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

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

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

Contents

- Overview
- 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 the components list
- Identify components by searching a mass spectral library
- 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 acquired on an LXQ[™] mass spectrometer (MS).

In this tutorial, you perform the following tasks:

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

thermoscientific

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

Demo data files

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

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

Check the connection to the mzCloud server

* To check the connection to the mzCloud server

- 4 F
- 1. Open the Mass Frontier application by double-clicking its desktop icon, ^{IIII}, or by choosing **Thermo Mass Frontier 8.1** > **Mass Frontier 8.1** from the Windows[™] Start menu.

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

Figure 1. Mass Frontier startup window

Inter Index Contract Contrac	9 () • »	Search	Mass Frontiar 8.1 Trial (Emoty)		TAX
Image: Current/ogram Image: Current/ogram Image: Current/ogram Image: Current/og	MG Start Home Modules & Tools 5	Search	mass from er o. f martempty		🥥 online 👻 🖪 🔹
Open Chomatogram Processor Curator Curator Curator Data Manager Metabolika	Chromatogram Curator Processor Manager	Structure Structure Batch Fragment SledgeHammer Editor Grid Generation	CH Li ¹² C O Formula Isotope MolGate Generator Pattern Search	on Mechanism Overview	
Open Image: Chromatogram Processor Curator Files Curator Files Data Manager Database Files Participation Participation <th>Mo</th> <th>dules 🔺</th> <th>Tools</th> <th>k</th> <th></th>	Mo	dules 🔺	Tools	k	
Chromatogram Processor Recent Data Files (C.MS) Corator Corator Files Data Manager Metabolika Metabolika Metabolika Metabolika Corator Gires Structure Files	Open				
Curator Curator Files Data Manager Database Files Fragments & Mechanisms Fragments & Mechanisms Metabolika Metabolika Editable collection of structures Editable collection of structures Generate fragments for a set of stru New Chromatogram Processor Curator Curator Data Manager Data Manager Data Manager Metabolika Metabolika	Chromatogram Processor Data Files (LC/MS)	Recent		Quick search	XIII Size: Large
Data Manager Data Manager Second Sec	Curator Curator Files				
Fragments & Mechanisms Programsts & Mechanisms Metabolika Metabolika Structure Gildor Structure Gildor Structure Gildor Structure Gildor Generate fragments of structures Generate fragments for a set of structures Chromatogram Processor Curator Curator Data Manager Data Manager Metabolika Metabolika Metabolika Metabolika Metabolika Metabolika Metabolika Metabolika	Data Manager Database Files				
Metabolika Metabolika Structure Editor Constation of structures Base Chromatogram Processor Convator Curator Data Manager Data Manager Metabolika Metabolika Metabolika Metabolika	Fragments & Mechanisms Fragments & Mechanisms				
Structure Editor Structure Files Structure Grid Structure Grid Batch Fragment Generation Generate fragments for a set of stru New Image: Chromatogram Processor Chromatogram Processor Curator Curator Data Manager Data Manager Metabolika Metabolika Metabolika Metabolika	Metabolika Metabolika				
Structure Grid Structure Grid Batch Fragment Generation Generate fragments for a set of stru New Image: Chromatogram Processor Chromatogram Processor Curator Curator Data Manager Data Manager Metabolika Metabolika Metabolika	Structure Editor Structure Files				
Batch Fragment Generation Generate fragments for a set of stru New Image: Chromatogram Processor Chromatogram Processor Curator Data Manager Data Manager Metabolika Metabolika Sucurur Editor	Structure Grid Editable collection of structures				
New Chromatogram Processor Chromatogram Processor Curator Data Manager Data Manager Metabolika Metabolika Sucture Editor	Batch Fragment Generation Generate fragments for a set of stru				
Chromatogram Processor Curator Curator Data Manager Data Manager Data Manager Metabolika Metabolika	New				
Curator Curator Data Manager Data Manager Data Manager Metabolika Metabolika	Chromatogram Processor Chromatogram Processor				
Data Manager Data Manager Metabolika Metabolika	Curator Curator				
Metabolika Metabolika Sucture Editor	Data Manager Data Manager				
Structure Editor	Metabolika Metabolika				
Structure Editor	Structure Editor Structure Editor				
Structure Grid	Structure Grid Structure Grid				
Satch Fragment Generation	Batch Fragment Generation Batch Fragment Generation				
SledgeHammer	SledgeHammer SledgeHammer				
Global Settings	Global Settings				
About	About	1			
Show this Window Next Time	Show this Window Next Time				

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

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

Application tab bar Status of the mzCloud Web site Search Mass Frontier 8.1 Trial [Empty] 7_ **X** ** 🤐 online 👻 📔 🗸 MB Start Home Modules & Tools Search New Document: Nev Chromatogram Processor Curato Data Manager Metabolika Open Import Ĵ,o հե Recent Chromatogram Metabolika Curato Data Manage Processor Save Structure Editor Structure Grid Batch Fragment Generatio SledgeHammer Global Settings Connection Che \mathbf{X} $\overline{\mathbf{N}}$ 仌 Help Batch Fragment Structure Editor Structure Grid SledgeHamme About Exit From the Start menu choose Connection Check

Start menu

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

The application verifies the connection.

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

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

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

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

Open and browse an data file

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

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

Follow these topics in order:

1. Open a raw data file for processing

To open the example raw data file

2. View information about the raw data file

Open a raw data file for processing

1. In the Modules & Tools toolbar, click Chromatogram Processor (Figure 3).

Start menu and application tab bar Figure 2.

example raw

Figure 3. Modules & Tools toolbar

MB Start Home	Modules	& Tools	Search						🇀 or	nline -
Chromatogram Processor	or Data Manager	Metabolika	Structure Editor	Structure Grid	Batch Fragment Generation	Fragments & Mechanisms	C H N O Formula Generator	Isotope Pattern	mzLogic Search	Periodic Table
43		Mo	dules					Too	ls	-

The Open Chromatogram dialog box opens.

2. Browse to the following folder, select Flavonoid_Stds_LXQ.raw, and click Open.

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

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

• The chromatogram data view at the upper left lists the scan data by scan stage and number.

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

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

Note To change the scale from absolute counts to relative intensity (versus the base mass spectrum peak), right-click the view and choose **Show Absolute Intensities**.

- The MS spectrum view (Spectrum Viewer) at the lower right displays the first scan in the raw data file.
- The command processor view at the lower left is empty, as you have not yet applied any actions to the chromatogram.



Figure 4. TIC chromatogram and scan number 1 for the selected raw data file

Note Large data files can take a significant time to load. The status bar at the bottom of the application window provides information about the loading progress, from reading the scan data to building the scan tree.

Tip To show or hide the views on a Chromatogram Processor page, click the following icons in the View group of the Chromatogram Processor toolbar:

- For the MS spectrum view, click the **Show MS Spectrum** icon, <u>L</u>.
- For the chromatogram data view, click the **Show Chromatogram Data** icon, \boxminus .
- For the command processor view, click the Show Command Processor icon, 🋸.

You cannot hide the chromatogram view.

View information about the raw data file

- To view information about the acquisition of a raw data file
- 1. Open the Flavonoid_Stds_LXQ.raw data file as described in the previous topic, "Open a raw data file for processing."
- 2. In the chromatogram view, click the Info tab.

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

TIC	2D Contour	3D	Info	Filter:	n‡z	All -
RAW fi	ile				Τ	C:\Users\Public\Documents\HighChem\Mass Frontier 8.1\Demo Data\Chromatograms\Flavonoid_Stds_LXQ.RAW
RAW fi	ile version					62
Creatio	on date					11/20/2007 5:31:01 AM
Modifie	ed date					11/20/2007 6:38:32 AM
Who cr	reated					LXQ
Who m	nodified					LXQ
Numbe	er of calibratio	ns				0
Numbe	er of time mod	ified				1
Numbe	er of instrumer	nts				3
Revisio	n					62
Instrum	nent model					LXQ
Instrum	nent name					LXQ
Serial n	number					LXQ10171
Softwa	re version					2.2
Units						None
Expecte	ed runTime					67
Filter m	nass precision					2
In Acqu	uisition					0
Comme	ent1					
Comme	ent2					
Max in	tensity					0
Max in	tegrated inten	sity				1440386.5
Tolerar	nce Unit					amu
Trailer	Extra Count					13416
Trailer	Extra Event Co	unt				13416
Tune d	lata Count					1
User La	abel					System.String[]
Mass re	esolution					0.500
Numbe	er of scans					13416
Scan ra	ange					1 - 13416
Time ra	ange				_	0.01 - 67.00
Mass ra	ange				_	50.0000 - 1080.0000
Device	Туре				_	MS
Instrum	nent Index				_	1
Barcod	le				_	
Barcod	le Status				_	NotRead
Calibra	ition File				+	
Calibra	ition Level				+	
Comme	ent				_	
Dilution	n Factor				+	1
Injectio	on Volume	91.			+	3 CAM-allandar also also Malla Lin 2007, 44 2007, 44 42, Canada
Instrum	nent Method F	пе			+	c:\xcalibur\methods\Kelly_Lin\2007-11\2007-11-3-6.meth
Istd An	nount				+	
Path	cing Mathead 5	ilo			+	c. Accalibul (baca)
Process Daw Eil	sing Method F	lie			+	11.10 - DAW
Cample	e warne				+	۲۱-۱-۱-۱۰-۱۰-۱۰-۱۰-۱۰-۱۰-۱۰-۱۰-۱۰-۱۰-۱۰-۱
Licorte	e weigilt				+	v
Compl-	n id				+	1
Sample	e iu				+	i Linknown
Sample	e type o viol				+	
Sample	e viai	ime			+	2
Sample	a row number	me			+	1
Sample	e row number a dilution facto	r			+	1
Instrum	nent methods				+	' Surveyor I C Pump, Surveyor AS, I TO, Surveyor PDA Plus
Sample	e name				+	18 ml Vial 5 travs 40 vials each
Junple	e nume					no na na suays to Yulo cuch

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

Detect components in LC/MS data

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

* To detect the components in the example file

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

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

- 2. To force the mass accuracy to 500 mmu do the following:
 - a. In the Actions group of the Chromatogram Processor toolbar, click the Force Accuracy icon, M.



The Force Tolerance dialog box opens.

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

Force Tolerance	4	×
ی 💾 🛤		
Accuracy:		
Ion Trap Mass Analyzers		
500.0 🗘 mmu 👻		
Preview Restore Accept Cancel		
Calculate command and show result in the Chromatogram P	roce	essor

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

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



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

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



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

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

The Joint Components Detection dialog box opens.

b. Browse to the following folder:

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

c. Select Flavonoid_Stds_LXQ.chpro_jcd and click Open.

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

Deconvolution			
Wizard			
Average & Minimal Peak Width			
Automatic Manual: Average: Average:	50 🗘 Min: 2 🗘	scans	
Power of Baseline Correction			
<u>~~</u>			
Smoothing Power			
<u> </u>	<u></u> J		
Component Overlapping Intensity			Moving the
<u> </u>		<u>×</u>	the probab
Intensity of Detected Component			peaks as se
<u></u>			——Moving the
Tree Branching			the detecti
Retention Time Range		66.007	
	Lt	00.997	
m/z kange			
154 89201 [-1]	(+)	943	

Figure 6. Detection settings from the selected CHPRO_JCD file

Moving the slider to the left decreases the probability of detecting overlapping peaks as separate peaks. Moving the slider to the left decreases the detection of low abundance peaks.

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

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

- In the chromatogram data view, the Components list appears above the MS1 Scans list. The components are displayed in ascending order by retention time [t_R (min)]. The number of detected components appears at the bottom of the view.
- A spectral tree appears on the left of the MS spectrum view. The Spectrum page displays the combined spectrum for the MS1 scans across the selected component's chromatographic peak.
- 7. Check the number of detected components.

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

8. To accept the components, click Accept.

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

Figure 7. Integrated TIC chromatogram with marked components



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

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

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

- To sort the components in descending order by abundance
- 1. Right-click the Abundance column and choose **Sort Descending**. Or, click the **Abundance** column heading until the arrow to the right of the Abundance points down.

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

Figure 8. Components sorted by abundance

ame	Scan	Precursor	Match	Match Name	t _R (min)	MS ⁿ	Abu 🗸 🗸	Ann	otation Sources
Components								ź↓	Sort Ascending
Component 14	9858	271.12			49.538	3	668,802	Z↓	Sort Descending
Component 10	6993	255.13			35.558	5	435,410		Clear Sorting
—Component 4	2559	446.91			13.357	5	346,339		Show Column Choose
Component 5	4091	432.99			21.039	4	299,572		Deet Fit
Component 3	2274	416.99			11.795	5	274,537	TAT	Best Fit
Component 11	7374	285.12			37.425	5	175,220		Best Fit (all columns)
Component 12	7605	475.01			38.598	5	164,965	T	Filter Editor
Component 7	5878	488.95			29.970	5	149,283		Show Search Panel
Component 6	5597	459.01			28.486	5	131,330	-	
Component 9	6479	532.96			32.994	5	113,039		
									Number of scans: 134
								Nu	mber of components:

Abundance column

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

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

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

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

-or-

- To sort by multiple columns:
 - a. Sort the first column.
 - b. Press the **SHIFT** key then click on the next column to sort. Repeat this step to sort by another column.

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

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

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

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

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

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

Identify components by searching a mass spectral library

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

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

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

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

Run an Identity search

Run an Identity search to identify the detected components.

Follow these procedures in order:

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

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

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



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

Figure 9. Default settings for the Component Search view

Component :	Search	# ×	
 × ≓ 	🛭 🔳 Show Details 🦑		
Search Type	Identity	• 🔅	——— Opens the Search Parameter
Library	mzCloud Reference	•	dialog box
	🥌 Search Selected	Search All	
Star	t by clicking button 'Search Select	ed' or 'Search All'	

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

Component S	iearch	4 ;
< × (=	I 📃 Show Details 🥏	
Search Type	Identity	- 🌣
Library	mzCloud Reference	
	mzCloud Libraries Altoprocessed Local Libraries Flavonoids_2023_03_14_0559	
	😌 Refresh	

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

- 3. (Optional) To review the default settings for an Identity search, do the following:
 - a. Click the settings icon, 🔅.

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

Figure 10. Default settings for an Identity search

Search Parame	ters			_ □
Search Type:	Identity			
Search Contain	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 I	straints Energy ive Energy Tolerance 0	3		
Tolerance	Factor 4.0 🗘			
Compound Cl	asses			•
			Restore Defaults	K Cance

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 Components list by the **Match** column in descending order.

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

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



ne	Scan	Precursor	Ma •	Match Name	te (min)	MS ⁿ	Abundance	Annotation Sources
Components								
Componer 14	9858	271.12	99	Genistein	49.538	3	668,802	Identity
Component 11	7374	285.12	94	Glycitein	37.425	5	175,220	Identity
Component 10	6993	255.13	83	Daidzein	35.558	5	435,410	Identity
Component 5	4091	432.99	10	Apigetrin	21.039	4	299,572	Identity
-Component 4	2559	446.91	10	Glycitin	13.357	5	346,339	Identity
Component 13	8672	519.04	0		43.929	5	29,637	Identity
Component 12	7605	475.01	0		38.598	5	164,965	Identity
Component 9	6479	532.96	0		32.994	5	113,039	Identity
Component 8	6424	503.03	0		32.693	5	28,849	Identity
Component 7	5878	488.95	0		29.970	5	149,283	Identity
Component 6	5597	459.01	0		28.486	5	131,330	Identity
Component 3	2274	416.99	0		11.795	5	274,537	Identity
Component 2	369	359.89	0		1.859	3	20,610	Identity
Component 1	248	240.59	0		1.243	5	31,934	Identity
MS1 Scans Product Scans								

* To review the library hits for a component

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

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

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



	Com	ponent S	earch				_ 🗆 X			
	1	×	Sh	ow Details						
	Sear	ch Type	Identity				- 🌣			
	Libra	iry	mzCloue	d Reference			•			
		· .	1	anda Calantad		Conrol All				
			M 36	arch Selected		- Search An				
	Mato	Natches for Component 14 using Identity profile								
		H	5	ID: 24		mzClou	d Reference			
	1	5	ha.	Genistein						
		C15H10	05	MM: 270.0528		Confidence: 98	3.9			
Expands the list of			Therm	0	NSI	MS ²	98.9			
matching spectra		± 🗘	Therm	0	ESI	MS ²	97.6			
matering operation		± 🗘	UC Da	vis	ESI	MS ²	86.9			
			\square	ID: 20		mzClou	d Reference			
	2	- T		Apigenin						
		C15H10O5		MM: 270.0528		Confidence: 48.9				
				ID: 6197	mzCloud Reference		d Reference			
	3	"		Galangin						
	1	он		- MM: 270.0528		Confidence: 47	2			
	H	C ₁₅ H ₁₀	05	ID: 002		mzClou	d Roforonco			
		in and	YOY	10. 302		mzciou	u Reference			
	4	St.	DH	Pelargonidin						
		C15H11	D 5	MM: 271.0601		Confidence: 47	.1			
	5	Spectral T	rees: 7, (Compounds: 4						
	XIC	NLC C	Compon	ent Search						

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

Ho	ome alt List						
	ID: 24	in				mz	Cloud Reference
	C15H10O5 MM: 27	70.0528			Cor	fidence: 98.9	
	🗉 😋 Thermo			NSI	MS		98.
	Matching Library Sp	ectra					
	Precursor m/z	MSn	Pos.	Activation	Analyzer	Confidence 🔻	Match Factor
	271.0601	2		CID-45	FT	98.9	99.
	271.0601	2	4	CID-60	FT	98.4	99
	271.0601	2	4	CID-70	FT	95.0	98.
	271.0601	2		CID-35	FT	85.2	95.
	🗉 😳 Thermo			ESI	MS		97.
	Matching Library Sp	ectra					
	Precursor m/z	MSn	Pos.	Activation	Analyzer	Confidence 🔻	Match Factor
	271.0601	2	A	CID-40	FT	97.6	99.
	271.0601	2	Α.	CID-50	FT	96.1	99.
	271.0601	2	A	CID-45	FT	95.8	98.
	271.0601	2		CID-35	FT	70.3	86.
	🗉 😳 UC Davis			ESI	MS		86.
	Matching Library Sp	ectra					
	Precursor m/z	MSn	Pos.	Activation	Analyzer	Confidence 🔻	Match Factor
	271.0601	2	A	CID-35	FT	86.9	96.
4	C1sH10Os MM: 27	in 70.0528			Cor	fidence: 48.9	Cloud Reference
	ID: 619)7 in				mz	Cloud Reference
	C ₁₅ H ₁₀ O ₅ MM: 27	20.0528			Cor	ftidence: 47.2 mz	Cloud Reference
ŀ	Pelargo	onidin					
	C15H11O5 MM: 27	71.0601		-	Cor	fidence: 47.1	

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

```
Component Search

✓ X I ➡ I ■ Show Details ◆
```

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

- On the left, the Result List pane matches the list in the Component Search view.
- On the top center, the Trees page shows the spectral tree for the component's query spectrum on the left and the spectral tree for the matching library compound on the right. Matching spectra are highlighted with red borders. In Figure 13, notice that only the MS² nodes contain spectra with red borders.
- On the bottom center, the Spectra Compare page displays the query spectrum in green, the best matching library spectrum in red, and a difference spectrum with peaks in three colors.
 - () Gray Matching peaks within the specified mass tolerance. The peak height is a measure of the intensity difference between the peak in the library spectrum and the peak in the query spectrum.
 - () Green Spectrum peaks in the query scan that are not present in the library spectrum.
 - () Red Spectrum peaks in the library spectrum that are not present in the query scan.
- On the right, the Compound Structure pane displays the structure of the library compound, and the Precursor Structure pane displays the structure of the precursor ion for the matching library spectrum.

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

Result List pane On the Trees tab, the red borders indicate matching MS2 spectra. Mass Frontier 8.1 Trial [Search Details - Component 14] 🗋 = » Search 7 _ B X 🧟 online 👻 📳 Start Home Modu Search AE CH₂O £ 82 إيتا انتل Library Query Spectrum - Spectrum Formula Structure leutral Compound Structure • Precursor Structure Posto sing Type im Pro oid Stds LXQ.RAW [Modified] A Search Details - Compo ent 14 × Frees Compound Info L orary Tree Info Library Spectrum Info Query Spectrum nfo Query Tree Info Compound Structure ID: 24 mzCloud Reference Library Genistein Querv 이 및 ④ 몸 0 9 🕀 🗄 MM: 270.052 Confidence: 98.9 C1sH10Os 🗉 🚺 Thermo NSI MS² 98.9 H 🚺 ESI MS² 97.6 Thermo H 🗘 ESI MS² 86.9 UC Davis D: 20 mzCloud Reference < 1/1 FT CID 35 NCE MS2 271.06 Combined St -> MM: 270.0528 C1sH10O Apigenin npare Library Spectrum Query Spectrum MM: 270.052 Confidence: 48.9 **`** @ ID: 6197 mzCloud Reference tR: 49.6457 • Scan No: 9889 • IT... 271.02 152.98 253.14 Query HighChem High Re 88.7 Galangin Mc2 [M + I Match Opt.Dot Product 97.0 MM: 270.052 Confidence: 47.2 NIST (Modified) 84.3 54.02 243.07 902 mzCloud Reference Identity 86.9 Similarity Forward 8.2 MS¹ Pelargonidin Library 153.01824 Similarity Revers 81.5 Spectral Trees: 7. Compounds: 4 m/z 271.06010 C14H110 Scan No: 2-148 • FTMS + c ESI d Full ms2 27

Difference spectrum with color-coded peaks

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

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



Library Spectrum

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



Figure 15. Annotated library spectrum for genistein

* To save the annotations for a set of components

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

Note The hits for components 10, 11, and 14 have relatively high match scores—whereas, the hits for components 4 and 5 have relatively low match scores.

Running a Tree Search, which searches for matching MSn spectra in addition to matching MS2 spectra, might provide a higher level of confidence that the hits for components 4 and 5 are correct.

2. In the Component Search view, click the Accept All Suggested Structures icon, ^ॐ.

Compone	nt Search		# ×
**	📰 📃 Show Details 🔶		
Se Accep	pt all suggested structures		• 🌣
,	Search Selected	Search All	
	Multiple	omponents selected	

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

Figure 16. Accepted annotations for the Identity search

ame	Scan	Precursor	Ma 🔻	Match Name	MS ⁿ	te (min)	Abundance	Annotatio	
Components						CR (miny			
Component 14	9858	271.12	99	Genistein	3	49.538	668,802	Identity	
Component 11	7374	285.12	94	Glycitein	5	37.425	175,220	Identity	Annotated componer
Component 10	6993	255.13	83	Daidzein	5	35.558	435,410	Identity	· · ·
Component 5	4091	432.99	10	Apigetrin	4	21.039	299,572	Identity	Identified companen
Component 4	2559	446.91	10	Glycitin	5	13.357	346,339	Identity	
Component 13	8672	519.04	0		5	43.929	29,637	Identity	with low match scor
Component 12	7605	475.01	0		5	38.598	164,965	Identity	
Component 9	6479	532.96	0		5	32.994	113,039	Identity	
Component 8	6424	503.03	0		5	32.693	28,849	Identity	
-Component 7	5878	488.95	0		5	29.970	149,283	Identity	
-Component 6	5597	459.01	0		5	28.486	131,330	Identity	
-Component 3	2274	416.99	0		5	11.795	274,537	Identity	
-Component 2	369	359.89	0		3	1.859	20,610	Identity	
Component 1	248	240.59	0		5	1.243	31,934	Identity	
MS1 Scans									
Product Scans									

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

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

Run a tree search If the data includes high-quality MSⁿ data, as does the example data file, run a Tree Search.

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

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

Figure 17 shows the default settings for a Tree Search.

Figure 17. Default settings for a Tree Search

Search Farann	eters			
Search Type:	Tree Search		•	
Search Contai	iner			4
Used Stag Libra Libra Libra O Libra	ges ary MS ² vs Component MS ² ary MS ² vs Component MS ⁿ ary MS ⁿ vs Component MS ² ary MS ⁿ vs Component MS ⁿ	Spectral Compare Type Identity Component Match Condition Aggregated Tree Mat		
Spectrum Cor Collision	nstraints Energy tive Energy Tolerance 0			
Mate Tolerance	ch Ion Activation Type			
Compound C	lasses			•
All				

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

3. Click OK.

- 4. In the Components Search view, click Search All.
- 5. When the search ends, sort the Components list in descending order by the Match column (Figure 18).

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

ma	Scan	Drocursor	Ma -	Match Namo	MCD		Abundanca	Appotatio	
Components	Scari	Flecuisor	Ivia	Match Name	IVIS	t _R (min)	Abundance	Annotatio	
Component 14	9858	271.12	99	Genistein	3	49.538	668,802	Identity	
Component 11	7374	285.12	94	Glycitein	5	37.425	175,220	Identity	
Component 10	6993	255.13	83	Daidzein	5	35.558	435,410	Identity	
Component 1	248	240.59	66	Proscaline	5	1.243	31,934	Tree Search	
-Component 5	4091	432.99	59	Genistin	4	21.039	299,572	Tree Search	
-Component 4	2559	446.91	58	Glycitin	5	13.357	346,339	Tree Search	
-Component 3	2274	416.99	10	Daidzin	5	11.795	274,537	Tree Search	
-Component 12	7605	475.01	8	N 223 Dichloro	5	38.598	164,965	Tree Search	
-Component 13	8672	519.04	0		5	43.929	29,637	Tree Search	
-Component 9	6479	532.96	0		5	32.994	113,039	Tree Search	
-Component 8	6424	503.03	0		5	32.693	28,849	Tree Search	
-Component 7	5878	488.95	0		5	29.970	149,283	Tree Search	
-Component 6	5597	459.01	0		5	28.486	131,330	Tree Search	
Component 2	369	359.89	0		3	1.859	20,610	Tree Search	
MS1 Scans									
Product Scans									

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

- 6. To inspect the matching spectra for component 4, do the following:
 - a. In the Components list, select **Component 4**.
 - b. In the Components Search view click Show Details.

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



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



Difference spectrum wit color-coded peaks

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

1	Chromatogram Processor - Flavonoid_Stds_	LXQ.RAW [Modified]	👫 Search Details - Component 4 🗙
Res	ult List		
	ID: 6137		mzCloud Reference
1	Glycitin		
	C ₂₂ H ₂₂ O ₁₀ MM: 446.1213		Tree Match: 58.2
	🗉 😋 Cayman	NSI	MS ² ;MS ³ ;MS ⁴ ;M 58.2

Expands the spectrum list

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

🕒 Cayman			NSI		MS ² ;MS ³ ;MS ⁴ ;M	58.2
Matching Library Sp	oectra					
Precursor m/z	MSn	Pos.	Activation	Analyze	Confidence 🔻	Match Factor
242.0574	5		CID-40	IT	98.8	97.5

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

Notice the large number of matching MSⁿ library spectra that contribute to the overall match score.

/latching Library Spe	ectra					
Precursor m/z	MSn 🔺	Pos.	Activation	Analyzer	Confidence	Match Facto
447.1286	2		CID-100	FT	10.0	1(
447.1286	2		CID-30	FT	10.0	1(
447.1286	2		HCD-30	FT	10.0	1(
447.1286	2		CID-55	FT	10.0	1(
447.1286	2		CID-20	FT	10.0	1(
447.1286	2		HCD-20	FT	10.0	9
447.1286	2		HCD-40	FT	10.0	9
447.1286	2		HCD-50	FT	9.8	9
447.1286	2		HCD-10	FT	9.4	1
447.1286	2		CID-15	FT	9.1	1
447.1286	2		HCD-60	FT	8.5	(
285.0757	3		CID-40	FT	95.9	9
285.0757	3		CID-45	FT	90.9	9
285.0757	3		CID-35	FT	90.7	9
285.0757	3		CID-60	FT	90.3	9
285.0757	3		CID-70	FT	79.9	<u>c</u>
285.0757	3		HCD-60	FT	68.3	5
285.0757	3		HCD-50	FT	49.8	1
285.0757	3		HCD-70	FT	45.9	t.
285.0757	3		CID-80	FT	9.7	(
285.0757	3		CID-30	FT	7.9	
270.0523	4		CID-65	FT	95.1	9
270.0523	4		CID-45	FT	92.6	9
270.0523	4		CID-75	FT	92.0	9
270.0523	4		CID-35	FT	91.4	9
270.0523	4		CID-60	FT	88.9	(
270.0523	4		HCD-50	FT	72.7	8
270.0523	4		CID-30	FT	69.8	(
270.0523	4		HCD-60	FT	67.2	5
270.0523	4		HCD-40	FT	45.8	
270.0523			HCD-70	FT	44.8	
270.0523			CID-70	FT	9.9	
270.0523			CID-80	FT	9.9	
242.0574	5		CID=40	IT	98.8	
242.0574	5		CID-60	IT	98.7	-
242.0574	5		CID-35	IT	98.6	
242.0374	5		CID-45	п	QR 2	
242.0574	5		CID-70	п	20.2 25 5	
242.0374	5		CID-35	FT	71 0	
242.0374	5		CID-80	IT	64.4	
242.0374			CID-30	IT	6/ 1	-
242.03/4	5		CID-30	ст	04.1 E0.5	
242.0374			CID-45	ГI IT	59.5	
242.0574	5			11 ET	57.4	
242.0574	5		HCD-60	FI ET	56.9	
242.05/4	5		HCD-70	F1	55.4	
242.0574	5	T	CID-30	FI	55.3	5

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

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

Cayman		NSI	1	MS ² ;MS ³ ;MS ⁴ ;M	58.2
latching Library Spe	ctra				
Precursor m/z	MSn 🔺 Pos	Activation	Analyze	er Confidence	Match Factor
447.1286	2	CID-100	FT	10.0	100.0
447.1286	2	CID-30	FT	10.0	100.0
447.1286	2	HCD-30	FT	10.0	100.0
447.1286	2 📕	CID-55	FT	10.0	100.0
447.1286	2	CID-20	FT	10.0	100.0
447.1286	2	HCD-20	FT	10.0	99.9
447.1286	2	HCD-40	FT	10.0	99.8

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

Figure 19. Matching MS² library spectrum for component 4



- 7. To accept the annotations for component 1 do the following:
 - a. In the Components list, select **Component 1**.
 - b. In the Components Search view, click the Accept All Suggested Structures icon, \checkmark .

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

Figure 20. Accepted annotations for the Tree Search

me	Scan	Precursor	Ma 🔻	Match Name	MS ⁿ	te (min)	Abundance	Annotation Sources	
Components									
Component 14	9858	271.12	99	Genistein	3	49.538	668,802	Identity	
Component 11	7374	285.12	94	Glycitein	5	37.425	175,220	Identity	
Component 10	6993	255.13	83	Daidzein	5	35.558	435,410	Identity	Annot
Component 1	248	240.59	66	Proscaline	5	1.243	31,934	Tree Search	-compo
Component 5	4091	432.99	59	Genistin	4	21.039	299,572	Tree Search	201101
Component 4	2559	446.91	58	Glycitin	5	13.357	346,339	Tree Search	
- Component 3	2274	416.99	10	Daidzin	5	11.795	274,537	Tree Search	
Component 12	7605	475.01	8	N 223 Dichloro	5	38.598	164,965	Tree Search	
-Component 13	8672	519.04	0		5	43.929	29,637	Tree Search	
-Component 9	6479	532.96	0		5	32.994	113,039	Tree Search	
Component 8	6424	503.03	0		5	32.693	28,849	Tree Search	
Component 7	5878	488.95	0		5	29.970	149,283	Tree Search	
-Component 6	5597	459.01	0		5	28.486	131,330	Tree Search	
Component 2	369	359.89	0		3	1.859	20,610	Tree Search	
MS1 Scans									
Product Scans									

Run an Identity Substructure search

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

- * To run a library search to find compounds with substructures that match the components
- 1. In the Components Search view on the Chromatogram Processor page, select **Identity Substructure** from the Search Type list.
- 2. To review the settings for an Identity Substructure search, click the settings icon, 🔅 .

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

Figure 21. Default settings for an Identity Substructure search

Search Type:	Identity Substructure	•	
Search Contai	iner		
Used Stag O Libra O Libra O Libra	ges ary MS ² vs Component MS ² ary MS ² vs Component MS ⁿ ary MS ⁿ vs Component MS ² ary MS ⁿ vs Component MS ⁿ	Spectral Compare Type Identity Component Match Condition Best Confidence Mate	
Collision Collision Rela Mate	Energy tive Energy Tolerance 0 ch Ion Activation Type		
Compound C	lasses		•

 $B_{\rm y}^{\rm I}$ default, an Identity Substructure search is constrained by the ion activation type.

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

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

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

me	Scan	Precursor	Ma 🔻	Match Name	MS ⁿ	t _R (min)	Abundance	Annotation Sources
Components								
Component 14	9858	271.12	99	Genistein	3	49.538	668,802	Identity
Component 11	7374	285.12	94	Glycitein	5	37.425	175,220	Identity
Component 10	6993	255.13	83	Daidzein	5	35.558	435,410	Identity
Component 1	248	240.59	66	Proscaline	5	1.243	31,934	Tree Search
Component 9	6479	532.96	99	Glycitin	5	32.994	113,039	Identity Substructure
Component 7	5878	488.95	99	Glycitin	5	29.970	149,283	Identity Substructure
Component 4	2559	446.91	99	Glycitin	5	13.357	346,339	Identity Substructure
Component 13	8672	519.04	98	Genistin	5	43.929	29,637	Identity Substructure
Component 12	7605	475.01	98	Genistin	5	38.598	164,965	Identity Substructure
Component 8	6424	503.03	98	2S 2'S 3R 3'R 7' 1	5	32.693	28,849	Identity Substructure
Component 5	4091	432.99	98	Genistin	4	21.039	299,572	Identity Substructure
Component 6	5597	459.01	97	2S 2'S 3R 3'R 7'	5	28.486	131,330	Identity Substructure
Component 3	2274	416.99	97	2S 2'S 3R 3'R 7'	5	11.795	274,537	Identity Substructure
Component 2	369	359.89	75	D Raffinose	3	1.859	20,610	Identity Substructure
MS1 Scans								
Product Scans								

- 5. Review the results for component 7 (m/z 488.95, $t_R(min)$ 29.97, a standard compound acetylgycitin) as follows:
 - a. In the Components list, select **Component** 7.
 - b. In the Component Search view, click **Show Details**.

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

- c. Make sure that the first compound—glycitin— is selected in the Result List at the left of the Search Details Component 7 page.
- d. On the Trees page at the top center of the Search Details Component 7 page (Figure 23), notice that there are no matching MS^2 spectra, but there are matching MS^3 , MS^4 , and MS^5 spectra.

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





The Spectra Compare page below the Trees page shows the matching MS⁵ spectra.

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

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

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

To run a subtree search

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

Figure 24 shows the default settings for a Subtree Search.

Figure 24. Default settings for a Subtree Search

Search Parame	rters			
Search Type:	Subtree Search			
Search Contai	ner			*
Used Stag Libra Libra Libra Eibra Spectrum Con	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 straints	Spectral Compare Type Identity Component Match Condition Aggregated Sub-Tree		
Collision I Relat Mate Tolerance	Energy ive Energy Tolerance 0 h Ion Activation Type Factor 4.0 \$	3		
Compound Cl	asses			•
			Restore Defaults	OK Cancel

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

Run a Subtree search

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

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

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

Figure 25. Subtree Search results for the example data file

ne	Scan	Precursor	Ma 🔻	Match Name	MS ⁿ	t _R (min)	Abundance	Annotation Sources
Components								
-Component 14	9858	271.12	99	Genistein	3	49.538	668,802	Identity
-Component 11	7374	285.12	94	Glycitein	5	37.425	175,220	Identity
-Component 10	6993	255.13	83	Daidzein	5	35.558	435,410	Identity
-Con gonent 1	248	240.59	66	Proscaline	5	1.243	31,934	Tree Search
Component 9	6479	532.96	99	Glycitin	5	32.994	113,039	Subtree Search
Component 7	5878	488.95	99	Glycitin	5	29.970	149,283	Subtree Search
Component 4	2559	446.91	99	Glycitin	5	13.357	346,339	Subtree Search
Component 13	8672	519.04	98	Genistin	5	43.929	29,637	Subtree Search
Component 12	7605	475.01	98	Genistin	5	38.598	164,965	Subtree Search
Component 2	369	359.89	75	D Raffinose	3	1.859	20,610	Subtree Search
Component 5	4091	432.99	72	Rhoifolin	4	21.039	299,572	Subtree Search
Component 3	2274	416.99	58	Zearalenone	5	11.795	274,537	Subtree Search
Component 6	5597	459.01	57	Zearalenone	5	28.486	131,330	Subtree Search
Component 8	6424	503.03	52	Zearalenone	5	32.693	28,849	Subtree Search
MS1 Scans								
Product Scans								

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

Search result summary

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

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

#	Precur sor <i>m/z</i>		Best compound hit (name and monoisotopic mass)										
		Identity		Tree Search		Identity Subst	ructure	Subtree Sear	ch				
1	240.59	-	_	Proscaline	239.15	Tentoxin	414.23	Proscaline	239.15				
2	359.89	-	_	-	_	D Raffinose	504.17	D Raffinose	504.17				
3	416.99	-	-	Daidzin	416.11	Compound name ^a	866.23	Zearalenone	318.15				
4	446.91	Glycitin	446.12	Glycitin	446.12	Glycitin	446.12	Glycitin	446.12				
5	432.99	Apigetrin	432.11	Genistin	432.11	Genistin	432.11	Rhoifolin	578.16				
6	459.01	-	-	-	_	Compound name ^a	866.23	Zearalenone	318.15				
7	488.95	-	_	-	_	Glycitin	446.12	Glycitin	446.12				
8	503.03	-	_	-	_	Compound name ^a	866.23	Zearalenone	318.15				

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

#	Precur sor <i>m/z</i>		Best compound hit (name and monoisotopic mass)											
		Identity		Tree Search		Identity Substru	icture	Subtree Search						
9	532.96	-	-	-	-	Glycitin	446.12	Glycitin	446.12					
10	255.13	Daidzein	254.06	Daidzein	254.06	Compound name ^a	866.23	Zearalenone	318.15					
11	285.12	Glycitein	284.07	Glycitein	284.07	Glycitein	284.07	Glycitin	446.12					
12	475.01	_	_	Compound name ^b	473.99	Genistin	432.11	Genistin	432.11					
13	519.03	-	_	-	_	Genistin	432.11	Genistin	432.11					
14	271.12	Genistein	270.05	Genistein	270.05	Genistein	270.05	Apigenin 7-0- glucuranide	446.08					

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

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

An mzLogic analysis combines mzCloud[™] spectral similarity searching (MS² and MSⁿ)and structure overlapping to rank putative structures. For nominal mass data, you cannot run a MolGate[™] search against any of the structure databases for retrieving possible structure candidates. You can only run an mzLogic analysis against a set of predefined structure candidates.

Follow these procedures in order:

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

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

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

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

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Spectral Library	mzCloud Reference	•
Adducts	Pos. [M + H] ⁺ • Neg. [M - H] •	
	👸 Rank More Options	
XIC NLC mzl	ogic	

3. Click More Options.

The Structure Database list and the Workspace list appear.

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

Identify a component by running an mzLogic analysis

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Chromatogr Processor	am Curator	Data Manager	Metabolika	Structure Editor	Structure Grid	Batch Fragment Generation	SledgeHammer	Formula Generator	Isotope Pattern	MolGate Search	Periodic Table	Reaction Mechanis Overview	sm		
			N	lodules						Too	ols				
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c. In the File group of the Structure Grid toolbar, click **Open**.

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MB Start	Home	Modules & To	ools	Search															🏩 online 🝷 📘
Open Save	Add New	Delete Delete	Select All	Edit	Cut	Copy P	aste Import InChi or SMILES	Frrors	m/z m/z	m/z m/z Range	Polarity	Structure	Clear filters	Card View	Table View	Add/Remove Columns	Structure	Structures	
File		Edit			A	Actions				1	Filters				Vie	ew	Se	nd To	
Open Adds one o	r more c	ards with structu	ires imp	orted fro	om one	or multip	le selected files												

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

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

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

Figure 26. Structure grid page



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

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

6. Click Rank.

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





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



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

ne	Scan	Precursor	Ma 🔻	Match Name	MS ⁿ	te (min)	Abundance	Annotation Sources
Components								
Component 14	9858	271.12	99	Genistein	3	49.538	668,802	Identity
Component 11	7374	285.12	94	Glycitein	5	37.425	175,220	Identity
Component 10	6993	255.13	83	Daidzein	5	35.558	435,410	Identity
-Component 1	248	240.59	66	Proscaline	5	1.243	31,934	Tree Search
Component 7	5878	488.95	60	DUBPGEJGGVZK	5	29.970	149,283	mzLogic
Component 9	6479	532.96	99	Glycitin	5	32.994	113,039	Subtree Search
Component 4	2559	446.91	99	Glycitin	5	13.357	346,339	Subtree Search
Component 13	8672	519.04	98	Genistin	5	43.929	29,637	Subtree Search
Component 12	7605	475.01	98	Genistin	5	38.598	164,965	Subtree Search
Component 2	369	359.89	75	D Raffinose	3	1.859	20,610	Subtree Search
Component 5	4091	432.99	72	Rhoifolin	4	21.039	299,572	Subtree Search
Component 3	2274	416.99	58	Zearalenone	5	11.795	274,537	Subtree Search
-Component 6	5597	459.01	57	Zearalenone	5	28.486	131,330	Subtree Search
Component 8	6424	503.03	52	Zearalenone	5	32.693	28,849	Subtree Search
VIS1 Scans								
Product Scans								

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

* To view the name of a structure candidate

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

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Adducts	Sub/Structure Search	1 - H1 ·	
	鷔 New SledgeHammer		
	🚣 New Metabolika	Aore Options	
	💫 New Structure Editor	400.05	
mzLogic resu	Structure Grid 1	ursor 488.95	
Cann	ot use stri Editable collect	ion of structures	

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

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

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

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

The acetyl (CH₃CO) moiety molar mass of 43g/mol.

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



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