Curating Spectra for a User Library

In the Mass Frontier™ 8.0 SR1 application, you use the Chromatogram Processor module to detect and identify components in your chromatographic data and the Curator module to create high-quality library entries for the identified components of interest.

This tutorial steps you through the component detection and mass spectra curation processes.

Contents

Prerequisites

To complete the procedures in this tutorial, your installation of the application must have the required software and licenses.

Required Software

This tutorial uses the Server Manager 3.0 application to create a user library. Make sure you have installations of both the Mass Frontier 8.0 SR1 and Server Manager 3.0 applications on your processing computer.

Required Licenses

This tutorial uses the Curator module to perform the curation process. Make sure you have licenses for all the Mass Frontier modules, including the Curator module.

Note Thermo Fisher Scientific provides the following types of licenses for the Mass Frontier 8.0 application:

- 60-day trial version—The trial version of the application includes licenses to all the modules, including the Curator module.
- Permanent versions—You can purchase the permanent version of the application with or without the Curator module license. Refer to the *Mass Frontier Release Notes* for more information.

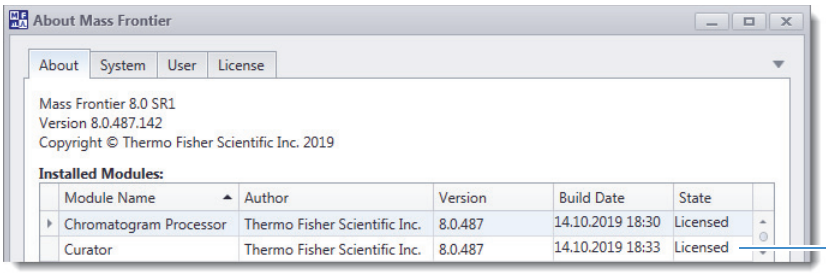
❖ To check the state of module licenses

1. From the application tab bar, click the **Start** tab and then choose **About**. See Figure 1 on page 2.

   The About Mass Frontier dialog box opens to the About page, which lists the installed modules.

2. Make sure that the Curator module is licensed.

Figure 1. About Mass Frontier dialog box



Demo Data Files

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

File	Description
Aceclofenac B02.raw	A raw data file acquired by infusing a solution of aceclofenac into an Orbitrap Fusion Lumos™ mass spectrometer. Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID).
Aceclofenac.mol	A structure file that contains the molecular structure for aceclofenac
Aceclofenac_Fragments.sdf	A structure file that contains fragment structures for the parent compound—aceclofenac
Aceclofenac.chpro_direct	Parameters file that contains the DICD parameter settings for the example raw data file
Aceclofenac.curator_srp	Curator parameters file for the Select Relevant Peaks action step

Creating a User Library

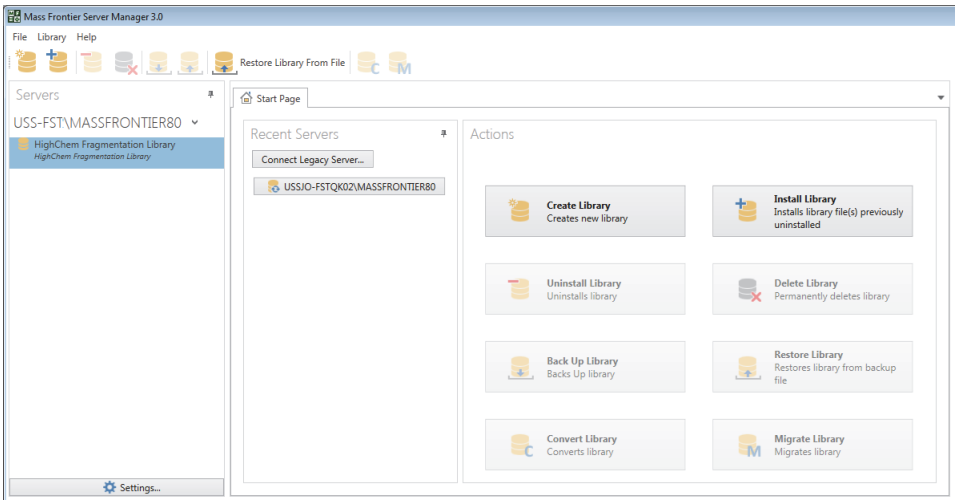
The Mass Frontier application uses a library service to connect to its mass spectral libraries. To add compound entries to a Mass Frontier user library, you must first create the library and connect the library service to it.

Use the Server Manager 3.0 application to create user libraries for your mass spectral data.

❖ To create a user library

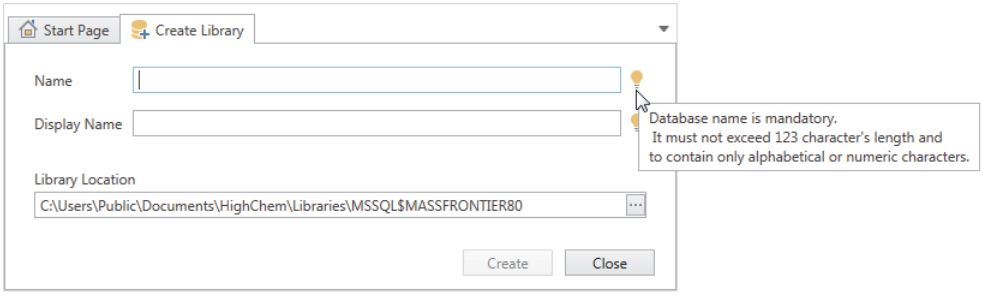
1. To open the Server Manager, do one of the following:
   - From the Windows™ Start menu, choose **Thermo Mass Frontier 8.0 > Mass Frontier Server Manager 3.0**.
   - From the desktop, double-click the **Mass Frontier Server 3.0** icon,  .

Figure 2. Server Manager 3.0 window



2. In the Actions pane, of the Start Page, click **Create Library**.

   The Create Library page opens. You must enter the database name in the Name box; the display name is optional.



3. For this tutorial, do the following:

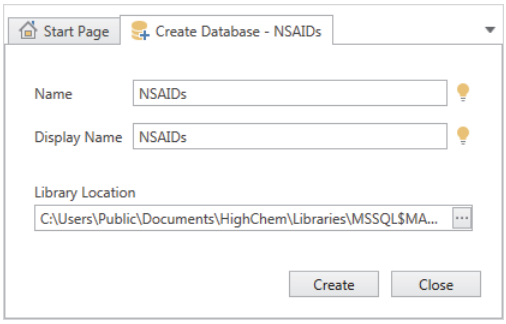
   a. In the Name box, type **NSAIDs**.

      The Create Library tab changes to the Create Database - *Database Name* tab.

   b. In the Display Name box, type **NSAIDs**.

> **Note** The database name and the display name can contain only alphabetical and numeric characters and cannot be more than 123 or 128 characters in length, respectively.

Figure 3. Database name and display name



4. Click **Create**.

5. Close the Server Manager application.

Opening a Raw Data File and Detecting Components

In the Mass Frontier application, you use the Chromatogram Processor module to read an Xcalibur™ RAW data file, detect components in the chromatographic data, and inspect the spectral tree for each detected component.

Follow these topics in order:

1. Opening a Raw Data File
2. Detecting Components
3. Reviewing the Detected Component

Opening a Raw Data File

❖ **To open a raw data file**

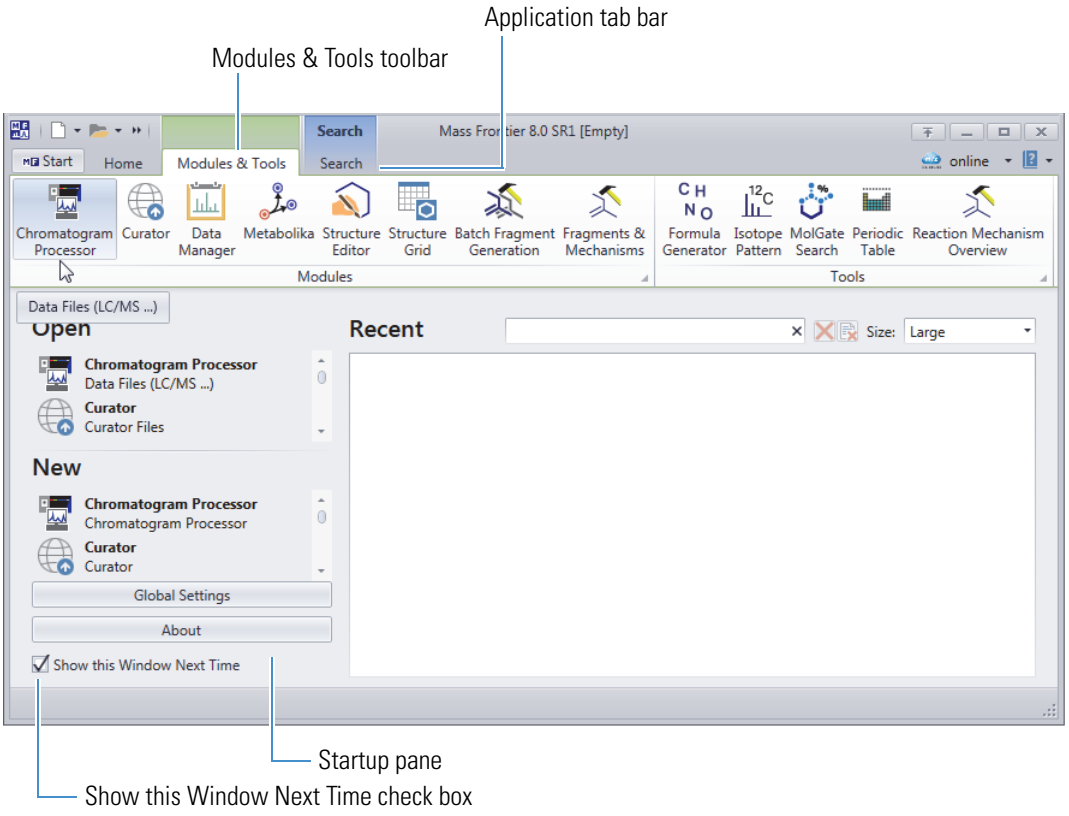
1. Open the Mass Frontier application by double-clicking its desktop icon,  , or by choosing **Thermo Mass Frontier 8.0 > Mass Frontier 8.0** from the Windows™ Start menu.

The first time you open the application after installing it, the application opens to the startup window with the Modules & Tools toolbar at the top and the startup pane at the left (Figure 4).

The application tab bar contains these four tabs:

- Start—Displays the Start menu.
- Home—Displays the toolbar for the active module or tool.
- Modules & Tools—Displays the Modules & Tools toolbar.
- Search—Displays the Search toolbar.

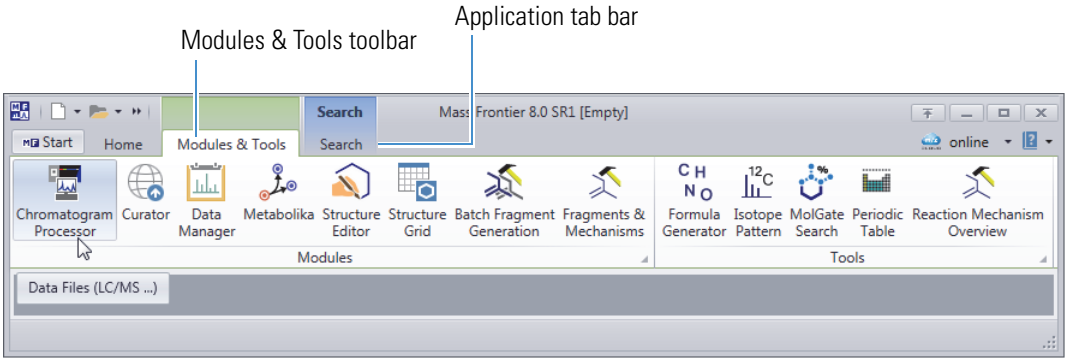
Figure 4. Mass Frontier startup window



Tip 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 (Figure 5).

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

Figure 5. Application window with the Modules & Tools toolbar displayed but without the startup pane



3. In the Open Chromatogram dialog box, do the following:

   a. Browse to the following folder:

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

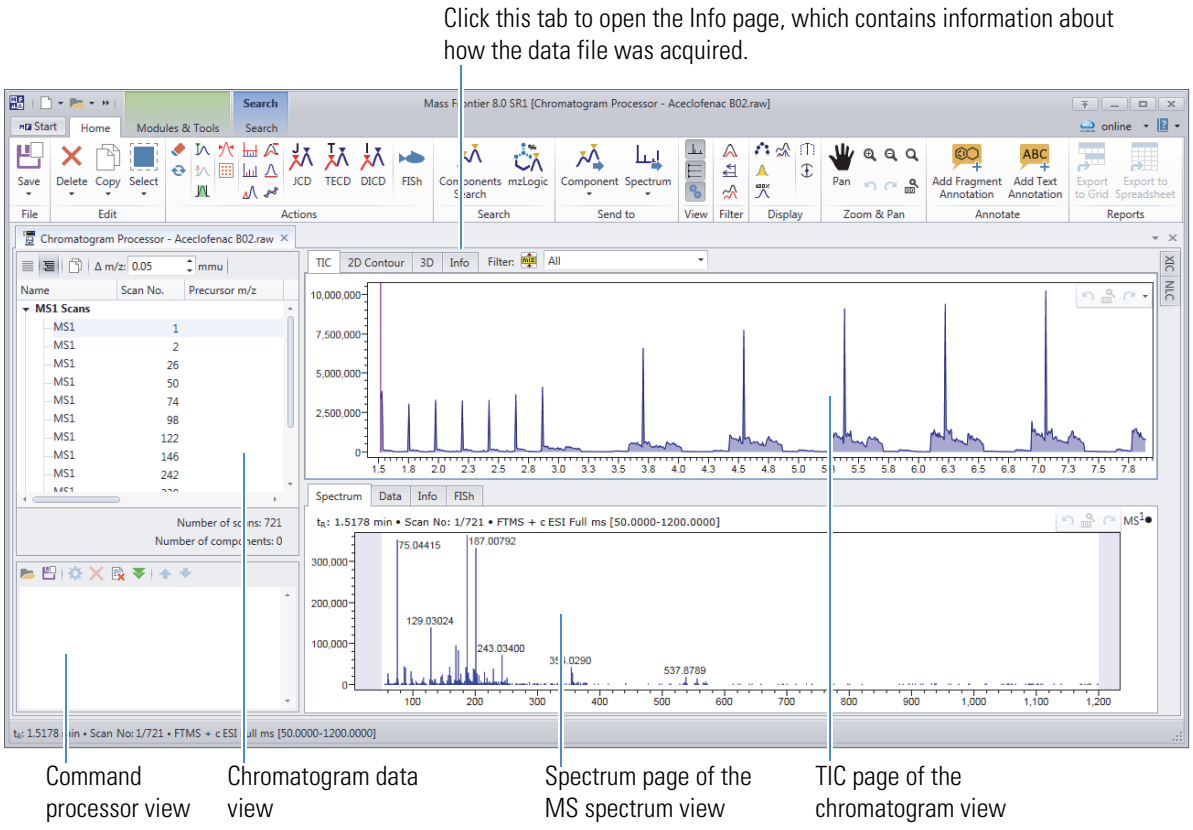
   b. Select the **Aceclofenac B02.raw** file and click **Open**.

> **Note** Aceclofenac, the compound that you are creating a curated spectral tree for, is a nonsteroidal anti-inflammatory drug (NSAID). The raw data file for this compound was acquired by direct injection (without chromatographic separation) into an Orbitrap Fusion Lumos mass spectrometer set to acquire data from 1.52 to 7.89 minutes after the run start time. For more information about the raw data file, open the Info page of the chromatogram view in the Chromatogram Processor window.

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

- On the upper left, the chromatogram data view lists the MS1 and product scans retrieved from the scan headers.
- On the lower left, the command processor view is empty, as you have not yet applied any actions to the chromatogram.
- On the upper right, the chromatogram view displays the total ion current (TIC) chromatogram.
- On the lower right, the MS spectrum view displays the scan selected in the chromatogram data view.

Figure 6. Chromatogram Processor page with the initial view for the selected raw data file



For a detailed description of the Chromatogram Processor module, refer to the *Mass Frontier 8.0 User Guide*.

Detecting Components

Use the Direct Infusion Component Detection algorithm to detect components in a solution that was infused into the mass spectrometer without any chromatographic separation.

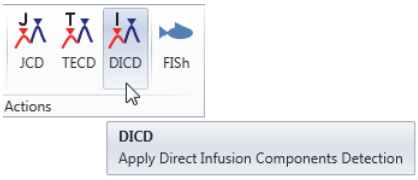
Tip In this tutorial, you are working with data from a direct infusion experiment and use the DICD algorithm for component detection.

For other data files, do the following:

- For a direct infusion experiment, use the Direct Infusion Component Detection (DICD) algorithm.
- For an LC/MS experiment, use the Joint Components Detection (JCD) algorithm.

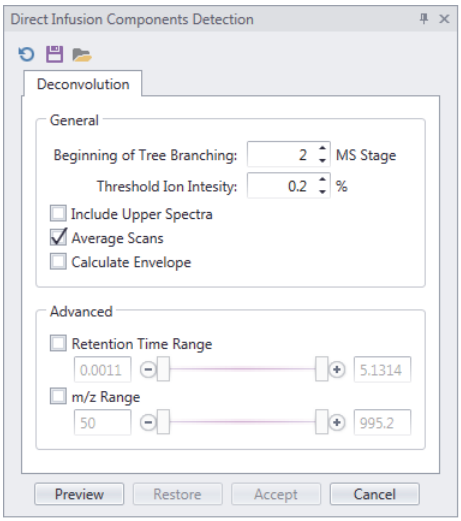
❖ To detect components in mass spectral data acquired with an infusion experiment

1. In the Actions group of the Chromatogram Processor toolbar, click **DICD** (Direct Infusion Component Detection).



The Direct Infusion Component Detection view opens at the right of the application window.

Figure 7. Default settings for the DICD algorithm



2. In the Direct Infusion Components Detection view, do the following:
   a. Click the **Reset** icon, , to make sure that all the parameters are set to their default settings.
   b. Click the **Load Parameters from a File** icon, .

      The Direct Infusion Components Detection dialog box opens to the Chromatograms folder (the last opened folder).

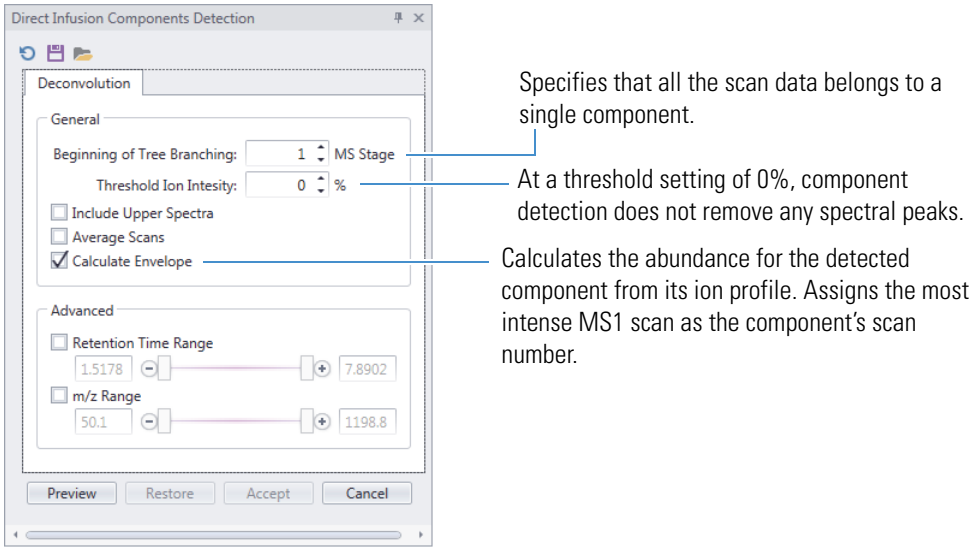
      drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Chromatograms

   c. Select the following Direct Infusion Parameters File, and then click **Open**.

      Aceclofenac.chpro_direct

The application applies the parameter settings from the file (Figure 8).

Figure 8. Modified parameter settings from the selected Direct Infusion Parameters File



Tip To create library entries for a custom spectral library, infuse a solution that contains only the compound of interest into the mass spectrometer.

To detect this compound in the raw data and create its initial spectral tree, Thermo Fisher Scientific recommends the following parameter settings:

- Beginning of Tree Branching: 1
- Threshold Ion Intensity: 0

  Use the Curator module to remove electronic and chemical noise peaks from a compound's spectral tree.
- Calculate Envelope: Selected

d. Click **Preview**.

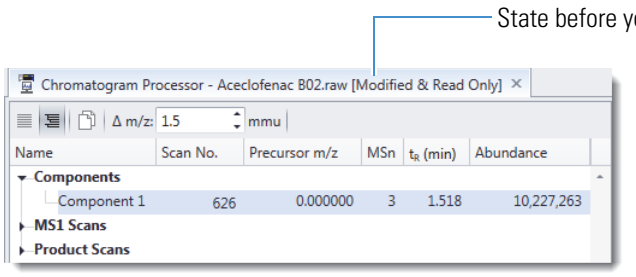
After the analysis finishes, a list of the detected components appears in the chromatogram data view, and a spectral tree for the detected component appears to the left of the Spectrum page.

The Components list includes information about the highest MS stage, retention time, and abundance for the detected component.

The spectral tree is a hierarchical representation of the data-dependent MS^n scans that consists of one or more nodes for each MS^n stage. Each node consists of spectra from the same precursor pathway, but with different collision energies, activation types, and isolation widths.

Note To reduce the number of individual spectra for each node, the application combines scans with the same precursor pathway, activation type (CID or HCD), collision energy, and isolation width (IW) into a single combined spectrum.

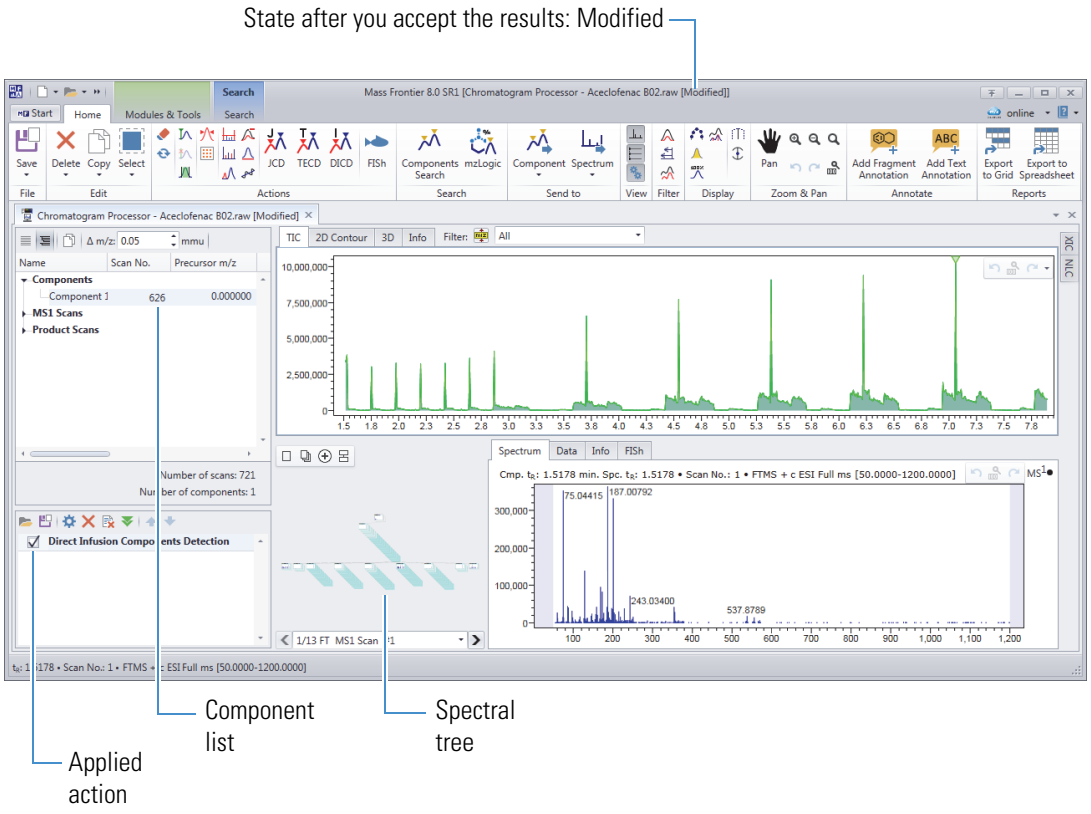
With the parameter settings shown in Figure 8 on page 7, the analysis detects one component in the example data file. Until you accept the results, the Chromatogram Processor window remains in the Modified & Read Only state.



e. To accept the results, click **Accept** in the Direct Infusion Components Detection view.

The DICD view closes, the Command Processor view lists Direct Infusion Component Detection as an applied action, and the state of the Chromatogram Processor window changes to Modified (Figure 9).

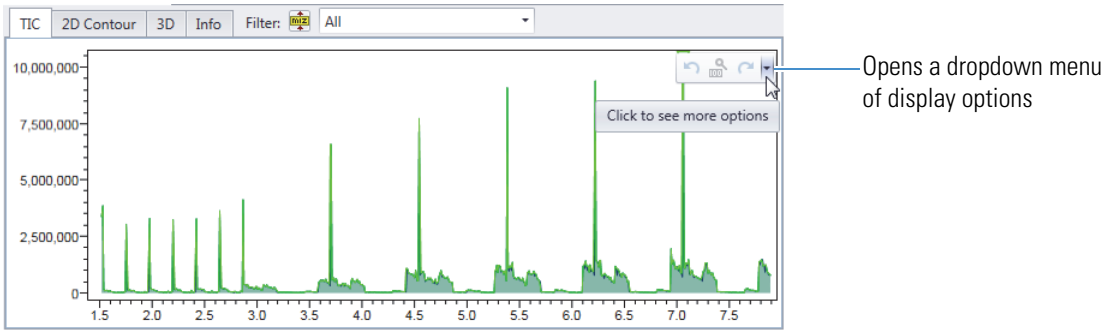
Figure 9. Chromatogram Processor window showing the total ion current trace (TIC) and spectral tree for the detected component



Reviewing the Detected Component

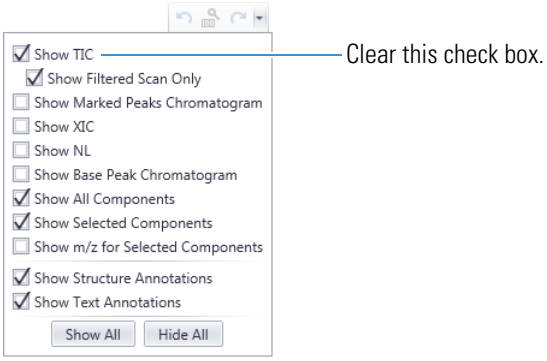
❖ To review the results for the detected component

1. Select the component in the Components list.
2. In the chromatogram view, open the display options menu by clicking the down arrow at the top right of the view.

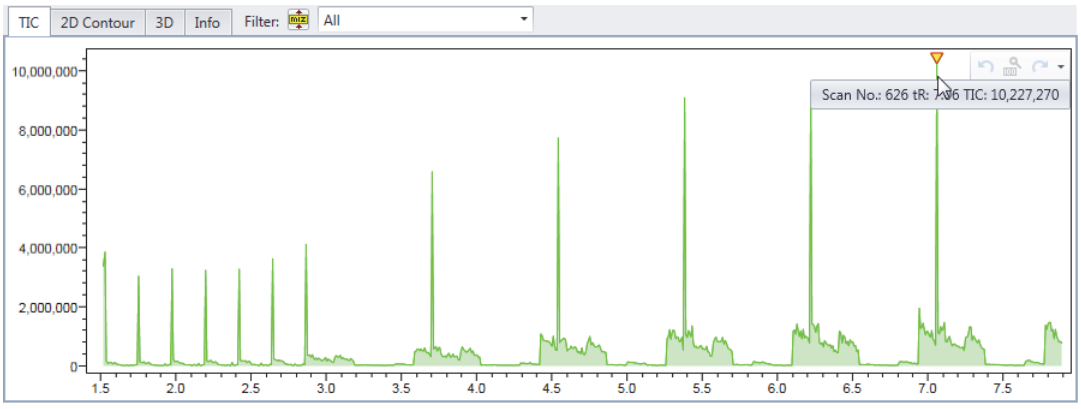


3. To display the trace for the component, clear the **Show TIC** check box in the display options menu.

Figure 10. Options menu for the TIC page of the chromatogram view

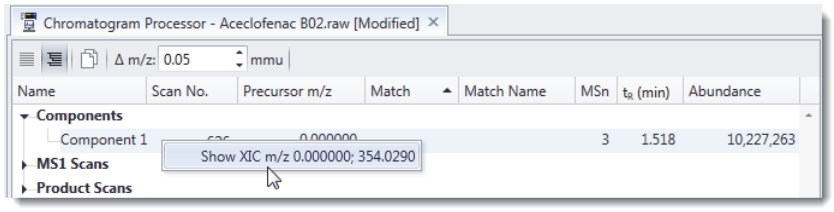


The component's trace appears by itself in the chromatogram view. Green is the default color for a component trace.

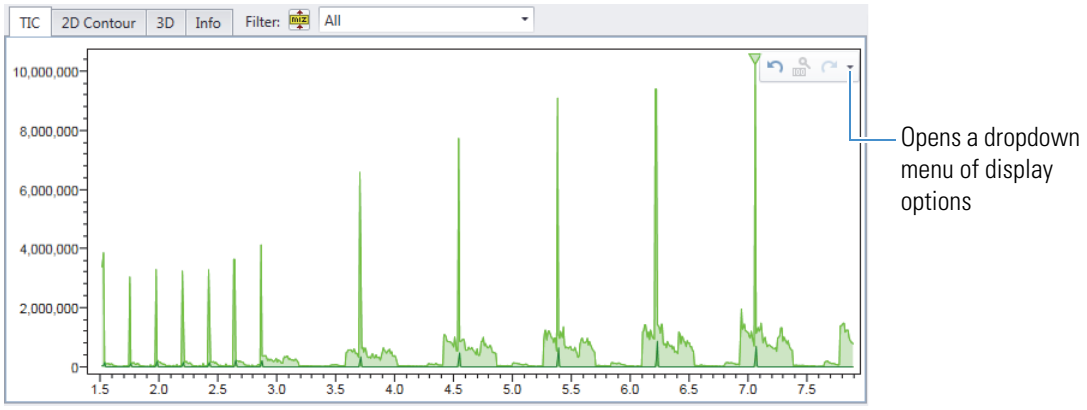


4. To view the component's XIC (extracted ion current trace), do the following:

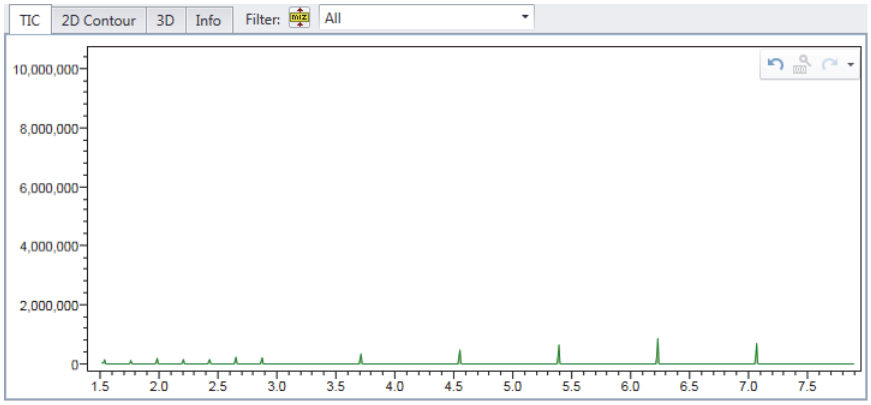
   a. In the chromatogram data view, right-click the component in the Components list and choose **Show XIC m/z 0.000000; 354.0290**.



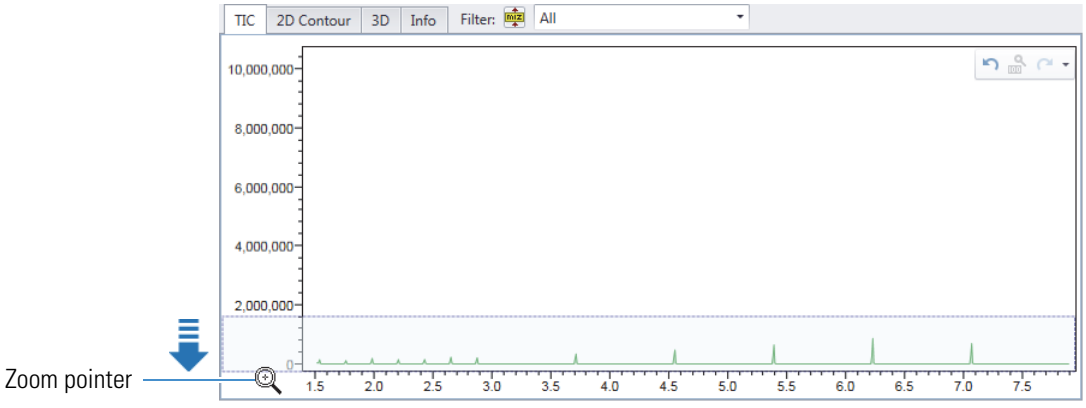
   The chromatogram view displays the XIC trace in green and the component's trace in lime green.



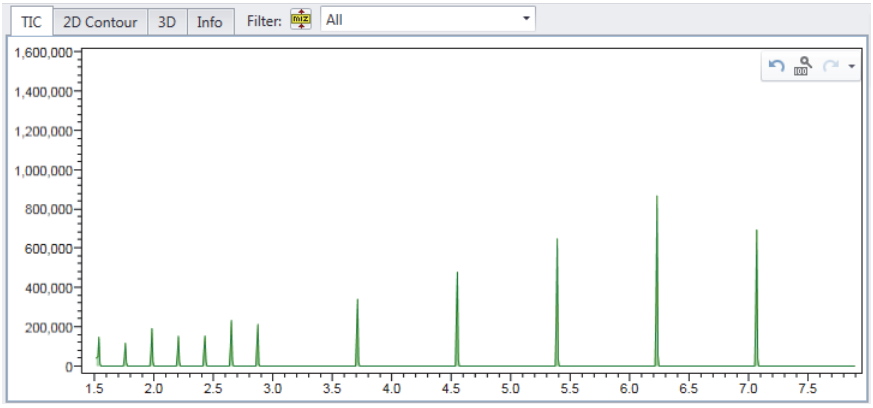
   b. To view the XIC trace by itself, open the display options menu and clear the **Show All Components** and **Show Selected Components** check boxes.



   c. To zoom in on the *y*-axis range of the XIC trace, drag the mouse pointer down the *y*-axis in the range that you want to view, from the highest value to the lowest value, and release.



This figure shows the magnified XIC trace on the TIC page of the chromatogram view.



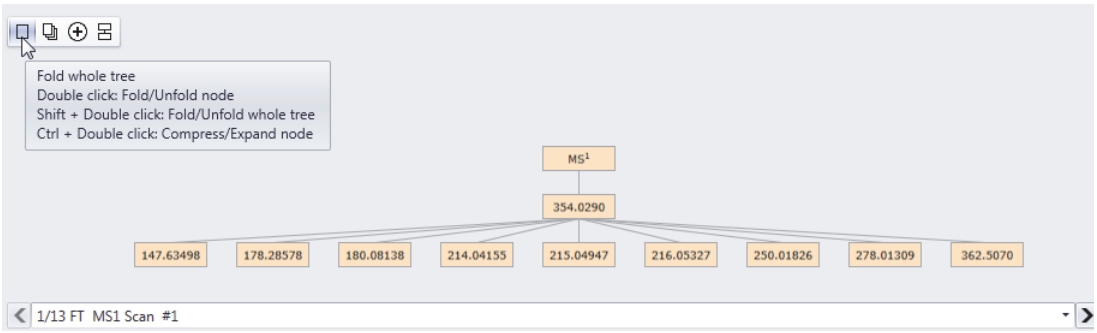
d. To undo the zoom, click the **Reset Zoom** icon, .

5. To review the component's spectral tree, do the following:

a. To view the folded tree, which shows only the precursor *m/z* value for each node, click the **Fold Whole Tree** icon, .

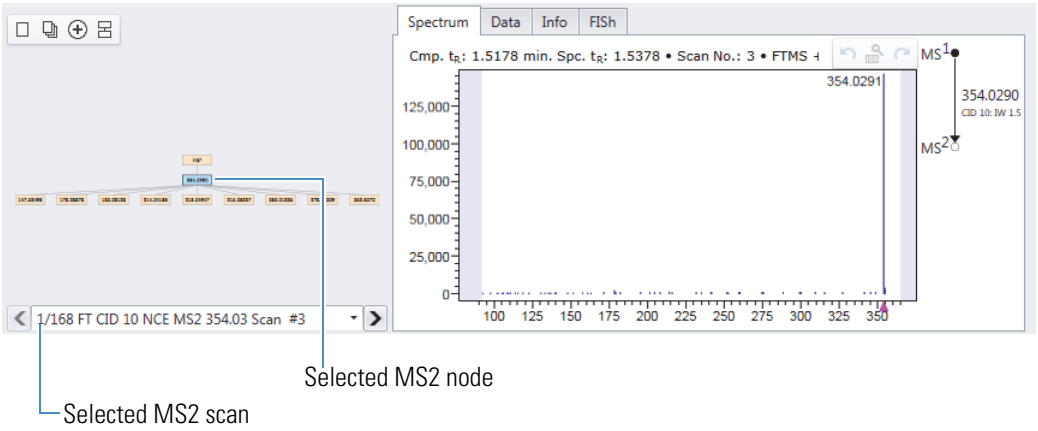
Figure 11 shows the folded tree. The tree contains one node for the MS^2 level and nine nodes for the MS^3 level.

Figure 11. Folded tree



b. To browse the scans for a node, click the node of interest in the tree, and then use the scan list below the tree to browse the scans on the Spectrum page to the right.

The selected node changes to blue and the scan list contains lists the scans for the selected node.



c. To return to the unfolded tree view, click the **Unfold Whole Tree** icon, .

Curating the Spectral Tree for a Component

To curate a component's spectral tree, follow these topics in order:

1. Sending the Component to the Curator Module and Specifying Its Structure
2. Selecting the Steps for the Curation Process
3. Selecting the Processing Mode
4. Using the Semi-Auto Mode to Step Through the Curation Process

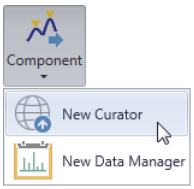
Sending the Component to the Curator Module and Specifying Its Structure

To curate a component's spectral tree, you send the component to the Curator module.

Note You can add the structure annotation to the component before or after you send the component to the Curator module by running a library search or an mzLogic analysis. In this tutorial, you add the structure annotation in the Curator module.

❖ **To send a component to the Curator module and specify its structure**

1. In the Components list of the Chromatogram Processor window, select the component.
2. In the Send To group of the Chromatogram Processor toolbar, click **Component** and select **New Curator**.



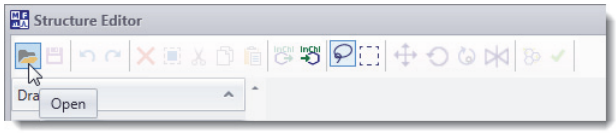
   This action sends the spectral tree for the selected component to the Curator module. A new instance of the Curator module opens as a tabbed page, but it needs a structure to continue processing.

3. At the prompt, click **OK**.

   The Structure Editor dialog box opens.

4. To import a structure for the selected component, do the following:

   a. In the Structure Editor dialog box, click the **Open** icon, .



   b. Browse to the following folder, select the **Aceclofenac.mol** file, and click **Open**.

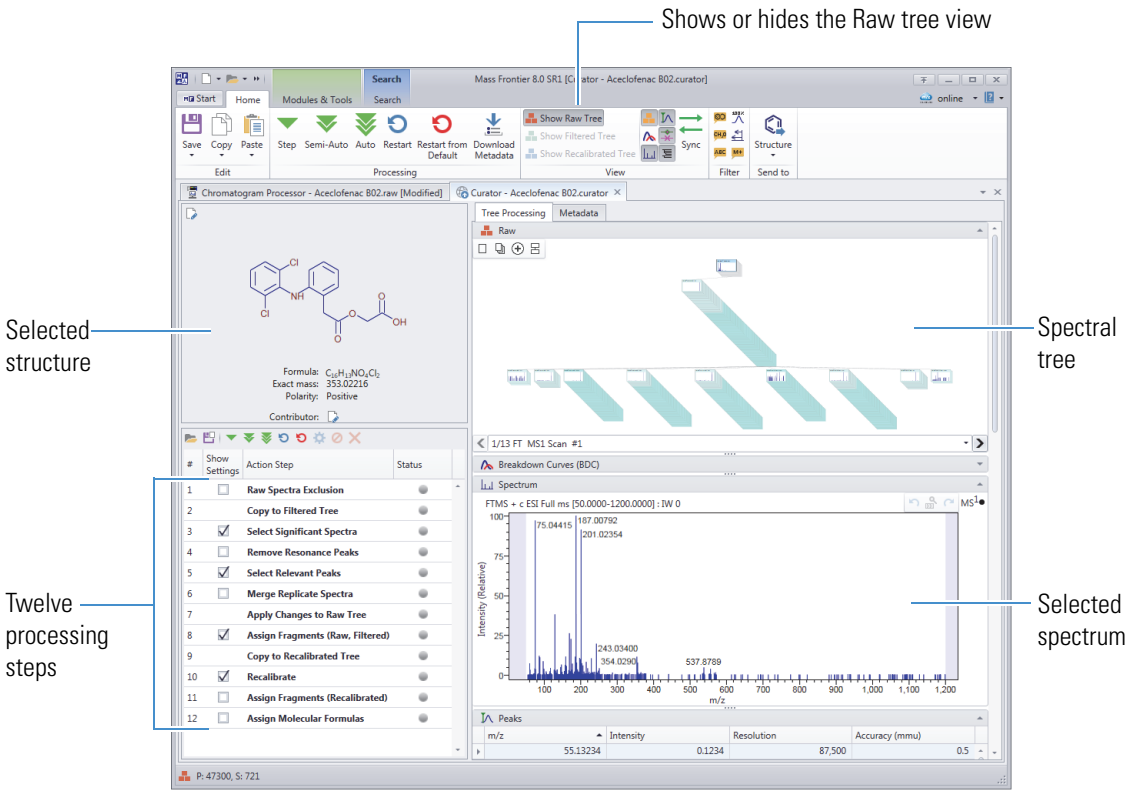
      drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Structures

      The two-dimensional structure appears in the Structure Editor's drawing area.

   c. Click **OK** to import the structure into the Curator's structure pane.

   On the Curator page, the selected structure appears in the upper left pane, and the raw spectral tree for the selected component appears in Raw tree view on the upper right (Figure 12 on page 13).

Figure 12. Curator page with the structure and spectral tree for the selected component



Tip Before or during the curation process, you can manually remove a node or spectrum from the raw spectral tree. To remove a node or spectrum from the tree, right-click the node or spectrum and choose **Delete Node** or **Delete Spectrum**, respectively.

Selecting the Steps for the Curation Process

By default, there are twelve steps to the curation process, and by default, the application displays the settings for four of these steps.

In this tutorial, you use the complete twelve-step process and view the settings of nine of these steps.

Tip To turn a step off or on, right-click the step and choose **Turn off/on the Selected Action**. The step becomes unavailable. To view the settings for a step, select the **Show Settings** check box to the left of the step to set the parameters during the curation process.

Selecting the Processing Mode

Table 1 describes the three modes for the curation process.

Review these modes, and then go to the next topic to begin the curation process in the semi-automatic mode.

Table 1. Processing modes (Sheet 1 of 2)

Processing mode	Description
Step	Runs the next step in the curation process.
	Use this mode to manually go through each step. The application prompts you to review and set the parameters settings at each of the selected steps.

Table 1. Processing modes (Sheet 2 of 2)

Processing mode	Description
Semi-Auto 	(Recommended) Starts a wizard that runs all the remaining action steps, with a pause between each step for previewing and optimizing the parameter settings. Use this mode to review and optimize the parameter settings at each of the selected steps during the curation process. Each settings dialog box has an Apply & Continue in Semi-Auto mode button.
Auto 	Automatically runs all the remaining action steps in the curation process by using the predefined parameter settings until it reaches step 8. Use this mode to automatically run through the curation process with the default settings or the settings from a Mass Frontier curator actions (.curator_act) file. **Note** Thermo Fisher Scientific recommends that you preview and optimize the parameter settings for each action step before using the Auto mode. After you optimize the settings, you can save the parameter settings to a .curator_act file for future use.

Using the Semi-Auto Mode to Step Through the Curation Process

The curation process refines the spectral tree for a library entry by doing the following:

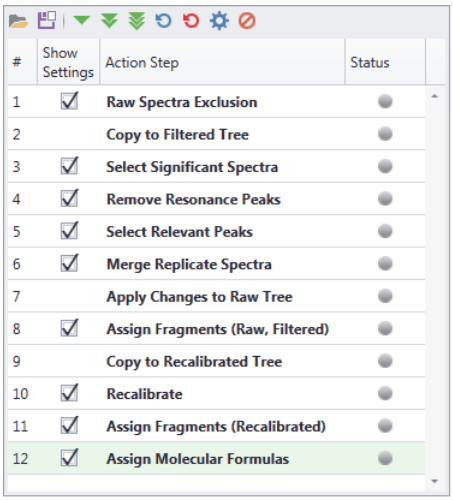
- Removes low-quality scans (node items) below a specified intensity or S/N threshold, and then refines each spectrum by removing FT artifacts and unexplained spectral peaks (Filtered tree view)
- Recalibrates the *m/z* values of the explained spectral peaks by using their adduct ion formulas. (Recalibrated tree view)

This tutorial shows you how to use the Semi-Auto mode to step through the curation process.

❖ To curate the spectral tree for the component using the semi-automatic mode

1. Select all the **Show Settings** check boxes in the action step pane.

Figure 13. Action step pane with all 12 check boxes selected

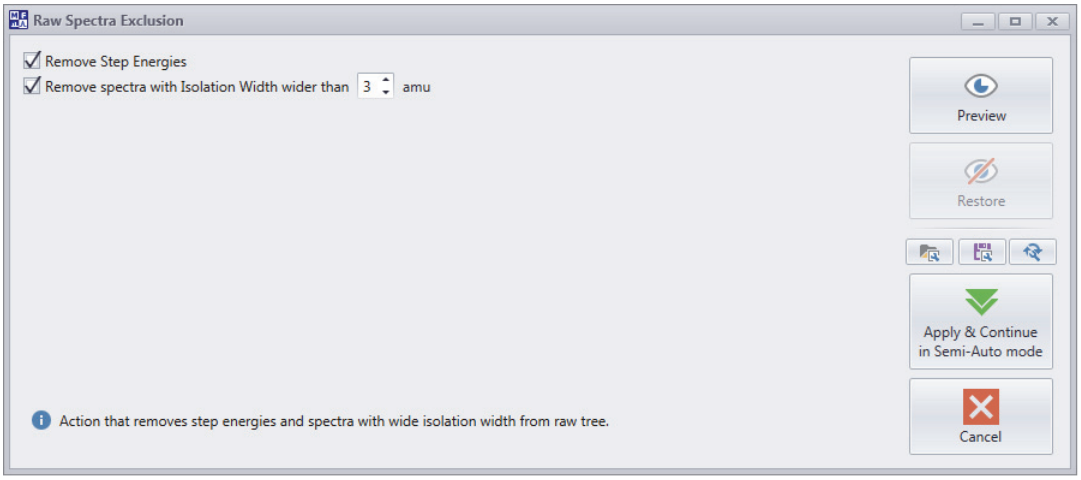


Starting the wizard

2. To start the wizard, click the **Semi-Auto** icon, .

   The Raw Spectra Exclusion dialog box opens.

Figure 14. Raw Spectra Exclusion dialog box for action step 1 with the default settings

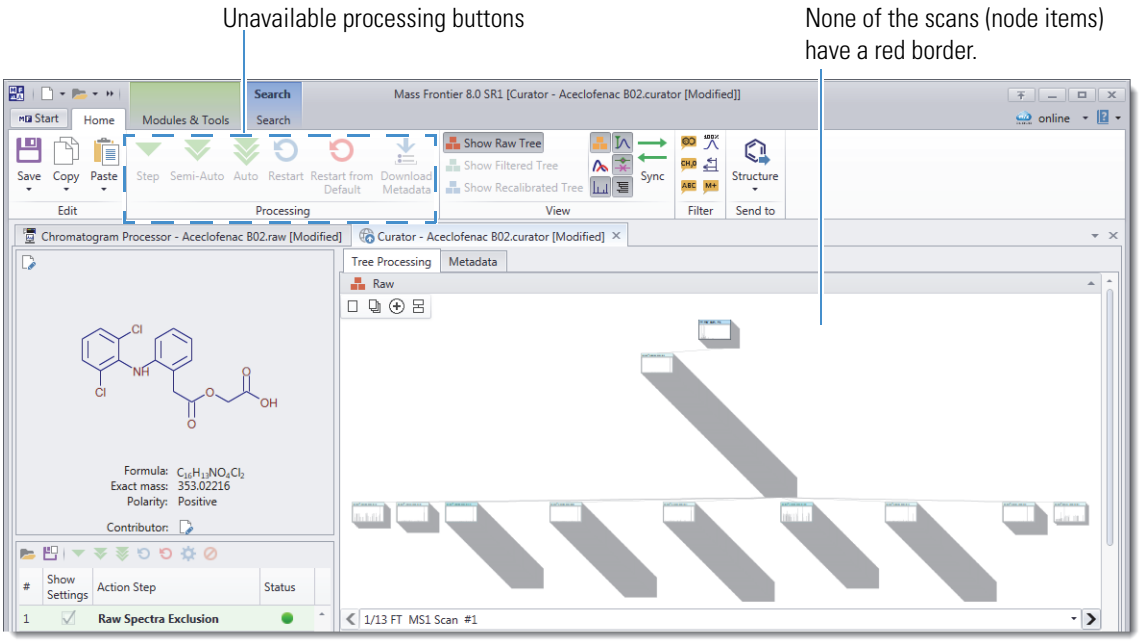


3. To temporarily apply the default parameter settings, click **Preview**.

   On the Tree Processing page, a red border indicates that a spectrum (node item) meets the removal criteria. None of the scans in the component's spectral tree meet the removal criteria (Figure 15).

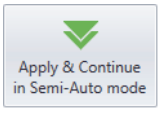
> **Note** The processing buttons on the Curator toolbar are unavailable. Clicking the Apply & Continue in Semi-Auto mode button on the wizard page is the only way to move forward through the curation steps.

Figure 15. None of the scans (node items) meet the removal criteria



Continue to action step 3

4. To apply the default settings and continue to the next step, click **Apply & Continue in Semi-Auto Mode** on the Raw Spectra Exclusion page of the wizard.



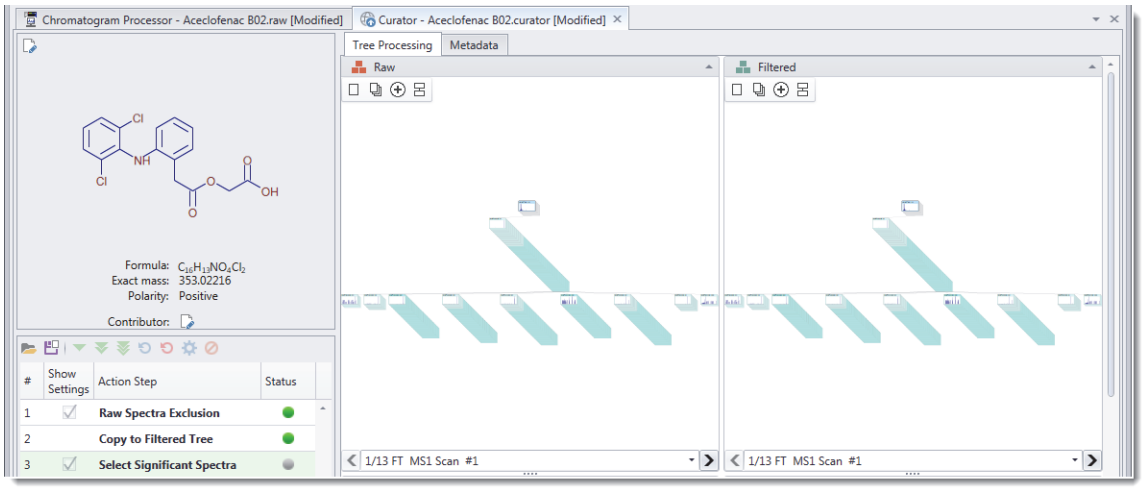
   The application automatically applies the Raw Spectra Exclusion and Copy to Filtered Tree Steps, and then opens the Select Significant Spectra dialog box (Figure 17 on page 16).

Figure 16 shows the Raw tree view and the Filtered tree view that open after the curation process begins (completes action step 2).

Tip You can use the buttons in the View group of the Curator toolbar to close or open the Raw tree and Filtered tree views or to synchronize the spectrum selections for these two views.

You can remove individual spectra or complete nodes from the Raw tree at any time during the curation process by right-clicking the Raw tree view and choosing **Delete Spectra** or **Delete Node**, respectively.

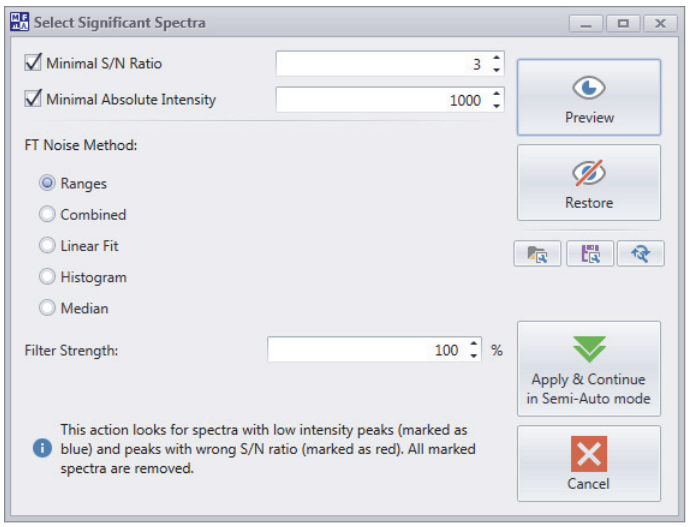
Figure 16. Status of the curation process following the first two steps



5. To remove spectra below a specified S/N threshold or intensity level, do the following:

   a. In the Select Significant Spectra dialog box, click **Preview**.

   Figure 17. Select Significant Spectra dialog box for action step 3 with the default settings



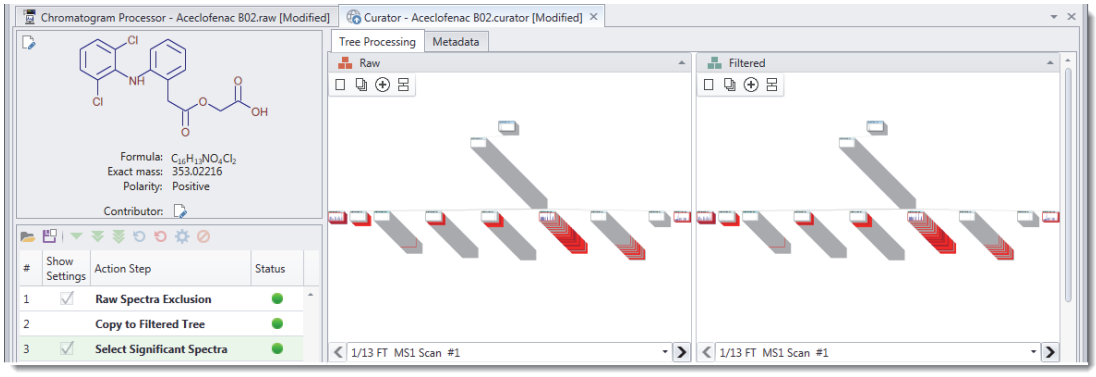
   b. Review the Raw tree view and the Filtered tree view on the Tree Processing page of the Curator window (Figure 18 on page 17).

On the Tree Processing page, a red or blue border indicates that a spectrum meets the removal criteria:

- A blue border indicates that the spectrum's base peak is below the specified minimum intensity level.
- A red border indicates a that the signal-to-noise level for the spectrum is below the specified threshold.

With the default thresholds of 3 for S/N and 1000 for the base peak (minimal absolute) intensity, you can see that some of the MS3 scans (node items) are highlighted for removal based on their S/N levels, and some of the MS3 scans are highlighted for removal based on the intensity of their base peaks (Figure 19 on page 17).

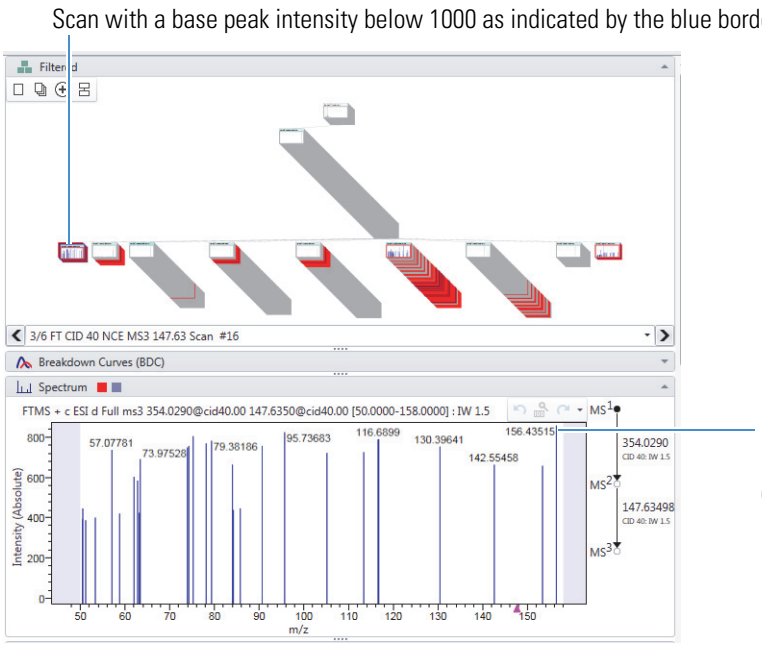
Figure 18. Scans selected for removal in action step 3 (node items with a red border)



You can view the individual scans for a node by selecting the node and then using the scan list below the spectral tree pane. There are quite a few low-intensity scans at the MS3 level.

Figure 19 shows a low-intensity scan for the precursor ion at *m/z* 147.63498.

Figure 19. Filtered tree with scans marked for removal, showing the spectrum for scan #16



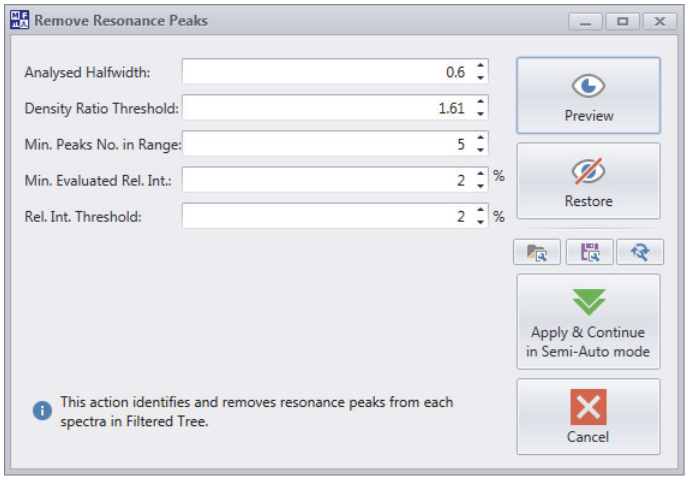
Tip To toggle the y-axis scale from relative intensity to absolute intensity, right-click the Spectrum pane and choose **Show Absolute Intensities**.

Continue to action step 4

c. To apply the default settings and move to the next step in the curation process, click **Apply & Continue in Semi-Auto Mode** to apply the default settings and move to action step 4 in the curation process.

The Remove Resonance Peaks dialog box opens.

Figure 20. Remove Resonance Peaks dialog box for action step 4 with the default settings



6. To identify and remove resonance peaks from the spectra, do the following:

   a. In the Remove Resonance Peaks dialog box, click **Preview**.

   b. In the Filtered tree view, review the spectra.

   If the application identifies an *m/z* peak as a resonance peak, it highlights the peak in red. With the default settings, the application does not find any resonance peaks in the spectra.

> **Note** Resonance peaks are *m/z* peaks generated by the Fourier Transform (FT) or Orbitrap analyzer, not *m/z* peaks for chemical entities in the infused sample.

Continue to action step 5

c. In the Remove Resonance Peaks dialog box, click **Apply & Continue in Semi-Auto Mode** to apply the default settings and move to action step 5.

The Select Relevant Peaks dialog box opens to the Basic view ().

> **Note** Use the Select Relevant Peaks dialog box to select the MS^1 adducts, the MS^1 neutral losses, and the MS^n collision cell adducts.

7. To remove the unexplained peaks from the spectra, do the following:

   a. In the Select Relevant Peaks dialog box, click **Advanced** to display all the parameters.

   These additional parameters appear (Figure 21 on page 19):

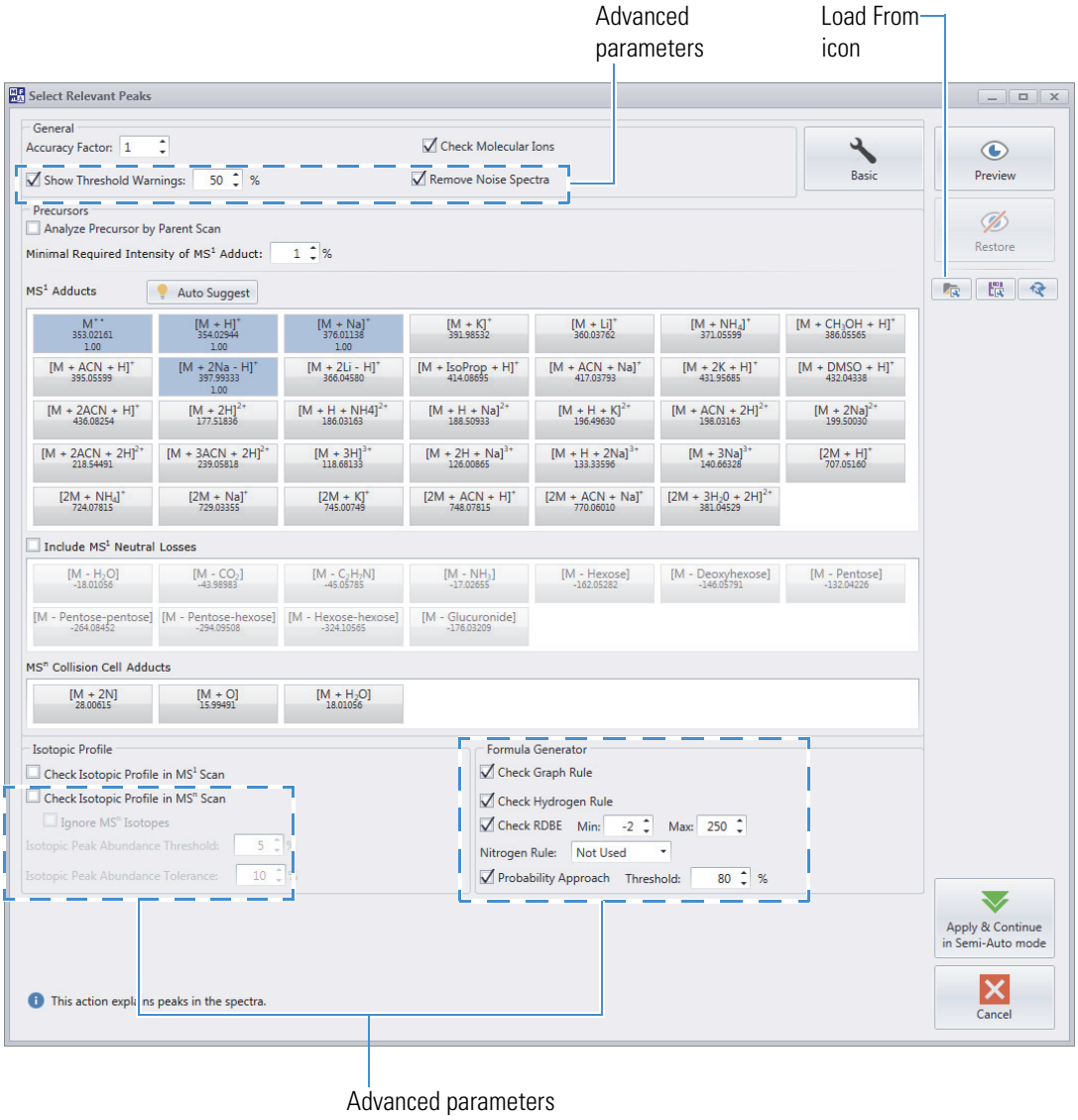
   - In the General area—Show Threshold Warnings and Remove Noise Spectra
   - In the Precursors area—Minimal Required Intensity of MS^1 Adduct
   - In the Isotope Profile area—Check Isotopic Profile in MS^n Scan
   - The Formula Generator area with the following parameters—Check Graph Rule, Check Hydrogen Rule, Check RDBE, Nitrogen Rule, Probability Approach, and Threshold

Note Use the parameters in the Formula Generator area to modify the set of predicted formulas that the application uses to explain the peaks in the spectral tree.

Tip The wizard automatically selects the appropriate MS1 Adducts for the spectral tree (Figure 21).

If you change the parameter settings, click **Auto Suggest** to automatically select suitable molecular ions.

Figure 21. Select Relevant Peaks dialog box for action step 5 with the advanced parameters displayed



b. To use the provided Select Relevant Peaks Parameter File, click the **Load From** icon,  . Then, in the Select Relevant Peaks dialog box, select **Aceclofenac B02.curator_srp**, and click **Open** (Figure 22 on page 20).

If the dialog box does not open to the following folder, browse to it:

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

Figure 22. Location of Select Relevant Peaks File

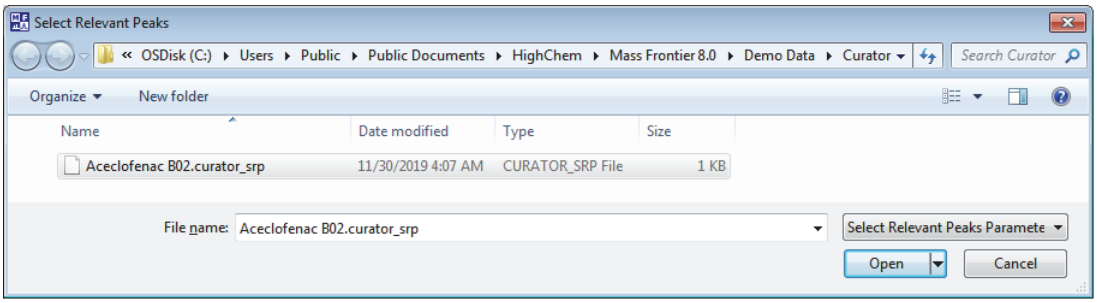
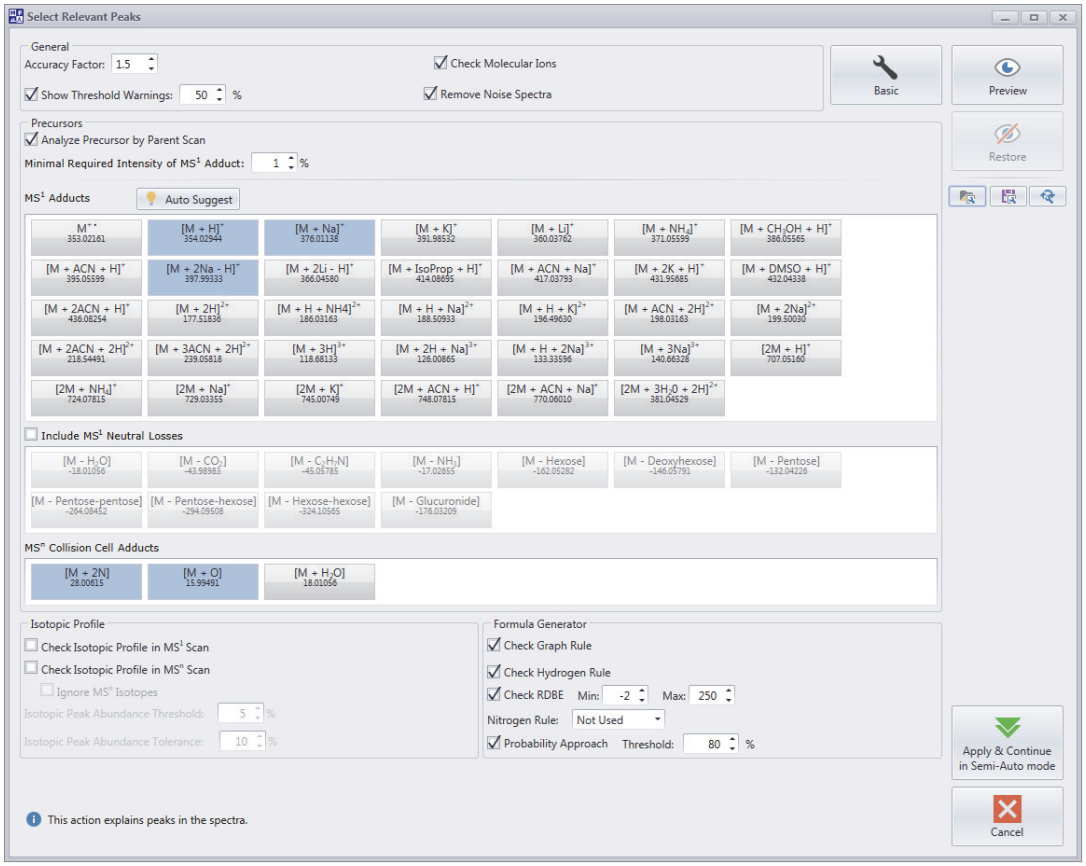


Figure 23 shows the modified settings from the selected parameter file:

- Accuracy Factor: 1.5
- Analyze Precursor by Parent Scan: Selected
- Selected MS^1 adducts: $[M+H]^+$, $[M+Na]^+$, $[M+2Na–H]^+$
- Selected MS^n Collision Cell Adducts: [M+2N] and [M+O]

Figure 23. Modified settings from the Select Relevant Peaks Parameter File



c. Click **Preview**.

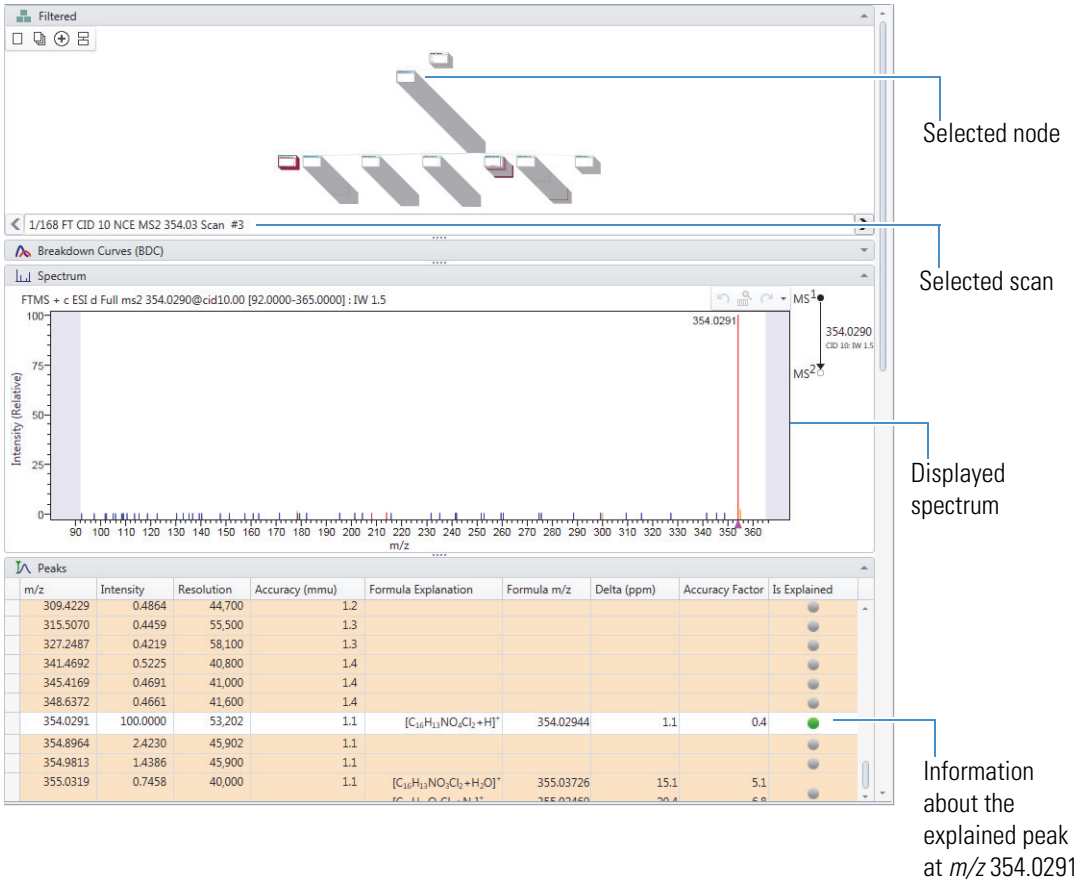
d. To view each spectrum in the Spectrum pane, click through the scans (node items) in the Filtered tree view.

The application color-codes the spectral peaks.

Color	Meaning
Red	Relevant peaks explained by the adducts and generated formulas
Blue	Unexplained peaks
Orange	Unexplained peaks with a relative intensity that is higher than the Show Threshold Warnings parameter setting. When a node item (spectrum) contains a number of unexplained peaks with a relatively high-intensity, the node item's border appears in a shade of red. As the number of unexplained peaks increases, the saturation of the red color increases.

Figure 24 shows a preview in the Spectrum pane for one of the MS^2 scans. The explained peaks are red and the unexplained peaks are blue.

Figure 24. Spectrum pane showing the explained and unexplained peaks for the spectrum selected in the Filtered tree view



Continue to action steps 6 & 7

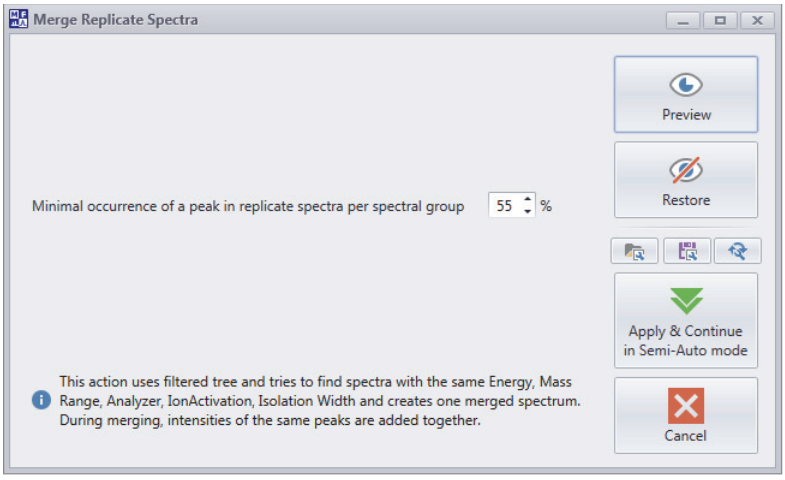
e. Click **Apply & Continue in Semi-Auto Mode**.

The application removes the unexplained peaks (in blue) from the spectra and continues to the next step.

The Merge Replicate Spectra dialog box opens (Figure 25 on page 22).

8. In the Merge Replicate Spectra dialog box, click **Preview**.

Figure 25. Merge Replicate Spectra dialog box for action step 6 with the default setting



Note The Merge Replicate Spectra step merges replicate scans into a single spectrum and adds the intensities of peaks with the same *m/z* value. Replicate spectra are spectra from the same analyzer, ion activation type, collision energy, isolation width, and mass range.

Figure 26 shows the filtered spectral tree before the merge step.

Figure 26. Filtered spectral tree before the merge step

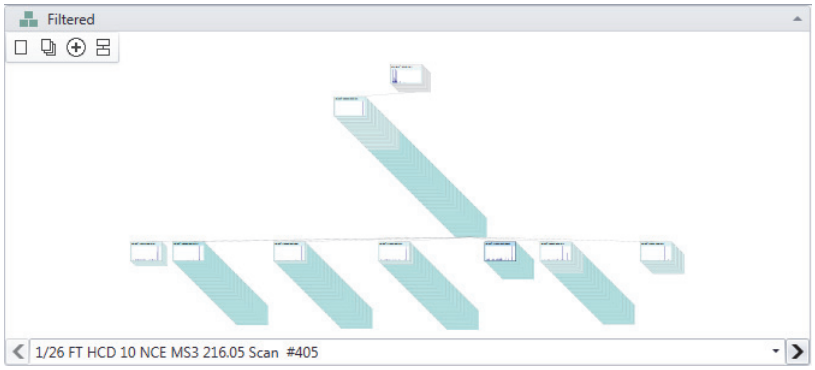
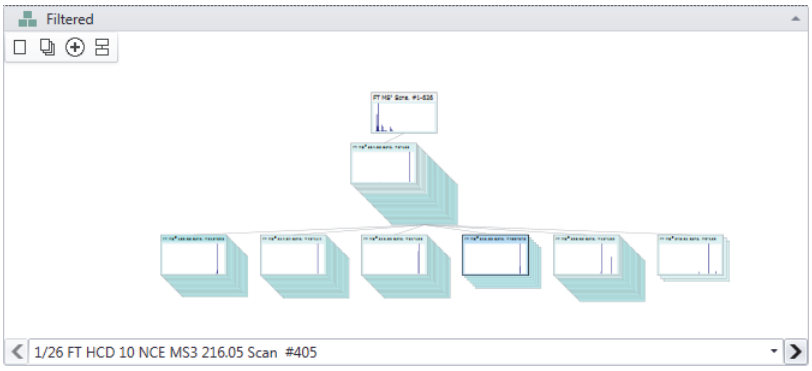


Figure 27 shows the filtered spectral tree after the merge step. Notice that the filtered tree contains one less MS3 node than the raw tree and far fewer scans (node items) per node.

Figure 27. Filtered spectral tree after the merge step



Continue to action steps 8 & 9

9. To apply the default setting for merging replicate spectra and continue the curation process, click **Apply & Continue in Semi-Auto Mode** (Figure 25 on page 22).

   The application automatically runs the Apply Changes to Raw Tree step, and the Assign Fragments (Raw, Filtered) dialog box opens.

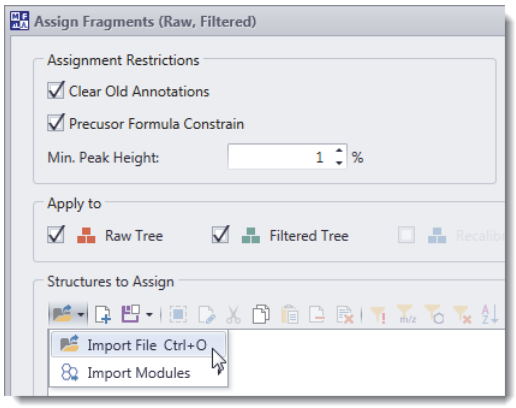
   Tip The fragment assignment step requires a fragment structure list. In this tutorial, you use an SDF file provided in the Demo Data folder.

   Outside this tutorial, if you have not already created an SDF file for the component you are curating, you can click **Structure > New Fragments & Mechanisms** in the Curator toolbar to send the component's structure to the Fragments & Mechanisms module for fragmentation. Click **Generate** to perform fragment prediction. After fragment prediction ends, return to the Assign Fragments dialog box, click the **Import** icon and select **Import Modules**, and then select the appropriate instance of the Fragments & Mechanisms module.

   If you have multiple compounds to curate, use the Batch Fragment Generation module to generate a separate SDF file for each submitted compound.

10. To assign fragment structures to the peaks in the filtered tree's spectra, do the following:

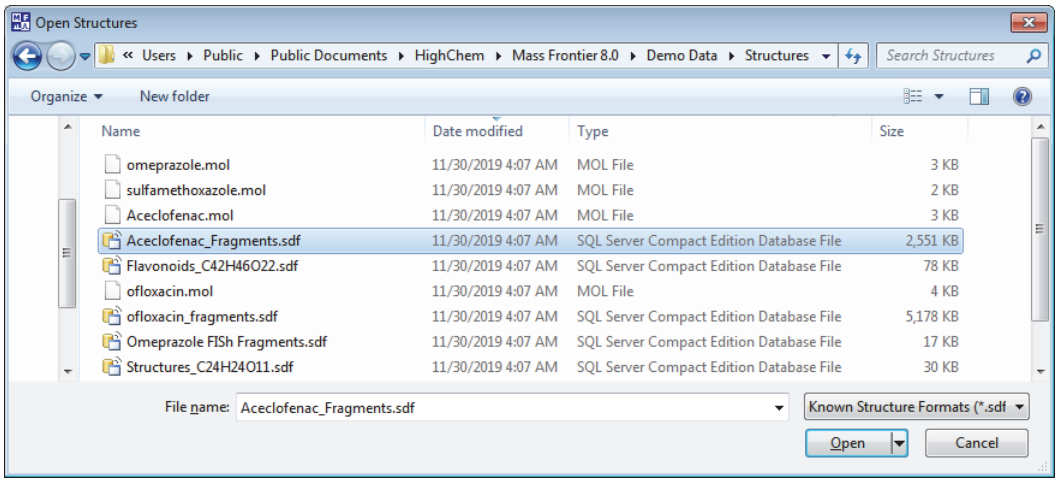
    a. In Structures to Assign area of the Assign Fragments (Raw, Filtered) dialog box, click the **Import** icon, , and select **Import File**.



    b. Browse to the following folder, select the **Aceclofenac_Fragments.sdf** file, and click **Open**.

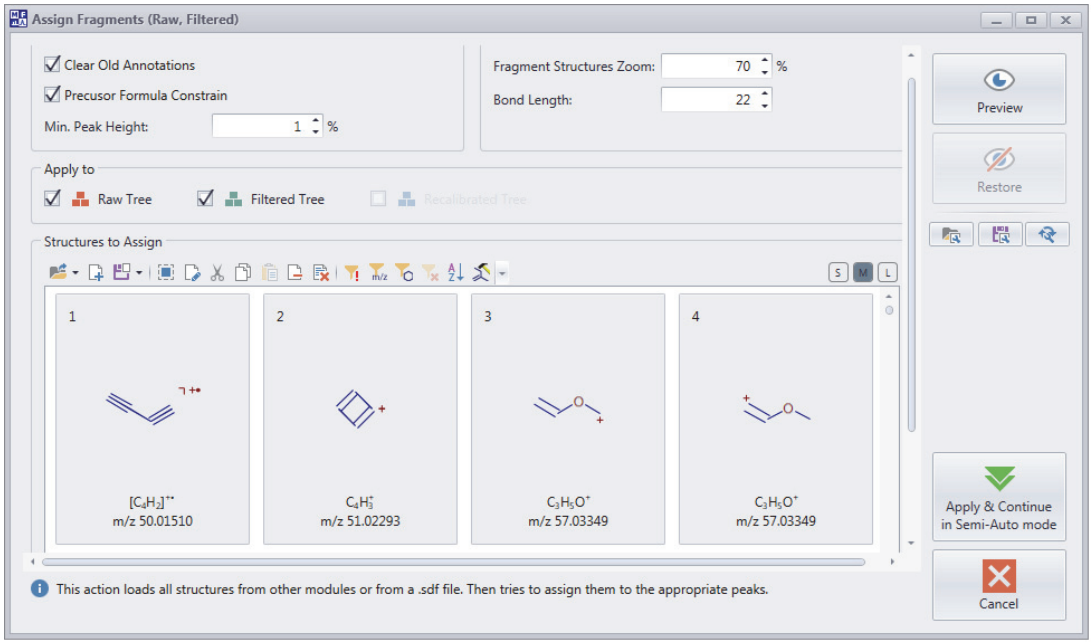
       drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.0\Demo Data\Structures

Figure 28. Location of the structure files



The fragment structures appear as cards in the Structures to Assign pane of the Assign Fragments (Raw, Filtered) dialog box.

Figure 29. Assign Fragments (Raw, Filtered) dialog box for action step 8 with imported structures

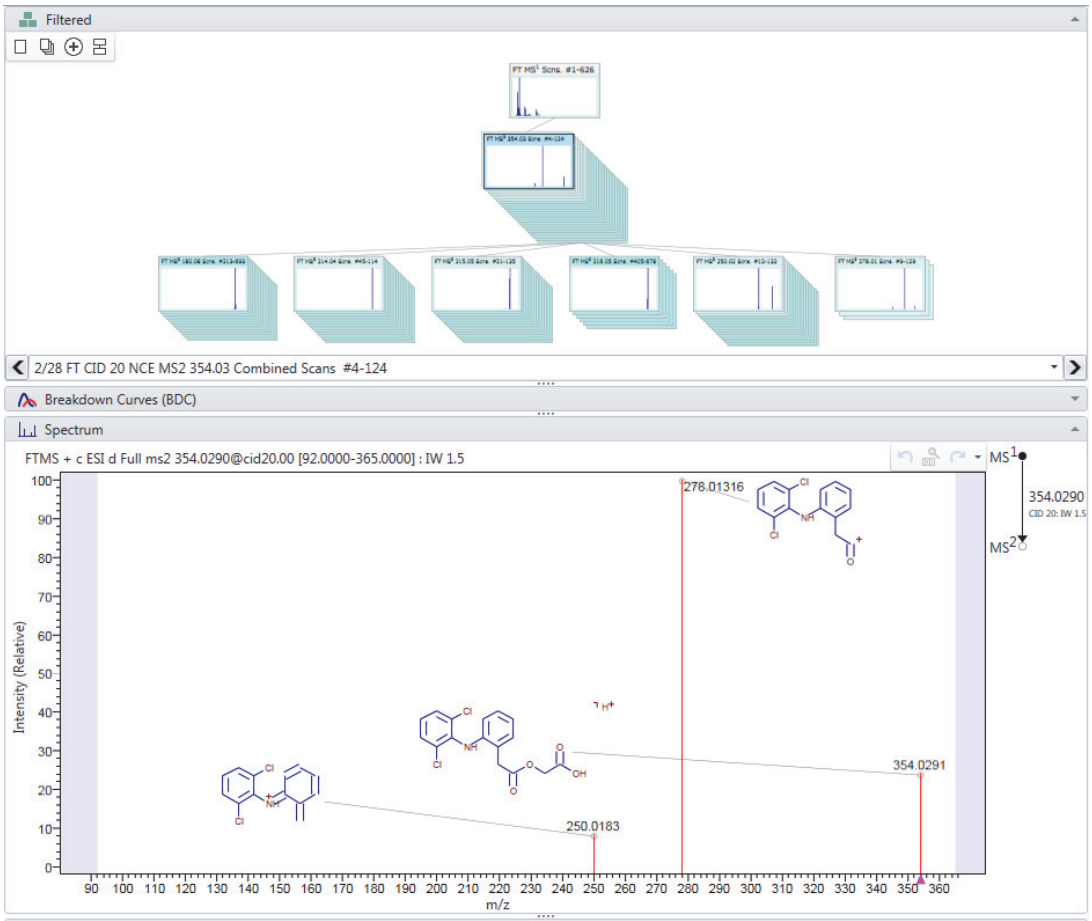


c. Click **Preview**.

On the Curator page, the Filtered tree view shows the explained structures as annotations on the selected spectrum (Figure 30 on page 25).

Tip If the Tree Processing page does not automatically update, click a card in the Structures to Assign area of the Assign Fragments page of the curator wizard.

Figure 30. Spectrum with structure annotations and a Peaks table with formula explanations



m/z	Intensity	Resolution	Accuracy (mmu)	Formula Explanation	Formula m/z	Delta (ppm)	Accuracy Factor	Is Explained
250.0183	8.2491	53,636	1.2	$C_{13}H_{10}NCl_2^+$	250.01848	0.8	0.2	
				$C_{16}H_7OCl^+$	250.01799	1.1	0.3	
278.01316	100.0000	58,768	0.9	$C_{14}H_{10}NOCl_2^+$	278.01340	0.8	0.3	
354.0291	24.0011	50,108	1.3	$[C_{16}H_{13}NO_4Cl_2+H]^+$	354.02944	1.0	0.3	

Continue to action step 10

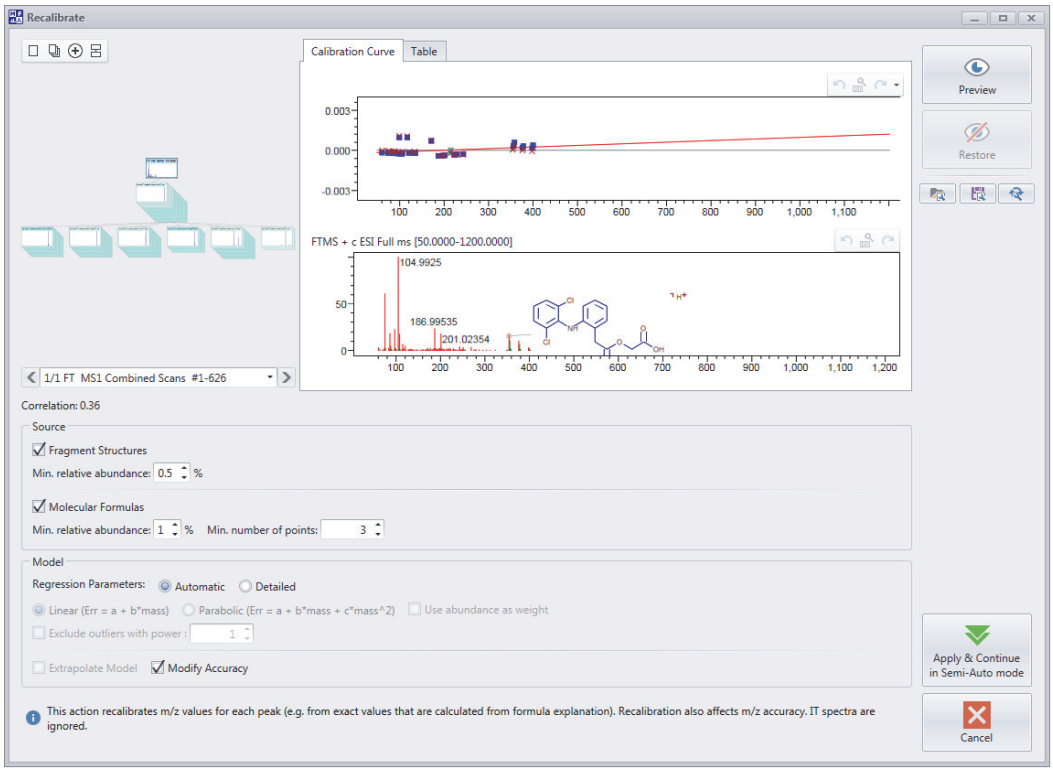
d. To assign the fragment structures to the appropriate peaks and move to the next step in the curation process, click **Apply & Continue in Semi-Auto Mode**.

The application automatically applies the Assign Fragments (Raw, Filtered) and Copy to Recalibrated Tree steps, and then opens the Recalibrate dialog box (Figure 31 on page 26).

11. To recalibrate the *m/z* values of the spectral peaks, do the following:

    a. In the Recalibrate dialog box, click through the spectral tree on the left and view the calibration curves on the right.

    Figure 31. Recalibrate dialog box for action step 10 with a preview of the calibration curve for the peaks the MS1 scan



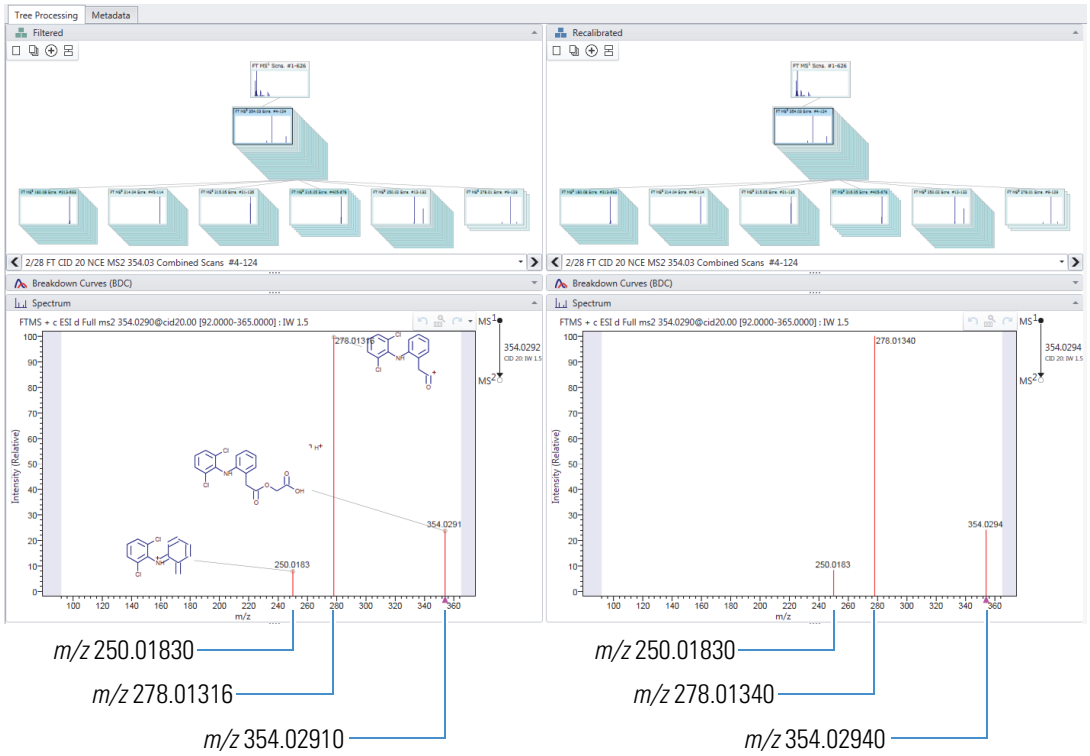
    b. Click **Preview**.

    c. On the Tree Processing page on the Curator page, review the recalibrated peaks.

> **Note** The Recalibrate step recalibrates the *m/z* value for each peak in the spectral tree by using the exact value for each peak, which it calculates from the peak's formula and fragment structure explanation, and the selected calibration model.

Figure 32 shows a filtered and annotated spectrum on the left with the original *m/z* values for each peak and a recalibrated spectrum on the right with the recalibrated *m/z* values for each peak.

Figure 32. Filtered and recalibrated MS2 spectrum



This table shows the mass difference between the spectral peaks in the filtered and recalibrated spectra for the MS2 spectrum for precursor *m/z* 354.0290 (combined scans 4–124)

Filtered tree (*m/z*)	Recalibrated tree (*m/z*)
250.01830	250.01830
278.01316	278.01340
354.02910	354.02940

Continue to action step 11

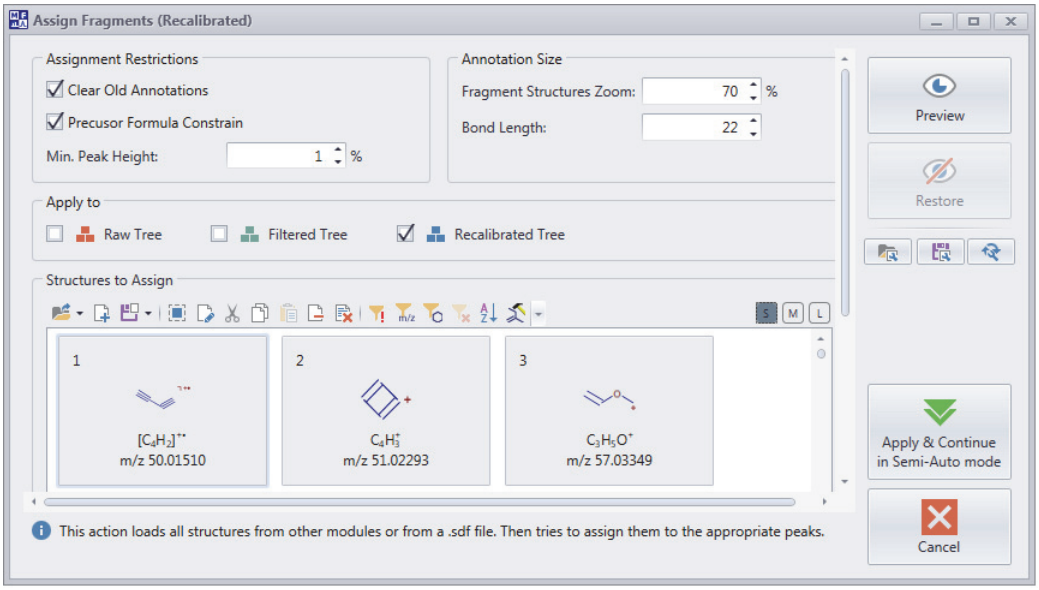
d. To apply the default settings and move to the next step in the curation process, click **Apply & Continue in Semi-Auto Mode**.

The Assign Fragments (Recalibrated) dialog box opens (Figure 33 on page 28).

Continue to action step 12

12. In the Assign Fragments (Recalibrated) dialog box, click **Apply & Continue in Semi-Auto Mode** to apply the default settings, assign fragment structures to the recalibrated tree, and move to the next step in the curation process.

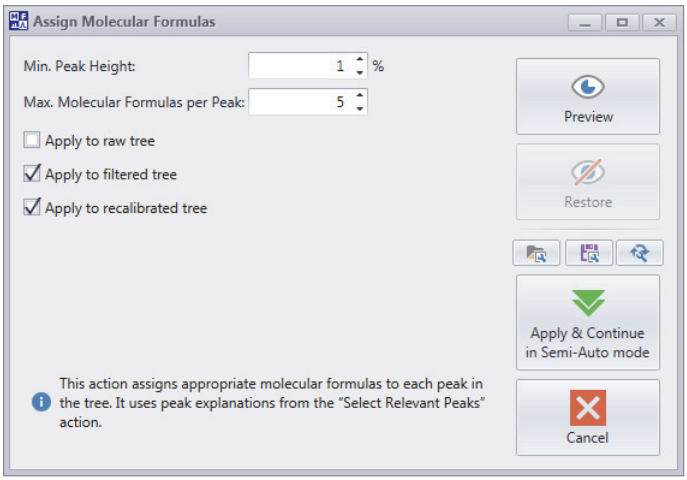
Figure 33. Assign Fragments (Recalibrated) dialog box for action step 11



The Assign Molecular Formulas dialog box opens (Figure 34).

13. To apply the default settings, assign molecular formulas to each peak in the tree, and complete the final step of the curation process, click **Apply & Continue in Semi-Auto Mode**.

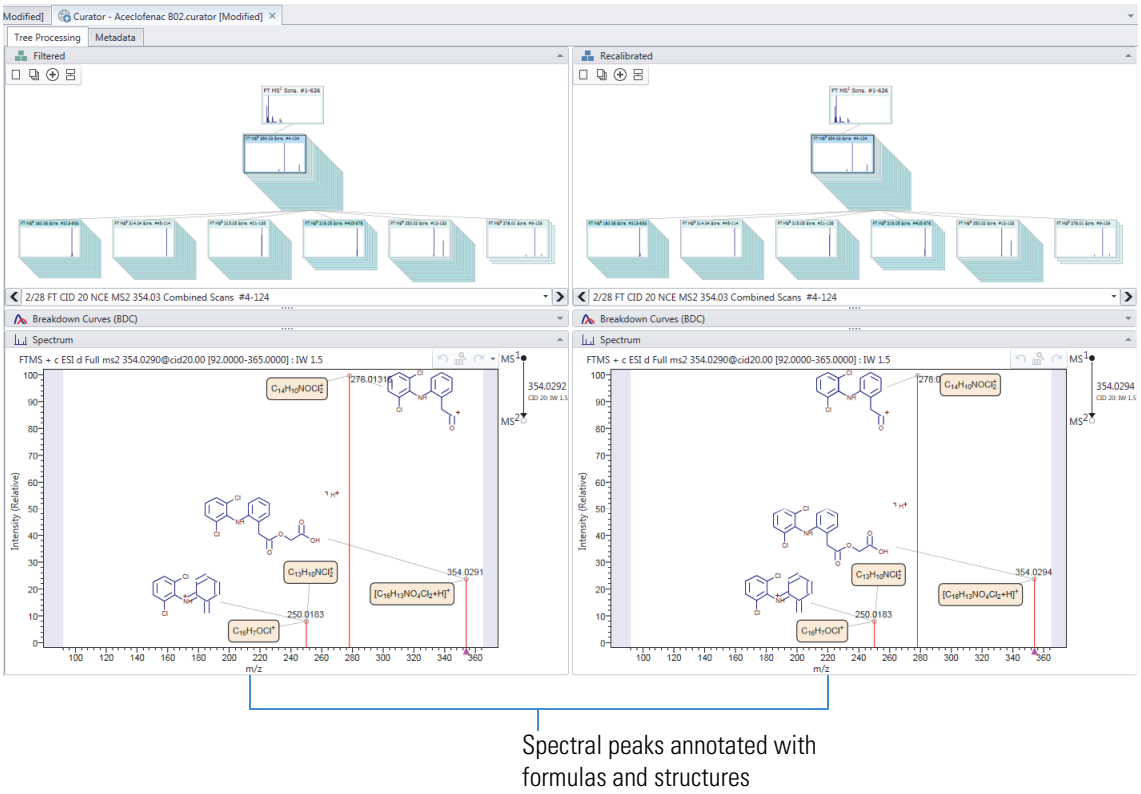
Figure 34. Assign Molecular Formulas dialog box for action step 12 with the default settings



Note The Assign Molecular Formulas step assigns the molecular formulas from the Select Relevant Peaks step to each peak in the tree.

On the Tree Processing page, structure and molecular formula annotations appear in both of the Spectrum views.

Figure 35. Tree Processing page with annotated spectral peaks in the Spectrum panes



Downloading Metadata from Public Sources

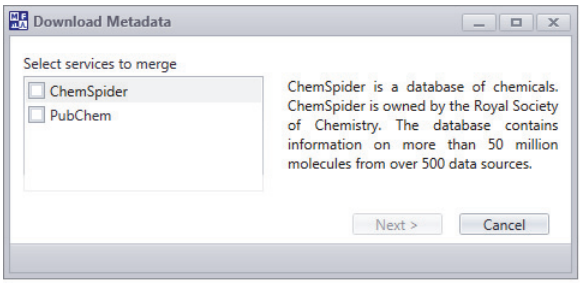
You can add supplementary metadata from public data sources like ChemSpider and PubChem to your library entries.

❖ To download metadata from PubChem

1. In the Processing group of the Curator toolbar, click **Download Metadata**.

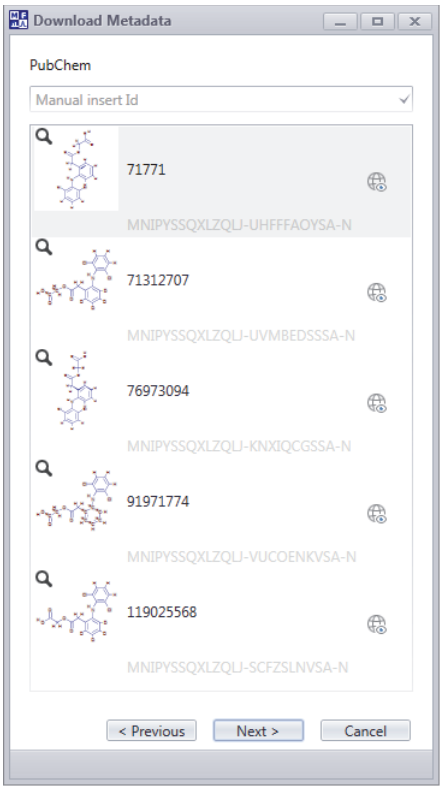
   The Download Metadata wizard opens (Figure 36).

   Figure 36. Download Metadata wizard



2. In the Download Metadata wizard, select the **PubChem** check box and click **Next**.

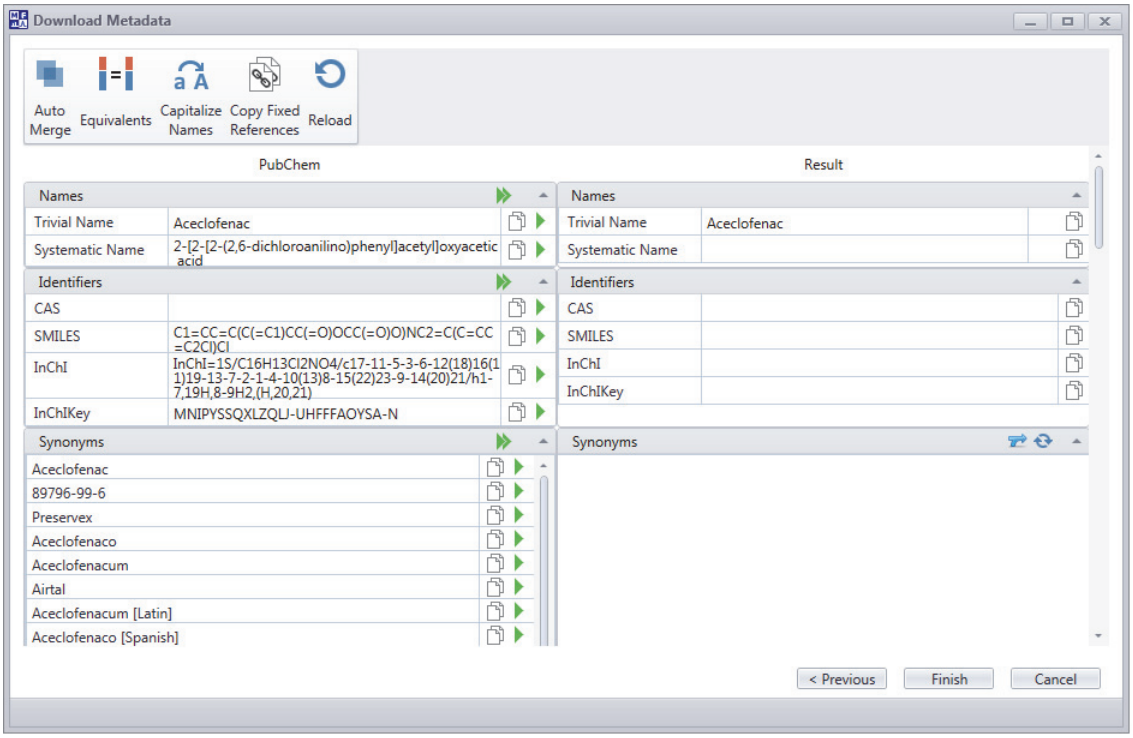
A list of matching PubChem entries appears.



3. In the list, select **71771,** and then click **Next.**

The final page of the wizard appears.

Figure 37. Final page of the Download Metadata wizard

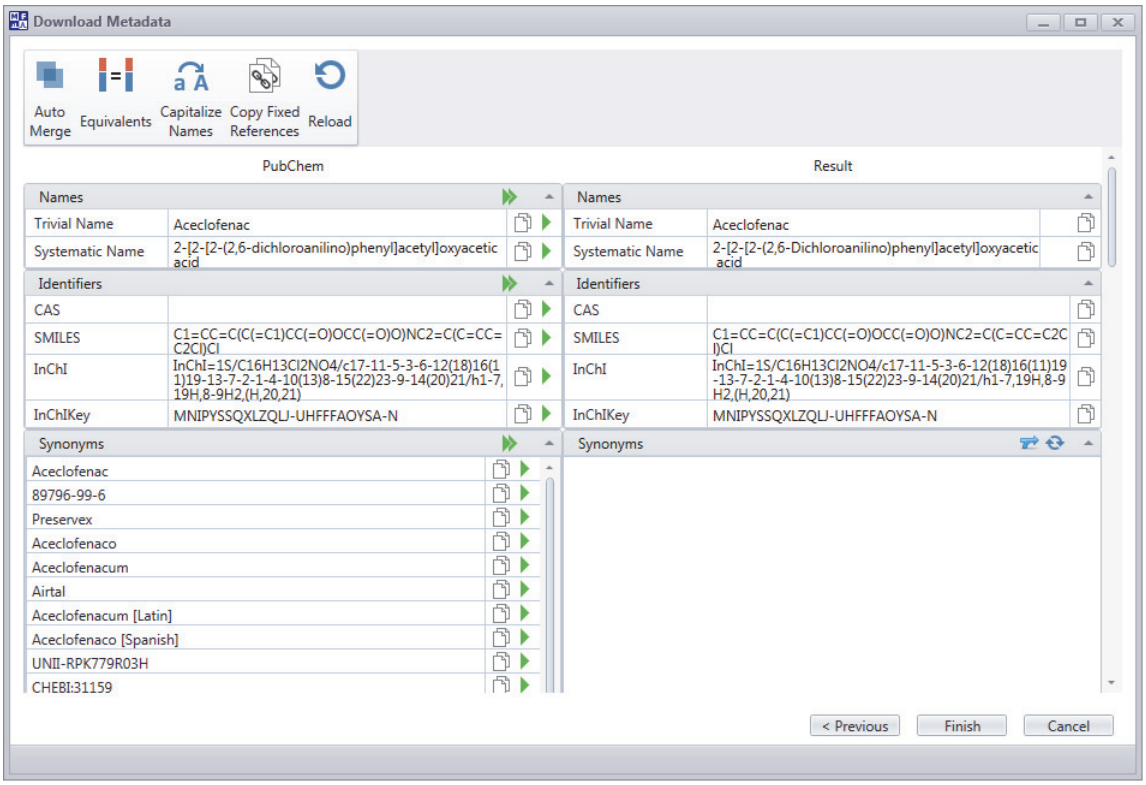


4. To copy an item from the Pub Chem column to the Result column, click the **Add Item** icon, ▸, to its right.

   Add the following items:

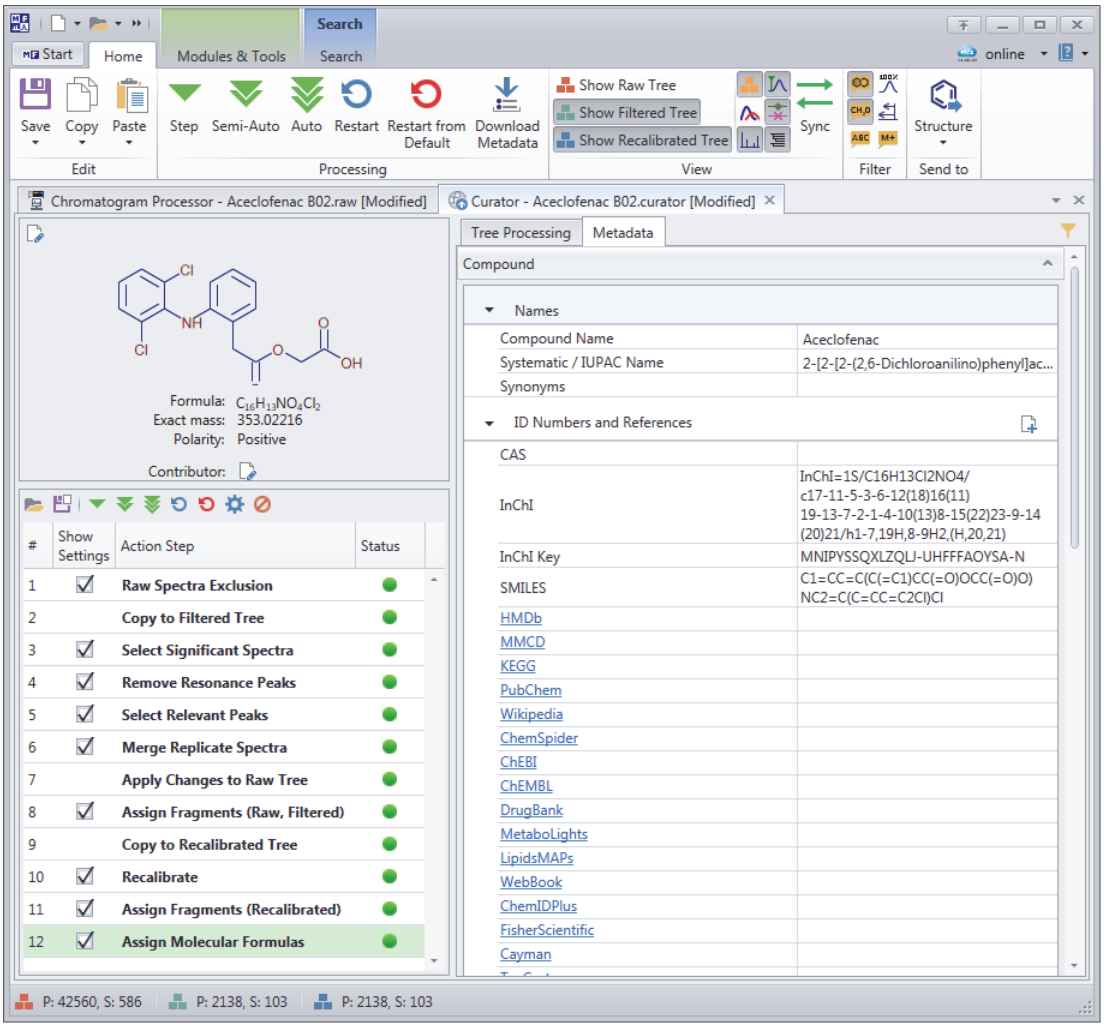
   - Systematic name
   - SMILES
   - InChI
   - InChIKey

Figure 38. Result column with added items



5. To import the metadata into your library entry, click **Finish**.
6. To skip searching for additional KEGG references, click **No** at the prompt.
7. To skip searching for additional Wikipedia references, click **No** at the prompt.
8. On the Curator page, click the **Metadata** tab to open the Metadata page and view the imported data (Figure 39).

Figure 39. Metadata page with metadata from the matching compound in the PubChem database



Saving the Curated Spectral Tree to a File

After completing the curation process, save the curated spectra for aceclofenac to a data file or a user library.

❖ To save the spectral tree record to a curator file

1. In the Curator toolbar, click **Save** and select **Save As**.

   The Curator Component File dialog box opens.

   By default, the File Name box displays the file name of the supplied structure file.

2. Locate the folder where you want to store the file, select the Curator file type, and click **Save**.

> **Note** You can save the curated record as a Mass Frontier Compound Data Container file (.mfcdc) or a Curator file (.curator).
>
> The Mass Frontier Compound Data Container file stores the processed spectral tree with its corresponding spectra, precursor *m/z*, and peak annotations. You can open this file type in the Data Manager module.
>
> The Curator file stores the processed spectral tree with its corresponding spectra, precursor *m/z*, and peak annotations and all the parameter settings for the completed action steps. You can open this file type in the Curator module.

Saving a Compound Entry with Curated Spectra to a User Library

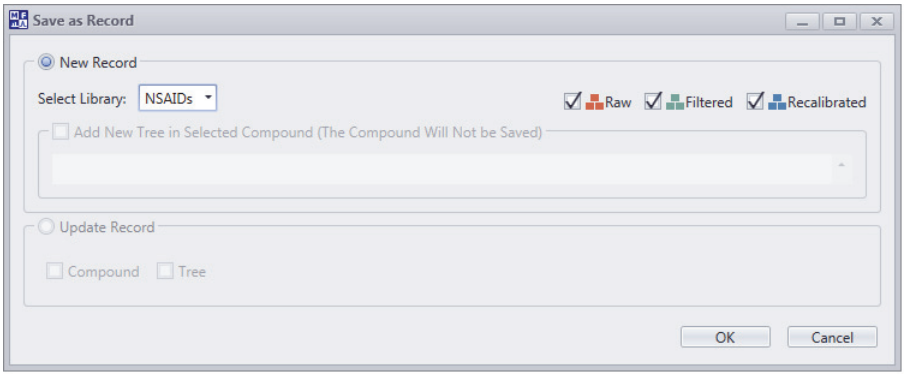
❖ To save the compound entry with curated spectra to a user library

1. In the Curator toolbar, click **Save** and select **Save to Library**.

   The Save as Record dialog box opens.

2. Select the **New Record** option, select the NSAIDs library for the new record, and then click **OK**.

Figure 40. Save as Record dialog box



Tip If the Select Library list does not contain the library you created, make sure that the library service is Started as follows:

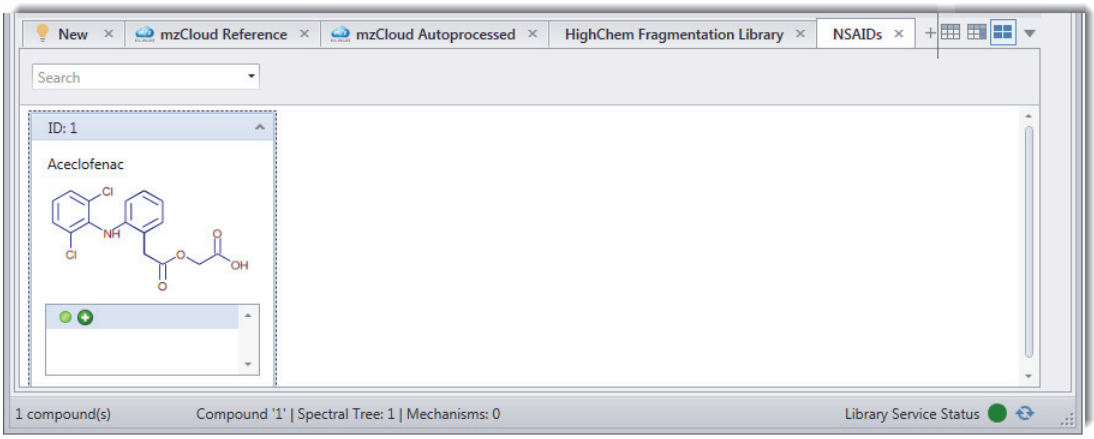
1. From Windows™ Start menu, open the Search Programs and Files box and type **Services**.
2. In the Programs list, select **Services**.
3. In the Services (Local) dialog box, right-click Mass Frontier Services and choose **Restart**.

3. At the prompt, click **OK**.
4. To view the record in the user library, in the **Modules & Tools** toolbar, click **Data Manager**.

   A new instance of the Data Manager module opens as a tabbed page.

5. In the tab bar in the middle of the Data Manager page, click the **NSAIDs** tab.

   The saved record appears in the library record view.



Tip If you do not see the saved record in your library, click **Reload** and select **Reload Compounds** to refresh the view.

Note After you complete this tutorial, you can delete the new user library.

Summary

To create and populate a custom mass spectral library with curated library spectra for your analytes of interest, follow these steps.

1. Infuse a relatively pure solution of the analyte into the mass spectrometer.
2. Open **Mass Frontier Server Manager 3.0**, create a library, and then close the server manager.
3. Open the raw data file in a Chromatogram Processor window.
4. Apply the DICD algorithm with the following parameter settings: Beginning of Tree Branching: 1, Threshold Ion Intensity: 0, and Calculate Envelope: Selected.
5. Do the following:
   a. (Optional) Run an identity search to annotate the component by clicking **Components Search** in the Search group of the Chromatogram Processor toolbar.
   b. Send the unannotated or annotated component to the Curator by clicking **Component > New Curator** in the Send To group of the Chromatogram Processor toolbar.
6. For an unannotated component, open a structure file in the Curator window.
7. Run the Curator action steps and modify the settings as appropriate.
8. Save the compound with its curated spectrum to your custom library by choosing **Save > Save to Library** from the Edit group of the Curator toolbar.

Trademarks