Mass Frontier 8.1 Tutorial to Identify Unknowns in Nominal-Mass, MSn Data by Running Library Searches and mzLogic Analyses

In the Mass Frontier™ 8.1 application, you use the Chromatogram Processor module to browse the information in Xcalibur™ RAW files from Thermo Scientific™ LC/MS systems. This information includes the chromatographic data, the mass spectral data, and some of the data acquisition parameters. In addition to browsing the raw data, you use the Chromatogram Processor module to detect and identify components in the chromatographic data.

This tutorial shows you how to run library searches using the mzCloud™ mass spectral database and an mzLogic™ analysis to identify the unknown components detected in the chromatogram from nominal mass data.

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

Contents

Overview

This tutorial uses a raw data file that contains data-dependent scans from an LC-ESI/MSn experiment acquired on an LXQ™ mass spectrometer (MS).

In this tutorial, you perform the following tasks:

1. Check your computer's connection to the mzCloud server.
2. Open an example data file, browse the chromatographic and mass spectral data, and review the acquisition information.
3. Detect components by applying the Joint Component Detection (JCD) algorithm for LC/MS data.
4. Sort the Components list.

Revision A B51001050

thermoscientific

5. Run mzCloud library searches for all the detected components and annotate each identified component with the name of its matching library compound.
6. Run an mzLogic analysis on a component that you cannot confidently identify with a library search.
7. Save the analysis results to a HighChem Chromatogram Format (HCCX) file.

Demo data files

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

File	Description
Flavonoid_Stds_LXQ.raw	A raw data file that includes 12 flavonoid standard compounds acquired with an LC-ESI/MSn experiment
Flavonoid_Stds_LXQ.chpro_jcd	A component detection file that contains custom component detection settings for the example data file
Structures_C24H24O11.sdf	A structure file that contains the structures for eight flavonoid compounds with the following chemical formula: $C_{24}H_{24}O_{11}$

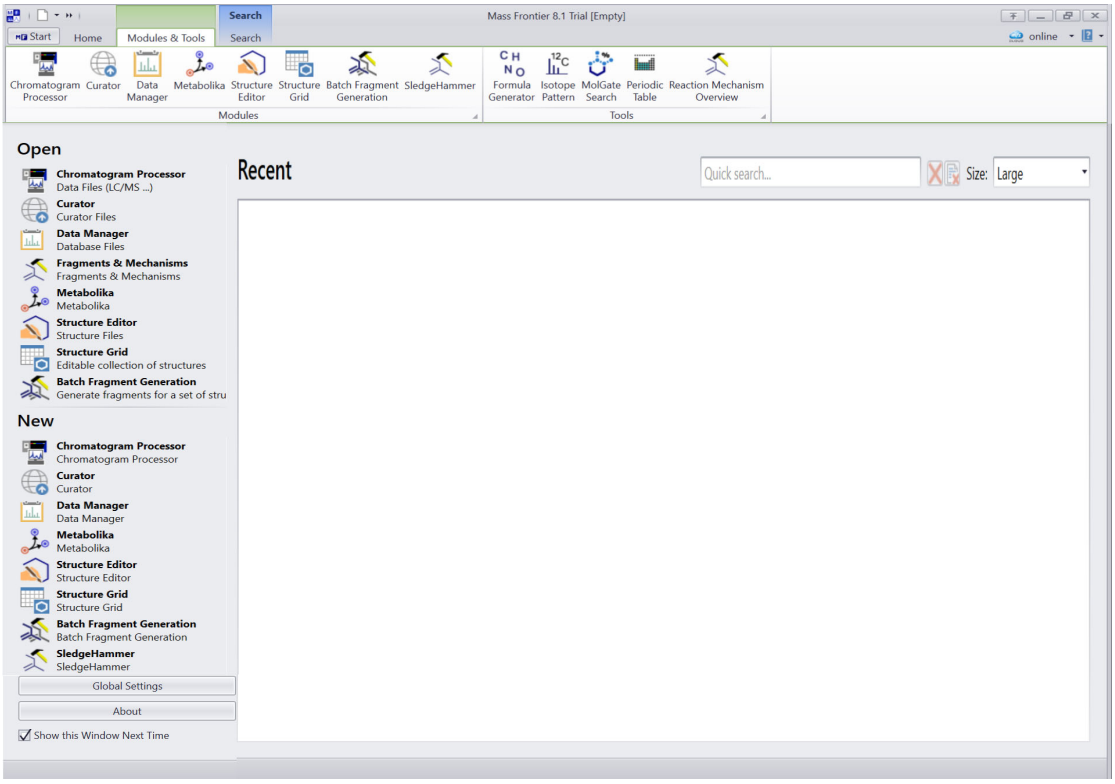
Check the connection to the mzCloud server

❖ To check the connection to the mzCloud server

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 (Figure 1).

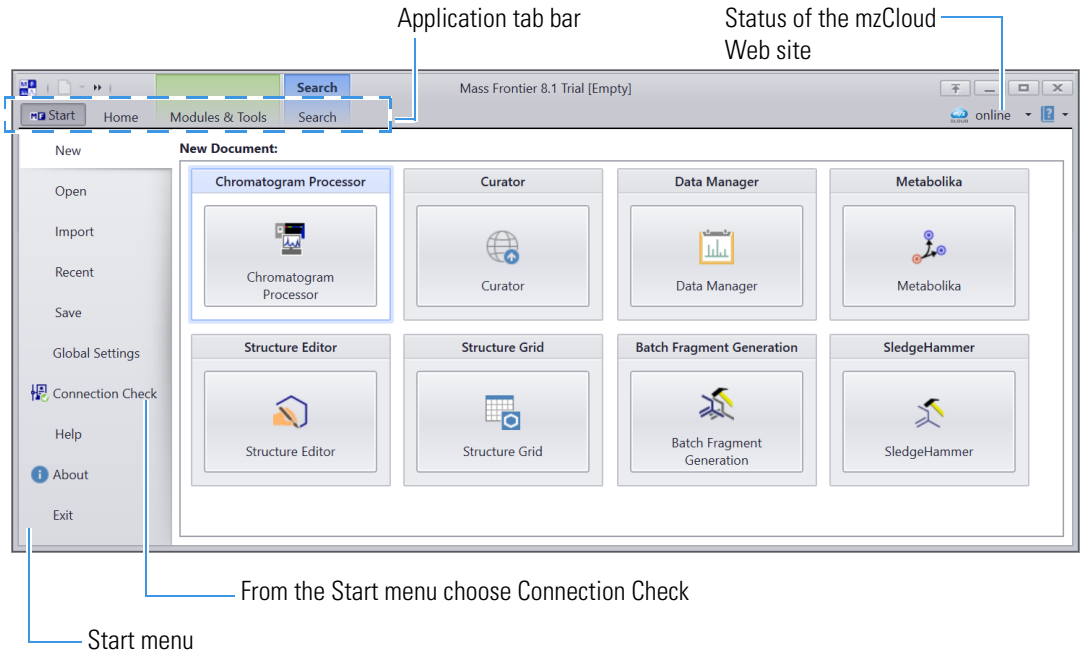
Figure 1. Mass Frontier startup window



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

2. From the application tab bar to open the Start menu, click the **Start** tab, and then choose **Connection Check** (Figure 2).

Figure 2. Start menu and application tab bar



3. In the Connection Check dialog box, click **Run**.

   The application verifies the connection.

4. If the connection check fails, check the computer's Internet connection and its access to various sites.

> **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).

> **Tip** Make sure the computer's clock is accurate within 5 minutes (refer to the instructions in the Release Notes).

Go to the next topic "Open and browse an example raw data file."

Open and browse an example raw data file

Use the Chromatogram Processor module to open raw data files, view the chromatographic and mass spectral data, detect and identify components, and review information about the data file.

> **Note** The Chromatogram Processor module recognizes Xcalibur RAW files from a Thermo Scientific MS, mzML files from a third-party MS, and HighChem Chromatogram Format files (.hccx).

Follow these topics in order:

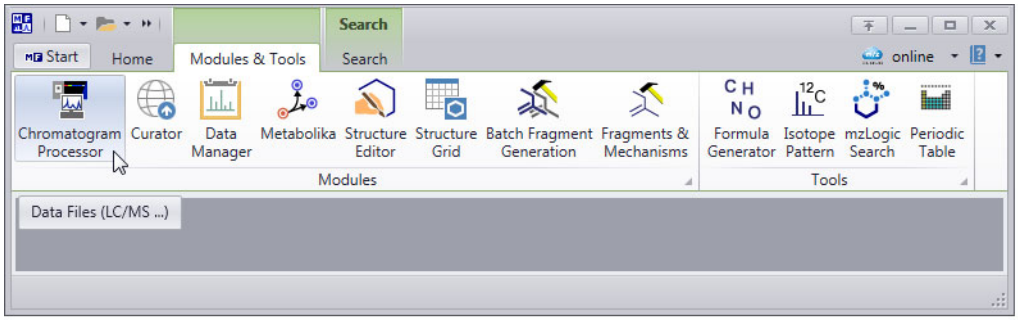
1. Open a raw data file for processing
2. View information about the raw data file

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).

Figure 3. Modules & Tools toolbar



The Open Chromatogram dialog box opens.

2. Browse to the following folder, select **Flavonoid_Stds_LXQ.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 stage and number.

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

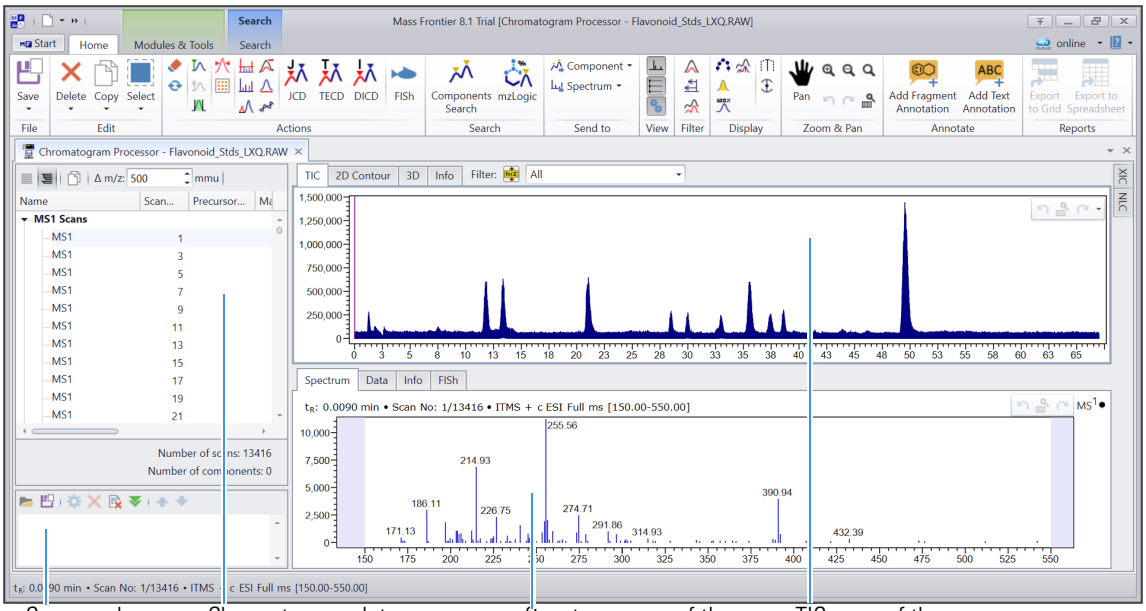
- The chromatogram view at the upper right displays the total ion current (TIC) chromatogram. The *y*-axis scale is set to absolute intensity.

  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 (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, as you have not yet applied any 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 group 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.

View information about the raw data file

❖ To view information about the acquisition of a raw data file

1. Open the Flavonoid_Stds_LXQ.raw data file as described in the previous topic, "Open a raw data file for processing."
2. In the chromatogram view, click the **Info** tab.

The Info page provides information about the MS used to acquire the raw data file.

TIC | 2D Contour | 3D | Info | Filter: All

RAW file	C:\Users\Public\Documents\HighChem\Mass Frontier 8.1\Demo Data\Chromatograms\Flavonoid_Stds_LXQ.RAW
RAW file version	62
Creation date	11/20/2007 5:31:01 AM
Modified date	11/20/2007 6:38:32 AM
Who created	LXQ
Who modified	LXQ
Number of calibrations	0
Number of time modified	1
Number of instruments	3
Revision	62
Instrument model	LXQ
Instrument name	LXQ
Serial number	LXQ10171
Software version	2.2
Units	None
Expected runTime	67
Filter mass precision	2
In Acquisition	0
Comment1	
Comment2	
Max intensity	0
Max integrated intensity	1440386.5
Tolerance Unit	amu
Trailer Extra Count	13416
Trailer Extra Event Count	13416
Tune data Count	1
User Label	System.String[]
Mass resolution	0.500
Number of scans	13416
Scan range	1 - 13416
Time range	0.01 - 67.00
Mass range	50.0000 - 1080.0000
Device Type	MS
Instrument Index	1
Barcode	
Barcode Status	NotRead
Calibration File	
Calibration Level	
Comment	
Dilution Factor	1
Injection Volume	3
Instrument Method File	C:\Xcalibur\methods\Kelly Lin\2007-11\2007-11-13-6.meth
Istd Amount	0
Path	C:\Xcalibur\Data\
Processing Method File	
Raw File Name	11-19-s.RAW
Sample Weight	0
User text	;;;;;;;;;;;;;;;;;;;;
Sample id	1
Sample type	Unknown
Sample vial	A:10
Sample injection volume	3
Sample row number	1
Sample dilution factor	1
Instrument methods	Surveyor LC Pump, Surveyor AS, LTQ, Surveyor PDA Plus
Sample name	1.8 ml Vial, 5 trays 40 vials each

Go to the next topic "Detect components in LC/MS data."

Detect components in LC/MS data

Use the Joint Component Detection (JCD) algorithm to detect the components in a chromatogram from an LC/MS experiment.

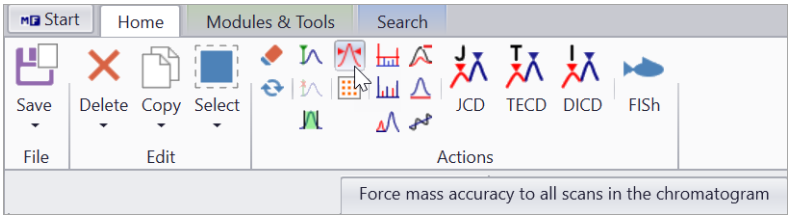
❖ To detect the components in the example file

1. Open the Flavonoid_Stds_LXQ.raw data file as described in the previous topic, "Open a raw data file for processing."

   Note By default the application uses the mass accuracy from the data. For the best component detection results for this data file, force the mass accuracy to 500 *mmu*.

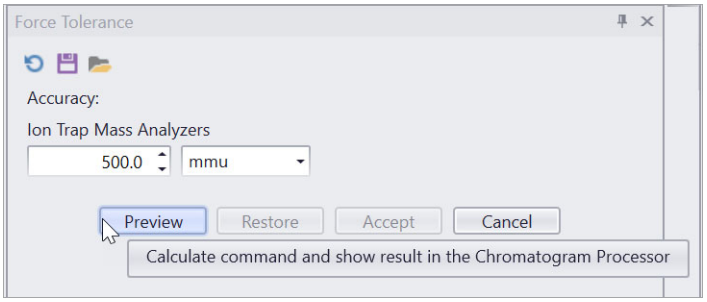
2. To force the mass accuracy to 500 *mmu* do the following:

   a. In the Actions group of the Chromatogram Processor toolbar, click the Force Accuracy icon, .

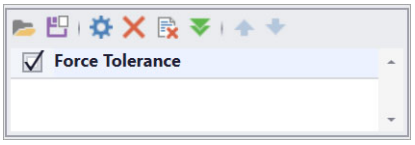
   The Force Tolerance dialog box opens.

   b. The Ion Trap Mass Analyzers setting is set to 500.0 *mmu*. To start processing, click **Preview**.

   c. After the processing finishes, to accept the settings click **Accept**.

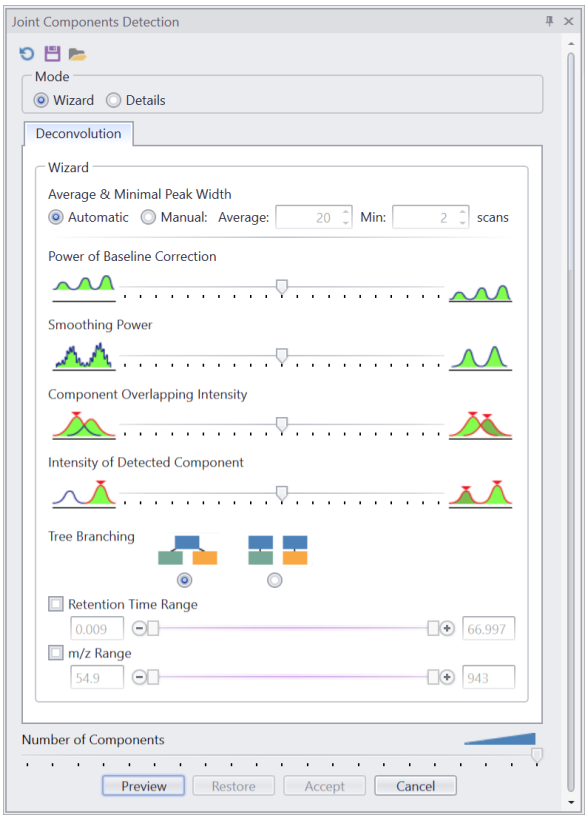
   In the command processor view, the force tolerance check box is checked.

3. In the Action group of the toolbar, click **JCD**.

   The Joint Components Detection view opens to the right of the chromatogram and MS spectrum views (Figure 5).

Figure 5. Default settings on the Wizard page for the JCD algorithm



4. Click the **Reset** icon, , to reset the parameters to their factory default values.
5. To load the detection parameters from an existing chpro_jcd file, do the following:
   a. Click the **Load Parameters** icon, .

      The Joint Components Detection dialog box opens.

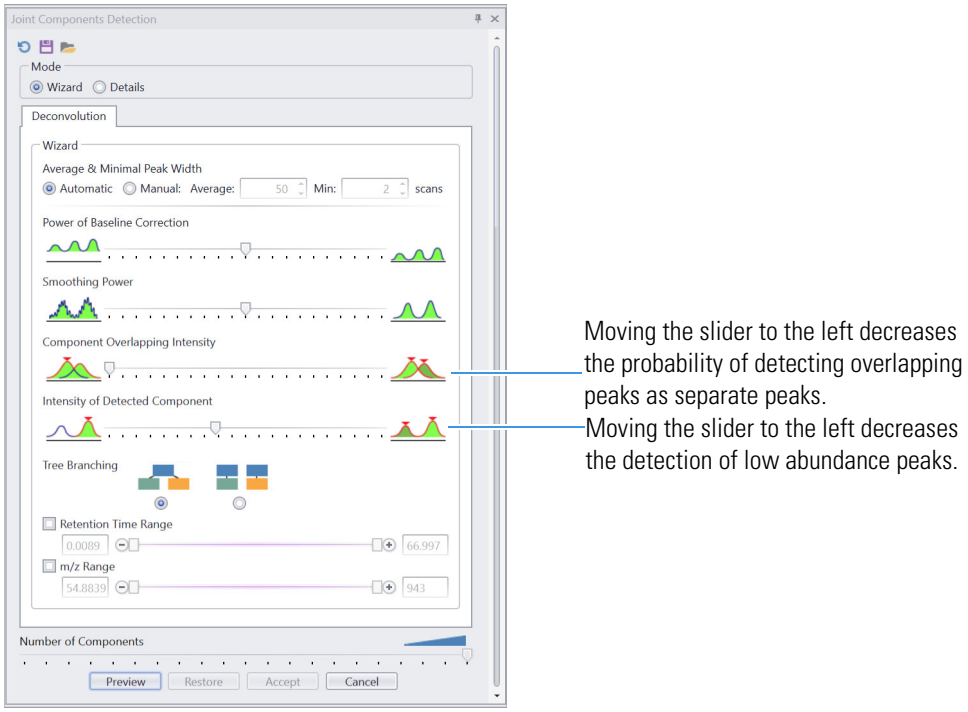
   b. Browse to the following folder:

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

   c. Select **Flavonoid_Stds_LXQ.chpro_jcd** and click **Open**.

      The Deconvolution area of the Joint Components Detection view displays the new detection settings (Figure 6). The Component Overlapping Intensity setting is set to the far left.

Figure 6. Detection settings from the selected CHPRO_JCD file



6. To start processing, click **Preview**.

   After the processing finishes, the following items appear (Figure 7):

   - In the chromatogram data view, the Components list appears above the MS1 Scans list. The components are displayed 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 chromatographic peak apexes of the detected components.
   - A spectral tree appears on the left of the MS spectrum view. The Spectrum page displays the combined spectrum for the MS1 scans across the selected component's chromatographic peak.

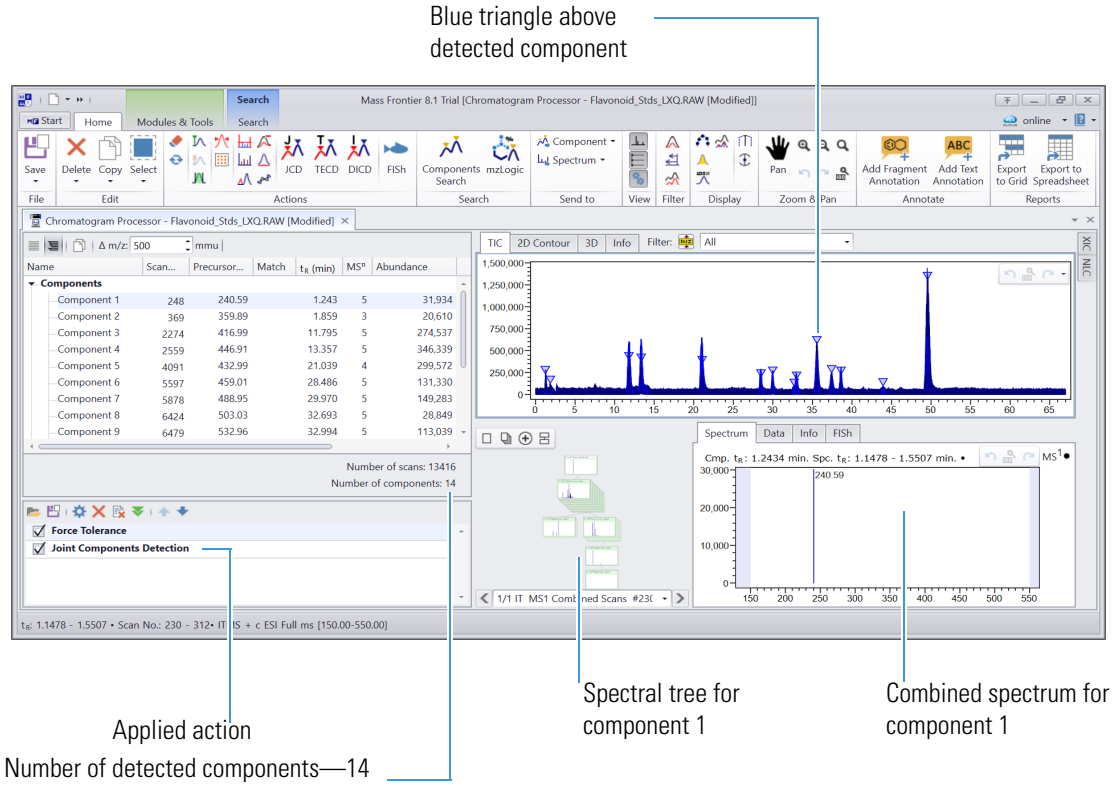
7. Check the number of detected components.

   With the detection settings in the selected CHPRO_JCD file, the application detects 14 components in the TIC chromatogram for the Flavonoid_Stds_LXQ.raw file.

8. To accept the components, click **Accept**.

   The Joint Components Detection view closes, and joint components detection appears as an applied action in the command processor view.

Figure 7. Integrated TIC chromatogram with marked components



Go to the next topic, "Sort the components list."

Sort the components list

In the chromatogram data view, you can sort the components by the scan no., precursor *m/z*, MSn, retention time, or abundance.

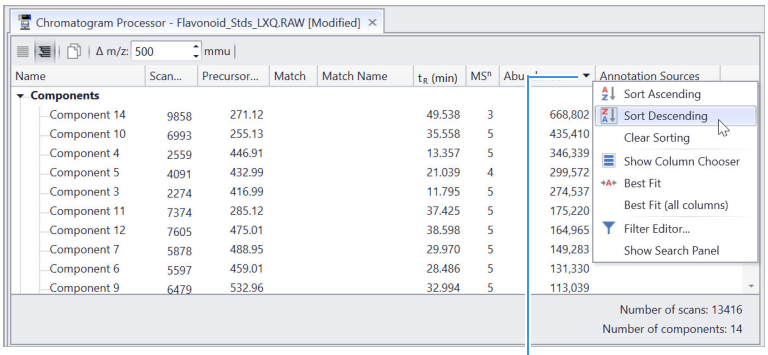
Note The chromatogram data view does not contain a Components list until you apply a component detection algorithm to the chromatographic data.

❖ To sort the components in descending order by abundance

1. Right-click the Abundance column and choose **Sort Descending**. Or, click the **Abundance** column heading until the arrow to the right of the Abundance points down.

   The higher-abundance components sort to the top (Figure 8).

Figure 8. Components sorted by abundance



2. Right-click the Abundance column and choose **Clear Sorting**.

Tip There are two ways to sort the columns in the Components list.

- To sort a single column, click the column heading until the desired arrow appears in the column (for descending, or for ascending), or right-click the column heading and choose a sort order.

  The arrow to the right of a column heading indicates that the column is sorted. When you clear the sorting for a column, the arrow disappears.

–or–

- To sort by multiple columns:

  a. Sort the first column.

  b. Press the **SHIFT** key then click on the next column to sort. Repeat this step to sort by another column.

Go to the next topic "Identify components by searching a mass spectral library."

Identify components by searching a mass spectral library

Table 1 describes the four search types that are available for nominal mass data. Only the Identity and Tree searches limit the hit results to compounds that match the unknown component's mass. The other two search types can help you determine whether an unknown component has a substructure in common with any of the compounds in the selected mass spectral libraries.

All four search types use the Identity spectral comparison algorithm to compare the query spectra to the library spectra. This means that the precursor *m/z* values for the comparison spectra must match.

Note The Mass Frontier application includes six search types, but the Similarity Forward and Similarity Reverse search types are unavailable for nominal mass data.

Table 1. Search types for nominal mass data (Sheet 1 of 2)

Search type	Used stages and constraints	Use	Confidence score
Identity	• Compares the MS2 library spectra against the MS2 query spectra. • The MS2 precursor ions must match.	Compound identification	Best Confidence Match

Table 1. Search types for nominal mass data, continued (Sheet 2 of 2)

Search type	Used stages and constraints	Use	Confidence score
Tree Search	• Compares any MSn library spectra against any MSn query spectra. • The MS2 precursors for the query spectrum and the library spectrum must match.	Compound identification with increased specificity	Aggregated Tree Match
Identity Substructure	• Compares any MSn library spectra against any MSn query spectra. • The precursor ions at any MSn must match.	Substructure identification	Best Confidence Match
Subtree Search	• Compares any MSn library spectra against any MSn query spectra. • The precursor ions at any MSn stage must match.	Substructure identification with increased sensitivity	Aggregated Sub-Tree Match

To familiarize yourself with the Components Search feature, in this tutorial follow these topics in order:

1. Run an Identity search
2. Run a tree search
3. Run an Identity Substructure search
4. Run a Subtree search
5. Search result summary

Run an Identity search

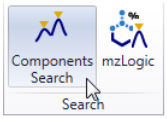
Run an Identity search to identify the detected components.

Follow these procedures in order:

1. To run an Identity search for all the components against the mzCloud library
2. To review the library hits for a component
3. (Optional) To annotate a library spectrum with fragment structures
4. To save the annotations for a set of components

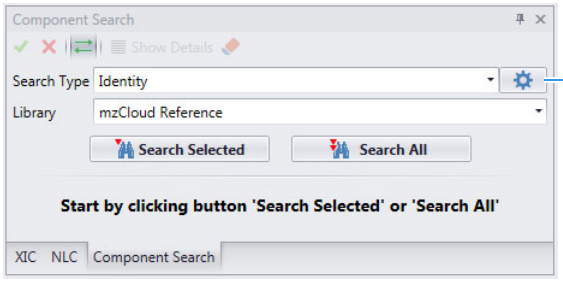
❖ To run an Identity search for all the components against the mzCloud library

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

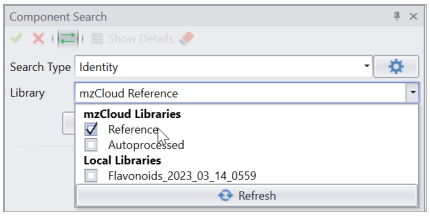


The Components Search view opens to the right of the chromatogram and MS spectrum views. By default, the Search Type is set to Identity (Figure 9).

Figure 9. Default settings for the Component Search view



2. If the Library box is empty or does not list the mzCloud Reference library, open the Library list and select the **Reference** check box.



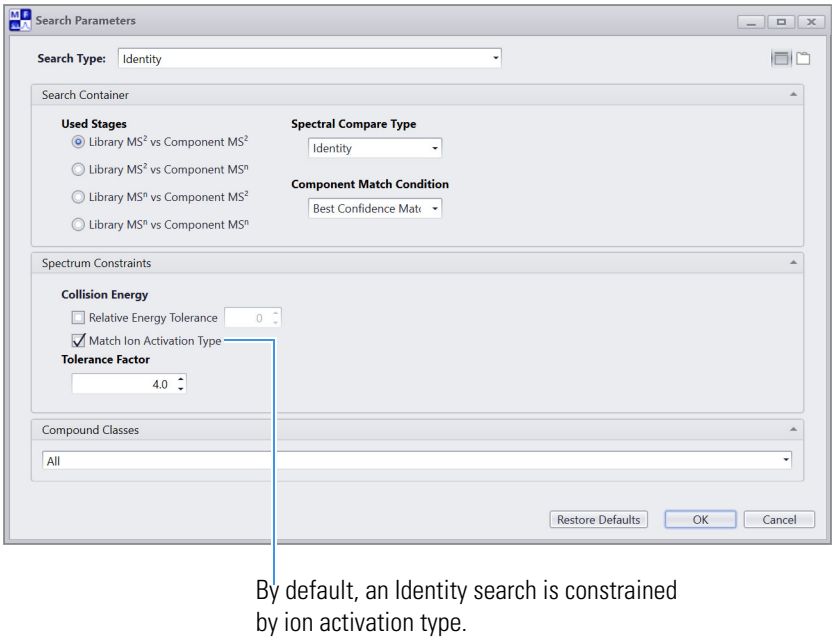
Tip If you recently added a user library, and it does not appear in the list, click **Refresh**.

3. (Optional) To review the default settings for an Identity search, do the following:

   a. Click the settings icon, .

   The Search Parameters dialog box opens with the Search Type set to Identity (Figure 10).

   Figure 10. Default settings for an Identity search



   b. Click **OK**.

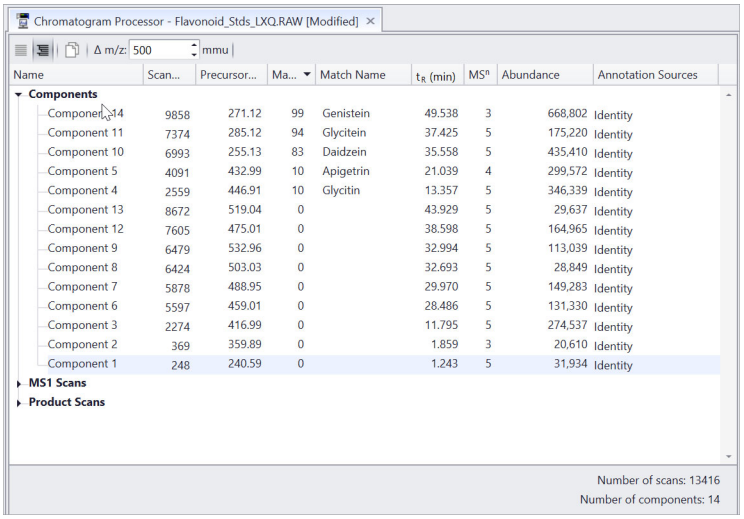
4. In the Component Search view, click **Search All**.

5. When the search ends, sort the Components list by the **Match** column in descending order.

   The library search finds matching compounds for five components (Figure 11).

Note Because the online mzCloud mass spectral database grows constantly, your search might return more matches.

Figure 11. Identity matches for the detected components in the flavonoid standards sample file (April 2023)



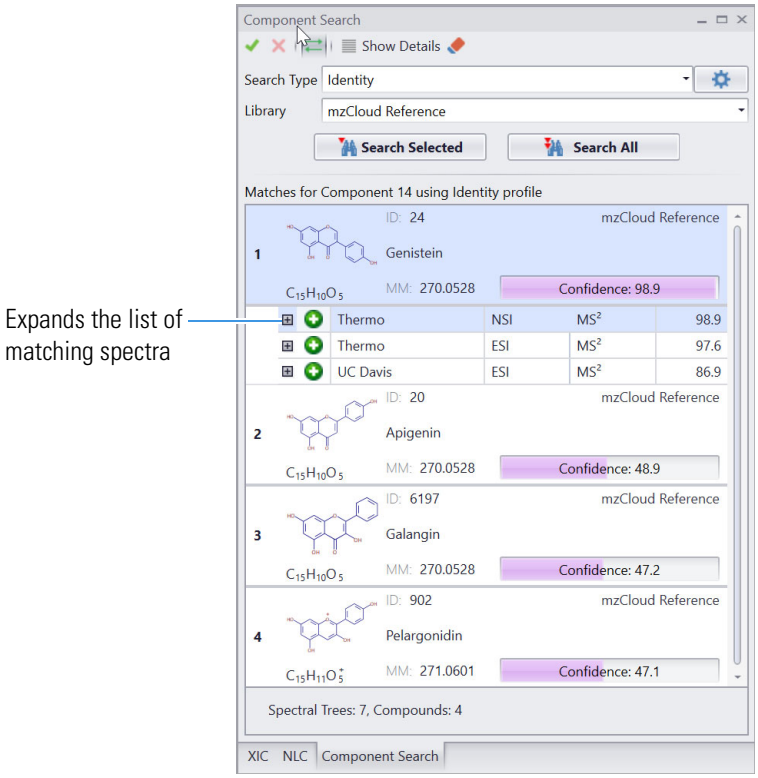
❖ To review the library hits for a component

1. In the chromatogram data view, select a component of interest in the Components list.

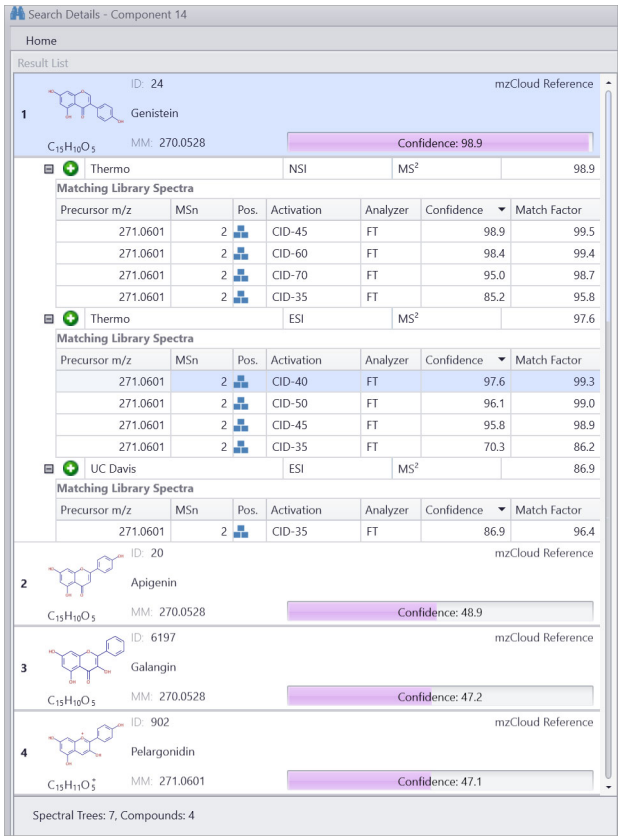
   For this tutorial, select **component 14**, the first component in the components list when it is sorted by the match score in descending order.

   The Component Search view shows the four hits in the mzCloud Reference library (Figure 12).

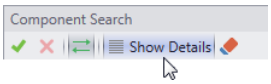
Figure 12. Component Search view showing the Identity search results for component 14



2. Expand the spectrum lists by clicking the expand icon, ⊞, and notice that the Identity search only returns compounds for the same precursor mass (within the specified mass tolerance) and that all the matching spectra are MS2 level fragmentation scans with the same ion activation type.



3. To view more details about the matching compounds, click **Show Details**.



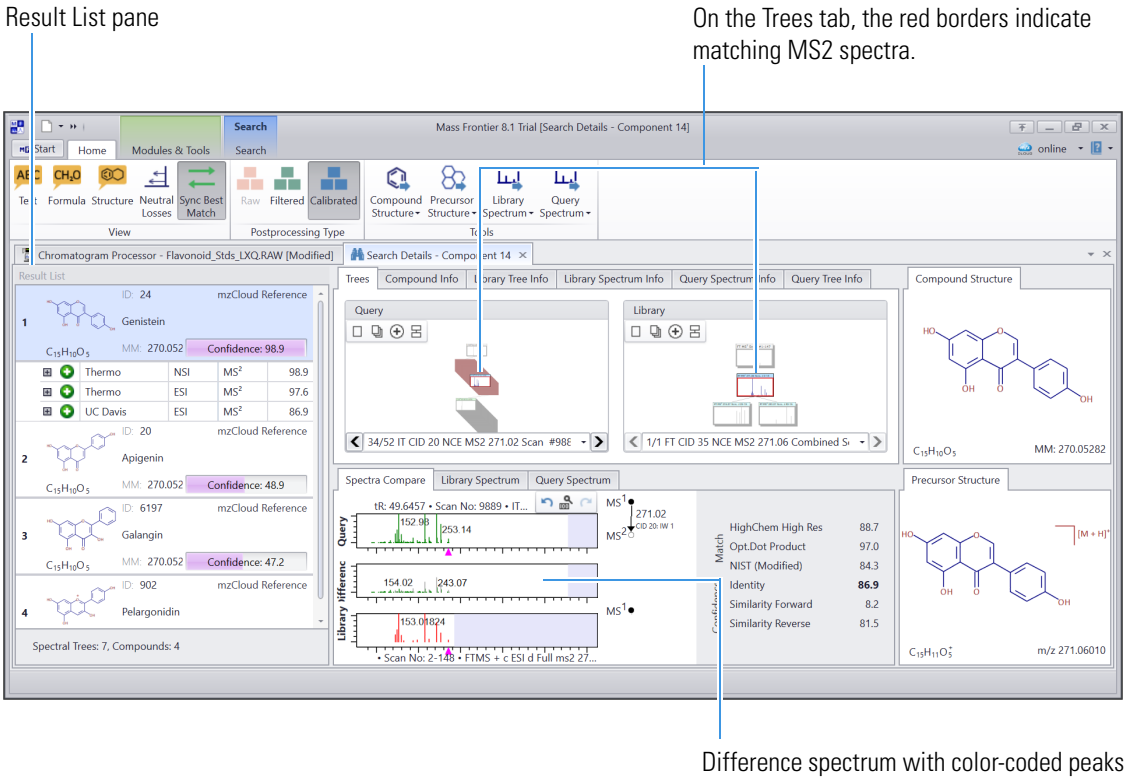
A Search Details page opens as a tabbed page (Figure 13):

- On the left, the Result List pane matches the list in the Component Search view.
- On the top center, the Trees page shows the spectral tree for the component's query spectrum on the left and the spectral tree for the matching library compound on the right. Matching spectra are highlighted with red borders. In Figure 13, notice that only the MS^2 nodes contain spectra with red borders.
- On the bottom center, the Spectra Compare page displays the query spectrum in green, the best matching library spectrum in red, and a difference spectrum with peaks in three colors.

Gray	Matching peaks within the specified mass tolerance. The peak height is a measure of the intensity difference between the peak in the library spectrum and the peak in the query spectrum.
Green	Spectrum peaks in the query scan that are not present in the library spectrum.
Red	Spectrum peaks in the library spectrum that are not present in the query scan.

- On the right, the Compound Structure pane displays the structure of the library compound, and the Precursor Structure pane displays the structure of the precursor ion for the matching library spectrum.

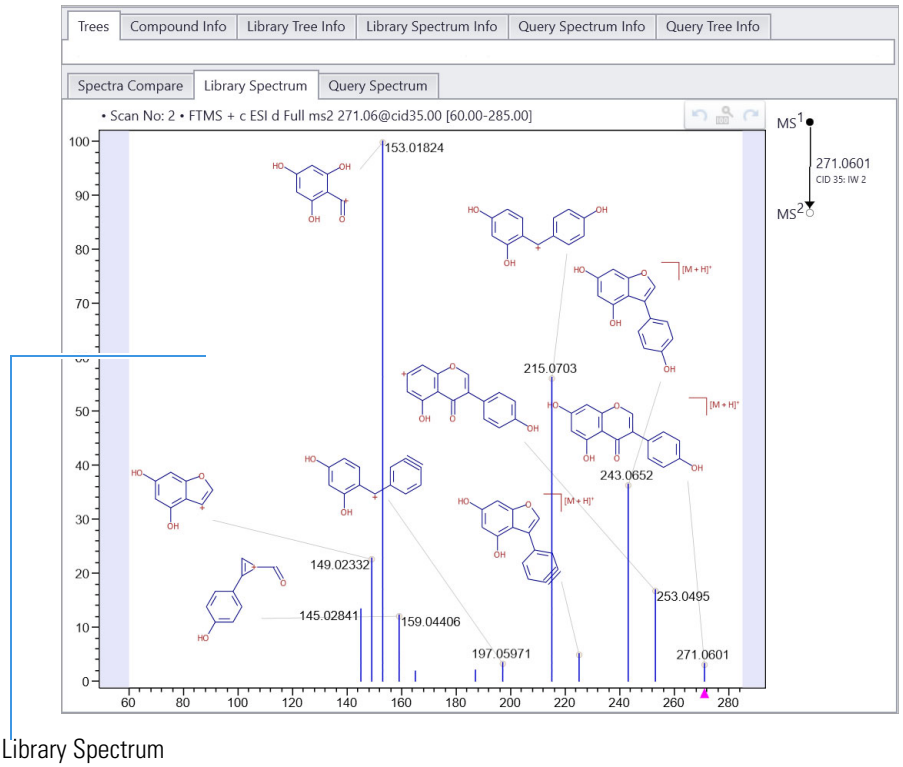
Figure 13. Search Details page for component 14 following an Identity search



❖ (Optional) To annotate a library spectrum with fragment structures

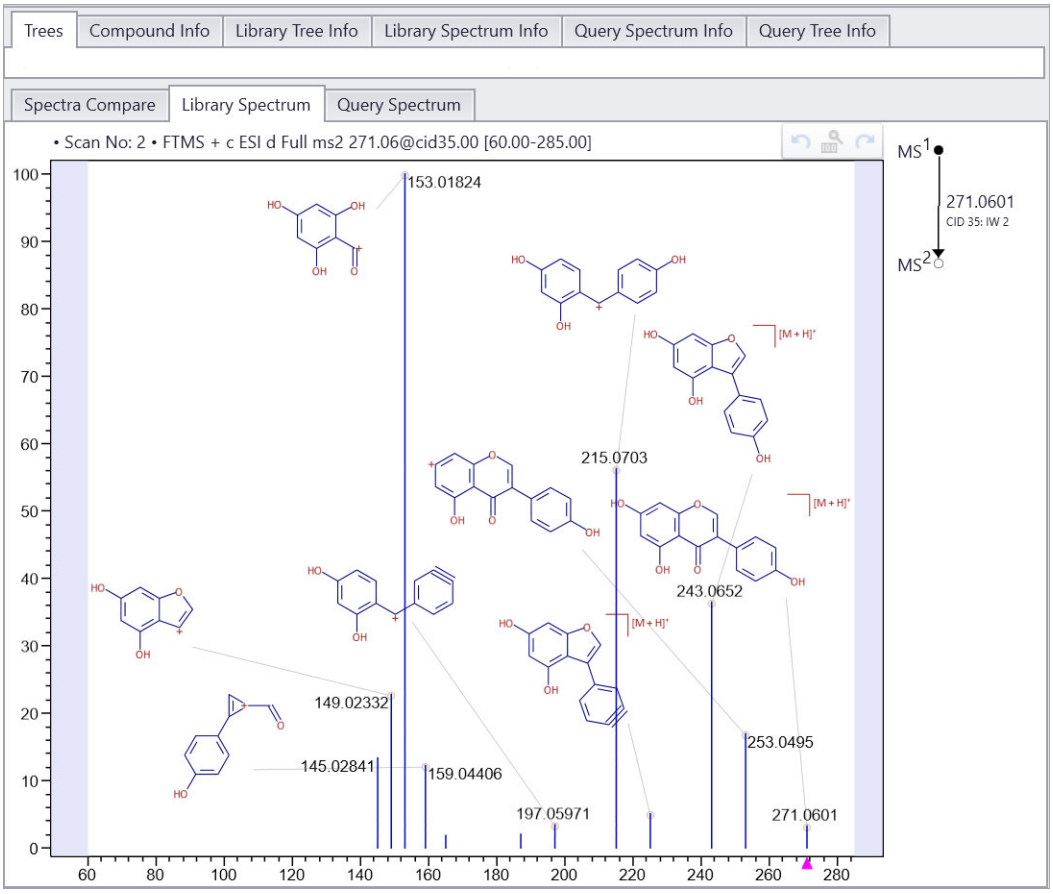
1. On the Search Details - Component 14 page, click the **Library Spectrum** tab to display the library spectrum by itself.
2. Right-click the Library Spectrum page and choose **Show Fragment Annotations** (Figure 14).

Figure 14. Shortcut menu (right-click menu) for the Library Spectrum page



The application annotates the spectral peaks with fragment structures (Figure 15).

Figure 15. Annotated library spectrum for genistein

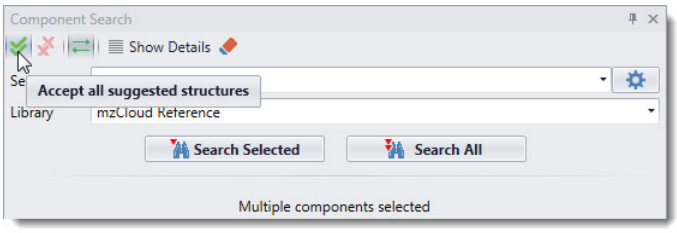


❖ To save the annotations for a set of components

1. On the Chromatogram Processor page, in the Components list, select the annotated components of interest. For this tutorial, select components **10, 11,** and **14**.

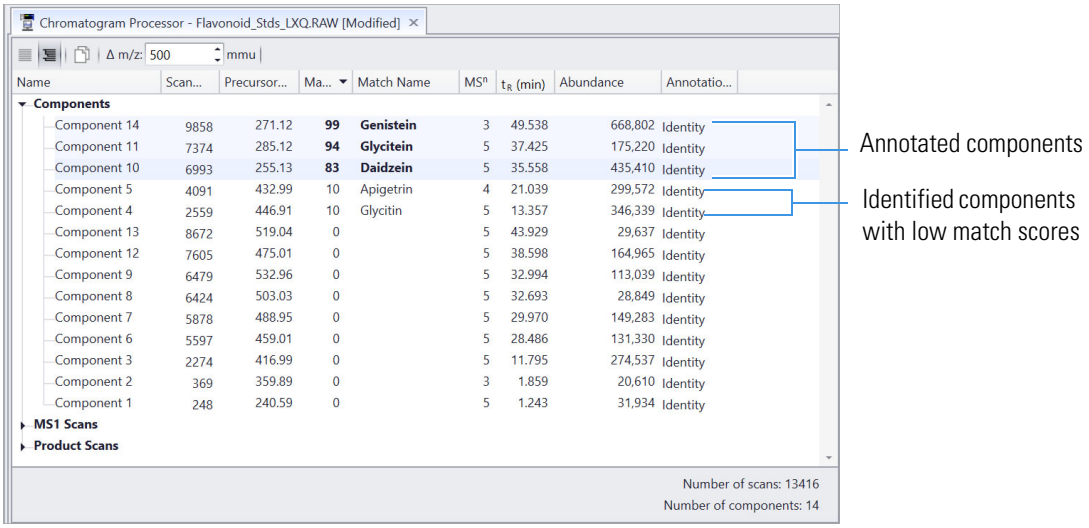
> **Note** The hits for components 10, 11, and 14 have relatively high match scores—whereas, the hits for components 4 and 5 have relatively low match scores.
>
> Running a Tree Search, which searches for matching MSn spectra in addition to matching MS2 spectra, might provide a higher level of confidence that the hits for components 4 and 5 are correct.

2. In the Component Search view, click the **Accept All Suggested Structures** icon, ✔.



The application assigns the compound names and annotation source to the selected components, and displays the assigned names and match scores in bold font (Figure 16).

Figure 16. Accepted annotations for the Identity search



Tip To unclutter the user interface, close the Search Details pages.

To confirm the hits for components 4 and 5, go the next topic "Run a tree search."

Run a tree search

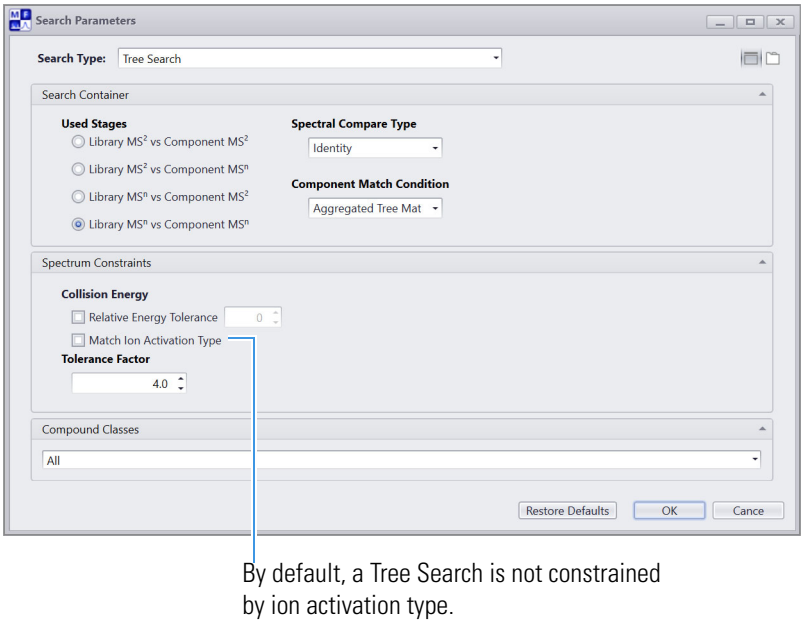
If the data includes high-quality MS^n data, as does the example data file, run a Tree Search.

❖ To run a tree search to find compounds with MS2 and MSn spectra that match the components

1. In the Components Search view on the Chromatogram Processor page, select **Tree Search** from the Search Type list.
2. To review the settings for a Tree Search, click the settings icon, ⚙.

   Figure 17 shows the default settings for a Tree Search.

   Figure 17. Default settings for a Tree Search



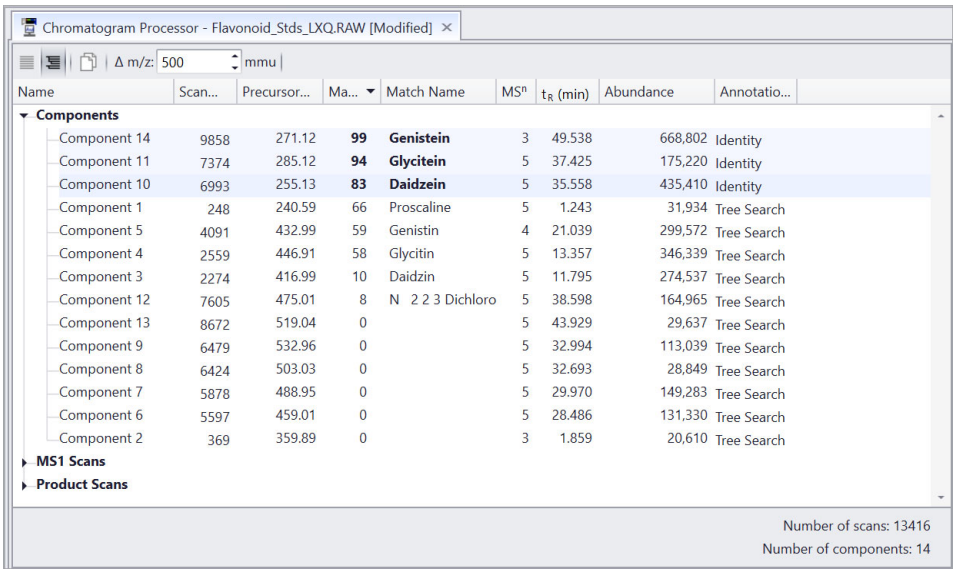
3. Click **OK**.

4. In the Components Search view, click **Search All**.

5. When the search ends, sort the Components list in descending order by the Match column (Figure 18).

   The Tree Search returns higher match scores for components 4 and 5 and matching compounds for components 1, 3, and 12.

   Figure 18. Tree Search results for the example data file (April 2023)

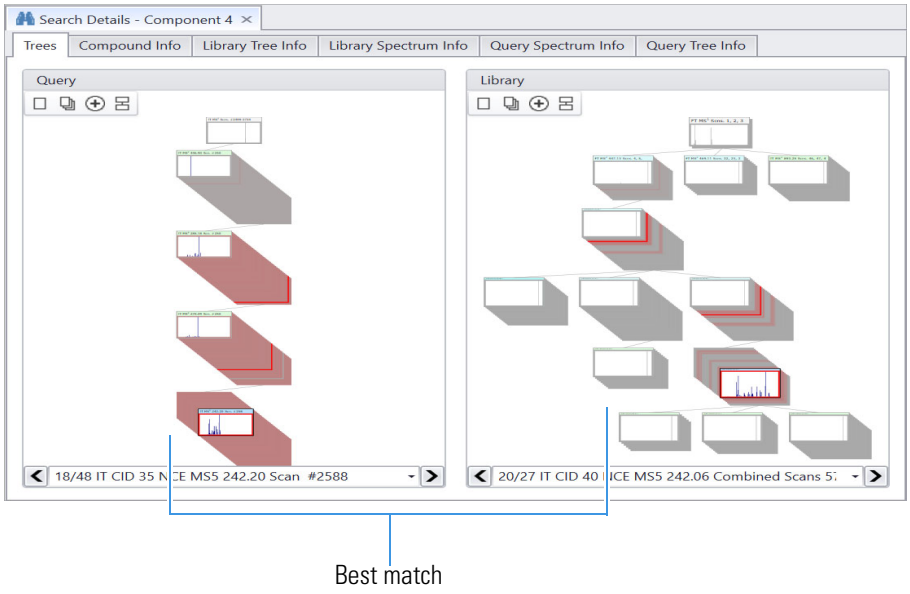


6. To inspect the matching spectra for component 4, do the following:

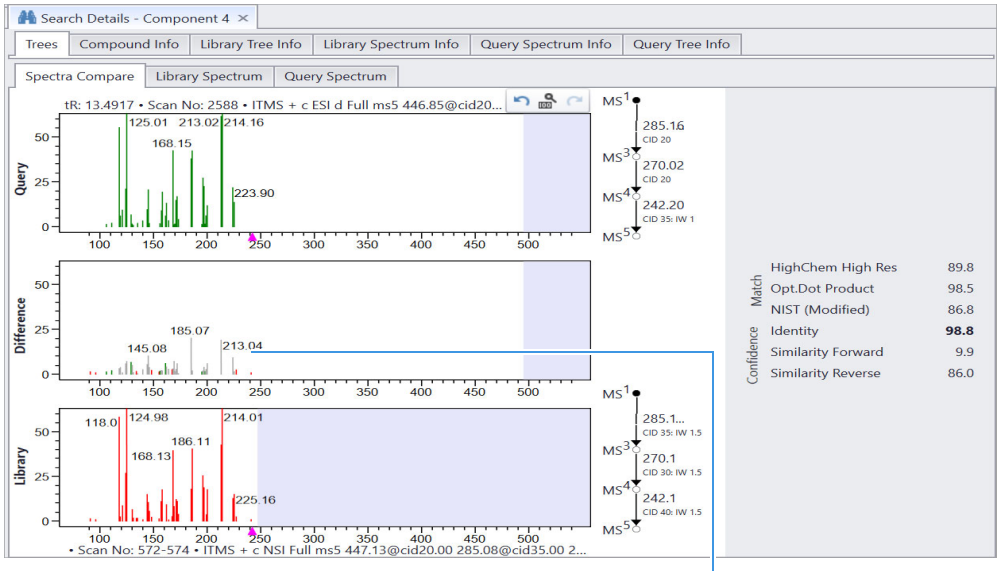
   a. In the Components list, select **Component 4**.

   b. In the Components Search view click **Show Details**.

      The Search Details - Component 4 page opens. At the top center of the Search Details page, the Trees page highlights the best matching spectra.

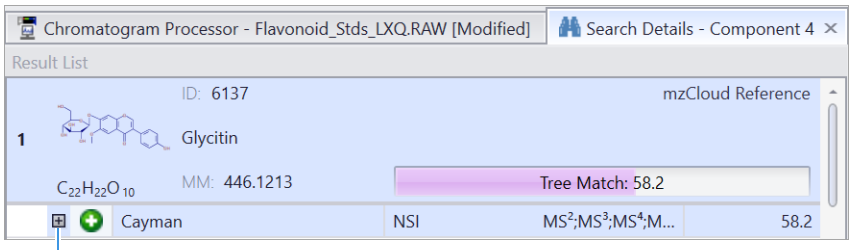


At the bottom center of the Components Search page, the Spectra Compare page displays a difference spectrum for the best matching spectra.



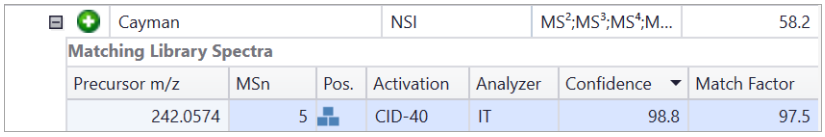
Difference spectrum with color-coded peaks

c. In the Result List pane on the left of the Component Search page, click the expand icon, ⊞, to expand the spectrum list.



Expands the spectrum list

The library spectrum with the highest Confidence and Match Factor appears at the top of the list.



d. To sort the list by the MS^n stage, click the **MSn** column heading.

Notice the large number of matching MS^n library spectra that contribute to the overall match score.

Cayman | NSI | $MS^2;MS^3;MS^4;M...$ | 58.2

Matching Library Spectra

Precursor m/z	MSn	Pos.	Activation	Analyzer	Confidence	Match Factor
447.1286	2		CID-100	FT	10.0	100.0
447.1286	2		CID-30	FT	10.0	100.0
447.1286	2		HCD-30	FT	10.0	100.0
447.1286	2		CID-55	FT	10.0	100.0
447.1286	2		CID-20	FT	10.0	100.0
447.1286	2		HCD-20	FT	10.0	99.9
447.1286	2		HCD-40	FT	10.0	99.8
447.1286	2		HCD-50	FT	9.8	95.0
447.1286	2		HCD-10	FT	9.4	88.5
447.1286	2		CID-15	FT	9.1	81.7
447.1286	2		HCD-60	FT	8.5	69.2
285.0757	3		CID-40	FT	95.9	98.9
285.0757	3		CID-45	FT	90.9	97.8
285.0757	3		CID-35	FT	90.7	97.7
285.0757	3		CID-60	FT	90.3	97.6
285.0757	3		CID-70	FT	79.9	97.2
285.0757	3		HCD-60	FT	68.3	84.8
285.0757	3		HCD-50	FT	49.8	79.6
285.0757	3		HCD-70	FT	45.9	58.2
285.0757	3		CID-80	FT	9.7	94.4
285.0757	3		CID-30	FT	7.9	58.2
270.0523	4		CID-65	FT	95.1	98.8
270.0523	4		CID-45	FT	92.6	99.4
270.0523	4		CID-75	FT	92.0	98.1
270.0523	4		CID-35	FT	91.4	99.3
270.0523	4		CID-60	FT	88.9	99.1
270.0523	4		HCD-50	FT	72.7	87.9
270.0523	4		CID-30	FT	69.8	93.9
270.0523	4		HCD-60	FT	67.2	83.9
270.0523	4		HCD-40	FT	45.8	57.7
270.0523	4		HCD-70	FT	44.8	52.0
270.0523	4		CID-70	FT	9.9	98.6
270.0523	4		CID-80	FT	9.9	97.4
242.0574	5		CID-40	IT	98.8	97.5
242.0574	5		CID-60	IT	98.7	97.2
242.0574	5		CID-35	IT	98.6	96.9
242.0574	5		CID-45	IT	98.2	95.1
242.0574	5		CID-70	IT	85.5	88.6
242.0574	5		CID-35	FT	71.8	91.4
242.0574	5		CID-80	IT	64.4	74.1
242.0574	5		CID-30	IT	64.1	73.9
242.0574	5		CID-45	FT	59.5	84.9
242.0574	5		CID-75	IT	57.4	66.1
242.0574	5		HCD-60	FT	56.9	64.4
242.0574	5		HCD-70	FT	55.4	58.8
242.0574	5		CID-30	FT	55.3	58.5
242.0574	5		CID-40	FT	39.4	91.0

Note In the example data file, all the fragmentation scans were acquired in the ion trap (IT mass analyzer) using the collision-induced dissociation (CID) ion activation method with a normalized collision energy of 20 for the lower MS^n stages and 35 for the MS^5 stage.

e. In the list of matching library spectra, select the **MSn = 2 scan with a CID-20 activation**.

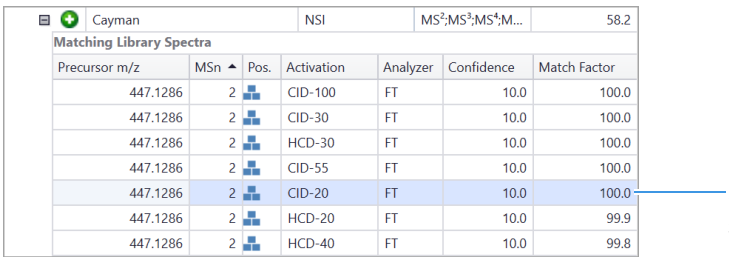
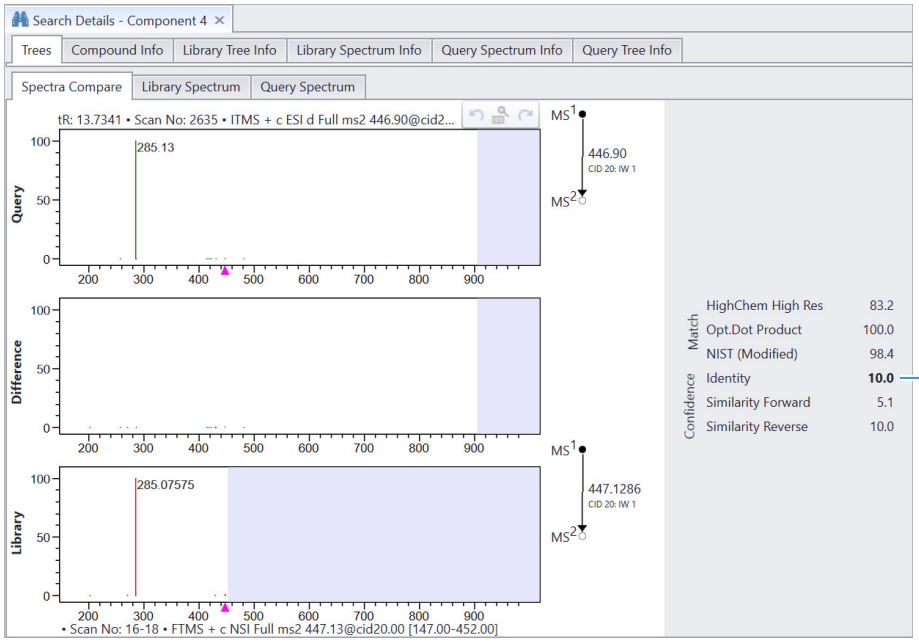


Figure 19 shows the low Confidence score for the comparison of the MS^2 query spectrum and the MS^2 library spectrum for glycitin. The MS^2 stage spectrum for glycitin has too few peaks for a good confidence match—that is, the MS^2 (CID 20) spectrum for glycitin has only one major fragment, which is not sufficient to confirm the identity of glycitin.

Figure 19. Matching MS^2 library spectrum for component 4



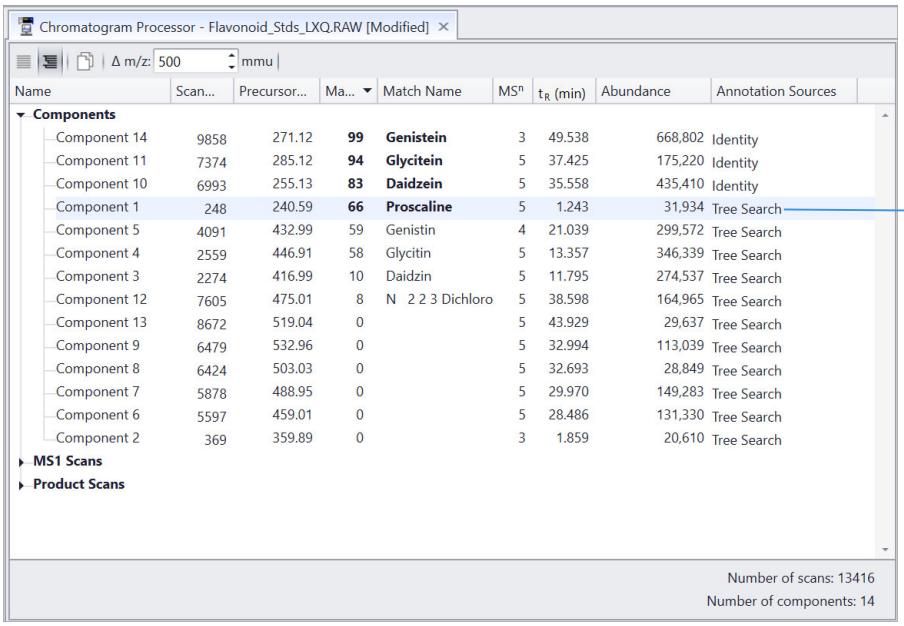
7. To accept the annotations for component 1 do the following:

   a. In the Components list, select **Component 1**.

   b. In the Components Search view, click the **Accept All Suggested Structures** icon, .

   The application assigns the compound names and annotation source to the selected components and displays the assigned names and match scores in bold font (Figure 20).

Figure 20. Accepted annotations for the Tree Search



Run an Identity Substructure search

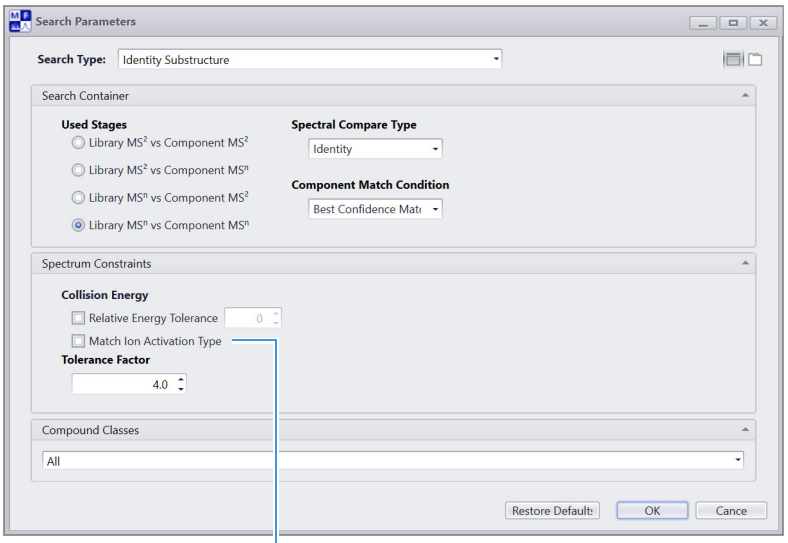
If an Identity search returns no results, run an Identity Substructure search to find matching substructures for the components of interest. An Identity Substructure search searches for matching spectra at any level in a component's spectral tree. The ion activation types must also match.

❖ To run a library search to find compounds with substructures that match the components

1. In the Components Search view on the Chromatogram Processor page, select **Identity Substructure** from the Search Type list.
2. To review the settings for an Identity Substructure search, click the settings icon, .

   Figure 21 shows the default settings for an Identity Substructure search.

   Figure 21. Default settings for an Identity Substructure search

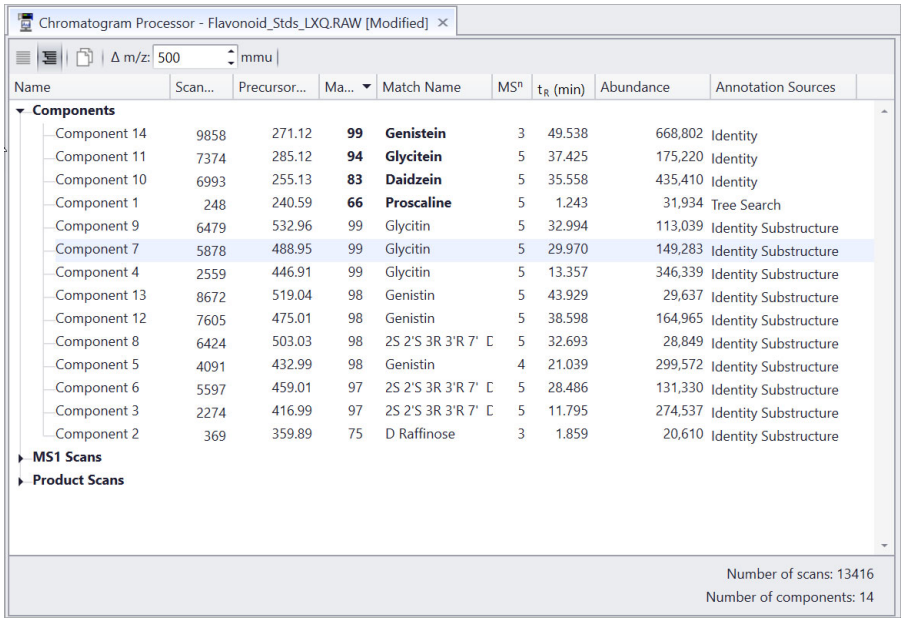


By default, an Identity Substructure search is constrained by the ion activation type.

3. Click **OK**.
4. In the Components Search view, click **Search All**.

   The application runs an Identity Substructure search for the unannotated components (Figure 22).

Figure 22. Results of an Identity Substructure search for the components in the example data file



5. Review the results for component 7 (*m/z* 488.95, t_R(min) 29.97, a standard compound - acetylgycitin) as follows:

   a. In the Components list, select **Component** 7.

   b. In the Component Search view, click **Show Details**.

      A Search Details - Component 7 page opens as a tabbed page.

   c. Make sure that the first compound—glycitin— is selected in the Result List at the left of the Search Details - Component 7 page.

   d. On the Trees page at the top center of the Search Details - Component 7 page (Figure 23), notice that there are no matching MS^2 spectra, but there are matching MS^3, MS^4, and MS^5 spectra.

      Figure 23. Trees for component 7 (query) and glycitin (library compound)

The Spectra Compare page below the Trees page shows the matching MS^5 spectra.

Glycitin and component 7 (acetylglycitin) share a common substructure.

Go to the next topic "Run a Subtree search."

Run a Subtree search

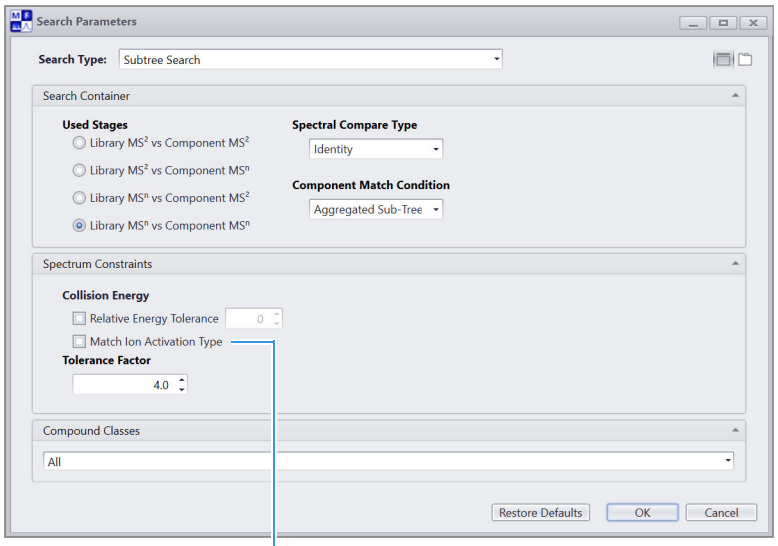
Similar to an Identity Substructure search, run a Subtree search for matching substructures. Refer to Table 1 for differences between an Identity Substructure and a Subtree search.

❖ To run a subtree search

1. In the Components Search view on the Chromatogram Processor page, select **Subtree Search** from the Search Type list.
2. To review the settings for a Subtree Search, click the settings icon, .

   Figure 24 shows the default settings for a Subtree Search.

Figure 24. Default settings for a Subtree Search



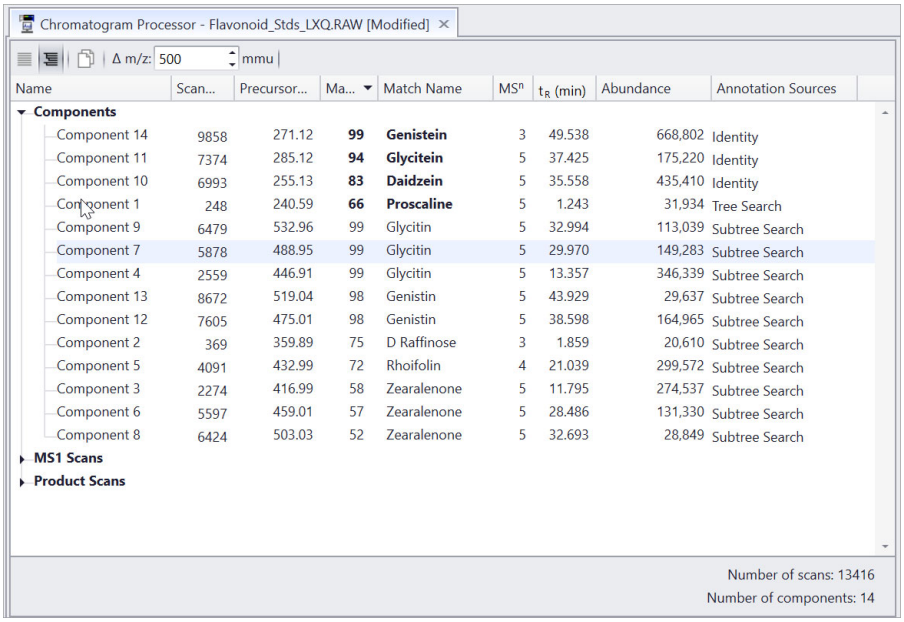
By default, a Subtree Search is not constrained by the ion activation type.

3. Click **OK**.
4. In the Components Search view, click **Search All**.
5. Review the search results in the Components list.

   For component 7, the Identity Substructure search and the Subtree Search return the same best matching spectrum from the mzCloud library.

   Figure 25 shows the results of the Subtree Search for the example data file.

Figure 25. Subtree Search results for the example data file



To identify component 7 (acetylglycitin), go to "Identify a component by running an mzLogic analysis" on page 28.

Search result summary

Table 2 lists the search results from the mzCloud library for the various search types (when you run the searches without accepting any of the structures).

Table 2. Best compound hits for the various search types (April 2023) (Sheet 1 of 2)

#	Precursor *m/z*	Best compound hit (name and monoisotopic mass)							
		Identity		Tree Search		Identity Substructure		Subtree Search	
1	240.59	–	–	Proscaline	239.15	Tentoxin	414.23	Proscaline	239.15
2	359.89	–	–	–	–	D Raffinose	504.17	D Raffinose	504.17
3	416.99	–	–	Daidzin	416.11	*Compound name* [a]	866.23	Zearalenone	318.15
4	446.91	Glycitin	446.12	Glycitin	446.12	Glycitin	446.12	Glycitin	446.12
5	432.99	Apigetrin	432.11	Genistin	432.11	Genistin	432.11	Rhoifolin	578.16
6	459.01	–	–	–	–	*Compound name* [a]	866.23	Zearalenone	318.15
7	488.95	–	–	–	–	Glycitin	446.12	Glycitin	446.12
8	503.03	–	–	–	–	*Compound name* [a]	866.23	Zearalenone	318.15

Table 2. Best compound hits for the various search types (April 2023), continued (Sheet 2 of 2)

#	Precursor *m/z*	Best compound hit (name and monoisotopic mass)							
		Identity		Tree Search		Identity Substructure		Subtree Search	
9	532.96	–	–	–	–	Glycitin	446.12	Glycitin	446.12
10	255.13	Daidzein	254.06	Daidzein	254.06	*Compound name* [a]	866.23	Zearalenone	318.15
11	285.12	Glycitein	284.07	Glycitein	284.07	Glycitein	284.07	Glycitin	446.12
12	475.01	–	–	*Compound name* [b]	473.99	Genistin	432.11	Genistin	432.11
13	519.03	–	–	–	–	Genistin	432.11	Genistin	432.11
14	271.12	Genistein	270.05	Genistein	270.05	Genistein	270.05	Apigenin 7-O-glucuranide	446.08

[a] (2S,2'S,3R,3'R)-7'-(beta-D-Glucopyranosyloxy)-5,5'-dihydroxy-2,2'-bis(4-hydroxyphenyl)-4,4'-dioxo-3,3',4,4'-tetrahydro-2H,2'H-3,3'-bichromen-7-yl beta-D-glucopyranoside

[b] N'(-[({[[[2-(2,3-Dichlorophenyl)-1,3-thiazol-4-yl]amino}carbonyl)oxy]-4-(trifluoromethyl) benzenecarboximid amide

Identify a component by running an mzLogic analysis

An mzLogic analysis combines mzCloud™ spectral similarity searching (MS^2 and MS^n)and structure overlapping to rank putative structures. For nominal mass data, you cannot run a MolGate™ search against any of the structure databases for retrieving possible structure candidates. You can only run an mzLogic analysis against a set of predefined structure candidates.

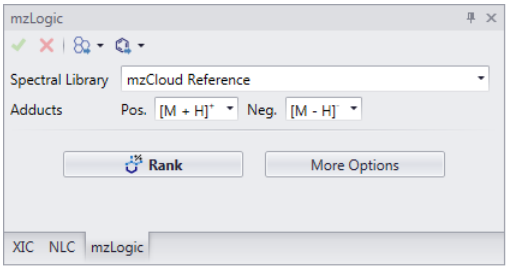
Follow these procedures in order:

1. To identify component 7 by running an mzLogic analysis against a list of structure candidates
2. To view the name of a structure candidate

❖ To identify component 7 by running an mzLogic analysis against a list of structure candidates

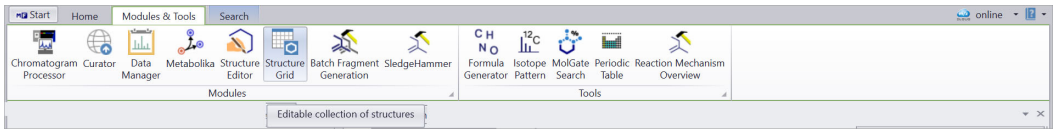
1. In the components list, select **Component 7**.
2. In the Search group of the Chromatogram Processor toolbar, click **mzLogic**.

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

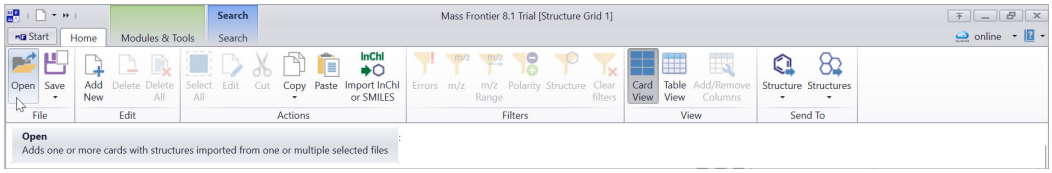


3. Click **More Options**.

   The Structure Database list and the Workspace list appear.
4. In the Structure Database drop down, deselect all databases and click **OK**.
5. To define the structure candidates, do the following:
   a. In the tab bar, click **Modules & Tools**.
   b. In the Modules group of the Modules & Tools toolbar, click **Structure Grid**.



c. In the File group of the Structure Grid toolbar, click **Open**.

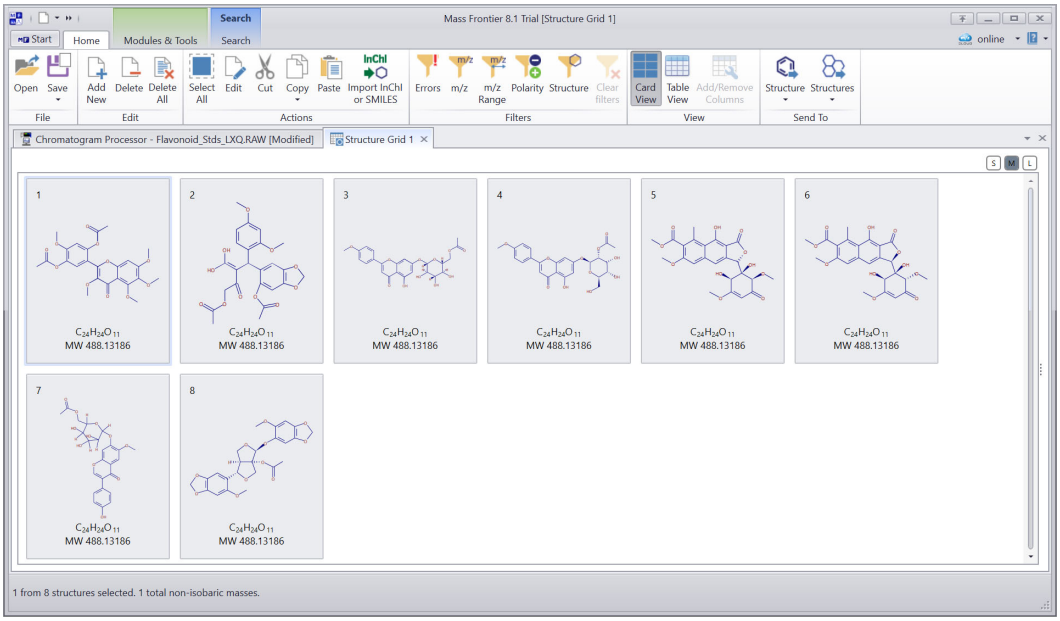


d. Browse to the following folder, select the **Structures_C24H24O11.sdf** file, and click **Open**.

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

Eight cards appear on the Structure Grid page (Figure 26).

Figure 26. Structure grid page



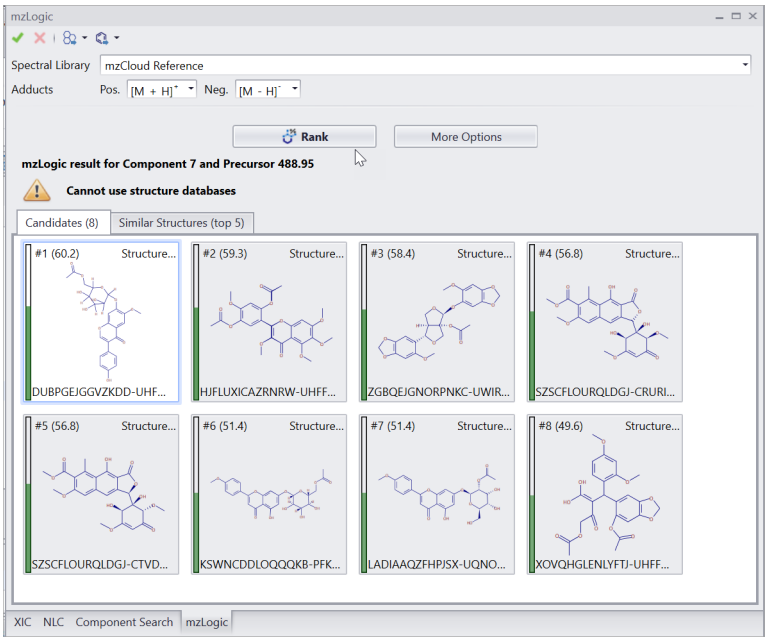
e. To return to the Chromatogram Processor page, click the **Chromatogram Processor - Flavonoid_Stds_LXQ.RAW [Modified]** tab.

The application automatically populates the Workspace list with the name of the latest Structure Grid page.

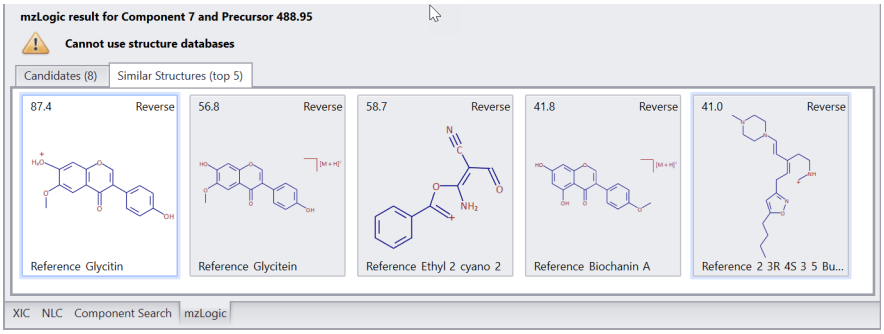
6. Click **Rank**.

The mzLogic application ranks the eight structure candidates in the Structure Grid (Figure 27).

Figure 27. mzLogic results for component 7

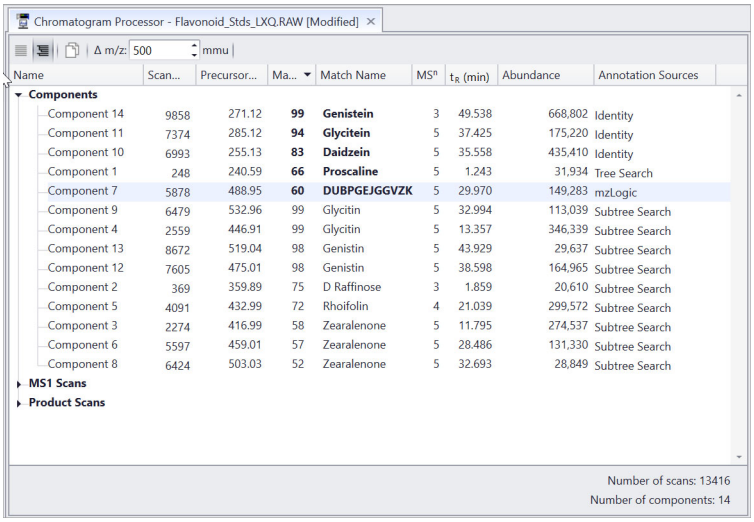


7. To view the similar structures that the analysis found in the mzCloud library, click the **Similar Structures** tab.



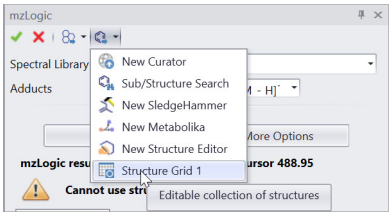
8. To annotate component 7, on the Candidates page of the mzLogic view, do the following:
   a. Select **card #1**.
   b. To accept the highest ranking structure as the annotation for component 7, click the **Accept Structure** icon, ✔, in the mzLogic view.

Component 7 moves to the top of the list, just below the previously annotated components.



❖ To view the name of a structure candidate

1. After running an mzLogic analysis on component 7, on the Candidates page of the mzLogic view, select **card #1**.
2. In the mzLogic toolbar, click the **Selected Candidate** icon, , and then select **Structure Grid 1**.



The application adds the selected structure to the selected Structure Grid page.

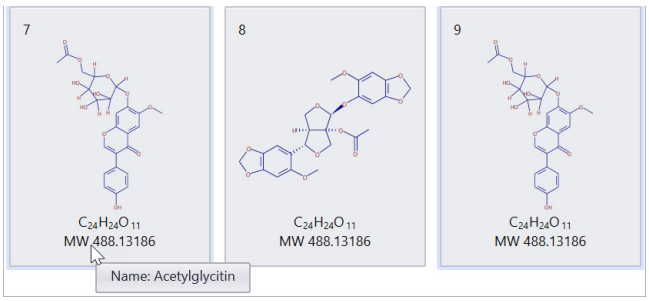
3. Click the **Structure Grid 1** tab to open the Structure Grid page.
4. Compare the structure on card # 9 (the structure ranked #1 by mzLogic) to the other structures.

   Card # 9 matches card # 7, which is acetylglycitin. For component 7, mzLogic ranks the correct structure with the highest mzLogic score.

5. Point on the matching structure to view its name—acetylglycitin—as a ToolTip (Figure 28).

   The acetyl (CH_3CO) moiety molar mass of 43g/mol.

Figure 28. Viewing a compound's name by pointing to its structure



Go to the next topic "Save the analysis results to an HCCX file."

Save the analysis 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** Save the intermediate component detection and annotation results to HCCX files so you can return to those results at a later time. This is helpful so you can return to a specific results state and then perform the same or different subsequent processing on the data.