Mass Frontier 8.1 Tutorial to Identify Unknowns in High-Resolution, Accurate-Mass 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 high-resolution, accurate-mass (HRAM) data.

IMPORTANT To run the mzCloud library searches and mzLogic analyses, 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.

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 data-acquisition settings.
3. Detect components by applying the Joint Component Detection (JCD) algorithm for LC/MS data.
4. Delete the components that are of no interest.
5. Run mzCloud library searches for detected components and annotate each identified component with the name of its matching library compound.
6. Run an mzLogic analysis for a component that you cannot confidently identify with a library search.
7. Save the analysis results to a HighChem Chromatogram Format (HCCX) file.

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Demo data files

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

File	Description
plant sample.raw	A raw data file acquired with an LC-ESI/MSn experiment using an Orbitrap™ ID-X™ Tribrid mass spectrometer (Figure 6). The MS3 data was triggered based on the Neutral Loss (NL) list.
JCD Plant Example.chpro_jcd	A component detection file that contains custom component detection settings for the example data file
Flavonoids_C39H50O24.sdf	A structure file that contains twelve flavonoids with the formula C39H50O24.

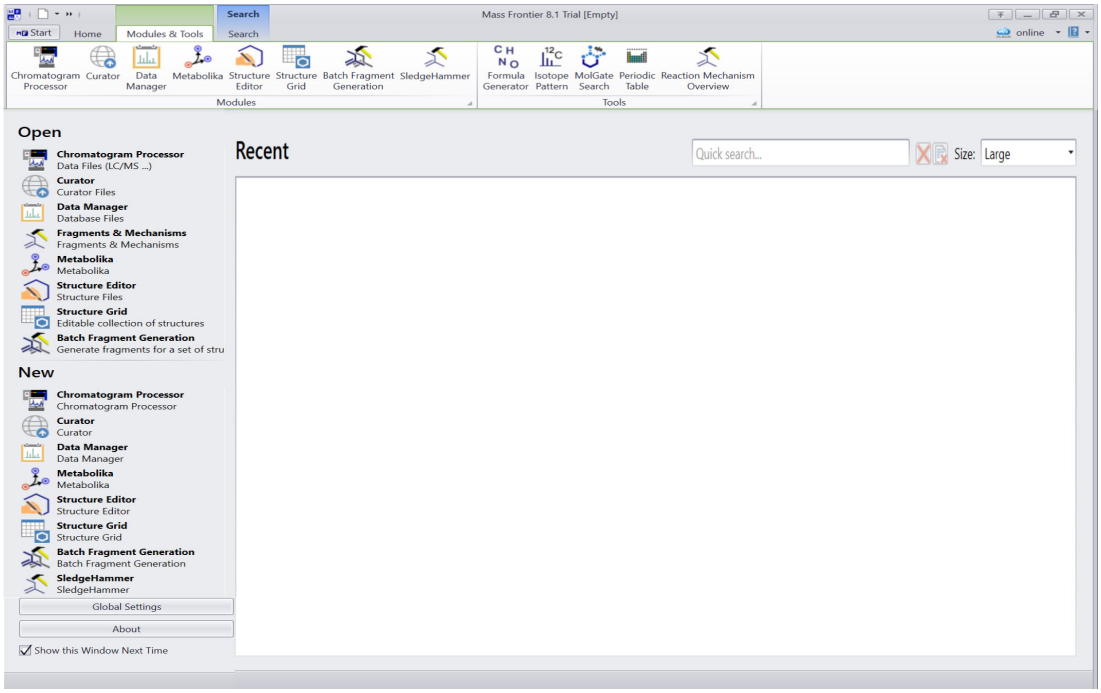
Check the connection to the mzCloud server

❖ 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.

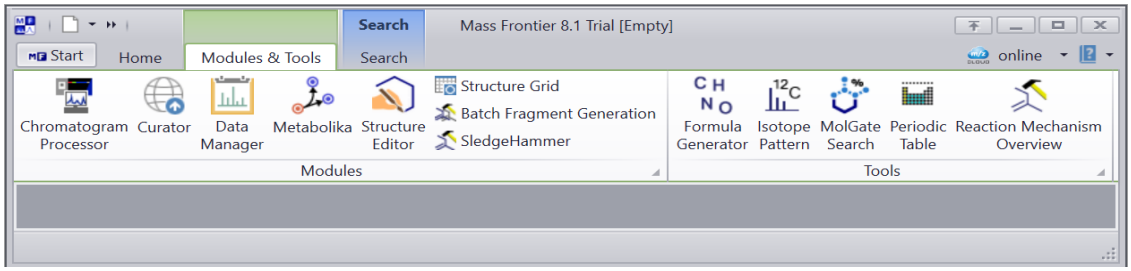
   The application opens to the Mass Frontier startup window (Figure 1) or the Modules & Tools toolbar (Figure 2).

Figure 1. Mass Frontier startup window



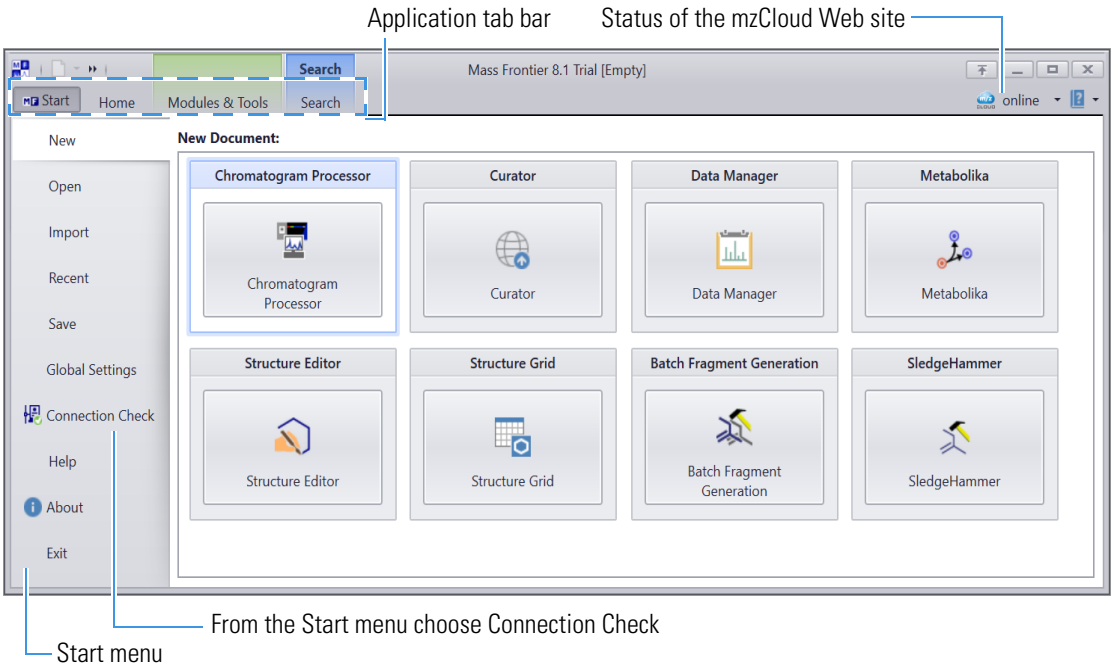
Note If you clear the Show this Window Next Time check box, the next time you open the application, it opens to the Modules & Tools toolbar.

Figure 2. Modules & Tools toolbar without the startup pane on the left



2. From the application tab bar to open the Start menu, click the **Start** tab, and then choose **Connection Check** (Figure 3).

Figure 3. Start menu and application tab bar



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

   The connection check verifies access to the mzCloud server.

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.

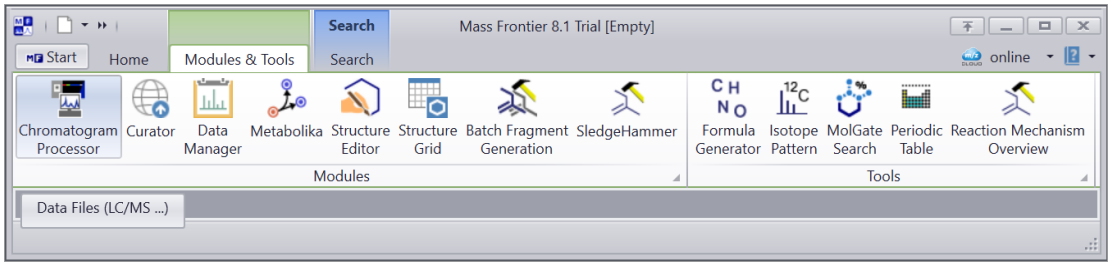
> **Note** The Chromatogram Processor module recognizes Xcalibur RAW files from a Thermo Scientific MS, mzML files from a third-party MS, and HighChem Chromatogram Format files (.hccx).

Open a raw data file for processing

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

1. In the Modules & Tools toolbar, click **Chromatogram Processor** (Figure 4).

Figure 4. Chromatogram Processor module in the Modules & Tools toolbar



The Open Chromatogram dialog box opens.

2. Browse to the following folder, select **plant sample.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 5):

- 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.

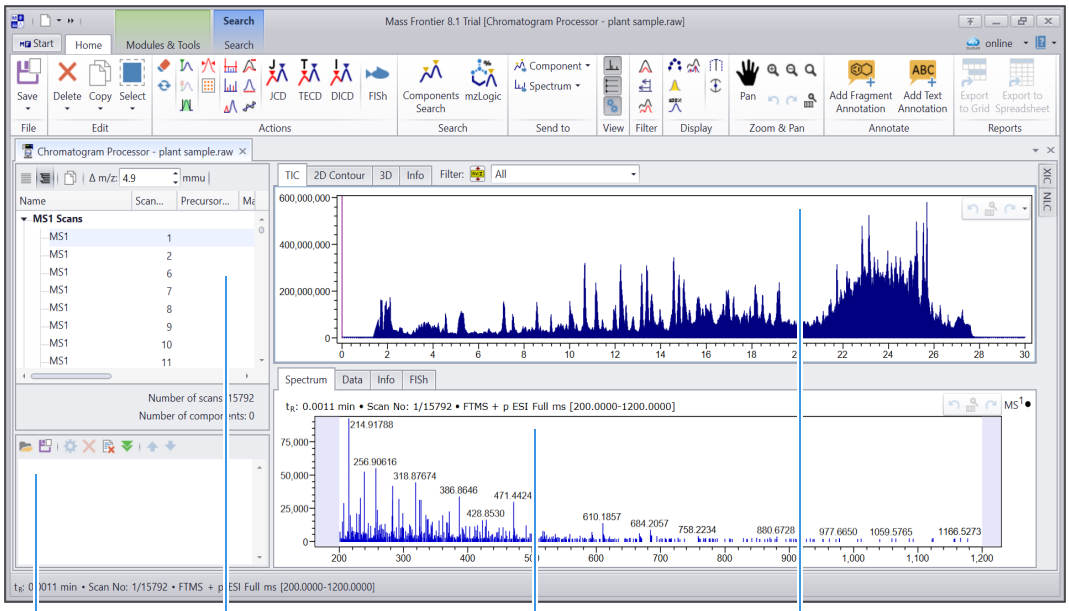
- The chromatogram view at the upper right displays the total ion current (TIC) chromatogram. The *y*-axis scale is set to absolute intensity.

  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 5. 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, .

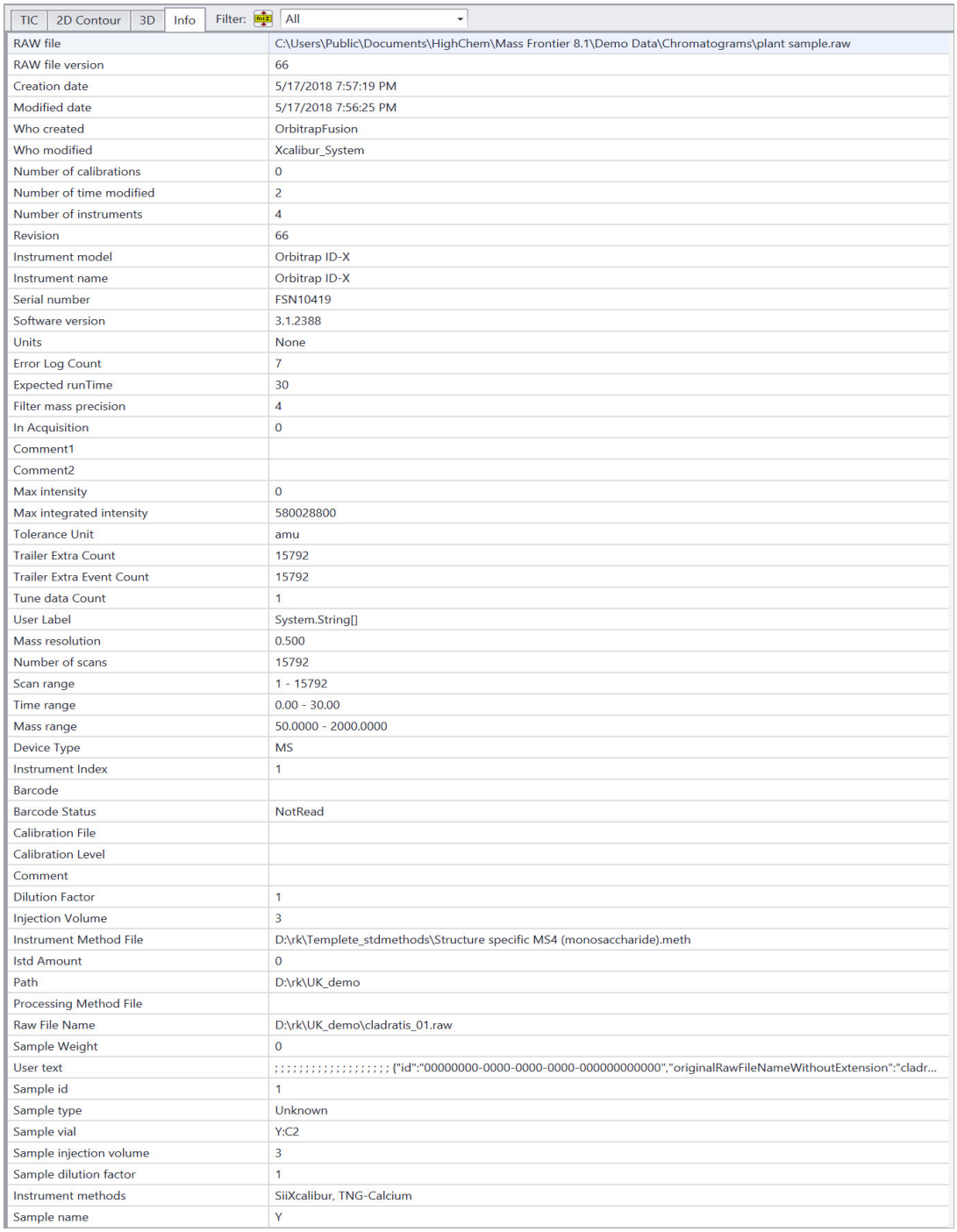
You cannot hide the chromatogram view.

View information about the raw data file

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

1. Open the plant sample.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 (Figure 6).

Figure 6. Information about the plant sample.raw file



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

RAW file	C:\Users\Public\Documents\HighChem\Mass Frontier 8.1\Demo Data\Chromatograms\plant sample.raw
RAW file version	66
Creation date	5/17/2018 7:57:19 PM
Modified date	5/17/2018 7:56:25 PM
Who created	OrbitrapFusion
Who modified	Xcalibur_System
Number of calibrations	0
Number of time modified	2
Number of instruments	4
Revision	66
Instrument model	Orbitrap ID-X
Instrument name	Orbitrap ID-X
Serial number	FSN10419
Software version	3.1.2388
Units	None
Error Log Count	7
Expected runTime	30
Filter mass precision	4
In Acquisition	0
Comment1	
Comment2	
Max intensity	0
Max integrated intensity	580028800
Tolerance Unit	amu
Trailer Extra Count	15792
Trailer Extra Event Count	15792
Tune data Count	1
User Label	System.String[]
Mass resolution	0.500
Number of scans	15792
Scan range	1 - 15792
Time range	0.00 - 30.00
Mass range	50.0000 - 2000.0000
Device Type	MS
Instrument Index	1
Barcode	
Barcode Status	NotRead
Calibration File	
Calibration Level	
Comment	
Dilution Factor	1
Injection Volume	3
Instrument Method File	D:\rk\Templete_stdmethods\Structure specific MS4 (monosaccharide).meth
Istd Amount	0
Path	D:\rk\UK_demo
Processing Method File	
Raw File Name	D:\rk\UK_demo\cladratis_01.raw
Sample Weight	0
User text	;;;;;;;;;;;;;;;;;;;; {"id":"00000000-0000-0000-0000-000000000000","originalRawFileNameWithoutExtension":"cladr...
Sample id	1
Sample type	Unknown
Sample vial	Y:C2
Sample injection volume	3
Sample dilution factor	1
Instrument methods	SiiXcalibur, TNG-Calcium
Sample name	Y

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

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

Detect components in LC/MS data

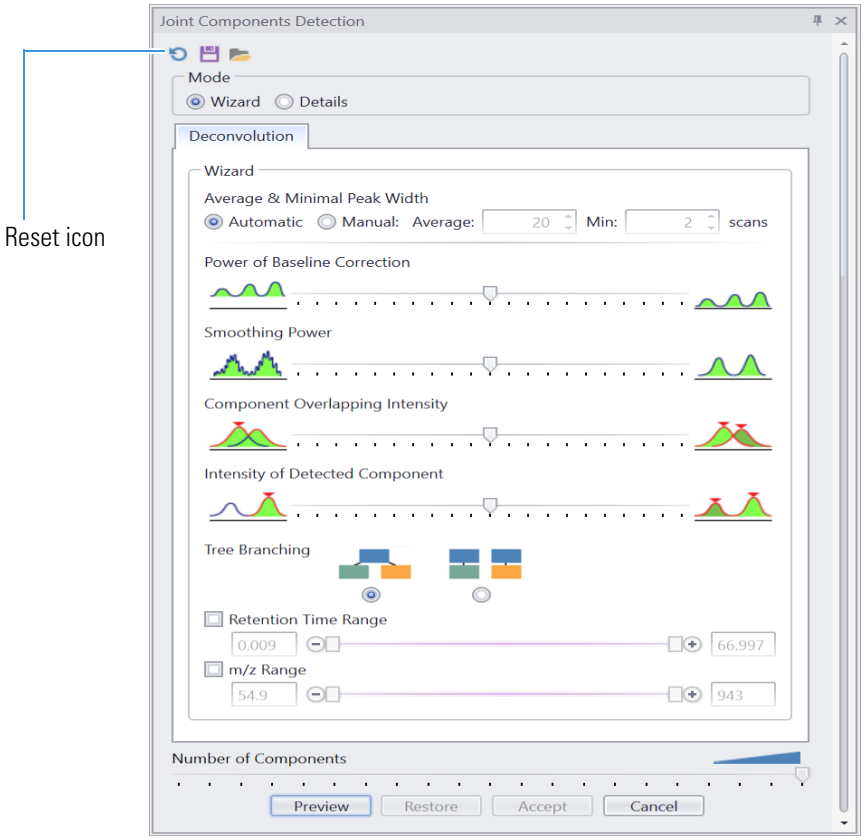
Use the Joint Component Detection (JCD) algorithm to detect the components in a chromatogram from an LC/MS experiment.

❖ To detect the components in the example file

1. Open the plant sample.raw data file as described in the previous topic, "Open a raw data file for processing."
2. 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 7).

   Figure 7. Default settings on the wizard page for the JCD algorithm



3. Click the **Reset** icon, , to reset the parameters to their factory default values.
4. 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.

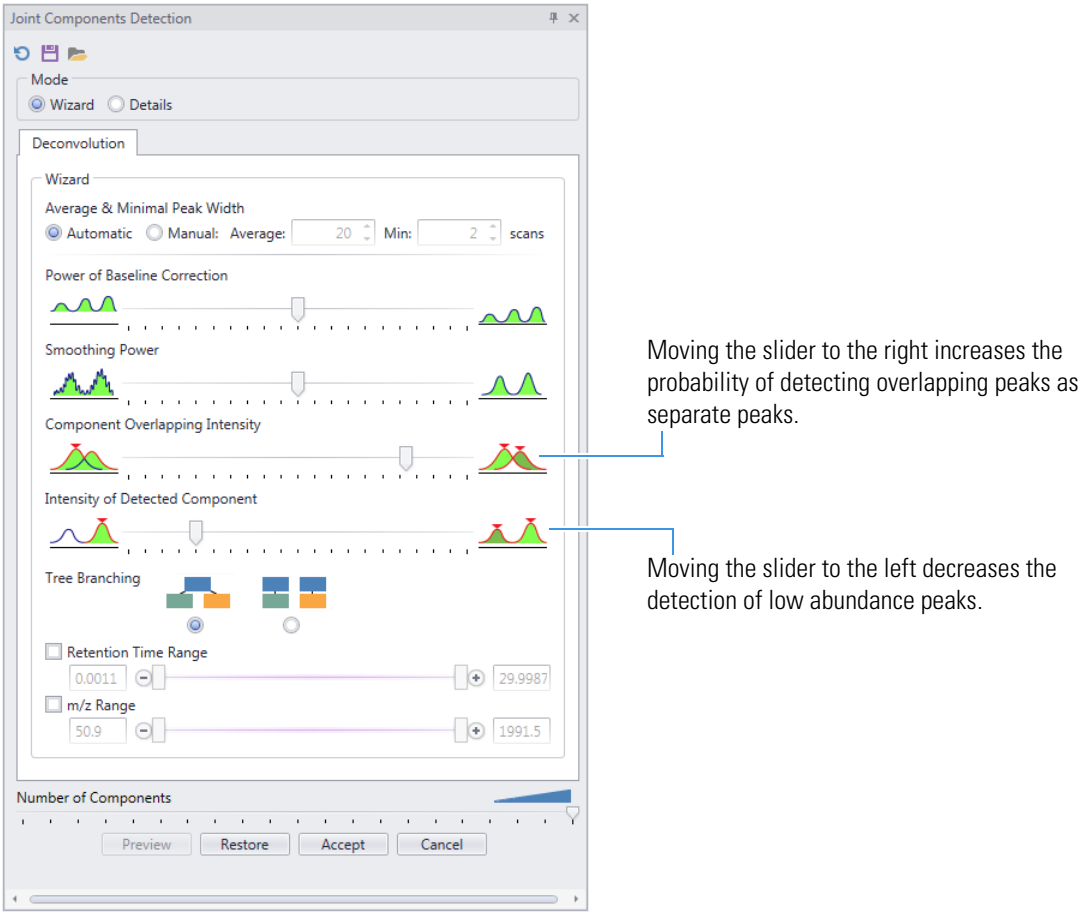
   b. Browse to the following folder:

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

   c. Select **JCD Plant Example.chpro_jcd** and click **Open**.

      The Deconvolution area of the Joint Components Detection view displays the new detection settings (Figure 8).

Figure 8. Detection settings from the selected CHPRO_JCD file

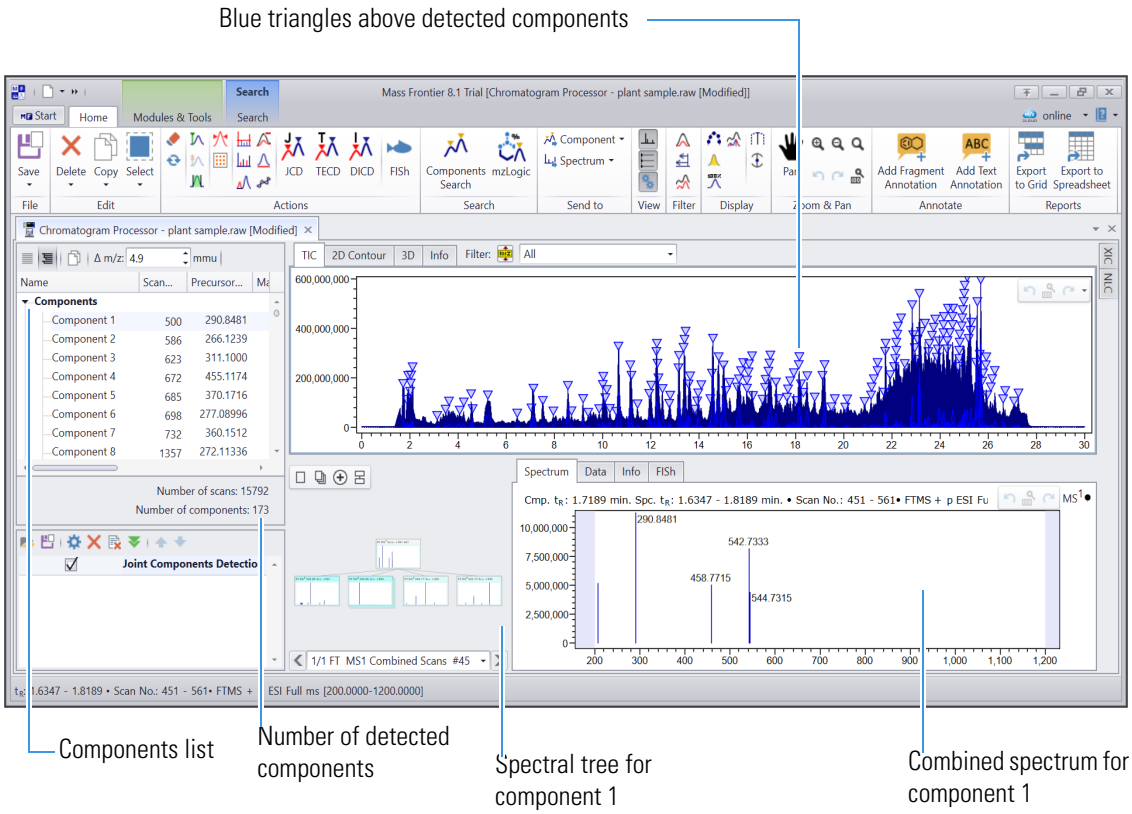


5. To start processing, click **Preview**.

   After the processing finishes, the following items appear (Figure 9):

   - 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 at 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.

Figure 9. Integrated TIC chromatogram with marked components



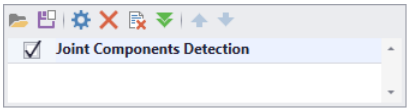
6. Check the number of detected components.

   With the detection settings in the selected chpro_jcd file, the application detects 173 components in the TIC chromatogram for the plant_sample.raw file.

7. 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 10).

Figure 10. Command processor view with one applied action—Joint Components Detection



Go to the next topic, "Sort and delete components in the components list."

Sort and delete components in the components list

Reduce the components in the components list by deleting the components that have only MS1 and MS2 scans to keep only the components of interest, flavonoids, which have associated neutral loss (NL) triggered MS3 fragmentation scans.

Sort the components by MSn stage

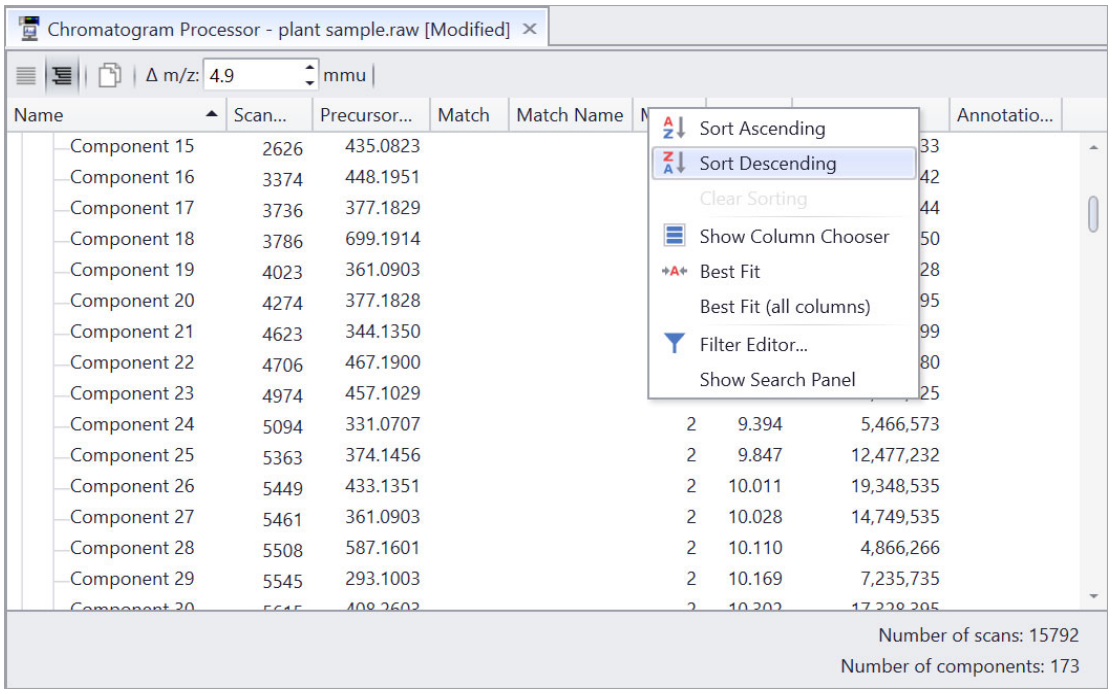
In the chromatogram data view, you can sort the components list by the column, such as the scan no., precursor *m/z*, retention time, MSn, or abundance.

❖ **To sort the components in descending order by MSn**

1. Click the **MSn** column heading until the arrow points down or right-click the MSn column heading and choose **Sort Descending**.

The higher-MSn components sort to the top (Figure 11).

Figure 11. Components sorted by MSn stage



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.

Delete components in the components list

The Components list contains MS1, MS2, and MS3 fragmentation scans (173 components in the list). The components of interest (Flavonoids) have MS3 fragmentation scans. To reduce the list to only the components of interest, delete components that only have MS1 and MS2 fragmentation scans.

❖ To delete components without MS3 scans

1. Select rows containing MS2 and MS1 scans.

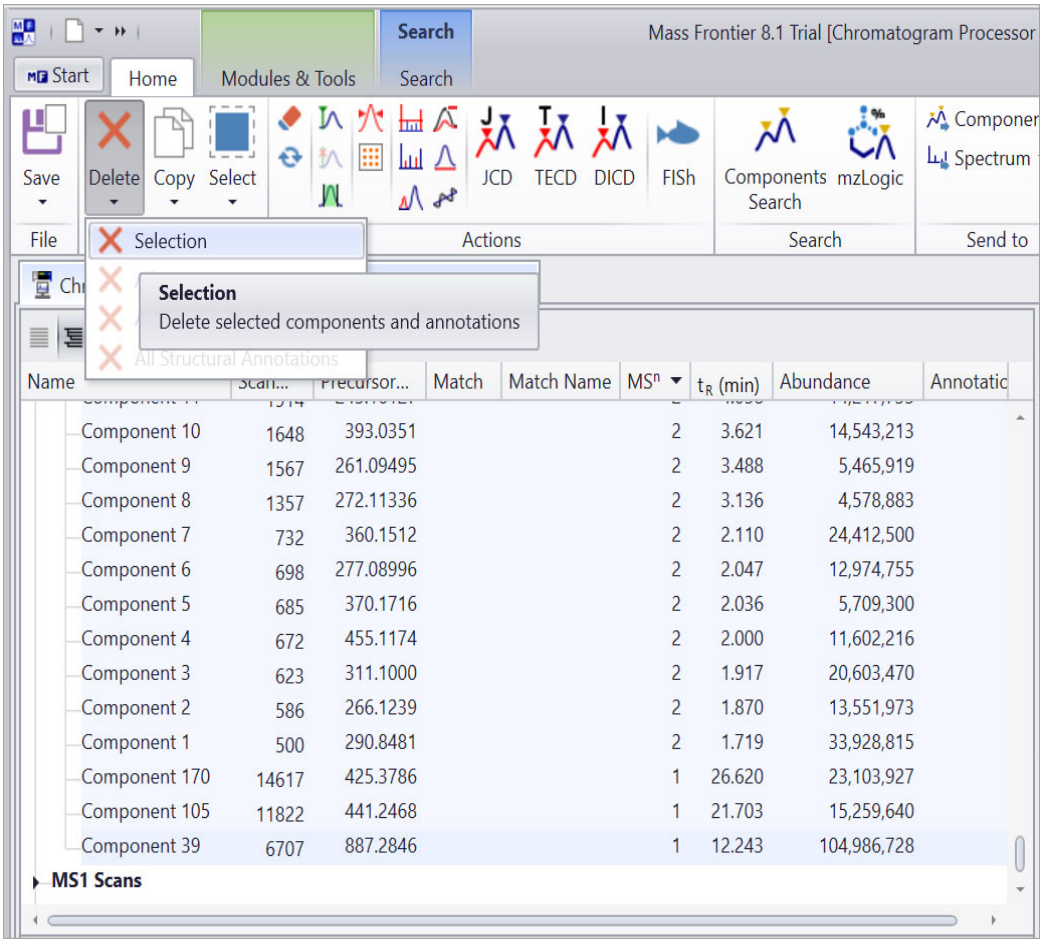
   a. Select the first row containing MS2 fragmentation scan data.

   b. Scroll down to the last row containing MS1 scan data.

   c. Press shift and select the last row containing the MS1 scan data (Figure 12).

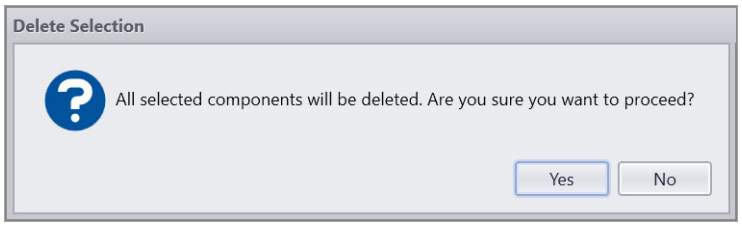
   All selected rows become highlighted.

Figure 12. Selected rows are highlighted



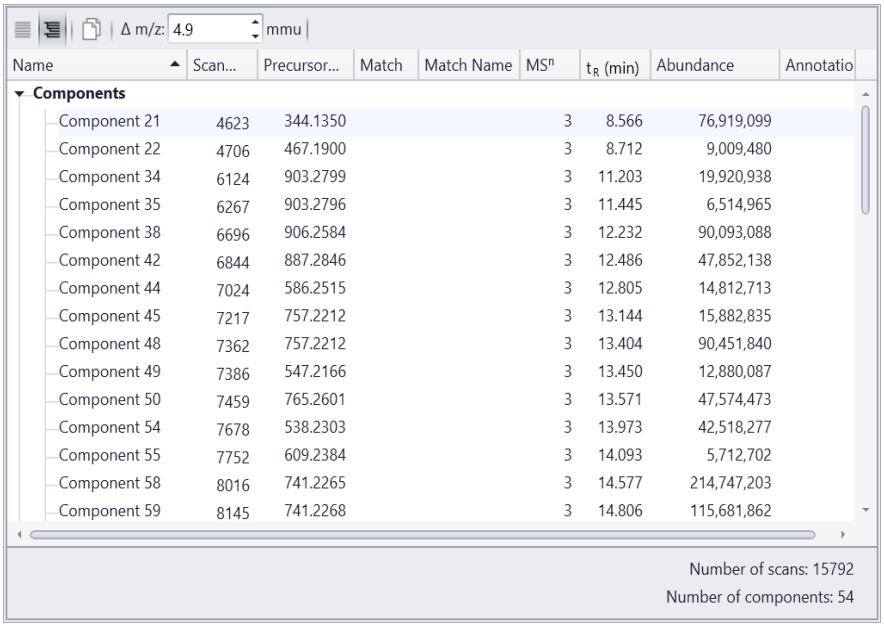
2. In the Edit group of the toolbar, click **Delete,** then in the drop down menu choose **Selection.** The Delete Selection dialog box opens, click **Yes** (Figure 13).

Figure 13. Delete Selection dialog box



Only components with MS3 fragmentation scans remain, 54 components (Figure 14).

Figure 14. Components with MS3 fragmentation scans



Go to the next topic, "Identify components by searching the mzCloud mass spectral database."

Identify components by searching the mzCloud mass spectral database

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

1. Run an identity search
2. Run a tree search
3. Run a Subtree search
4. Run an Identity Substructure search

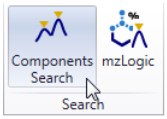
Note If you have not already done so, follow the previous topics in this tutorial to open the example data file, apply the JCD algorithm, delete the components without MS3 scans, so only the components with MS3 fragmentation scans are in the Components list.

Run an identity search

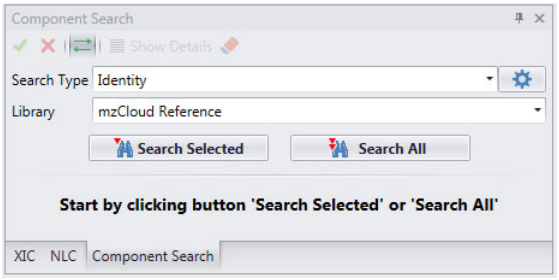
Run an identity search to find matching compounds in the selected mass spectral libraries for the unknown components.

❖ **To run an identity search for all the components against the mzCloud mass spectral data base**

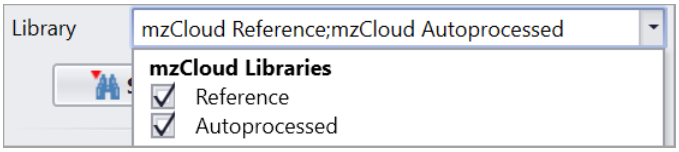
1. In the Search group of the Chromatogram Processor toolbar, click **Components Search**.



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



2. In the Library box open the Library list and select the **Reference** and **Autoprocessed** check boxes.



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

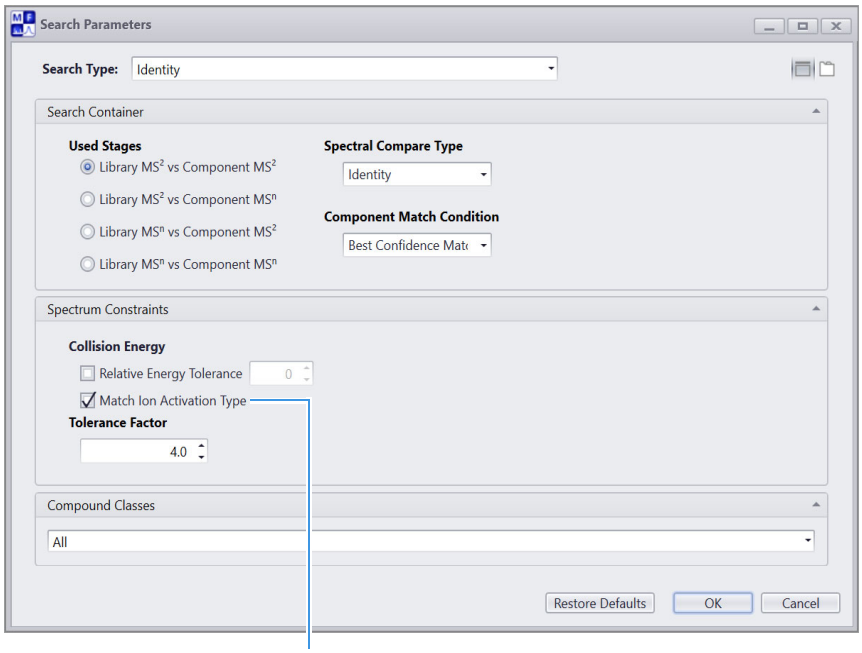
3. (Optional) To review the default settings for an Identity search, do the following:

   a. Click the **Settings** icon, .

   The Search Parameters dialog box opens with the Search Type set to Identity (Figure 15).

   Note An Identity search searches the selected mass spectral libraries for spectra that match the data-dependent MS2 spectra associated with the selected component. It compares each MS2 query spectrum to MS2 spectra for the same precursor *m/z* and ion activation type in the selected libraries and uses the Best Confidence Match algorithm to calculate the match score.

Figure 15. Default settings for an Identity search



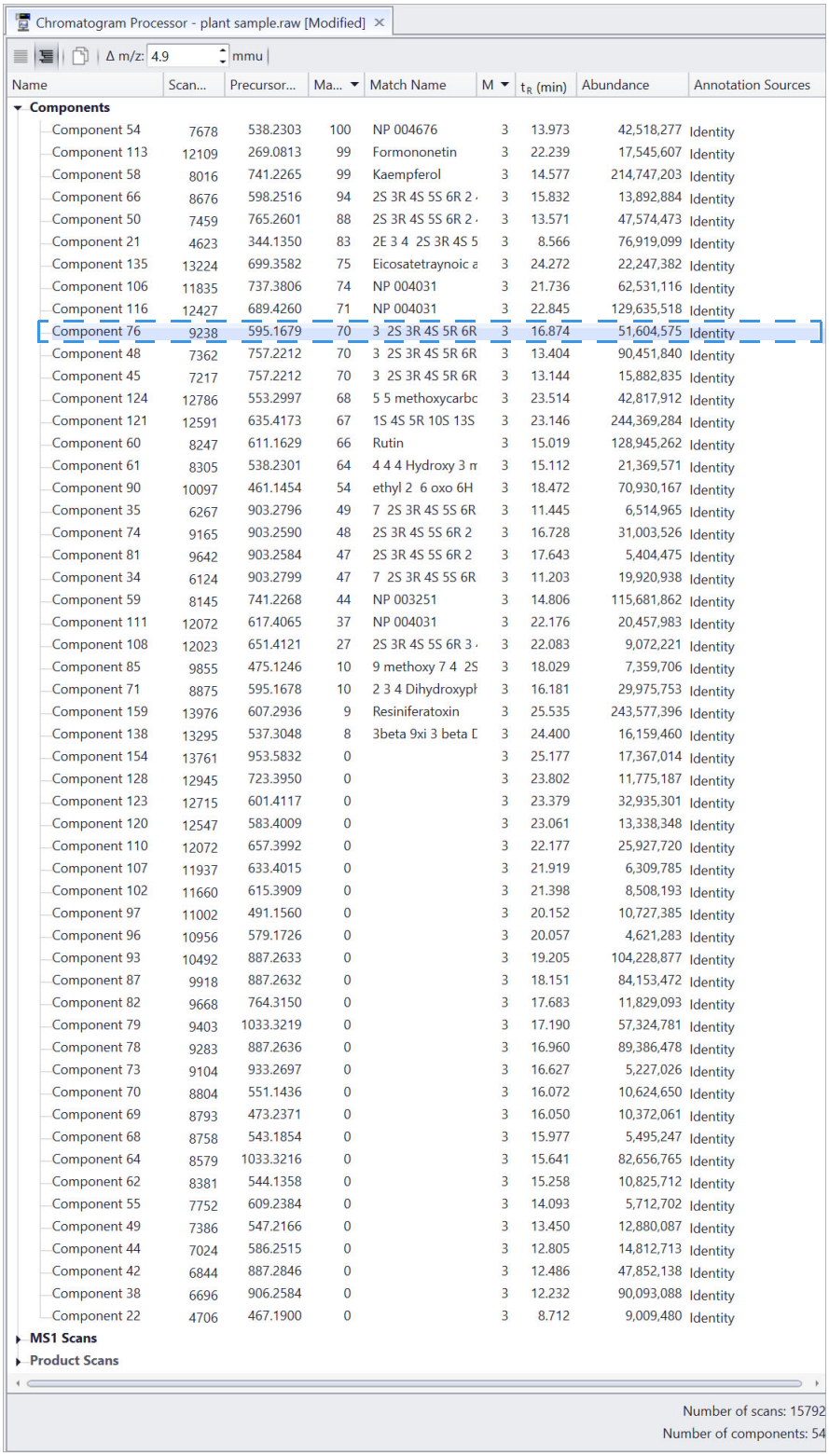
b. Click **OK**.

4. In the Component Search view, click **Search All**.
5. When the search ends, sort the chromatogram data view by the **Match** column in descending order.

   The library search finds matching compounds for 28 components (Figure 16).

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

Figure 16. Identity matches for the detected components in the plant sample file (April 2023)



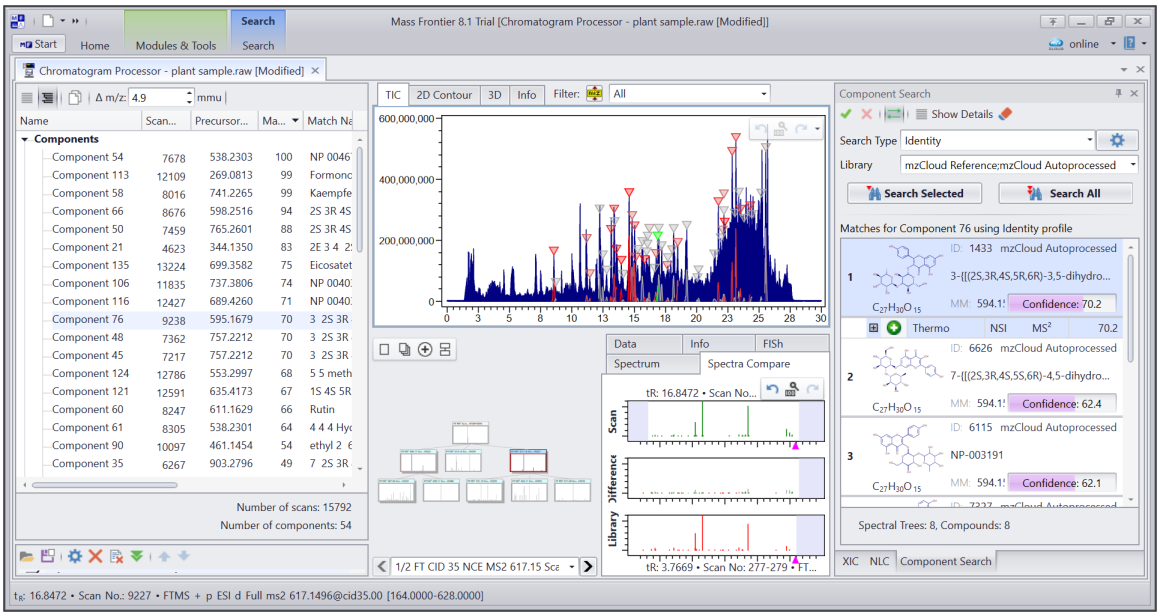
Name	Scan...	Precursor...	Ma...	Match Name	M	t_R (min)	Abundance	Annotation Sources
Components								
Component 54	7678	538.2303	100	NP 004676	3	13.973	42,518,277	Identity
Component 113	12109	269.0813	99	Formononetin	3	22.239	17,545,607	Identity
Component 58	8016	741.2265	99	Kaempferol	3	14.577	214,747,203	Identity
Component 66	8676	598.2516	94	2S 3R 4S 5S 6R 2	3	15.832	13,892,884	Identity
Component 50	7459	765.2601	88	2S 3R 4S 5S 6R 2	3	13.571	47,574,473	Identity
Component 21	4623	344.1350	83	2E 3 4 2S 3R 4S 5	3	8.566	76,919,099	Identity
Component 135	13224	699.3582	75	Eicosatetraynoic a	3	24.272	22,247,382	Identity
Component 106	11835	737.3806	74	NP 004031	3	21.736	62,531,116	Identity
Component 116	12427	689.4260	71	NP 004031	3	22.845	129,635,518	Identity
Component 76	9238	595.1679	70	3 2S 3R 4S 5R 6R	3	16.874	51,604,575	Identity
Component 48	7362	757.2212	70	3 2S 3R 4S 5R 6R	3	13.404	90,451,840	Identity
Component 45	7217	757.2212	70	3 2S 3R 4S 5R 6R	3	13.144	15,882,835	Identity
Component 124	12786	553.2997	68	5 5 methoxycarbc	3	23.514	42,817,912	Identity
Component 121	12591	635.4173	67	1S 4S 5R 10S 13S	3	23.146	244,369,284	Identity
Component 60	8247	611.1629	66	Rutin	3	15.019	128,945,262	Identity
Component 61	8305	538.2301	64	4 4 4 Hydroxy 3 m	3	15.112	21,369,571	Identity
Component 90	10097	461.1454	54	ethyl 2 6 oxo 6H	3	18.472	70,930,167	Identity
Component 35	6267	903.2796	49	7 2S 3R 4S 5S 6R	3	11.445	6,514,965	Identity
Component 74	9165	903.2590	48	2S 3R 4S 5S 6R 2	3	16.728	31,003,526	Identity
Component 81	9642	903.2584	47	2S 3R 4S 5S 6R 2	3	17.643	5,404,475	Identity
Component 34	6124	903.2799	47	7 2S 3R 4S 5S 6R	3	11.203	19,920,938	Identity
Component 59	8145	741.2268	44	NP 003251	3	14.806	115,681,862	Identity
Component 111	12072	617.4065	37	NP 004031	3	22.176	20,457,983	Identity
Component 108	12023	651.4121	27	2S 3R 4S 5S 6R 3	3	22.083	9,072,221	Identity
Component 85	9855	475.1246	10	9 methoxy 7 4 2S	3	18.029	7,359,706	Identity
Component 71	8875	595.1678	10	2 3 4 Dihydroxyph	3	16.181	29,975,753	Identity
Component 159	13976	607.2936	9	Resiniferatoxin	3	25.535	243,577,396	Identity
Component 138	13295	537.3048	8	3beta 9xi 3 beta D	3	24.400	16,159,460	Identity
Component 154	13761	953.5832	0		3	25.177	17,367,014	Identity
Component 128	12945	723.3950	0		3	23.802	11,775,187	Identity
Component 123	12715	601.4117	0		3	23.379	32,935,301	Identity
Component 120	12547	583.4009	0		3	23.061	13,338,348	Identity
Component 110	12072	657.3992	0		3	22.177	25,927,720	Identity
Component 107	11937	633.4015	0		3	21.919	6,309,785	Identity
Component 102	11660	615.3909	0		3	21.398	8,508,193	Identity
Component 97	11002	491.1560	0		3	20.152	10,727,385	Identity
Component 96	10956	579.1726	0		3	20.057	4,621,283	Identity
Component 93	10492	887.2633	0		3	19.205	104,228,877	Identity
Component 87	9918	887.2632	0		3	18.151	84,153,472	Identity
Component 82	9668	764.3150	0		3	17.683	11,829,093	Identity
Component 79	9403	1033.3219	0		3	17.190	57,324,781	Identity
Component 78	9283	887.2636	0		3	16.960	89,386,478	Identity
Component 73	9104	933.2697	0		3	16.627	5,227,026	Identity
Component 70	8804	551.1436	0		3	16.072	10,624,650	Identity
Component 69	8793	473.2371	0		3	16.050	10,372,061	Identity
Component 68	8758	543.1854	0		3	15.977	5,495,247	Identity
Component 64	8579	1033.3216	0		3	15.641	82,656,765	Identity
Component 62	8381	544.1358	0		3	15.258	10,825,712	Identity
Component 55	7752	609.2384	0		3	14.093	5,712,702	Identity
Component 49	7386	547.2166	0		3	13.450	12,880,087	Identity
Component 44	7024	586.2515	0		3	12.805	14,812,713	Identity
Component 42	6844	887.2846	0		3	12.486	47,852,138	Identity
Component 38	6696	906.2584	0		3	12.232	90,093,088	Identity
Component 22	4706	467.1900	0		3	8.712	9,009,480	Identity
MS1 Scans								
Product Scans								

For this tutorial, choose component 76 to demonstrate the difference between an Identity search and a Tree search.

Note A Tree search (MSn versus MSn) is more sensitive compared to an Identity search (MS2 versus MS2) when the spectral tree contains MSn (n>2).

6. Review the library hits for a component, by doing the following:

   a. In the chromatogram data view, select the component of interest, **Component 76**.

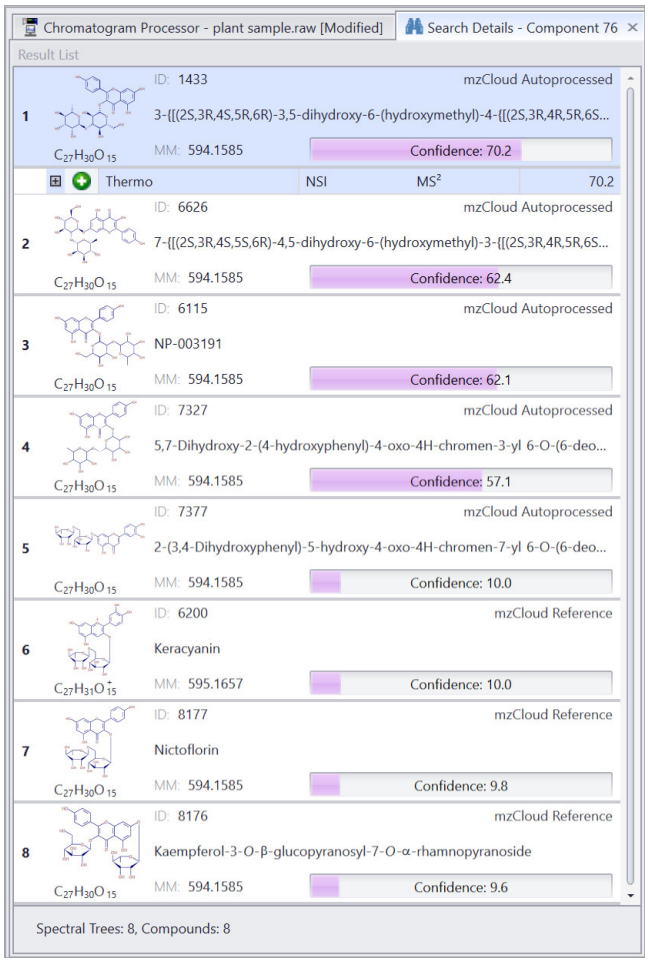


The Component Search view shows eight hits (Figure 17):

- Five hits in the mzCloud Autoprocessed library
- Three hits in the mzCloud Reference library

The mzCloud hit with ID: 1433 is ranked #1with a confidence score of 70.2.

Figure 17. Component Search view showing the Identity search results for component 76

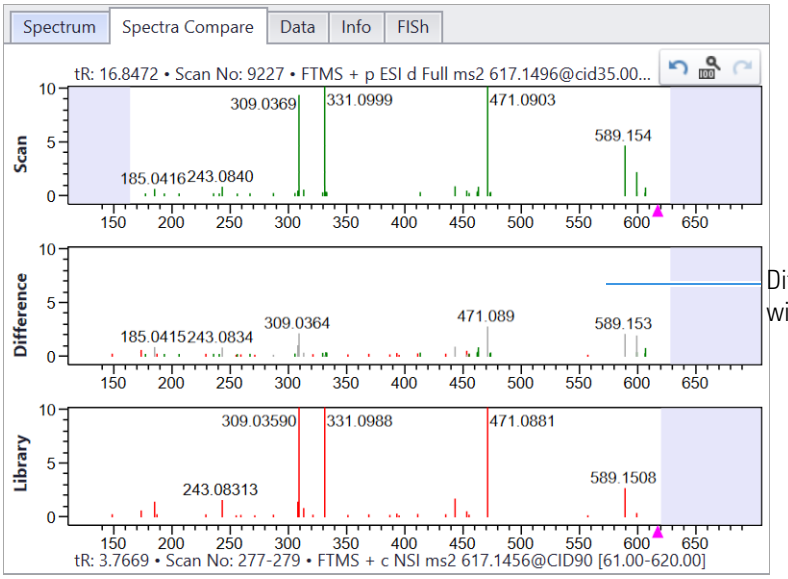


b. To review the spectra, on the Chromatogram Processor - plant sample page, click the Spectra Compare tab.

In the MS spectrum view, the Spectra Compare page displays the query spectrum in green, the best matching library spectrum in red, and a difference spectrum with peaks in these three colors (Figure 18):

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 scan.
Green ( )	Spectral peaks in the query scan that are not present in the library spectrum.
Red ( )	Spectral peaks in the library spectrum that are not present in the query scan.

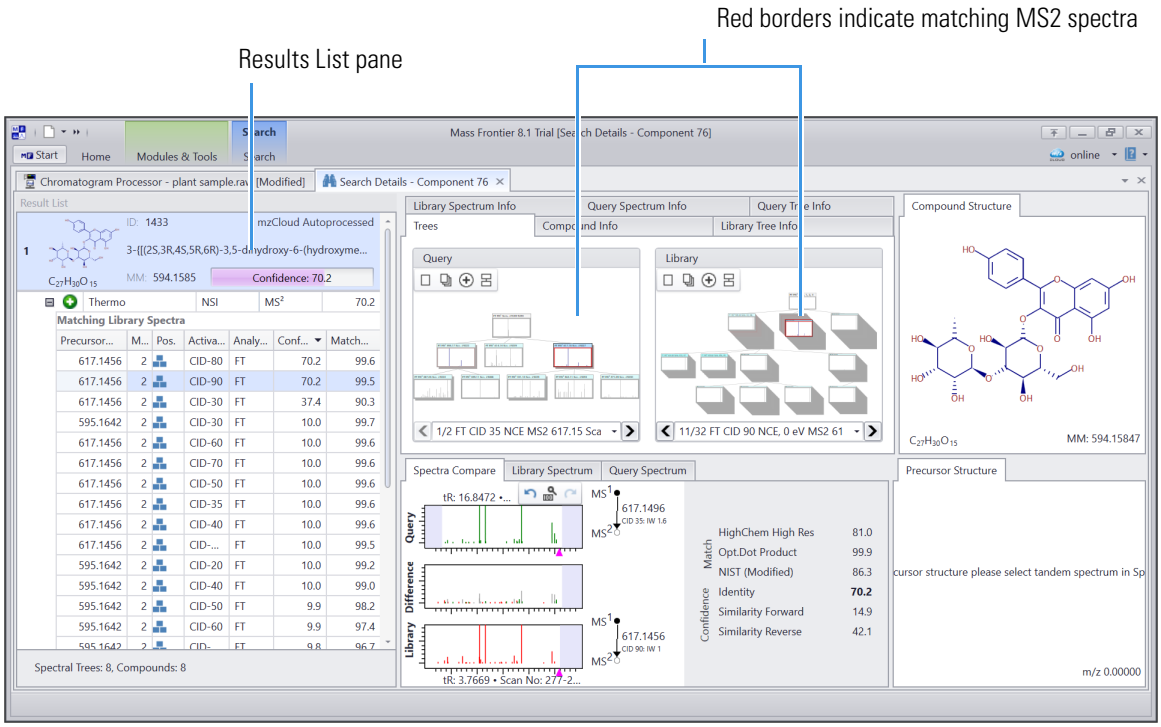
Figure 18. Spectra Compare page with the best matching library spectrum for component 76



c. To view more details about the matching compounds, click **Show Details** in the Component Search toolbar.

A Search Details - Component 76 window opens as a tabbed page (Figure 19). The Identity search is based on the matching MS2 precursor ion of 617.1496 *m/z* between the query and library compound.

Figure 19. Search Details page for component 76 following an Identity search



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

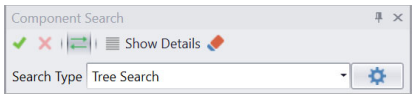
Run a tree search

Run a tree search (MSn versus MSn) as it is more sensitive compared to an Identity search (MS2 versus MS2) when the spectral tree contains MSn (n>2) data.

Note Because a Tree search is not restricted by ion activation type, it might return different results than an Identity search.

❖ To run a tree search to find compounds that match the components

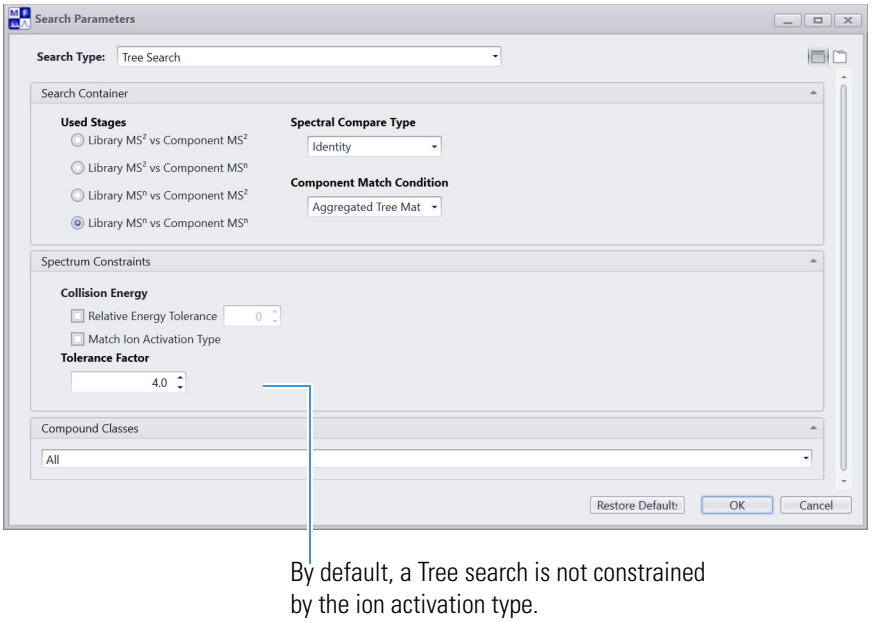
1. In the Components Search view on the Chromatogram Processor page, select **Tree Search** from the Search Type list.



2. To review the settings for a Tree search, click the settings icon,  .

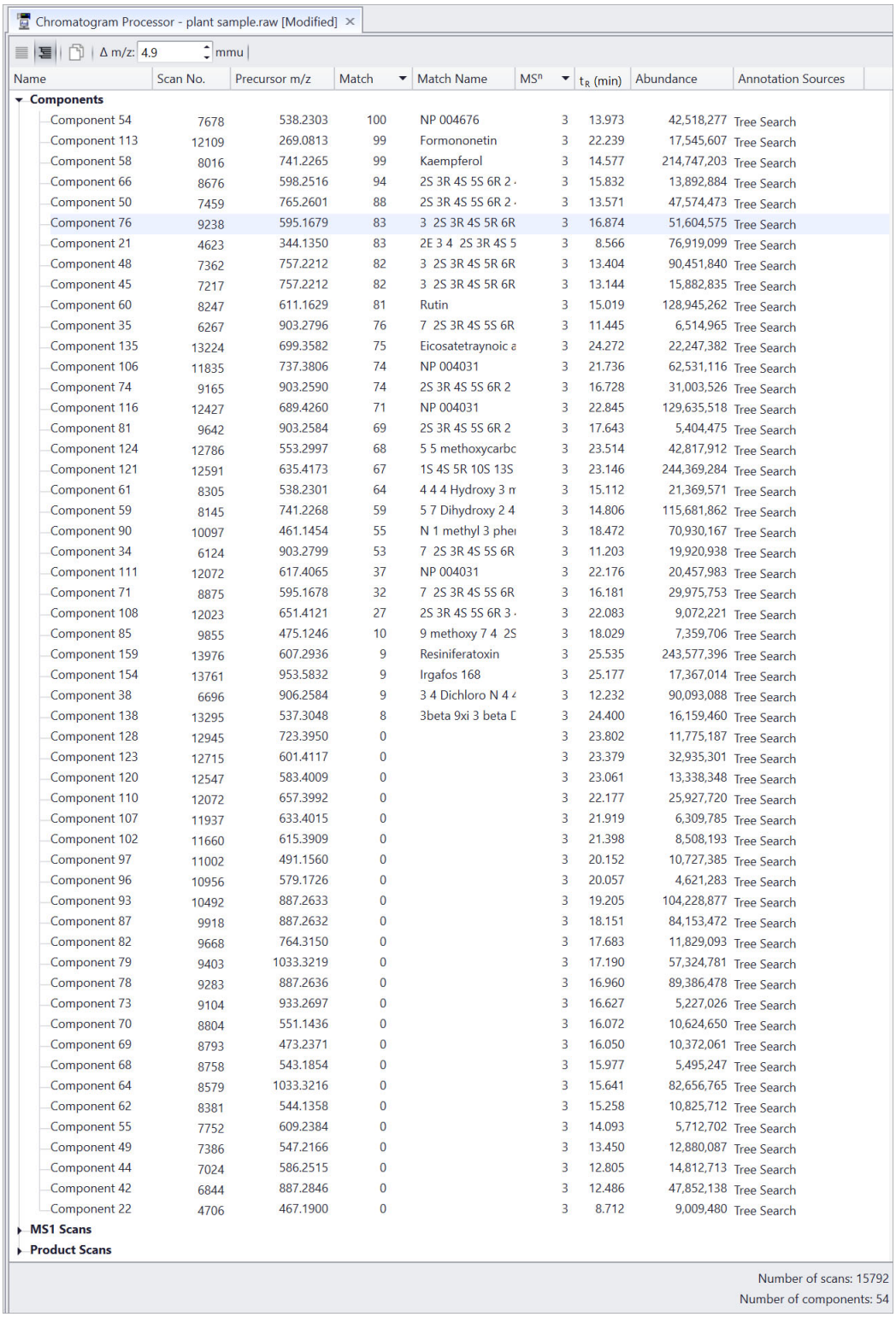
   Figure 20 shows the default settings for a Tree search.

Figure 20. Default settings for a Tree search



3. Click **OK**.
4. In the Components Search view, click **Search All**.
5. When the search ends, in the chromatogram data view sort the Tree search results by Match (Figure 21).

Figure 21. Tree Search results sorted by Match (April 2023)



Chromatogram Processor - plant sample.raw [Modified]

Δ m/z: 4.9 mmu

Name	Scan No.	Precursor m/z	Match	Match Name	MS^n	t_R (min)	Abundance	Annotation Sources
Components								
Component 54	7678	538.2303	100	NP 004676	3	13.973	42,518,277	Tree Search
Component 113	12109	269.0813	99	Formononetin	3	22.239	17,545,607	Tree Search
Component 58	8016	741.2265	99	Kaempferol	3	14.577	214,747,203	Tree Search
Component 66	8676	598.2516	94	2S 3R 4S 5S 6R 2 ·	3	15.832	13,892,884	Tree Search
Component 50	7459	765.2601	88	2S 3R 4S 5S 6R 2 ·	3	13.571	47,574,473	Tree Search
Component 76	9238	595.1679	83	3 2S 3R 4S 5R 6R	3	16.874	51,604,575	Tree Search
Component 21	4623	344.1350	83	2E 3 4 2S 3R 4S 5	3	8.566	76,919,099	Tree Search
Component 48	7362	757.2212	82	3 2S 3R 4S 5R 6R	3	13.404	90,451,840	Tree Search
Component 45	7217	757.2212	82	3 2S 3R 4S 5R 6R	3	13.144	15,882,835	Tree Search
Component 60	8247	611.1629	81	Rutin	3	15.019	128,945,262	Tree Search
Component 35	6267	903.2796	76	7 2S 3R 4S 5S 6R	3	11.445	6,514,965	Tree Search
Component 135	13224	699.3582	75	Eicosatetraynoic a	3	24.272	22,247,382	Tree Search
Component 106	11835	737.3806	74	NP 004031	3	21.736	62,531,116	Tree Search
Component 74	9165	903.2590	74	2S 3R 4S 5S 6R 2	3	16.728	31,003,526	Tree Search
Component 116	12427	689.4260	71	NP 004031	3	22.845	129,635,518	Tree Search
Component 81	9642	903.2584	69	2S 3R 4S 5S 6R 2	3	17.643	5,404,475	Tree Search
Component 124	12786	553.2997	68	5 5 methoxycarbc	3	23.514	42,817,912	Tree Search
Component 121	12591	635.4173	67	1S 4S 5R 10S 13S	3	23.146	244,369,284	Tree Search
Component 61	8305	538.2301	64	4 4 4 Hydroxy 3 n	3	15.112	21,369,571	Tree Search
Component 59	8145	741.2268	59	5 7 Dihydroxy 2 4	3	14.806	115,681,862	Tree Search
Component 90	10097	461.1454	55	N 1 methyl 3 phe	3	18.472	70,930,167	Tree Search
Component 34	6124	903.2799	53	7 2S 3R 4S 5S 6R	3	11.203	19,920,938	Tree Search
Component 111	12072	617.4065	37	NP 004031	3	22.176	20,457,983	Tree Search
Component 71	8875	595.1678	32	7 2S 3R 4S 5S 6R	3	16.181	29,975,753	Tree Search
Component 108	12023	651.4121	27	2S 3R 4S 5S 6R 3 ·	3	22.083	9,072,221	Tree Search
Component 85	9855	475.1246	10	9 methoxy 7 4 2S	3	18.029	7,359,706	Tree Search
Component 159	13976	607.2936	9	Resiniferatoxin	3	25.535	243,577,396	Tree Search
Component 154	13761	953.5832	9	Irgafos 168	3	25.177	17,367,014	Tree Search
Component 38	6696	906.2584	9	3 4 Dichloro N 4 4	3	12.232	90,093,088	Tree Search
Component 138	13295	537.3048	8	3beta 9xi 3 beta D	3	24.400	16,159,460	Tree Search
Component 128	12945	723.3950	0		3	23.802	11,775,187	Tree Search
Component 123	12715	601.4117	0		3	23.379	32,935,301	Tree Search
Component 120	12547	583.4009	0		3	23.061	13,338,348	Tree Search
Component 110	12072	657.3992	0		3	22.177	25,927,720	Tree Search
Component 107	11937	633.4015	0		3	21.919	6,309,785	Tree Search
Component 102	11660	615.3909	0		3	21.398	8,508,193	Tree Search
Component 97	11002	491.1560	0		3	20.152	10,727,385	Tree Search
Component 96	10956	579.1726	0		3	20.057	4,621,283	Tree Search
Component 93	10492	887.2633	0		3	19.205	104,228,877	Tree Search
Component 87	9918	887.2632	0		3	18.151	84,153,472	Tree Search
Component 82	9668	764.3150	0		3	17.683	11,829,093	Tree Search
Component 79	9403	1033.3219	0		3	17.190	57,324,781	Tree Search
Component 78	9283	887.2636	0		3	16.960	89,386,478	Tree Search
Component 73	9104	933.2697	0		3	16.627	5,227,026	Tree Search
Component 70	8804	551.1436	0		3	16.072	10,624,650	Tree Search
Component 69	8793	473.2371	0		3	16.050	10,372,061	Tree Search
Component 68	8758	543.1854	0		3	15.977	5,495,247	Tree Search
Component 64	8579	1033.3216	0		3	15.641	82,656,765	Tree Search
Component 62	8381	544.1358	0		3	15.258	10,825,712	Tree Search
Component 55	7752	609.2384	0		3	14.093	5,712,702	Tree Search
Component 49	7386	547.2166	0		3	13.450	12,880,087	Tree Search
Component 44	7024	586.2515	0		3	12.805	14,812,713	Tree Search
Component 42	6844	887.2846	0		3	12.486	47,852,138	Tree Search
Component 22	4706	467.1900	0		3	8.712	9,009,480	Tree Search
MS1 Scans								
Product Scans								

Number of scans: 15792
Number of components: 54

6. Review the results from the Tree search for component 76 (Figure 22 and Figure 23):
   - The Tree search ranks mzCloud ID: 1433 with a Tree Match score of 82.7

Figure 22. Tree search results for component 76 showing the matching score

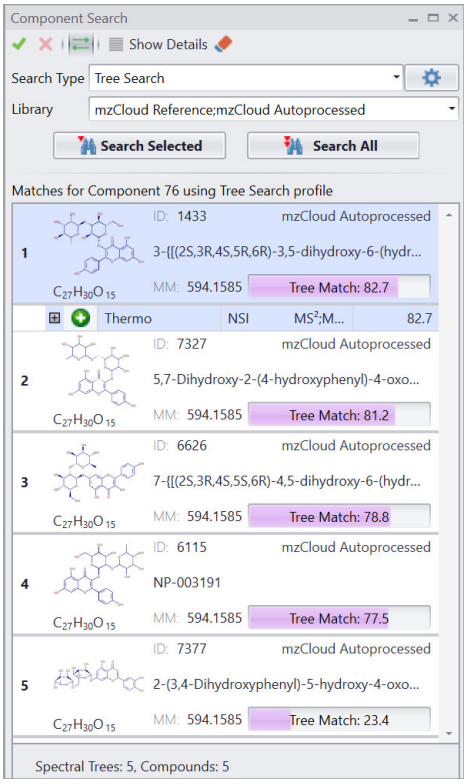
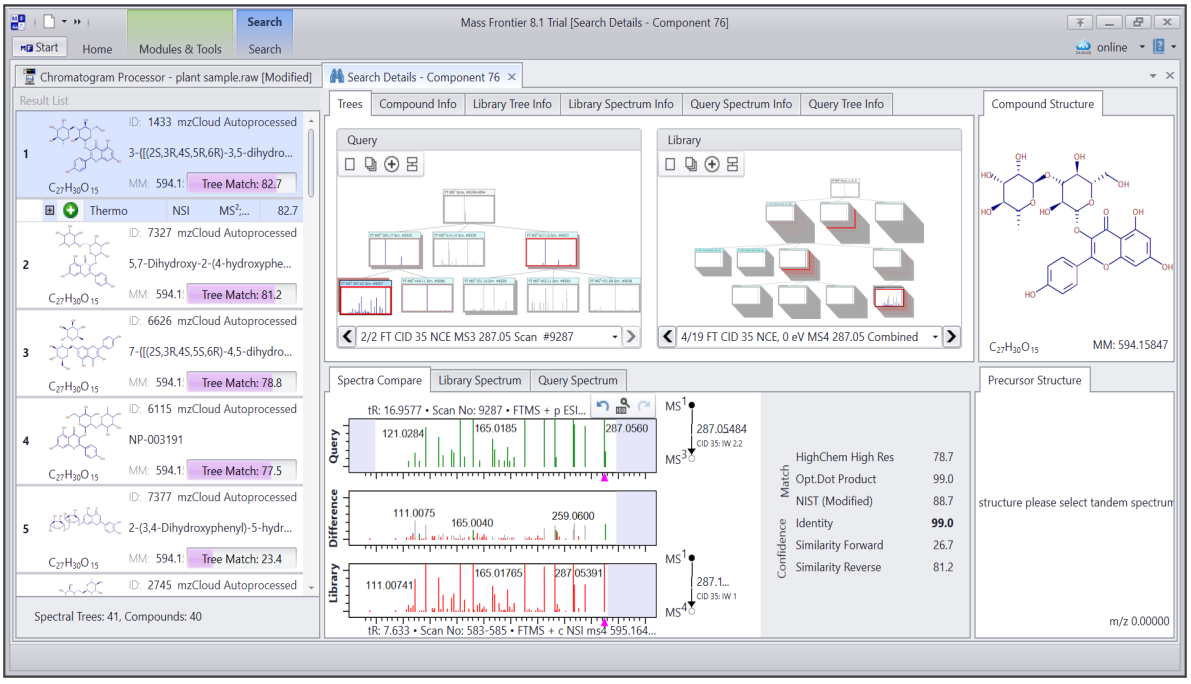


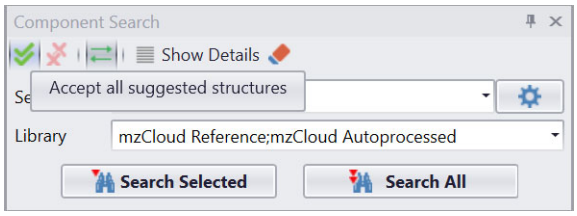
Figure 23. Search Details page for component 76 following a Tree search



7. For this tutorial, accept all Tree search hits with scores greater than 60.

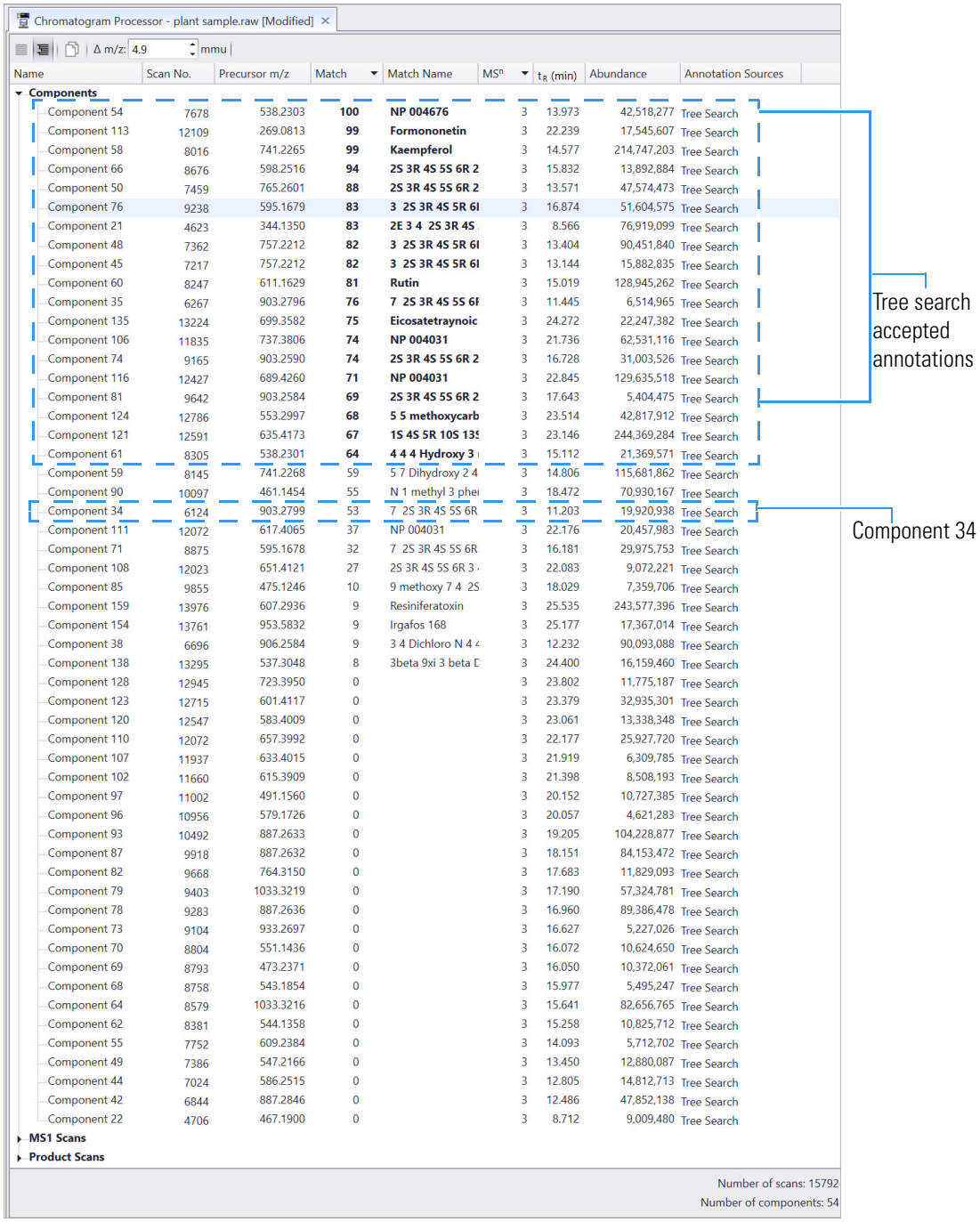
   a. In the chromatogram data view of the Chromatogram Processor window, select all components with a Match score of 60 and greater.

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



The selected components are annotated. The match name and match score for these components become bold (Figure 24).

Figure 24. Tree search results showing accepted annotations



To identify the remaining components, use the Subtree search or Identity Substructure search to match the substructures between query unknowns and library compounds.

Component 34 has a poor match score of 53, scores < 59 are considered to be poor, (Figure 24). You cannot identify this component using the Identity or Tree searches. However, you can find library compounds with matching substructures. You can use the Subtree search to identify a partial structure of the compound.

Run a Subtree search

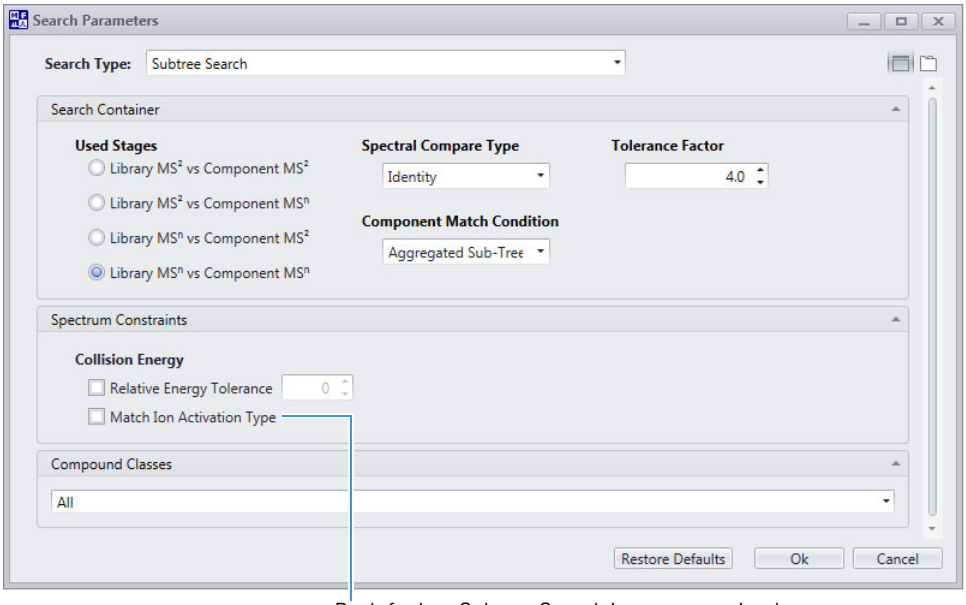
Running a Subtree search you can find matches that the Identity and Tree searches did not find. The subtree search matches using MSn versus MSn data.

❖ 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 25 shows the default settings for a Subtree search.

   Figure 25. Default settings for a Subtree search



> **Note** A Subtree search searches for matching spectra for all the data-dependent MSn scans associated with the selected component. It compares each query scan to scans for all the MSn stages in the library and uses the Aggregated Sub-Tree algorithm to calculate the match score.

3. Click **OK** to close the Search Parameters dialog box.
4. In the Components Search view, click **Search All**.

   The application runs a Subtree Search for the unannotated components (Figure 26).

Figure 26. Results of a Subtree Search for the components detected in the plant sample

As an example, component 34 with the Subtree search result has a match score of 99 for the substructure (Figure 26). Additionally, you can perform a substructure search. Refer to Table 1 to understand the differences between these two search types.

Run an Identity Substructure search

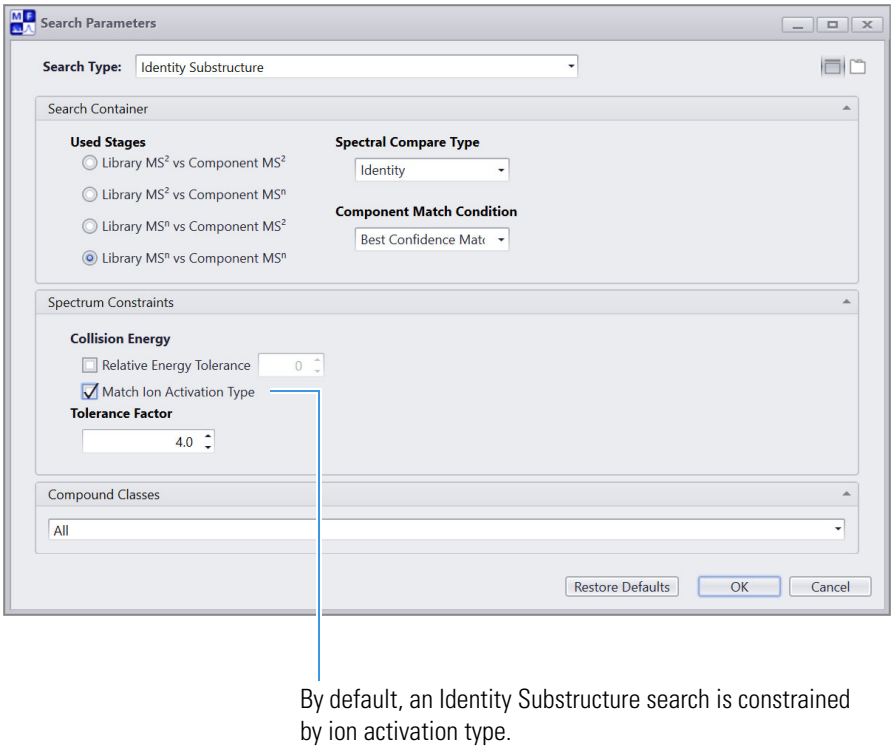
An Identity Substructure search matches using MSn versus MSn data. The Identity Substructure search is constrained by ion activation type whereas the Subtree search is not constrained by ion activation type. Run an Identity Substructure search to find matching substructures for the components of interest.

❖ 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 27 shows the default settings for an Identity Substructure search.

   Figure 27. Default settings for an Identity Substructure search



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

Note An Identity Substructure search searches for all the data-dependent MS^n scans associated with the selected component. It compares each query scan to library scans for the same precursor *m/z* at any MS^n stage and uses the Best Confidence Match algorithm to calculate the match score.

3. Click **OK** to close the Search Parameters dialog box.
4. In the Components Search view, click **Search All**.

   The application runs an Identity Substructure search for the unannotated components (Figure 28).

Figure 28. Identity Substructure search results

Chromatogram Processor - plant sample.raw [Modified]

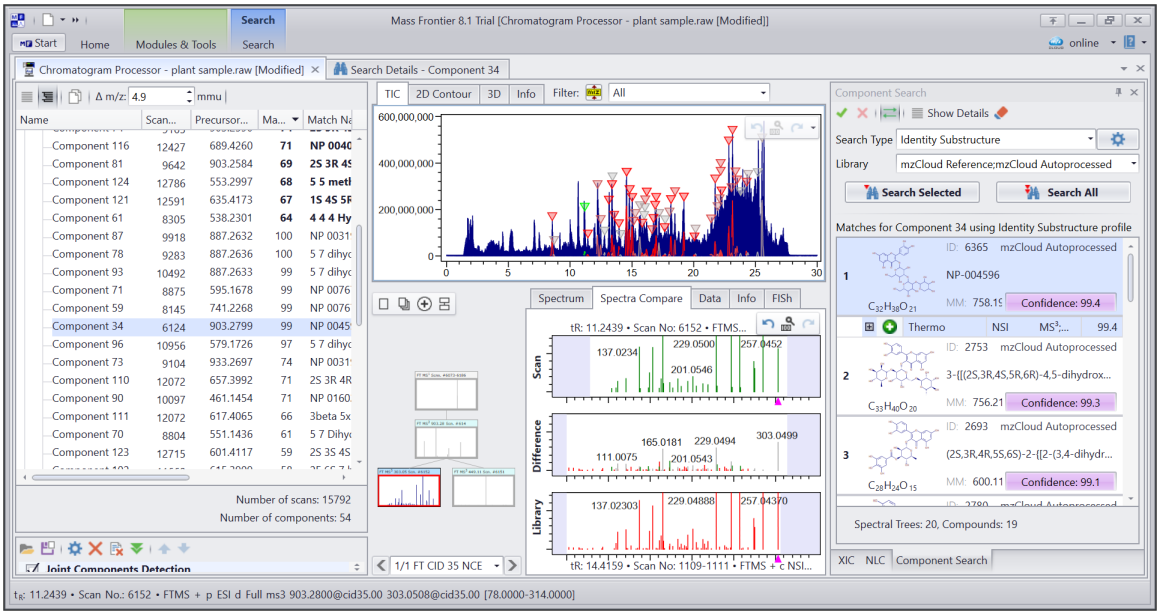
Δ m/z: 4.9 mmu

Name	Scan No.	Precursor m/z	Match	Match Name	MS^n	t_R (min)	Abundance	Annotation Sources
Components								
Component 54	7678	538.2303	**100**	**NP 004676**	3	13.973	42,518,277	Tree Search
Component 113	12109	269.0813	**99**	**Formononetin**	3	22.239	17,545,607	Tree Search
Component 58	8016	741.2265	**99**	**Kaempferol**	3	14.577	214,747,203	Tree Search
Component 66	8676	598.2516	**94**	**2S 3R 4S 5S 6R 2**	3	15.832	13,892,884	Tree Search
Component 50	7459	765.2601	**88**	**2S 3R 4S 5S 6R 2**	3	13.571	47,574,473	Tree Search
Component 76	9238	595.1679	**83**	**3 2S 3R 4S 5R 6I**	3	16.874	51,604,575	Tree Search
Component 21	4623	344.1350	**83**	**2E 3 4 2S 3R 4S**	3	8.566	76,919,099	Tree Search
Component 48	7362	757.2212	**82**	**3 2S 3R 4S 5R 6I**	3	13.404	90,451,840	Tree Search
Component 45	7217	757.2212	**82**	**3 2S 3R 4S 5R 6I**	3	13.144	15,882,835	Tree Search
Component 60	8247	611.1629	**81**	**Rutin**	3	15.019	128,945,262	Tree Search
Component 35	6267	903.2796	**76**	**7 2S 3R 4S 5S 6R**	3	11.445	6,514,965	Tree Search
Component 135	13224	699.3582	**75**	**Eicosatetraynoic**	3	24.272	22,247,382	Tree Search
Component 106	11835	737.3806	**74**	**NP 004031**	3	21.736	62,531,116	Tree Search
Component 74	9165	903.2590	**74**	**2S 3R 4S 5S 6R 2**	3	16.728	31,003,526	Tree Search
Component 116	12427	689.4260	**71**	**NP 004031**	3	22.845	129,635,518	Tree Search
Component 81	9642	903.2584	**69**	**2S 3R 4S 5S 6R 2**	3	17.643	5,404,475	Tree Search
Component 124	12786	553.2997	**68**	**5 5 methoxycarb**	3	23.514	42,817,912	Tree Search
Component 121	12591	635.4173	**67**	**1S 4S 5R 10S 13S**	3	23.146	244,369,284	Tree Search
Component 61	8305	538.2301	**64**	**4 4 4 Hydroxy 3**	3	15.112	21,369,571	Tree Search
Component 87	9918	887.2632	100	NP 003191	3	18.151	84,153,472	Identity Substructure
Component 78	9283	887.2636	100	5 7 dihydroxy 2 4	3	16.960	89,386,478	Identity Substructure
Component 96	10956	579.1726	99	Biochanin A	3	20.057	4,621,283	Identity Substructure
Component 93	10492	887.2633	99	5 7 dihydroxy 2 4	3	19.205	104,228,877	Identity Substructure
Component 71	8875	595.1678	99	NP 007676	3	16.181	29,975,753	Identity Substructure
Component 59	8145	741.2268	99	NP 007676	3	14.806	115,681,862	Identity Substructure
Component 34	6124	903.2799	99	NP 004596	3	11.203	19,920,938	Identity Substructure
Component 73	9104	933.2697	74	NP 003191	3	16.627	5,227,026	Identity Substructure
Component 110	12072	657.3992	71	2S 3R 4R 5R 6S 2	3	22.177	25,927,720	Identity Substructure
Component 90	10097	461.1454	71	NP 016028	3	18.472	70,930,167	Identity Substructure
Component 42	6844	887.2846	68	Robinin	3	12.486	47,852,138	Identity Substructure
Component 111	12072	617.4065	66	3beta 5xi 9xi 16al	3	22.176	20,457,983	Identity Substructure
Component 70	8804	551.1436	61	5 7 Dihydroxy 2 4	3	16.072	10,624,650	Identity Substructure
Component 123	12715	601.4117	59	2S 3S 4S 5R 6R 6	3	23.379	32,935,301	Identity Substructure
Component 102	11660	615.3909	58	3 5 9 16 21 22 28	3	21.398	8,508,193	Identity Substructure
Component 120	12547	583.4009	56	2S 3S 4S 5R 6R 6	3	23.061	13,338,348	Identity Substructure
Component 154	13761	953.5832	47	UR 144 N 2 hydro	3	25.177	17,367,014	Identity Substructure
Component 108	12023	651.4121	46	24E 12 15 Dihydrc	3	22.083	9,072,221	Identity Substructure
Component 38	6696	906.2584	45	Kaempferol 3 Gal	3	12.232	90,093,088	Identity Substructure
Component 107	11937	633.4015	36	24E 12 15 Dihydrc	3	21.919	6,309,785	Identity Substructure
Component 62	8381	544.1358	36	2S 3R 4S 5S 6R 2	3	15.258	10,825,712	Identity Substructure
Component 85	9855	475.1246	34	5 Hydroxy 2 4 hyc	3	18.029	7,359,706	Identity Substructure
Component 22	4706	467.1900	25	Methyl 3 3 methy	3	8.712	9,009,480	Identity Substructure
Component 97	11002	491.1560	10	5 4 5 dihydroxy 6	3	20.152	10,727,385	Identity Substructure
Component 79	9403	1033.3219	10	Kaempferitrin	3	17.190	57,324,781	Identity Substructure
Component 64	8579	1033.3216	10	Kaempferitrin	3	15.641	82,656,765	Identity Substructure
Component 159	13976	607.2936	9	Resiniferatoxin	3	25.535	243,577,396	Identity Substructure
Component 138	13295	537.3048	8	3beta 9xi 3 beta D	3	24.400	16,159,460	Identity Substructure
Component 128	12945	723.3950	0		3	23.802	11,775,187	Identity Substructure
Component 82	9668	764.3150	0		3	17.683	11,829,093	Identity Substructure
Component 69	8793	473.2371	0		3	16.050	10,372,061	Identity Substructure
Component 68	8758	543.1854	0		3	15.977	5,495,247	Identity Substructure
Component 55	7752	609.2384	0		3	14.093	5,712,702	Identity Substructure
Component 49	7386	547.2166	0		3	13.450	12,880,087	Identity Substructure
Component 44	7024	586.2515	0		3	12.805	14,812,713	Identity Substructure
MS1 Scans								
Product Scans								

Number of scans: 15792
Number of components: 54

5. To review the results for component 34 in the Chromatogram Processor - plant sample.raw view, in the components list, select **Component 34**(Figure 29).

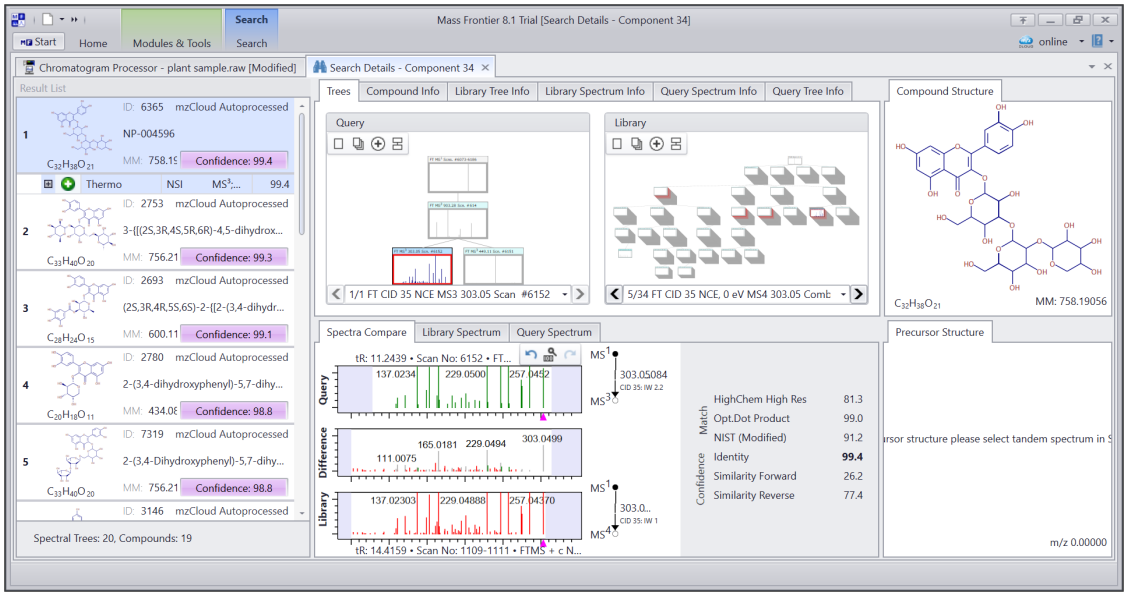
Figure 29. Results of an Identity Substructure search for component 34 in the plant sample



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

A Search Details - Component 34 page opens as a tabbed page (Figure 30). The Result List shows the best matching library spectrum for component 34 with hits from the mzCloud Autoprocessed and mzCloud Reference libraries.

Figure 30. Search Details page for component 34 showing information for the first hit result

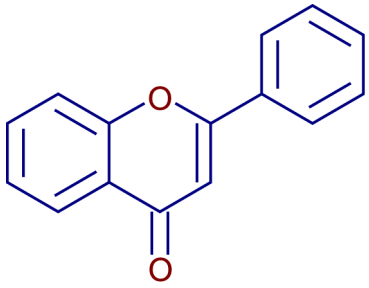


7. In the Search Details page for component 34, review the Spectra Compare view (Figure 31).

Figure 31. Substructure search results for component 34 showing the Spectra Compare page

The Spectra Compare view (Figure 31) shows the best matching library spectrum for component 34 with of 303.05 m/z. substructure containing the basic C6-C3-C6 flavone backbone.

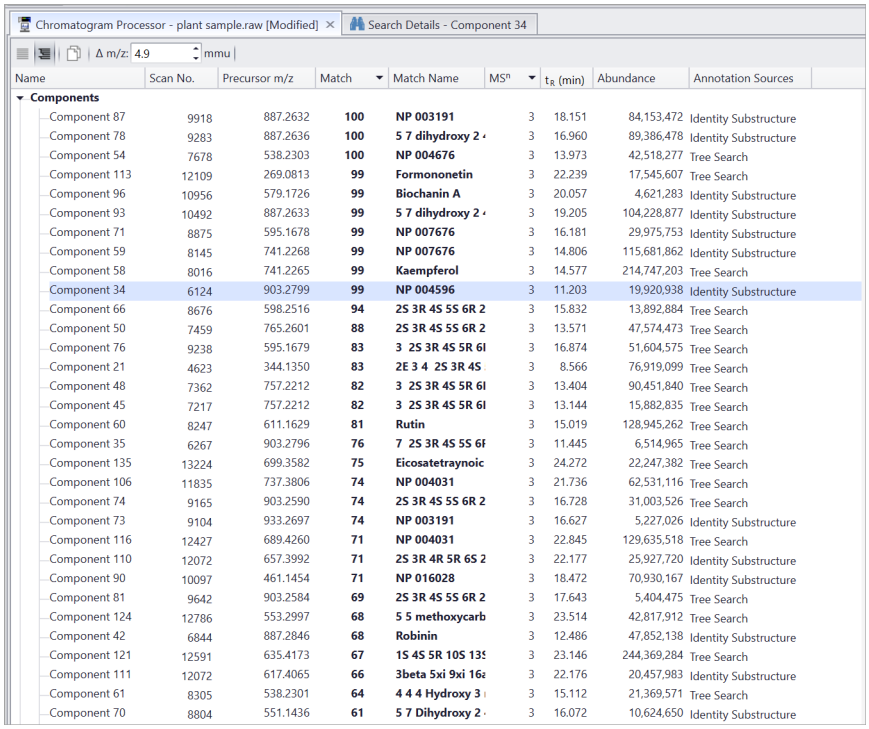
Figure 32. Flavone backbone



The Substructure search helps to identify unknowns when the compound is not in the library. Based on the substructure match, component 34 is a flavonoid.

8. Accept all the Identity Substructure matches with scores > 60 (Figure 33).

Figure 33. Components annotated with Identity Substructure matches



Name	Scan No.	Precursor m/z	Match	Match Name	MS^n	t_R (min)	Abundance	Annotation Sources
Components								
Component 87	9918	887.2632	**100**	**NP 003191**	3	18.151	84,153,472	Identity Substructure
Component 78	9283	887.2636	**100**	**5 7 dihydroxy 2**	3	16.960	89,386,478	Identity Substructure
Component 54	7678	538.2303	**100**	**NP 004676**	3	13.973	42,518,277	Tree Search
Component 113	12109	269.0813	**99**	**Formononetin**	3	22.239	17,545,607	Tree Search
Component 96	10956	579.1726	**99**	**Biochanin A**	3	20.057	4,621,283	Identity Substructure
Component 93	10492	887.2633	**99**	**5 7 dihydroxy 2**	3	19.205	104,228,877	Identity Substructure
Component 71	8875	595.1678	**99**	**NP 007676**	3	16.181	29,975,753	Identity Substructure
Component 59	8145	741.2268	**99**	**NP 007676**	3	14.806	115,681,862	Identity Substructure
Component 58	8016	741.2265	**99**	**Kaempferol**	3	14.577	214,747,203	Tree Search
Component 34	6124	903.2799	**99**	**NP 004596**	3	11.203	19,920,938	Identity Substructure
Component 66	8676	598.2516	**94**	**2S 3R 4S 5S 6R 2**	3	15.832	13,892,884	Tree Search
Component 50	7459	765.2601	**88**	**2S 3R 4S 5S 6R 2**	3	13.571	47,574,473	Tree Search
Component 76	9238	595.1679	**83**	**3 2S 3R 4S 5R 6I**	3	16.874	51,604,575	Tree Search
Component 21	4623	344.1350	**83**	**2E 3 4 2S 3R 4S**	3	8.566	76,919,099	Tree Search
Component 48	7362	757.2212	**82**	**3 2S 3R 4S 5R 6I**	3	13.404	90,451,840	Tree Search
Component 45	7217	757.2212	**82**	**3 2S 3R 4S 5R 6I**	3	13.144	15,882,835	Tree Search
Component 60	8247	611.1629	**81**	**Rutin**	3	15.019	128,945,262	Tree Search
Component 35	6267	903.2796	**76**	**7 2S 3R 4S 5S 6I**	3	11.445	6,514,965	Tree Search
Component 135	13224	699.3582	**75**	**Eicosatetraynoic**	3	24.272	22,247,382	Tree Search
Component 106	11835	737.3806	**74**	**NP 004031**	3	21.736	62,531,116	Tree Search
Component 74	9165	903.2590	**74**	**2S 3R 4S 5S 6R 2**	3	16.728	31,003,526	Tree Search
Component 73	9104	933.2697	**74**	**NP 003191**	3	16.627	5,227,026	Identity Substructure
Component 116	12427	689.4260	**71**	**NP 004031**	3	22.845	129,635,518	Tree Search
Component 110	12072	657.3992	**71**	**2S 3R 4R 5R 6S 2**	3	22.177	25,927,720	Identity Substructure
Component 90	10097	461.1454	**71**	**NP 016028**	3	18.472	70,930,167	Identity Substructure
Component 81	9642	903.2584	**69**	**2S 3R 4S 5S 6R 2**	3	17.643	5,404,475	Tree Search
Component 124	12786	553.2997	**68**	**5 5 methoxycarb**	3	23.514	42,817,912	Tree Search
Component 42	6844	887.2846	**68**	**Robinin**	3	12.486	47,852,138	Identity Substructure
Component 121	12591	635.4173	**67**	**1S 4S 5R 10S 13S**	3	23.146	244,369,284	Tree Search
Component 111	12072	617.4065	**66**	**3beta 5xi 9xi 16a**	3	22.176	20,457,983	Identity Substructure
Component 61	8305	538.2301	**64**	**4 4 4 Hydroxy 3**	3	15.112	21,369,571	Tree Search
Component 70	8804	551.1436	**61**	**5 7 Dihydroxy 2**	3	16.072	10,624,650	Identity Substructure

9. To review the Tree and Identity Substructure results by search type and in descending Match order, sort by multiple columns. First sort on the Annotation Sources column in descending order, then sort on the Match column in descending order (Figure 34).

   a. Sort the Annotation Sources column by clicking the column heading until the ▾ arrow appears in the column, or right-click the column heading and choose a sort order.

   b. Press the Shift-key then click on the Match column heading until the ▾ arrow appears in the column.

Figure 34. Tree and Identity Substructure search results

Library search types summary

Table 1 provides a brief description of the library search types.

Table 1. Library search types (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 must match.	Compound identification	Best Confidence Match
Identity Substructure	• Compares any MSn library spectra against any MSn query spectra. • The precursor ions at any MS^n stage must match.	Substructure identification	Best Confidence Match

Table 1. Library search types, continued (Sheet 2 of 2)

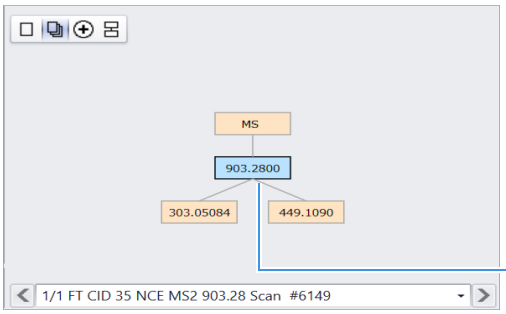
Search type	Used stages and constraints	Use	Confidence score
Similarity (Forward and Reverse)	• Compares the MS2 library spectra against the MS2 query spectra. • The MS2 precursor ions do not have to match.	Finding structurally similar compounds	Best Confidence Match
Tree Search	• Compares any MSn library 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
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

Identify a component by running an mzLogic analysis

For ranking structure candidates of unknowns, mzLogic uses a novel algorithm. An mzLogic analysis combines mzCloud™ spectral similarity searching (MS^2 and MS^n) and substructure overlapping to rank putative structures.

❖ To identify a component by running an mzLogic analysis

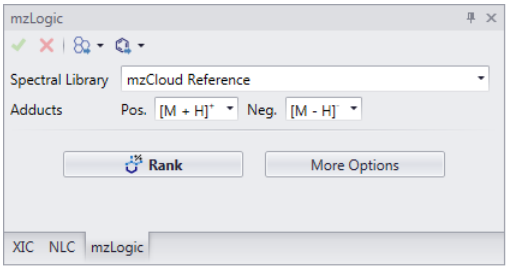
1. In the Components list of the chromatogram data view, select **Component 34** (*m/z* 903.2799).
2. In the MS spectrum view, click the ***m/z* 903.2799 MS2 node.**



Tip To view the folded tree, which shows only the precursor *m/z* value for each node, click the **Fold Whole Tree** icon, ☐. To return to the unfolded tree view, click the **Unfold Whole Tree** icon, ⧉.

3. 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.



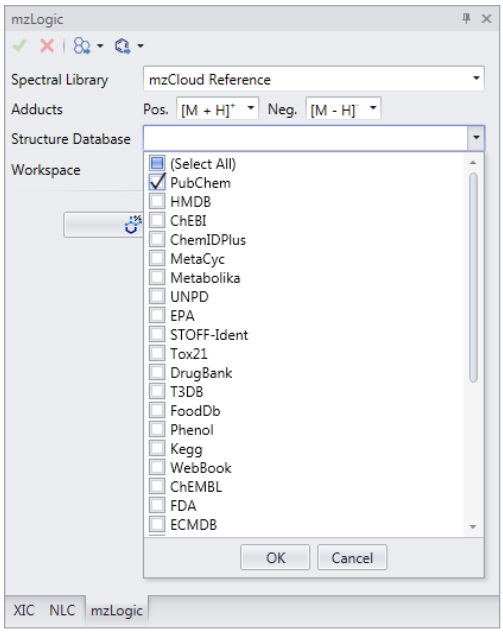
4. Click **More Options**.

The Structure Database list and the Workspace list appear. The default selection for the structure database is All except PubChem.

5. Change the selection to PubChem as follows:

   a. Open the Structure Database list.

   b. Clear the **Select All** check box and select the **PubChem** check box.

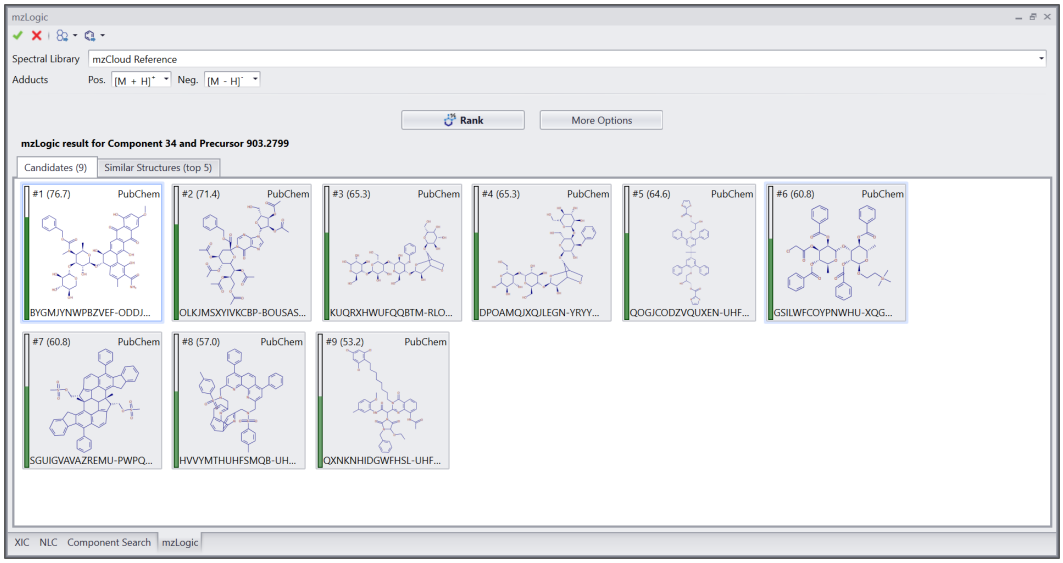


   c. Click **OK**.

6. In the mzLogic view, click **Rank**.

   The mzLogic analysis finds nine candidates, but none of these candidates is a flavonoid (Figure 35). To identify component 34, we must add a custom list of structures to the search.

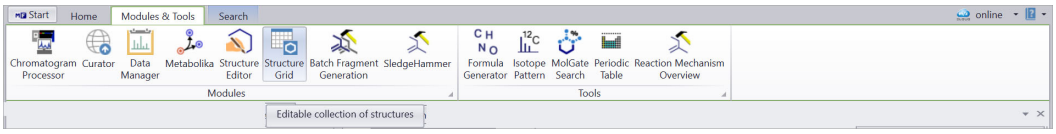
Figure 35. mzLogic results for component 34 with structures from PubChem



7. To specify a custom list of structures, do the following:

   a. In the application tab bar, click the **Modules & Tools** tab.

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



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

c. In the File group of the Structure Grid toolbar, click **Open** (Figure 36). Then, browse to the following folder, select the **Flavonoids_C39H50O24.sdf** structure file, and click **Open**.

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

The SDF file populates the grid with 7 structure cards (Figure 36).

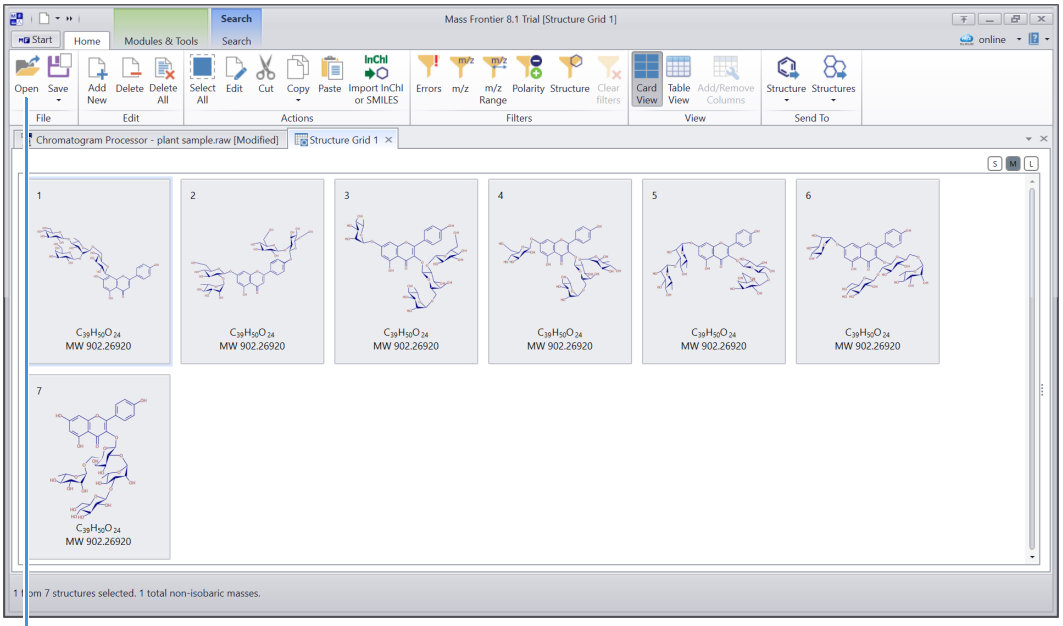
Note Th flavonoids structure file contains structures provided by the Arita Lab National Institute of Genetics, Japan.

The Flavonoid structure file **Flavonoids_C39H50O24.sdf** was taken from the Flavonoid database (http://metabolomics.jp/wiki/Category:FL).

Tip If you install the Thermo Scientific Compound Discoverer™ 3.0 or later application, you can access the Arita Lab Flavonoid Structure Database mass list, which currently contains 6961 flavonoid structures.

Compound Discoverer is a qualitative data-processing application that uses accurate mass data, isotope pattern matching, and mass spectral library searches for the structural identification of small molecules. It provides an integrated set of libraries, databases, and statistical analysis tools linked in customizable workflows for the identification of unknowns, the determination of differences between samples, and the elucidation of biological pathways.

Figure 36. Structure grid populated with structures from the selected structure file



File Open icon

d. To return to the Chromatogram Processor page, click the **Chromatogram Processor – *plant sample.raw*** tab.

The application automatically populates the Workspace list with the name of the latest Structure Grid page (Figure 37).

8. Click **Rank**.

The mzLogic analysis ranks all the supplied structures (Figure 37).

Figure 37. Ranking for 16 structure candidates

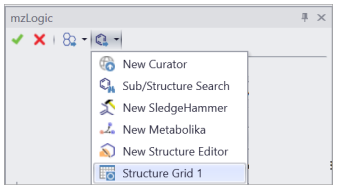
9. To annotate component 34 (precursor *m/z* 903.2799), do the following:

a. Select card #1 in the Candidates tab, the highest ranked structure candidate.

b. To accept this structure as the annotation for component 34, click the **Accept Structure** icon, ✔, in the mzLogic view.

Component 34 moves up in the list (Figure 39).

c. To send card #1 to the structure grid, click the dropdown for the selected candidates and choose the Structure Grid that has the 12 flavonoid candidates.



In the Structure Grid, compare card #8 with the 7 structure candidates. Card #8 matches card #10. Point on card #3 to view the name of the compound, which is FL5FAAGA0026 (Figure 38).

Figure 38. Pointing on a card to view the compound name

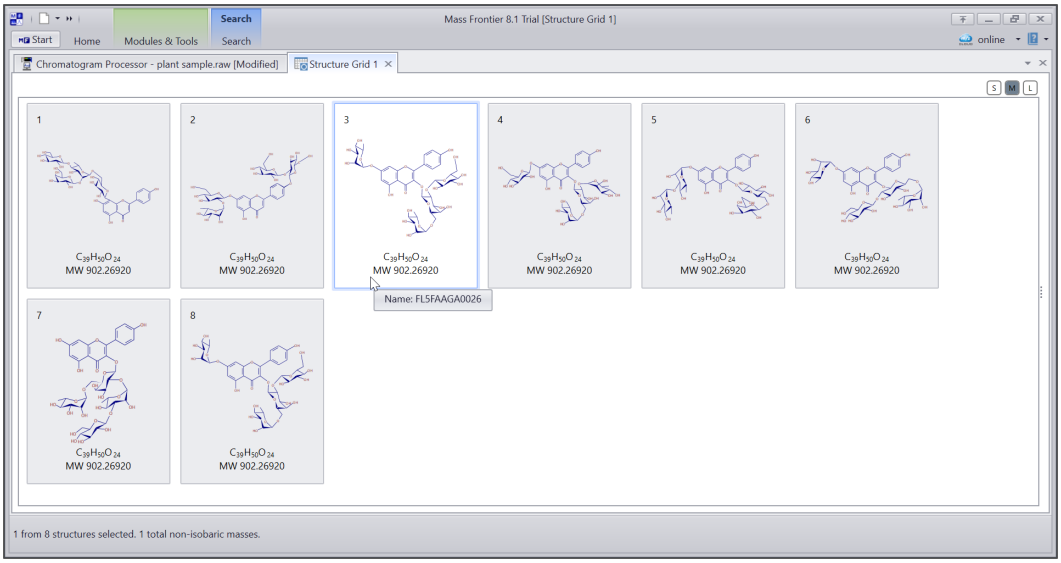


Figure 39. Accepted annotations for component 34

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.