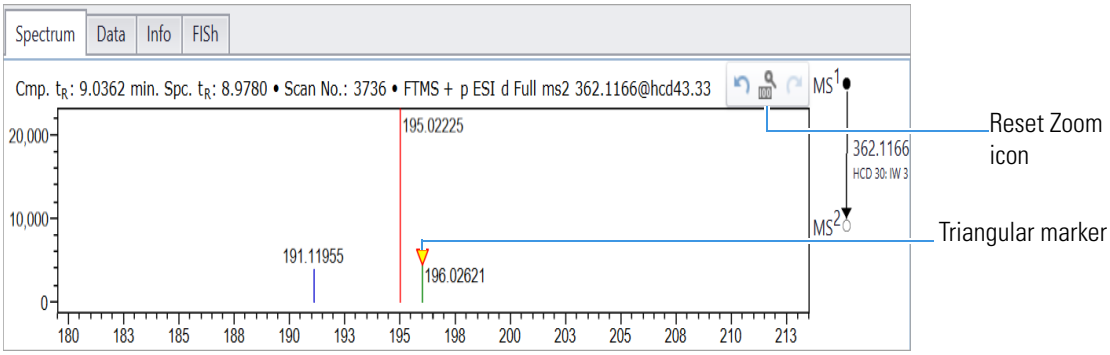
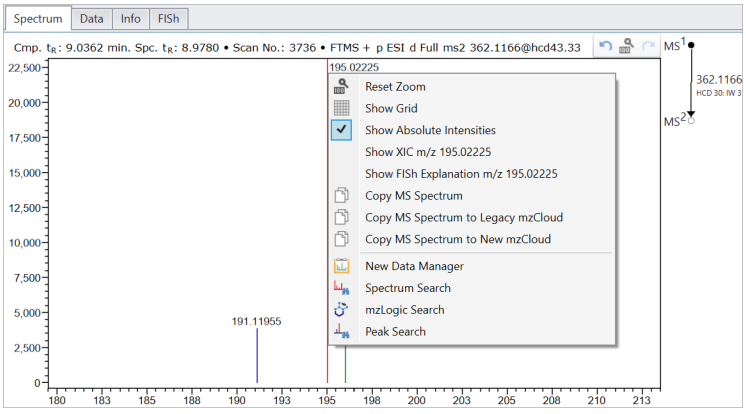
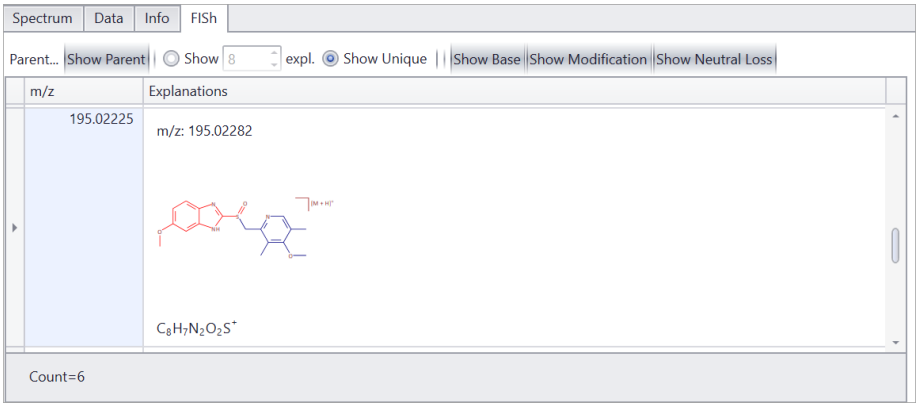


Figure 17. Spectrum shortcut menu



The FISh page opens in the spectrum view and displays the most likely explanation for the product ion (Figure 18). The count number at the bottom of the page is the total number of explanations in the list (with Show Base, Show Modification, and Show Neutral Loss turned on).

Figure 18. Parent (precursor ion) view on the FISh page

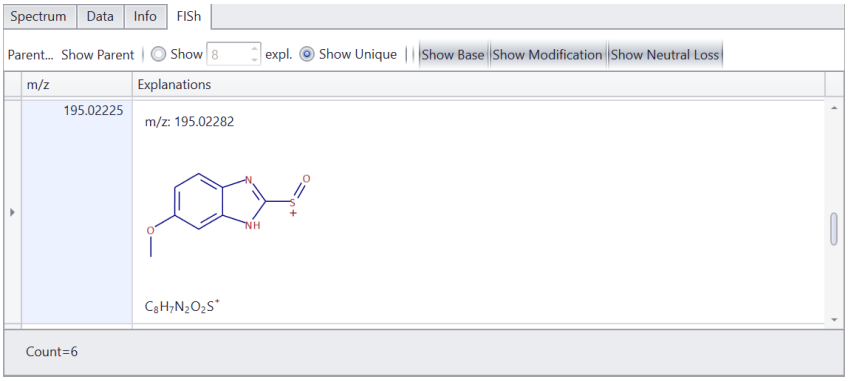


5. To view the fragment structure by itself, click **Show Parent**.

Note Clicking Show Parent toggles the displayed structure between these views:

- The parent structure (precursor ion) with the bonds for the fragment structure (product ion) shown in red
- The fragment structure by itself

Figure 19. Fragment structure (product ion) view on the FISh page of the spectrum view



6. In the MS spectrum view, click the **Spectrum** tab to display the Spectrum page. Then, to reset the zoom level, click the **Reset Zoom** icon, , to the right of the scan header (Figure 16).

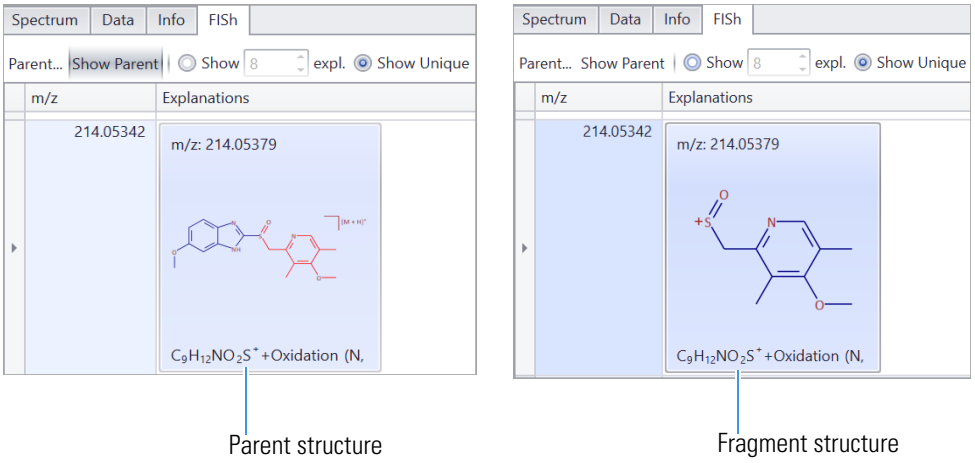
7. To display the explanations for a shifted peak (a fragment with the specified modification), do the following:

   a. On the Spectrum page, zoom in on the *m/z* 188-218 range on the *x* axis.

   b. Point to the light-blue peak at *m/z* 214.05342 until the triangular marker appears. Then, right-click and choose **Show FISh Explanation *m/z* 214.05342**.

Figure 20 shows the "Show Parent" view on the left and the "fragment" view on the right for *m/z* 214.05342 ( ).

Figure 20. FISh explanation for *m/z* 214.05342 (modification = oxidation)



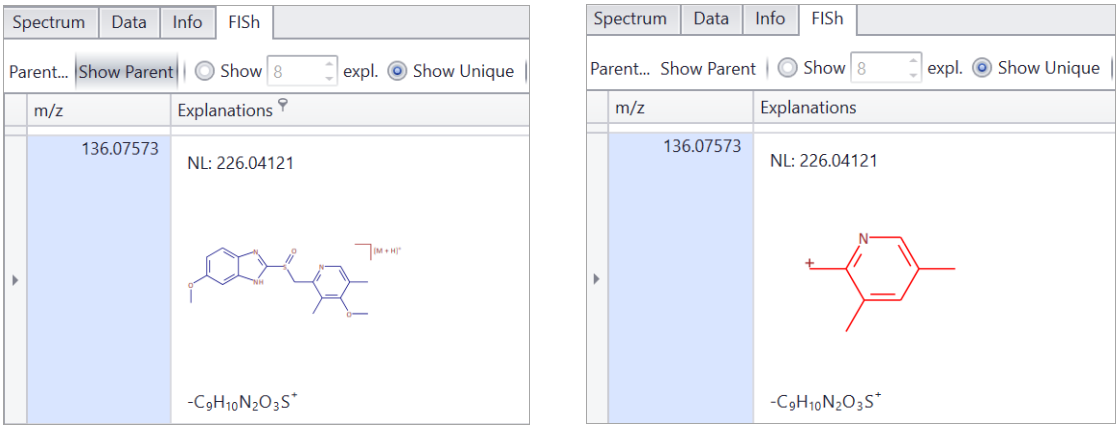
8. To display the explanations for a neutral loss peak (a fragment produced by a neutral loss), do the following:

   a. On the Spectrum page, reset the zoom level, and then zoom in on the *m/z* 133-138 range on the *x* axis.

   b. Point to the lime green peak at *m/z* 136.07573 until the triangular marker appears. Then, right-click and choose **Show FISh Explanation m/z 136.07573.**

Figure 21 shows the Show Parent view on the left and the fragment view on the right for *m/z* 136.07573 ( ).

Figure 21. FISh explanation for *m/z* 136.07573 (fragment from a neutral loss)



Note The application calculates neutral losses by subtracting the *m/z* value for a fragment structure from the highest *m/z* value for a structure in the fragment list on the FISh Model page.

Identify detected components

From the Chromatogram Processor module, you can run a library search to identify the detected components. For this tutorial, run an Identity search against the mzCloud™ mass spectral database. This online database contains entries for omeprazole (the analyte in this tutorial) and its impurities.

IMPORTANT To search the online mzCloud mass spectral database, your processing computer must be connected to the Internet and have unblocked access to the mzCloud server.

❖ To identify components by running a library search

1. (Optional) Run a connection check as follows:
   a. From the application tab bar, click the **Start** tab to open the Start menu, and then click **Connection Check**.
   b. In the Connection Check dialog box, click **Run**.

      The application verifies the connection.
   c. If the connection is successful, go to the next step of this procedure.
   d. If the application cannot connect to the mzCloud server, check the computer's Internet connection and its access to various sites. Also, make sure that the computer is synchronized with Internet time.

   Note Occasionally, the mzCloud Web site goes offline. When this happens, the mzCloud status readback to the right of the application tab bar changes from Online to Offline (in red).

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

   The Component Search view opens to the right of the chromatogram and MS spectrum views.

3. In the Search Type list, select **Identity**.
4. In the Library list, select the **Reference** check box under mzCloud libraries.
5. To run a search for selected components, select the components in the chromatogram data view. For this tutorial, select **Component 5** in the Components list. Then, in the Component Search view, click **Search Selected**.

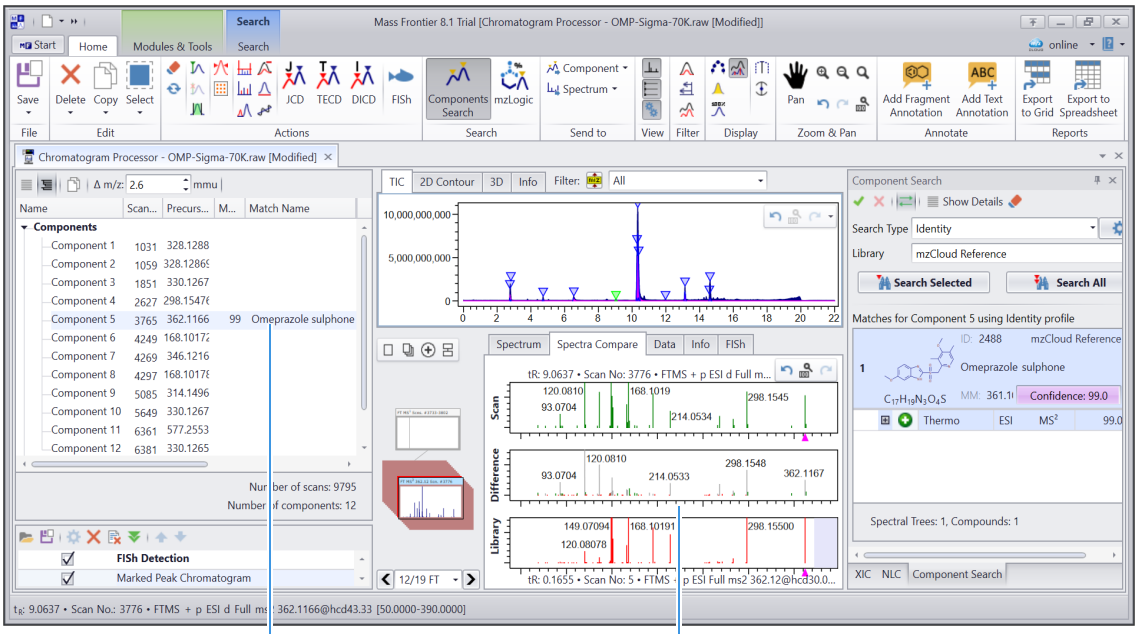
   Note If you do not select a component, the application runs the search on component 1.

   If the search finds a matching precursor *m/z* and spectrum in the spectral library, the following items appear:

   - The match score and compound name appear in the Match column of the chromatogram data view.
   - The Spectra Compare page opens in the MS spectrum view.
   - Information about the matching mzCloud entry appears in the Component Search view.

   Figure 22 shows the search result for component 5 against the mzCloud Reference library.

Figure 22. Search result for component 5



6. To run a search for all the detected components with MS^2 scans, click **Search All**.

Figure 23 shows the result of an mzCloud identity search run in March 2023 against the mzCloud Reference library. As new compounds are constantly being added to the mzCloud library, your results might differ.

Figure 23. Search All results for the mzCloud Reference library

Chromatogram Processor - OMP-Sigma-70K.raw [Modified]

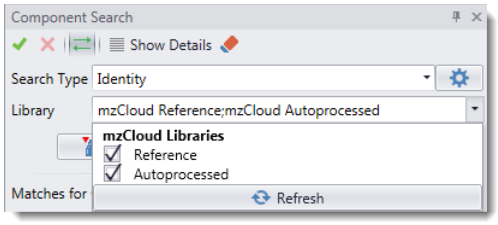
Δ m/z: 2.6 mmu

Name	Scan No.	Precursor m/z	Match	Match Name	MS^n	t_R (min)	Abundance	Annotation...
Components								
Component 1	1031	328.1288	0		2	2.755	365,553,101	Identity
Component 2	1059	328.12869	0		2	2.810	122,906,072	Identity
Component 3	1851	330.1267	0		2	4.721	180,540,890	Identity
Component 4	2627	298.15476	9	N 2 Morpholinophenyl N phenylurea	2	6.539	346,244,570	Identity
Component 5	3765	362.1166	99	Omeprazole sulphone	2	9.036	67,631,854	Identity
Component 6	4249	168.10172	59	Anhydroecgonine	2	10.286	338,070,356	Identity
Component 7	4269	346.1216	65	Omeprazole	2	10.329	7,825,801,948	Identity
Component 8	4297	168.10178	62	Anhydroecgonine	2	10.386	394,281,870	Identity
Component 9	5085	314.1496	36	4 2 Methoxyphenyl 1 3 nitropyridin 2 yl piperidine	2	11.976	39,920,517	Identity
Component 10	5649	330.1267	0		2	13.131	1,131,626,807	Identity
Component 11	6361	577.2553	0		2	14.573	43,385,971	Identity
Component 12	6381	330.1265	0		2	14.613	332,538,271	Identity

7. To search both the mzCloud Reference and Autoprocessed libraries, do the following in the Component Search view:

   a. Open the Library list.

   b. Select the **Reference** and **Autoprocessed** check boxes.

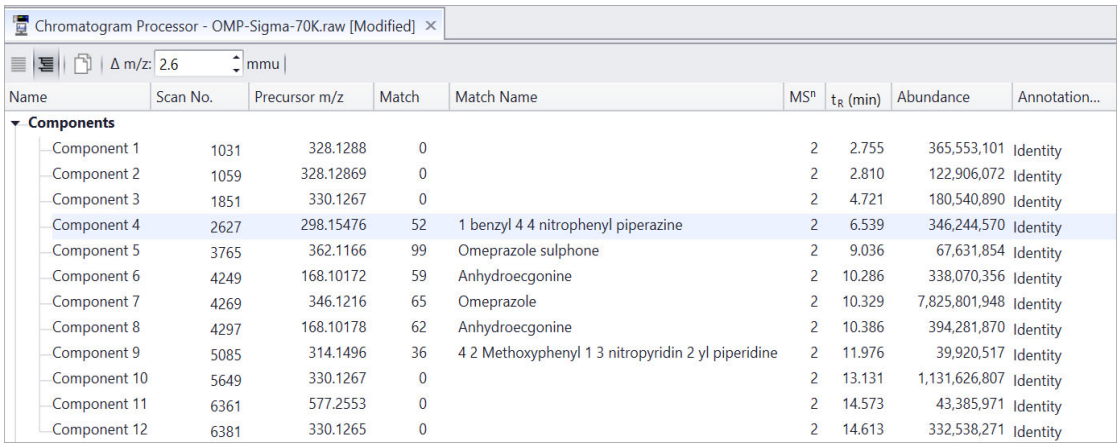


   c. Click **Search All**.

> **Note** The Reference library contains compounds with spectra that have been fully curated by mass spectrometry specialists. The Autoprocessed library contains compounds with spectra that have only been automatically processed with a curation software application.

The result for component 4 changed because the search found a compound with a higher match score in the Autoprocessed library.

Figure 24. Search All results for the mzCloud Reference and Autoprocessed libraries



Name	Scan No.	Precursor m/z	Match	Match Name	MS^n	t_R (min)	Abundance	Annotation...
Components								
Component 1	1031	328.1288	0		2	2.755	365,553,101	Identity
Component 2	1059	328.12869	0		2	2.810	122,906,072	Identity
Component 3	1851	330.1267	0		2	4.721	180,540,890	Identity
Component 4	2627	298.15476	52	1 benzyl 4 4 nitrophenyl piperazine	2	6.539	346,244,570	Identity
Component 5	3765	362.1166	99	Omeprazole sulphone	2	9.036	67,631,854	Identity
Component 6	4249	168.10172	59	Anhydroecgonine	2	10.286	338,070,356	Identity
Component 7	4269	346.1216	65	Omeprazole	2	10.329	7,825,801,948	Identity
Component 8	4297	168.10178	62	Anhydroecgonine	2	10.386	394,281,870	Identity
Component 9	5085	314.1496	36	4 2 Methoxyphenyl 1 3 nitropyridin 2 yl piperidine	2	11.976	39,920,517	Identity
Component 10	5649	330.1267	0		2	13.131	1,131,626,807	Identity
Component 11	6361	577.2553	0		2	14.573	43,385,971	Identity
Component 12	6381	330.1265	0		2	14.613	332,538,271	Identity

Save the results to an HCCX file

You can save the component detection and component annotation results to an HCCX file.

❖ To save the results to an HCCX file

1. In the File group of the Chromatogram Processor toolbar, click **Save**, and then click **Chromatogram As**.

2. Select a file location, name the file, and click **Save**.

> **Tip** You can save the intermediate and final component detection and annotation results to HCCX files so you can return to those results at a later time. Saving HCCX files lets you return to a specific results state, and then perform different processing actions on the data.

Create and edit a fragments list

In this tutorial, you used a predefined fragments list to create a FISh model for the analyte of interest. This topic shows you how to create a fragments list with an appropriate set of fragments for the analyte of interest in this tutorial—omeprazole.

> **Note** To create a fragments list, you can use the Batch Fragment Generation wizard or the SledgeHammer module.

Follow these topics in order:

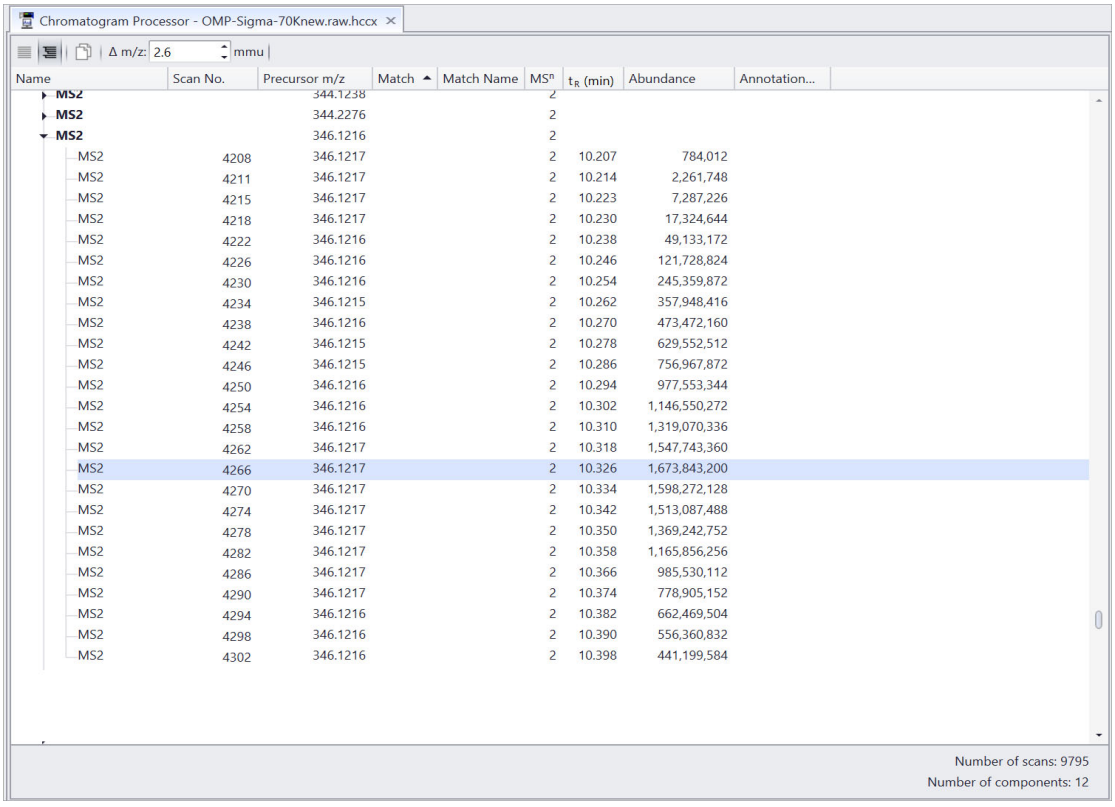
1. create a fragments list and save it to an SDF file
2. Edit a fragments list

create a fragments list and save it to an SDF file

❖ **To create a fragments list and save it to an SDF file**

1. On the Spectrum page of the MS spectrum view, display the highest abundance MS2 scan for omeprazole (precursor *m/z* 346) by selecting this scan from the MS2 Scans list in the chromatogram data view.

   Figure 25. Selecting scan no. 4266—the highest abundance MS2 scan for omeprazole

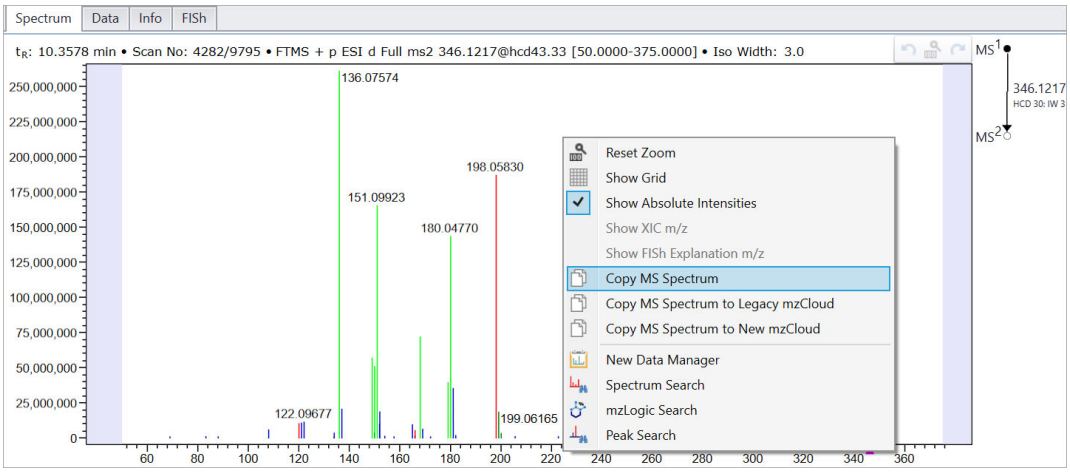


Name	Scan No.	Precursor m/z	Match	Match Name	MS^n	t_R (min)	Abundance	Annotation...
MS2		344.1238			2			
MS2		344.2276			2			
MS2		346.1216			2			
MS2	4208	346.1217			2	10.207	784,012	
MS2	4211	346.1217			2	10.214	2,261,748	
MS2	4215	346.1217			2	10.223	7,287,226	
MS2	4218	346.1217			2	10.230	17,324,644	
MS2	4222	346.1216			2	10.238	49,133,172	
MS2	4226	346.1216			2	10.246	121,728,824	
MS2	4230	346.1216			2	10.254	245,359,872	
MS2	4234	346.1215			2	10.262	357,948,416	
MS2	4238	346.1216			2	10.270	473,472,160	
MS2	4242	346.1215			2	10.278	629,552,512	
MS2	4246	346.1215			2	10.286	756,967,872	
MS2	4250	346.1216			2	10.294	977,553,344	
MS2	4254	346.1216			2	10.302	1,146,550,272	
MS2	4258	346.1216			2	10.310	1,319,070,336	
MS2	4262	346.1217			2	10.318	1,547,743,360	
MS2	4266	346.1217			2	10.326	1,673,843,200	
MS2	4270	346.1217			2	10.334	1,598,272,128	
MS2	4274	346.1217			2	10.342	1,513,087,488	
MS2	4278	346.1217			2	10.350	1,369,242,752	
MS2	4282	346.1217			2	10.358	1,165,856,256	
MS2	4286	346.1217			2	10.366	985,530,112	
MS2	4290	346.1217			2	10.374	778,905,152	
MS2	4294	346.1216			2	10.382	662,469,504	
MS2	4298	346.1216			2	10.390	556,360,832	
MS2	4302	346.1216			2	10.398	441,199,584	

   The spectrum appears on the Spectrum page (Figure 26 on page 21).

2. Right-click the spectrum and choose **Copy MS Spectrum**.

Figure 26. Scan no. 4266



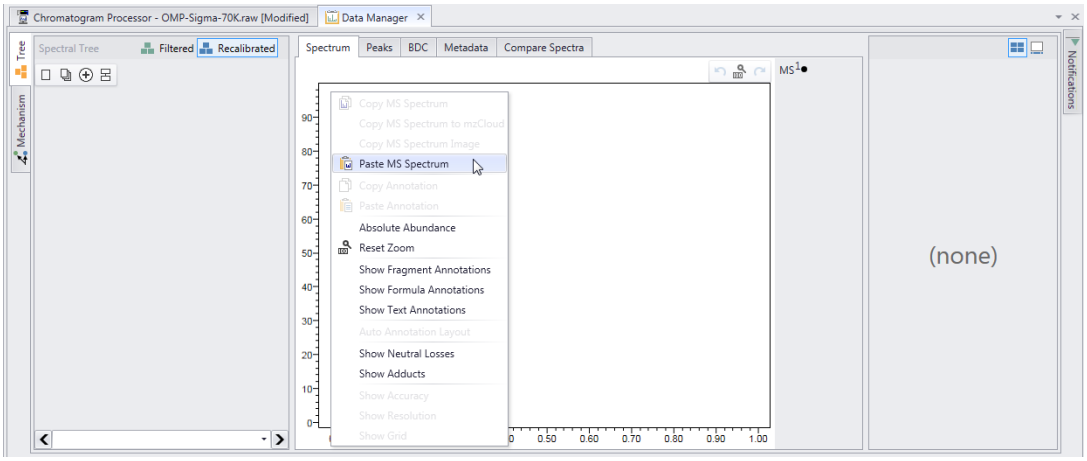
The application copies the spectrum to the Clipboard.

3. In the Modules & Tools toolbar, click **Data Manager**.

   A Data Manager window opens.

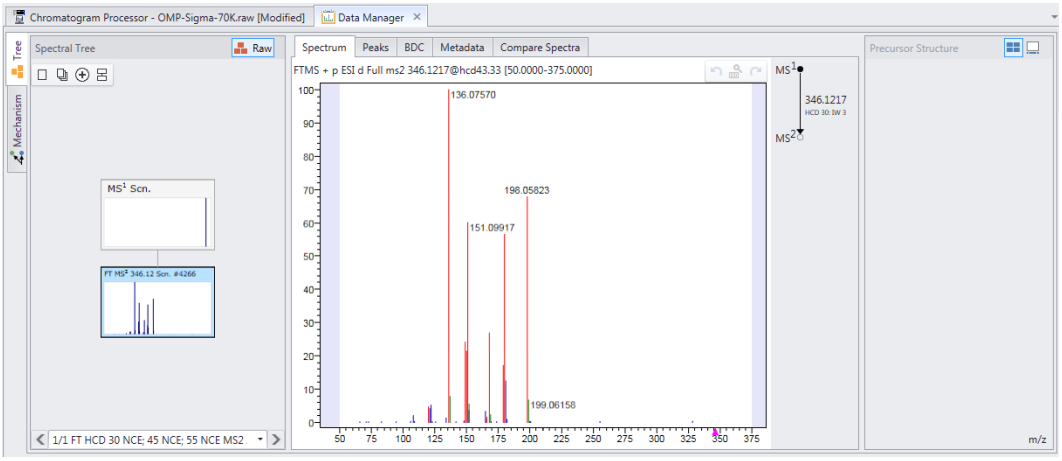
4. In the Data Manager window, right-click the Spectrum page and choose **Paste MS Spectrum**.

Figure 27. Pasting an MS Spectrum from the Clipboard to a Data Manager window



The spectrum appears on the Spectrum page and the tree for this spectrum appears in the Spectral Tree pane (Figure 28 on page 22).

Figure 28. Pasted spectrum



5. To specify the precursor structure for this spectrum, do the following:

   a. Double-click the **Precursor Structure** pane.

      The Structure Editor opens.

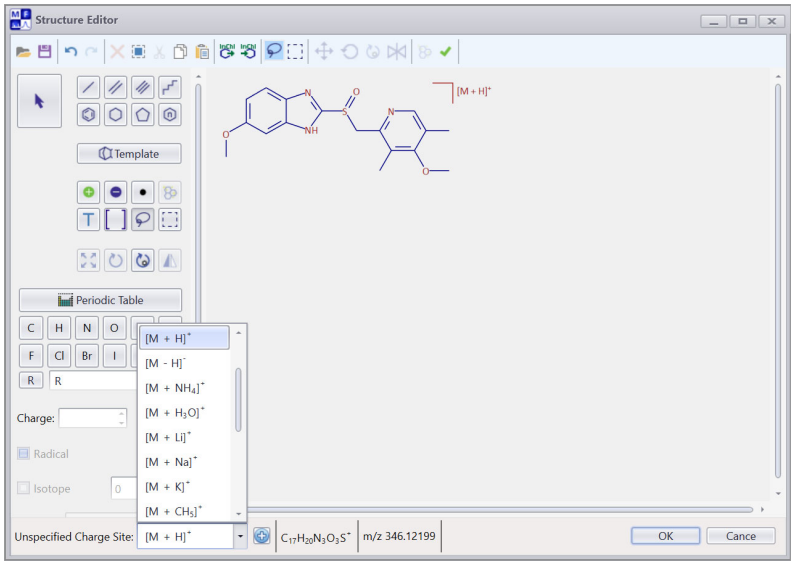
   b. Click the **Open** icon, , and select the omeprazole.mol file in the following folder, and open the file.

      drive:\Users\Public\Public Documents\Highchem\Mass Frontier 8.11\Demo Data\Structures

      The structure for omeprazole appears in the drawing area of the Structure Editor.

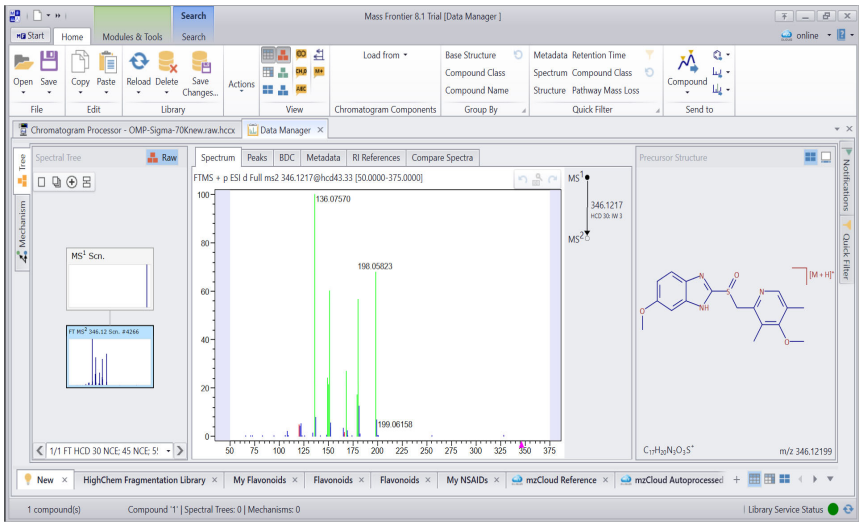
   c. To add a charge to the structure, select **[M + H]$^+$** from the dropdown Unspecified Charge Site list.

      Figure 29. Adding a charge to omeprazole



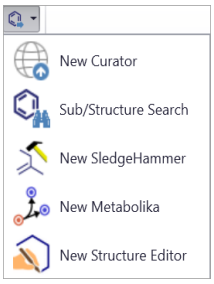
   d. Click **OK**.

The protonated ion of omeprazole appears in the Precursor Structure pane.



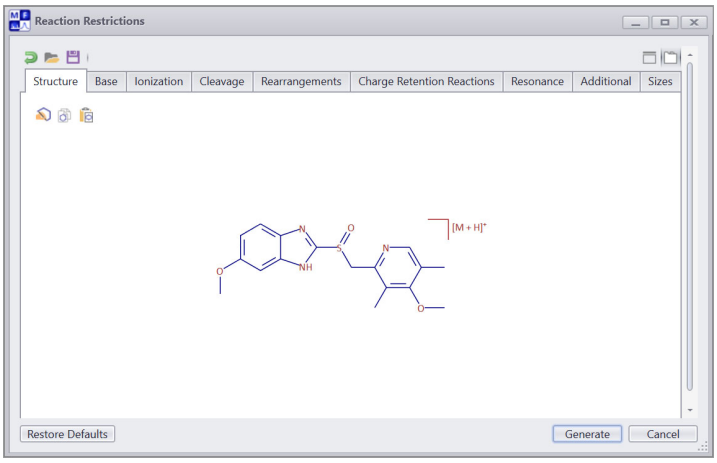
6. To generate the fragments for this structure, do the following:

   a. In the Send To group of the Data Manager toolbar, click **Structure** and select **New SledgeHammer** from the dropdown list.



   The Reaction Restrictions dialog box opens, showing the protonated ion of omeprazole on the Structure page.

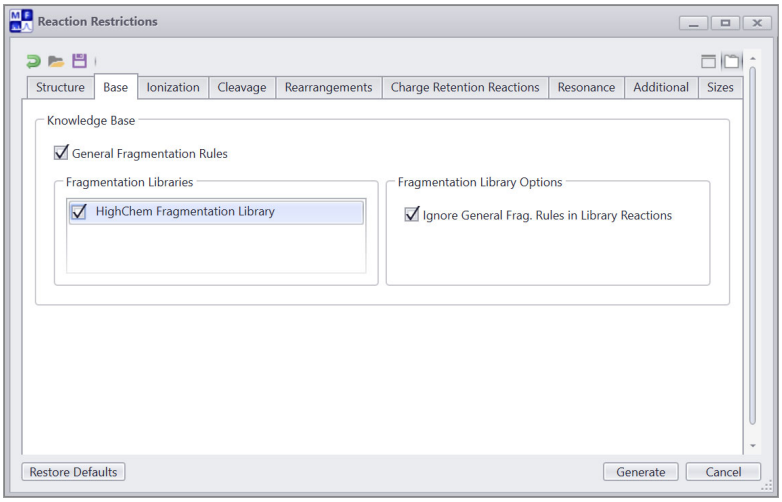
   Figure 30. Reaction Restrictions dialog box with the protonated ion of omeprazole



   b. Click the **Base** tab to open the Base page.

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

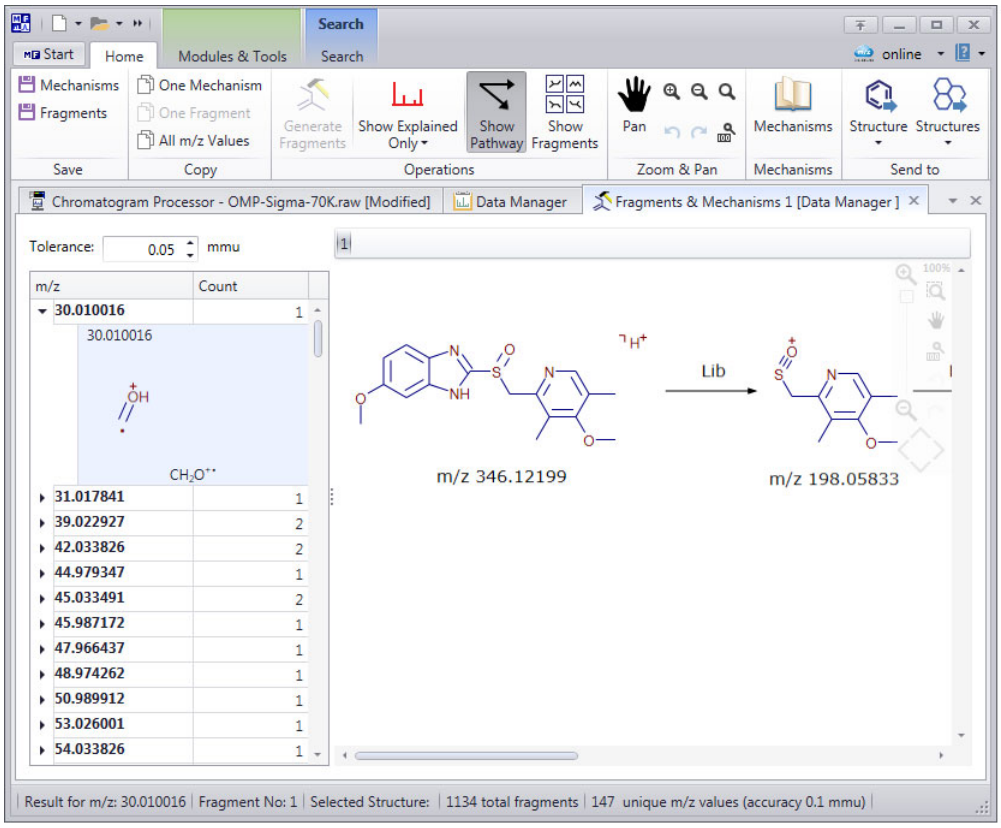
Figure 31. Base page



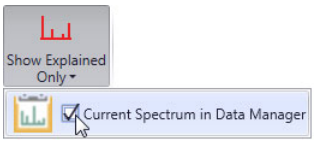
d. Click **Generate**.

The Fragment Generation progress box appears. When the fragmentation finishes, a new SledgeHammer window opens as a tabbed page.

Figure 32. SledgeHammer page with 1134 fragments for 147 unique *m/z* values

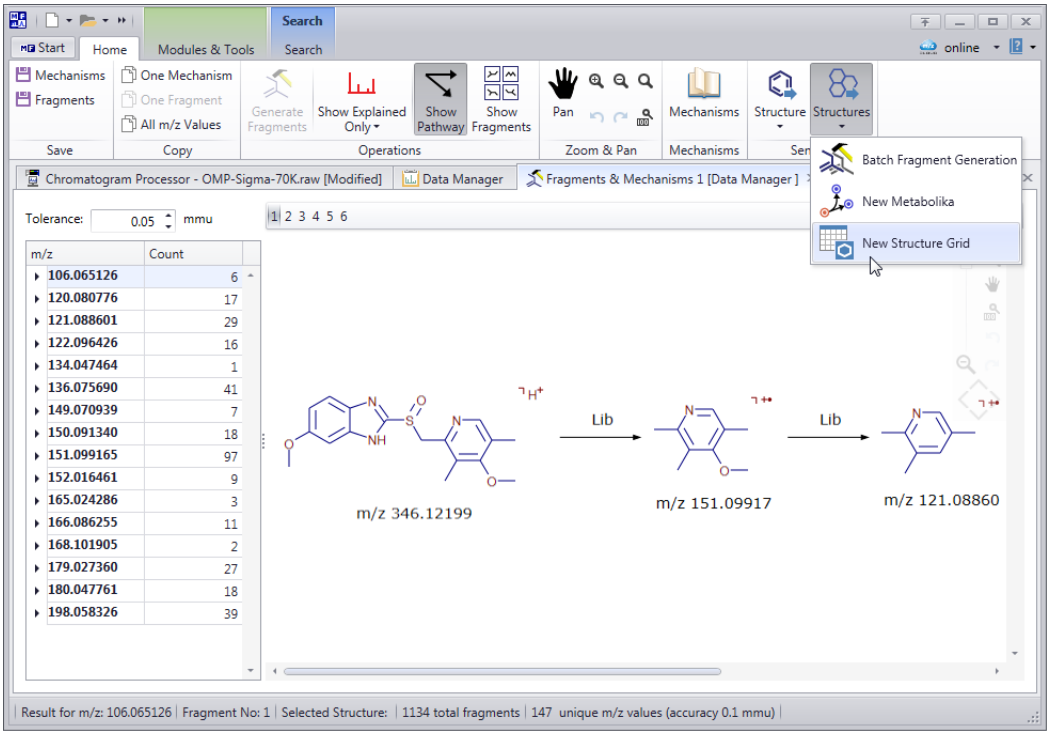


7. To reduce the number of fragments to those explained by the FISh peaks, in the Operations group of the Fragments & Mechanisms toolbar, click **Show Explained Only** and select the **Current Spectrum in Data Manager** check box.



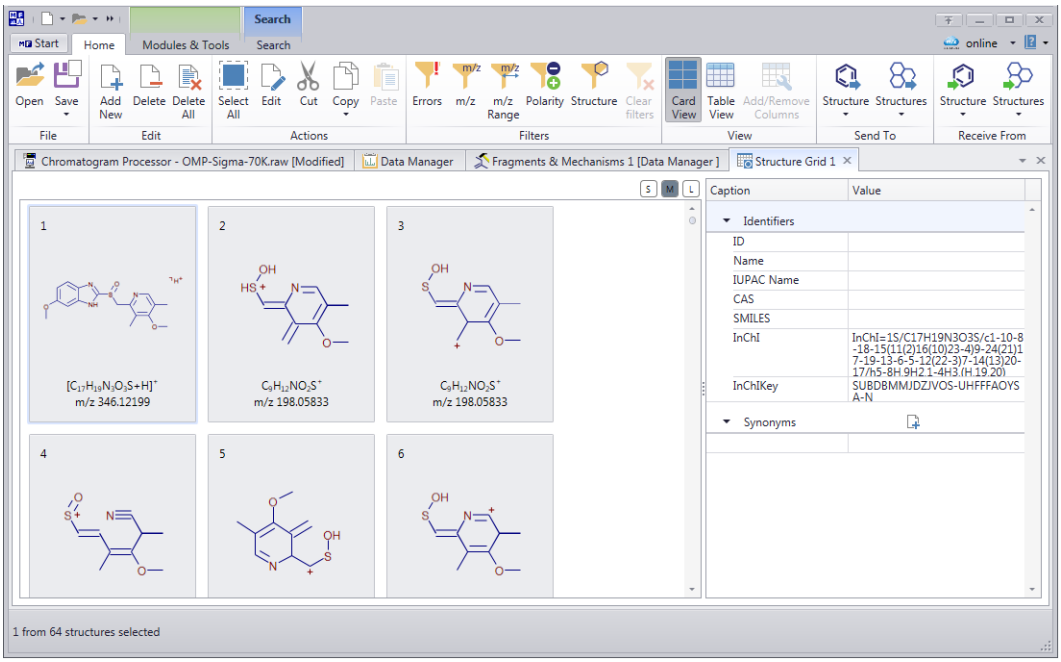
The number of unique *m/z* values decreases to 16.

8. To send the structures to a new Structure Grid, in the Send To group of the Fragments & Mechanisms toolbar, click **Structures**, and then select **New Structure Grid** from the dropdown list.



The new Structure Grid opens as a tabbed page (Figure 33 on page 26).

Figure 33. Structure Grid with fragment structures in the 106 –198 *m/z* range



9. Save the structures to a structure file as follows:

   a. In the Structure Grid toolbar, click **Save**, and then select **All** from the dropdown list.

   b. In the Save Structures dialog box, name the file and select its folder.

   c. Record the file name and location for this structure file, as the next topic describes how to reopen the file and edit it.

Edit a fragments list

This procedure describes how to edit a list of fragments.

❖ To edit a fragments list

1. In the Modules & Tools toolbar, click **Structure Grid**.

   A new instance of the Structure Grid module opens as a tabbed document.

2. In the Structure Grid toolbar, click **Open**.

3. Browse to the SDF file that you created in the previous topic, "create a fragments list and save it to an SDF file," and click **Open**.

4. If you are editing the structure list that you created in the previous procedure, skip step 5 and step 6.

> **Note** In the previous procedure, you created a structure grid from a set of FISh fragments, so the grid does not include any low *m/z* value fragments or fragments that do not match the parent structure.

5. In the Structure Grid toolbar, use the filters and the Delete button to remove structures that can lead to false positives:
   - Filter out fragments with low *m/z* values.
   - Delete fragments that do not match any part of the parent structure.
6. In the Structure Grid toolbar, use the Delete button to Remove duplicates.
7. If the fragments list does not include the parent precursor ion, add it. To add the protonated ion of omeprazole (*m/z* 346) for the tutorial example, do the following:
   a. In the Structure Grid toolbar, click **Add New**.

      The Structure Editor opens.

   b. In the Structure Editor, click the **Open** icon,  , select the omeprazole.mol file, and click **Open**.

   c. Protonate the omeprazole structure by adding an unspecified charge site (select **H^+**).

   d. Click **OK**.

   Note The FISh analysis uses the parent precursor ion for the Mark Precursors feature and for calculating the neutral loss masses.
8. In the toolbar, click **Save**, and then click **All**.
9. Locate the appropriate folder, name the edited fragments list, and click **Save**.