

Compound Discoverer 3.1 Metabolism Tutorial

If you are unfamiliar with the Thermo Compound Discoverer™ 3.1 application, follow the topics in this tutorial to set up a new study and a new analysis, process a set of example Xcalibur™ RAW files, review the targeted (expected) and untargeted (detected) compounds that the analysis found, and print a report.

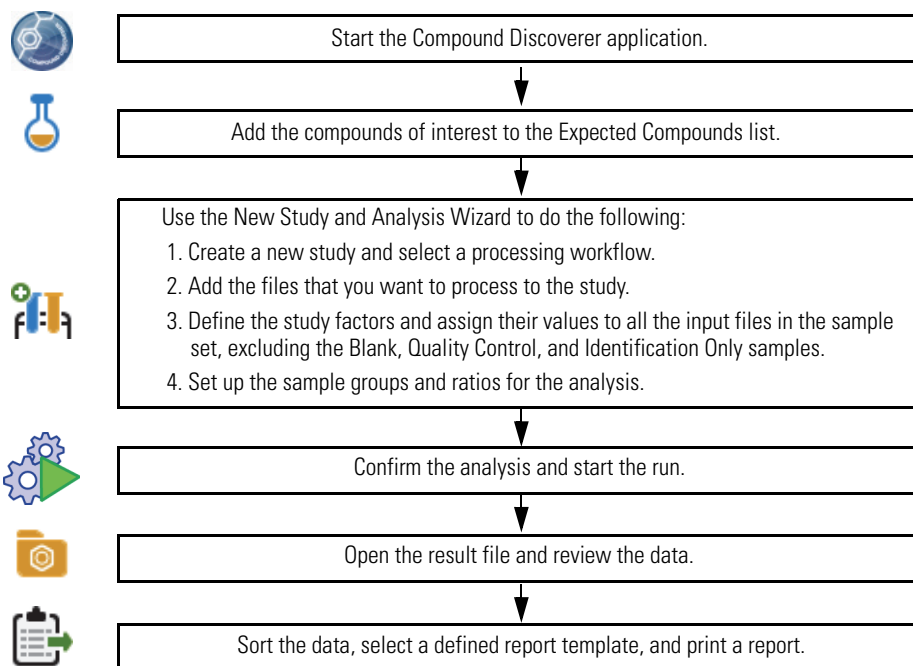
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Overview

In the Compound Discoverer application, data processing—the analysis of a set of raw data files to extract information about the sample set—takes place within the study environment. To create a practice study, use the example Xcalibur RAW files. These files are provided in the following folder on the key-shaped USB drive in the software media kit: Example Studies\Omeprazole Metabolism Study. Copy the Omeprazole Metabolism Study folder to your data processing computer.

This figure shows the typical workflow for a targeted analysis.



Starting the Application

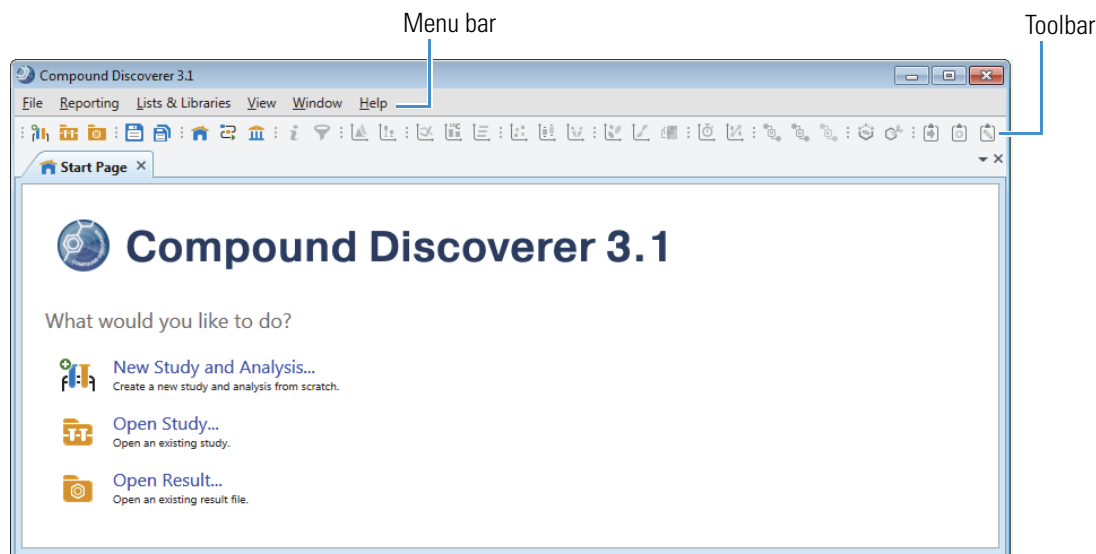
❖ To start the application

- From the taskbar, choose **Start > All Programs (or Programs) > Thermo Compound Discoverer 3.1**.

–or–

- From the computer desktop, double-click the **Compound Discoverer** icon, .

The application opens to the Start Page. By default, the toolbar icons are relatively small.



Enlarging the Toolbar Icons


❖ To enlarge the toolbar icons

Right-click the toolbar and choose **Show Large Icons**.

Accessing Help

The application provides Help for the views, tabbed pages, and dialog boxes.

❖ To open the Help topic for a specific view, tabbed page, or dialog box

1. Open the view, tabbed page, or dialog box.
2. Place the mouse cursor anywhere in the opened view, tabbed page, or dialog box.
3. On the computer keyboard, press the F1 key. 

For targeted analyses of known compounds and their transformation products, you must add the compounds to the Expected Compounds list.

Tip If you are **not** using the omeprazole example data set for this tutorial, follow the instructions in this topic to add the compounds you are studying to the Expected Compounds list. This list already includes omeprazole.


Adding Compounds to the Expected Compounds List

❖ To add a compound to the Expected Compounds list

1. (Optional) Download a 2D structure file for the compound from the Internet. For example, download the structure for caffeine from the ChemSpider™ database.
2. From the menu bar, choose **List & Libraries > Expected Compounds**.
The Expected Compounds view opens.
3. Click **New**.
The Compound Editor dialog box opens.
4. To add the compound's structure, do one of the following:
 - a. If you do not have a structure file for the compound, click **ChemSpider**.
The ChemSpider Search dialog box opens.
 - b. In the Input box, type the name, formula, molecular weight, or ChemSpider ID (CSID) for the compound.

- c. Click **Search**.
- d. Select one of the hits and click **Select**.

–or–

- a. If you have a structure file for the compound, in the Compound Editor toolbar, click the **Load Structure from Disk** icon, .

The Open Structure dialog box opens.

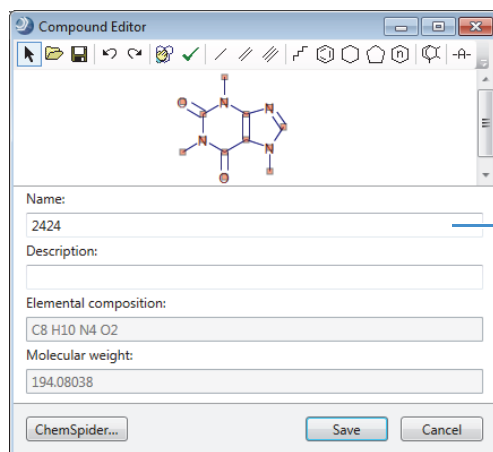
- b. In the Known Structure Formats list, select the format of the structure file.

The Compound Editor dialog box supports the following file formats:

- MOL Format (.mol)
- Compressed Structure (.mcs)
- Template (.tml)

- c. Select the structure file of interest and click **Open**.

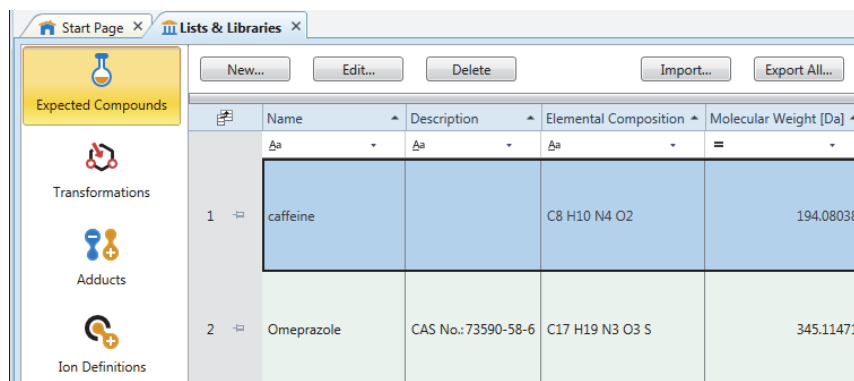
The chemical structure appears in the drawing pane of the Compound Editor dialog box, and the application automatically populates the Elemental Composition and Molecular Weight boxes. The application also populates the Name box with the CSID or the file name of the structure file.



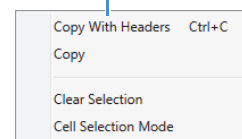
Replace the ChemSpider ID number with the compound name.


5. If necessary, type a different name for the compound.
6. Click **Save** to add the compound to the Expected Compounds list.

The new compound appears in the list.



Shortcut menu



7. To close the Lists & Libraries page, click the close icon, , on its tab.

Note To delete a compound, right-click the table to open the shortcut menu and turn off the Cell Selection Mode if it is on (). Then select the compound of interest and click **Delete**.

Setting Up a New Study and a New Analysis

Naming the Study and Selecting Its Storage Location

To create a new study, you must use the New Study and Analysis wizard. As its name implies, you can also use the wizard to set up a new analysis. Or, you can start a new analysis from within an existing study.

Follow these topics to set up a new study and a new analysis:

1. [Naming the Study and Selecting Its Storage Location](#)
2. [Selecting a Processing Workflow for the Analysis](#)
3. [Adding Input Files to the Study](#)
4. [Setting Up the Experimental Variables](#)
5. [Grouping the Samples By Study Variable](#)
6. [Troubleshooting the Analysis](#)

Use the Study Name and Processing Workflow page of the wizard to name the study and select the top-level folder for storing studies of this type.


❖ To name the new study and select the top-level folder for studies of this type

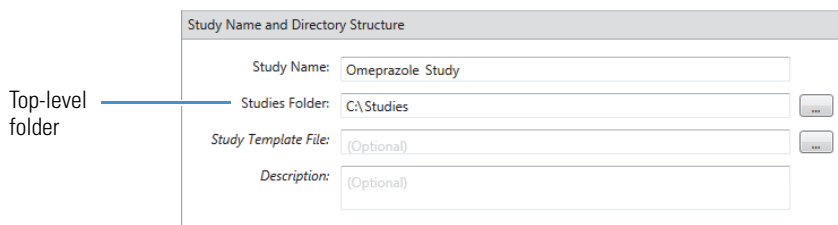
1. From the menu bar, choose **File > New Study and New Analysis**.

The New Study and Analysis Wizard opens.

2. Click **Next** to open the Study Name and Processing Workflow page.

The first time you open the wizard, the top-level folder for storing studies is undefined.

3. In the Study Name box, type a name for the new study (the study folder for this study shares the same name).
 - For a metabolism study with the omeprazole example files, type **Omeprazole Study**.
 - For a study that includes your own Xcalibur RAW files, type an appropriate name.
4. Select the folder where you want to store your Compound Discoverer studies as follows:
 - a. Click the browse icon, , next to the Studies Folder box.
 - b. Browse to a location on your local disk drive or network.
 - c. Click **New Folder** and name the new folder **Studies**.

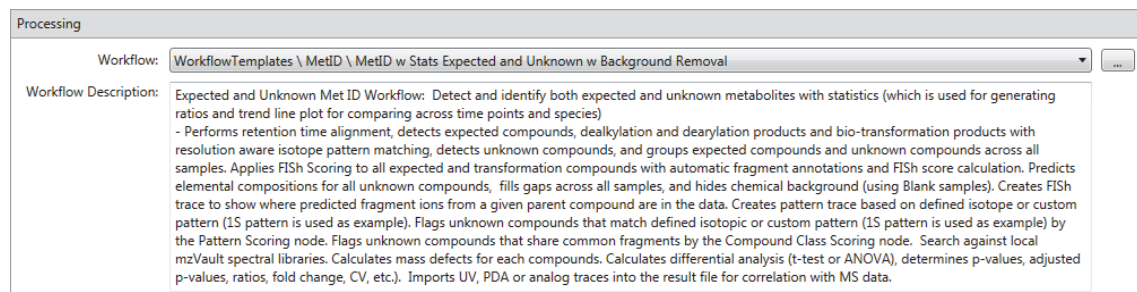


Continue on the Study Name and Processing Workflow page of the wizard to select a processing workflow for the analysis.

❖ To select the processing workflow

1. In the Workflow list in the Processing area, select the following processing workflow:

Workflow Templates\MetID\MetID w Stats Expected and Unknown w Background Removal



2. Read the description of the processing workflow, and then click **Next** to open the Input File Selection page of the wizard.

Selecting a Processing Workflow for the Analysis

Adding Input Files to the Study

Go to the next topic, [“Adding Input Files to the Study.”](#)

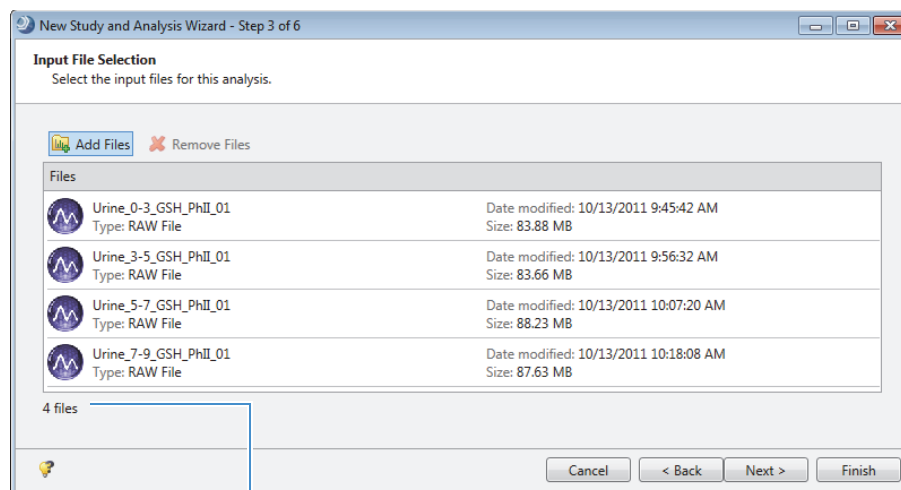
❖ To add input files to the study

1. On the Input File Selection page of the wizard, click **Add Files**.
2. Browse to the folder where you copied the example data.

drive:\Example Studies\Omeprazole Metabolism Study

3. Select all the raw data files in this folder and click **Open**.

The file names of the selected files appear in the Files box, the number of files that you selected appears below this box, and the Next button becomes available.



Number of selected files

4. Click **Next** to open the Input File Characterization page of the wizard.

To define the study factors, go to the next topic, [“Setting Up the Experimental Variables.”](#)

Setting Up the Experimental Variables

The sample type and study factor values for each sample are experimental variables. A study factor is an experimental variable that might have a statistically significant effect on the sample population being studied.

In this tutorial, you are comparing the compounds in urine samples collected at different time points from a subject dosed with the drug omeprazole. The time points are 3, 5, 7 and 9 hrs after dosage.

Note Omeprazole is a proton pump inhibitor that blocks the production of acid by the stomach. It is metabolized in the liver and excreted as metabolites in the urine within 3 to 6 hours after oral ingestion. Its primary metabolites are omeprazole sulfone and 5-hydroxyomeprazole.

❖ To set up the experimental variables for the example data set

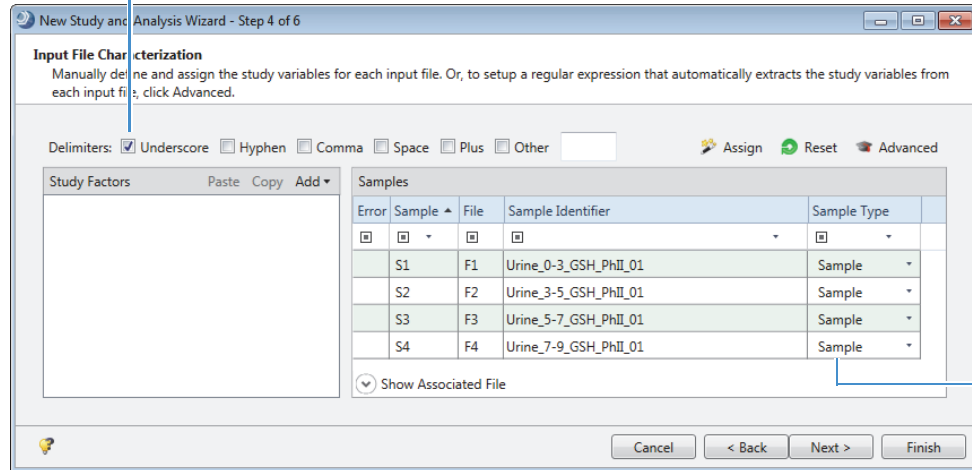
Note The study variables include the sample types and study factor values.

1. For the example data set, select the **Underscore** check box for the file name delimiter.

The time points study variable is included in the file names of the example data set, and underscore symbols separate the time point text (for example, 0-3) from the other text in the file name (Urine_0-3_GSH_PhII_01).

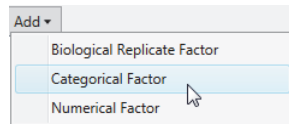
This figure shows the newly added samples in the Samples area. Sample is the default sample type. The Underscore check box is selected as the file name delimiter.

Underscore selected as the file name delimiter



Default sample type

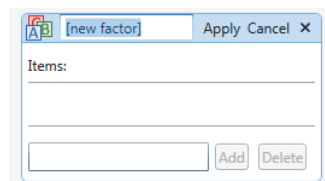
2. In the Study Factors area, choose **Add > Categorical Factor**.



- Tip** If you are not working with the omeprazole data set, select the study factor type as follows:
- To define study factors that include alphanumeric items, use the categorical study factor editor.
 - To define study factors that include only numbers, use the numerical study factor editor.
 - To set up a nested experiment, use the biological replicate factor for one of the study factors.

To open the Help topic about setting up the study factors and sample types, press the F1 key.

The categorical factor editor appears in the Study Factors area with the [new factor] text selected.



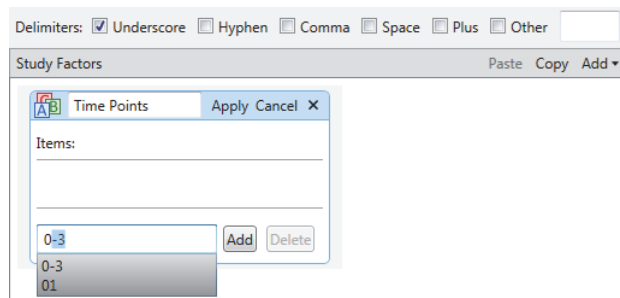
3. To replace the [New Factor] text, type the factor name, **Time Points**.

4. For each item that you want to add to the Items list (0-3, 3-5, 5-7, and 7-9), do the following:

a. In the Items box (next to the Add button), begin typing a factor item, for example, **0-3**.

If the file name contains a character delimiter and you selected the delimiter's check box, the editor displays a selection list as you type. Otherwise, you must type all the characters for the item.

Finish typing the appropriate alphanumeric text or select an item from the list.



The Add button becomes available.

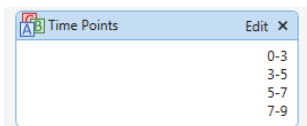
b. Click **Add**.

The current item appears in the Items list.

Add the remaining time points, 3-5, 5-7, and 7-9.

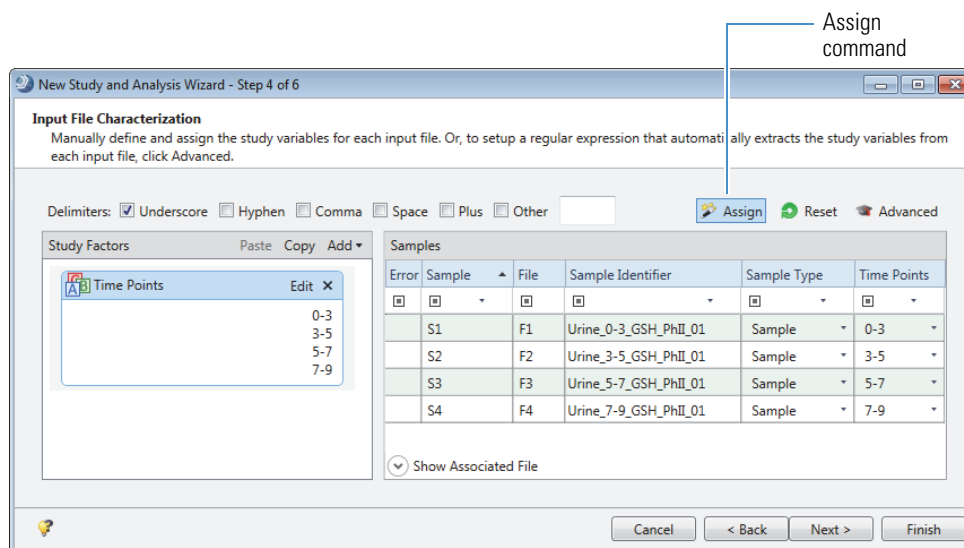
5. To save the study factor, click **Apply**.

The factor editor collapses to show only the study factor name and items list. The items appear in ascending order.



6. Click **Assign**.

Note The application assigns the Blank sample type to any sample that begins with Blank (or that contains Blank as delimited text, for example, Solvent_Blank with Blank preceded by the selected delimiter). The sample set for this tutorial does not include blanks.



7. For the example data set, keep the default assignments of **Sample**.

For other data sets, select the sample types as needed from the list.

Tip To select a row in the Samples table, click the **Error**, **File**, or **Sample Identifier** columns. Do not click the Sample Type or *Study Factor* columns.

Or, use the shortcut menu as follows (see [Figure 1](#) on [page 8](#)):

- To select the same sample type for a consecutive sample range, hold down the SHIFT key and select the rows of interest. Then, right-click and choose **Set Sample Type To > Sample Type**.
- To select the same sample type for non-consecutive samples, hold down the CTRL key and select the rows of interest. Then, right-click and choose **Set Sample Type To > Sample Type**.

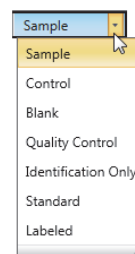
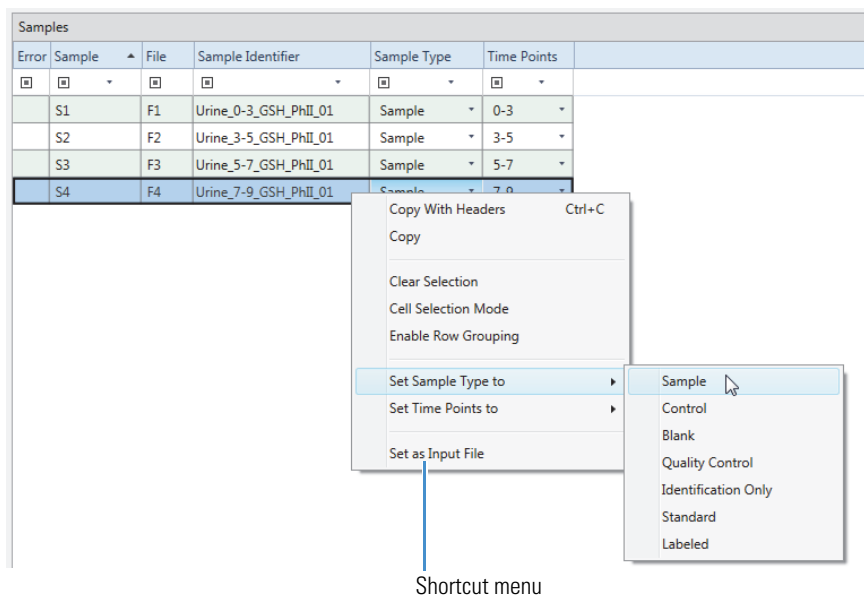


Figure 1. Samples pane shortcut menu



The sample type affects the processing actions.

Table 1. Sample types

Sample type	Processing actions
Sample, Control, and Standard	<p>Detects the components in the sample. When the analysis and the processing workflow include grouping, the application reports the group areas for these sample types by study factor.</p> <p>Does not differentiate between the Sample, Control, and Standard sample types, which means that you can use these sample types interchangeably. Thermo Fisher Scientific recommends that you use the Control and Standard sample types for labeling or grouping purposes as needed.</p> <p>IMPORTANT When you want to group the samples by one or more study factors, you must define the study factor items (or values) for the Sample, Control, and Standard sample types. Failing to define the study factors for these sample types generates error messages on the Sample Groups and Ratios page.</p>
Blank	Detects the components in the sample. When the processing workflow includes one or both Mark Background Compound nodes, the application marks these components as background compounds.
Quality Control	<p>Detects the components in the sample. When the processing workflow includes the Normalize Areas node, the application does the following:</p> <ul style="list-style-type: none"> Fits the areas of the compounds in the QC samples to linear or cubic spline curves. Runs a batch normalization of the non-QC samples against these curves.
Identification Only	Does not report the chromatographic peak areas for the sample components (chromatographic peaks with the same MW×RT dimensions). The application uses the sample’s data-dependent fragmentation (DDA) scans for component identification when the processing workflow includes the Group Unknown Compounds node.
Labeled	Not used for targeted analyses.

- Click **Next** to open the Sample Groups and Ratios page.

The Study Variables area contains check boxes for the study variables—File, Sample Type, and the user-defined study factors.

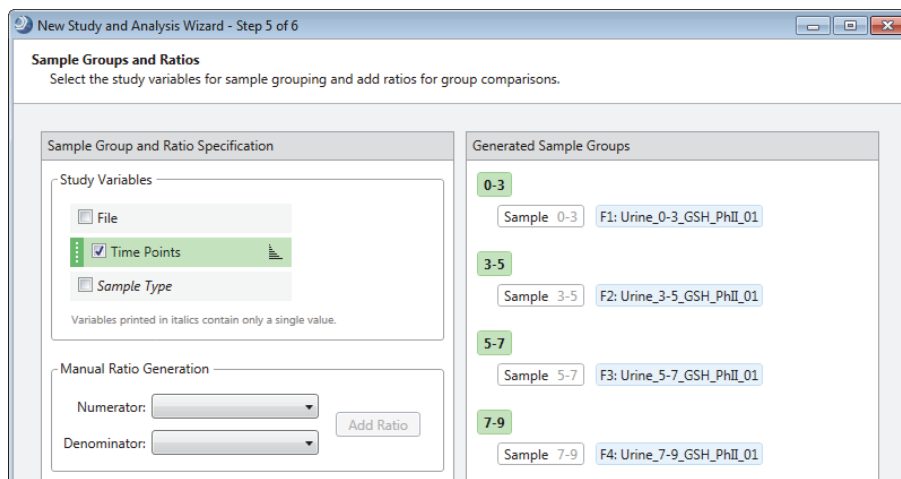
Go to the next topic, “[Grouping the Samples By Study Variable.](#)”

Grouping the Samples By Study Variable

❖ To set up the sample groups for the data set

1. On the Sample Groups and Ratios page, select the **Time Points** check box in the Study Variables area.

The generated sample groups appear in the Generated Sample Groups pane. The application generates a “not assigned” (n/a) group for samples without an assigned value for the Time Points study factor. For the example data, there is no n/a group.



2. Do one of the following:
 - If you are working with the omeprazole example files, go [step 4](#).
 - If you are **not** working with the omeprazole data set, and error messages appear when you select the study variables for the analysis, go to [step 3](#). If no errors appear, go to [step 4](#).
3. If you are not working with the example data set, and an error message appears in the Generated Sample Groups pane, do the following:
 - a. Check whether any of these sample types are part of an n/a sample group in the Generated Sample Groups pane on the Samples Groups and Ratios page:
 - Sample
 - Standard
 - Control

IMPORTANT When you select a study factor in the Study Variables area, the Sample, Quality Control, and Control sample types must have assigned study factor values. Only Blanks and Identification Only samples can have an n/a study factor value.

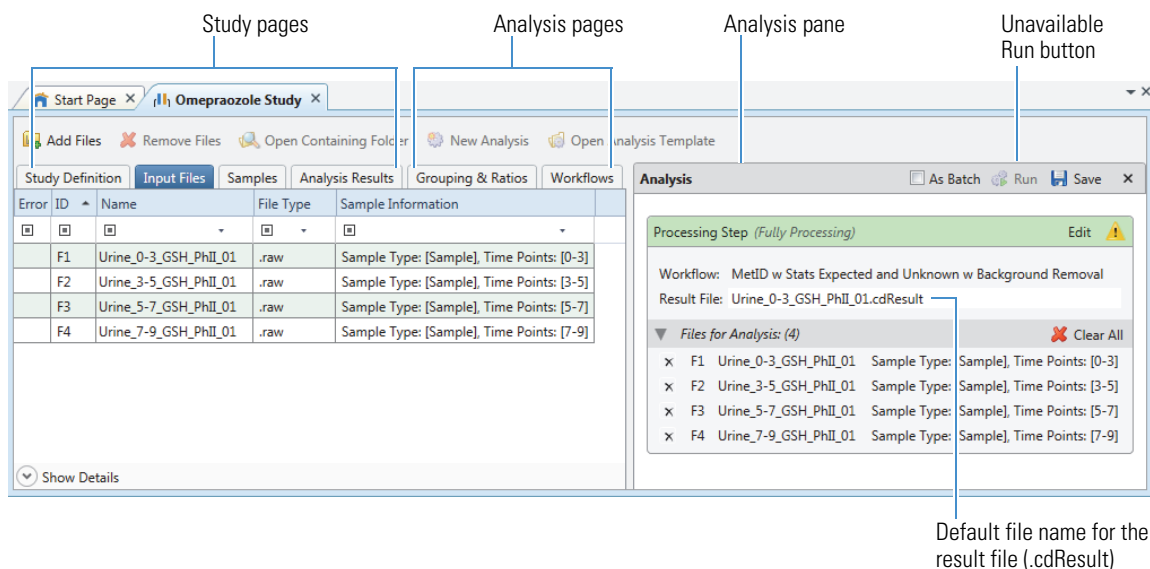
If you are working with a different data set and you do not assign study factor values to one or more of the Sample, Control, or Standard samples, an error message (❗) appears and the n/a group is highlighted in red.

- b. To assign values to the Sample, Standard, or Control samples, click **Back** to return to the Input File Characterization page. Then, select study factor values for all the samples except for the Blank and Identification Only samples.
 - c. Return to the Sample Groups and Ratios page.
 - d. In the Study Variables area, select the study variables of interest.
 - e. In the Generated Samples Groups pane, make sure that the n/a group only includes the Blank samples.
4. Click **Finish**.

The study—Omeprazole Study—opens as a tabbed document at the left of the application window, and the Analysis pane opens to the right of the study.

The study consists of four tabbed pages: Study Definition, Input Files, Samples, and Analysis Results. The two tabs—Grouping & Ratios and Workflows—to the right of the study pages are part of the analysis. If you close the Analysis pane, these two tabs also close.

The Analysis pane lists the selected processing workflow, the name of the result file (which is based on the first input file), and the selected raw data files. If the analysis is valid, the Run button is green.



Note Because the example data set contains only one sample per group and the statistical analysis tools require at least two samples per group, this tutorial does not describe how to set up ratios. For information about setting up ratios and using the application's statistical views, refer to the Help, the metabolomics tutorial, or the extractables and leachables tutorial.

Go to the next topic, “[Troubleshooting the Analysis.](#)”

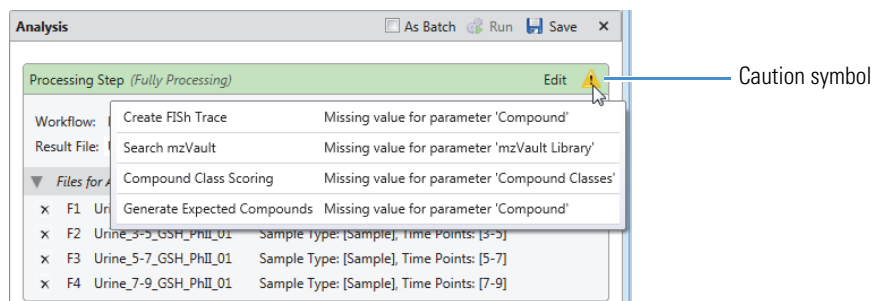
Troubleshooting the Analysis

A Caution symbol to the right of Processing Step in the Analysis pane warns you that the analysis contains errors. You cannot submit the run until you fix the errors.

❖ To troubleshoot the analysis and fix the errors

1. In the Analysis pane, point to the Caution symbol. Or, click the Workflows tab and review the messages in the Current Workflow Issues pane at the bottom of the Workflows page.

The application displays explanations for the analysis errors.



Current Workflow Issues			
Node Name	Issue Description	Parameter Name	Value
Create FISH Trace	Missing value for parameter 'Compound'	Compound	
Search mzVault	Missing value for parameter 'mzVault Library'	mzVault Library	
Compound Class Scoring	Missing value for parameter 'Compound Classes'	Compound Classes	
Generate Expected Compounds	Missing value for parameter 'Compound'	Compound	

2. To fix the analysis errors, go to “[Customizing the Processing Workflow.](#)”

For an analysis of one or more known compounds and their transformation products, you must specify the target compounds in the processing workflow template. For the MetID w Stats Expected and Unknown w Background Removal processing workflow, you must also select the parent compound for the Create FISH Trace node, the spectrum database file for the Search mzVault node, and the compound class file for the Compound Class Scoring node.

Tip When customizing a processing workflow template, consider doing the following:

- Basing the Ions list in the Generate Expected Compounds node and the Preferred Ions list in the Group Expected Compounds node on the sample matrix or other experimental factors
- Basing the Base Ions for the Detect Compounds node and the Preferred Ions for the Group Compounds node on the experimental factors
- Changing the Min. Peak Intensity setting in the Find Expected Compounds node and the Detect Compounds node to a threshold that is consistent with the mass spectrometer where you acquired the data
- Changing the Sample/Blank ratio threshold in the Mark Background Compounds node

To customize the processing workflow for the example data set, see these topics:

- [Specifying the Target Compounds and Adduct Ions](#)
- [Specifying the Parent Compound for the Create FISH Trace Node](#)
- [Specifying the mzVault Database Files for the Search mzVault Node](#)
- [Specifying the Compound Class Library for the Compound Class Scoring Node](#)
- (Optional) [Modifying the Specified Isotope Patterns](#)

The default Expected Compounds list includes omeprazole—the target compound for the example data set.

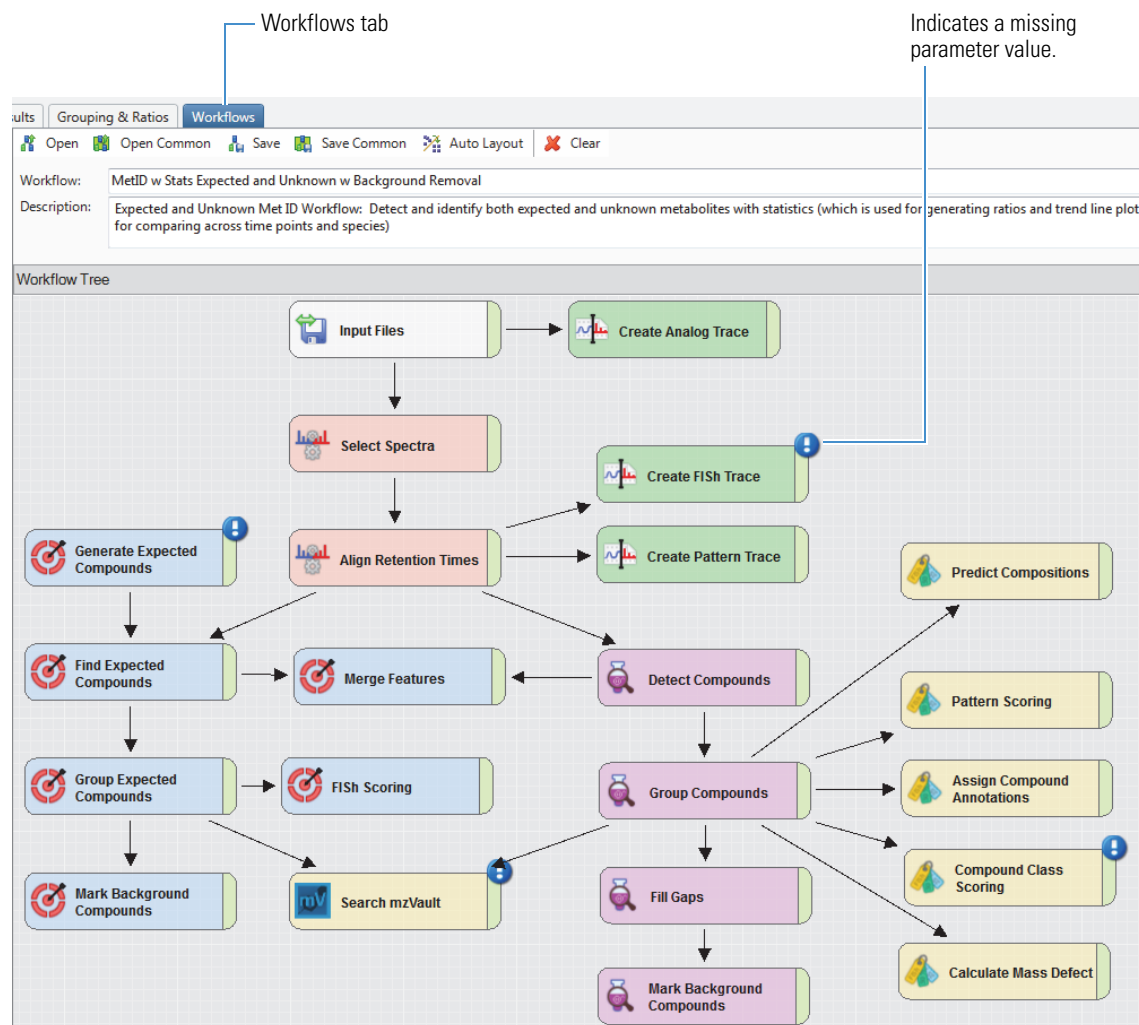
IMPORTANT If you are following this tutorial with a different data set and you have not added the target compounds to the Expected Compounds list, add the compounds to the list as described in [“Adding Compounds to the Expected Compounds List”](#) on page 2.

In the Compound Discoverer 3.1 or later application, you can leave the analysis open as you update any of the lists under Lists & Libraries.

❖ **To specify the target compound for the example data set**

1. Click the **Workflows** tab to open the Workflows page.

This figure shows the Workflows page with the selected processing workflow. The exclamation marks in the upper right corner of the Generate Expected Compound node, Search mzVault node, Create FISh Trace node, and Compound Class Scoring node indicate that these nodes are missing parameter values.



2. In the Workflow Tree pane, select the **Generate Expected Compounds** node.

The Parameters page for the Generate Expected Compounds node opens to the left. In the selected processing workflow template, Apply Dearylation has been set to True, and all the Phase I and Phase II reactions have been selected.

Parameters page

Parameters of 'Generate Expected Compounds'

Show Advanced Parameters

- 1. **Compound Selection**
 - Compounds
- 2. **Dealkylation**
 - Apply Dealkylation: True
 - Apply Dearylation: True
 - Max. # Steps: 1
 - Min. Mass [Da]: 150
- 3. **Transformations**
 - Phase I: Dehydration (H₂O →); Desaturation (C=C → C=C); Acetylation (H → C₂H₃O); Arginine (C → C₂H₃N₂O₂)
 - Phase II: Acetylation (H → C₂H₃O); Arginine (C → C₂H₃N₂O₂)
 - Others
 - Max. # Phase II: 1
 - Max. # All Steps: 3
- 4. **Ionization**
 - Ions: [M+H]⁺; [M-H]⁻

Workflow Tree

Workflow: MetID w Stats Expected and Unknown w Background Removal

Description: Expected and Unknown Met.ID Workflow: Detect and identify both expected and unknown metabolites with statistics (which is used for generating ratios and trend line plot for comparing across time points and species)

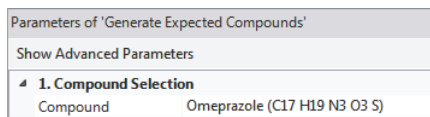
The Workflow Tree shows the 'Generate Expected Compounds' node selected, with 'Find Expected Compounds' and 'Mark Background Compounds' nodes below it.

Specifying the Parent Compound for the Create FISH Trace Node

3. Do one of the following:

- For the example data, in the Compound box, type **O** for omeprazole.

If Omeprazole is the only compound in the list that begins with an O, it appears in the box. Otherwise, continue typing the next letter in the name until the compound's name appears.



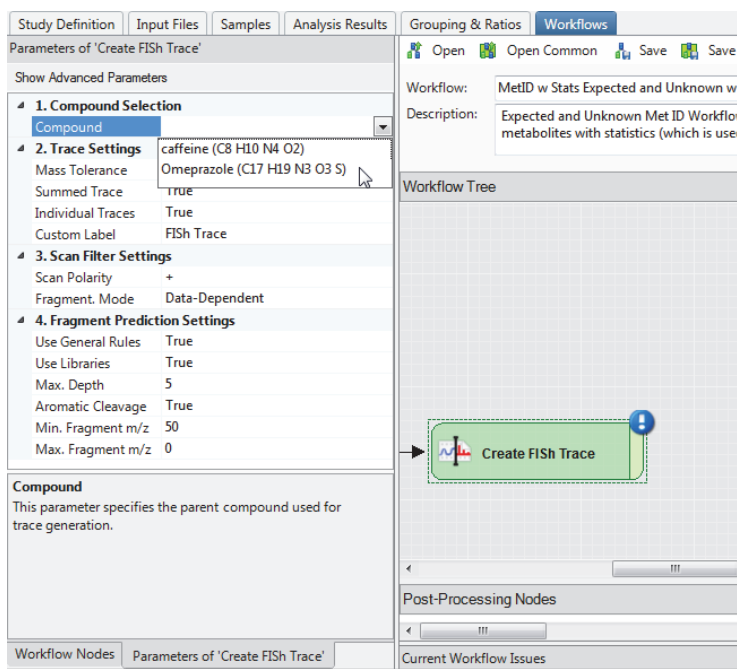
- For a different data set, add the target compounds to the Expected Compounds list, and then select these compounds in the Generate Expected Compounds node. To target multiple compounds with a different set of transformation rules for each compound, add an additional Generate Expected Compounds node for each set of transformation rules, and then connect these nodes to the Find Expected Compounds node.

To continue fixing the validation issues for the MetID w Stats Expected and Unknown w Background Removal processing workflow, go to the next topic, [“Specifying the Parent Compound for the Create FISH Trace Node.”](#)

The Create FISH Trace node uses the observed and theoretical fragments for a compound along with a list of fragmentation rules and fragmentation libraries to search the spectral data in the input files for fragments related to the compound of interest. It uses the matched fragments to create a fragment based total ion current chromatogram that you can use to visualize regions with fragment ions related to the parent compound.

❖ To specify the compound for the Create FISH Trace node

1. On the Workflows page, select the Create FISH Trace node.
2. On the Parameters of Create FISH Trace page, select the parent compound for the trace.
 - For the example data, select omeprazole and keep the default settings for the Scan Polarity and Fragment Mode parameters.



- If you are not working with the example data set, match the following parameter settings in the Create FISH Trace node to your data set: Scan Polarity (positive or negative) and Fragmentation Mode (Data-Dependent or Data Independent).

To continue fixing the validation issues for the MetID w Stats Expected and Unknown w Background Removal processing workflow, go to the next topic, [“Specifying the Parent Compound for the Create FISH Trace Node.”](#)

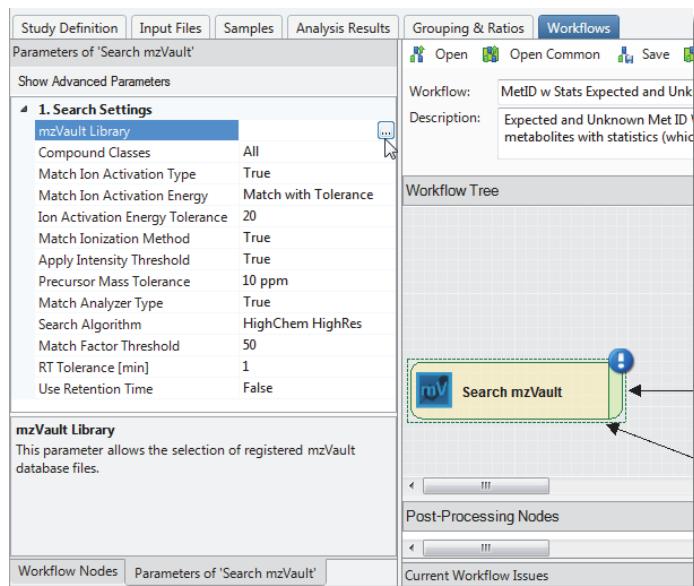
Specifying the mzVault Database Files for the Search mzVault Node

The Search mzVault node searches the selected mzVault databases for scans that match the scans associated with each expected or detected compound.

❖ To specify the mzVault database for the Search mzVault node

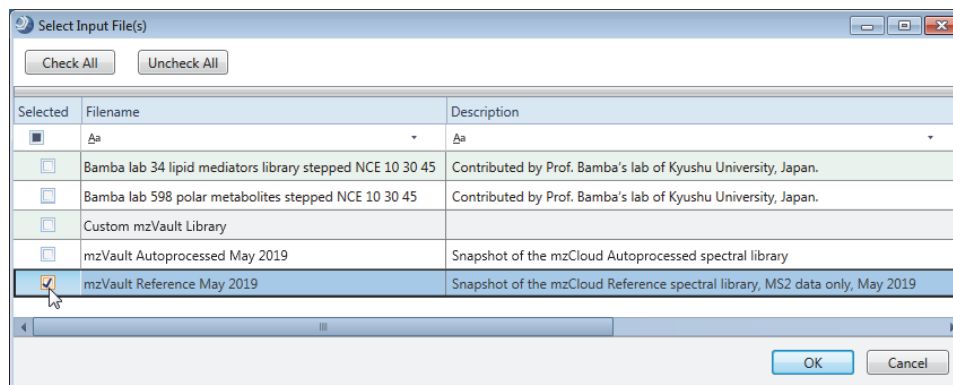
1. On the Workflows page, select the Search mzVault node.
2. On the Parameters of Search mzVault page, click the **mzVault Library** box, and then click the browse icon to the right of this box.

The Select Input Files dialog box opens.



3. In the Select Input Files dialog box, select the check box for the mzVault Reference May 2019 spectral database.

Tip If you working with your own data set, consider creating your own mzVault libraries with the mzVault 2.2 application. For information about importing mzVault libraries into the Compound Discoverer application, choose **Lists & Libraries > Spectral Libraries** from the menu bar, and then press the F1 key on your computer keyboard to open the Help topic.



4. Click **OK**.

To continue fixing the validation issues for the MetID w Stats Expected and Unknown w Background Removal processing workflow template, go to the next topic, “[Specifying the Compound Class Library for the Compound Class Scoring Node.](#)”

The Compound Class Scoring node scores the detected compounds against a set of fragment ions commonly present in the fragmentation scans for a compound class. The node compares the ions (m/z values) detected in the fragmentation scans to the fragments in the selected compound class libraries.

Specifying the Compound Class Library for the Compound Class Scoring Node

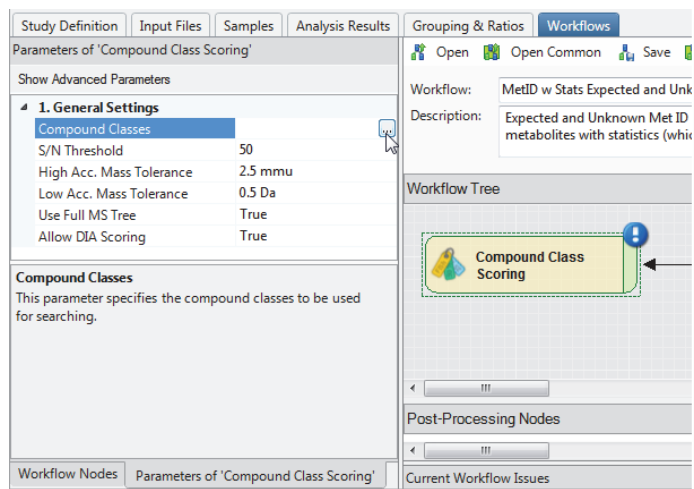
Note The Compound Discoverer 3.1 application comes with a compound class library for omeprazole, which is the compound of interest in this tutorial.

Tip If you are not working with the example data set, delete the Compound Class Scoring node from the processing workflow. Or, create compound class libraries for your targeted compounds.

For information about adding compound class libraries to the Compound Class list, choose **Lists & Libraries > Compound Classes** from the application menu. Then, press the F1 key.

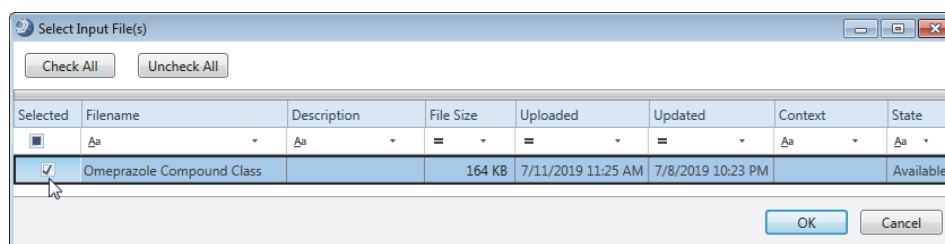
❖ **To specify the compound class library for the example data files**

1. On the Workflows page, select the **Compound Class Scoring** node.
2. On the Parameters of Compound Class Scoring page, click the **Compound Classes** box, and then click the browse icon to the right of this box.



The Select Input Files dialog box opens.

3. Select the check box to the left of the Omeprazole Compound Class file.



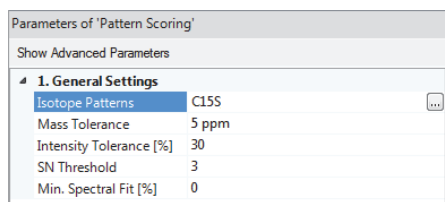
4. Click **OK**.

Modifying the Specified Isotope Patterns

The MetID w Stats Expected and Unknown w Background Removal processing workflow template includes the Pattern Scoring node and the Create Pattern Trace node. The Create Pattern Trace node can create a trace based on only one isotope pattern; whereas, the Pattern Scoring node can search for multiple isotope patterns.

The processing workflow templates that come with the application (and that include one or both of these nodes) specify an isotope pattern at a resolution of 60 000 for the following elemental composition: C15S.

Figure 2. Parameter settings for the Pattern Scoring node in the MetID workflow templates



To optimize the node's settings for your experimental data, enter the appropriate elemental compositions for your targeted compounds. For example, the targeted compound in the example data is omeprazole (C₁₇ H₁₉ N₃ O₃ S). Appropriate elemental compositions for omeprazole's metabolites might include C₁₅S, C₁₆S, and C₁₇S, and so on.

In addition to entering the appropriate compositions, match the Resolution setting to the instrument method used to acquire the sample data.

❖ **To specify the parameter settings for the Pattern Scoring node for the example data**

1. On the Workflows page, select the **Compound Class Scoring node**.

1. On the Parameters page, click the Isotope Patterns box, and then click the browse icon, .

The Pattern List Editor opens.

2. To modify the isotope pattern for C₁₅S, do the following:

a. Select **row 1** and click **Edit Pattern**.

The Isotope Ratio Editor opens.

b. Type **70 000** in the Resolution box, and then click **OK**.

3. To add the isotope pattern for C₁₇S, do the following:

a. In the Pattern List Editor, click **Add Patterns**.

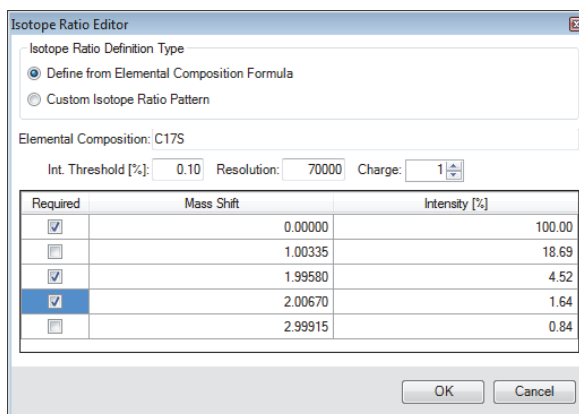
The Isotope Ratio Editor opens.

b. In the Elemental Composition box, type **C₁₇S**.

c. In the Resolution box, type **70 000**.

d. Set up the required isotope pattern as follows:

- Clear the check box for the A1 peak (mass shift of 1.00335).
- Select the check box for the A2 peak (mass shift of 1.99580).
- Select the check box for the A2 peak (mass shift of 2.00670).



Required	Mass Shift	Intensity [%]
<input checked="" type="checkbox"/>	0.00000	100.00
<input type="checkbox"/>	1.00335	18.69
<input checked="" type="checkbox"/>	1.99580	4.52
<input checked="" type="checkbox"/>	2.00670	1.64
<input type="checkbox"/>	2.99915	0.84

A0 peak for the monoisotopic ion with only the C₁₂ isotope of carbon and the S₃₂ isotope of sulfur

A2 peak for ion with one sulfur-34 atom

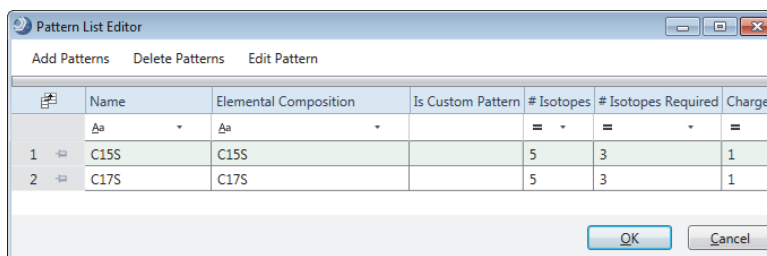
A2 peak for ion with two carbon-13 atoms

4. Click **OK**.

5. In the Pattern List Editor, do the following:

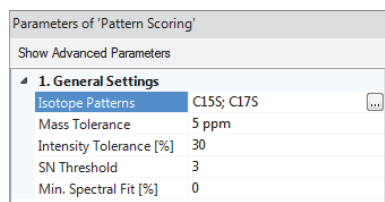
a. Verify that the editor lists the C₁₅S and C₁₇S elemental compositions and that the number of required peaks for both of these compositions is 3.

b. Click **OK**.



	Name	Elemental Composition	Is Custom Pattern	# Isotopes	# Isotopes Required	Charge
1	C15S	C15S		5	3	1
2	C17S	C17S		5	3	1

- Verify that the Isotope Patterns parameter for the Pattern Scoring node lists the following: C15S; C17S.



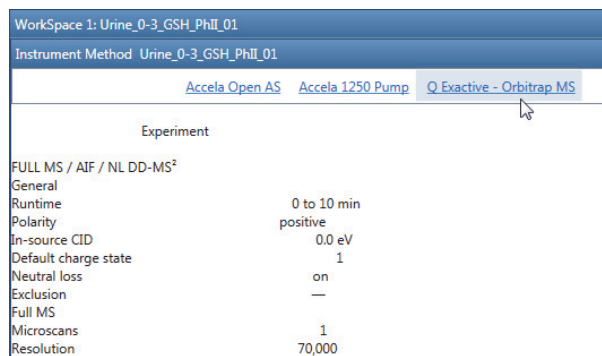
The experimental m/z values of the A2 peaks must be within 5 ppm and their relative intensity must be within 30% of the theoretical isotope pattern.

Table 2. Theoretical isotope patterns at a resolution of 70 000

Elemental composition	Peak	Mass Shift	Relative Intensity	Relative Intensity Range
C15 S	A0 monoisotopic	0.00000	100.00	N/A
	A2 (one ^{34}S atoms)	1.99580	4.52	3.16 to 5.88
	A2 (two ^{13}C atoms)	2.00670	1.27	0.89 to 1.65
C17 S	A0 monoisotopic	0.00000	100.00	N/A
	A2 (one ^{34}S atoms)	1.99580	4.52	3.84 to 5.20
	A2 (two ^{13}C atoms)	2.00670	1.64	1.15 to 2.13

❖ **To check the resolution of the full scan data in the RAW data files**

- Open the RAW data file in the FreeStyle™ application.
- In the Workspace Options toolbar, click **Instrument Method**.
- In the Instrument Method view, select the *Mass Spectrometer* page.



Go to “Submitting the Analysis to the Job Queue.”

For the omeprazole study, set up the analysis as described on the previous pages of this tutorial.

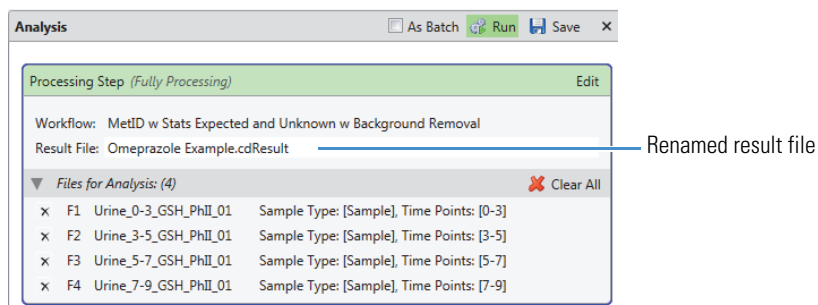
❖ **To submit the analysis to the job queue**

- To create one result file for the input files set, leave the **As Batch** check box clear.

By default, the application uses the name of the first input file as the result file name.

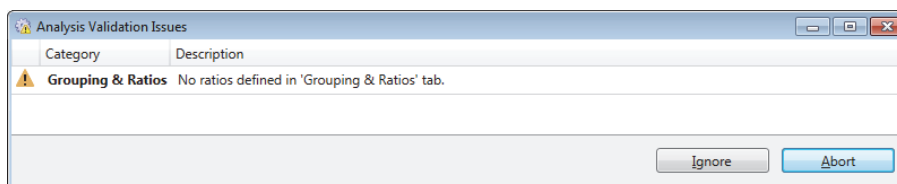
Note If you select the As Batch check box, the application creates a separate result file for each input file, which means that it cannot compare the results across the data set.

2. In the Result File box, rename the result file **Omeprazole Example**.



3. Click **Run** to submit the analysis to the job queue.

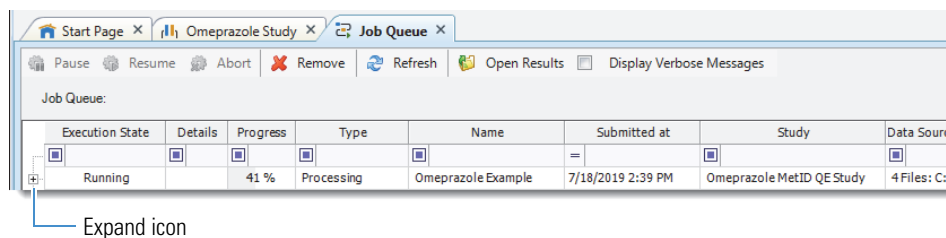
Because the processing workflow includes the Differential Analysis node and you have not defined any ratios for the analysis, the Analysis Validation Issues dialog box opens.




4. Click **Ignore**.

Note Because a differential analysis requires a minimum of two data points per group and the data set includes only one sample per group, the Differential Analysis node cannot calculate the p-values for the sample group regardless of whether you define ratios for the analysis.

The Job Queue page opens to the right of the study tab.



5. To view the processing messages, click the expand icon, , to the left of the processing job. You can ignore the warning messages that are highlighted in yellow.

Note During the run, the nodes generate the following warning messages, which you can ignore:

- The input files do not contain any UV data, so the Create Analog Trace node generates a warning message for each input file.
- The data set does not include any blank samples, so the Mark Background Compounds node generates a warning message that no blank files were specified for the analysis.
- You did not define any ratios for the analysis, so the Differential Analysis node generates a warning message about the missing ratios.

Leave the Job Queue page open and go to the next topic [“Opening the Result File and Reviewing the Default Layout.”](#)

Opening the Result File and Reviewing the Default Layout

Opening the Result File

You can open a result file from multiple locations: the Job Queue page, the Analysis Results page of a study, the Start Page, or the menu bar. The result file opens as a tabbed document in the application window (see [Figure 3](#)). For information about all the ways you can open a result file, refer to the Help.

Tip If you did not reprocess the example data set, do the following:

1. Open the result file provided on the Compound Discoverer 3.1 USB key:

Example Studies\Omeprazole Metabolism Study\Omeprazole MetID QE Example.cdResult

2. From the menu bar, choose **Window > Reset Layout**.

To open the result file and review the default layout, see these topics:

- [Opening the Result File](#)
- [Default Layout for a Result Page](#)

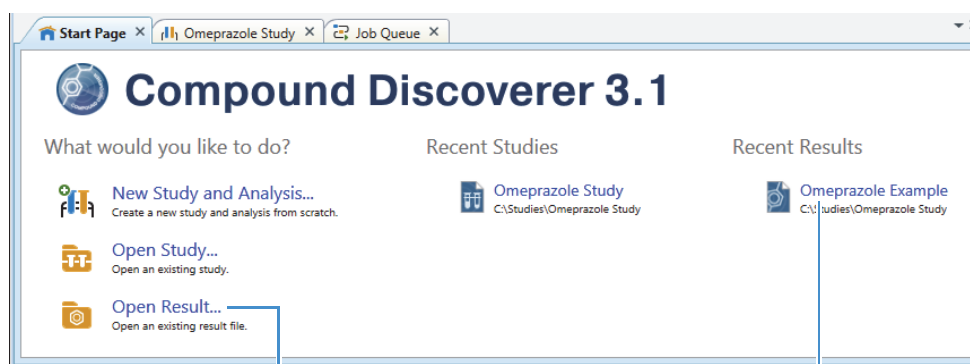
❖ To open the result file generated by the analysis

Do one of the following:

- If the Job Queue page is open, double-click the completed run.
- If the study is open, click the **Analysis Results** tab, and then double-click the link to the completed run.

–or–

- If you closed the study after the run completed, under Recent Results on the Start Page, click the result file name under Recent Results.



Opens a dialog box to locate and open a result file.

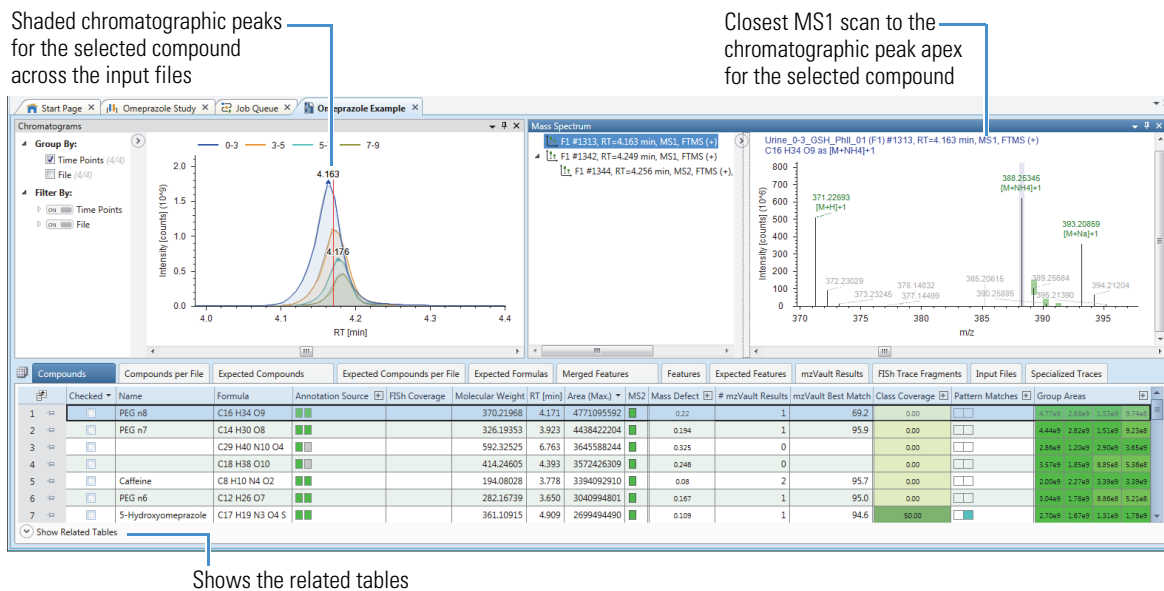
Opens the Omeprazole Example result file.

Default Layout for a Result Page

The factory default layout for a result file includes the following items ([Figure 3](#) on [page 20](#)):

- A page tab with the result file name.
 - A Chromatograms view on the top left that is populated with XIC traces for the compound in the first row of the Expected Compounds table. The view automatically zooms in to the start and end points of the chromatographic peak for the compound, and the area under the peak is shaded.
 - A Mass Spectrum view on the top right that is populated with the MS1 scan (for a preferred ion) that is closest to the chromatographic peak apex for the selected compound across the input files. The spectrum tree to the left includes the MS1 scans and the fragmentation scans for the preferred ions that were acquired within the following retention time window:
 - The chromatographic peak apex for the selected compound \pm peak width at half maximum (FWHM)
- or–
- The Start and end points of the chromatographic peak, as determined by the peak detection algorithm
- A set of tabbed main tables below the two graphical views.
 - A collapsed area for the related tables below the main tables.

Figure 3. Default layout for the result file generated by the processing workflow



The selected processing workflow creates the following main tables from left to right.

Table 3. Result tables for the selected processing workflow template

• Compounds	Lists all the compounds that the Detect Compounds node detected across the input file set beginning with the compound with the largest chromatographic peak area in any of the input files.
• Compounds per File	Lists the compounds that the Detect Compounds node detected in each input file.
• Expected Compounds	Lists all the expected compounds that the Expected Compounds node found in one or more of the input files, and groups the compounds by molecular weight and retention time.
• Expected Compounds per File	Lists all the expected compounds that the Expected Compounds node found in each input file (Xcalibur RAW file). The Group Expected Compounds node groups the compounds by molecular weight and retention time.
• Expected Formulas	Lists the expected formulas that the Find Expected Compounds node searched against. The expected formulas list is the output from the Generate Expected Compounds node.
• Features	Lists the adduct ions that the Detect Compounds node detected across all the input files.
• Merged Features	Lists the chromatographic peaks with the same $m/z \times RT$ dimensions that the analysis found in one or more of the input files. When the processing workflow includes both of these nodes—Find Expected Compounds and Detect Unknown Compounds—the Merge Feature node merges the features in this table and adds the Ion Status column.
• Expected Features	Lists the features that the Find Expected Compounds node found across all the input files.
• mzVault Results	Lists all the compounds that the mzVault search identified across the input files. The compounds are listed in order of the best match score between the query spectra and the matching spectra in the selected mzVault database.
• FISh Trace Fragments	Lists the structures of the expected fragments and the summed intensities of the matching adduct ions. The Create FISh Trace node generates a list of expected fragments (adduct ions) for the user-specified compound.
• Input Files	Lists the input files for the analysis.
• Specialized Traces	For the selected MetID processing workflow, this table lists the traces generated by the Create FISh Trace node and the Create Pattern Trace node.

Working with the Result Tables

Viewing the Number of Items in a Table

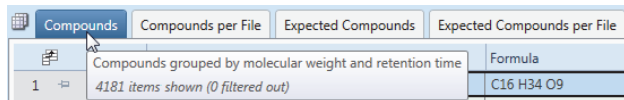
The following topics describe how to sort and filter the result tables:

- Viewing the Number of Items in a Table
- Sorting the Result Tables
- Filtering the Tables
- Displaying or Hiding Result Table Columns
- Viewing Related Information about an Item in a Main Result Table

❖ To view the total number of items in a result table

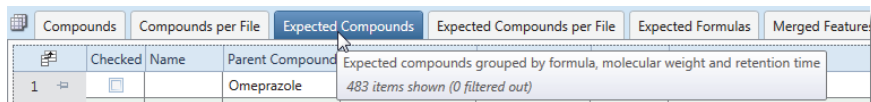
Point to its tab.

For this analysis, the Compounds table contains 4181 compounds.



Compounds grouped by molecular weight and retention time		Formula
1		C16 H34 O9

The Expected Compounds table contains 483 compounds.



Checked	Name	Parent Compound	Expected compounds grouped by formula, molecular weight and retention time
<input type="checkbox"/>		Omeprazole	

Sorting the Result Tables

❖ To sort a result table by a single table column


Click the column header. Click the column header a second time to reverse the sort order.

- Descending order (▲)
- Ascending order (▼)

❖ To sort a table by multiple columns

Hold down the CTRL key and click the column header of the first column and the column headers of any additional columns that you want to sort by.

❖ To sort a table by a column with multiple sub-columns

1. Click the expand icon, , to the right of the column heading to display the vertical headings for the sub-columns.
2. Select the sub-column that you want to sort by.
An asterisk appears at the top of the vertical heading.
3. Click the column heading.

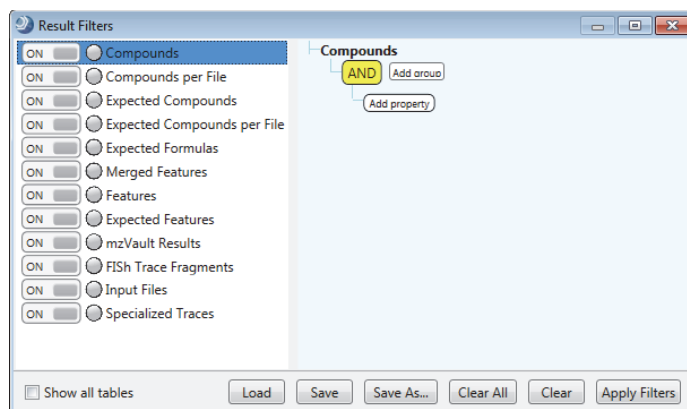
Filtering the Tables

Use the Result Filters view to create filters for data reduction.

❖ To filter the Compounds table by the maximum chromatographic peak area of each compound

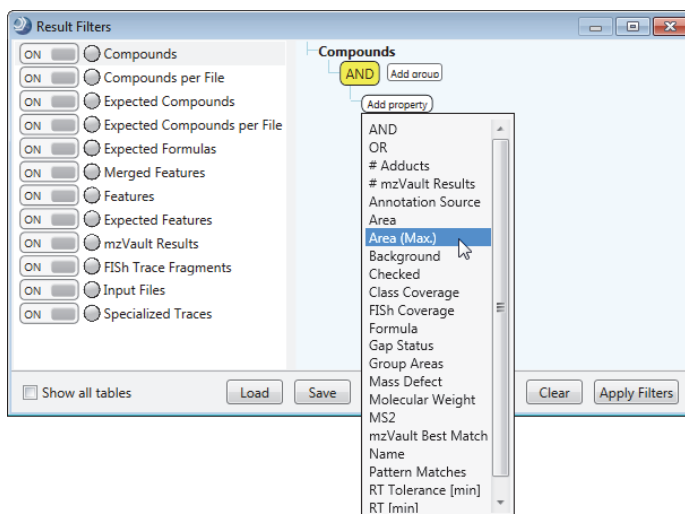
1. From the application menu bar, choose **View > Result Filters**.
The Result Filters view opens as a floating window.

2. In the list of tables on the right, select the Compounds table if it is not already selected.

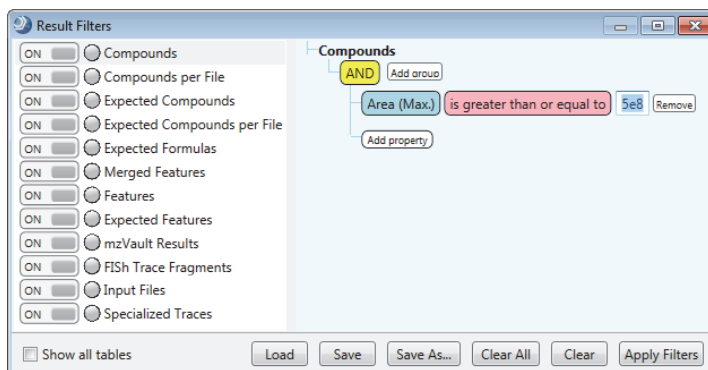


3. In the right pane, add a filter that hides compounds with maximum peak areas below 500 000 000 counts as follows:

- a. Click **Add Property** and select **Area (Max.)** from the list. (The maximum area for each compound is the largest chromatographic peak for this compound across the input file set.)

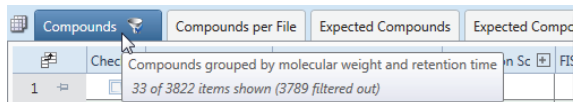


- b. Click the (pink) relation list and select **Is Greater Than or Equal To**.
c. Type **5e8** (or **500 000 000**) in the box next to the relation box.



- d. Click **Apply Filters**.


The Compounds tab has a filter icon and the table now displays only 33 entries.



Displaying or Hiding Result Table Columns

By default, some of the result table columns are hidden. You can choose to hide or display any of the columns in a result table.

❖ To display or hide a column

1. Click the icon, , in the upper left corner of the result table.
The Field Choose dialog box for the result table opens.
2. Select the check boxes for columns that you want to display and clear the check boxes for columns that you want to hide.

Viewing Related Information about an Item in a Main Result Table

The related tables below the opened main table contain related information about the selected item in the main table.

❖ To open a related table for a selected item

1. Select the item in the main table. For example, in the example result file, select row 4 (Caffeine) in the main Compounds table.
2. Click **Show Related Tables** at the bottom of the application window.
3. Click the tab for the related table of interest. For example, click the **mzVault Results** tab to open the mzVault Results table and view the compound matches for caffeine.

Reviewing the Untargeted Compounds

To review the results for the untargeted compounds detected by the Detect Compounds node, see these topics:

- [Filtering the Compounds Table by the Peak Areas](#)
- [Reviewing the Results from the Compound Class Scoring Node](#)
- [Reviewing the Results from the Pattern Scoring Node](#)
- [Comparing the Chromatograms for Two Compounds](#)
- [Comparing the Trendline Plots for Two Compounds](#)
- [Viewing a Mass Defect Plot](#)

Filtering the Compounds Table by the Peak Areas

❖ To filter the Compounds table by the chromatographic peak areas

1. Click the **Compounds** tab if the Compounds table is not open.
2. Filter the Compounds table by the chromatographic peak area [Area (Max.)]. Limit the table entries to compounds with chromatographic peak areas greater than or equal to 5e8. See [“Filtering the Tables”](#) on [page 21](#).
Only 36 compounds are now visible in the Compounds table for the example result file.

The processing workflow for this tutorial included the Compound Class Scoring node with the selection of a compound class (fragmentation) library for omeprazole.

❖ To review the class coverage for detected compounds reported in the Compounds table

1. If you have not already filtered the Compounds table by the maximum chromatographic peak area, filter it now.
2. Sort the Compounds table in descending order by the Area (Max.) column.
3. In the Compounds table, select **row 7** (5-hydroxyomeprazole).
4. Click Show Related Tables at the bottom of the application window.
5. In the set of related tables, click the **Compounds Class Matches** tab.
6. Select the entry in the Compound Class Matches table (see [Figure 4](#) on [page 24](#)).

Note To display the Compounds table in the allotted space below, several of the columns are hidden.

Reviewing the Results from the Compound Class Scoring Node

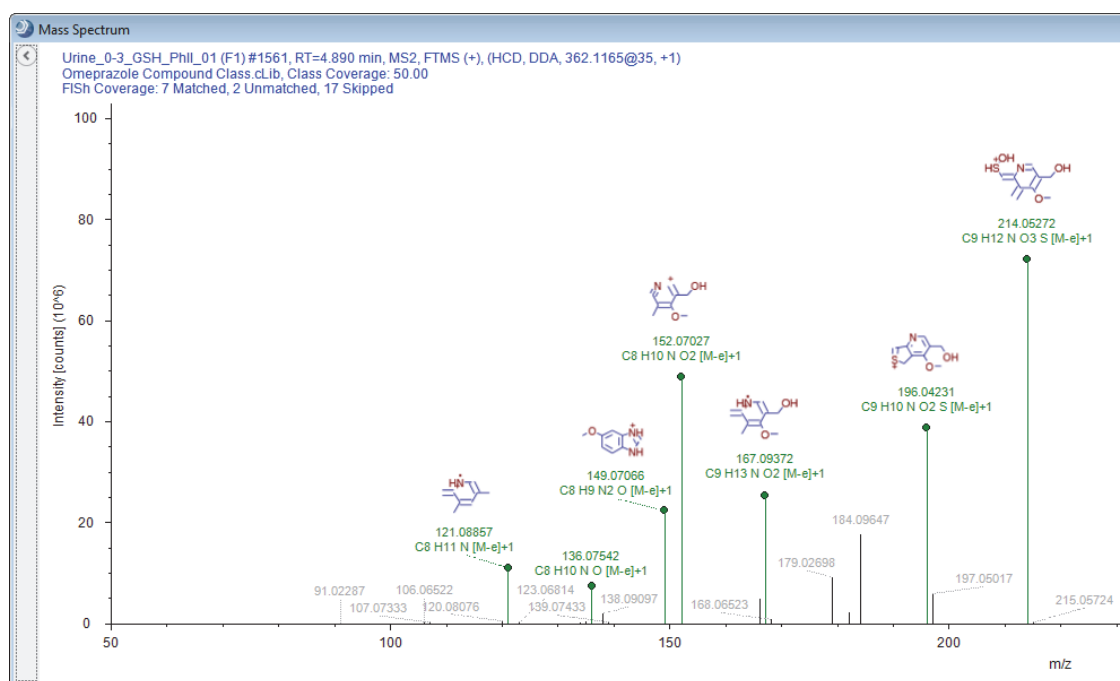
Figure 4. Compound Class Matches table for 5-hydroxyomeprazole

Compounds		Compounds per File	Expected Compounds	Expected Compounds per File	Expected Formulas	Merged Features	Features	Expected Features	
Checked	Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max.)	MS2	Class Coverage	Pattern Matches
<input type="checkbox"/>	PEG n8	C16 H34 O9	■ ■	370.21968	4.171	4771095592	■	0.00	<input type="checkbox"/>
<input type="checkbox"/>	PEG n7	C14 H30 O8	■ ■	326.19353	3.923	4438422204	■	0.00	<input type="checkbox"/>
<input type="checkbox"/>		C29 H40 N10 O4	■ ■	592.32525	6.763	3645588244	■	0.00	<input type="checkbox"/>
<input type="checkbox"/>		C18 H38 O10	■ ■	414.24605	4.393	3572426309	■	0.00	<input type="checkbox"/>
<input type="checkbox"/>	Caffeine	C8 H10 N4 O2	■ ■	194.08028	3.778	3394092910	■	0.00	<input type="checkbox"/>
<input type="checkbox"/>	PEG n6	C12 H26 O7	■ ■	282.16739	3.650	3040994801	■	0.00	<input type="checkbox"/>
<input checked="" type="checkbox"/>	5-Hydroxyomeprazole	C17 H19 N3 O4 S	■ ■	361.10915	4.909	2699494490	■	50.00	<input checked="" type="checkbox"/>

Checked	Name	Description	FISH Coverage	Class Coverage	# Matched Fr.	# Missed Fr.
<input checked="" type="checkbox"/>	Omeprazole Compound Class.Lib		77.78	50.00	7	7

In the Mass Spectrum view, each matching fragment for the selected compound is highlighted in green and annotated with its structure.

Figure 5. Data-dependent fragmentation scan for 5-hydroxyomeprazole with structure annotations for matching fragments



Reviewing the Results from the Pattern Scoring Node

❖ To review the results of the Pattern Scoring node

1. If you have not already filtered the Compounds table by the chromatographic peak area [Area (Max.)], filter it now. Limit the table entries to compounds with chromatographic peak areas greater than or equal to 5e8 (see “Filtering the Tables” on page 21).
2. Sort the Compounds table by the C17S subcolumn of the Pattern Matches column in descending order (see “To sort a table by a column with multiple sub-columns” on page 21).
3. Select **row 1** (Esomeprazole).

Note Esomeprazole is an isomer of omeprazole.

4. In the related tables, click the **Matched Patterns** tab. Then, select the entry in the Matched Patterns table (see Figure 6 on page 25).

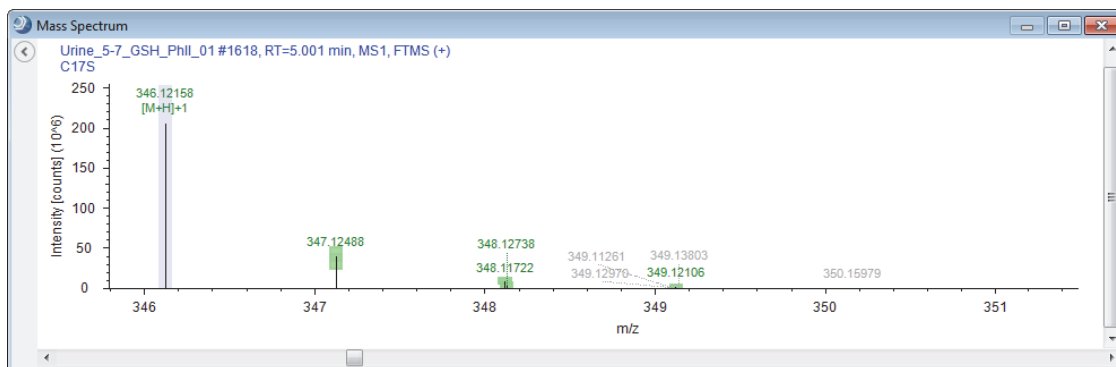
Note To display the Compounds table in the allotted space below, several of the columns are hidden.

Figure 6. Compounds table sorted by pattern matches for C17S

Compounds										
Checked	Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max.)	MS2	Class Coverage	Pattern Matches	
<input checked="" type="checkbox"/>	Esomeprazole	C17 H19 N3 O3 S		345.11424	5.003	866917990		14.29	<input checked="" type="checkbox"/>	C17S
<input type="checkbox"/>		C17 H19 N3 O5 S		377.10405	5.508	1183484546		21.43	<input type="checkbox"/>	
<input type="checkbox"/>		C17 H17 N3 O3 S		343.09872	4.942	1479181577		0.00	<input type="checkbox"/>	
<input type="checkbox"/>	5-Hydroxyomeprazole	C17 H19 N3 O4 S		361.10915	4.909	2699494490		50.00	<input type="checkbox"/>	
<input type="checkbox"/>		C16 H19 N O6 S		353.09292	6.544	689397452		0.00	<input type="checkbox"/>	
<input type="checkbox"/>		C17 H17 N3 O4 S		359.09351	4.663	1032708463		7.14	<input type="checkbox"/>	
<input type="checkbox"/>		C16 H17 N3 O2 S		315.10372	4.850	672976559		14.29	<input type="checkbox"/>	
<input type="checkbox"/>		C17 H19 N3 O6 S2		425.07099	4.525	653396483		0.00	<input type="checkbox"/>	
<input type="checkbox"/>		C26 H44 N10 O2 F		590.31011	6.797	773629566		0.00	<input type="checkbox"/>	
<input type="checkbox"/>		C24 H29 N5 O5		467.21527	6.494	795682648		0.00	<input type="checkbox"/>	

Hide Related Tables						
Structure Proposals	Compounds per File	Predicted Compositions	Merged Features	Compound Class Matches	mzVault Results	Matched Patterns
Checked	Name	SFit [%]	Pattern Cov. [%]	# Matched Iso.	Study File ID	
<input checked="" type="checkbox"/>	C17S	74	100.00	5	F3	
<input type="checkbox"/>	C15S	64	100.00	5	F3	

The Mass Spectrum view displays the matching isotopes. The monoisotopic adduct ion is highlighted in lavender, and the other matching isotopes are highlighted in green.



Go to the next topic, “[Comparing the Chromatograms for Two Compounds.](#)”

Comparing the Chromatograms for Two Compounds

The untargeted portion of the analysis detected the parent compound, esomeprazole, and one of its oxidation products, 5-hydroxyomeprazole.

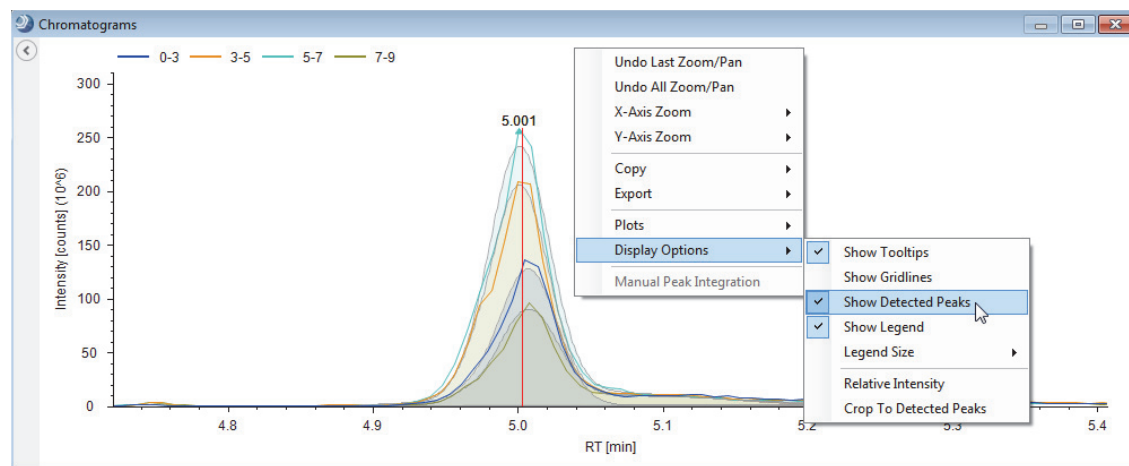
❖ To compare the chromatograms for esomeprazole and 5-hydroxyomeprazole

1. If you have not already sorted the Compounds table by the C17S subcolumn of the Pattern Matches column in descending order, sort it now.
2. In the Compounds table, select **row 1** (Esomeprazole).

Note Esomeprazole is an isomer of omeprazole.

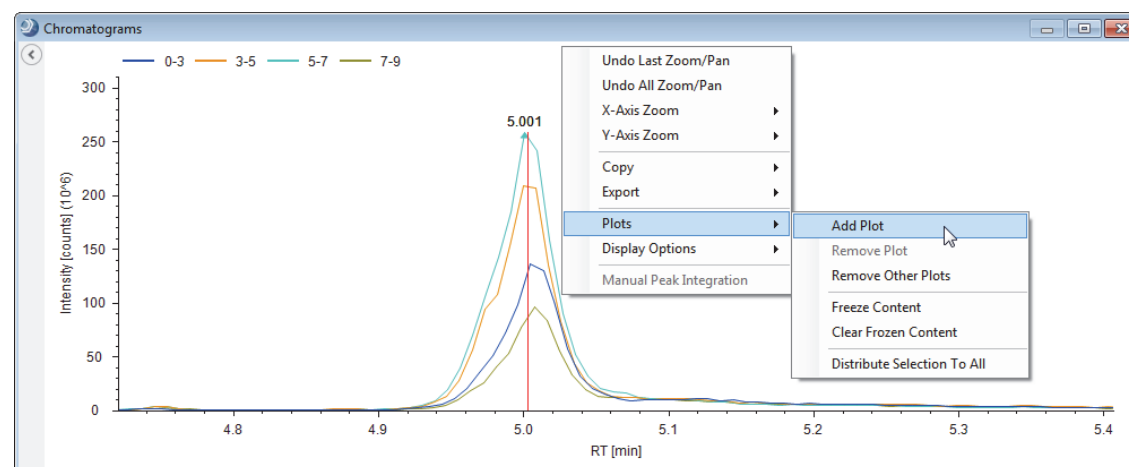
3. To view the chromatograms for the four time points more clearly, right-click the Chromatograms view and choose **Display Options > Show Detected Peaks** (Figure 7 on page 26).

Figure 7. Shaded peaks for esomeprazole



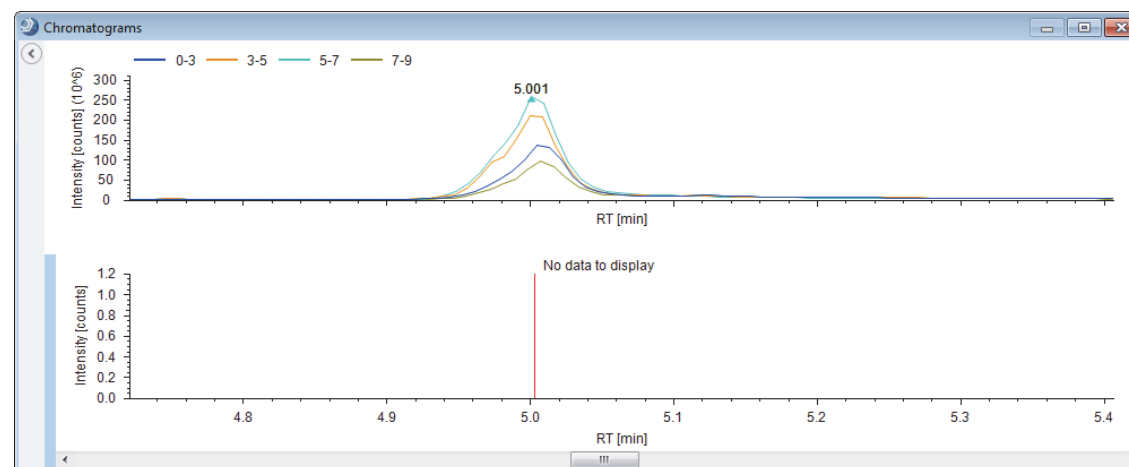
4. To add a plot below the current plot, right-click the Chromatograms view and choose **Plots > Add Plot**.

Figure 8. Unshaded peaks for esomeprazole



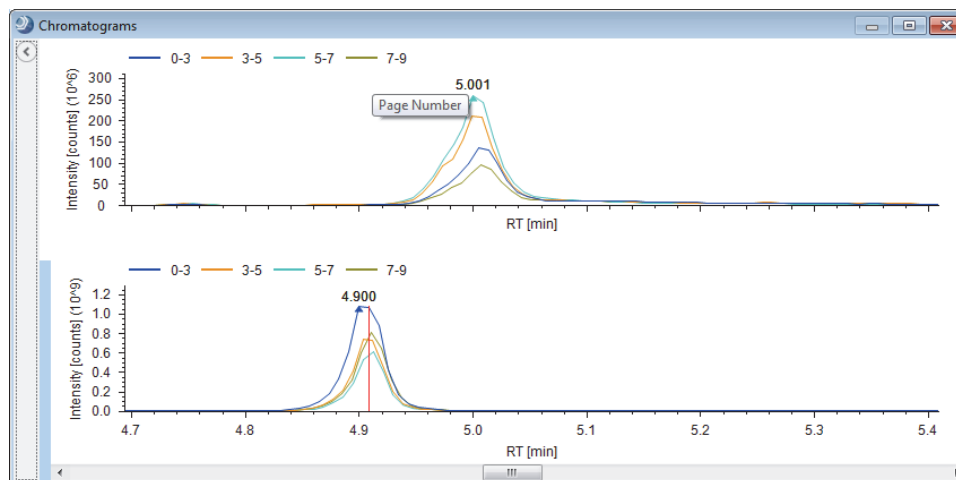
An empty plot appears below the current plot. The blue bar at the left of the plot indicates that the plot is active.

Figure 9. Two plots



5. In the Compounds table, select **row 5** (5-Hydroxyomeprazole).

The chromatograms for 5-Hydroxyomeprazole appear below those for Esomeprazole.



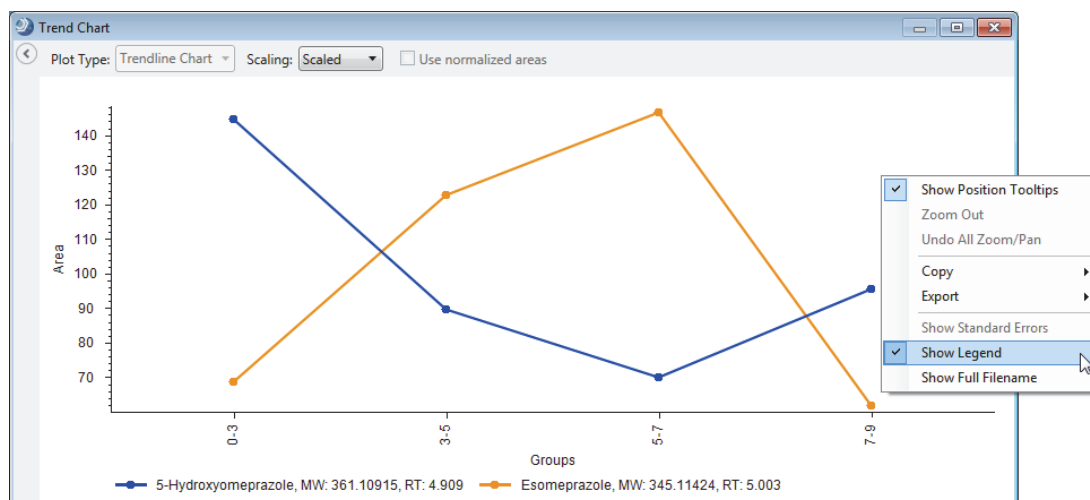
Go to the next topic, “[Comparing the Trendline Plots for Two Compounds.](#)”

Comparing the Trendline Plots for Two Compounds

❖ To compare the trendline plots for 5-hydroxyomeprazole and esomeprazole

1. If you have not already sorted the Compounds table by the C17S subcolumn of the Pattern Matches column in descending order, sort it now.
2. From the application menu bar, choose **View > Trend Chart**.
The Trend Chart view opens to the right of the result tables.
3. Hold down the CNTRL key and select **row 1** and **row 5** in the Compounds table.
4. Right-click the Trend Chart view and choose **Show Legend**.

Note The sampling points for this study represent the last dosage of omeprazole taken by the test subject after several previous dosages. The samples taken at 3, 5, and 7 hrs show a delay in the oxidation of omeprazole due to enzyme inhibition by its metabolites. After 7 hrs, the subject begins to metabolize the current dosage of omeprazole.



Go to the next topic, “[Viewing a Mass Defect Plot.](#)”

Viewing a Mass Defect Plot

You can view a mass defect plot for the compounds in the Compounds table or the Expected Compounds table. In this tutorial, you inspect a plot of the Kendrick mass defect for the following Kendrick formula: C₂H₄O.

Follow this procedure to set up a mass defect plot that highlights polyethylene glycols.

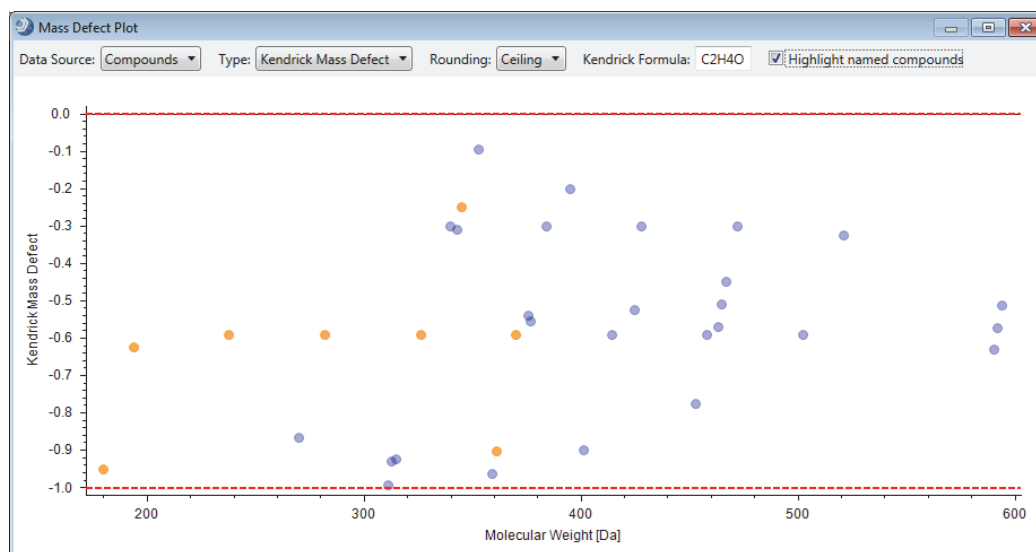
Note Polyethylene glycols are commonly detected in urine samples.

❖ To set up a mass defect plot for a Kendrick formula

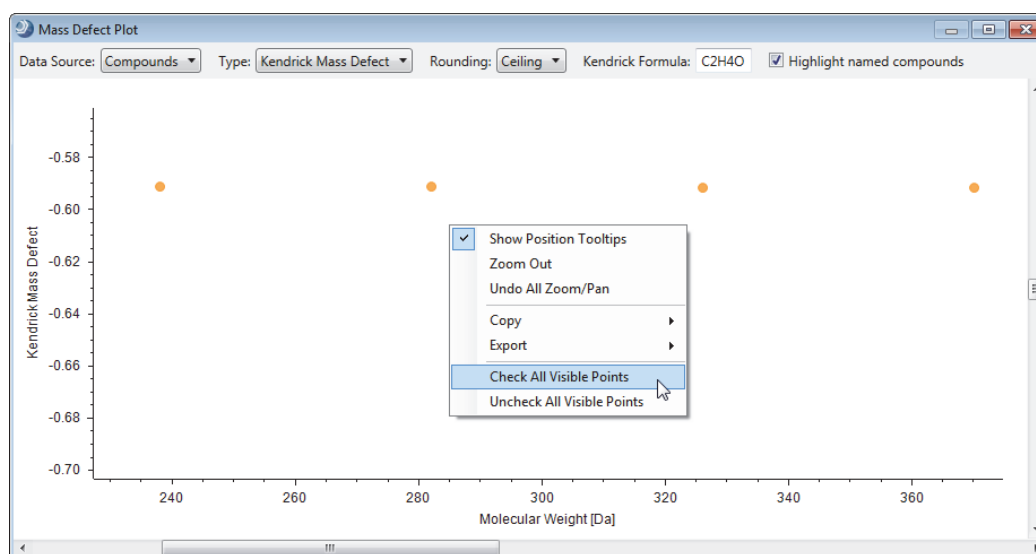
1. From the application menu bar, choose **View > Mass Defect Plot**.

The Mass Defect Plot opens to the right of the result tables.

2. In the Kendrick Formula box, type C₂H₄O.
3. Select the **Highlight Named Compounds** check box.



4. Zoom in on the four named compounds that form a horizontal line.
5. Right-click the plot and choose **Check All Visible Points**.



6. Sort the Compounds table by the Name column in descending order.

7. Notice that the four PEG compounds are checked. Clear these check boxes.

Compounds		Checked	Name
1	<input checked="" type="checkbox"/>	PEG n8	
2	<input checked="" type="checkbox"/>	PEG n7	
3	<input checked="" type="checkbox"/>	PEG n6	
4	<input checked="" type="checkbox"/>	PEG n5	
5	<input type="checkbox"/>	Paraxanthine	

Go to the next topic, “[Reviewing the Targeted Compounds.](#)”

Reviewing the Targeted Compounds

The Expected Compounds table lists the compounds found by the Find Expected Compounds node, which searches the data for the adduct ions specified by the Generate Expected Compounds node. The Group Expected Compounds node groups the compounds found across the input files by their molecular weight, formula, transformations, and retention time.

To review the targeted (expected) compounds, follow these topics in order:

1. [Filtering the Expected Compounds Table by Peak Area](#)
2. [Sorting the Expected Compounds by Their Transformations](#)
3. [Reviewing the Mass Spectra for the Expected Compounds](#)
4. [Determining the Most Probable Explanation for Each Expected Compound](#)
5. [Naming and Proposing Structures for the Expected Compounds](#)

❖ To filter the Expected Compounds table by the chromatographic peak areas

1. Click the **Expected Compounds** tab
2. Filter the Expected Compounds table by the chromatographic peak area [Area (Max.)]. Limit the table entries to compounds with chromatographic peak areas greater than or equal to 5e8. See “[Filtering the Tables](#)” on [page 21](#). Only 13 compounds are now visible in the Expected Compounds table for the example result file.

❖ To bring the parent compound to the top of the Expected Compounds table

1. In the Expected Compounds table, do the following:
 - a. Hold down the CNTRL key, and do the following:
 - Click the **Dealkylated** column heading until the table is sorted in ascending order—that is, until all the compounds that are not the product of a dealkylation reaction move to the top.
 - Click **Transformations** column heading until the table is sorted in ascending order by the Transformations (alphabetical and number).

This figure shows the Expected Compounds table sorted by the Dealkylated and Transformations columns. The parent compound, with no dealkylation reactions and no transformations, is at the top of the table.

Compounds		Checked	Name	Parent Compound	Formula	Molecular Weight	Dealkylated	Transformations
1	<input type="checkbox"/>	Parent	Omeprazole	C17 H19 N3 O3 S	345.11471			
2	<input type="checkbox"/>		Omeprazole	C17 H19 N3 O3	313.14264		Dehydration, Reduction, Thiourea to Urea	
3	<input type="checkbox"/>		Omeprazole	C17 H17 N3 O3 S	343.09906		Desaturation	
4	<input type="checkbox"/>		Omeprazole	C17 H17 N3 O4 S	359.09398		Desaturation, Oxidation	
5	<input type="checkbox"/>		Omeprazole	C23 H27 N3 O9 S	521.14680		Glucuronide Conjugation	
6	<input type="checkbox"/>		Omeprazole	C17 H19 N3 O4 S	361.10963		Oxidation	
7	<input type="checkbox"/>		Omeprazole	C17 H19 N3 O5 S	377.10454		Oxidation, Oxidation	
8	<input type="checkbox"/>		Omeprazole	C17 H19 N3 O6 S2	425.07153		Sulfation	
9	<input type="checkbox"/>		Omeprazole	C17 H19 N3 O4 S	361.10963	X	Dehydration	
10	<input type="checkbox"/>		Omeprazole	C17 H17 N3 O3 S	343.09906	X	Dehydration, Dehydration	
11	<input type="checkbox"/>		Omeprazole	C17 H17 N3 O4 S	359.09398	X	Dehydration, Desaturation	
12	<input type="checkbox"/>		Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	
13	<input type="checkbox"/>		Omeprazole	C17 H19 N3 O5 S	377.10454	X	Desaturation	

Go to the next topic, “[Reviewing the Mass Spectra for the Expected Compounds.](#)”

Filtering the Expected Compounds Table by Peak Area

Sorting the Expected Compounds by Their Transformations

Reviewing the Mass Spectra for the Expected Compounds

Inspecting the Isotope Pattern in the Full MS Scan

Use the Mass Spectrum view to view the isotopic pattern in the full MS scans for a selected compound and the FISh annotations in the compound's fragmentation scans.

To review the mass spectra, see these topics:

- [Inspecting the Isotope Pattern in the Full MS Scan](#)
- [Viewing the Fragmentation Scans](#)
- [FISh Coverage Score](#)

❖ To inspect the isotope pattern for a selected compound

1. Select the first row in the sorted Expected Compounds table (with omeprazole sorted to the first row).
2. In the Mass Spectrum view, inspect the compound's isotope pattern in the full MS scan.

Note This tutorial uses the following terminology for isotope patterns:

- A0—The spectrum peak for the monoisotopic ion or the leftmost isotopic ion.
- A1—The M + 1 ion approximately one mass unit greater than the peak for the monoisotopic ion, for example, an ion where one ^{13}C atom replaces one ^{12}C atom.
- A2—The M + 2 ion, approximately two mass units greater than the peak for the monoisotopic ion, for example, an ion where one ^{34}S atom replaces one ^{32}S atom and two ^{13}C atoms replace two ^{12}C atoms.
- A3—The M + 3 ion, approximately three mass units greater than the peak for the monoisotopic ion, for example, an ion where one ^{34}S atom replaces one ^{32}S atom and one ^{13}C atom replaces one ^{12}C atom.

This figure shows the isotope pattern for the protonated ion of omeprazole.



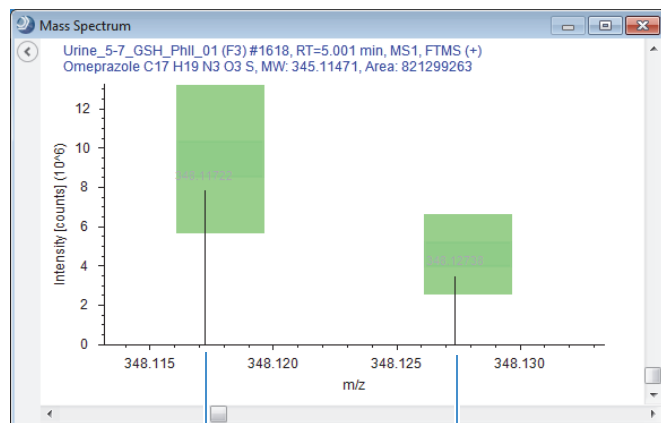
A0 peak for the monoisotopic protonated ion

A1 peak for isotope with one carbon-13 atom

Two A2 peaks

One A3 peak

This figure shows the resolution of the A2 isotopes at m/z 348.1.



A2 peak for the (M + 2) ion with two carbon-13 atoms

A2 peak for the (M + 2) ion with one sulfur-34 atom

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.

Table 4. Color-coding for isotope patterns

Color	Meaning
() Lavender	The labeled centroid is the monoisotopic mass of the expected adduct ion. The x -axis position and the width of the bar reflect the expected m/z value of the centroid and the user-specified mass tolerance, respectively. The Find Expected Compounds node searches for the monoisotopic peaks associated with the selected ion species, for example, $[M+H]+1$, $[M+Na]+1$, and so on. In this tutorial, the selected processing workflow only generates a list of expected compounds for the protonated adduct ion, $[M+H]+1$.
() Green	The labeled centroid matches the delta mass and relative intensity of the theoretical isotope pattern. When you zoom in on the matching centroid, the x -axis position and width of the rectangle reflect the expected m/z value of the centroid and the user-specified mass tolerance, respectively. The y -axis position and height of the rectangle reflect the expected relative intensity of the centroid and the user-specified intensity tolerance, respectively.
() 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 measured baseline noise (determined by the Fourier transform mass spectrometry (FTMS) mass analyzer).

Viewing the Fragmentation Scans

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 as follows:

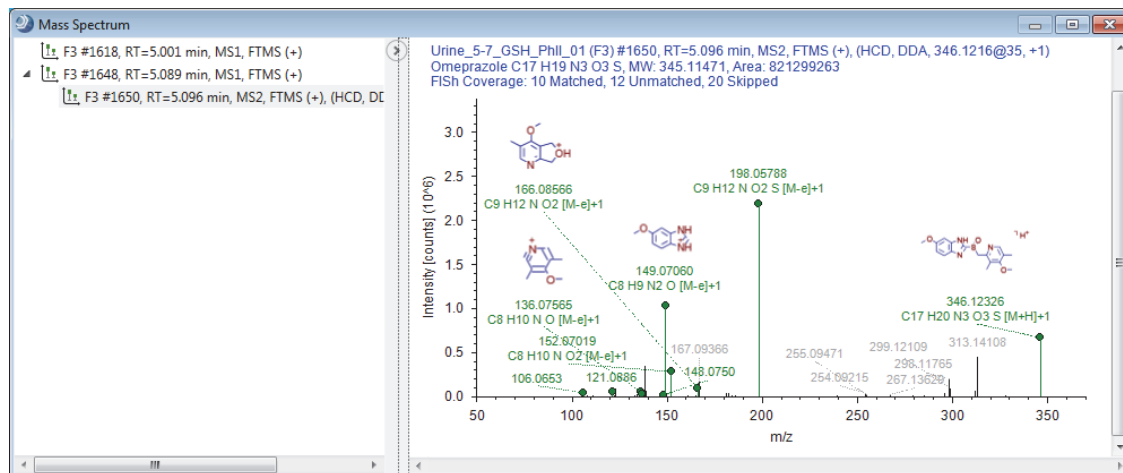
- Direct match—(●) Green
- Shifted match—(●) Blue

❖ To compare the fragmentation spectra of a metabolite and the parent compound

1. Select the first row in the sorted Expected Compounds table (with omeprazole sorted to the first row).
2. In the spectral tree pane of the Mass Spectrum view, select an MS2, DDA scan, for example, scan #1650.

The annotated fragmentation scan appears in the right pane.

Figure 10. Annotations for scan #1650 generated by the FISH Scoring node during data processing

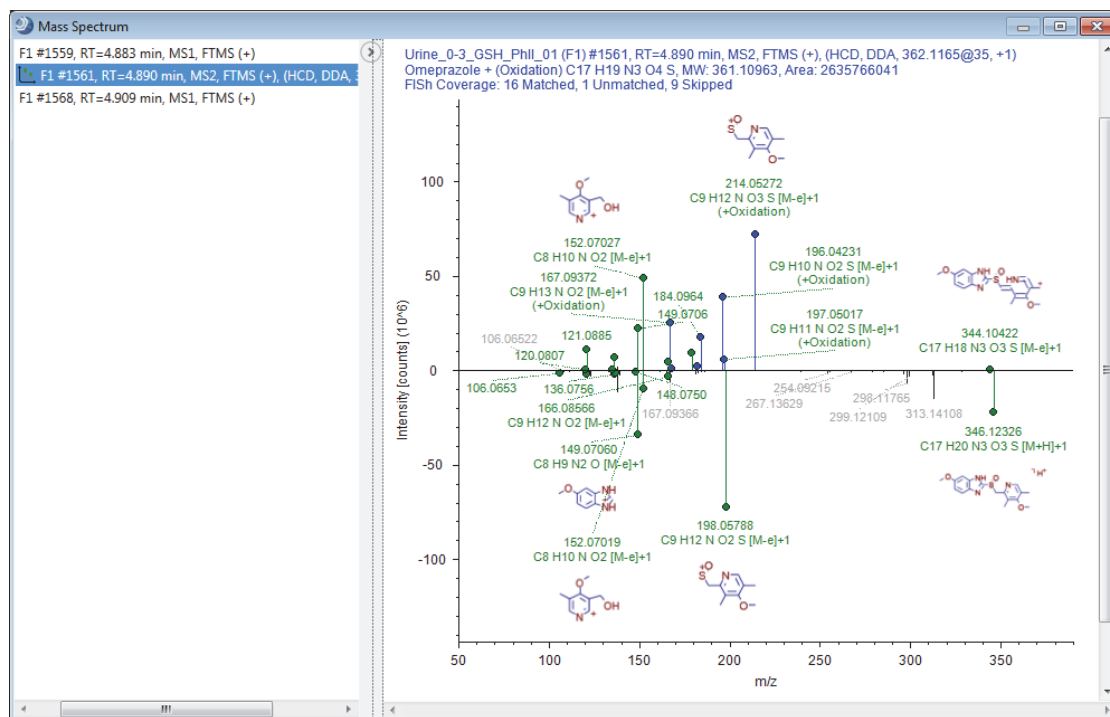


Note The FISH Scoring node cannot calculate a FISH Coverage score for compounds that lack DDA scans.

3. Right-click the Mass Spectrum view and choose **Show Library Spectra As Reference** to turn this feature off.
4. Right-click the Mass Spectrum view and choose **Use As Reference**.
5. In the Expected Compounds table, select **row 5** (oxidation product).

A mirror plot appears in the Mass Spectrum view. The MS2 spectrum for omeprazole is on the bottom and the MS2 spectrum for the metabolite is on the top.

This figure shows a mirror plot with the data-dependent fragmentation scan for omeprazole set as the reference.



Tip Use the color coded FISH fragments to elucidate the structure for the putative metabolites. Typically the smallest shifted and largest unshifted fragments help to localize the site of transformation.

In a targeted workflow where you connect the Group Expected Compounds 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 (matched + unmatched) centroids represents the number of centroids in the fragmentation scan that are above the user-specified signal-to-noise threshold (not skipped).

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 compounds with DDA scans in the Expected Compounds table.

❖ To display only the most probable explanation for each compound

1. If you have not already filtered the Expected Compounds table by the chromatographic peak area [Area (Max.)], do so now. Limit the table entries to compounds with chromatographic peak areas greater than or equal to 5e8. See “Filtering the Tables” on page 21.
2. Sort the Expected Compounds table by the Area (Max.) column in descending order.
3. For each replicated compound (same MW×RT dimensions), select the check box for the most probable explanation. In addition, select the check box for each unique compound.

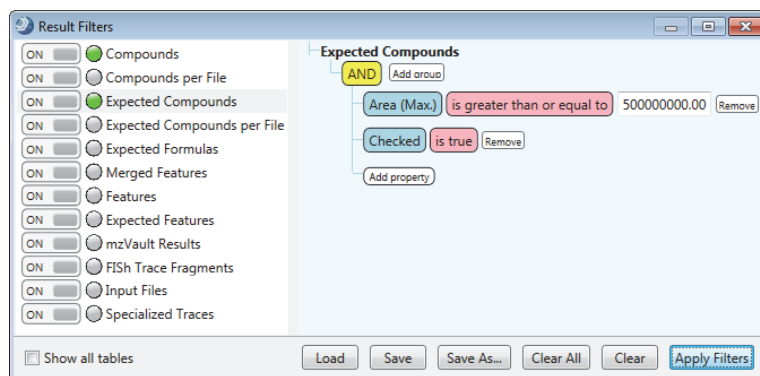
When the application returns multiple explanations for the same compound (same MW×RT dimensions), consider the following criteria to determine the correct explanation:

- Compare the FISH Coverage score for each explanation. The probability that an explanation is correct increases as the FISH Coverage score increases.
- Compare the reaction pathway for each explanation. The correct explanation is often the one with the fewest steps.

This figure shows the selection of the explanations with the higher FISH coverage score.

Comounds		Comounds per File		Expected Comounds		Expected Comounds per File		Expected Formulas		Merged Features		Features		Expected	
	Checked	Name	Parent Compound	Formula	Molecular Weight	Dealkylated	Transformations	RT [min]	FISH Coverage	Area (Max.)					
1	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H19 N3 O4 S	361.10963		Oxidation	4.909	94.12	2635766041					
2	<input type="checkbox"/>	Omeprazole	Omeprazole	C17 H19 N3 O4 S	361.10963	X	Dehydration	4.909	35.29	2635766041					
3	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H17 N3 O3 S	343.09906		Desaturation	4.939	35.00	1406494099					
4	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H17 N3 O3 S	343.09906	X	Dehydration, Dehydration	4.939	25.00	1406494099					
5	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H19 N3 O5 S	377.10454		Oxidation, Oxidation	5.507	64.29	1133779890					
6	<input type="checkbox"/>	Omeprazole	Omeprazole	C17 H19 N3 O5 S	377.10454	X	Desaturation	5.507	28.57	1133779890					
7	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H17 N3 O4 S	359.09398		Desaturation, Oxidation	4.663	46.67	1049339358					
8	<input type="checkbox"/>	Omeprazole	Omeprazole	C17 H17 N3 O4 S	359.09398	X	Dehydration, Desaturation	4.663	26.67	1049339358					
9	<input checked="" type="checkbox"/>	Parent	Omeprazole	C17 H19 N3 O3 S	345.11471			5.004	45.45	821299263					
10	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H19 N3 O6 S2	425.07153		Sulfation	4.525	23.33	620465272					
11	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	4.850	75.00	591615532					
12	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C23 H27 N3 O9 S	521.14680		Glucuronide Conjugation	3.960	47.37	508034037					
13	<input checked="" type="checkbox"/>	Omeprazole	Omeprazole	C17 H19 N3 O3	313.14264		Dehydration, Reduction, Thiourea to Urea	4.794	81.82	503325249					

- In the Result Filters view, click **Add Property** and select **Checked** from the list.



- Click **Apply Filters**.

The Expected Compounds table now displays only nine compounds.

Go to the next topic, “Viewing the Dealkylation Product for a Compound.”

Viewing the Dealkylation Product for a Compound

❖ To view the dealkylation product for a compound with additional transformations

- In the Expected Compounds table, select a compound that is the product of a dealkylation reaction and one or more transformations.
- For example, select **row 7** (C16 H17 N3 O2 S).

Figure 11. Related structures table for dealkylation product

Expected Compounds										
Checked	Name	Parent Compound	Formula	Molecular Weight	Dealkylated	Transformations	RT [min]	FISH Coverage	Area (Max.)	
<input checked="" type="checkbox"/>	Omeprazole		C17 H19 N3 O4 S	361.10963		Oxidation	4.909	94.12	2635766041	
<input checked="" type="checkbox"/>	Omeprazole		C17 H17 N3 O3 S	343.09906		Desaturation	4.939	35.00	1406494099	
<input checked="" type="checkbox"/>	Omeprazole		C17 H19 N3 O5 S	377.10454		Oxidation, Oxidation	5.507	64.29	1133779890	
<input checked="" type="checkbox"/>	Omeprazole		C17 H17 N3 O4 S	359.09398		Desaturation, Oxidation	4.663	46.67	1049339358	
<input checked="" type="checkbox"/>	Omeprazole	Parent	C17 H19 N3 O3 S	345.11471			5.004	45.45	821299263	
<input checked="" type="checkbox"/>	Omeprazole		C17 H19 N3 O6 S2	425.07153		Sulfation	4.525	23.33	620465272	
<input checked="" type="checkbox"/>	Omeprazole		C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	4.850	75.00	591615532	
<input checked="" type="checkbox"/>	Omeprazole		C23 H27 N3 O9 S	521.14680		Glucuronide Conjugation	3.960	47.37	508034037	
<input checked="" type="checkbox"/>	Omeprazole		C17 H19 N3 O3	313.14264		Dehydration, Reduction, Thiourea to Urea	4.794	81.82	503325249	

Structure Proposals										
Checked	Parent Compound	Formula	Molecular Weight	Dealkylated	Transformations	Composition Char	RT [min]	FWHM [min]	Best SFit [%]	Max. # MI
<input checked="" type="checkbox"/>	Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	-(C H2 O)	4.850	0.041	71	5
<input type="checkbox"/>	Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	-(C H2 O)	4.848	0.041	73	5
<input type="checkbox"/>	Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	-(C H2 O)	4.855	0.039	66	5
<input type="checkbox"/>	Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	-(C H2 O)	4.849	0.040	81	5

Related Structures						
Checked	Parent Compound	Formula	Molecular Weight	Dealkylated	Composition Char	Structure
<input type="checkbox"/>	Omeprazole	C16 H17 N3 O3 S	331.09906	X	-(C H2)	
<input type="checkbox"/>	Omeprazole	C16 H17 N3 O3 S	331.09906	X	-(C H2)	

Parent structures related to
2 items shown (0 filtered out)

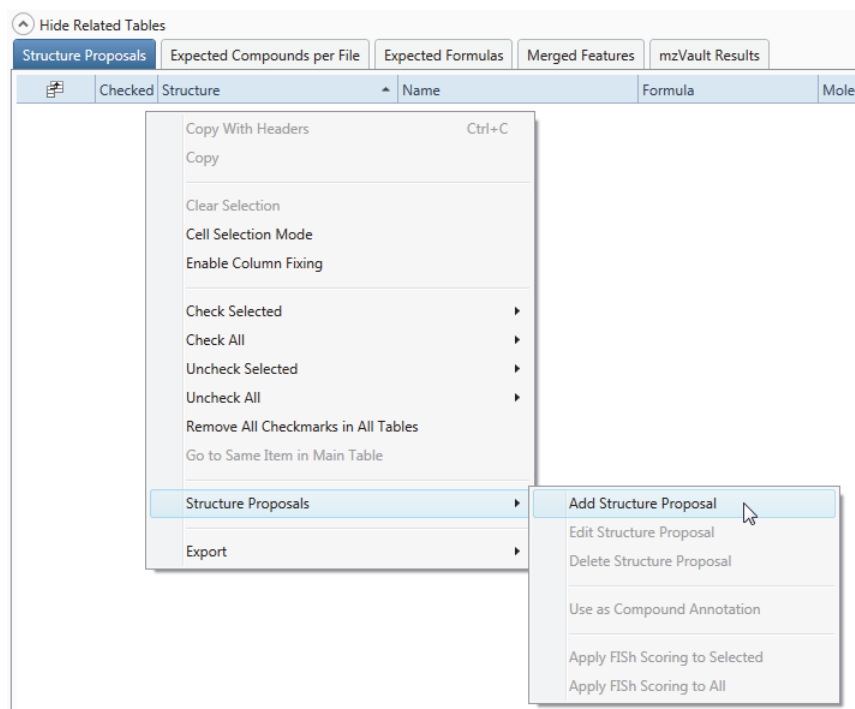
Go to the next topic, “Naming and Proposing Structures for the Expected Compounds.”

Naming and Proposing Structures for the Expected Compounds

You can add names and structures for expected compounds to the result file.

❖ To populate the Name and Structure columns in the Expected Compounds table


1. In the Expected Compounds table, right-click the row for omeprazole—row 5 when sorted in descending order by Area (Max.).
2. Click **Show Related Tables**, and then click the **Structure Proposals** tab.
3. Right-click the empty Structure Proposals table and choose **Add Structure Proposal**.



A new row with the formula and MW of the expected compound appears in the Structure Proposals table.

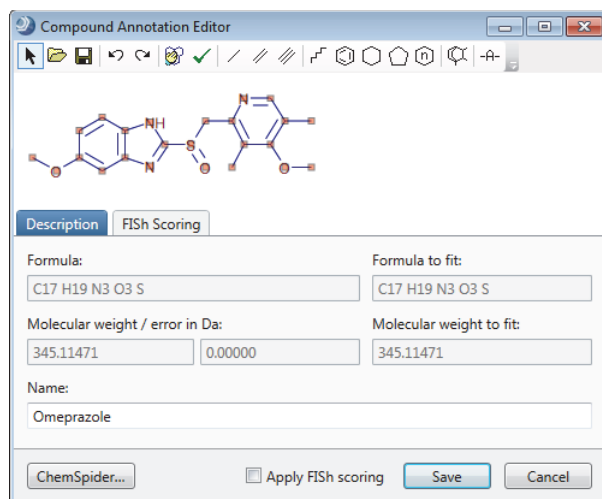
The screenshot shows the 'Structure Proposals' table with a new row added. The table has columns: Checked, Structure, Name, Formula, Molecular Weight, FISH Coverage, and Comments. The new row has the following data:


Checked	Structure	Name	Formula	Molecular Weight	FISH Coverage	Comments
1			C17 H19 N3 O3 S	345.11471		

4. Double-click the row in the Structure Proposals table.
The Compound Annotation Editor opens to the Description page.
5. To assign a structure to this custom annotation, do one of the following:
 - Click the **Load Structure from Disk** icon, , and open the Omeprazole.mol file that you copied to your processing computer.
 - Use the drawing tools to draw a structure. To open the Help for the Compound Annotation Editor, press the F1 key.
 - If your computer has Internet access, click **ChemSpider**. When the search finishes, select a structure from the list.

The application checks whether the calculated exact mass of the structure matches the molecular weight and the elemental composition (if available) of the selected peak within the mass tolerance (for XIC trace creation) that you specified in the Find Expected Compounds node.


6. In the Name box, type **Omeprazole**.



7. Run the FISH scoring algorithm as follows:
- Select the **Apply FISH Scoring** check box.
 - Click **Save**.
- The structure appears in the Structure column of the Structure Proposals table, and the FISH Scoring Queue opens. The application recalculates the FISH Coverage score and modifies the fragmentation spectra annotations by using the proposed structure.
8. In the Structure Proposals table, right-click the row for your new structure proposal and choose **Structure Proposals > Use As Compound Annotation**.
- Omeprazole appears in the Name column of row 5 (MW 345.11471).
9. To view the structure in the Expected Compounds table, display the Structure column as follows:
- Click the **Field Chooser** icon, .
 - Select the **Structure** check box.
10. If necessary, to restore the original processing results, right-click the newly annotated row and choose **Reset Compound Annotation**.
- The application removes the structure and name annotations and restores the original FISH Coverage score.
11. Repeat steps 2 through 8 for row 1 (MW 361.10963). To annotate the compound, use the 5-hydroxyomeprazole.mol file that you copied to your processing computer, and then name the compound 5-hydroxyomeprazole.

If an unidentified compound in the Compounds table matches the expected isotope pattern, use its related Merged Features and Expected Compounds tables to determine its possible transformation path.

❖ To find unexpected metabolites

- Click the **Compounds** tab.
- If you have not already filtered the Compounds table by the following filter do so now—Area (Max.) is greater than or equal to 5e8.
The Compound table is now reduced to 36 visible compounds.
- Click the expand icon, , to the right of the Pattern Matches column heading, select **C17S**, and then sort the Compounds table in descending order by the **Pattern Matches** column.
- Select **row 2** (C17 H19 N3 O5 S).
- In the primary set of related tables, click the **Merge Features** tab.
- Compare the Find Expected Compounds column to the Detect Compounds column.
- Select an entry in the Merged Features table where both nodes found the compound. In addition, select the check box for this entry.

8. In the secondary set of related tables, click the **Expected Compounds** tab.
9. Review the transformations.
10. Select the check box for most probable explanation.

This figure shows the selection of the Oxidation, Oxidation transformation as the most probable explanation for C17H19N3O5S in the Compounds table.

Checked	Name	Formula	Annotation Source	FISH Coverage	Molecular Weight	RT [min]	Area (Max.)
<input type="checkbox"/>	Esomeprazole	C17 H19 N3 O3 S			345.11424	5.003	866917990
<input type="checkbox"/>		C17 H19 N3 O5 S			377.10405	5.508	1183484546

Checked	Name	Apex m/z	RT [min]	Max. Area	Ion Conflict Status	Find Expected Compounds	Detect Compounds
<input checked="" type="checkbox"/>		378.11133	5.507	1174933584	■■■■	■■■■	■■■■
<input type="checkbox"/>		400.09317	5.507	8444432	■■■■	■■■■	■■■■

Checked	Name	Parent Compound	Formula	Molecular Weight	Dealkylated	Transformations	# mzVault Results	Composition Change
<input checked="" type="checkbox"/>		Omeprazole	C17 H19 N3 O5 S	377.10454		Oxidation, Oxidation	0	+(O2)

The layout of the result file includes the location of the graphical views, the columns and rows that you want to display in the result tables, the filter set, and the Group By and Filter By settings.

❖ **To save the current layout of the result file**

With the result file selected as the active page, do one of the following:

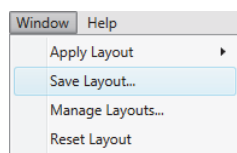
- In the toolbar, click the **Save the Currently Active Item** icon, .

–or–

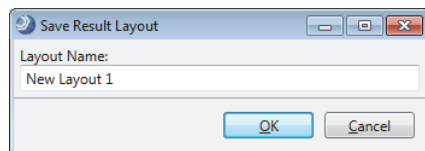
- From the menu bar, choose **File > Save** (save the currently active item).

❖ **To save the current layout of the result file to a layout file**

1. With the result file selected as the active page, in the menu bar choose **Window > Save Layout**.



2. Name the layout and click **OK**.



❖ **To restore the default layout to a result file**

1. With the result file selected as the active page, in the menu bar choose **Window > Reset Layout**.
2. Click **Yes** to confirm the action.

Saving or Resetting the Result File's Layout

Compound Discoverer reports are based on the result tables and their associated chromatograms and mass spectra.

Before you print a report, review the result table that you want to include in the report. Filter the table as described in “Determining the Most Probable Explanation for Each Expected Compound.” Sort the table by the column of interest, for example, by retention time (see “Sorting the Result Tables” on page 21).

Note For information about creating and editing your own custom report templates, refer to the *Compound Discoverer 3.1 Reporting Quick Start*, the reporting chapter in the *Compound Discoverer 3.1 User Guide*, or the Help.

With the current reporting tool, you can add graphs for chromatogram traces and the MS1 and MS2 spectra to the report template. You cannot add graphs for the statistical plots.

Follow these procedures to print reports:

- To create a report by using the defined report template for the Expected Compounds table
- To print the report
- To export the contents of a report to a file

❖ **To create a report by using the defined report template for the Expected Compounds table**


1. On the result file page, click the **Expected Compounds** tab.
2. From the menu bar, choose **Reporting > Create Report**.

The Open Design Template dialog box opens.

3. Select the **Expected Compounds No Graphs A4.cdReportTemplate** file and click **Open**.

The application resolves the report and displays thumbnails in the Page Thumbnails pane to the left.

❖ **To print the report**

1. In the Current Page/Total Number of Pages box, check how many pages are in the report.
2. In the reporting toolbar, click the **Print** icon, , make the appropriate selections in the Print dialog box, including the print range and any advanced settings, and click **OK**.

❖ **To export the contents of a report to a file**

1. In the reporting toolbar, choose **Export > File Type**.
2. Select the directory where you want to store the file, name the file, and click **Save**.

To create an inclusion list of m/z values for specific adduct ions, follow these topics in order:

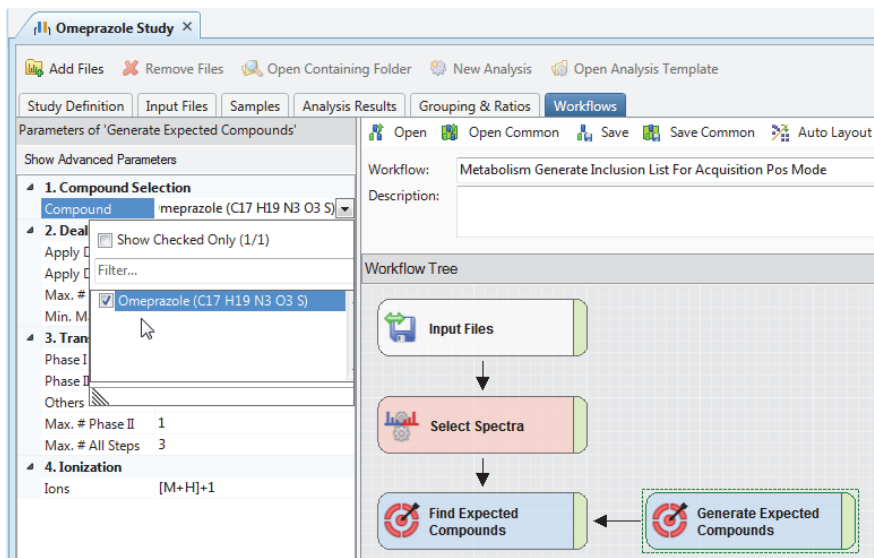
1. [Generating a Formulas List](#)
2. [Exporting the Contents of a Result Table to a Spreadsheet](#)
3. [Converting a Molecular Weight List to a Mass-to-Charge List](#)

❖ **To generate a list of formulas for the targeted compounds and their transformation products**

1. Add the compounds of interest to your Expected Compounds list (“Adding Compounds to the Expected Compounds List” on page 2).
2. Start a new analysis from the existing study as follows:
 - a. Click the **Omeprazole Study** tab.
 - b. In the study command bar, click **New Analysis**.
3. Click the **Workflows** tab.

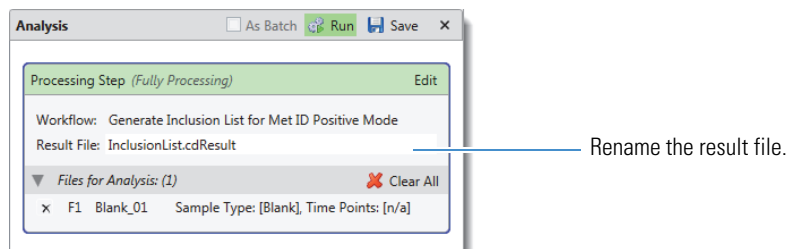
4. Select the processing workflow as follows:
 - a. Click **Open Common** in the command bar.
 - b. Open the **Workflow Templates** folder.
 - c. Open the **MetID** folder.
 - d. Select the following processing workflow:

MetID Generate Inclusion List for Acquisition Pos Mode.cdProcessingWF
 - e. Click **Open**.
5. Customize the processing workflow as follows:
 - a. In the Workflow Tree area, select the **Generate Expected Compounds** node.
 - b. In the Compound list, select the compounds of interest.



6. Drag any input file from the Input Files page to the Files for Analysis area of the Analysis pane.

All analyses require input files; however, this analysis does not use the raw data to generate chromatograms or spectral trees. It only generates a list of expected formulas for the selected compounds and their transformation products.
7. Rename the result file.



8. Click **Run**.

The Job Queue page opens.

Go to the next topic


Follow this procedure to export the contents of the Expected Formulas table to a spreadsheet.

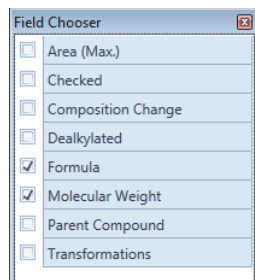
❖ **To export the formulas to a spreadsheet**

1. When the run is complete, on the Job Queue page, double-click the result file.

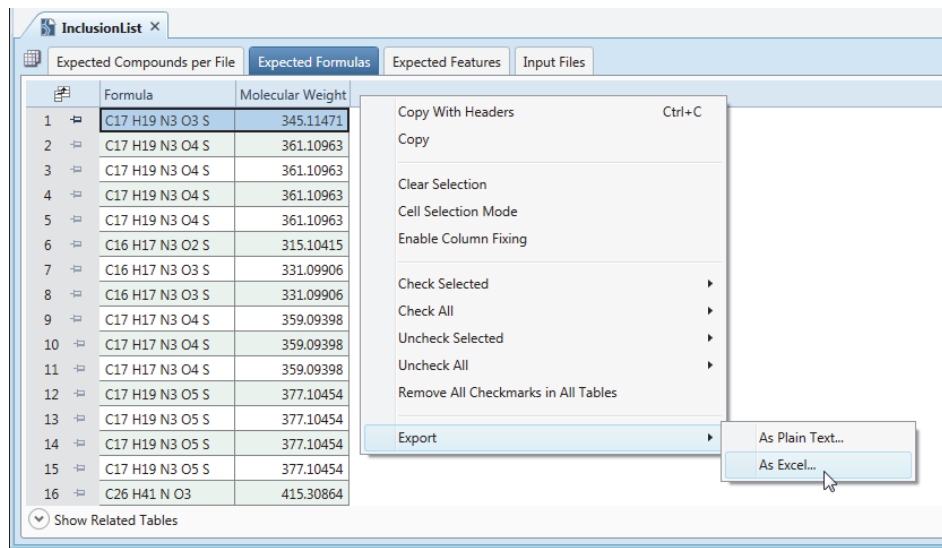
The result file opens as a tabbed page.
2. On the result file page, click the **Expected Formulas** tab.

Exporting the Contents of a Result Table to a Spreadsheet

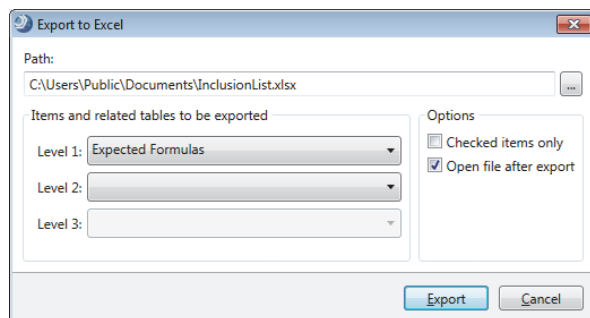
- Open the Field Chooser dialog box for the Expected Formulas table (click its **Field Chooser** icon, ) and clear all the check boxes, except those for Formula and Molecular weight.



- Right-click the table and choose **Export > As Excel**.



- In the Export To Excel dialog box, select a folder, name the file, and click **Export**.



- At the prompt, click **OK**.

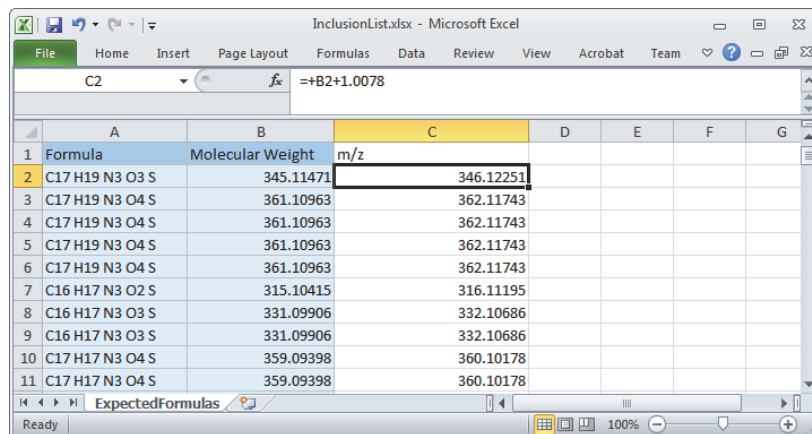
Converting a Molecular Weight List to a Mass-to-Charge List

Follow this procedure to convert a list of molecular weights to a list of mass-to-charge values. Use the following procedure to convert the molecular weights for neutral compounds to the mass-to-charge values for the protonated adduct ions.

❖ To convert the spreadsheet to an inclusion list for data acquisition

- Open the exported spreadsheet in a spreadsheet application.
The Molecular Weight column displays the molecular weight of the neutral compound.
- Name the third column **m/z**.

- To display the molecular weight of the protonated ion of each compound, copy the following formula down the third column: **+B2+1.00728**.



The screenshot shows a Microsoft Excel spreadsheet titled "InclusionList.xlsx". The spreadsheet has three columns: A (Formula), B (Molecular Weight), and C (m/z). The formula bar shows the formula $=+B2+1.00728$ entered in cell C2. The data in the spreadsheet is as follows:

	A	B	C	D	E	F	G
1	Formula	Molecular Weight	m/z				
2	C17H19N3O3S	345.11471	346.12251				
3	C17H19N3O4S	361.10963	362.11743				
4	C17H19N3O4S	361.10963	362.11743				
5	C17H19N3O4S	361.10963	362.11743				
6	C17H19N3O4S	361.10963	362.11743				
7	C16H17N3O2S	315.10415	316.11195				
8	C16H17N3O3S	331.09906	332.10686				
9	C16H17N3O3S	331.09906	332.10686				
10	C17H17N3O4S	359.09398	360.10178				
11	C17H17N3O4S	359.09398	360.10178				

- Check the cell format and verify that the number of decimal places is appropriate for your MS.
- Save the modified spreadsheet.

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