

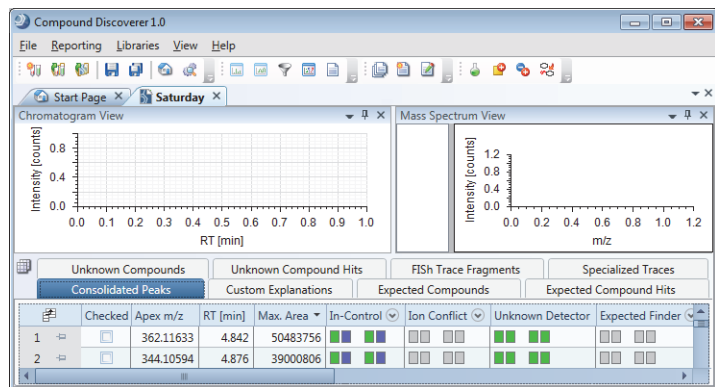
Compound Discoverer 1.0 Data Review Quick Start

This guide shows you how to review the graphical and tabular data in Compound Discoverer™ result files (.cdResult). You can review the data without an active software license.

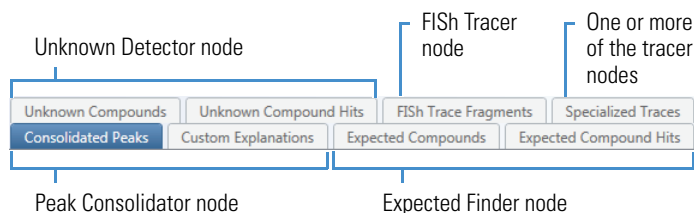
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A result file opens as a tabbed document with three main views: the Chromatogram View, the Mass Spectrum View, and a set of up to ten master tables. The Chromatogram View and Mass Spectrum View are independent graphical views; that is, working in one view does not affect the other view. The graphical views are empty until you select a row in one of these tables: Consolidated Peaks, Expected Compound Hits, Unknown Compound Hits, Expected Compounds, Unknown Compounds, Specialized Traces, or FISH Trace Fragments.



Depending on the processing workflow, the result page contains one or more of the following master tables.



The Custom Explanations table appears when the workflow contains the Peak Consolidator node; however, the table is empty until you create a custom explanation for an expected compound hit or unknown compound hit.

The Manual Peaks table (not shown) appears when you add a manual peak to a trace in the Specialized Traces table (see “[To integrate chromatographic peaks manually](#)” on page 2).

By default, the Adducts table (not shown) is hidden.

Opening a Result File

There are several ways to open a result file.

- From the Compound Discoverer menu bar, choose **File > Open Result**.
- On the Start Page, click the result file name, which is displayed in blue hypertext.

If the Start Page is closed, open it by choosing **View > Start Page** from the Compound Discoverer menu bar.

- From the Job Queue page, double-click a completed run.

If the Job Queue page is closed, open it by choosing **View > Job Queue** from the Compound Discoverer menu bar.

- From the Analysis Results page of a study, select the completed run of interest and click **Open Result** in the Analysis Results command bar.

Working with the Floating Views

You can change the following views to floating windows: the Chromatogram View, Mass Spectrum View, Result Filters view, Scatter Chart view, and Summaries view.

❖ To enlarge the views

1. Double-click the view's title bar to make it a floating window.
2. Drag the view to a second monitor.
3. Enlarge the view by dragging its edges.

Working with the Chromatogram View

The Chromatogram View is empty until you select a row in one of these tables: Consolidated Peaks, Expected Compounds, Expected Compound Hits, Unknown Compounds, Unknown Compound Hits, FISH Trace Fragments, or Specialized Traces.

Follow these procedures as needed:

- [To view a chromatogram](#)
- [To overlay chromatograms in one plot](#)
- [To integrate chromatographic peaks manually](#)
- [To apply a shortcut menu command in the Chromatogram View](#)

❖ To view a chromatogram

Do one of the following:

- Click the **Consolidated Peaks** tab and select a row.

The Chromatogram View displays the chromatographic peaks detected by both the Unknown Detector node and the Expected Finder node.

- Click the **Expected Compound Hits** tab or the **Unknown Compound Hits** tab and select a row.

The Chromatogram View displays the integrated chromatographic peak for the selected table row.

- Click the **Expected Compounds** tab or the **Unknown Compounds** tab and select a row.

The Chromatogram View displays a composite trace of all the chromatographic peaks detected for the molecular weight listed in the selected table row.

- Click the **FISH Trace Fragments** tab and select a row.

The Chromatogram View displays a trace for the structure displayed in the selected table row. The application displays the FISH Trace Fragments table when you select True for Individual Traces in the FISH Tracer node.

- Click the **Specialized Traces** tab, and then select a row.

Depending on the processing workflow, the Specialized Traces table can contain the following traces:

- A Mass Tracer node can generate any of these traces: extracted ion (mass range) chromatogram (XIC), base peak chromatogram (BPC), or total ion chromatogram (TIC).
- An Analog Tracer node can generate any of these traces: a UV-Vis trace from a UV-Vis or PDA detector, up to three traces from a PDA detector, or an analog trace from an LC detector that is connected to one of the analog input channels of a Thermo Scientific mass spectrometer.
- A Pattern Tracer node generates a TIC trace by summing the mass spectral peaks (across the entire scan) that match the user-defined isotope pattern.
- A FISH Tracer node generates the following:
 - A summed FISH trace of all the matching fragment ion scans (DDF or AIF) when you select True in the Summed Trace list.
 - An individual FISH trace for each fragment ion when you select True in the Individual Traces list. To view the individual trace for each fragment ion, see the FISH Trace Fragments table.

❖ To overlay chromatograms in one plot

1. Select a table row.
2. Do one of the following:
 - Hold down the SHIFT key and select the last row in the contiguous range that you want to overlay.
 - Hold down the CTRL key and individually select the rows that you want to overlay.
 - Hold down the SHIFT key and use the Down Arrow key to select a range of rows.

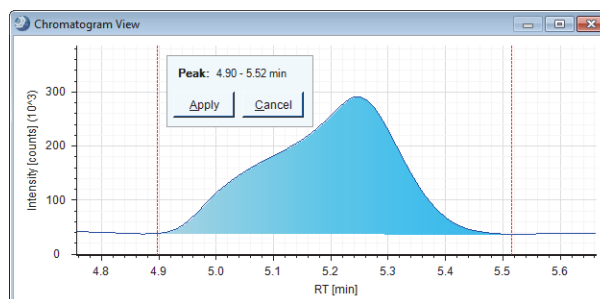
❖ To integrate chromatographic peaks manually

1. In the Specialized Traces table, select the trace of interest.
2. Right-click the **Chromatogram View** and choose **Manual Peak Integration** from the shortcut menu.

Two red dashed lines appear.
3. Drag one line to the beginning of the chromatographic peak and the other line to the end of the chromatographic peak.

The application highlights the integrated peak area in blue.
4. Move the cursor over the peak.

A pop-up box appears with the selected retention time range and Apply and Cancel buttons.



5. To add the manual peak to the Manual Peaks table, click **Apply**, or press the A key on the keyboard.

If the result file did not already contain a Manual Peaks table, the new table appears in the master table tab set. The Manual Peaks table contains a row for the new manual peak.

❖ To apply a shortcut menu command in the Chromatogram View

Right-click the **Chromatogram View** and choose a command from the shortcut menu (Table 1).

Table 1. Chromatogram View—Shortcut menu commands (Sheet 1 of 2)

Command	Description
Undo Last Zoom/Pan	Undoes the last zoom or pan movement.
Undo All Zoom/Pan	Zooms out to the full data acquisition time for the chromatogram on the x axis and the height of the largest chromatographic peak on the y axis.
Keep Zoom	Maintains the same zoom range, as you select components with different peak areas. Overrides the Zoom to Detected Peaks command as you select different table rows.
Copy Image	Copies an image of the Chromatogram View (including the legend) to the Clipboard. You can paste the image into a Microsoft Office document as a raster image or into a vector-drawing program as a vector image.
Copy Points	Copies the data as a two-column list of data points and the legend. The first column lists the retention time and the second column lists either the relative intensity or the counts.

Table 1. Chromatogram View—Shortcut menu commands (Sheet 2 of 2)

Command	Description
Save Image As	Opens the Save As dialog box where you can specify a file name and save the contents of the Chromatogram View as one of these selectable image formats: EME, PNG, GIF, JPG, TIFF, or BMP. The PNG, GIF, JPG, TIFF, and BMP formats are raster images.
Save Points As	Opens the Save As dialog box where you can specify a file name and save the contents of the Chromatogram View as a text file. The default file name is Chart.txt.
Show ToolTips	Displays a pop-up box with information about the trace when you place the cursor, +, over a chromatographic peak.
Show Gridlines	Adds grid lines to the Chromatogram View.
Show Detected Peaks	Shades the integrated area within the detected chromatographic peaks a light gray. Turning off this command removes the shading.
Show Legend	Displays a legend at the top of the view. The displayed information depends on the selected trace.
Legend Size	Specifies the selected number (1–10) of legend lines that you want the application to display.
Relative Intensity	Displays the <i>y</i> -axis scale as relative intensity (0–100%).
Zoom to Detected Peaks	Zooms in on the detected peak or peaks.
Add Plot	Adds an empty, active plot to the Chromatogram View. Only the screen size limits the maximum number of displayed plots. When you reach the screen's limit, the Chromatogram View appears to be empty and the following message appears—Not enough space for drawing chart properly.
Remove Plot	Removes the active plot, which has a gray border. Adding more than one plot activates this command.
Remove All Plots	Removes all of the plots in the Chromatogram View.
Freeze Content	Keeps the chromatogram of the currently selected row in the view when you select another row.
Clear Frozen Content	Clears the frozen chromatogram from the view.
Manual Peak Integration	Use to add a manual peak to a specialized trace. Adding a manual peak adds the Manual Peaks table to a result file. Selecting a trace in the Specialized Traces table activates this command.

Working with the Mass Spectrum View

The Mass Spectrum View contains a spectral tree on the left and the mass spectrum of the selected scan on the right.

In addition to the MS1 scans, the spectral tree also displays matching data-dependent (DDF) scans and all ions fragmentation (AIF) scans. When the processing workflow includes the FISH Scoring node, you can view the annotated fragment structures in the Mass Spectrum View for a selected fragmentation scan (DDF or AIF).

Follow these procedures as needed:

- To display a mass spectrum
- To zoom in or out on the *x* axis of the Mass Spectrum View
- To apply a shortcut menu command in the Mass Spectrum View
- To interpret the color coding in the full MS scans for the Expected Compound Hits
- To view the matching fragment structures predicted by the FISH Scoring node
- To create a mirror plot

❖ To display a mass spectrum

Select a row in the Expected Compound Hits table or the Unknown Compound Hits table.

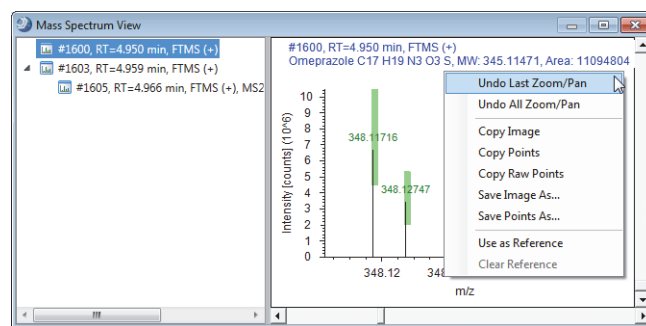
The Mass Spectrum View displays the matching spectral tree and a zoomed-in view of the full MS scan.

❖ To zoom in or out on the *x* axis of the Mass Spectrum View

- To zoom in, drag the cursor to the right over the *m/z* range of interest.
- To zoom out, drag the cursor to the left over the *m/z* range of interest.

❖ To apply a shortcut menu command in the Mass Spectrum View

Right-click the **Mass Spectrum View** and choose a command from the shortcut menu (Table 2).

**Table 2.** Mass Spectrum View—Shortcut menu commands (Sheet 1 of 2)





Command	Description
Undo Last Zoom/Pan	Undoes the last zoom or pan movement.
Undo All Zoom/Pan	Zooms out to the full scan range.
Copy Image	Copies the mass spectrum as a bitmap (raster) image to the Clipboard.

Table 2. Mass Spectrum View—Shortcut menu commands (Sheet 2 of 2)

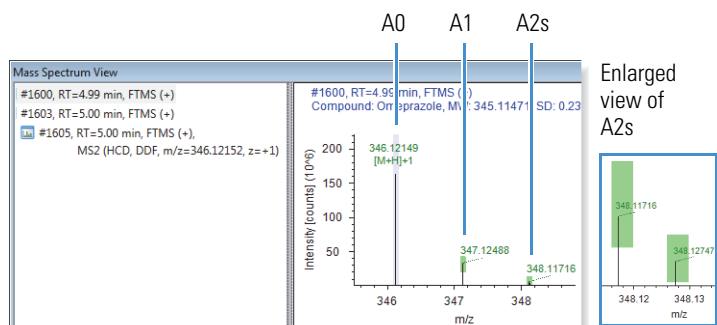
Command	Description
Copy Points	Copies the data points and the scan header for the selected scan to the Clipboard. Also copies the FISH annotations.
Copy Raw Points	Copies the data points for the selected scan to the Clipboard. Use this command to copy points to a library search application.
Save Image As	Opens the Save As dialog box, where you can type a name and select a file type for the current scan. The available file types are BMP, EMT, GIF, JPG, PNG, and TIF.
Save Points As	Opens the Save As dialog box, where you can type a name and select a file type for the current scan. The available file type is TXT.
Use as Reference	Creates a mirror plot that initially consists of the currently selected scan. When you select another scan, the reference scan remains in the $-y$ -axis portion of the graph, and the new scan appears in the $+y$ -axis portion of the graph.
Clear Reference	Removes the reference plot from the Mass Spectrum View.

❖ To interpret the color coding in the full MS scans for the Expected Compound Hits

The full MS scan shows the isotope pattern for the detected compound. Colored rectangles highlight the mass spectrum peaks (centroids) that match the theoretical isotope pattern.

Color	Meaning
	Lavender The labeled centroid is the monoisotopic mass of the expected compound ion.
	Green The labeled centroid matches the delta mass and relative intensity of the theoretical isotope pattern.
	Red The expected centroid for this m/z value is missing or its intensity does not fall within the tolerance range for the theoretical isotope pattern.
	Light blue The centroid for this m/z value might be missing because its theoretical intensity is at the level of the baseline noise.

This figure shows a full MS scan with four matching isotopes for omeprazole (C₁₇ H₁₉ N₃ O₃ S). The second A2 isotope is not visible at the normal zoom level.



Mass spectrum peak	Delta mass	Relative intensity
A0 monoisotopic protonated molecule	0	100.0%
A1 protonated molecule with one ¹³ C	1.00339	19.0% ^a
A2 protonated molecule with one ³⁴ S	1.99567	4.4% ^b
A2 protonated molecule with two ¹³ C	2.00603	1.7%



^a Carbon is an X + 1 element. The relative abundance of the stable ¹³C isotope is 1.1%. The relative intensity of the A1 isotopic peak is approximately equal to 1.1n_c, where n_c is the number of carbon atoms.

^b Sulfur is an X + 2 element. The relative abundance of the stable ³⁴S isotope is 4.22%. The relative intensity of the A2 isotopic peak (one ³⁴S) is approximately equal to 4.4n_s, where n_s is the number of sulfur atoms.

❖ To view the matching fragment structures predicted by the FISH Scoring node

1. Enlarge the view (see “To enlarge the views” on page 1).
2. In the spectral tree, select an MS/MS or higher scan.

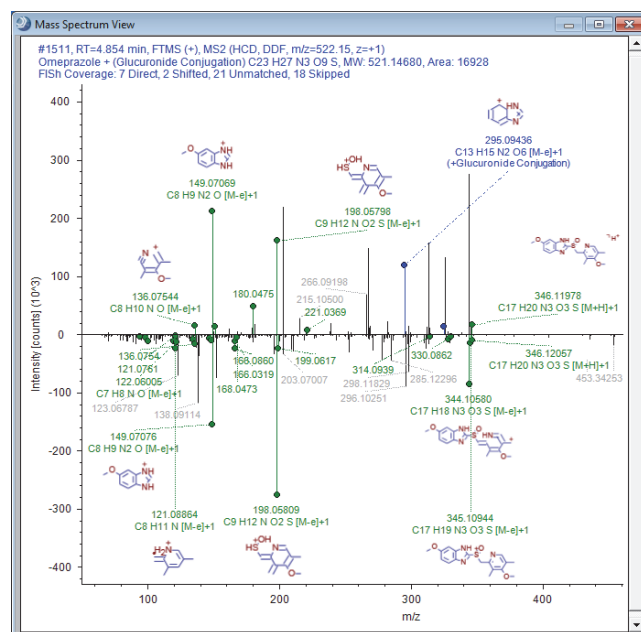
The FISH Scoring node annotates centroids that match the m/z value of a theoretical fragment ion with its theoretical structure and color codes the centroids in a fragmentation scan.

Color	Meaning
	Green Direct match
	Blue Shifted match

❖ To create a mirror plot

1. In the spectral tree pane, select the scan that you want to use as a reference scan.
2. Right-click the **Mass Spectrum View** and choose **Use as Reference** from the shortcut menu.
3. Select a scan for the compound that you want to compare.

This figure shows a mirror plot for omeprazole and one of its glucuronide conjugation products. The omeprazole fragmentation scan (reference scan) is on the bottom.



Reviewing the Tabular Data

Follow these procedures as needed:

- To sort the table columns
- To create, apply, and save data reduction filters
- To save the current filter settings for reuse
- To decide whether an unknown compound is a compound of interest

❖ To sort the table columns

- To sort a column, click the column header. Click the column header a second time to reverse the sort order.
- To sort multiple columns, click the column header of the first column you want to sort by. Then, while holding down the CTRL key, click the column header of the second column that you want to sort by.

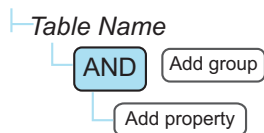
❖ To create, apply, and save data reduction filters

1. From the Compound Discoverer menu, choose **View > Result Filters**.

The Result Filters view opens as a floating window and displays the filter tree for the current table. Each table has its own set of filter conditions. Filtering one table has no effect on any other table.

2. In the left pane of the view, select a table of interest.

The table name appears in the right pane. The following selection tree appears below the table name.



3. Set up a filter condition as follows:

- a. Click **Add Property**.

A list that includes the columns in the selected table and the AND and OR logic selections appears.

- b. Select the property (table column) that you want to filter by.

An expression box with a default selection and an empty value box appear to the right of the selected property. In addition, another Add Property button appears below the filter expression that you are currently editing.

- c. Select an expression and type a value in the value box if applicable. To view only the checked table rows, select **Checked** and **Is True** (**Checked** **is true**).

4. Repeat for each filter condition that you want to apply to the current table. Repeat steps for each result table that you want to filter.

5. To apply the filter conditions, click **Apply Filters**.

❖ To save the current filter settings for reuse


1. In the Result Filters window, click **Save** or **Save As**.

The Save Filter dialog box opens.

2. Select an appropriate directory folder.

3. Type a name in the File Name box and click **Save**.

The file name extension is .filterset.

Tip Depending on the workflow, the Consolidated Peaks table contains up to five status columns. Each status column contains two or more parallel columns of colored rectangles, with one column for each input file. To view the file names of the input files, click the  icon to the right of the column header.

❖ To decide whether an unknown compound is a compound of interest

1. Click the **Consolidated Peaks** tab.
2. Select a row of interest.
3. Check whether the unknown compound is found by only the Unknown Detector by comparing the results in the Expected Finder and Unknown Detector columns.

Consolidated Peaks table Unknown Detector and Expected Finder columns

State	Meaning
<input type="checkbox"/> Gray	No match found
<input checked="" type="checkbox"/> Green	Single match found
<input type="checkbox"/> Red	Multiple matches found

4. Check whether there are any ion conflicts between the Expected Finder and Unknown Detector node by reviewing the Ion Conflict column.

Consolidated Peaks table— Ion Conflict column

State	Meaning
<input type="checkbox"/> Gray	No data to compare, as the related node did not detect this ion.
<input checked="" type="checkbox"/> Green	No conflict, as both nodes assigned the same ion to this chromatographic peak.
<input type="checkbox"/> Orange	The Unknown Detector node did not detect this ion, so the expected compound hit for this component might be in doubt.
<input type="checkbox"/> Red	Either the two nodes assigned different ions or one of the nodes assigned more than one ion to this <i>m/z</i> value and retention time.

5. Check whether a peak of interest is found in the associated control sample by reviewing the In-Control column.

Consolidated Peaks table – In-Control column

State	Sample type	Meaning
<input type="checkbox"/> Gray	Sample or Control	Not found in the current file
<input checked="" type="checkbox"/> Green	Sample	Found in both the current file and the control file
<input type="checkbox"/> Purple	Control	Found in the current file, which is a control file
<input type="checkbox"/> Orange	Sample	Found but outside the control limits
<input type="checkbox"/> White	Sample	Found in the current file but not in the control file

6. To show the related tables, click **Show Related Tables**.
7. Click the **Unknown Compound Hits** tab.

8. Select an unknown compound hit that is not in the control and that has a relatively high area count. View the chromatographic trace and the scan information.
9. To decide whether the chromatographic peak comes from a metabolite of the parent compound, compare it against the FISh, Pattern, and analog (UV or PDA) traces for the parent compound as follows:
 - a. Add a plot to the Chromatogram View by right-clicking the view and choosing **Add Plot** from the shortcut menu.
 - b. Click the **Specialized Traces** tab and select the FISh trace for the sample file. Check whether the FISh trace contains a chromatographic peak at the same retention time.
 - c. Add another plot to the Chromatogram View.
 - d. Select a Pattern Trace and check whether it contains a peak at the same retention time.
 - e. Add another plot to the Chromatogram View.
 - f. Overlay the UV or PDA traces for the samples and the controls. Check whether the UV or PDA traces contain chromatographic peaks at the same retention time as the mass chromatogram.

Adding Custom Explanations

Use the Custom Explanations table to assign structures to compounds of interest in the Expected Compound Hits and Unknown Compound Hits tables and to run the FISh scoring algorithm on the proposed structures.

❖ To add custom explanations

1. In the Expected Compound Hits or Unknown Compound Hits table, right-click the row of interest and choose **Add to Custom Explanations** from the shortcut menu.
2. Click the **Custom Explanations** tab to display the Custom Explanations table.
The table displays a row for your recent addition.
3. To edit the information for the new row, right-click the row and choose **Edit** from the shortcut menu.
The Custom Explanation Editor opens.
4. To assign a name for this custom explanation, type a name in the Name box.
5. To assign a structure to this custom explanation, open a MOL file or use the drawing tools to draw a structure.
The application checks whether the calculated exact mass of the structure matches the molecular weight of the selected peak within the mass tolerance (for XIC trace creation) that was specified in the Expected Finder or Unknown Detector node. If applicable, it also checks whether the proposed elemental composition matches the original formula.
6. Rerun the FISh scoring algorithm as follows:
 - a. Click the **FISh Scoring** tab.
 - b. Select the **Apply FISh Scoring** check box.
 - c. Select the **Use Fragmentation Libraries** check box.
 - d. Click **Save**.

Using the proposed structure, the application recalculates the FISh Coverage score and annotates the fragmentation spectra.

Understanding the FISh Coverage Score

In a targeted workflow where you connect the Expected Finder node to the FISh Scoring node, the FISh Scoring node creates a list of expected fragments for each found expected compound. It then attempts to match the fragment structures to the centroids in the fragmentation scans of the precursor ions.

When only one fragmentation scan follows a precursor ion scan, the node calculates the FISh coverage score as follows:

$$\text{FISh coverage score} = \frac{\# \text{ matched centroids}}{\# \text{ used centroids}} \times 100$$

where:

- # matched centroids represents the number of matched centroids.
- # used centroids represents the number of centroids in the fragmentation scan that are above the user-specified signal-to-noise threshold.

When a precursor scan is followed by more than one fragmentation scan, the node calculates a composite score as follows:

$$\text{FISh coverage score} = \frac{(\sum_{\text{per all scans}} \# \text{ matched centroids})}{(\sum_{\text{per all scans}} \# \text{ used centroids})} \times 100$$

The FISh Scoring node annotates the centroids in the fragmentation scans with the matching fragment structures. It also provides a FISh Coverage score for data-dependent scans in the Mass Spectrum View legend and a Best FISh Coverage score in the Expected Compound Hits table.

Reviewing the Processing Workflow

❖ To review the processing workflow for a result file

1. From the Compound Discoverer menu bar, choose **View > Result Summaries**.
The Summaries view appears on the result page.
2. Click the **Workflow Summary** tab and review the processing workflow.

Saving the Result File Layout

Before you close a result file, save the changes that you made to the layout if you want the application to remember the changes.

❖ To save the layout of a result file

- From the Compound Discoverer menu bar, choose **File > Save**.
The application saves the docked positions of the Mass Spectrum View, Chromatogram View, Result Summaries view, and Scatter Chart view; the rearrangement of the table columns; and the applied filter settings.

Trademarks

Compound Discoverer is a trademark of Thermo Fisher Scientific Inc. in the United States.

Fragment Ion Search (FISh) is a trademark of HighChem Ltd. in the Slovak Republic.