Mass Frontier 8.1 tutorial to Detect Structurally-Related Compounds with the FISh Algorithm

This tutorial describes how to detect structurally-related compounds by running a Fragment Ion Search[™] (FISh) analysis. Applying a FISh filter to a chromatogram removes all the spectral peaks except for the defined FISh model peaks. You can use this feature to extract spectral peaks for structurally-related features, for example, compounds related to the parent drug in metabolite ID, API impurity ID, or compounds that share a common list of fragments.

Contents

- Demo data files
- Check the global settings
- Run and Review a FISh analysis
- Identify detected components
- Save the results to an HCCX file
- Create and edit a fragments list

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

File	Description
OMP-Sigma-70K. raw	A raw data file acquired with an LC-ESI/MS/MS experiment using an Orbitrap [™] QExactive [™] Plus MS. The sample solution was prepared from an omeprazole standard. Omeprazole is a therapeutic drug for acid reflux.
Omeprazole FISh Fragments.sdf	A structure file that contains twelve fragment structures for the parent compound—omeprazole
OMP-Sigma-70K.chpro.jcd	A component detection file that contains the component detection settings for an LC/MS experiment
Omeprazole.mol	A structure file that contains the structure for omeprazole—the analyte of interest in this tutorial

Check the global settings

Before you begin this tutorial, check the global settings for the mass accuracy and color-coding of the spectrum peaks.

* To restore the default settings for mass accuracy and spectrum peak color-coding

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

The application opens to the Mass Frontier startup window or the Modules & Tools toolbar.

- 2. Do one of the following:
 - From the Mass Frontier startup window, click Global Settings.

Figure 1. Mass Frontier startup window

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Show this Window Next Time			

Click to open the Global Settings dialog box.

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

-or-

• From the application tab bar, click the **Start** tab to display the Start menu, and then choose **Global Settings**.

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The Global Settings dialog box opens (Figure 2).

In the left pane, under Layout, click MS Spectrum, and then click Restore Default.
 Figure 2. Global Settings dialog box – MS Spectrum view with the default settings

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D Chromatogram D Chromatogram ragments & Generation Ietabolika	Colors Peaks: Base Peaks:	#FF0000CD •	Product Explained Peaks: Undefined Peaks:	#FF8A2BE2	
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arameters A	Mass Diff./ Modif.:	#FF87CEEB *	Mark 2:	Green	•
lare Toloranco	Neutral Loss:	#FF00FF00 *	Mark 3:	#FFA9A9A9	•
eaction Restrictions	MSn Adduct Peak:	#FFB8860B -	Mass Range:	#FFE6E6FA	•

4. In the left pane under Parameters, click Mass Tolerance, and then click Restore Default.

Main ibrany Santica	Mass Accuracy				*			
nzCloud	Mass Accuracy of Experimental Data							
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2D Chromatogram	O User Defined (use accu	iracy of calculat	ed data)					
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IMPORTANT Make sure that the Determine from Source option (default) is selected for the mass accuracy of the experimental data; a user-defined setting for mass accuracy can change the number of detected components.

Run and Review a FISh analysis

To run a FISh analysis and review the results, follow these topics in order:

- 1. Open a raw data file for processing
- 2. Apply a fragment ion search filter
- 3. View the FISh trace
- 4. Review the detected componentsTo open the example raw data file

Open a raw data file for processing

1. In the Modules & Tools toolbar, click Chromatogram Processor.



Home Wouldes	& Tools Search					👜 online
Chraatogram Curator Data	Metabolika Structure	Structure Batch Fr	agment SledgeHammer	CH NO Formula Isotop	e MolGate Periodic	Reaction Mech
Processor Manager	Editor	Grid Gener	ration	Generator Patter	n Search Table Tools	Overviev
Data Files (LC/MS)						

Modules & Tools toolbar

2. In the Open Chromatogram dialog box, browse to the following folder, select **OMP-Sigma-70K.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 level and number.

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

• 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 at the lower right displays the first scan in the raw data file.
- The command processor view at the lower left is empty until you apply actions to the chromatogram.





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 area of the Chromatogram Processor toolbar:

- For the MS spectrum view, click the **Show MS Spectrum** icon, <u>II</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.

To apply a fragment ion search filter to the TIC chromatogram

Note The fragment ion search filter that you specify for FISh detection is called the FISh model.

1. In the Actions group of the Chromatogram Processor toolbar, click FISh.

M G Sta	art Home Modu	les & Tools Search										
Save	Delete Copy Select	× ↓ ∧ ↓ ◆ △ □ □ ∧ × ∧ ↓ □	JCD TECD	FISh	Components Search	mzLogic	Component	Spectrum		≪ ₩ %	^: ☆ ▲ [∭]	П Ф
File	Edit	A	ctions		Searc	h	Send	to	View	Filter	Displa	зу

FISh button

The Model page of the FISh Detection view opens to the right of the chromatogram and spectrum views (Figure 5).

Note You use the Model page to set up the FISh model for the fragment ion search.

Apply a fragment ion search filter

Figure 5. Model page of the FISh Detection view with the default settings

	FISh Detection	Ψ×						
	S Image: Second secon	Î						
Restores the default parameter settings on the Options page	Create FISh Model Polarity: Positive Negative Available Sources Chromatogram Processor - OMP-Sigma-70							
Fragments option— Opens the FISh Filter:Input Fragments dialog box	Generated From Structure Geneted From Structure Geneted From Structure Generated From							
	Modifications (empty) Edit Save FISh Model (after calculation) Component Sensitivity Preview Restore Accept Cancel	•						

- 2. In the Fragments area, set up the fragments list as follows:
 - a. Select the **Fragments** option.

Fragments (empty)

The FISh Filter: Input Fragments dialog box opens.

Tip To edit the fragment options after closing the FISh Filter: Input Fragments dialog box, click **Edit**.

b. Click the **Import** icon, ¹⁶, and then click **Import File**.

FISh filter: Input Fragments	_ □ ×
🐸 - 📮 🖽 - I 🏽 🕞 🗶 🗗 🛍 🖻 🗟 I 🦷 🏧 🏹 🛠 🕵 🛃 -	S M L
Import File Ctrl+O	
Positive charged fragments will be incerted. Neutral and penative charged for	agments will be ignored
Positive charged fragments will be inserted. Neutral and negative charged fr 0 structures will be inserted and 0 structures will be ignored.	agments will be ignored.

c. Browse to the following folder, select Omeprazole FISh Fragments.sdf, and click Open.

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

Twelve structures appear in the dialog box (Figure 6). The structure with the highest m/z value (m/z 346.12199) is the protonated ion of omeprazole.

Note For a FISh analysis, make sure that the fragments list includes the analyte's precursor ion.



Figure 6. Fragment structures in the Omeprazole FISh Fragments.sdf file

d. Click OK.

The number of fragments to use appears to the right of the Fragments option.



- 3. Add one modification to the FISh model as follows:
 - a. On the Model page of the FISh Detection view, select the Modifications check box.

Modifications (empty)	
Edit	

The FISh Modifications dialog box opens.

Tip To edit the FISh modifications after closing the FISh Modifications dialog box, click Edit.

b. In the Predefined Modifications area, select the **Oxidation** (+O) check box, and then click **Add** (Figure 7).

Figure 7. FISh Modifications dialog box with one modification

re ar	defined me: Pl	Modifications					Working N	Aodifications				
		Name	Formula	Shift				Name	Formula	Shift		
		Ethyl to carboxylic acid	+02-CH4	15.958!	*	Add		Oxidation (N, S), aliphat	+0	15.994	-	
	\checkmark	Oxidation (N, S), aliphati	+0	15.994		Add All Remove						
		Demethylation + 2x hyd	+02-CH2	17.974:	•	Remove All						ОК

c. Click OK.

The number of modifications to consider appears to the right of the Modifications check box.

Modifications (1) Number of Edit...

4. To reset the parameter settings on the Options page, click the **Reset** icon, **S**.

Note You use the Options page to specify the component detection algorithm and which spectral peaks you want the application to use to build the FISh chromatogram.

5. Click the **Options** tab to open the Options page (Figure 8). Keep the default settings for selecting which spectral peaks contribute to the ion current at each time point in the FISh chromatogram.

Figure 8. Options page of the FISh Detection view

	FISh Detection # ×	
Identifies the spectral peaks that correspond to the FISh model fragments.	HSb Detection # x Model Options Filtering Target Mark Peaks Model Options Filtering Target Mark Peaks Detect Components Joint Component Detection Options Filter Scans Filter Scans Filter Precursors Apply to Top Stage Only Remove Data Dependent Scans Mark Precursors Sensitivity Isotopes Use Moncisotopic Peaks Only, All other peaks are ignored. This option provides the fastest processing. Mark the Moncisotopic Peaks Only, All other peaks are ignored. This option provides the fastest processing. Mark the Moncisotopic Peaks Only. All other peaks are ignored. This option provides the fastest processing. Mark the Moncisotopic Peaks Only. All other peaks are ignored. This option provides the fastes processing. Mark the Moncisotopic Pattern when available. If a moncisotopic peak is recognized, the application includes other existing isotopes in the result. Missed isotopes do not influence the recognition of the peaks. This option produces the highest signal and can produce false position of the peaks. This option produces the longest to process. The Mass Fron application changes the option to Mark Isotopic Pattern when available for data-dependent scans. Constraints Image Mark Eliminated Abund: 100 % % Mark Eliminated Abund:	— Opens the JCD dialog box.
	Component Sensitivity	

- 6. Set up component detection as follows:
 - a. On the Options page of the FISh Detection view, click **Options**.

The JCD dialog box opens (Figure 9).

By default, all the sliders in the Deconvolution pane of the JCD view are set to the middle.

- b. In the JCD dialog box, click Load.
- c. Browse to the following folder, select the OMP-Sigma-70K.chpro.jcd file, and click Open.

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

The selected .chpro.jcd file moves the Intensity of Detected Component slider to the left, which increases the minimum intensity threshold for a component and typically decreases the number of detected components. It also slightly changes the Component Overlapping Intensity setting.

CD	×	
Mode © Wizard O Details	OK Cancel	
Deconvolution	Default	
Wizard	Save	
Average Peak Width	Load	
Automatic Manual: 20 scans		
Power of Baseline Correction		
Smoothing Power		
Component Overlapping Intensity		
		— Modified settin
Intensity of Detected Component		
Tree Branching		
· · · · · ·		
Retention Time Range		
m/z Range		

Figure 9. Component detection settings (from selected file)

- d. Click OK.
- 7. In the FISh Detection view, click **Preview**.
- 8. After the analysis finishes, click Accept.

The command processor view lists the FISh Detection filter as an applied action. A list of detected components appears in the chromatogram data view, and the blue triangles indicate the detected components.





Applied actions in the command processor view

Number of detected components in the chromatogram data view—12

View the FISh trace

Use the options menu to display selected chromatograms.

To view the FISh chromatogram

Open the options menu and clear the **Show TIC**, **Show All Components**, and **Show Selected Components** check boxes.



A FISh chromatogram is a TIC chromatogram that is calculated from the identified (marked) m/z peaks for the specified FISh model. By default, the application displays calculated TIC chromatograms in pink (Figure 11).

Tip You can change the color settings for chromatogram in the 2D Chromatogram view of the Global Settings dialog box.





Review the detected components

* To inspect the spectra for a detected component

1. In the Components list, select **Component 5** (m/z 362.1166 at 9.036 min).

Figure 12. Chromatogram data view with a list of 12 components

Name	Scan	Precursor	Match	Match Name	MSn	t- (min)	Abundance	Annotatio		
▼ Components	0 currin		materi			CR (IIIII)	, ibuildance			-
Component 1	1031	328.1288			2	2.755	365,553,101			
Component 2	1059	328.12869			2	2.810	122,906,072			
Component 3	1851	330.1267			2	4.721	180,540,890			
Component 4	2627	298.15476			2	6.539	346,244,570			
Component 5	3765	362.1166			2	9.036	67,631,854			
Component 6	4249	168.10172			2	10.286	338,070,356			
-Component 7	4269	346.1216			2	10.329	7,855,607,334			
-Component 8	4297	168.10178			2	10.386	394,281,870			
-Component 9	5085	314.1496			2	11.976	39,920,517			
-Component 10	5649	330.1267			2	13.131	1,131,626,807			
-Component 11	6361	577.2553			2	14.573	43,385,971			
Component 12	6381	330.1265			2	14.613	332,538,271			
MS1 Scans										
Product Scans										
										-
								Number	of scans: 979	95
								Number of c	omponents: '	12

A combined scan for the MS1 scan stage appears on the Spectrum page of the MS spectrum view (Figure 13).

TIC 2D Contour 3D Info Filter: 🙀	All
	Spectrum Data Info FISh
	Cmp. t _R : 9.0362 min. Spc. t _R : 8.9709 - 9.1164 min. • Sca
	362.1166
	60,000,000
	55,000,000
FT MS ¹ Scns. #3733-3802	50,000,000
	45,000,000
	40,000,000
FT MS ² 362.12 Scn. #3736	35,000,000
	30,000,000
	25,000,000
	20,000,000
	15,000,000
	10,000,000
	5,000,000
	0-1
▲ 1/1 FT MS1 Combined Scans #37 •	200 400 600 800 1,000 1,200 1,400

Figure 13. MS spectrum view open to the Spectrum page

To display the first MS² scan for the selected component, click the MS² node of the spectrum tree.
 Figure 14. Spectrum page showing scan no. 3736 at full scale



On the Spectrum page, each FISh peak is color-coded:

- () Explained Red indicates that the peak matches a specified fragment.
 - () Mass Diff./Modified Light blue indicates that the peak matches a shifted fragment for a specified modification.
 - Lime green indicates that the peak matches a specified fragment from a neutral loss.
- () Isotope explained peak Green indicates that the peak matches an isotopic ion for a specified fragment.

The unexplained peaks are a royal blue .

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()Neutral Loss

3. To zoom in on a specific peak, drag the pointer from left to right across a small range of m/z values on the *x* axis.

This figure shows scan no. 3736 with the selection of the m/z 191–196 range on the x axis.

Figure 15. Scan no. 3736 showing the selection of the *m/z* 191–196 range on the *x* axis



4. To show the explanation for a FISh peak, point to the peak until the triangular marker appears (Figure 16). Then, right-click and choose **Show FISh Explanation** *m/z Value* (Figure 17).

Figure 16 shows the spectrum peak for m/z 195.0219 (). The peak is red, which indicates that its m/z value matches one of the fragments in the user-specified fragments list (see card 8 in Figure 6).

Figure 16. Triangular marker that appears when you point to a peak



Figure 17. Spectrum shortcut menu



The FISh page opens in the spectrum view and displays the most likely explanation for the product ion (Figure 18). The count number at the bottom of the page is the total number of explanations in the list (with Show Base, Show Modification, and Show Neutral Loss turned on).

Figure 18. Parent (precursor ion) view on the FISh page

S	pectrum Data	Info FISh	
Pa	arent Show Paren	t 🔘 Show 8 🔅 expl. (a) Show Unique Show Base Show Modification Show Neutral Loss	
	m/z	Explanations	
	195.02225	m/z: 195.02282	*
Þ			0
		C ₈ H ₇ N ₂ O ₂ S*	-
	Count=6		

5. To view the fragment structure by itself, click Show Parent.

Note Clicking Show Parent toggles the displayed structure between these views:

- The parent structure (precursor ion) with the bonds for the fragment structure (product ion) shown in red
- The fragment structure by itself

Figure 19. Fragment structure (product ion) view on the FISh page of the spectrum view

S	pectrum Data	Info FISh										
Pi	Parent Show Parent 🔘 Show 🛛 🔅 expl. 🐵 Show Unique Show Base Show Modification Show Neutral Loss											
	m/z	Explanations										
	195.02225	m/z: 195.02282	*									
•			0									
		$C_8H_7N_2O_2S^+$	÷									
	Count=6											

- 6. In the MS spectrum view, click the **Spectrum** tab to display the Spectrum page. Then, to reset the zoom level, click the **Reset Zoom** icon, and, to the right of the scan header (Figure 16).
- 7. To display the explanations for a shifted peak (a fragment with the specified modification), do the following:
 - a. On the Spectrum page, zoom in on the m/z 188-218 range on the x axis.
 - b. Point to the light-blue peak at *m/z* 214.05342 until the triangular marker appears. Then, right-click and choose **Show FISh Explanation** *m/z* **214.05342**.

Figure 20 shows the "Show Parent" view on the left and the "fragment" view on the right for m/z 214.05342 (

Spectrum	Data Info FISh	Spectrum Data Info FISh
Parent Sho	w Parent 🔘 Show 8 📫 expl. 💿 Show Unique	Parent Show Parent 🔘 Show 🛛 🗘 expl. 💿 Show Unique
m/z	Explanations	m/z Explanations
214	1.05342 m/z: 214.05379	214.05342 m/z: 214.05379
	$C_9H_{12}NO_2S^+$ +Oxidation (N,	C ₉ H ₁₂ NO ₂ S ⁺ +Oxidation (N,
	Parent structure	Fragment structure

Figure 20. FISh explanation for m/z 214.05342 (modification = oxidation)

- 8. To display the explanations for a neutral loss peak (a fragment produced by a neutral loss), do the following:
 - a. On the Spectrum page, reset the zoom level, and then zoom in on the m/z 133-138 range on the x axis.
 - b. Point to the lime green peak at *m*/*z* 136.07573 until the triangular marker appears. Then, right-click and choose **Show FISh Explanation m**/*z* 136.07573.

Figure 21 shows the Show Parent view on the left and the fragment view on the right for m/z 136.07573 (

Figure 21. FISh explanation for m/z 136.07573 (fragment from a neutral loss)



Note The application calculates neutral losses by subtracting the m/z value for a fragment structure from the highest m/z value for a structure in the fragment list on the FISh Model page.

Identify detected components

From the Chromatogram Processor module, you can run a library search to identify the detected components. For this tutorial, run an Identity search against the mzCloud[™] mass spectral database. This online database contains entries for omeprazole (the analyte in this tutorial) and its impurities.

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

To identify components by running a library search

- 1. (Optional) Run a connection check as follows:
 - a. From the application tab bar, click the **Start** tab to open the Start menu, and then click **Connection Check**.
 - b. In the Connection Check dialog box, click Run.

The application verifies the connection.

- c. If the connection is successful, go to the next step of this procedure.
- d. If the application cannot connect to the mzCloud server, check the computer's Internet connection and its access to various sites. Also, make sure that the computer is synchronized with Internet time.

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).

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

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

- 3. In the Search Type list, select **Identity**.
- 4. In the Library list, select the Reference check box under mzCloud libraries.
- 5. To run a search for selected components, select the components in the chromatogram data view. For this tutorial, select **Component 5** in the Components list. Then, in the Component Search view, click **Search Selected**.

Note If you do not select a component, the application runs the search on component 1.

If the search finds a matching precursor m/z and spectrum in the spectral library, the following items appear:

- The match score and compound name appear in the Match column of the chromatogram data view.
- The Spectra Compare page opens in the MS spectrum view.
- Information about the matching mzCloud entry appears in the Component Search view.

Figure 22 shows the search result for component 5 against the mzCloud Reference library.



Figure 22. Search result for component 5

6. To run a search for all the detected components with MS² scans, click Search All.

Figure 23 shows the result of an mzCloud identity search run in March 2023 against the mzCloud Reference library. As new compounds are constantly being added to the mzCloud library, your results might differ.

Figure 23.	Search All results for the mzCloud Reference lib	rary
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🔄 Chromatogram	n Processor - OMP	-Sigma-70K.raw [Mc	odified] ×					
■ 3 5 4	m/z: 2.6	mmu						
Name	Scan No.	Scan No. Precursor m/z		Match Name		t _R (min)	Abundance	Annotation
 Components 								
-Component	1 1031	328.1288	0		2	2.755	365,553,101	Identity
-Component	2 1059	328.12869	0		2	2.810	122,906,072	Identity
-Component	3 1851	330.1267	0		2	4.721	180,540,890	Identity
-Component	4 2627	298.15476	9	N 2 Morpholinophenyl N phenylurea	2	6.539	346,244,570	Identity
-Component	5 3765	362.1166	99	Omeprazole sulphone	2	9.036	67,631,854	Identity
-Component	6 4249	168.10172	59	Anhydroecgonine	2	10.286	338,070,356	Identity
-Component	7 4269	346.1216	65	Omeprazole	2	10.329	7,825,801,948	Identity
-Component	8 4297	168.10178	62	Anhydroecgonine	2	10.386	394,281,870	Identity
-Component	9 5085	314.1496	36	4 2 Methoxyphenyl 1 3 nitropyridin 2 yl piperidine	2	11.976	39,920,517	Identity
-Component	10 5649	330.1267	0		2	13.131	1,131,626,807	Identity
-Component	: 11 6361	577.2553	0		2	14.573	43,385,971	Identity
Component	: 12 6381	330.1265	0		2	14.613	332,538,271	Identity

- 7. To search both the mzCloud Reference and Autoprocessed libraries, do the following in the Component Search view:
 - a. Open the Library list.
 - b. Select the Reference and Autoprocessed check boxes.

Component	Ψ×	
 ✓ × ≓ 	📄 Show Details 🔶	
Search Type	Identity	- 🌣
Library	mzCloud Reference;mzCloud Autoprocessed	-
	mzCloud Libraries Reference Autoprocessed	
Matches for	😌 Refresh	

c. Click Search All.

Note The Reference library contains compounds with spectra that have been fully curated by mass spectrometry specialists. The Autoprocessed library contains compounds with spectra that have only been automatically processed with a curation software application.

The result for component 4 changed because the search found a compound with a higher match score in the Autoprocessed library.

Figure 24. Search All results for the mzCloud Reference and Autoprocessed libraries

📱 Chromatogram Pr	ocessor - OMP-	Sigma-70K.raw [Mc	odified] \times					
📃 🧏 🖒 Δ m/z	2.6	mmu						
Name	Scan No.	Precursor m/z	Match	Match Name	MS ⁿ	t _R (min)	Abundance	Annotation
 Components 								
-Component 1	1031	328.1288	0		2	2.755	365,553,101	Identity
Component 2	1059	328.12869	0		2	2.810	122,906,072	Identity
Component 3	1851	330.1267	0		2	4.721	180,540,890	Identity
Component 4	2627	298.15476	52	1 benzyl 4 4 nitrophenyl piperazine	2	6.539	346,244,570	Identity
Component 5	3765	362.1166	99	Omeprazole sulphone	2	9.036	67,631,854	Identity
Component 6	4249	168.10172	59	Anhydroecgonine	2	10.286	338,070,356	Identity
Component 7	4269	346.1216	65	Omeprazole	2	10.329	7,825,801,948	Identity
Component 8	4297	168.10178	62	Anhydroecgonine	2	10.386	394,281,870	Identity
Component 9	5085	314.1496	36	4 2 Methoxyphenyl 1 3 nitropyridin 2 yl piperidine	2	11.976	39,920,517	Identity
Component 10	5649	330.1267	0		2	13.131	1,131,626,807	Identity
Component 11	6361	577.2553	0		2	14.573	43,385,971	Identity
Component 12	6381	330.1265	0		2	14.613	332,538,271	Identity

Save the 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 You can save the intermediate and final component detection and annotation results to HCCX files so you can return to those results at a later time. Saving HCCX files lets you return to a specific results state, and then perform different processing actions on the data.

Create and edit a fragments list

In this tutorial, you used a predefined fragments list to create a FISh model for the analyte of interest. This topic shows you how to create a fragments list with an appropriate set of fragments for the analyte of interest in this tutorial—omeprazole.

Note To create a fragments list, you can use the Batch Fragment Generation wizard or the SledgeHammer module.

Follow these topics in order:

- 1. create a fragments list and save it to an SDF file
- 2. Edit a fragments list

* To create a fragments list and save it to an SDF file

1. On the Spectrum page of the MS spectrum view, display the highest abundance MS2 scan for omeprazole (precursor m/z 346) by selecting this scan from the MS2 Scans list in the chromatogram data view.

Figure 25. Selecting scan no. 4266—the highest abundance MS2 scan for omeprazole

100 C	Scan No.	Precursor m/z	Match 🔺 Match Nam	e MS ⁿ	t _e (min)	Abundance	Annotation	
MS2		344.1238		2				
MS2		344.2276		2				
 MS2 		346.1216		2				
_MS2	4208	346.1217		2	10.207	784,012		
MS2	4211	346.1217		2	10.214	2,261,748		
MS2	4215	346.1217		2	10.223	7,287,226		
MS2	4218	346.1217		2	10.230	17,324,644		
-MS2	4222	346.1216		2	10.238	49,133,172		
MS2	4226	346.1216		2	10.246	121,728,824		
MS2	4230	346.1216		2	10.254	245,359,872		
MS2	4234	346.1215		2	10.262	357,948,416		
MS2	4238	346.1216		2	10.270	473,472,160		
MS2	4242	346.1215		2	10.278	629,552,512		
MS2	4246	346.1215		2	10.286	756,967,872		
MS2	4250	346.1216		2	10.294	977,553,344		
MS2	4254	346.1216		2	10.302	1,146,550,272		
MS2	4258	346.1216		2	10.310	1,319,070,336		
MS2	4262	346.1217		2	10.318	1,547,743,360		
MS2	4266	346.1217		2	10.326	1,673,843,200		
MS2	4270	346.1217		2	10.334	1,598,272,128		
MS2	4274	346.1217		2	10.342	1,513,087,488		
MS2	4278	346.1217		2	10.350	1,369,242,752		
MS2	4282	346.1217		2	10.358	1,165,856,256		
MS2	4286	346.1217		2	10.366	985,530,112		
MS2	4290	346.1217		2	10.374	778,905,152		
MS2	4294	346.1216		2	10.382	662,469,504		
MS2	4298	346.1216		2	10.390	556,360,832		
		246 1216		2	10 398	441 199 584		

The spectrum appears on the Spectrum page (Figure 26 on page 21).

2. Right-click the spectrum and choose Copy MS Spectrum.

create a fragments list and save it to an SDF file



The application copies the spectrum to the Clipboard.

3. In the Modules & Tools toolbar, click Data Manager.

A Data Manager window opens.

In the Data Manager window, right-click the Spectrum page and choose Paste MS Spectrum.
 Figure 27. Pasting an MS Spectrum from the Clipboard to a Data Manager window

5	Chromatogram Processor - OMP-Sigma-70K.raw [Mod	ified]	Ĩ	Data	Manag	jer ×							Ŧ	~
e	Spectral Tree 📑 Filtered 📑 Recalibrated	Spe	ectru	m	Peaks	BDC	Meta	data	Compare Spectra					Z
	□ 및 ⊕ 몸									5 00 0	MS ¹ •			otificat
chanism		90-	(<u>බ</u> ි ර			rum rum to n	nzClou	d					tions
A. Me		80		្រ	opy Mi aste M	S Spectr	rum Ima	ge N						
		70-		30	opy Ar	notatio	n	13						
		60		A D	aste Ar bsolute	e Abunc	dance							
		50-	α	R R	eset Zo	oom	A					(none)		
		40		SI	now Fo	ormula A	Annotati	ons						
		30-		SI	now Te uto An	ext Anno notatio	otations n Layout							
		20-		Sł	now N	eutral Lo	osses							
		10		SI	now Ac	dducts curacy								
		0												
	< ->								0 0.50 0.60 0.70	0.80 0.90 1.00				

The spectrum appears on the Spectrum page and the tree for this spectrum appears in the Spectral Tree pane (Figure 28 on page 22).



lee	Spectral Tree Raw	Spectrum	Peaks	BDC	Metadata	Compare Spectra	Precursor Structure
	□ 및 ⊕ 몸	FTMS + p ES	iI d Full m	s2 346.1	217@hcd43	33 [50.0000-375.0000] 5 🔊 🐣 C MS ¹ •	
4° Mechanism	MS ¹ Sen.	100- 90- 70- 60- 50- 40- 30- 20-			136.0757	D 346.1217 HC3 30 RW 3 198.05823 39917	
	1/1 FT HCD 30 NCE; 45 NCE; 55 NCE MS2.	10- 0- 50	75	L	25 150	199.06158 175 200 225 260 275 300 325 350 375	m/z

- 5. To specify the precursor structure for this spectrum, do the following:
 - a. Double-click the **Precursor Structure** pane.

The Structure Editor opens.

b. Click the **Open** icon, **>**, and select the omeprazole.mol file in the following folder, and open the file.

drive:\Users\Public\Public Documents\Highchem\Mass Frontier 8.11\Demo Data\Structures

The structure for omeprazole appears in the drawing area of the Structure Editor.

c. To add a charge to the structure, select $[M + H]^+$ from the dropdown Unspecified Charge Site list.

Structure Editor	_ - ×
▶ 🖻 🔊 ୯ 🗙 🗊 📦 🛱 🦉 🛱 🗭 🔂 🕹 🕬 🔊 🗸	
	Î
CTemplate	
Imperiodic Table C H N 0 (M+H)*** * F Cl Br 1 (M-H)*** * 1 R R (M+NH_1)*** (M+NH_2)*** * 1	
Charge: [M + H ₃ O]*	
Radical [M + Na]*	
□ Isotope 0 [M + K]*	•
	,
Unspecified Charge Site: $[M + H]^*$ \bullet $\textcircled{0}$ $C_{17}H_{20}N_3O_3S^*$ m/z 346.12199 OK	Cance

Figure 29. Adding a charge to omeprazole

d. Click OK.

The protonated ion of omeprazole appears in the Precursor Structure pane.

8					Searc	ch					Mass Fron	tier 8.1 Tria	I [Data Manager	1						<u></u>	
MCBS	tart	Home	Mod	ules & Tools	Searc	ch														🤬 onlin	ne 🔹 📔
Oper	Save	Copy	Paste	Reload Del	ete Sa	ave Anges	ctions		80 전 HA MH KC	Change	Load from	•	Base Structure Compound Cla Compound Na	iss me	Metada Spectru Structur	ta Retention m Compound e Pathway M	ime Class ass Loss	Compound	а. ц. ц.		
	Chrome	togram [CMD Sigm	a 70Kpm			vier	«	Chiom	atogram co	inponents	Group by			QUICK FIILE		a senu t	0		
걸	unroma	itogram F	rocesso	r - OMP-Sigm	a-70knev	w.raw.nco		ata Maha	ger ×												
8	Spectra	al Tree		🛻 R.	aw	Spectrum	n Peak	s BDC	Metad	ata Ri	References	Compa	e Spectra				Pro	cursor Structure			
🍝 Mechanism	Q	MS ¹ :	30 NCE	##256 :45 NCE; 5!		TMS + p E 100 60 	SI d Full		136 136 125 15	07570 07570 0 175	198.05823 199.05823 199.06 200 2 × Flavor	158 250 10003	275 300 S	1		MS ¹ 346.1 HCD 30 MS ²	217 w3	nH ₂₀ N ₂ O ₃ 5*	>	n/z 346:	M+H ¹
1	compoi	und(s)		Compound	11 Spec	ctral Trees	:0 Med	hanisms: 0											Lib	rary Service Sta	tus 🔵

- 6. To generate the fragments for this structure, do the following:
 - a. In the Send To group of the Data Manager toolbar, click **Structure** and select **New SledgeHammer** from the dropdown list.



The Reaction Restrictions dialog box opens, showing the protonated ion of omeprazole on the Structure page.

Figure 30. Reaction Restrictions dialog box with the protonated ion of omeprazole

Reaction	Restricti	ons					-	. – ×
۵ 📥 🕻	1							
Structure	Base	Ionization	Cleavage	Rearrangements	Charge Retention Reactions	Resonance	Additional	Sizes
A 0 1	⁶		o fil					
Restore De	aults					(ienerate	Cancel

- b. Click the **Base** tab to open the Base page.
- c. Select the Use HighChem Fragmentation Library check box.

Figure 31. Base page

Structure	Base	Ionization	Cleavage	Rearrangements	ts Charge Retention Reactions Resonance Additional Sizes								
- Knowled	lge Base heral Frag	gmentation Re	ules		<u></u>								
Frag	nentatio	n Libraries	ation Library		Fragmentation Library Options								
	HighCh	em Fragment	ation clorary		Ignore General Frag. Ru	iles in Library	Reactions						
	HighCh	em Fragment			v ignore veneral rrag. ku		Reactions						

d. Click Generate.

The Fragment Generation progress box appears. When the fragmentation finishes, a new SledgeHammer window opens as a tabbed page.

Figure 32. SledgeHammer page with 1134 fragments for 147 unique *m/z* values

🗄 🗋 🕶 📂 🕶	**	Sear	ch						Ŧ>
ME Start Hor	me Modules 8	Tools Sea	rch						🤮 online 🔹 📘
Mechanisms Fragments	One Mechani One Fragmen All m/z Values	t Generate Fragments	Show Explaine Only +	ed Show Pathway	가 도 Show Fragments	Pan	<u>ର</u> ସ ପ ଜ୍ୟ ପ	Mechanisms	Structure Structure
Save	Сору		Operat	tions		Zo	om & Pan	Mechanisms	Send to
Chromatog	am Processor - ON	1P-Sigma-70K.ra	w [Modified]	Data M	anager 🔰	Fragm	ents & Mecha	nisms 1 [Data M	Manager] × 🔹
Tolerance:	0.05 🗘 mmu	1						,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
m/z	Count								Q 100% *
+ 30.010016		1 *							
1	ÓН / сн₂о⁺*		о ⁻ ~ ~ ~	NH ⊆	2199	_	10	•	.05833
> 31.017841		1							
39.022927		2							
+ 42.033826		2							
• 44.979347		1							
+ 45.033491		2							
+ 45.987172		1							
+ 47.966437		1							
+ 48.974262		1							
NU 989917		1							
52 026001									
> 53.026001									*

7. To reduce the number of fragments to those explained by the FISh peaks, in the Operations group of the Fragments & Mechanisms toolbar, click **Show Explained Only** and select the **Current Spectrum in Data Manager** check box.



The number of unique m/z values decreases to 16.

8. To send the structures to a new Structure Grid, in the Send To group of the Fragments & Mechanisms toolbar, click **Structures**, and then select **New Structure Grid** from the dropdown list.



The new Structure Grid opens as a tabbed page (Figure 33 on page 26).

Figure 33. Structure Grid with fragment structures in the 106 – 198 m/z range

🔢 I 🗋 🕶 📂 🕶	me Modules &	Search								∓ _ □ × ∴ online • 2 •
Open Save	Add Delete Delete	Select Edit	Cut Copy Paste	Frrors m/z m/z Rang	Polarity s	Structure Clear filters	Card View	Table Add/Remove View Columns	Structure Structures	Structure Structures
File	Edit	4	Actions		Filters			View	Send To	Receive From
Chromatog	ram Processor - OM	-Sigma-70K.raw [[Modified] 🔟 Data	a Manager 🛛 鷔 Frag	ments & M	echanisms 1 (Data	Manag	er] 🛛 🐻 Structure Gr	id 1 ×	- ×
						S	ML	Caption	Value	
1		2		1			ô	 Identifiers 		-
-		-						ID		
		ОН		OH				Name		
	-19 N-1++	HS+	N	S N				IUPAC Name		
P NH		\=<			-			CAS		
	/ b-	1	<u> </u>	+ 0-	-			InChI	InChI=1S/C17H -18-15(11(2)16(7-19-13-6-5-12 17/b5-8H 9H2 1	19N3O3S/c1-10-8 10)23-4)9-24(21)1 (22-3)7-14(13)20- -4H3 (H 19 20)
[C ₁₇ H ₁₉ m/z	3N3O3S+H] ⁺ 346.12199	C ₉ H ₁₂ N m/z 198	NO ₂ S ⁺ 8.05833	C ₉ H ₁₂ NO ₂ S ⁺ m/z 198.05833				InChIKey	SUBDBMMJDZJ A-N	VOS-UHFFFAOYS
								 Synonyms 	G.	
4		5		5						
s+		N	OH +		-		*			
1 from 64 structu	res selected									

- 9. Save the structures to a structure file as follows:
 - a. In the Structure Grid toolbar, click Save, and then select All from the dropdown list.



- b. In the Save Structures dialog box, name the file and select its folder.
- c. Record the file name and location for this structure file, as the next topic describes how to reopen the file and edit it.

This procedure describes how to edit a list of fragments.

✤ To edit a fragments list

1. In the Modules & Tools toolbar, click Structure Grid.

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

- 2. In the Structure Grid toolbar, click **Open**.
- 3. Browse to the SDF file that you created in the previous topic, "create a fragments list and save it to an SDF file," and click **Open**.
- 4. If you are editing the structure list that you created in the previous procedure, skip step 5 and step 6.

Note In the previous procedure, you created a structure grid from a set of FISh fragments, so the grid does not include any low m/z value fragments or fragments that do not match the parent structure.

Edit a fragments list

- 5. In the Structure Grid toolbar, use the filters and the Delete button to remove structures that can lead to false positives:
 - Filter out fragments with low *m/z* values.
 - Delete fragments that do not match any part of the parent structure.
- 6. In the Structure Grid toolbar, use the Delete button to Remove duplicates.
- 7. If the fragments list does not include the parent precursor ion, add it. To add the protonated ion of omeprazole (m/z 346) for the tutorial example, do the following:
 - a. In the Structure Grid toolbar, click **Add New**.

The Structure Editor opens.

- b. In the Structure Editor, click the **Open** icon, 📥, select the omeprazole.mol file, and click **Open**.
- c. Protonate the omeprazole structure by adding an unspecified charge site (select H^+).
- d. Click OK.

Note The FISh analysis uses the parent precursor ion for the Mark Precursors feature and for calculating the neutral loss masses.

- 8. In the toolbar, click Save, and then click All.
- 9. Locate the appropriate folder, name the edited fragments list, and click Save.
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