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
- Demo data files
- Check the connection to the mzCloud server
- Open and browse an example raw data file
- Detect components in LC/MS data
- Sort and delete components in the components list
- Identify components by searching the mzCloud mass spectral database
- Identify a component by running an mzLogic analysis
- Save the analysis results to an HCCX file

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.

thermoscientific

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.

* To check the connection to the mzCloud server

Check the connection to the mzCloud server

 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).

🔡 i 🗋 * » i 🔰 S	Search	Mass Frontier 8.1 Trial [Empty]	7 _ 8 ×
Ha Start Home Modules & Tools S	Search		🔬 online 👻 🔢 👻
Chromatogram Curator Data Metabolika S Processor Mo	Structure Structure Batch Fragment SledgeHammer Grid Generation	Formula Isotope MolGate Periodic Reaction Mechanism Generator Pattern Search Table Overview	
Open			
Chromatogram Processor Data Files (LC/MS)	Recent	Quick search	🗙 💽 Size: Large 🔹
Curator Curator Files			
Data Manager Database Files			
Fragments & Mechanisms Fragments & Mechanisms			
Metabolika Metabolika			
Structure Editor Structure Files			
Structure Grid Editable collection of structures			
Batch Fragment Generation Generate fragments for a set of stru			
New			
Chromatogram Processor Chromatogram Processor			
Curator Curator			
Data Manager Data Manager			
Metabolika Metabolika			
Structure Editor Structure Editor			
Structure Grid Structure Grid			
Batch Fragment Generation Batch Fragment Generation			
SledgeHammer SledgeHammer			
Global Settings			
About]		
Show this Window Next Time			

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

🔛 🗋 🕶 H		Search	Mass Frontier 8.1 Trial [Empty]					★ _ □	×
MG Start Home	Modules & Tools	Search						👜 online 🝷	2 -
Chromatogram Curato Processor	or Data Metabolik Manager	a Structure Editor	Structure Grid Structure Grid Setch Fragment Generation SledgeHammer	C H N O Formula Generator	lsotope Pattern	MolGate Search	Periodic Table	Reaction Mechani Overview	ism
	Modu	les	4			То	ols		- 4
									.:

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

□ - » I	Search	Mass Frontier 8.1 Tria	l [Empty]	Ŧ
Start Home Mo	odules & Tools Search			in online
New N	New Document:			
Open	Chromatogram Processor	Curator	Data Manager	Metabolika
Import				20
Recent	Chromatogram Processor	Curator	Data Manager	Metabolika
Save Global Settings	Structure Editor	Structure Grid	Batch Fragment Generation	SledgeHammer
Connection Check Help			Ratch Fragment	×
About	Structure Editor	Structure Grid	Generation	SledgeHammer
Exit				

Start menu

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."

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).

* To open the example raw data file

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

Open and browse an example raw data file

Open a raw data file for processing

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

🔛 🗋 🕶 🕨			Search		Mass Frontier 8.1	Trial [Empty]					Ŧ	x
Ma Start Home	Modules	& Tools	Search								🧀 online 👻	? -
	<u>ىلىك</u>		\mathbf{i}		1	×	Сн NO	1 ² C	$\ddot{\mathbf{v}}$		×	
Chromatogram Curato Processor	r Data Manager	Metabolika	Structure Editor	Structure Grid	Batch Fragment Generation	SledgeHammer	Formula Generator	lsotope Pattern	MolGate Search	Periodic Table	Reaction Mechani Overview	sm
		N	1odules						Тос	ols		- 1
Data Files (LC/MS)												
												.::

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, <u>L</u>.
- For the chromatogram data view, click the **Show Chromatogram Data** icon, \square .
- For the command processor view, click the Show Command Processor icon, ⁵/₈.

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).

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
	Nana
Error Log Count	7
Error Log Count	7
Expected run lime	50
riiter 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	2
Instrument Method File	D) rb/ Tamplata_stdmathade) Structure_specific MS4 (manasascharida) math
	D. (K(Templete_stamethods)structure specific Mis4 (monosacchande).meth
Paul	
Processing Method File	
Kaw File Name	U:\rk\UK_demo\cladratis_U1.raw
Sample Weight	0
User text	;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
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

Figure 6. Information about the plant sample.raw file

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).



	Joint Components Detection	# ×
	♥ ➡ ► Mode Wizard ○ Details	- Î
	Deconvolution	
	Wizard Average & Minimal Peak Width Automatic Manual: Average: 20 Min: 2 scans	
Reset icon	Power of Baseline Correction	
	Smoothing Power	
	Component Overlapping Intensity	
	Intensity of Detected Component	
	Tree Branching	
	Retention Time Range	
	54.9 ● 943	
	Number of Components	
	Preview Restore Accept Cancel	·

- 3. Click the **Reset** icon, **O**, 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).

int Components Detection 4 ×	
) 🗒 📂	
Mode	
Deconvolution	
Wizard	
Average & Minimal Peak Width	
O Automatic ○ Manual: Average: 20 ↓ Min: 2 ↓ scans	
Power of Baseline Correction	
Smoothing Power	Moving the slider to the right increases the
	probability of detecting overlapping peaks of
	probability of detecting overlapping peaks a
Component Overlapping Intensity	separate peaks.
Intensity of Detected Component	
· · · · · · · · · · · · · · · · · · ·	
Tree Branching	Moving the slider to the left decreases the
	detection of low abundance peaks.
O	
Retention Time Range	
0.0011 • 29.9987	
m/z Range	
50.9 • 1991.5	
lumber of Components	
Preview Restore Accept Cancel	

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



Blue triangles above detected 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

📂 💾 🌣 🗙 🕵 🔻 🛧 🔸	
Joint Components Detection	*
	Ŧ

Go to the next topic, "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.

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**.

Sort and delete components in the components list

> Sort the components by MSn stage

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

Figure 11. Components sorted by MSn stage

Chrom	atogram Proce	ssor - plan	it sample.raw	Modified	4] ×						
≣ ≣ [Δ m/z: 4.	9	mmu								
Name	•	Scan	Precursor	Match	Match Name	N AI	Sort Ascend	ing		Annotatio	
Con	nponent 15	2626	435.0823			Z	Cart Daaraa	alta a	33		÷.
Con	nponent 16	3374	448.1951			A	Sort Descen	aing	42		
-Con	nponent 17	3736	377.1829						44		
Con	nponent 18	3786	699.1914				Show Colun	nn Chooser	50		0
Con	nponent 19	4023	361.0903			+A+	Best Fit		28		
Con	nponent 20	4274	377.1828				Best Fit (all o	columns)	95		
_Con	nponent 21	4623	344.1350				Ciltor Editor	,	99		
Con	nponent 22	4706	467.1900			1	Filter Eultor.		80		
Con	nponent 23	4974	457.1029				Show Search	n Panel	25		
Con	nponent 24	5094	331.0707				2 9.394	5,466,	573		
Con	nponent 25	5363	374.1456				2 9.847	12,477,	232		
Con	nponent 26	5449	433.1351				2 10.011	19,348,	535		
Con	nponent 27	5461	361.0903				2 10.028	14,749,	535		
Con	nponent 28	5508	587.1601				2 10.110	4,866,	266		
_Con	nponent 29	5545	293.1003				2 10.169	7,235,	735		
Con	nnonont 20	FCAF	100 2602				2 10 202	17 200	205		*
								Nur	nber	of scans: 1579	92
								Number	of c	omponents: 17	73

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.

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.

Delete components in the components list

L ave Delete Copy Sel	ect		N N N N N N N N N N N N N N N N N N N	X X		TD FIS	n Comp	oonents mzLogic arch	🏹 Compone 止 Spectrum
File 🗙 Selection			Acti	ons				Search	Send to
Chi X Delete se	lected com	ponents and	annotatio	ons					
Vame All Structura	Scall	Precursor	Match	Mato	h Name	MS ⁿ 🔻	t _R (min)	Abundance	Annotatic
Component 10	16/18	393.0351				2	3.621	14.543.213	-
-Component 9	1567	261.09495				2	3.488	5,465,919	
Component 8	1357	272.11336				2	3.136	4,578,883	
Component 7	732	360.1512				2	2.110	24,412,500	
Component 6	698	277.08996				2	2.047	12,974,755	
Component 5	685	370.1716				2	2.036	5,709,300	
Component 4	672	455.1174				2	2.000	11,602,216	
Component 3	623	311.1000				2	1.917	20,603,470	
Component 2	586	266.1239				2	1.870	13,551,973	
Component 1	500	290.8481				2	1.719	33,928,815	
Component 170	14617	425.3786				1	26.620	23,103,927	
Component 105	11822	441.2468				1	21.703	15,259,640	
Component 39	6707	887,2846				1	12.243	104,986,728	0

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
------------	------------	----------	---------------	-------

vame	▲ Scan	Precursor	Match	Match Name	MS ⁿ	t _R (min)	Abundance	Annotatio
 Components 								
Component 21	4623	344.1350			3	8.566	76,919,099	
Component 22	4706	467.1900			3	8.712	9,009,480	
-Component 34	6124	903.2799			3	11.203	19,920,938	
—Component 35	6267	903.2796			3	11.445	6,514,965	
-Component 38	6696	906.2584			3	12.232	90,093,088	
Component 42	6844	887.2846			3	12.486	47,852,138	
-Component 44	7024	586.2515			3	12.805	14,812,713	
-Component 45	7217	757.2212			3	13.144	15,882,835	
—Component 48	7362	757.2212			3	13.404	90,451,840	
—Component 49	7386	547.2166			3	13.450	12,880,087	
-Component 50	7459	765.2601			3	13.571	47,574,473	
-Component 54	7678	538.2303			3	13.973	42,518,277	
-Component 55	7752	609.2384			3	14.093	5,712,702	
	8016	741.2265			3	14.577	214,747,203	
-Component 58					2	11006	115 601 060	

Go to the next topic, "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.



Identify

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

Component	Search		Ψ×
< × ≓	🗐 📰 Show Details 🥏		
Search Type	Identity	•	\$
Library	mzCloud Reference		•
	Search Selected	March All	
Star	by clicking button 'Search S	elected' or 'Search All'	
XIC NLC	Component Search		

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

Library	mzCloud Reference;mzCloud Autoprocessed	-
***	mzCloud Libraries ✓ Reference ✓ Autoprocessed	

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

Search Parame	ters			_ – ×
Search Type:	Identity		*	
Search Contair	ner			*
Used Stag Eibra Libra Libra Libra	es ry MS ² vs Component MS ² ry MS ² vs Component MS ⁿ ry MS ⁿ vs Component MS ² ry MS ⁿ vs Component MS ⁿ	Spectral Compare Type Identity • Component Match Condition Best Confidence Matc •		
Spectrum Con Collision E Relat	straints inergy ive Energy Tolerance 0 1 h Ion Activation Type	1		A
Compound Cla All	A.0 2			*
			Restore Defaults	OK Cancel

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

- 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.

	Δ 11/2. 4				and a second				
ie		Scan	Precursor	Ma ▼	Match Name	м •	t _R (min)	Abundance	Annotation Source
omponen	ts	1.100	520.0202	100		2	42.072	10 540 077	27. AND
Compor	ient 54	7678	538.2303	100	NP 004676	3	13.973	42,518,277	Identity
Compor	nent 113	12109	269.0813	99	Formononetin	3	22.239	17,545,607	Identity
Compor	ient 58	8016	741.2265	99	Kaempferol	5	14.577	214,747,203	Identity
_Compor	ient 66	8676	765 2601	94	25 3R 45 55 6R 2 4	3	13.832	13,892,884	Identity
Compor	ient 30	7459	244 1250	00	25 5K 45 55 0K 2 4	2	0.566	47,574,475	Identity
Compor	ient 21	4623	600 2502	75	Eicocatatraynaica	2	24 272	22 247 202	Identity
Compor	opt 106	13224	737 3806	73	NIP 004031	3	24.272	62 531 116	Identity
Compor	ont 116	11835	689.4260	74	NP 004031	3	22.7.50	129 635 518	Identity
Compor	ont 76	0220	595 1679	70	3 25 38 45 58 68	3	16.974	51 604 575	Identity
Compor	ent 48	9238	757 2212	70	3 25 3R 45 5R 6R	3	13,404	90,451,840	Identity
Compor	opt 45	7302	757 2212	70	3 25 38 45 58 68	3	13 1//	15 882 835	Identity
Compor	ont 124	1217	553 2007	68	5 5 mothow/carbo	3	23 514	12,002,033	Identity
Compor	ont 121	12/00	635 /173	67	15 45 5R 105 135	3	23.146	244 369 284	Identity
Compor	ent 60	0247	611 1629	66	Rutin	3	15 019	128 945 262	Identity
Compor	ent 61	0247	538 2301	64	4 4 4 Hydroxy 3 m	3	15.013	21 369 571	Identity
Compor	nent 90	10007	461 1454	54	ethyl 2 6 oxo 6H	3	18 472	70 930 167	Identity
Compor	ent 35	6267	903 2796	49	7 25 3R 45 55 6R	3	11 445	6 514 965	Identity
Compor	nent 74	0207	903 2590	48	25 3R 45 55 6R 2	3	16 728	31 003 526	Identity
Compor	ent 81	9105	903 2584	47	25 3R 45 55 6R 2	3	17 643	5 404 475	Identity
Compor	ent 34	6124	903 2799	47	7 25 3R 45 55 6R	3	11 203	19 920 938	Identity
Compor	nent 59	81/15	741,2268	44	NP 003251	3	14.806	115.681.862	Identity
Compor	nent 111	12072	617,4065	37	NP 004031	3	22,176	20,457,983	Identity
Compor	ent 108	12072	651,4121	27	25 3R 45 55 6R 3	3	22.083	9.072.221	Identity
-Compor	nent 85	9855	475.1246	10	9 methoxy 7 4 25	3	18.029	7.359.706	Identity
Compor	nent 71	8875	595,1678	10	2 3 4 Dihydroxyph	3	16,181	29.975.753	Identity
Compor	nent 159	13976	607.2936	9	Resiniferatoxin	3	25.535	243,577,396	Identity
Compor	nent 138	13295	537.3048	8	3beta 9xi 3 beta D	3	24.400	16,159,460	Identity
Compor	nent 154	13761	953.5832	0		3	25.177	17,367,014	Identity
Compor	nent 128	12945	723.3950	0		3	23.802	11,775,187	Identity
Compor	nent 123	12715	601.4117	0		3	23.379	32,935,301	Identity
Compor	nent 120	12547	583.4009	0		3	23.061	13,338,348	Identity
Compor	nent 110	12072	657.3992	0		3	22.177	25,927,720	Identity
Compor	nent 107	11937	633.4015	0		3	21.919	6,309,785	Identity
_Compor	nent 102	11660	615.3909	0		3	21.398	8,508,193	Identity
Compor	nent 97	11002	491.1560	0		3	20.152	10,727,385	Identity
Compor	nent 96	10956	579.1726	0		3	20.057	4,621,283	Identity
Compor	nent 93	10492	887.2633	0		3	19.205	104,228,877	Identity
Compor	nent 87	9918	887.2632	0		3	18.151	84,153,472	Identity
Compor	nent 82	9668	764.3150	0		3	17.683	11,829,093	Identity
Compor	nent 79	9403	1033.3219	0		3	17.190	57,324,781	Identity
Compor	nent 78	9283	887.2636	0		3	16.960	89,386,478	Identity
Compor	nent 73	9104	933.2697	0		3	16.627	5,227,026	Identity
Compor	nent 70	8804	551.1436	0		3	16.072	10,624,650	Identity
Compor	nent 69	8793	473.2371	0		3	16.050	10,372,061	Identity
Compor	nent 68	8758	543.1854	0		3	15.977	5,495,247	Identity
Compor	nent 64	8579	1033.3216	0		3	15.641	82,656,765	Identity
Compor	nent 62	8381	544.1358	0		3	15.258	10,825,712	Identity
Compor	nent 55	7752	609.2384	0		3	14.093	5,712,702	Identity
Compor	nent 49	7386	547.2166	0		3	13.450	12,880,087	Identity
-Compor	nent 44	7024	586.2515	0		3	12.805	14,812,713	Identity
-Compor	nent 42	6844	887.2846	0		3	12.486	47,852,138	Identity
Compor	nent 38	6696	906.2584	0		3	12.232	90,093,088	Identity
Compor	nent 22	4706	467.1900	0		3	8.712	9,009,480	Identity
IS1 Scans									
roduct Sca	ans								

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

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.

2	🗋 🕶 🗰 (Sea	irch		Mass Frontier 8.1 Trial [Chromatogram Proc	essor - plant sample.raw [Modified]]	₹_ ₽ ×
MB S	itart Home	Modules &	Tools Sea	arch				🛁 online 👻 🖪 🕶
	Chromatogram Pr	ocessor - plar	nt sample.raw	Modified] ×			- ×
	5 Δ m/z:	4.9	mmu			TIC 2D Contour 3D Info Filter:	All -	Component Search 📮 🗙
Nan	ne la	Scan	Precursor	Ma 🔻	Match Na	600,000,000		🖌 🗙 I 🚞 I 🗮 Show Details 🥏
- (omponents				÷.	1		Search Type Identity 👻 🔅
	-Component 54	7678	538.2303	100	NP 0046		Y II	Library mzCloud Reference mzCloud Autoprocessed
	-Component 11	3 12109	269.0813	99	Formonc	400,000,000-		cloury mzcloud kelelence, mzcloud Autoprocessed
	-Component 58	8016	741.2265	99	Kaempfe		▼	🥻 Search Selected 🛛 👬 Search All
	-Component 66	8676	598.2516	94	2S 3R 4S			
	-Component 50	7459	765.2601	88	2S 3R 4S	200.000.000		Matches for Component 76 using Identity profile
	-Component 21	4623	344.1350	83	2E 3 4 2: U			D: 1433 mzCloud Autoprocessed
	Component 13	> 13224	727 2006	75	ElCOSatet		M ₩ M M X M X M M M M M M M M M M M M M	1 3-{[(2S,3R,4S,5R,6R)-3,5-dihydro
	Component 116	11835	689,4260	74	NP 0040			
	Component 76	9 12427	595.1679	70	3 25 3R	0 3 5 8 10	13 15 18 20 23 25 28 30	C ₂₇ H ₃₀ O ₁₅ MM: 594.1: Confidence: 70.2
	Component 48	7362	757.2212	70	3 2S 3R			E 🖸 Thermo NSI MS ² 70.2
	Component 45	7217	757.2212	70	3 2S 3R	□ 및 ④ 몸	Data Into FISh	D: 6626 mzCloud Autoprocessed
	-Component 124	12786	553.2997	68	5 5 meth		Spectrum Spectra Compare	2 7-(((2S.3R.4S.5S.6R)-4.5-dihydro
	-Component 12	1 12591	635.4173	67	1S 4S 5R		tR: 16.8472 • Scan No 🌇 📸 🦳	- ····
	-Component 60	8247	611.1629	66	Rutin		s	C ₂₇ H ₃₀ O ₁₅ MM: 594.1! Confidence: 62.4
	-Component 61	8305	538.2301	64	4 4 4 Hyc	1.07 1.0 200 0.00	S	D: 6115 mzCloud Autoprocessed
	-Component 90	10097	461.1454	54	ethyl 2 6			3 MILLE NP-003191
	-Component 35	6267	903.2796	49	7 2S 3R 🖕		e en c	
• =					+		in the second se	C ₂₇ H ₃₀ O ₁₅ MM: 594.1! Confidence: 62.1
			Nun	nber of so	ans: 15792		2	CY ID: 7227 msCloud Autonrococcod
			Numbe	r of comp	oonents: 54		June 1	Spectral Trees: 8, Compounds: 8
	u . 🛪 🗸 📾	× 1 A 4					² 3	
		* I T Y				1/2 FT CID 35 NCE MS2 617.15 Sca	tR: 3.7669 • Scan No: 277-279 • FT	XIC NLC Component Search
t _R : 16	.8472 • Scan No.:	9227 • FTMS	+ p ESI d Fu	ll ms2 61	7.1496@cid35	.00 [164.0000-628.0000]		

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.





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.

Component	Search	平	×
< × (=	🛛 📰 Show Details 🔶		
Search Type	Tree Search	- 🌣	

2. To review the settings for a Tree search, click the settings icon, $\overset{\circ}{\mathbf{x}}$.

Figure 20 shows the default settings for a Tree search.

Figure 20. Default settings for a Tree search

Search Type:	Tree Search	-	
Search Contai	ner		*
Used Stag Libra Libra Libra O Libra	ry MS ² vs Component MS ² ry MS ² vs Component MS ⁿ ry MS ⁿ vs Component MS ² ry MS ⁿ vs Component MS ⁿ	Spectral Compare Type Identity Component Match Condition Aggregated Tree Mat	
Spectrum Con Collision I Relat Mato Tolerance	straints Energy ive Energy Tolerance th Ion Activation Type Factor 4.0	:	*
Compound Cl	asses		
		Deste	ara Dafaulti OK Cancel

 B_{y}^{i} default, a Tree search is not constrained by the ion activation type.

- 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)
------------	-------------	----------------	----------	--------------

톨 🖄 Δ m/z:	4.9 🗘 m	imu						
ie	Scan No.	Precursor m/z	Match	▼ Match Name MS	sn 👻	t _R (min)	Abundance	Annotation Sources
omponents								
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 m	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 phei	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 E	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
IS1 Scans								
roduct Scans								

- 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



~)	🗙 I 🚞 I 📃 SI	how Details ,					
Searc	h Type Tree Se	arch	- 🌣				
Libra	ry mzClou	d Reference;mzClo	oud Autoprocessed				
	🕌 Searc	h Selected	🕌 Search All				
Matc	hes for Compor	ent 76 using Tree !	Search profile				
	-1-1-1-	ID: 1433	mzCloud Autoprocessed				
1	and the	3-{[(2S,3R,4S,5R,	.6R)-3,5-dihydroxy-6-(hydr				
	C ₂₇ H ₃₀ O ₁₅	MM: 594.1585	Tree Match: 82.7				
	🗉 😳 Thern	no NSI	MS ² ;M 82.7				
	Tr. I.	ID: 7327	mzCloud Autoprocessed				
2	dit.	5,7-Dihydroxy-2	5,7-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo				
	C ₂₇ H ₃₀ O ₁₅	MM: 594.1585	Tree Match: 81.2				
	- <u>I</u>	ID: 6626	mzCloud Autoprocessed				
3	1000	7-{[(2S,3R,4S,5S,	6R)-4,5-dihydroxy-6-(hydr				
	C27H30O15	MM: 594.1585	Tree Match: 78.8				
	-i-l-	ID: 6115	mzCloud Autoprocessed				
4	in a	NP-003191					
	C27H30O15	MM: 594.1585	Tree Match: 77.5				
		ID: 7377	mzCloud Autoprocessed				
5 55500		2-(3,4-Dihydrox	yphenyl)-5-hydroxy-4-oxo				
	6 11 0	MM: 594.1585	Tree Match: 23.4				





- 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, 💆 .

Compone	nt Search		Ψ×
\$	📰 🛛 📰 Show Details 🦑		
Se Accep	ot all suggested structures	•	₽
Library	mzCloud Reference;mz	Cloud Autoprocessed	•
	A Search Selected	M Search All	

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

[[]] Δ m/z:	4.9 🗘 m	mu									
	Scan No.	Precursor m/z	Match 🔹	Match Name	MS ⁿ	•	t _R (min)	Abundance	Annotation	Sources	
nponents						_				-	
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	1.1	
Component 66	8676	598.2516	94	2S 3R 4S 5S 6R 2		3	15.832	13,892,884	Tree Search	1	
Component 50	7459	765.2601	88	25 3R 45 55 6R 2		3	13.571	47,574,473	Tree Search		
Component 76	9238	595.1679	83	3 25 3R 45 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 6		3	13.144	15,882,835	Tree Search		
Component 60	8247	611.1629	81	Rutin		3	15.019	128,945,262	Tree Search	1.1	т
Component 35	6267	903.2796	76	7 25 3R 45 55 6F		3	11.445	6,514,965	Tree Search	1	Iree s
Component 135	13224	699.3582	75	Eicosatetraynoic		3	24.272	22,247,382	Tree Search	1	accen
Component 106	11835	737.3806	74	NP 004031		3	21.736	62,531,116	Tree Search	1	00000
Component /4	9165	903.2590	74	2S 3R 4S 5S 6R 2		3	16.728	31,003,526	Tree Search		annot
Component 116	12427	689.4260	71	NP 004031		3	22.845	129,635,518	Tree Search		
Component 81	9642	903.2584	69	25 3R 45 55 6R 2		3	17.643	5,404,475	Iree Search		
Component 124	12786	553.2997	68	5 5 methoxycarb		3	23.514	42,817,912	Tree Search	1.1	
Component 121	12591	635.4173	67	15 45 5R 105 135		3	23.146	244,369,284	Tree Search	1	
Component 61	8305	538.2301	64	4 4 4 Hydroxy 3	_	3	15.112	21,369,571	Tree Search		
Component 59	8145	/41.2268	59	5 / Dihydroxy 2 4		3	14.806	115,681,862	Tree Search		
Component 90	10097	461.1454	55	N 1 methyl 3 pher	_	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	1	0
Component 111	12072	617.4065	37	NP 004031		3	22.176	20,457,983	Tree Search		Compon
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	25 3R 45 55 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	Resiniteratoxin		3	25.535	243,577,396	Tree Search		
Component 154	13761	953.5832	9	Irgatos 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 L		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	0,309,785	Iree Search		
Component 102	11660	615.3909	0			3	21.398	8,508,193	Iree Search		
Component 97	11002	491.1500	0			2	20.152	10,727,385	Iree Search		
Component 96	10956	007.0622	0			3	20.057	4,021,283	Iree Search		
Component 93	10492	007.2033	0			2	19.205	04 152 472	Iree Search		
Component 87	9918	367.2032	0			2	17,000	64,155,472	Iree Search		
Component 82	9668	/04.3150	0			3	17.083	11,829,093	Iree Search		
Component 79	9403	1033.3219	0			3	17.190	57,324,781	Iree Search		
Component 78	9283	022.2030	0			2	10.900	69,386,478	Iree Search		
Component 75	9104	955.2097	0			2	16.072	3,227,020	Tree Search		
Component 70	8804	331.1430	0			2	16.072	10,024,030	Tree Search		
Component 69	8793	4/3.23/1	0			3	16.050	10,372,001	Iree Search		
Component 68	8/58	1022 2216	0			2	15.977	03,493,247	Iree Search		
Component 64	8579	1055.5210	0			2	15.041	02,030,703	Tree Search		
Component 62	8381	544.1358	0			3	13.238	10,825,712	Tree Search		
Component 55	//52	609.2384	0			2	12 450	12,000,007	Tree Search		
Component 49	7386	547.2166	0			3	13.450	12,880,087	Iree Search		
Component 44	7024	586.2515	U			3	12.805	14,812,/13	Iree Search		
Component 42	6844	887.2846	0			3	12.486	47,852,138	Iree Search		
Component 22	4706	467.1900	0			3	8./12	9,009,480	Tree Search		
1 Scans											
duct Scans											

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.

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

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

Search Paramete	ers			_ 0
Search Type:	Subtree Search		•	
Search Contain	ner			*
Used Stag Libra Libra Libra O Libra	ry MS ² vs Component MS ² ry MS ² vs Component MS ⁿ ry MS ⁿ vs Component MS ² ry MS ⁿ vs Component MS ⁿ	Spectral Compare Type Identity Component Match Condition Aggregated Sub-Tree	Tolerance Factor	
Spectrum Con Collision I Relat Matc	straints Energy ive Energy Tolerance			*
Compound Cla	asses			•
			Restore Defaults Ok	Cancel
		By default, a Subtree Sear	ch is not constrained	

by ion activation type.

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).

톨 []] Δ m/z: 4.9	📮 mmu									
e s	Scan No. P	recursor m/z	Match	Match Name	∕IS ⁿ ▼	t _R (min)	Abundance	Annotation Sources		
omponents										
-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 25 3R 45 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 6F	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	25 3R 45 55 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	15 45 5R 105 135	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 96	10956	579.1726	99	Biochanin A	3	20.057	4,621,283	Subtree Search		
Component 93	10492	887.2633	99	2S 3R 4S 5S 6R 2	3	19.205	104,228,877	Subtree Search		
Component 87	9918	887.2632	99	2S 3R 4S 5S 6R 2	3	18.151	84,153,472	Subtree Search		
Component 78	9283	887.2636	99	2S 3R 4S 5S 6R 2	3	16.960	89,386,478	Subtree Search	_	
Component 34	6124	903.2799	99	3 2S 3R 4S 5R 6R	3	11.203	19,920,938	Subtree Search		
Component 71	8875	595.1678	98	Robinin	3	16.181	29,975,753	Subtree Search		
Component 59	8145	741.2268	97	Robinin	3	14.806	115,681,862	Subtree Search	Comp	
Component 73	9104	933.2697	74	NP 003191	3	16.627	5,227,026	Subtree Search	Comb	001
Component 110	12072	657.3992	71	2S 3R 4R 5R 6S 2	3	22.177	25,927,720	Subtree Search		
Component 42	6844	887.2846	68	Robinin	3	12.486	47,852,138	Subtree Search		
Component 111	12072	617.4065	66	3beta 5xi 9xi 16alı	3	22.176	20,457,983	Subtree Search		
Component 70	8804	551.1436	61	5 7 Dihydroxy 2 4	3	16.072	10,624,650	Subtree Search		
Component 123	12715	601.4117	59	2S 3S 4S 5R 6R 6	3	23.379	32,935,301	Subtree Search		
Component 102	11660	615.3909	58	3 5 9 16 21 22 28	3	21.398	8,508,193	Subtree Search		
Component 120	12547	583.4009	56	2S 3S 4S 5R 6R 6	3	23.061	13,338,348	Subtree Search		
Component 90	10097	461.1454	55	NP 019984	3	18.472	70,930,167	Subtree Search		
Component 154	13761	953.5832	47	UR 144 N 2 hydro	3	25.177	17,367,014	Subtree Search		
Component 108	12023	651.4121	46	24E 12 15 Dihydro	3	22.083	9,072,221	Subtree Search		
Component 38	6696	906.2584	45	Kaempferol 3 Gala	3	12.232	90,093,088	Subtree Search		
Component 107	11937	633.4015	36	24E 12 15 Dihydrc	3	21.919	6,309,785	Subtree Search		
Component 62	8381	544.1358	36	2S 3R 4S 5S 6R 2	3	15.258	10,825,712	Subtree Search		
Component 85	9855	475.1246	34	5 Hydroxy 2 4 hyc	3	18.029	7,359,706	Subtree Search		
Component 22	4706	467.1900	28	Methyl 3 3 methy	3	8.712	9,009,480	Subtree Search		
Component 97	11002	491.1560	10	5 4 5 dihydroxy 6	3	20.152	10,727,385	Subtree Search		
Component 79	9403	1033.3219	10	Kaempferitrin	3	17.190	57,324,781	Subtree Search		
Component 64	8579	1033.3216	10	Kaempferitrin	3	15.641	82,656,765	Subtree Search		
Component 159	13976	607.2936	9	Resiniferatoxin	3	25.535	243,577,396	Subtree Search		
Component 138	13295	537.3048	8	3beta 9xi 3 beta E	3	24.400	16,159,460	Subtree Search		
Component 128	12945	723.3950	0		3	23.802	11,775,187	Subtree Search		
Component 82	9668	764.3150	0		3	17.683	11,829,093	Subtree Search		
Component 69	8793	473.2371	0		3	16.050	10,372,061	Subtree Search		
Component 68	8758	543.1854	0		3	15.977	5,495,247	Subtree Search		
Component 55	7752	609.2384	0		3	14.093	5,712,702	Subtree Search		
Component 49	7386	547.2166	0		3	13.450	12,880,087	Subtree Search		
Component 44	7024	586.2515	0		3	12.805	14,812,713	Subtree Search		
S1 Scans										
roduct Scans										

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

Search Type:	Identity Substructure		•	
Search Contai	ner			
Used Stag Libra Libra Libra O Libra	ry MS ² vs Component MS ² ry MS ² vs Component MS ⁿ ry MS ⁿ vs Component MS ² ry MS ⁿ vs Component MS ⁿ	Spectral Compare Type Identity • Component Match Condition Best Confidence Matc •		
Spectrum Cor Collision Rela Mate Tolerance	Instraints Energy ive Energy Tolerance the Ion Activation Type Factor 4.0 C]		
	asses			

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 result	S
riyure 28.	identity Substructure search result	2

	Chromatogram Proc	essor - plant sa	mple.raw [Modified	i] ×						
	🔄 🔂 Δ m/z: 4	1.9 🗘 mi	mu							
Nam	e	Scan No.	Precursor m/z	Match	 Match Name 	MS ⁿ	•	te (min)	Abundance	Annotation Sources
- C	omponents							- K (
	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	25 3R 45 55 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 6	il 👘	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 6	61	3	13.404	90,451,840	Tree Search
	Component 45	7217	757.2212	82	3 2S 3R 4S 5R 6	il i	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 6	F	3	11.445	6,514,965	Tree Search
	Component 135	13224	699.3582	75	Eicosatetraynoi	0	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 /4	9165	903.2590	74	25 3R 45 55 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	25 3K 45 55 6K	2	3	17.043	5,404,475	Tree Search
	Component 124	12/86	555.2997	67	15 AS ED 105 12	с с	2	23.314	42,017,912	Tree Search
	Component 121	12591	635.4173	67	15 45 5K 105 15	2	2	23.140	244,309,284	Tree Search
	Component 87	0010	887 2632	100	NP 003191		3	18 151	84 153 472	Iree Search
	Component 78	0202	887 2636	100	5.7 dibydroxy 2.4	0	3	16.960	89 386 478	Identity Substructure
	Component 96	9205	579 1726	99	Biochanin A		3	20.057	4 621 283	Identity Substructure
	Component 93	10/192	887.2633	99	5 7 dihydroxy 2 4	L	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 16a	lj -	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	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 hydr	0	3	25.177	17,367,014	Identity Substructure
	Component 108	12023	651.4121	46	24E 12 15 Dihydr	rc	3	22.083	9,072,221	Identity Substructure
	Component 38	6696	906.2584	45	Kaempferol 3 Ga	lć	3	12.232	90,093,088	Identity Substructure
	Component 107	11937	633.4015	36	24E 12 15 Dinydr	°C	3	21.919	6,309,785	Identity Substructure
	Component 62	8381	344.1338	30	25 3K 45 55 6K 2		3	10.208	10,825,712	Identity Substructure
	Component 22	9855	475.1246	25	Mothyl 3.3 mothy	C .	3	9 712	9,009,480	Identity Substructure
	Component 97	4/06	407.1900	10	5 4 5 dihydroxy	6	2	20 152	10 727 385	Identity Substructure
	-Component 79	0402	1033 3219	10	Kaempferitrin	~	2	17 190	57 324 781	Identity Substructure
	Component 64	9403	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	C	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
► M	IS1 Scans									
► Pr	roduct 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).

Search ____» Mass Frontier 8.1 Trial (Chromatogram Processor - plant sample.raw (Modified)) 주 _ 문 × MD Start Home Modules & Tools Search 🤐 online 🔹 📔 🔹 Chromatogram Processor - plant sample, raw [Modified] × 🧥 Search Details - Component 34 TIC 2D Contour 3D Info Filter: 🙀 All 📃 🔄 | 🔁 | Δ m/z: 4.9 🗘 mmu | 🗸 🗙 🖂 📄 Show Details 🤞 600,000,000 Scan... Precursor... Ma... - Match Na ÷ Search Type Identity Substructure 71 NP 0040 -Component 116 12427 689.4260 903.2584 25 3R 45 Library mzCloud Reference;mzCl Component 81 9642 69 00,000,00 Component 124 12786 553.2997 68 5 5 metł No. 10 Search Selected 🕌 Search All Component 121 635.4173 67 1S 4S 5F 12591 200,000,000 Component 61 8305 538.2301 64 444 Hv Matches for Component 34 using Identity Substructure profile Component 87 887.2632 100 NP 0031 9918 ID: 6365 mzCloud Autoprocessed Component 78 9283 887 2636 100 5.7 diby 887.2633 5 7 dihyc 20 Component 93 10492 99 25 NP-004596 Component 71 595 1678 99 NP 0076 8875 Spectrum Spectra Compare Data Info FISh MM: 758.19 Confidence: 99.4 ㅁ 및 🕀 몸 C32H38O21 Component 59 8145 741.2268 99 NP 0076 Component 34 6124 903.2799 99 NP 0045 tR: 11.2439 • Scan No: 6152 • FTMS **`** 🖻 🗉 😳 Thermo NSI MS³;... 99.4 ID: 2753 mzCloud Autoprocessed Component 96 10956 579.1726 97 5 7 dihvc 229.050 137.0234 Component 73 933.2697 74 NP 0031 Scan 9104 Component 110 12072 657.3992 71 2S 3R 4R l ddiadailau d Component 90 461,1454 71 NP 0160 10097 C₃₃H₄₀O₂₀ MM: 756.21 Confidence: 99.3 ID: 2693 mzCloud Autoprocessed Component 111 617 4065 66 3beta 5v 12072 551.1436 61 5 7 Dihyo Component 70 8804 303.0499 165.0181 229.0494 Component 123 601.4117 59 2S 3S 4S 12715 Differ (25,3R,4R,55,65)-2-{[2-(3,4-dihydr. 111.0075 075 201.0543 MM: 600.11 Confidence: 99.1 C₂₈H₂₄O₁₅ Number of scans: 15792 257.04370 D: 2700 mrCloud A ibrary 137.02303 Number of components: 54 Spectral Trees: 20, Compounds: 19 📂 💾 I 🌣 🗙 💽 🔻 I 🛧 🔹 XIC NLC Component Search < 1/1 FT CID 35 NCE -> tR: 14.4159 • Scan No: 1109-1111 • FTMS + c NSI. Joint Components Detection ie: 11.2439 • Scan No.: 6152 • FTMS + p ESI d Full ms3 903.2800@cid35.00 303.0508@cid35.00 [78.0000-314.0000]

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.





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).

🔙 🔁 Δ m/z:	4.9 🗘 m	mu								
ame	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	4	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	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 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		25 3R 45 55 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 6	i i	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 6	i i	3	13.404	90,451,840	Tree Search
-Component 45	7217	757.2212	82		3 2S 3R 4S 5R 6	i i	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 6	F	3	11.445	6,514,965	Tree Search
-Component 135	13224	699.3582	75		Eicosatetraynoi		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		25 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		25 3R 45 55 6R	2	3	17.643	5,404,475	Tree Search
Component 124	12786	553.2997	68		5 5 methoxycar	Ь	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		15 45 5R 105 13	5	3	23.146	244,369,284	Tree Search
-Component 111	12072	617.4065	66		3beta 5xi 9xi 16	a	3	22.176	20,457,983	Identity Substructure
Component 61	8305	538.2301	64		4 4 4 Hydroxy 3	i	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

Figure 33. Components annotated with Identity Substructure matches

- 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.

		nu j							
	Scan No.	Precursor m/z	Match	 Match Name 	MS ⁿ		t _R (min)	Abundance	Annotation Sourc
mponents		F30 3303	100	ND 004CTC		2	12 072	42 540 277	T
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	Kaempterol		3	14.577	214,747,203	Tree Search
Component 66	8676	598.2516	94	25 3R 45 55 6R	2	3	15.832	13,892,884	Tree Search
Component 50	7459	765.2601	00	25 3R 45 55 6R	2	3	13.371	47,574,473	Iree Search
Component 76	9238	595.1679	83	3 25 3R 45 5R 0	1	3	16.874	51,604,575	Tree Search
Component 21	4623	344.1350	83	2E 3 4 25 3R 45		3	000.8	76,919,099	Iree Search
Component 46	/362	757.2212	02	3 23 3R 43 5R		2	13.404	90,451,640	Iree Search
Component 45	/21/	131.2212	02	5 25 5K 45 5K (4	2	15.144	138.045.363	Iree Search
Component 60	8247	011.1029	76			3	11.445	128,945,262	Iree Search
Component 35	6267	905.2790	70	7 23 38 43 33 6		2	24.272	0,514,905	Iree Search
Component 155	13224	727 2000	75	Elcosatetraynol		2	24.272	22,247,302	Iree Search
Component 106	11835	/3/.3606	74	NP 004051		2	21.730	02,551,110	Iree Search
Component 74	9165	903.2590	74	25 3K 45 55 6K	2	3	10.728	31,003,520	Iree Search
Component 116	12427	003.4200		NF 004031		2	17 6 43	129,053,316 E 404 47E	Tree Search
Component 124	9642	505.2584	69	23 3K 43 35 6K	<u>-</u>	3	23 51/	3,404,475	Tree Search
Component 124	12786	555.2997	00	5 5 methoxycar		2	23.314	42,017,912	Iree Search
Component 121	12591	030.4173	6/	15 45 5K 105 1	-	3	23.140	244,309,284	Iree Search
Component 07	8305	007 2622	100	4 4 4 Hydroxy 3	10	2	10.112	21,509,371	Iree Search
Component 87	9918	007.2032	100	NP 003191		2	16.050	04,135,472	Identity Substructure
Component 78	9283	667.2030	100	5 / dinydroxy 2		2	20.057	09,500,470	Identity Substructure
Component 98	10956	007 3633	99	E 7 dibudress 7		2	10.2057	4,021,203	Identity Substructure
Component 71	10492	505 1670	99	5 7 ulliyuroxy 2	•	2	16 101	20 075 752	Identity Substructure
Component 71	8875	741 2260	99	NP 007676		2	14 906	115 601 063	Identity Substructure
Component 34	8145	002 2700	99	NP 00/070		2	11 202	10,000,002	Identity Substructure
Component 73	0124	033 2607	74	NP 003191		3	16.627	5 227 026	Identity Substructure
Component 110	9104	657 2002	74	25 3D 4D 5D 65	2	2	22 177	25 927 720	Identity Substructure
Component 90	10007	461 1454	71	NP 016028		3	18 472	70 930 167	Identity Substructure
Component 42	6044	887 2846	68	Robinin		3	12.486	47 852 138	Identity Substructure
Component 111	12072	617.4065	66	3beta 5vi 9vi 16	3	3	22 176	20 457 983	Identity Substructure
Component 70	0004	551 1436	61	5 7 Dibydroxy 2		3	16.072	10 624 650	Identity Substructure
Component 123	12715	601 4117	59	25 35 45 5R 6R 6		3	23 379	32 935 301	Identity Substructure
Component 102	12/13	615 3909	58	3 5 9 16 21 22 2	1	3	21 308	8 508 193	Identity Substructure
Component 120	12547	583.4009	56	25 35 45 5R 6R 6		3	23.061	13 338 348	Identity Substructure
Component 154	12347	953 5832	47	UR 144 N 2 hvdr	n	3	25 177	17 367 014	Identity Substructure
Component 108	12022	651 4121	46	24E 12 15 Dibyd	·	3	22 083	9.072.221	Identity Substructure
Component 38	6606	906 2584	45	Kaempferol 3 Ga	• 6	3	12 232	90.093.088	Identity Substructure
Component 107	11027	633.4015	36	24E 12 15 Dibyd	Y .	3	21 919	6 309 785	Identity Substructure
Component 62	8381	544,1358	36	25 3R 45 55 6R 2		3	15,258	10,825,712	Identity Substructure
Component 85	0955	475 1246	34	5 Hydroxy 2.4 by	c	3	18 029	7 359 706	Identity Substructure
Component 22	4706	467,1900	25	Methyl 3 3 meth	,	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	13205	537,3048	8	3beta 9xi 3 beta	C	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	9793	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	7004	586,2515	0			3	12.805	14.812 713	Identity Substructure
S1 Scans	1024	00012010	0						actuary substructure
oduct Scans									

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 MSⁿ 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 (MS² and MSⁿ) 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.

Image: MS 903.2800	
	Selected node

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

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.

mzLogic			ųх
🗸 🗙 8a - 1	Q •		
Spectral Library	mzCloud Refere	nce	•
Adducts	Pos. [M + H]*	• Neg. [M - H] •	
	🖑 Rank	More Options	
XIC NLC mzL	ogic		

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.

mzLogic		Ψ×
🗸 🗙 8a - 🕼	•	
Spectral Library	mzCloud Reference	•
Adducts	Pos. [M + H] ⁺ * Neg. [M - H] *	
Structure Database		-
Workspace	Gelect All) VebChem HMDB ChEBI ChemIDPlus MetaCyc Metabolika UNPD EPA STOFF-Ident Tox21 DrugBank T3DB FoodDb Phenol Kegg WebBook ChEMBL FDA ECMDB OK Cancel	Î
XIC NLC mzLog	ic	

- 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

mzLogic								- 6 >
🗸 🗙 i 82 - Q								
Spectral Library	mzCloud Reference	9						*
Adducts P	os. [M + H]* *	Neg. [M - H]**						
mat onic result t	for Component 34	1 and Procursor 903 27	00	👸 Rank	More Op	tions		
Candidates (9)	Similar Structure	es (top 5)						
#1 (76.7)	PubChem	PU PU PU PU PU PU PU PU PU PU PU PU PU P	bChem #3 (65.3)	PubChem	#4 (65.3) PubChem	PubChem	6 (60.8) PubChem	
#7 (60.8)	PubChem	#8 (57.0) Pub	Chem #9 (53.2)	PubChem				
XIC NLC Comp	onent Search mz	Logic						

- 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.

mzLogic	Ψ×
✓ × 82 - 62 -	
🔞 New Curator	
Sub/Structure Search	
💉 New SledgeHammer	
🦾 New Metabolika	
New Structure Editor	
Structure Grid 1	:

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).







	Scan	Precursor	Ma 👻	Match Name	MSP	to (min)	Abundance	Annot
mponents	Jocurt					r (mm)	- as an our rec	. windt
Component 54	7678	538.2303	100	NP 004676	3	13.973	42,518,277	Tree Sea
Component 113	12109	269.0813	99	Formononetin	3	22.239	17,545,607	Tree Sea
Component 58	8016	741.2265	99	Kaempferol	3	14.577	214,747,203	Tree Sea
Component 66	8676	598.2516	94	2S 3R 4S 5S 6R 2 4 1S 3aR 4S 6aR 4 4 hyd	3	15.832	13,892,884	Tree Sea
Component 50	7459	765.2601	88	2S 3R 4S 5S 6R 2 4 1R 3aR 4R 6aR 4 3 5 d	3	13.571	47,574,473	Tree Sea
Component 76	9238	595.1679	83	3 25 3R 4S 5R 6R 3 5 dihydroxy 6 hydroxy	3	16.874	51,604,575	Tree Sea
Component 21	4623	344.1350	83	2E 3 4 2S 3R 4S 5S 6R 3 4 5 trihydroxy 6 ł	3	8.566	76,919,099	Tree Sea
Component 48	7362	757.2212	82	3 25 3R 45 5R 6R 4 5 dihydroxy 3 2S 3R 4	3	13.404	90,451,840	Tree Sea
Component 45	7217	757.2212	82	3 2S 3R 4S 5R 6R 4 5 dihydroxy 3 2S 3R 4	3	13.144	15,882,835	Tree Sea
Component 60	8247	611.1629	81	Rutin	3	15.019	128,945,262	Tree Sea
Component 35	6267	903.2796	76	7 2S 3R 4S 5S 6R 4 5 dihydroxy 6 hydroxy	3	11.445	6,514,965	Tree Sea
Component 135	13224	699.3582	75	Eicosatetraynoic acid	3	24.272	22,247,382	Tree Sea
Component 106	11835	737.3806	74	NP 004031	3	21.736	62,531,116	Tree Sea
Component 74	9165	903.2590	74	25 3R 45 55 6R 2 3 2S 3R 4S 5S 6R 4 5 dil	3	16.728	31,003,526	Tree Sea
Component 116	12427	689.4260	71	NP 004031	3	22.845	129,635,518	Tree Sea
Component 81	9642	903.2584	69	25 3R 4S 5S 6R 2 3 2S 3R 4S 5S 6R 4 5 dil	3	17.643	5,404,475	Tree Sea
Component 124	12786	553.2997	68	5 5 methoxycarbonyl 5 8a dimethyl 2 met	3	23.514	42,817,912	Tree Sea
Component 121	12591	635.4173	67	15 45 5R 10S 13S 17S 19S 20R 10 hydroxy	3	23.146	244,369,284	Tree Sea
Component 61	8305	538.2301	64	4 4 4 Hydroxy 3 methoxyphenyl tetrahydr	3	15.112	21,369,571	Tree Sea
Component 34	6124	903.2799	87	CGBWBDPNSXERKW-YHGJYWGMSA-N	3	11.203	19,920,938	mzLogic
Component 87	9918	887.2632	100	NP 003191	3	18.151	84,153,472	Identity 5
Component 78	9283	887.2636	100	5 7 dihydroxy 2 4 hydroxyphenyl 3 2S 3R	3	16.960	89,386,478	Identity 5
Component 93	10492	887.2633	99	5 7 dihydroxy 2 4 hydroxyphenyl 3 2S 3R	3	19.205	104,228,877	Identity 5
Component 71	8875	595.1678	99	NP 007676	3	16.181	29,975,753	Identity !
Component 59	8145	741.2268	99	NP 007676	3	14.806	115,681,862	Identity 5
Component 96	10956	579.1726	97	5 7 dihydroxy 3 4 methoxyphenyl 4H chrc	3	20.057	4,621,283	Identity !
Component 73	9104	933.2697	74	NP 003191	3	16.627	5,227,026	Identity 5
Component 110	12072	657.3992	71	2S 3R 4R 5R 6S 2 2R 3R 4S 5R 6R 4 2S 3R	3	22.177	25,927,720	Identity !
Component 90	10097	461.1454	71	NP 016028	3	18.472	70,930,167	Identity 5
Component 111	12072	617.4065	66	3beta 5xi 9xi 16alpha 18xi 22alpha 3 beta	3	22.176	20,457,983	Identity 5
Component 70	8804	551.1436	61	5 7 Dihydroxy 2 4 hydroxyphenyl 4 oxo 4ł	3	16.072	10,624,650	Identity !
Component 123	12715	601.4117	59	25 35 45 5R 6R 6 35 6aR 6bS 8aR 9R 12aS 1	3	23.379	32,935,301	Identity 5
Component 102	11660	615.3909	58	2E 6S 7 hydroxy 2 methyl 6 2S 11S 14S 15S .	3	21.398	8,508,193	Identity !
Component 120	12547	583.4009	56	25 35 45 5R 6R 6 35 6aR 6bS 8aR 9R 12aS 1-	3	23.061	13,338,348	Identity !
Component 108	12023	651.4121	46	24E 12 15 Dihydroxy 3 pentopyranosyloxy 9	3	22.083	9,072,221	Identity !
Component 38	6696	906.2584	45	Kaempterol 3 Galactoside 6 Rhamnoside 3	3	12.232	90,093,088	Identity :
Component 42	6844	632,4015	43	Kaempterol 3 Galactoside 6 Knamnoside 3	2	12,480	47,852,138	Identity !
Component 107	11937	635.4015	20	24E 12 15 Dinyuroxy 5 periopyranosyloxy 9	2	15 359	10,005,703	Identity :
Component 95	8381	475 1246	33	NP 002943	2	18.029	7 359 706	Identity !
Component 07	11002	491 1560	10	5 4 5 dihydroxy 6 hydroxymethyl 3 3 4 5 tril	3	20 152	10 727 285	Identity !
Component 79	0402	1033 3219	10	Kaempferitrin	2	17 190	57 324 781	Identity :
Component 64	9403	1033 3216	10	Kaemoferitrin	3	15.641	82 656 765	Identity !
Component 22	4706	467 1900	9	7' hydroxy 6' methoxy 2' methyl 3' 4' 6 8 tetr	2	8 712	9 009 480	Identity :
Component 159	13976	607,2936	8	7 15 bis 3 4 dimethoxyphenyl 19 ova 4 18 di	3	25.535	243.577 396	Identity :
Component 138	13205	537 3048	8	3beta 9xi 3 beta D Glucopyranosylovy 14 by	3	24 400	16 159 460	Identity :
Component 154	13761	953,5832	0		3	25.177	17.367.014	Identity :
Component 128	120/5	723 3950	0		3	23.802	11,775 187	Identity :
Component 82	9669	764.3150	0		3	17.683	11.829.093	Identity :
Component 69	9000	473,2371	0		2	16.050	10.372.061	Identity :
Component 68	8750	543,1854	ő		3	15.977	5,495 247	Identity 9
Component 55	7753	609,2384	0		3	14.093	5,712,702	Identity :
Component 49	7396	547,2166	0		3	13,450	12.880 087	Identity :
Component 44	7024	586,2515	0		3	12.805	14.812.713	Identity :
1 Scans	1024	5002010			5	12.000	14,012,715	identity :
aluat Canaa								

Save the analysis results to an HCCX file	You can save the component detection and component annotation 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.					
Trademarks	Mass Frontier, MolGate, mzCloud, and mzLogic are trademarks of Thermo Fisher Scientific Inc. in the United States.					
	Compound Discoverer, ID-X, Orbitrap, and Xcalibur are registered trademarks of Thermo Fisher Scientific Inc. in the United States.					
	All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.					