Mass Frontier 8.1 tutorial to Curate Spectral Trees for User Libraries

In the Mass Frontier[™] 8.1 software, you use the Chromatogram Processor module to detect and identify components in your chromatographic data and the Curator module to create high-quality library entries for the identified components of interest.

This tutorial steps you through the component detection and mass spectra curation processes.

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Prerequisites

To complete the procedures in this tutorial, your installation of the application must have the required software and licenses.

Required This tutorial uses the Server Manager 8.1 application to create a user library. Make sure you have software installations of both the Mass Frontier 8.1 and Server Manager 8.1 applications on your processing computer.

Required This tutorial uses the Curator module to perform the curation process. Make sure you have licenses for all licenses the Mass Frontier modules, including the Curator module.

> Note Thermo Fisher Scientific provides the following types of licenses for the Mass Frontier 8.1 application:

- 60-day trial version—The trial version of the application includes licenses to all the modules, including the Curator module.
- Permanent versions—You can purchase the permanent version of the application with or without the Curator module license. Refer to the Mass Frontier Release Notes for more information.

To check the state of module licenses

1. From the application tab bar, click the **Start** tab and then choose **About**. See Figure 1 on page 2.

The About Mass Frontier dialog box opens to the About page, which lists the installed modules.

2. Make sure that the Curator module is licensed.

Figure 1. About Mass Frontier dialog box

M N	Ab	oout Mass Frontier					x	
	Ab	out System User Lic	ense				-	
	Ma Ver © The Ins	ss Frontier 8.1 sion 8.1.71.0 1998 - 2023 Thermo Fisher S ermo Fisher Scientific Inc. talled Modules:	icientific Inc.					Check the state of
		Module Name	Author	Version	Build Date	State		the Curator license
		Chromatogram Processor	Thermo Fisher Scientific	8.1.71	01.03.2023 18:04	Licensed	-	
	÷.	Curator	Thermo Fisher Scientific	8.1.71	01.03.2023 18:07	Licensed		
		Data Manager	Thermo Fisher Scientific	8.1.71	01.03.2023 18:06	Licensed		

Demo data files

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

File	Description
Aceclofenac B02.raw	A raw data file acquired by infusing a solution of aceclofenac into an Orbitrap Fusion Lumos [™] mass spectrometer. Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID).
Aceclofenac.mol	A structure file that contains the molecular structure for aceclofenac
Aceclofenac_Fragments.sdf	A structure file that contains fragment structures for the parent compound—aceclofenac
Aceclofenac.chpro_direct	Parameters file that contains the DICD parameter settings for the example raw data file
Aceclofenac.curator_srp	Curator parameters file for the Select Relevant Peaks action step

Create a user library

The Mass Frontier application uses a library service to connect to its mass spectral libraries. To add compound entries to a Mass Frontier user library, you must first create the library and connect the library service to it.

Use the Server Manager 8.1 application to create user libraries for your mass spectral data.

✤ To create a user library

- 1. To open the Server Manager, do one of the following:
 - From the Windows[™] Start menu, choose **Thermo Mass Frontier 8.1 > Mass Frontier Server Manager 8.1**.
 - From the desktop, double-click the **Mass Frontier Server 8.1** icon,

Mass Frontier Server Manager 8.1					- 0	×
File Library Help						
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Servers #				Properties		ą
TF-791741361871\PGMA	🙆 Start Page			▼ 🗄 🗮 Search		
HighChem Fragmentation Lib HighChem Fragmentation Library	D 10 8			✓ Server Info		
Elavanoida	Recent Servers *	Actions		Server Name	TF-7917413	_
My Flavanoids	Connect Legacy Server			Server Version	15.1.0	_
Steroids	TE-701741361871\DGMASSER			Edition		_
My Steroids		Croste Library	Delete Library	Is Full-text En	V	
Antibiotics		Creates new library	Permanently deletes library	Library mo	4000	
Antibiotics				Kind	Fragmentati	-
				Created By	High Chem	
		Back Up Library	Restore Library	▶ Name	HighChem F	
		Backs Up library	file	▶ Display Name	HighChem F	. 0
				Description		
				Status	Online	
		Migrate Library		Is User Acces	\checkmark	
		Migrates library		Is Copy to Cli		
				Is Read Only	\checkmark	
				Location	Data: C:\Use	
				> Size	1351.32 MB	
Settings				record Count	7/28/2022	
💐 Error List				4	112012022	
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0 orrors						

2. In the Actions pane, of the Start Page, click Create Library.

The Create Library page opens. You must enter the database name in the Name box; the display name is optional.

🗟 Start Page	异 Create Library	•
Name	Enter name	Database name is mandatory.
Display Name		It must not exceed 123 character's length and to contain only alphabetical or numeric characters.
Library Locatio	1	
C:\Users\Publi	c\Documents\HighChem\Libraries\PGSQL\$MASSFRONTIER8	ER81
	Cre	Close

- 3. For this tutorial, do the following:
 - a. In the Name box, type NSAIDs.

The Create Library tab changes to the Create Database - Database Name tab.

b. In the Display Name box, type NSAIDs.

Note The database name and the display name can contain only alphabetical and numeric characters and cannot be more than 123 or 128 characters in length, respectively.

Figure 3. Database name and display name

Name	NSAIDs
Display Name	NSAIDs
Library Locatio	n

- 4. Click Create.
- 5. Close the Server Manager application.

In the Mass Frontier application, you use the Chromatogram Processor module to read an Xcalibur™ RAW data file, detect components in the chromatographic data, and inspect the spectral tree for each detected component.

Follow these topics in order:

- 1. Open a raw data file
- 2. Detect components
- 3. Review the detected component

To open a raw data file

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 first time you open the application after installing it, the application opens to the startup window with the Modules & Tools toolbar at the top and the startup pane at the left (Figure 4).

The application tab bar contains these four tabs:

- Start—Displays the Start menu.
- Home—Displays the toolbar for the active module or tool.
- Modules & Tools—Displays the Modules & Tools toolbar.
- Search—Displays the Search toolbar.

Open a raw data file and detect components

Open a raw data file

Application tab bar									
Modules & Tool	s toolbar								
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Chromatogram Processor	E Structure Grid Satch Fragme SledgeHamm	d ent Generation er	Formula Generator	C 😚 🖬	ic Reaction Mechanism Overview				
Modules Open Image: Chromatogram Processor Data Files (LC/MS) Curator Curator Files New Image: Chromatogram Processor Chromatogram Processor Curator Curator Curator Curator Global Settings About Image: Show this Window Next Time	6 0	earch		Iools Size:	Large				
Show this Window Next Tr	up pane				.#				

Tip 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 (Figure 5).

2. In the Modules & Tools toolbar, click **Chromatogram Processor**.

Figure 5. Application window with the Modules & Tools toolbar displayed but without the startup pane

Moc	Appli	ication tab	bar					
■ + · · · · · Mas	Aodules & Tools	Search Search					T	- I2 -
Chromatogram Process Curator	sor 🕹 Metabolika 🔊 Structure E	átor ∱ rid	C H N (Form Genera	ula Isotope ator Pattern	MolGate Search	Periodic Table	Reaction Mecha Overview	anism
Mod	ules	×			Тос	ols		*

- 3. In the Open Chromatogram dialog box, do the following:
 - a. Browse to the following folder:

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

b. Select the Aceclofenac B02.raw file and click Open.

Note Aceclofenac, the compound that you are creating a curated spectral tree for, is a nonsteroidal anti-inflammatory drug (NSAID). The raw data file for this compound was acquired by direct injection (without chromatographic separation) into an Orbitrap Fusion Lumos mass spectrometer set to acquire data from 1.52 to 7.89 minutes after the run start time. For more information about the raw data file, open the Info page of the chromatogram view in the Chromatogram Processor window.

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

- On the upper left, the chromatogram data view lists the MS1 and product scans retrieved from the scan headers.
- On the lower left, the command processor view is empty, as you have not yet applied any actions to the chromatogram.
- On the upper right, the chromatogram view displays the total ion current (TIC) chromatogram.
- On the lower right, the MS spectrum view displays the scan selected in the chromatogram data view.

Figure 6. Chromatogram Processor page with the initial view for the selected raw data file

Click this tab to open the Info page, which contains information about how the data file was acquired.



For a detailed description of the Chromatogram Processor module, refer to the *Mass Frontier 8.1 User Guide*.

Detect components Use the Direct Infusion Component Detection algorithm to detect components in a solution that was infused into the mass spectrometer without any chromatographic separation. **Tip** In this tutorial, you are working with data from a direct infusion experiment and use the DICD algorithm for component detection.

For other data files, do the following:

- For a direct infusion experiment, use the Direct Infusion Component Detection (DICD) algorithm.
- For an LC/MS experiment, use the Joint Components Detection (JCD) algorithm.

To detect components in mass spectral data acquired with an infusion experiment

1. In the Actions group of the Chromatogram Processor toolbar, click **DICD** (Direct Infusion Component Detection).

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JCD	TECD	DICD	FISh
Actions		3	
		DICE) y Direct I

The Direct Infusion Component Detection view opens at the right of the application window.

Figure 7. Default settings for the DICD algorithm

Direct Infusion Components Detection	щ	×
🔊 💾 📂		
General		
Beginning of Tree Branching: 2 🗘 MS Stage		
Threshold Ion Intesity: 0.2 🗘 %		
Include Upper Spectra Average Scans Calculate Envelope		
Advanced		
Retention Time Range 0.0011 0 10		
50 € 995.2		
Preview Restore Accept Cancel		

- 2. In the Direct Infusion Components Detection view, do the following:
 - a. Click the **Reset** icon, **O**, to make sure that all the parameters are set to their default settings.
 - b. Click the Load Parameters from a File icon, 🔤.

The Direct Infusion Components Detection dialog box opens to the Chromatograms folder (the last opened folder).

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

c. Select the following Direct Infusion Parameters File, and then click Open.

Aceclofenac.chpro_direct

The application applies the parameter settings from the file (Figure 8).

Figure 8. Modified parameter settings from the selected Direct Infusion Parameters File

Tip To create library entries for a custom spectral library, infuse a solution that contains only the compound of interest into the mass spectrometer.

To detect this compound in the raw data and create its initial spectral tree, Thermo Fisher Scientific recommends the following parameter settings:

- Beginning of Tree Branching: 1
- Threshold Ion Intensity: 0

Use the Curator module to remove electronic and chemical noise peaks from a compound's spectral tree.

• Calculate Envelope: Selected

d. Click Preview.

After the analysis finishes, a list of the detected components appears in the chromatogram data view, and a spectral tree for the detected component appears to the left of the Spectrum page.

The Components list includes information about the highest MS stage, retention time, and abundance for the detected component.

The spectral tree is a hierarchical representation of the data-dependent MSⁿ scans that consists of one or more nodes for each MSⁿ stage. Each node consists of spectra from the same precursor pathway, but with different collision energies, activation types, and isolation widths.

Note To reduce the number of individual spectra for each node, the application combines scans with the same precursor pathway, activation type (CID or HCD), collision energy, and isolation width (IW) into a single combined spectrum.

With the parameter settings shown in Figure 8 on page 8, the analysis detects one component in the example data file. Until you accept the results, the Chromatogram Processor window remains in the Modified & Read Only state.



e. To accept the results, click **Accept** in the Direct Infusion Components Detection view.

The DICD view closes, the Command Processor view lists Direct Infusion Component Detection as an applied action, and the state of the Chromatogram Processor window changes to Modified (Figure 9).

Figure 9. Chromatogram Processor window showing the total ion current trace (TIC) and spectral tree for the detected component



State after you accept the results: Modified -

Review the detected component

* To review the results for the detected component

- 1. Select the component in the Components list.
- 2. In the chromatogram view, open the display options menu by clicking the down arrow at the top right of the view.



To display the trace for the component, clear the Show TIC check box in the display options menu.
 Figure 10. Options menu for the TIC page of the chromatogram view



The component's trace appears by itself in the chromatogram view. Green is the default color for a component trace.



- 4. To view the component's XIC (extracted ion current trace), do the following:
 - a. In the chromatogram data view, right-click the component in the Components list and choose **Show XIC m/z 0.000000; 354.0290**.



The chromatogram view displays the XIC trace in green and the component's trace in lime green.



b. To view the XIC trace by itself, open the display options menu and clear the **Show TIC**, **Show All Components**, and **Show Selected Components** check boxes.



c. To zoom in on the *y*-axis range of the XIC trace, drag the mouse pointer down the *y*-axis in the range that you want to view, from the highest value to the lowest value, and release.



TIC	2D Contour	3D	Info	Filter: 📑	All			-					
1,600,	000											h	n 🔍 (~ +
1,200,	000												
1,000,	000												
800,	000												
600,	000												
400,	000-				1								
200,													
	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5

This figure shows the magnified XIC trace on the TIC page of the chromatogram view.

- d. To undo the zoom, click the **Reset Zoom** icon, \mathbb{B}^{3} .
- 5. To review the component's spectral tree, do the following:
 - a. To view the folded tree, which shows only the precursor m/z value for each node, click the **Fold** Whole Tree icon, \Box .

Figure 11 shows the folded tree. The tree contains one node for the MS^2 level and nine nodes for the MS^3 level.

Figure 11. Folded tree

Fold whole tree Double click: Fold/Unfold node Shift + Double click: Fold/Unfold whole tree Ctrl + Double click: Compress/Expand node				
		MS1		
	3	354.0290		
147.63498 178.28578	180.08138 214.04155 2	15.04947 216.05327	250.01826 278.01309	362.5070
< 1/13 FT MS1 Scan #1				-)

b. To browse the scans for a node, click the node of interest in the tree, and then use the scan list below the tree to browse the scans on the Spectrum page to the right.

The selected node changes to blue and the scan list contains lists the scans for the selected node.



c. To return to the unfolded tree view, click the **Unfold Whole Tree** icon, \square .

Curate the spectral tree for a component

- To curate a component's spectral tree, follow these topics in order:
- 1. Send the component to the Curator module and specify its structure
- 2. Select the steps for the curation process
- 3. Select the processing mode
- 4. Use the semi-auto mode to step through the curation process

To curate a component's spectral tree, you send the component to the Curator module.

Note You can add the structure annotation to the component before or after you send the component to the Curator module by running a library search or an mzLogic analysis. In this tutorial, you add the structure annotation in the Curator module.

To send a component to the Curator module and specify its structure

- 1. In the Components list of the Chromatogram Processor window, select the component.
- 2. In the Send To group of the Chromatogram Processor toolbar, click **Component** and select **New Curator**.



This action sends the spectral tree for the selected component to the Curator module. A new instance of the Curator module opens as a tabbed page, but it needs a structure to continue processing.

3. At the prompt, click OK.

The Structure Editor dialog box opens.

- 4. To import a structure for the selected component, do the following:
 - a. In the Structure Editor dialog box, click the **Open** icon, 📂.



b. Browse to the following folder, select the Aceclofenac.mol file, and click Open.

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

The two-dimensional structure appears in the Structure Editor's drawing area.

c. Click **OK** to import the structure into the Curator's structure pane.

On the Curator page, the selected structure appears in the upper left pane, and the raw spectral tree for the selected component appears in Raw tree view on the upper right (Figure 12 on page 14).

Send the component to the Curator module and specify its structure



Figure 12. Curator page with the structure and spectral tree for the selected component

review and set the parameters settings at each of the selected steps.

Table 1. Processing modes (Sheet 2 of 2)

Processing mode	Description
Semi-Auto	(Recommended) Starts a wizard that runs all the remaining action steps, with a pause between each step for previewing and optimizing the parameter settings.
\checkmark	Use this mode to review and optimize the parameter settings at each of the selected steps during the curation process. Each settings dialog box has an Apply & Continue in Semi-Auto mode button.
	Automatically runs all the remaining action steps in the curation process by using the predefined parameter settings until it reaches step 8.
Auto	Use this mode to automatically run through the curation process with the default settings or the settings from a Mass Frontier curator actions (.curator_act) file.
V	Note Thermo Fisher Scientific recommends that you preview and optimize the parameter settings for each action step before using the Auto mode. After you optimize the settings, you can save the parameter settings to a .curator_act file for future use.

Use the semi-auto mode to step through the curation process The curation process refines the spectral tree for a library entry by doing the following:

- Removes low-quality scans (node items) below a specified intensity or S/N threshold, and then refines each spectrum by removing FT artifacts and unexplained spectral peaks (Filtered tree view)
- Recalibrates the *m/z* values of the explained spectral peaks by using their adduct ion formulas. (Recalibrated tree view)

This tutorial shows you how to use the Semi-Auto mode to step through the curation process.

* To curate the spectral tree for the component using the semi-automatic mode

1. Select all the **Show Settings** check boxes in the action step pane.

Figure 13. Action step pane with all 12 check boxes selected

Þ	만 🔻	₹ § 9 9 🌣 🖉		
#	Show Settings	Action Step	Status	
1	\checkmark	Raw Spectra Exclusion		^
2		Copy to Filtered Tree		
3	\checkmark	Select Significant Spectra		
4	\checkmark	Remove Resonance Peaks		
5	\checkmark	Select Relevant Peaks		
6	\checkmark	Merge Replicate Spectra		
7		Apply Changes to Raw Tree		
8	\checkmark	Assign Fragments (Raw, Filtered)		
9		Copy to Recalibrated Tree		
10	\checkmark	Recalibrate		
11	\checkmark	Assign Fragments (Recalibrated)		
12	\checkmark	Assign Molecular Formulas		
				-

Start the wizard

2. To start the wizard, click the **Semi-Auto** icon, $\mathbf{\nabla}$.

The Raw Spectra Exclusion dialog box opens.

Figure 14. Raw Spectra Exclusion dialog box for action step 1 with the default settings

Raw Spectra Exclusion	
Remove Step Energies	
Remove spectra with Isolation Width wider than 3 C amu	Preview
	Restore
	Apply & Continue in Semi-Auto mode
1 Action that removes step energies and spectra with wide isolation width from raw tree.	Cancel

3. To temporarily apply the default parameter settings, click Preview.

On the Tree Processing page, a red border indicates that a spectrum (node item) meets the removal criteria. None of the scans in the component's spectral tree meet the removal criteria (Figure 15).

Note The processing buttons on the Curator toolbar are unavailable. Clicking the Apply & Continue in Semi-Auto mode button on the wizard page is the only way to move forward through the curation steps.

Figure 15. None of the scans (node items) meet the removal criteria



Continue to action step 3

4. To apply the default settings and continue to the next step, click **Apply & Continue in Semi-Auto Mode** on the Raw Spectra Exclusion page of the wizard.



The application automatically applies the Raw Spectra Exclusion and Copy to Filtered Tree Steps, and then opens the Select Significant Spectra dialog box (Figure 17 on page 17).

Figure 16 shows the Raw tree view and the Filtered tree view that open after the curation process begins (completes action step 2).

Tip You can use the buttons in the View group of the Curator toolbar to close or open the Raw tree and Filtered tree views or to synchronize the spectrum selections for these two views.

You can remove individual spectra or complete nodes from the Raw tree at any time during the curation process by right-clicking the Raw tree view and choosing **Delete Spectra** or **Delete Node**, respectively.

Figure 16. Status of the curation process following the first two steps

📱 Chromatogram Processor - Aceclofenac B02.raw [Modified]	🗞 Curator - Aceclofenac B02.curator [Modified] 🗴
₽\$0	Tree Processing Metadata
	🛔 Raw 🔺 🛔 Filtered 🔺
Formula: C ₁₈ H ₁₉ Cl ₂ NO ₄	
Exact mass: 353.02216 Polarity: Positive	
Contributor: 🍃	
© ‡ ८ ८ ३ ≈ ∞ । 🗄 🛋	
# Show Action Step Stat	
1 🗹 Raw Spectra Exclusion	Û
2 Copy to Filtered Tree	
3 Select Significant Spectra	▼ 1/13 FT MS1 Scan #1 ▼ ▼ ▼ ▼

- 5. To remove spectra below a specified S/N threshold or intensity level, do the following:
 - a. In the Select Significant Spectra dialog box, click **Preview**.

Figure 17. Select Significant Spectra dialog box for action step 3 with the default settings

A server as Factors		
Minimal S/N Ratio	1 • Aut	Proviow
🗹 Minimal Absolute Intensity		Treview
FT Noise Method:		Ø
Ranges		Kestore
Combined		
🔘 Linear Fit		
O Histogram		
🔘 Median		
Filter Strength:	100 🕽 %	Apply & Continue in Semi-Auto mode
This action looks for spectra wit blue) and peaks with wrong S/N spectra are removed.	h low intensity peaks (marked as I ratio (marked as red). All marked	Cancel

b. Review the Raw tree view and the Filtered tree view on the Tree Processing page of the Curator window (Figure 18 on page 18).

On the Tree Processing page, a red or blue border indicates that a spectrum meets the removal criteria:

- A blue border indicates that the spectrum's base peak is below the specified minimum intensity level.
- A red border indicates a that the signal-to-noise level for the spectrum is below the specified threshold.

With the default thresholds of 3 for S/N and 1000 for the base peak (minimal absolute) intensity, you can see that some of the MS3 scans (node items) are highlighted for removal based on their S/N levels, and some of the MS3 scans are highlighted for removal based on the intensity of their base peaks (Figure 19 on page 18).

Figure 18. Scans selected for removal in action step 3 (node items with a red border)



You can view the individual scans for a node by selecting the node and then using the scan list below the spectral tree pane. There are quite a few low-intensity scans at the MS3 level.

Figure 19 shows a low-intensity scan for the precursor ion at m/z 147.63498.

Figure 19. Filtered tree with scans marked for removal, showing the spectrum for scan #16

Scan with a base peak intensity below 1000 as indicated by the blue border



In scan #16, the base peak at m/z 156.43515 is below 1000 counts.

Tip To toggle the y-axis scale from relative intensity to absolute intensity, right-click the Spectrum pane and choose **Show Absolute Intensities**.

Continue to action step 4

c. To apply the default settings and move to the next step in the curation process, click **Apply & Continue in Semi-Auto Mode** to apply the default settings and move to action step 4 in the curation process.

The Remove Resonance Peaks dialog box opens.

Figure 20. Remove Resonance Peaks dialog box for action step 4 with the default settings



- 6. To identify and remove resonance peaks from the spectra, do the following:
 - a. In the Remove Resonance Peaks dialog box, click Preview.
 - b. In the Filtered tree view, review the spectra.

If the application identifies an m/z peak as a resonance peak, it highlights the peak in red. With the default settings, the application does not find any resonance peaks in the spectra.

Note Resonance peaks are m/z peaks generated by the Fourier Transform (FT) or Orbitrap analyzer, not m/z peaks for chemical entities in the infused sample.

c. In the Remove Resonance Peaks dialog box, click **Apply & Continue in Semi-Auto Mode** to apply the default settings and move to action step 5.

The Select Relevant Peaks dialog box opens to the Basic view ().

Note Use the Select Relevant Peaks dialog box to select the MS¹ adducts, the MS¹ neutral losses, and the MSⁿ collision cell adducts.

- 7. To remove the unexplained peaks from the spectra, do the following:
 - a. In the Select Relevant Peaks dialog box, click Advanced to display all the parameters.

These additional parameters appear (Figure 21 on page 20):

- In the General area—Show Threshold Warnings and Remove Noise Spectra
- In the Precursors area—Minimal Required Intensity of MS¹ Adduct
- In the Isotope Profile area—Check Isotopic Profile in MSⁿ Scan

Continue to action step 5

• The Formula Generator area with the following parameters—Check Graph Rule, Check Hydrogen Rule, Check RDBE, Nitrogen Rule, Probability Approach, and Threshold

Note Use the parameters in the Formula Generator area to modify the set of predicted formulas that the application uses to explain the peaks in the spectral tree.

Tip The wizard automatically selects the appropriate MS1 Adducts for the spectral tree (Figure 21).

If you change the parameter settings, click **Auto Suggest** to automatically select suitable molecular ions.

Advanced Load Fromparameters icon Select Relevant Peaks _ 8 × - General Check Molecular Ions Accuracy Factor: 1 ٢ Show Threshold Warnings: 50 🗘 % Remove Noise Spectra Basi Preview 1 Precursors Analyze Precursor by Parent Scan Minimal Required Intensity of MS¹ Adduct: 1 🗘 % ha 🗄 🛠 MS¹ Adducts 🕴 Auto Suggest [M + CH3OH + H]* [M + ACN + H]* M⁺ ' 353.02161 [M + H]* [M + Na] 376.01138 [M + K]⁴ [M + Li]* [M + NH₄] [M + 2Li - H]* [M + 2Na - H]* [M + ACN + Na] [M + IsoProp + H]* [M + 2K + H] [M + DMSO + H] [M + 2ACN + H]* [M + 2H]² 177,51836 [M + H + Na]² [M + H + NH4]²⁺ $[M + H + K]^{2}$ [M + ACN + 2H]² [M + 2Na]² [M + 2ACN + 2H]2+ [M + 3ACN + 2H]2+ [M + 3H]³ [M + 2H + Na]3+ [M + H + 2Na]3+ [M + 3Na]3 [2M + H]* [2M + NH4]* [2M + Na] [2M + K]* [2M + ACN + H]* [2M + ACN + Na]* [2M + 3H₂0 + 2H]² 381.04529 Include MS¹ Neutral Losses [M - C2H7N] [M - H₂O] [M - CO₂] [M - NH3] [M - Hexose] [M - Deoxyhexose] [M - Pentose] [M - Pentose-pentose -264.08452 [M - Pentose-hexose] [M - Hexose-hexose] [M - Glucuronide] MSⁿ Collision Cell Adducts [M + 0] [M + H₂O] [M + 2N] Formula Generator Check Isotopic Profile in MS¹ Scan 🗹 Check Graph Rule l Check Isotopic Profile in MSⁿ Scan Check Hydrogen Rule 🗹 Check RDBE Min: -2 🗘 Max: 250 🗘 $\overline{\mathbf{v}}$ I Nitrogen Rule: Not Used Apply & Continue . Semi-Auto mode Probability Approach Threshold: 80 1 % X 1 This action explains p taks in the spectra. Cance

Figure 21. Select Relevant Peaks dialog box for action step 5 with the advanced parameters displayed

Advanced parameters

 b. To use the provided Select Relevant Peaks Parameter File, click the Load From icon, Then, in the Select Relevant Peaks dialog box, select Aceclofenac B02.curator_srp, and click Open (Figure 22 on page 21).

If the dialog box does not open to the following folder, browse to it:

drive:\Users\Public\Public Documents\HighChem\Mass Frontier 8.1\Demo Data\Curator Figure 22. Location of Select Relevant Peaks File

Select Relevant Peaks					×
$\leftarrow \rightarrow \land \uparrow$	📜 « Users > Public > Public Docume	ents > HighChem > Mass Frontier 8.1 >	Demo Data > Curator	~ C	♀ Search Curator
Organize • New fo	older				≣ • 💷 💡
✓	Name	Date modified	Туре	Size	
> 🛄 Desktop	Aceclofenac B02.curator_srp	2/11/2023 4:13 PM	CURATOR_SRP File	1 KB	
> 🧧 Documents					
> 🛓 Downloads					
N 🕰 Musia					
File	name: Aceclofenac B02.curator_srp			~	Select Relevant Peaks Parameter $ \! \! \! \! \! \! \! \! \! \! \! \! \! \! \! \! \! $
					Open Cancel

Figure 23 shows the modified settings from the selected parameter file:

- Accuracy Factor: 1.5
- Analyze Precursor by Parent Scan: Selected
- Selected MS¹ adducts: [M+H]⁺, [M+Na]⁺, [M+2Na-H]⁺
- Selected MSⁿ Collision Cell Adducts: [M+2N] and [M+O]

Figure 23. Modified settings from the Select Relevant Peaks Parameter File

Select Relevant Peaks											
General Accuracy Factor: 1.5	:		🗹 Check	Molecular Ions			4				
Show Threshold War	rnings: 50 🗘 %	ngs: 50 🗘 %	is: 50 🗘 %		☑ Remove Noise Spectra		🗹 Remove Noise Spectra			Basic	Preview
Precursors Analyze Precursor by Inimal Required Inter	/ Parent Scan nsity of MS ¹ Adduct:	1 🗘 %						Ø Restore			
IS ¹ Adducts	🌻 Auto Suggest										
M ⁺⁺ 353.02161	[M + H] ⁺ 354.02944	[M + Na] ⁺ 376.01138	[M + K]* 391.98532	[M + Li] ⁺ 360.03762	[M + NH ₄] ⁺ 371.05599	[M + CH ₃ OH + H]' 386.05565					
[M + ACN + H]* 395.05599	[M + 2Na - H] ⁺ 397.99333	[M + 2Li - H] ⁺ 366.04580	[M + IsoProp + H]*	[M + ACN + Na]* 417.03793	[M + 2K + H] ⁺ 431.95685	[M + DMSO + H]* 432.04338					
[M + 2ACN + H] ⁺ 436.08254	[M + 2H] ²⁺ 177.51836	[M + H + NH4] ²⁺ 186.03163	[M + H + Na] ²⁺ 188.50933	[M + H + K] ²⁺ 196.49630	[M + ACN + 2H] ²⁺ 198.03163	[M + 2Na] ²⁺ 199.50030					
[M + 2ACN + 2H] ²⁺ 218.54491	[M + 3ACN + 2H] ²⁺ 239.05818	[M + 3H] ³⁺ 118.68133	[M + 2H + Na] ³⁺ 126.00865	[M + H + 2Na] ³⁺ 133.33596	[M + 3Na] ³⁺ 140.66328	[2M + H] ⁺ 707.05160					
[2M + NH4] ⁺ 724.07815	[2M + Na] ⁺ 729.03355	[2M + K]* 745.00749	[2M + ACN + H] ⁺ 748.07815	[2M + ACN + Na]*	[2M + 3H ₂ 0 + 2H] ²⁺ 381.04529		-				
Include MS ¹ Neutra	l Losses										
[M - H ₂ O] -18.01056	[M - CO ₂] -43.98983	[M - C ₂ H ₇ N] -45.05785	[M - NH ₃] -17.02655	[M - Hexose] -162.05282	[M - Deoxyhexose] -146.05791	[M - Pentose] -132.04226					
[M - Pentose-pentose] -264.08452	[M - Pentose-hexose] -294.09508	[M - Hexose-hexose] -324.10565	[M - Glucuronide] -176.03209								
S ⁿ Collision Cell Addu	ıcts										
[M + 2N] 28.00615	[M + O] 15.99491	[M + H ₂ O] 18.01056									
sotopic Profile Check Isotopic Profil Check Isotopic Profil	e in MS ¹ Scan e in MS" Scan pes			Formula Generator Check Graph Rule Check Hydrogen Rul	e 2 * May 250	•					
	e Threshold: 5			Nitrogen Rule: Not U	sed • Threshold: 80	\$ %		Apply & Continu			
This action explains	s peaks in the spectra.							In Semi-Auto moc			

c. Click Preview.

d. To view each spectrum in the Spectrum pane, click through the scans (node items) in the Filtered tree view.

The application color-codes the spectral peaks.

Color	Meaning
Red	Relevant peaks explained by the adducts and generated formulas
Blue	Unexplained peaks
Orange	Unexplained peaks with a relative intensity that is higher than the Show Threshold Warnings parameter setting. When a node item (spectrum) contains a number of unexplained peaks with a relatively high-intensity, the node item's border appears in a shade of red. As the number of unexplained peaks increases, the saturation of the red color increases.

Figure 24 shows a preview in the Spectrum pane for one of the MS² scans. The explained peaks are red and the unexplained peaks are blue.





Continue to action steps 6 & 7

e. Click Apply & Continue in Semi-Auto Mode.

The application removes the unexplained peaks (in blue) from the spectra and continues to the next step.

The Merge Replicate Spectra dialog box opens (Figure 25 on page 23).

8. In the Merge Replicate Spectra dialog box, click **Preview**.

Figure 25. Merge Replicate Spectra dialog box for action step 6 with the default setting

Merge Replicate Spectra	_ – ×
Minimal occurrence of a peak in replicate spectra per spectral group 55 36 TIC threshold 50 36	© Preview
Accuracy Factor: 1.5	Ø Restore
	Apply & Continue in Semi-Auto mode
This action uses filtered tree and tries to find spectra with the same Energy, Mass Range, Analyzer, IonActivation, Isolation Width and creates one merged spectrum. During merging, intensities of the same peaks are added together.	Cancel

Note The Merge Replicate Spectra step merges replicate scans into a single spectrum and adds the intensities of peaks with the same m/z value. Replicate spectra are spectra from the same analyzer, ion activation type, collision energy, isolation width, and mass range.

Figure 26 shows the filtered spectral tree before the merge step.

Figure 26. Filtered spectral tree before the merge step



Figure 27 shows the filtered spectral tree after the merge step. Notice that the filtered tree contains one less MS3 node than the raw tree and far fewer scans (node items) per node.



Figure 27. Filtered spectral tree after the merge step



9. To apply the default setting for merging replicate spectra and continue the curation process, click **Apply & Continue in Semi-Auto Mode** (Figure 25 on page 23).

The application automatically runs the Apply Changes to Raw Tree step, and the Assign Fragments (Raw, Filtered) dialog box opens.

Tip The fragment assignment step requires a fragment structure list. In this tutorial, you use an SDF file provided in the Demo Data folder.

Outside this tutorial, if you have not already created an SDF file for the component you are curating, you can click **Structure > New SledgeHammer** in the Curator toolbar to send the component's structure to the Fragments & Mechanisms module for fragmentation. Click **Generate** to perform fragment prediction. After fragment prediction ends, return to the Assign Fragments dialog box, click the **Import** icon and select **Import Modules**, and then select the appropriate instance of the SledgeHammer module.

If you have multiple compounds to curate, use the Batch Fragment Generation module to generate a separate SDF file for each submitted compound.

- 10. To assign fragment structures to the peaks in the filtered tree's spectra, do the following:
 - a. In Structures to Assign area of the Assign Fragments (Raw, Filtered) dialog box, click the **Import** icon, ≝ -, and select **Import File**.

Accuracy Factor: 1.5 C Ø Clear Old Annotations Precusor Formula Constrain Min. Peak Height: 1 C %	Annotation Size Fragment Structures Zoom: 70 \$% Bond Length: 22 \$	Preview
Apply to Apply to Apply to Structures to Assign Market Processor Structures to Assign Market Processor Structures to Assign Market Processor Structures to Assign Market Processor Structures to Assign Structures to Assign Structur	 ■ Recalibrated Tree ■ To To To 24 S ■ L 	
Import File (Ctrl+O) Adds one or more cards with structures imp	ported from one or multiple selected files.	▼

b. Browse to the following folder, select the Aceclofenac_Fragments.sdf file, and click Open.

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

Figure 28. Location of the structure files

Open Structures				>
\leftarrow \rightarrow \checkmark \uparrow 📜 $<$ HighChem $>$	Mass Frontier 8.1 > Demo Data	> Structures	~ C	Search Structures
Organize 🔹 New folder				≣ • 💷
Name	Date modified	Туре	Size	
Aceclofenac.mol	2/11/2023 5:13 PM	MOL File	3 KB	
🖞 Aceclofenac_Fragments	2/11/2023 5:13 PM	SQL Server Compact	2,618 KB	
🚹 Flavonoids_C42H46O22	2/11/2023 5:13 PM	SQL Server Compact	80 KB	
] ofloxacin.mol	2/11/2023 5:13 PM	MOL File	4 KB	
省 ofloxacin_fragments	2/11/2023 5:13 PM	SQL Server Compact	5,304 KB	
File name: Aceclofenac_	Fragments		~	Known Structure Formats (*.sdf, $ imes $
				Open Cancel

The fragment structures appear as cards in the Structures to Assign pane of the Assign Fragments (Raw, Filtered) dialog box.

Figure 29. Assign Fragments (Raw, Filtered) dialog box for action step 8 with imported structures



c. Click Preview.

On the Curator page, the Filtered tree view shows the explained structures as annotations on the selected spectrum (Figure 30 on page 26).

Tip If the Tree Processing page does not automatically update, click a card in the Structures to Assign area of the Assign Fragments page of the curator wizard.







d. To assign the fragment structures to the appropriate peaks and move to the next step in the curation process, click **Apply & Continue in Semi-Auto Mode**.

The application automatically applies the Assign Fragments (Raw, Filtered) and Copy to Recalibrated Tree steps, and then opens the Recalibrate dialog box (Figure 31 on page 27).

- 11. To recalibrate the m/z values of the spectral peaks, do the following:
 - a. In the Recalibrate dialog box, click through the spectral tree on the left and view the calibration curves on the right.



Figure 31. Recalibrate dialog box for action step 10 with a preview of the calibration curve for the peaks the MS1 scan

b. Click **Preview**.

c. On the Tree Processing page on the Curator page, review the recalibrated peaks.

Note The Recalibrate step recalibrates the m/z value for each peak in the spectral tree by using the exact value for each peak, which it calculates from the peak's formula and fragment structure explanation, and the selected calibration model.

Figure 32 shows a filtered and annotated spectrum on the left with the original m/z values for each peak and a recalibrated spectrum on the right with the recalibrated m/z values for each peak.





This table shows the mass difference between the spectral peaks in the filtered and recalibrated spectra for the MS2 spectrum for precursor m/z 354.0290 (combined scans 4–124)

Filtered tree (<i>m/z</i>)	Recalibrated tree (<i>m/z</i>)
250.01830	250.01830
278.01316	278.01340
354.02910	354.02940



d. To apply the default settings and move to the next step in the curation process, click **Apply & Continue in Semi-Auto Mode**.

The Assign Fragments (Recalibrated) dialog box opens (Figure 33 on page 29).



12. In the Assign Fragments (Recalibrated) dialog box, click **Apply & Continue in Semi-Auto Mode** to apply the default settings, assign fragment structures to the recalibrated tree, and move to the next step in the curation process.

Figure 33. Assign Fragments (Recalibrated) dialog box for action step 11

Assignment Restrictions		Annotation Size	*	[
Clear Old Annotations		Fragment Structures Zoom:	70 🗘 %	٢
Precusor Formula Constrai	n	Bond Length:	22	Preview
/lin. Peak Height:	1 🗘 %			(h)
pply to				Restore
Raw Tree	Filtered Tree	Recalibrated Tree		
Raw Tree	📕 Filtered Tree 🗹	Recalibrated Tree		
 	Filtered Tree	■ Recalibrated Tree To Te	SML	
Raw Tree	Filtered Tree	Recalibrated Tree	s M L	
Raw Tree	Filtered Tree	Recalibrated Tree	M L	
Raw Tree	Filtered Tree	Recalibrated Tree	S M L	
	Filtered Tree ✓ Filtered Tree ✓ C+Hi C+Hi m(r 51 0220	Recalibrated Tree	S M L	Apply & Continu

The Assign Molecular Formulas dialog box opens (Figure 34).

13. To apply the default settings, assign molecular formulas to each peak in the tree, and complete the final step of the curation process, click **Apply & Continue in Semi-Auto Mode**.

Figure 34. Assign Molecular Formulas dialog box for action step 12 with the default settings

🖁 Assign Molecular Formulas		
Min. Peak Height:	1 🗘 %	0
Max. Molecular Formulas per Peak:	5 🗘	Preview
Apply to raw tree		
🗹 Apply to filtered tree		Ø
Apply to recalibrated tree		Restore
		Apply & Continue in Semi-Auto mode
This action assigns appropriate mo the tree. It uses peak explanations action.	lecular formulas to each peak in from the "Select Relevant Peaks"	Cancel

Note The Assign Molecular Formulas step assigns the molecular formulas from the Select Relevant Peaks step to each peak in the tree.

On the Tree Processing page, structure and molecular formula annotations appear in both of the Spectrum views.





Download metadata from public sources

You can add supplementary metadata from public data sources like ChemSpider and PubChem to your library entries.

To download metadata from PubChem

1. In the Processing group of the Curator toolbar, click **Download Metadata**.

The Download Metadata wizard opens (Figure 36).

Figure 36. Download Metadata wiza

Select services to merge	
ChemSpider	ChemSpider is a database of chemicals
PubChem	ChemSpider is owned by the Royal Society of Chemistry. The database contains information on more than 50 millior molecules from over 500 data sources.
	Next > Cancel

2. In the Download Metadata wizard, select the **PubChem** check box and click **Next**.

A list of matching PubChem entries appears.



3. In the list, select 71771, and then click Next.

The final page of the wizard appears.

Figure 37. Final page of the Download Metadata wizard

Download Metada	ita				_ □
Auto Merge	Capitalize Copy Fixed Names References Reload				
	PubChem			Result	
Names		▶ -	Names		*
Trivial Name	Aceclofenac	D •	Trivial Name	Aceclofenac	Ď
Systematic Name	2-[2-[2-(2,6-dichloroanilino)phenyl]ac	etyl]oxyacetic 📑 🕨	Systematic Name		ß
Identifiers		- 🕷	Identifiers		*
CAS		L. 1	CAS		Ē
SMILES	C1=CC=C(C(=C1)CC(=O)OCC(=O)O)N	IC2=C(C=CC	SMILES		۲
InChi	=C2CI)CI InChI=1S/C16H13Cl2NO4/c17-11-5-3	-6-12(18)16(1	InChI		۲ì
	1)19-13-7-2-1-4-10(13)8-15(22)23-9-: 7.19H.8-9H2.(H.20.21)	14(20)21/h1-	InChIKey		 N
InChIKey	MNIPYSSQXLZQLJ-UHFFFAOYSA-N	D >			
Synonyms		» - «	Synonyms		₽ ↔ -
Aceclofenac		D 🕨 📩			
39796-99-6					
Preservex					
Aceclofenaco					
Aceclofenacum					
Airtal					
Aceclofenacum [Lat	tin]				
Aceclofenaco [Span	ushj				
				< Previous	Finish Cancel

4. To copy an item from the Pub Chem column to the Result column, click the **Add Item** icon, ▶, to its right.

Add the following items:

- Systematic name
- SMILES
- InChI
- InChIKey

Figure 38. Result column with added items

Download Metada	ta			_	
Auto Merge Equivalents	Capitalize Copy Fixed Names References Reload				
	PubChem			Result	
Names		* •	Names		*
Trivial Name	Aceclofenac	3)	Trivial Name	Aceclofenac	ß
Systematic Name	2-[2-[2-(2,6-dichloroanilino)phenyl]acetyl]oxyacetic acid	ß	Systematic Name	2-[2-[2-(2,6-Dichloroanilino)phenyl]acetyl]oxyacetic acid	ß
Identifiers		> -	Identifiers		
CAS		5)	CAS		ß
SMILES	C1=CC=C(C(=C1)CC(=O)OCC(=O)O)NC2=C(C=CC= C2CI)CI	ß	SMILES	C1=CC=C(C(=C1)CC(=O)OCC(=O)O)NC2=C(C=CC=C2C) I)Cl	6
InChI	InChi=1S/C16H13Cl2NO4/c17-11-5-3-6-12(18)16(1 1)19-13-7-2-1-4-10(13)8-15(22)23-9-14(20)21/h1-7, 19H.8-9H2 (H.20.21)	ß 🕨	InChI	InChI=1S/C16H13Cl2NO4/c17-11-5-3-6-12(18)16(11)19 -13-7-2-1-4-10(13)8-15(22)23-9-14(20)21/h1-7,19H,8-9 H2.(H.20,21)	6
InChIKey	MNIPYSSQXLZQLJ-UHFFFAOYSA-N	D >	InChIKey	MNIPYSSQXLZQLJ-UHFFFAOYSA-N	ß
Synonyms		> -	Synonyms	72 😯	
Aceclofenac	Ć	1) -			
39796-99-6) 🕨 📗			
Preservex) 🕨			
Aceclofenaco) 🕨 📗			
Aceclofenacum) 🕨 📗			
Airtal) 🕨 📗			
Aceclofenacum [Lat	in]) 🕨			
Aceclofenaco [Span	ish]				
UNII-RPK779R03H) 🕨			
CHEBI:31159	میا ا	ð 🕨 📗			
				< Previous Finish Ca	incel

- 5. To import the metadata into your library entry, click Finish.
- 6. To skip searching for additional KEGG references, click No at the prompt.
- 7. To skip searching for additional Wikipedia references, click No at the prompt.
- 8. On the Curator page, click the **Metadata** tab to open the Metadata page and view the imported data (Figure 39).



Figure 39. Metadata page with metadata from the matching compound in the PubChem database

Save the curated spectral tree to a file After completing the curation process, save the curated spectra for aceclofenac to a data file or a user library.

* To save the spectral tree record to a curator file

1. In the Curator toolbar, click Save and select Save As.

The Curator Component File dialog box opens.

By default, the File Name box displays the file name of the supplied structure file.

2. Locate the folder where you want to store the file, select the Curator file type, and click Save.

Note You can save the curated record as a Mass Frontier Compound Data Container file (.mfcdc) or a Curator file (.curator).

The Mass Frontier Compound Data Container file stores the processed spectral tree with its corresponding spectra, precursor m/z, and peak annotations. You can open this file type in the Data Manager module.

The Curator file stores the processed spectral tree with its corresponding spectra, precursor m/z, and peak annotations and all the parameter settings for the completed action steps. You can open this file type in the Curator module.

Save a compound entry with curated spectra to a user library

- * To save the compound entry with curated spectra to a user library
- 1. In the Curator toolbar, click Save and select Save to Library.
 - The Save as Record dialog box opens.
- Select the New Record option, select the NSAIDs library for the new record, and then click OK.
 Figure 40. Save as Record dialog box

Save as Record			_ □ ×
New Record			
Add New Tree in Selected Compound (The Compound Will Not be Saved)	Raw	Filtered	Recalibrated
O Update Record			
Compound Tree			
		OK	Cancel

Tip If the Select Library list does not contain the library you created, make sure that the library service is Started as follows:

- 1. From Windows[™] Start menu, open the Search Programs and Files box and type Services.
- 2. In the Programs list, select Services.
- 3. In the Services (Local) dialog box, right-click Mass Frontier Services and choose Restart.
- 3. At the prompt, click **OK**.
- 4. To view the record in the user library, in the Modules & Tools toolbar, click Data Manager.

A new instance of the Data Manager module opens as a tabbed page.

5. In the tab bar in the middle of the Data Manager page, click the NSAIDs tab.

The saved record appears in the library record view.

🜻 New 🗴 🤐 mzCloud Reference 🗴 🤐 mzCloud Autoprocessed 🗴 🛛 HighChem Fragmentation Library 🗴	NSAIDs × + ⊞ ⊞ ■ ▼
Search	
ID: 1 ^	î
Aceclofenac	
1 compound(s) Compound '1' Spectral Tree: 1 Mechanisms: 0	Library Service Status 🔵 😔 📑

Tip If you do not see the saved record in your library, click **Reload** and select **Reload Compounds** to refresh the view.

	Note After you complete this tutorial, you can delete the new user library.
Summary	To create and populate a custom mass spectral library with curated library spectra for your analytes of interest, follow these steps.
	1. Infuse a relatively pure solution of the analyte into the mass spectrometer.
	2. Open Mass Frontier Server Manager 8.1, create a library, and then close the server manager.
	3. Open the raw data file in a Chromatogram Processor window.
	4. Apply the DICD algorithm with the following parameter settings: Beginning of Tree Branching: 1, Threshold Ion Intensity: 0, and Calculate Envelope: Selected.
	5. Do the following:
	a. (Optional) Run an identity search to annotate the component by clicking Components Search in the Search group of the Chromatogram Processor toolbar.
	 b. Send the unannotated or annotated component to the Curator by clicking Component > New Curator in the Send To group of the Chromatogram Processor toolbar.
	6. For an unannotated component, open a structure file in the Curator window.
	7. Run the Curator action steps and modify the settings as appropriate.
	8. Save the compound with its curated spectrum to your custom library by choosing Save > Save to Library from the Edit group of the Curator toolbar.
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	Xcalibur is a registered trademark of Thermo Fisher Scientific Inc. in the United States.
	KEGG is a registered trademark of Kanehisa, Minoru (an individual). Wikipedia is a registered trademark of the Wikimedia Foundation, Inc.
	All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.