Compound Discoverer 3.2 Metabolism Tutorial

To familiarize yourself with the Thermo Compound Discoverer™ 3.2 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.

Contents

Overview

Before you open the application and begin this tutorial, review the following topics:

- Location of the example files
- Tutorial summary
- The Help system

Location of the example files

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\LC\Met ID\Omeprazole Study

Tip You can also download these example files from the Compound Discoverer 3.2 Product Download page of the LSMS Software Download and Licensing Portal website.

https://thermo.flexnetoperations.com

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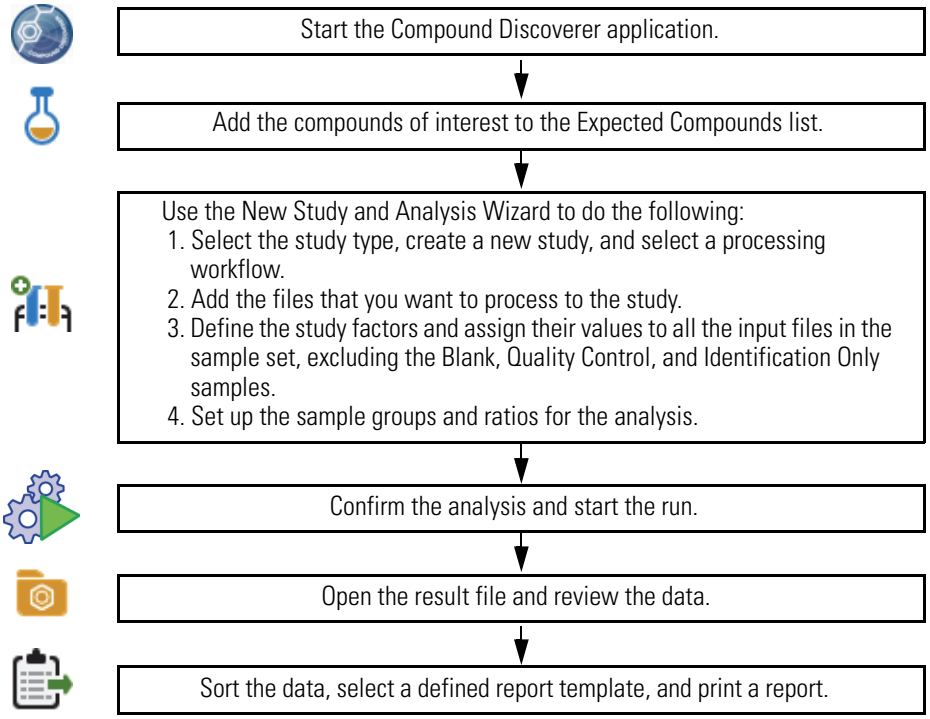
Copy the Met ID folder to your data processing computer.

This folder contains the following files.

File name	Description
Omeprazole Study.cdStudy	Compound Discoverer study file with the study information for the Xcalibur RAW files
Omeprazole Example.cdResult	Compound Discoverer result file from an analysis with a Met ID processing workflow without a node for naming the detected compounds
Omeprazole Example with ID.cdResult	Compound Discoverer result file from reprocessing the Omeprazole Example.cdResult file after adding a node for naming the detected compounds
Omeprazole.mol	Structure file
Urine_0-3_GSH_PhII_01.raw	Xcalibur raw data file from a Q Exactive mass spectrometer (MS)
Urine_3-5_GSH_PhII_01.raw	Xcalibur raw data file from a Q Exactive MS
Urine_5-7_GSH_PhII_01.raw	Xcalibur raw data file from a Q Exactive MS
Urine_7-9_GSH_PhII_01.raw	Xcalibur raw data file from a Q Exactive MS

Tutorial summary

This flowchart summarizes the overall workflow for a targeted analysis of expected compounds.



The Help system

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. 

Start the application

❖ To start the Compound Discoverer application

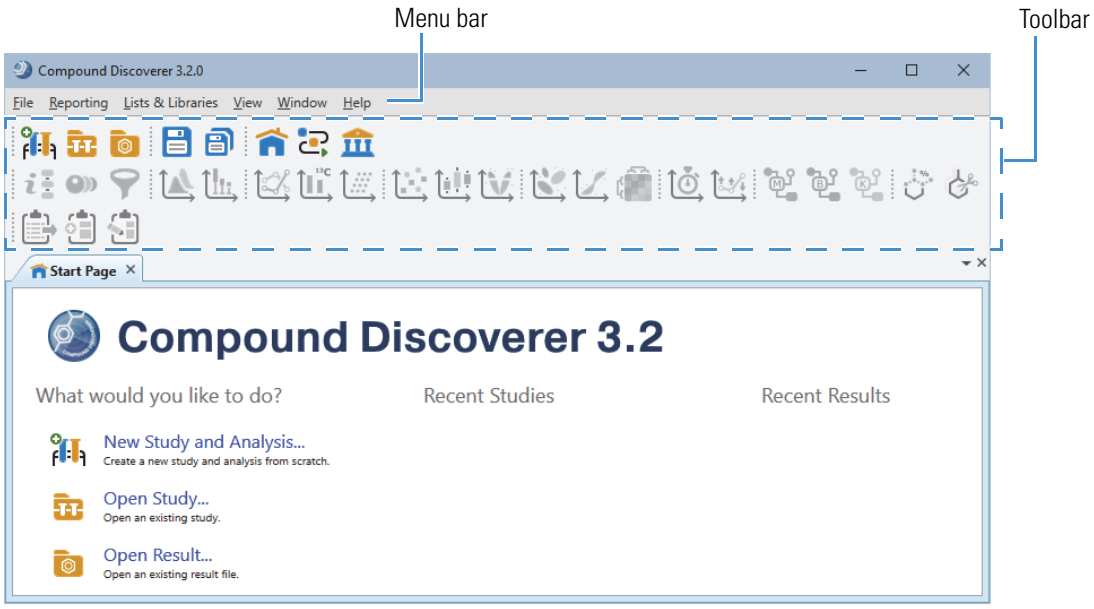
- From the taskbar, choose **Start > All Programs** (or **Programs**) **> Thermo Compound Discoverer 3.2**.

–or–

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

The application opens to the Start Page (Figure 1).

Figure 1. Start Page



Add your target compounds to the Expected Compounds list

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

Tip If you are using the omeprazole example data set, you do not need to add the target compound, as this library already includes omeprazole. To use a different data set for this tutorial, follow the instructions in this topic to add the compounds you are studying to the Expected Compounds library.

❖ To add a compound to the Expected Compounds library

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 structure for a compound, use one of the following methods:

   a. If you do not have a structure file for the compound, click **ChemSpider** at the bottom right of the dialog box.

      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, click the **Load Structure from Disk** icon, , in the Compound Editor toolbar,

The Open Structure dialog box opens.

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

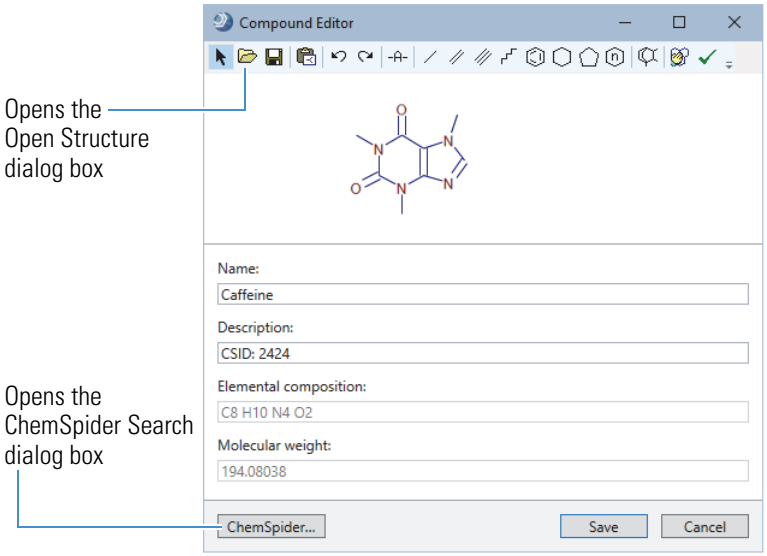
The Compound Editor dialog box supports the following file formats:

- 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. For a ChemSpider search, the application also populates the Description box with the CSID number of the compound and the Name box with its name (Figure 2).

Figure 2. Compound Editor dialog box with the structure of caffeine returned by a ChemSpider search

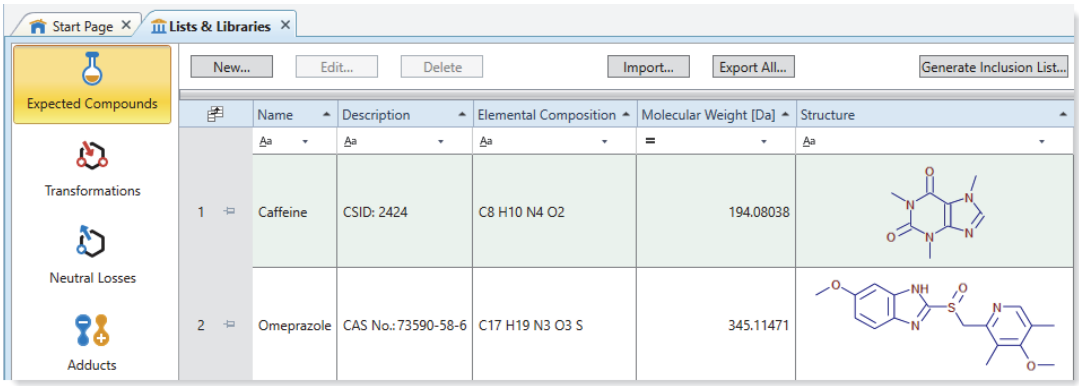


5. When applicable, type a different name for the compound.

6. Click **Save** to add the compound to the Expected Compounds library.

The new compound appears in the library (Figure 3).

Figure 3. Lists & Libraries page with the Expected Compounds library displayed



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

Note To delete a compound, select the compound in the library and click **Delete**.

Set up a new study and a new analysis

To create a new study, you must use the New Study and Analysis wizard.

To set up a new study and a new analysis, follow these steps:

1. Open the New Study and Analysis Wizard
2. Select the study type, specify the directory folder, and name the new study
3. Select a processing workflow for the analysis
4. Add the input files to the study
5. Specify the experimental variables
6. Group the samples in this tutorial by their collection time
7. Troubleshoot the analysis

Open the New Study and Analysis Wizard

❖ **To open the New Study and Analysis Wizard**

Do one of the following:

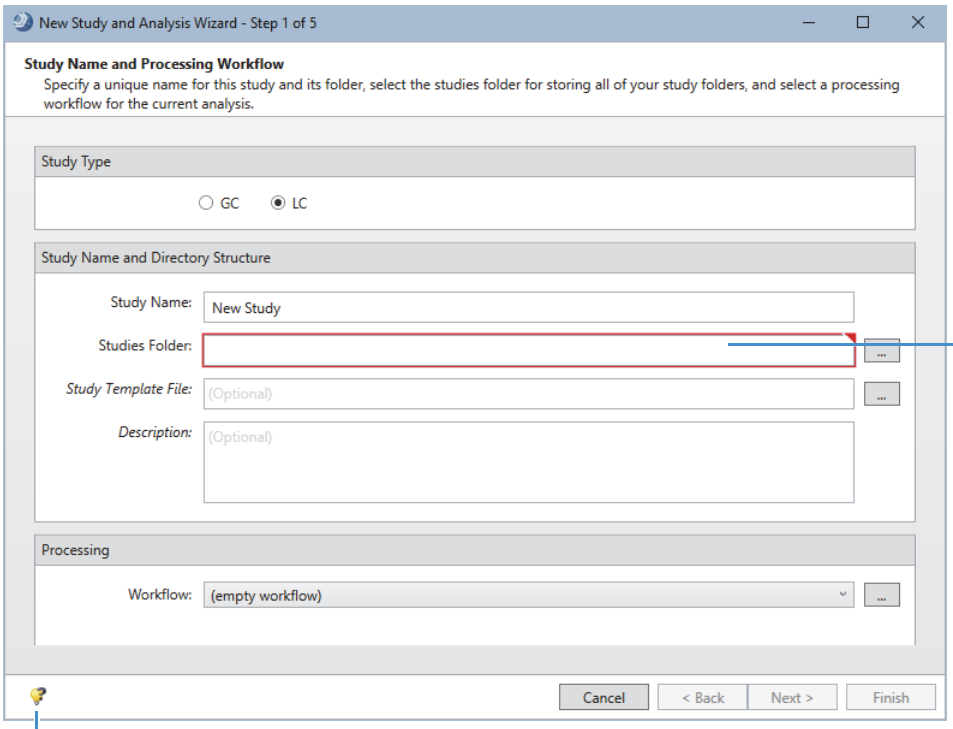
- From the menu bar, choose **File > New Study and Analysis**.
- From the application toolbar, click the **Create a New Study** icon, .
- On the Start Page, click the **New Study and Analysis** link in the What Would You Like to Do? area.

The New Study and Analysis Wizard opens to the Study Name and Processing Workflow page (Figure 4).

The first time you open the wizard, the Studies Folder is undefined. After you select the directory folder for your Compound Discoverer studies, the application remembers the location. You can store all your studies in one directory or create new directory folders as needed.

Clicking the light bulb icon (or), at the bottom left of the wizard opens or closes the wizard's embedded instructions.

Figure 4. Study Name and Processing Workflow page of the New Study and Analysis Wizard



Go to the next task to "Select the study type, specify the directory folder, and name the new study."

Select the study type, specify the directory folder, and name the new study

❖ To select the study type, specify the study folder, and name the new study

1. In the Study Type area on the Study Name and Processing Workflow page of the wizard (Figure 4), select the **LC** option if it is not already selected.

   The application stores this selection until you change it.

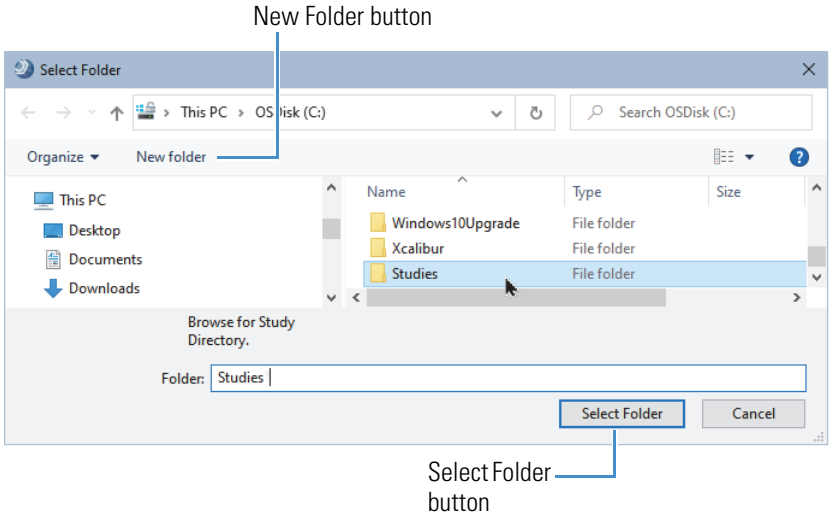
   Note There are two types of studies: GC for gas chromatography-mass spectrometry data and LC for liquid chromatography-mass spectrometry data.

2. In the Study Name and Directory Structure area, select the studies folder as follows:

   a. Click the **browse** icon, ..., next to the Studies Folder box.

      The Select Folder dialog box opens (Figure 5).

Figure 5. Select Folder dialog box

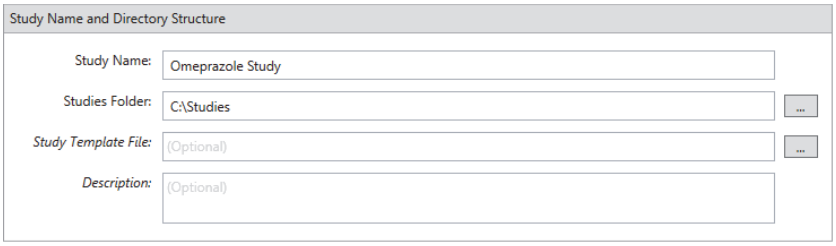


b. Browse to the directory where you want to store your studies.

c. Click **New Folder**.

d. Name the new folder **Studies**, select it, and then click **Select Folder**.

3. In the Study Name and Directory Structure area, type **Omeprazole Study** in the Study Name box.



Note When you create a new study, the application creates a new study folder with the same name and stores the study file (.cdStudy) in the new folder and the new study folder in the specified studies folder.

Stay on this page of the wizard and go the next topic to "Select a processing workflow for the analysis."

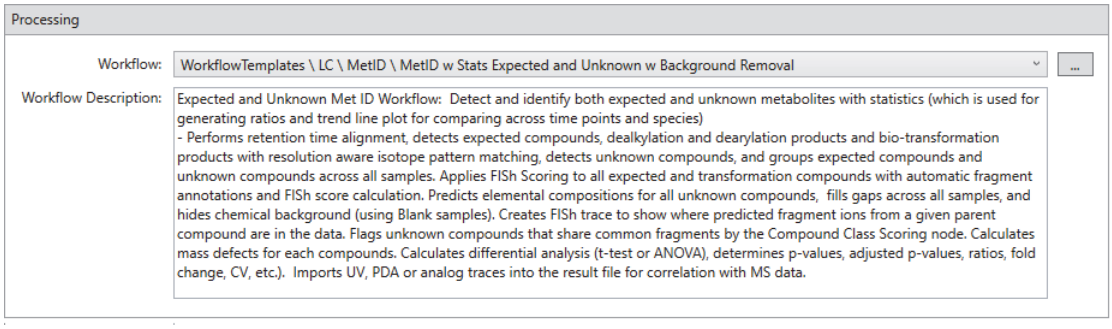
Select a processing workflow for the analysis

❖ To select the processing workflow

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

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

   A description of the processing workflow appears in the Workflow Description box.



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

Go to the next topic to "Add the input files to the study."

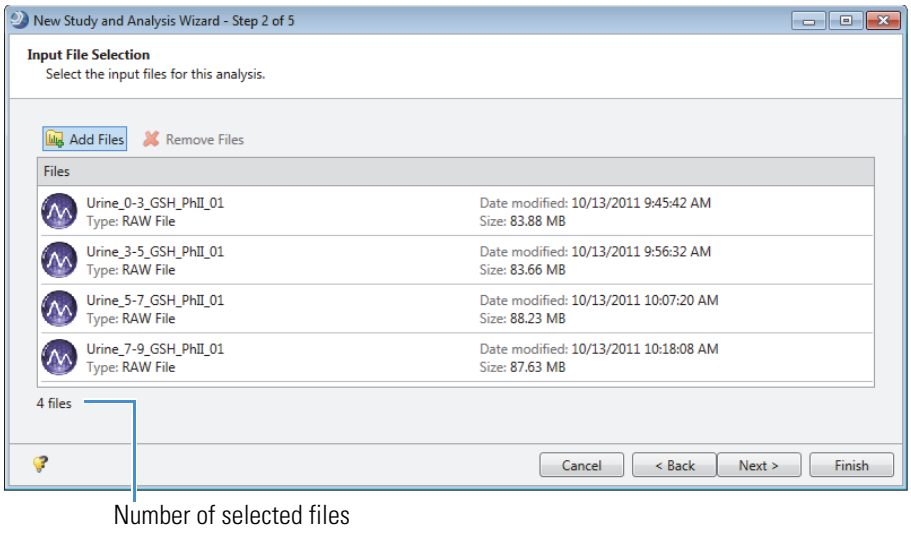
Add the input files to the study

❖ 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\ LC\Met ID\Omeprazole 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.



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

Go to the next topic to "Specify the experimental variables."

Specify 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, 7and 9 hours 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 specify the study variables for the example data set

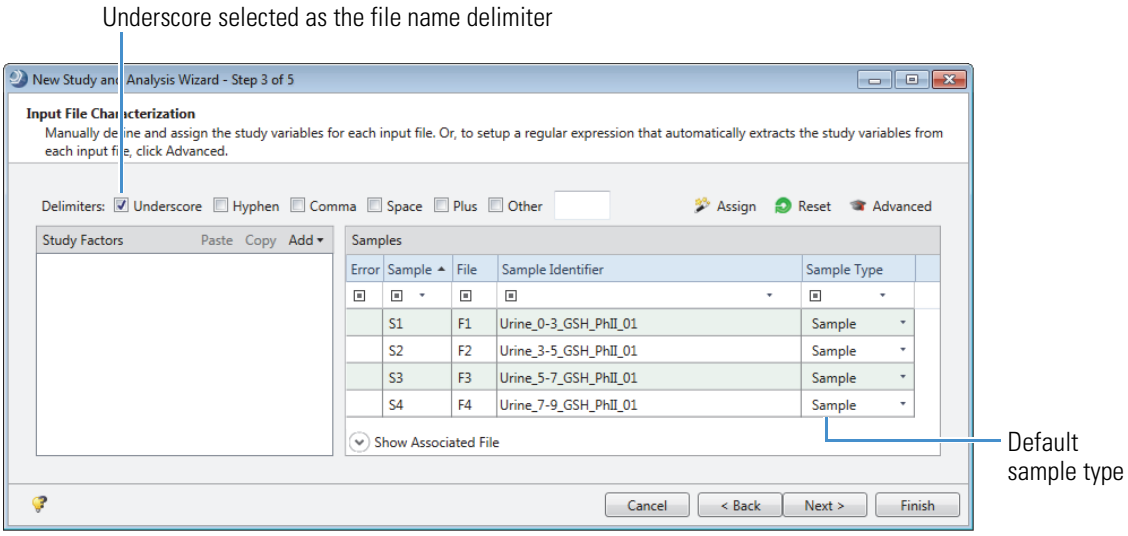
The study variables include the sample types and study factor values.

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

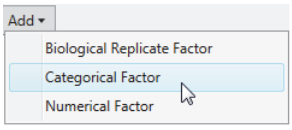
   The time points study variable is included in the file names of the example data set, and underscore symbols separate the time point text (for example, 0-3) from the other text in the file name (Urine_0-3_GSH_PhII_01).

Figure 6 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.

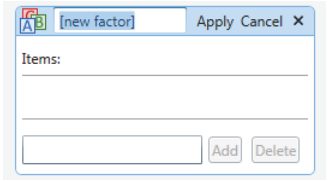
Figure 6. Underscore check box selected



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



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



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 for a non-quantifiable feature, use the categorical study factor editor.
- To define study factors for quantifiable features that are provided as numeric values, use the numerical study factor editor.
- To set up a nested experiment, use the biological replicate factor for one of the study factors.

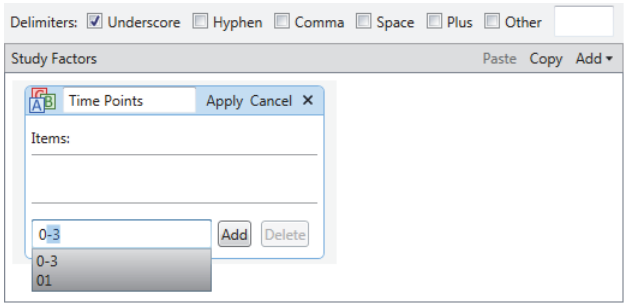
To open the Help topic about setting up the study factors and sample types, press the F1 key.

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** (Figure 7).

      If the file name contains a character delimiter and you selected the check box for the delimiter, 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.

Figure 7. Typing the categorical item



The Add button becomes available.

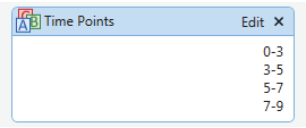
b. Click **Add**.

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.

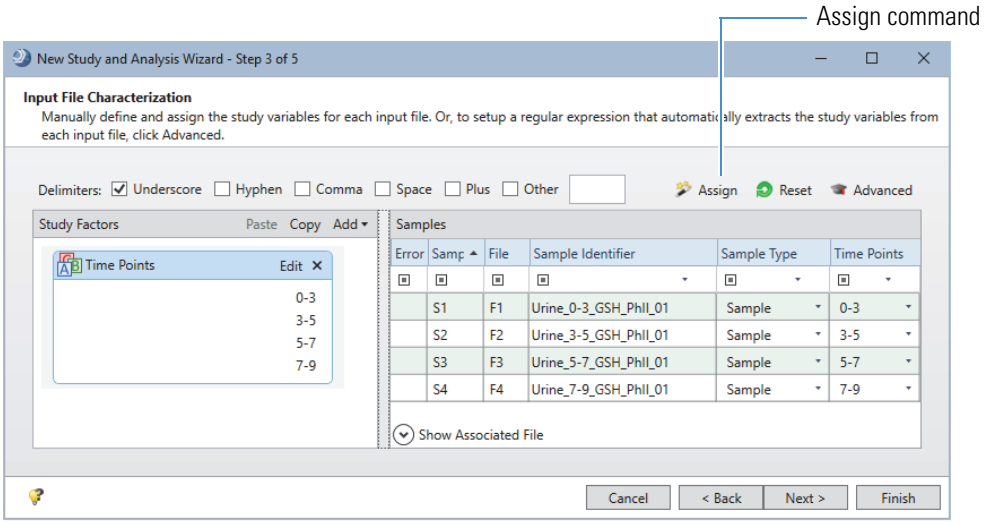


6. Click **Assign**.

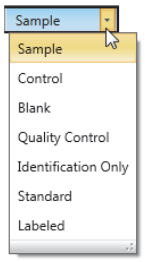
Note The application assigns the Blank sample type to any sample that begins with Blank (or that contains Blank as delimited text, for example, Solvent_Blank with Blank preceded by the selected delimiter). The sample set for this tutorial does not include blanks.

The wizard automatically assigns the study factors to the samples.

Figure 8. Samples with assigned study factor values



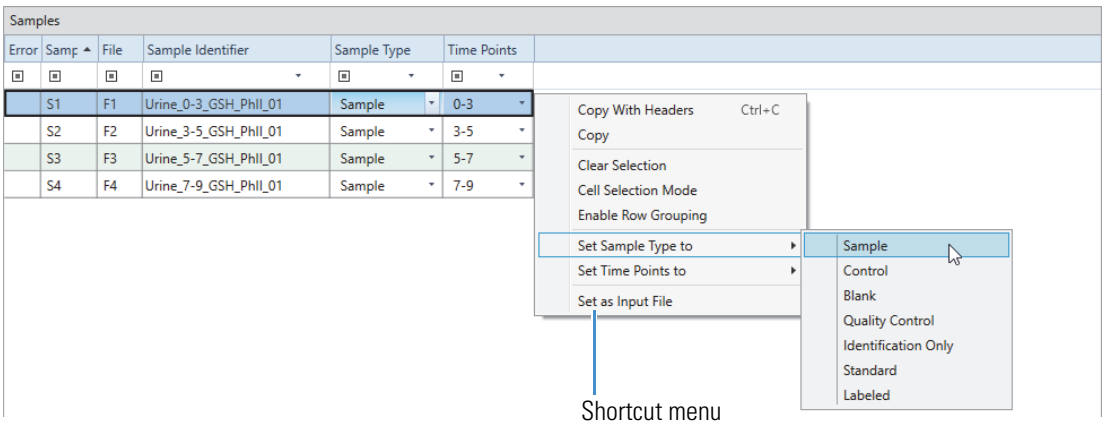
7. For the example data set, keep the default assignments of **Sample**.



   For other data sets, select the sample types as follows.

   - To change the sample type for a single sample, click the down arrow and select the sample type from the dropdown list.
   - 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 9. Samples pane shortcut menu



The sample type affects the processing actions (Table 1).

Table 1. Sample types (Sheet 1 of 2)

Sample type	Processing actions
Sample, Control, and Standard	Detects the components in the sample. When the analysis includes study groups, the application reports the group areas for these sample types by study factor. The application 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.
	IMPORTANT To group 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.
Blank	Detects the components in the sample. When the processing workflow includes Mark Background Compound nodes, the application marks these components as background compounds.
Quality Control	Detects the components in the sample. When the processing workflow includes a QC correction node and the analysis includes QC samples, the application does the following: • Fits the areas of the compounds in the QC samples to linear or cubic spline curves. Or, runs a Random Forest analysis (new Apply SERRF QC Correction (beta) node on the compound areas. • Runs a batch normalization of the non-QC samples against these curves.

Table 1. Sample types (Sheet 2 of 2)

Sample type	Processing actions
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.

8. 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.

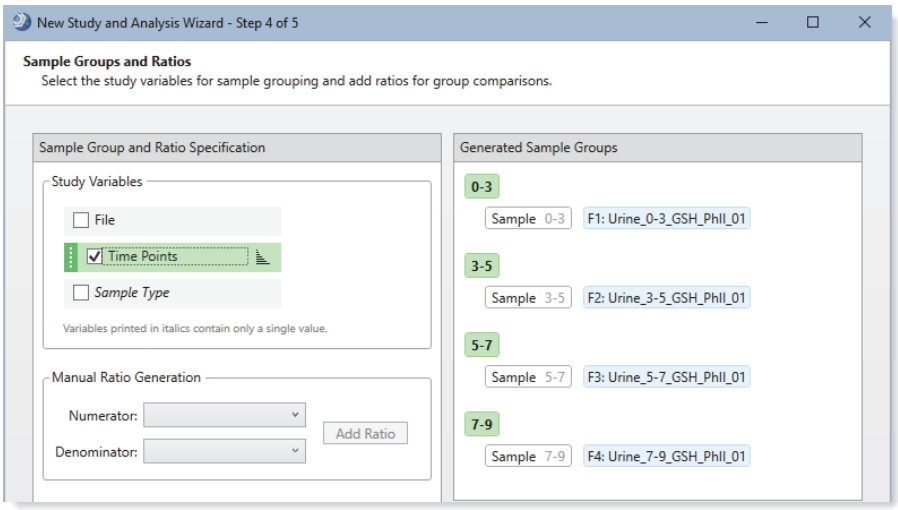
Go to the next topic to "Group the samples in this tutorial by their collection time."

Group the samples in this tutorial by their collection time

❖ 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. 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.

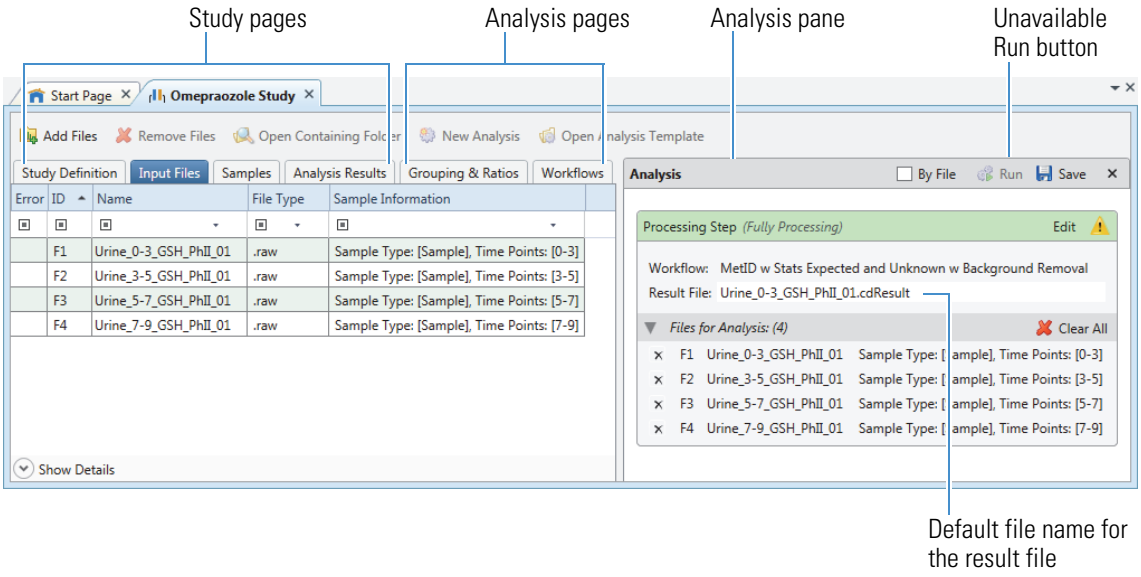
3. Click **Finish**.

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 (see Figure 10).

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.

Figure 10. New study page and new analysis



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 statistical views, refer to the Help, the metabolomics tutorial, or the extractables and leachables tutorial.

Go to the next topic to "Troubleshoot the analysis."

Troubleshoot 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

In the Analysis pane, point to the Caution symbol (Figure 11). Or, click the Workflows tab and review the messages in the Current Workflow Issues pane at the bottom of the Workflows page (Figure 12).

The application displays explanations for the analysis errors.

Figure 11. Analysis pane with Caution symbol to the right of the Edit button

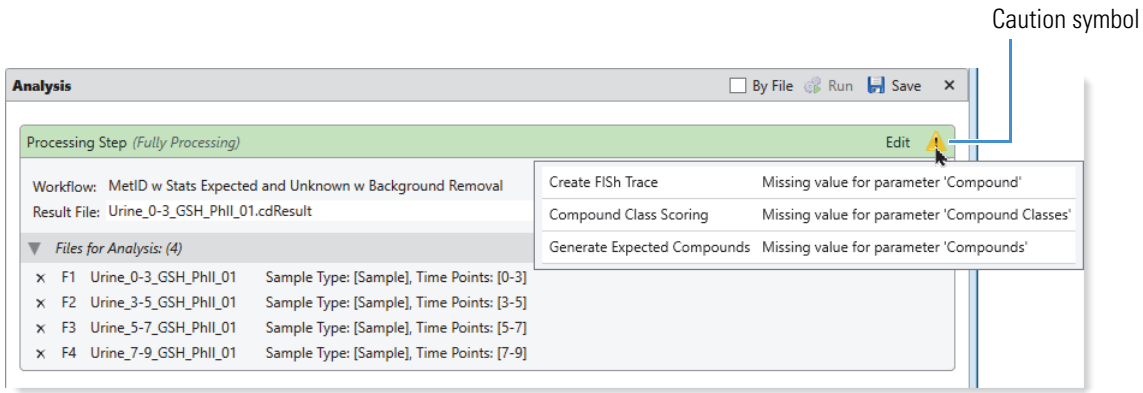


Figure 12. Current Workflow Issues pane at the bottom of the Workflows page

Current Workflow Issues

Node Name	Issue Description	Parameter Name	Value
Create FISh Trace	Missing value for parameter 'Compound'	Compound	
Compound Class Scoring	Missing value for parameter 'Compound Classes'	Compound Classes	
Generate Expected Compounds	Missing value for parameter 'Compound'	Compound	

All of the errors are due to missing values in the workflow nodes. To fix the analysis errors, go to the next topic to "Customize the processing workflow."

Customize the processing workflow

For an analysis of one or more target 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 and the compound class file for the Compound Class Scoring node.

Tip When customizing a processing workflow template, do the following:

- Base 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.
- Base the Base Ions for the Detect Compounds node and the Preferred Ions for the Group Compounds node on the experimental factors.
- Change 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.

To customize the processing workflow for the example data set, do the following:

- Specify the target compounds and adduct ions
- Specify the parent compound for the Create FISh Trace node
- Specify the compound class library for the Compound Class Scoring node

Specify the target compounds and adduct ions

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 "Add your target compounds to the Expected Compounds list" on page 3.

❖ To specify the target compound for the example data set

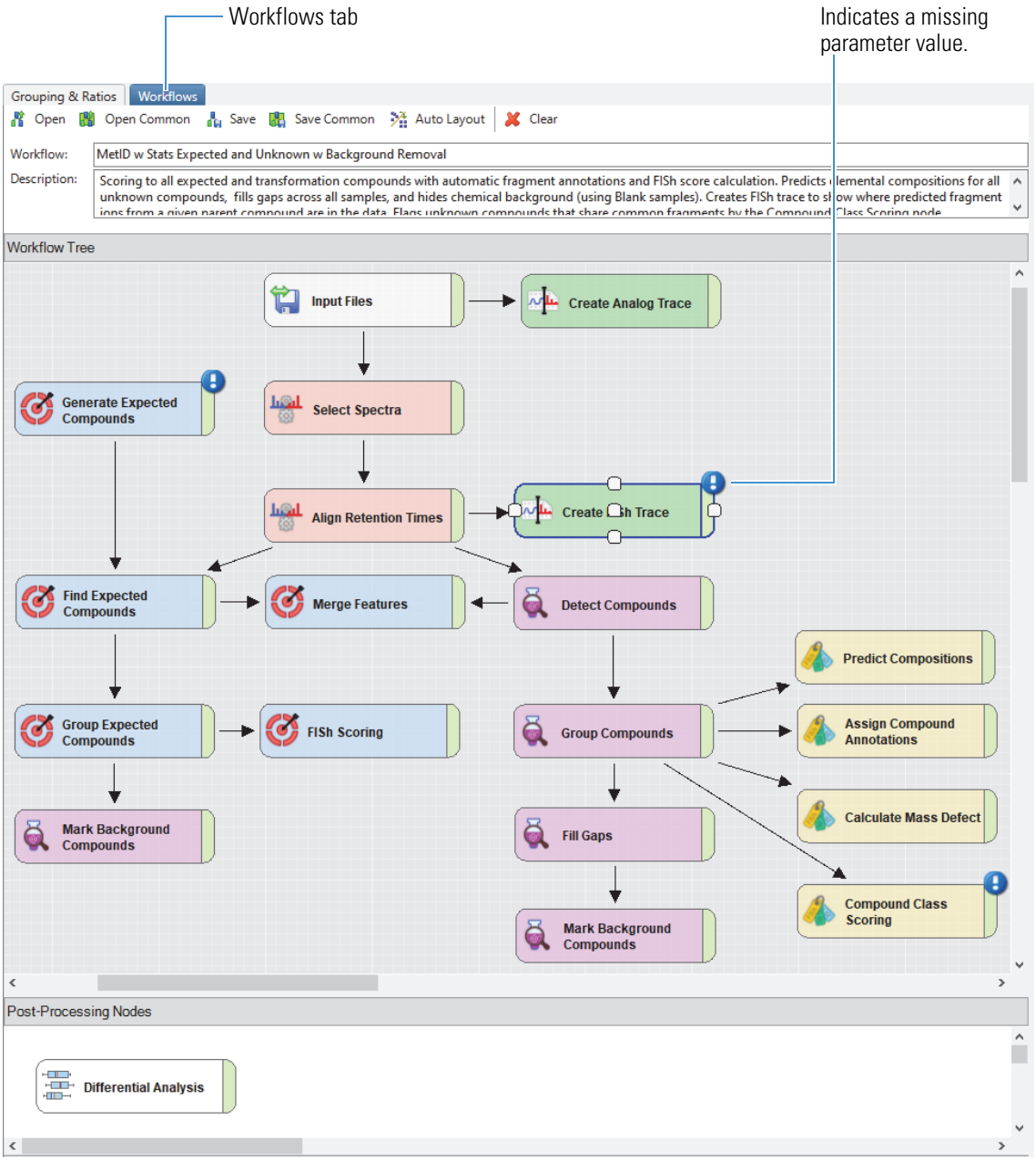
1. Click the **Workflows** tab to open the Workflows page.

   Figure 13 shows the Workflows page with the selected processing workflow.

   The exclamation marks () in the upper right corner of the following nodes indicate that these nodes are missing parameter values:

   - Generate Expected Compound node
   - Create FISh Trace node
   - Compound Class Scoring node

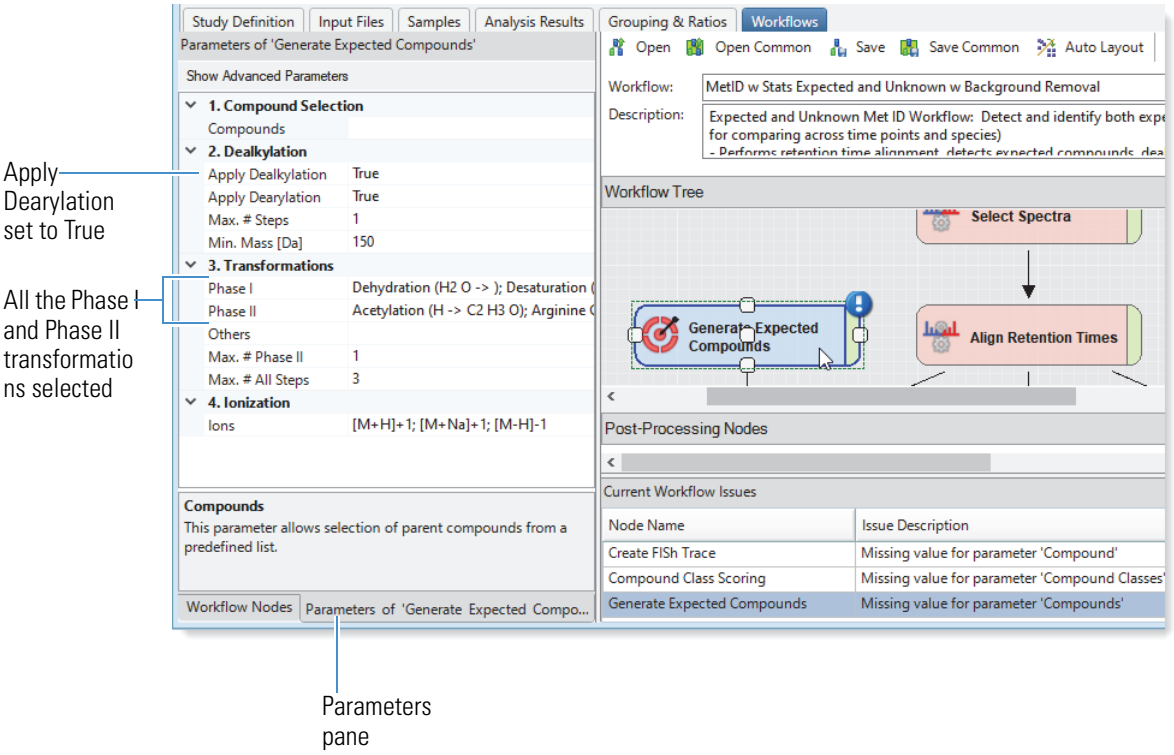
Figure 13. Processing workflow nodes for the selected processing workflow template



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

   The Parameters pane for the Generate Expected Compounds node opens to the left. In the selected processing workflow template, Apply Dearylation is set to True, and all the Phase 1 and Phase II reactions are selected.

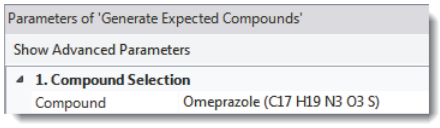
Figure 14. Generate Expected Compounds node



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 to "Specify the parent compound for the Create FISh Trace node."

Specify 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.

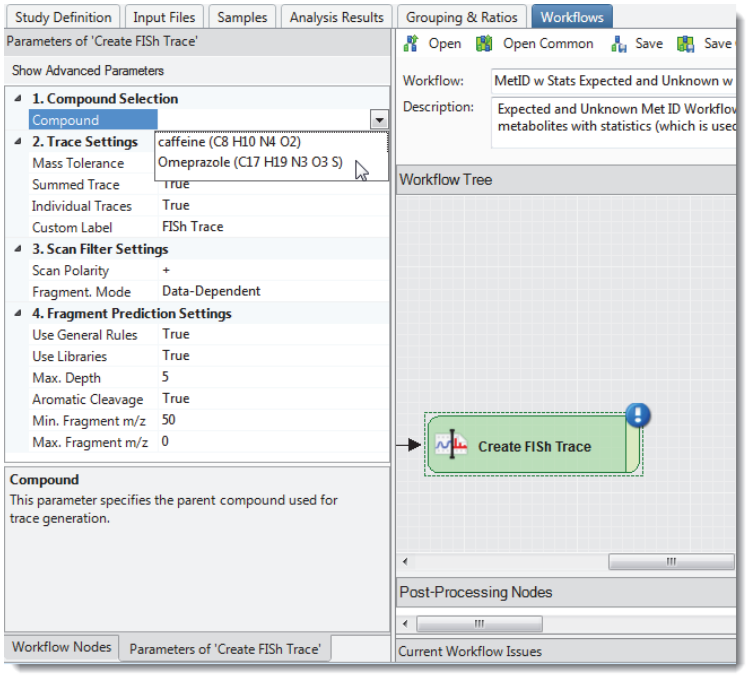
❖ To specify the compound for the Create FISh Trace node

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.

   Figure 15. Create FISh Trace node



- 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 template, go to the next topic to "Specify the compound class library for the Compound Class Scoring node."

Specify 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.

Note The Compound Discoverer 3.2 application comes with a compound class library for omeprazole, which is the compound of interest in this tutorial.

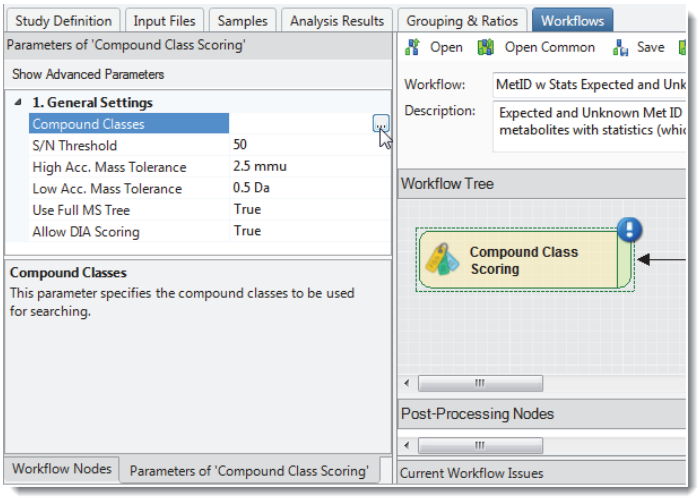
Tip If you are not working with the example data set, delete the Compound Class Scoring node from the processing workflow. Or, create compound class libraries for your targeted compounds.

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

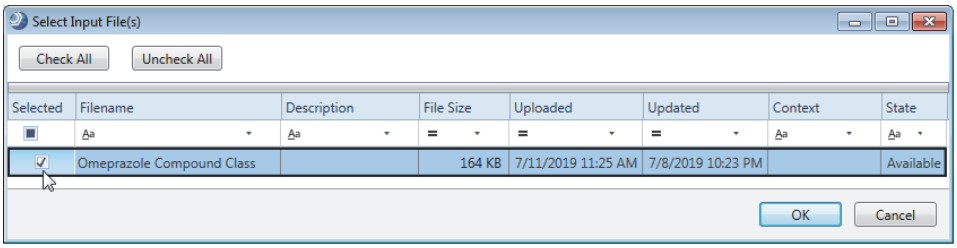
1. On the Workflows page, select the **Compound Class Scoring** node.
2. On the Parameters of Compound Class Scoring page, click the Compound Classes box, and then click the **browse** icon to the right of this box (Figure 16).

Figure 16. Compound Class Scoring node



The Select Input Files dialog box opens.

3. Select the check box for the Omeprazole Compound Class file.



4. Click **OK**.

Go to the next topic to "Submit the analysis to the job queue."

Submit the analysis to the job queue

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

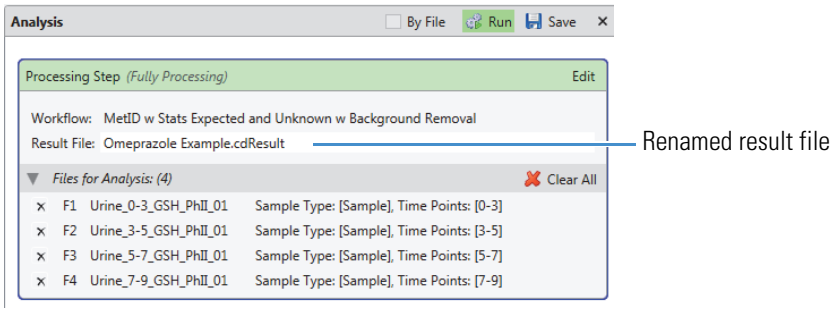
❖ To submit the analysis to the job queue

1. To create one result file for the input files set, leave the **By File** check box clear.

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

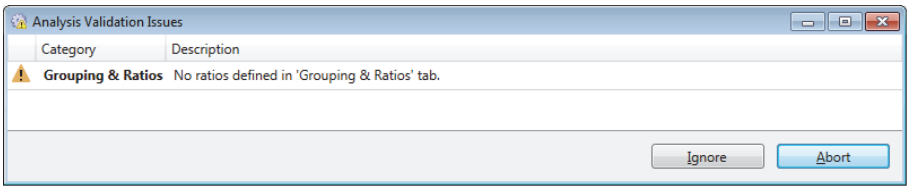
> **Note** If you select the By File check box, the application creates a separate result file for each input file, which means that it cannot compare the results across the data set.

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



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

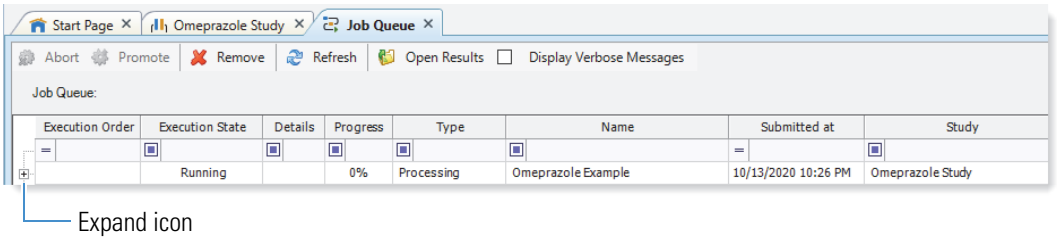
   Because the processing workflow includes the Differential Analysis node and you have not defined any ratios for the analysis, the Analysis Validation Issues dialog box opens.



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 "Open the result file and review its layout and other layout options."

Open the result file and review its layout and other layout options

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 17). 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 3.2 USB key:
>
>   **Example Studies\LC\Met ID\Omeprazole Example.cdResult**
>
> 2. From the menu bar, choose **Window > Reset Layout**.

To open the result file and review the default layout, do the following:

1. Open the result file
2. Review the default layout for the result page

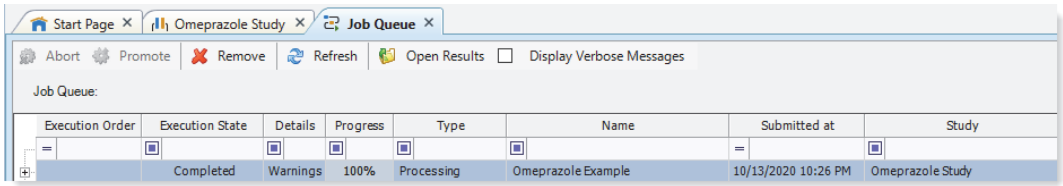
For general information about modifying the layout of a result page, see "Common layout modifications for the result tables and views" on page 23.

Open the result file

❖ **To open the result file generated by the analysis**

Do one of the following:

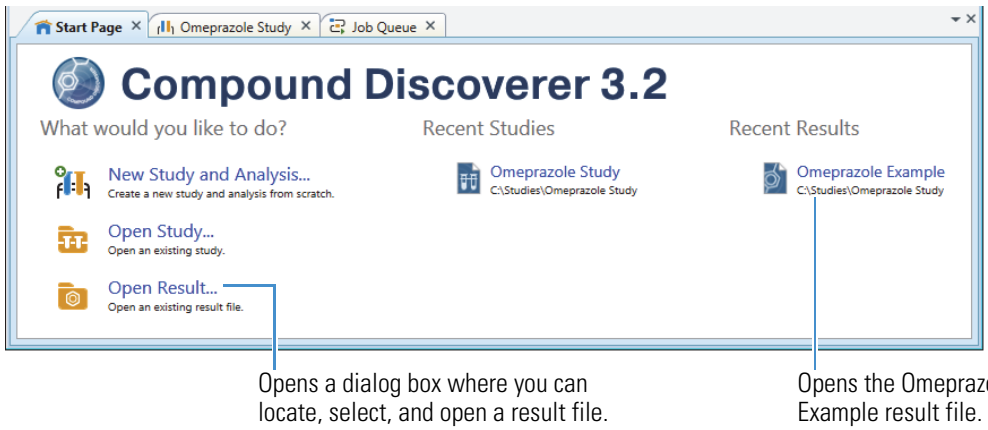
- If the Job Queue page is open, double-click the completed run.



- If the Omeprazole Study page is open, click the **Analysis Results** tab. Then, double-click the link to the completed analysis.

–or–

- If you closed the study after the run completed, click the result file name under Recent Results on the Start Page. If the Start Page is closed, open it by choosing **View > Start Page** from the menu bar.



To identify the unknown compounds by name go to the following topic to "Modify the processing workflow and partially reprocess the analysis" on page 24.

Review the default layout for the result page

The factory default layout for a result file includes the following items (Figure 17):

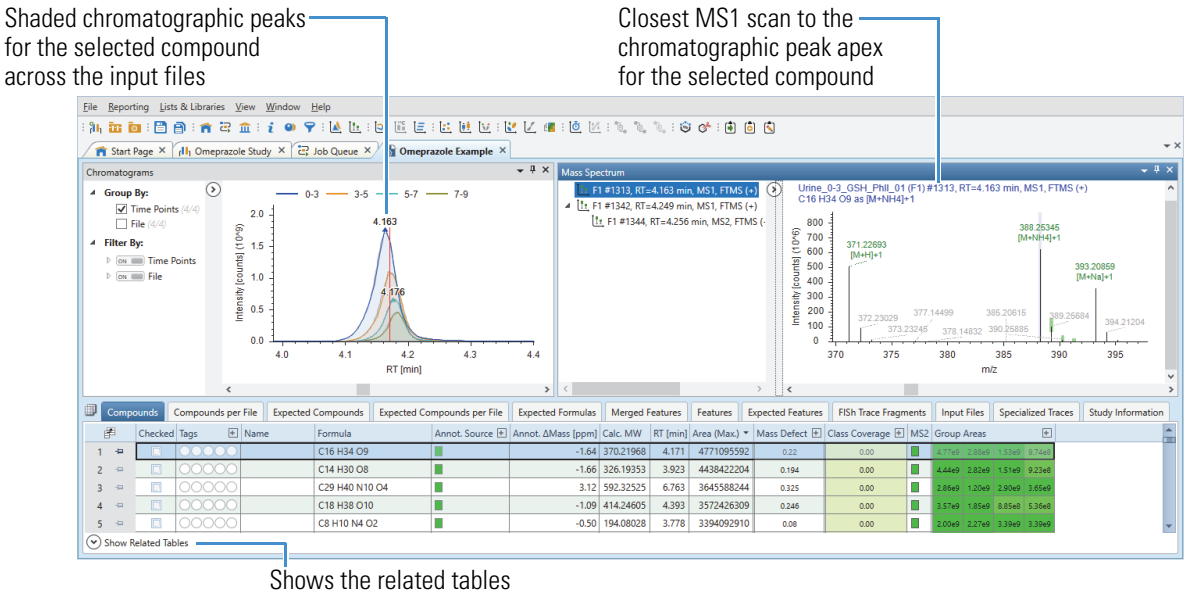
- A page tab with the result file name.
- A Chromatograms view on the top left that is populated with XIC traces for the compound in the first row of the Expected Compounds table. The view automatically zooms in to the start and end points of the chromatographic peak for the compound, and the area under 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 ± peak width at half maximum (FWHM)

  –or–

  - The Start and end points of the chromatographic peak
- A set of tabbed main tables below the two graphical views.
- A collapsed area for the related tables below the main tables.

Figure 17. Default layout for the result file generated by the processing workflow



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

Table 2. Result tables for the selected processing workflow template (Sheet 1 of 2)

• 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.

Table 2. Result tables for the selected processing workflow template (Sheet 2 of 2)

• Merged Features	Lists the chromatographic peaks with the same *m/z* × 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.
• Features	Lists the adduct ions detected by the Detect Compounds node across all the input files.
• Expected Features	Lists the features found by the Find Expected Compounds node across all the input files.
• 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.
• Study Information	Displays information about the samples, study groups, and ratios for the set of processed input files. The sample information includes the sample type and file name of each input file.

Common layout modifications for the result tables and views

Table 3 describes common layout modifications for the result tables and views in a result file.

Table 3. Common layout modifications (Sheet 1 of 2)

To do this	Do the following
Show or hide a table column	Open the Field Chooser for a table by clicking the icon,  , in the upper left corner of the table. To display a column, select its check box. To hide a column, clear its check box.
Show or hide a table	Open the Select Table Visibility dialog box by clicking the icon,  , at the left of the table tabs.
Close a view	Click the close icon, ✕, in the upper right corner of the view.
Open a view	In the application menu bar, choose **View > Specific View**. Or, in the toolbar, click the icon for the view.
Expand the header for a column with multiple subcolumns	Click the expand icon, , to the right of the heading.
Freeze a column to the left side of the table	Right-click the table and choose **Enable Column Fixing**. Then, click the pin icon to the right of the column heading.
Pin a row to the top of the result table.	Click the pin icon to the right of the row number (unpinned, , or pinned, )
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 (▼)

Table 3. Common layout modifications (Sheet 2 of 2)

To do this	Do the following
Sort a table by two columns.	Click the column header of the first column once or twice until the column is sorted in the order you want. Then, hold down the CTRL key and click the column header of the second column until the column is sorted in the order you want.
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. 3. An asterisk appears at the top of the vertical heading. 4. Click the column heading.
Reset the result page layout.	From the application menu bar, choose **Window > Reset Layout**.

You can also change the relative location of the table columns and views. Or, float the views and drag them to another monitor. For more details, refer to the following Help topics:

"Modifying the result page layout" and "Common operations for manipulating data tables"

Tip To find a topic in the Help system if you know its name, do the following:

1. In the Contents pane of the Help system, enter the topic's name in quotes in the search box.
2. Click **List Topics**.
3. In the Select Topic to Display list, select the topic of interest and click **Display**.

To identify the unknown compounds by name go to the following topic to "Modify the processing workflow and partially reprocess the analysis" on page 24.

Modify the processing workflow and partially reprocess the analysis

You can modify the parameter settings for any of the scoring nodes, mapping nodes, or search nodes without reprocessing the entire workflow—that is, without reprocessing the core peak detection and grouping nodes.

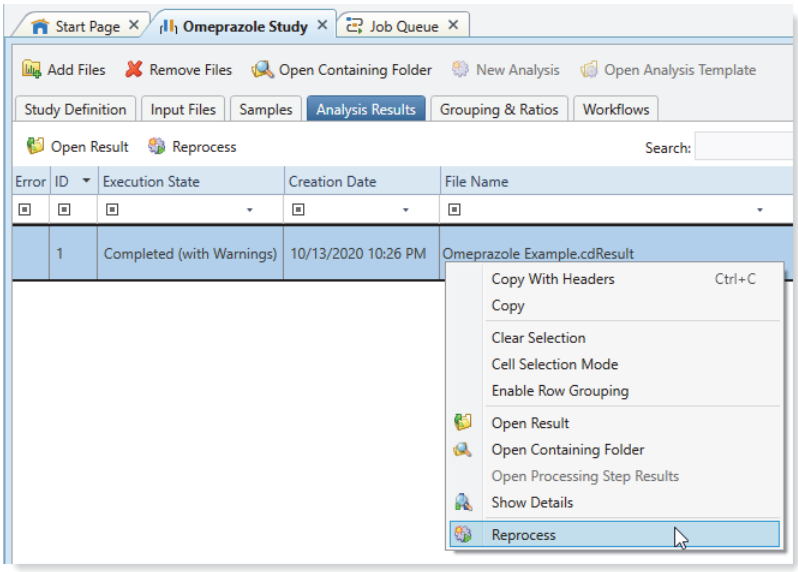
To identify compounds by using MS/MS database matching, add the Search mzVault node to the processing workflow. Then, partially reprocess the analysis.

Note The Search mzVault node searches for matching entries in the local mzCloud Offline databases. Your computer does not need access to the Internet to search these databases.

❖ To add the Search mzVault node to the processing workflow and reprocess the data

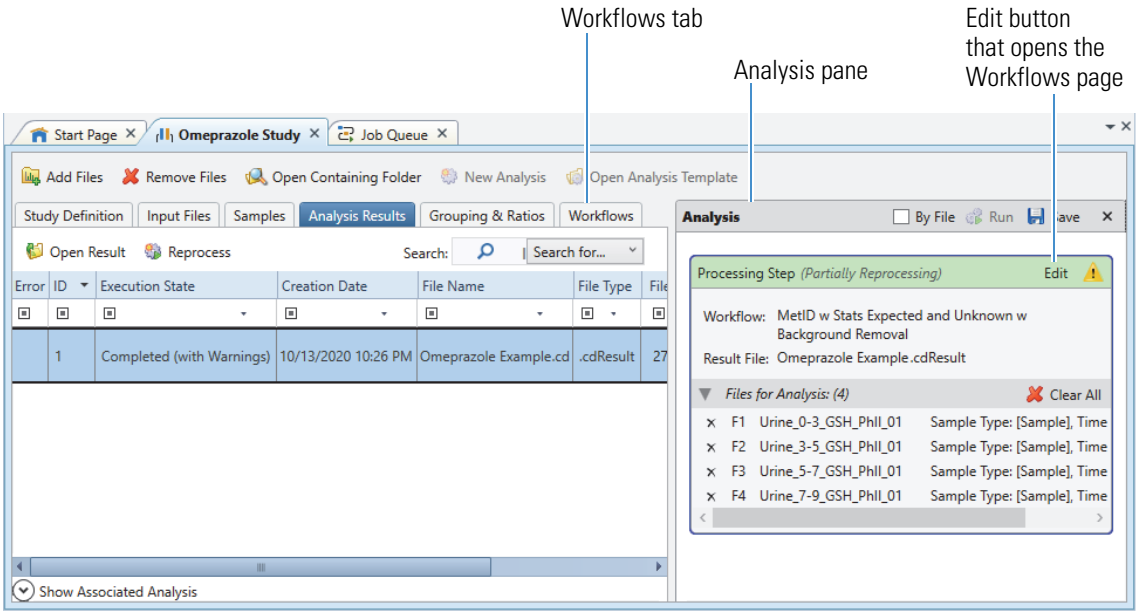
1. Close the Omeprazole Example result file by clicking the Close icon, ✕, on the result page tab.
2. Open the Omeprazole Study page by doing one of the following:
   - If the Omeprazole Study file is open, click the **Omeprazole Study** tab.
   - If the Omeprazole Study file is closed, click the **Omeprazole Study** link under Recent Studies on the Start Page. Or, choose **File > Open Study** from the menu bar and locate the study.
3. On the Omeprazole Study page, click the **Analysis Results** tab.
4. On the Analysis Results page, right-click the analysis and choose **Reprocess**.

Figure 18. Analysis Result page with the result file selected



The Grouping & Ratios and Workflows tabs appear to the right of the study tabs, and the Analysis pane opens to the right of the tabbed pages.

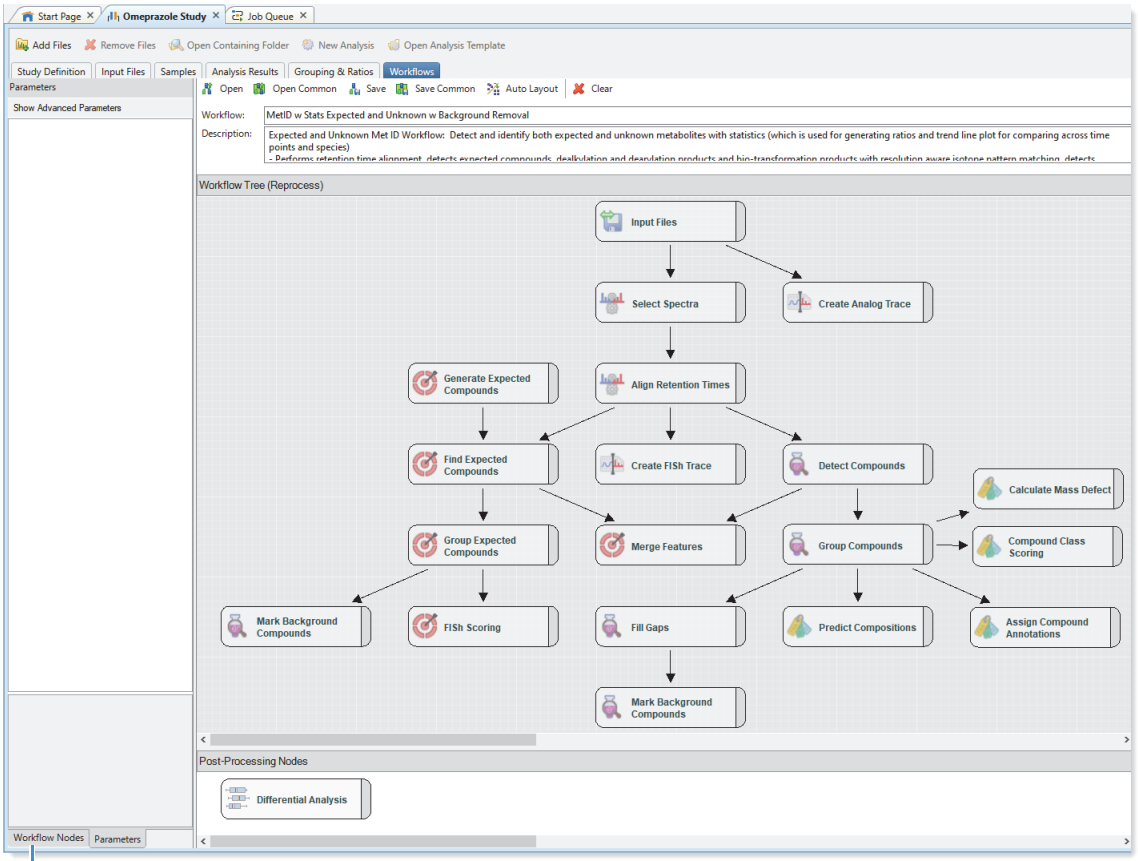
Figure 19. Omeprazole Study page with a reopened analysis



5. In the Analysis pane, click **Edit**.

   The Workflows page opens (Figure 20).

Figure 20. Workflow Tree (Reprocess) pane with unmodified workflow nodes from the selected analysis

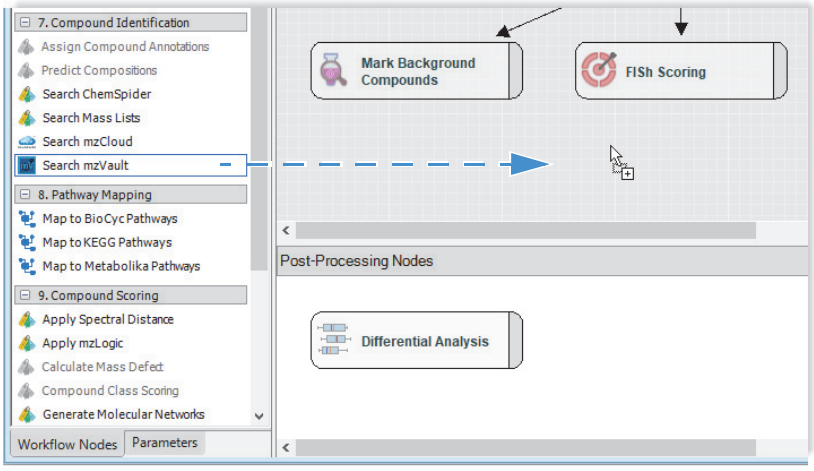


Workflow Nodes tab

6. If the Workflow Nodes pane is closed, click the **Workflow Nodes** tab at the bottom left of the application window.

7. Add the Search mzVault node to the processing workflow by dragging it from the Workflow nodes pane to the Workflow Tree (Reprocess) pane.

Figure 21. Dragging the Search mzVault node from the Workflow nodes pane to the Workflow Tree (Reprocess) pane



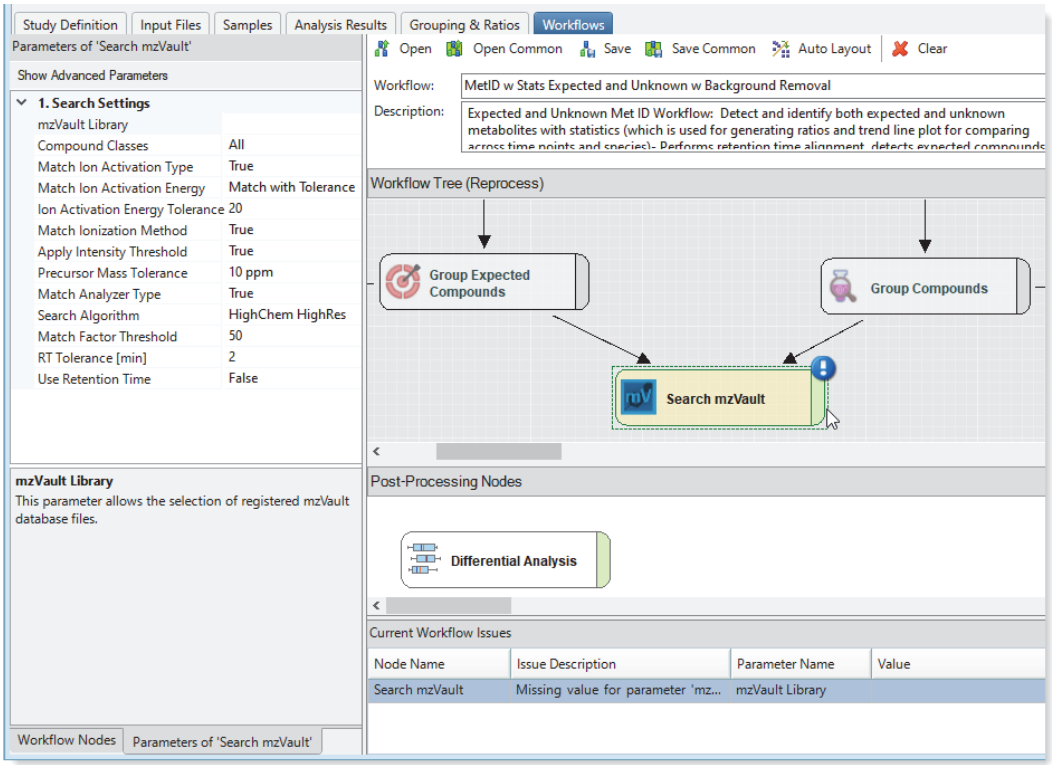
The Group Compounds and Group Expected Compounds nodes automatically connect to the Search mzVault node. The Current Workflow Issues area displays an issue for the Search mzVault node. You must select a library to search.

8. Select the mzVault library for this tutorial as follows:

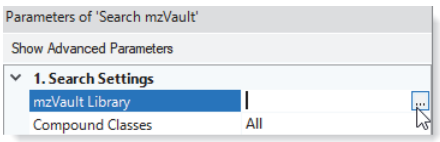
   a. In the Workflow Tree (Reprocess) pane, select the **Search mzVault** node.

      The Parameters of Search mzVault pane appears at the left (Figure 22).

      Figure 22. Modified processing workflow with recently added Search mzVault node



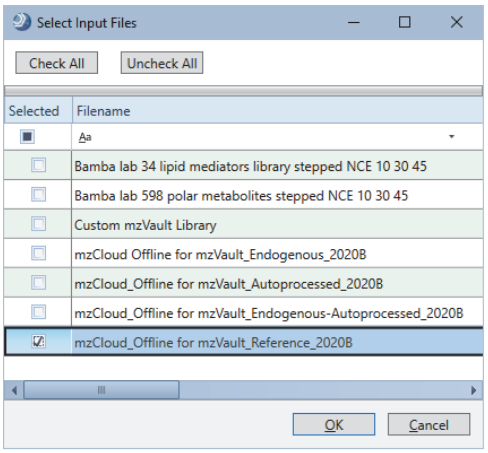
   b. Click the box to the right of mzVault Library. Then, click the **browse** icon, ...



      The Select Input Files dialog box opens.

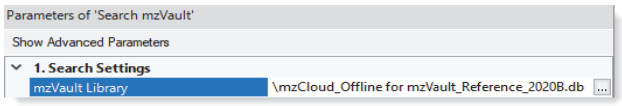
   c. Select the check box for the mzCloud_Offline for mzVault_Reference_2020B file (Figure 23).

      Figure 23. Select Input Files dialog box



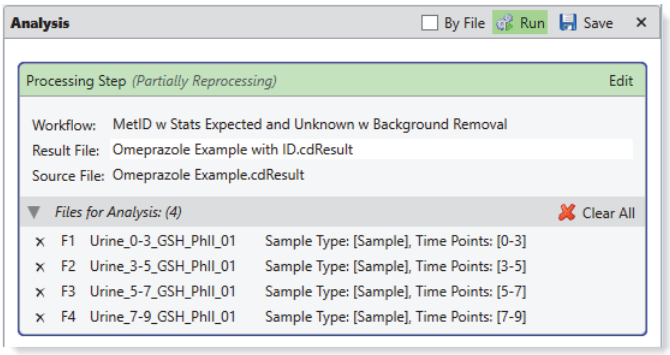
d. Click **OK**.

The name of the selected library appears in the mzVault Library box, and the Current Workflow Issues pane closes.



9. In the Analysis pane, rename the result file by appending **with ID** to the original file name in the Result File box.

Figure 24. Renamed result file



10. Click **Run**.
11. At the warning prompt, do the following:
    a. If you did not close the result file before you clicked Run, click **Abort**, close the result file, and click **Run** again.
    b. At the Grouping and Ratios warning prompt, click **Ignore**.

    Reprocessing starts.

When the run is completed, go to the next topic to "Review the results from the untargeted analysis."

Review the results from the untargeted analysis

The processing workflow that you selected in the wizard performed a targeted analysis for omeprazole and its transformation products and an untargeted analysis for the detection of unknown compounds.

To review the results from the untargeted analysis, see these topics:

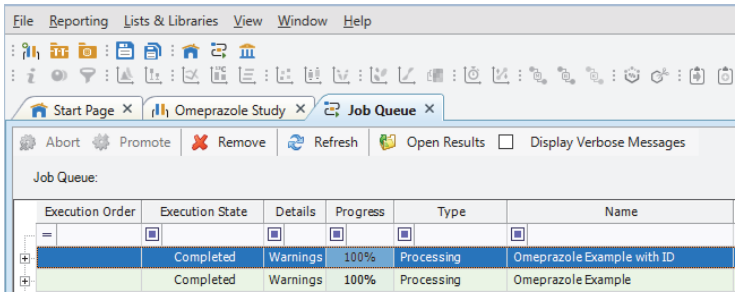
- Open the new result file from reprocessing
- View the number of number of compounds in the Compounds table
- Filter the Compounds table to reduce the number of compounds to review
- Review the results from the Compound Class Scoring node
- Compare the chromatograms for two compounds
- Compare the trend line plots for two compounds
- View a mass defect plot

Open the new result file from reprocessing

❖ To open the new result file from reprocessing

1. Do one of the following:

   - Double-click the Omeprazole Example with ID run on the Job Queue page.



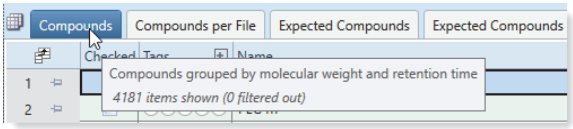
   - Open the Omeprazole Example with ID.cdResult file in the Example Study folder that you copied from the media or downloaded from the LSMS Software Download and Licensing Portal website. Then, choose **Window > Reset Layout** from the application menu bar.

View the number of number of compounds in the Compounds table

❖ To view the total number of compounds in the Compounds table

Point to its tab.

For this analysis, the Compounds table contains 4181 compounds.



Filter the Compounds table to reduce the number of compounds to review

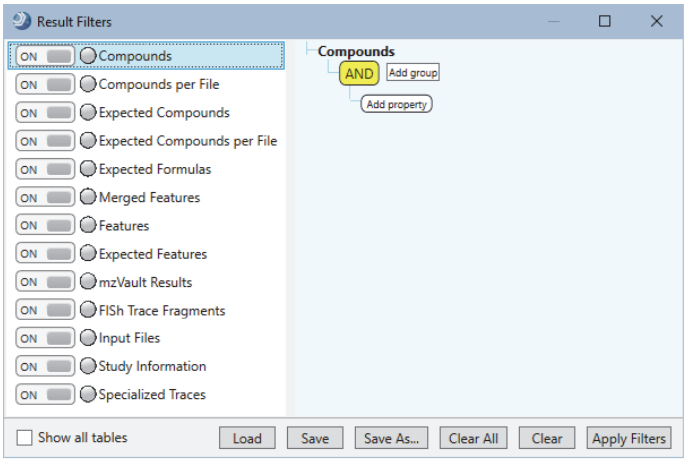
To reduce the number of compounds to review, filter out the low-level compounds.

❖ To filter the Compounds table by the chromatographic peak areas

1. Open the Omeprazole Example with ID.cdResult file, if it is not currently open.
2. If the Compounds table is not open, click the **Compounds** tab.
3. From the application menu bar, choose **View > Result Filters**.

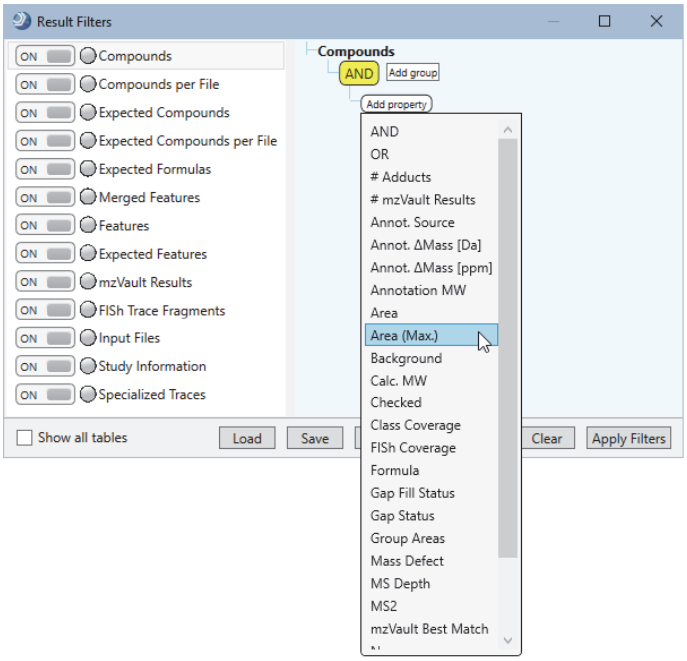
   The Result Filters view opens as a floating window.

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

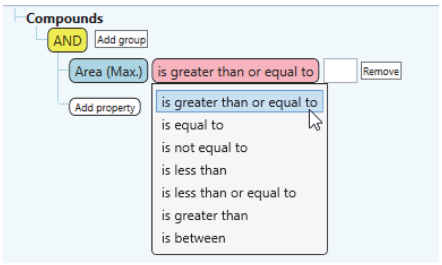


5. In the right pane, add a filter that hides compounds with maximum peak areas below 500 000 000 counts as follows:

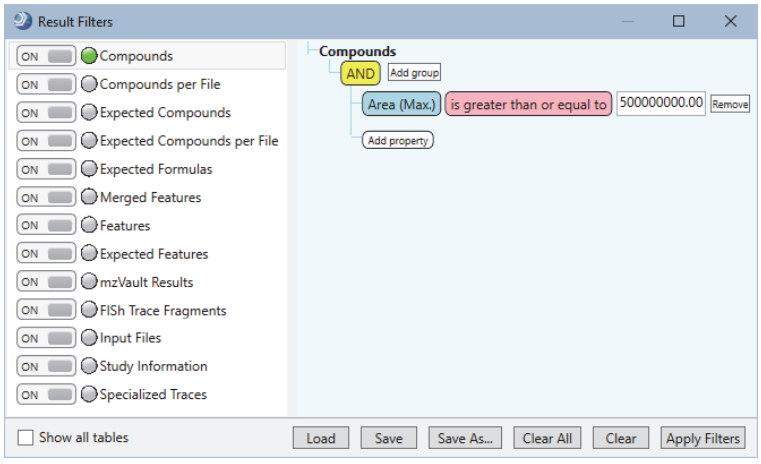
   a. Click **Add Property** and select **Area (Max.)** from the list. (The maximum area for each compound is the largest chromatographic peak for this compound across the input file set.)



   b. Click the (pink) operation list and select **Is Greater Than or Equal To**.



   c. Type **5e8** (or **500 000 000**) in the box next to the operation.



   d. Click **Apply Filters**.

   The Compounds tab has a filter icon and the table now displays only 36 entries. 4145 compounds are currently hidden.

Review the results from the Compound Class Scoring node

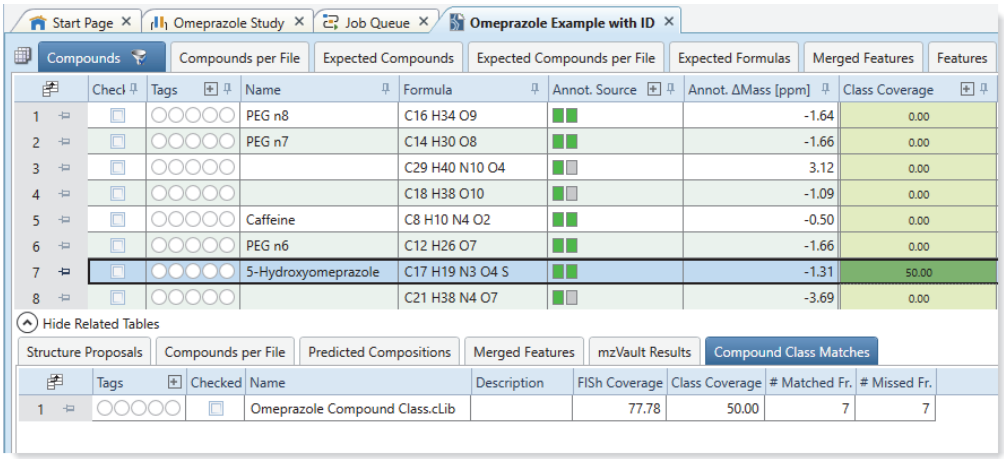
The processing workflow for this tutorial included the Compound Class Scoring node with the selection of a compound class (fragmentation) library for omeprazole.

❖ 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 Area (Max.) column, filter it now (see "Filter the Compounds table to reduce the number of compounds to review" on page 29).
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 left of the result page.
5. In the set of related tables, click the **Compounds Class Matches** tab.
6. Select the entry in the Compound Class Matches table.

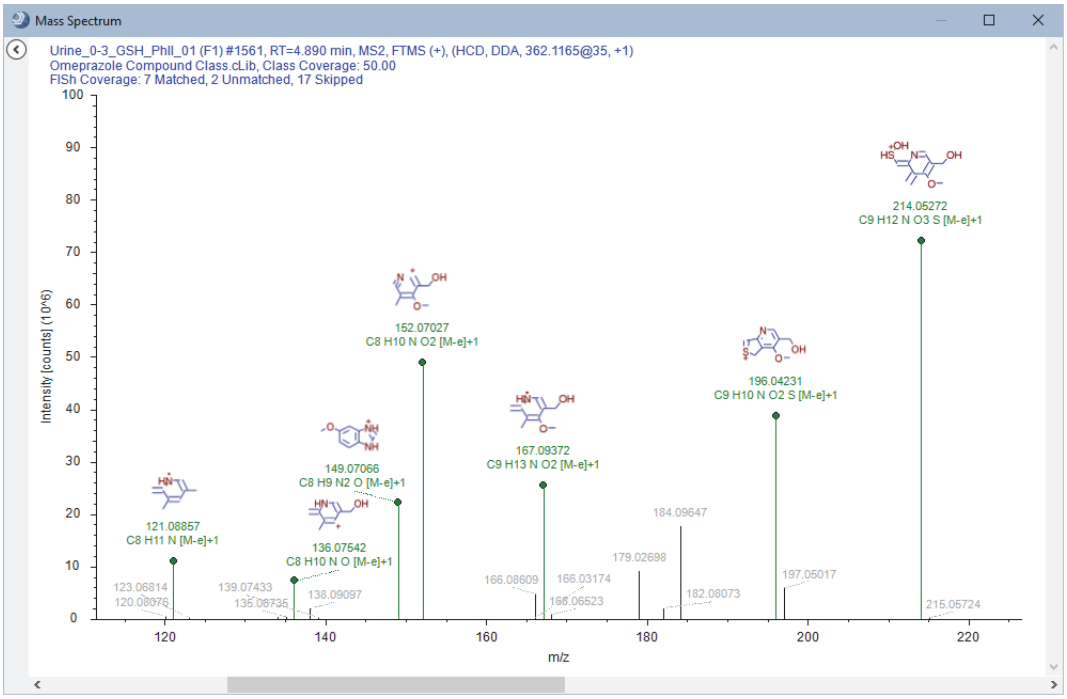
Note To fit the columns in the alloted space in Figure 25, column fixing was enabled.

Figure 25. Compound Class Matches table for 5-hydroxyomeprazole



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

Figure 26. Data-dependent fragmentation scan for 5-hydroxyomeprazole with structure annotations for matching fragments



Go to the next topic to "Compare the chromatograms for two compounds."

Compare the chromatograms for two compounds

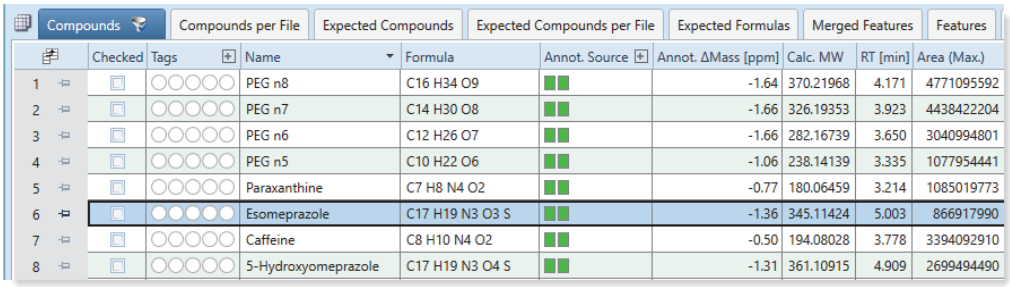
The untargeted portion of the analysis detected the parent compound, esomeprazole, and one of its oxidation products, 5-hydroxyomeprazole.

Note Esomeprazole is an isomer of omeprazole.

❖ To compare the chromatograms for esomeprazole and 5-hydroxyomeprazole

1. Sort the Compounds table by the Name column in descending order (see "Common layout modifications for the result tables and views" on page 23).
2. In the Compounds table, select **row 6** (Esomeprazole).

   Figure 27. Filtered Compounds table sorted by the Name column in descending order

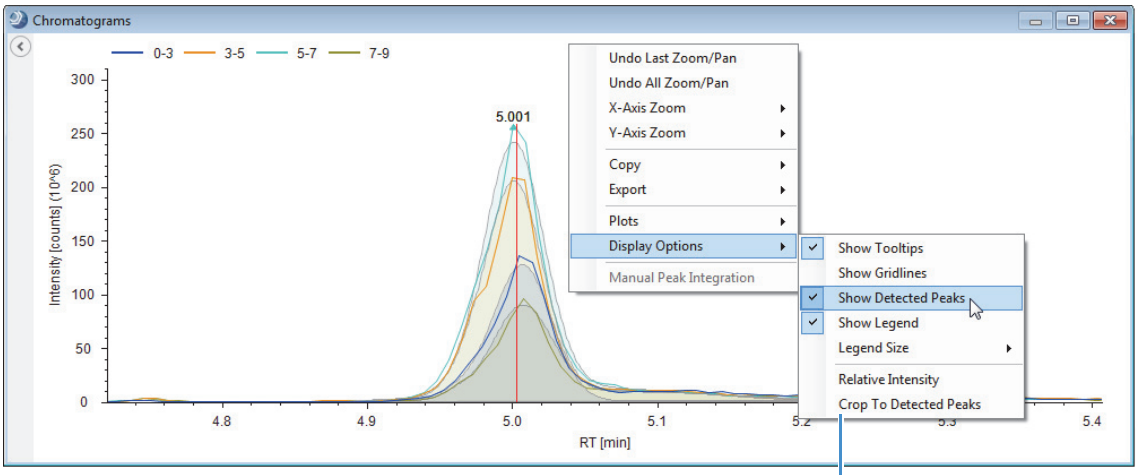


Compounds | Compounds per File | Expected Compounds | Expected Compounds per File | Expected Formulas | Merged Features | Features

	Checked	Tags	Name	Formula	Annot. Source	Annot. ΔMass [ppm]	Calc. MW	RT [min]	Area (Max.)
1	☐	○○○○○	PEG n8	C16 H34 O9	■■	-1.64	370.21968	4.171	4771095592
2	☐	○○○○○	PEG n7	C14 H30 O8	■■	-1.66	326.19353	3.923	4438422204
3	☐	○○○○○	PEG n6	C12 H26 O7	■■	-1.66	282.16739	3.650	3040994801
4	☐	○○○○○	PEG n5	C10 H22 O6	■■	-1.06	238.14139	3.335	1077954441
5	☐	○○○○○	Paraxanthine	C7 H8 N4 O2	■■	-0.77	180.06459	3.214	1085019773
6	☐	○○○○○	Esomeprazole	C17 H19 N3 O3 S	■■	-1.36	345.11424	5.003	866917990
7	☐	○○○○○	Caffeine	C8 H10 N4 O2	■■	-0.50	194.08028	3.778	3394092910
8	☐	○○○○○	5-Hydroxyomeprazole	C17 H19 N3 O4 S	■■	-1.31	361.10915	4.909	2699494490

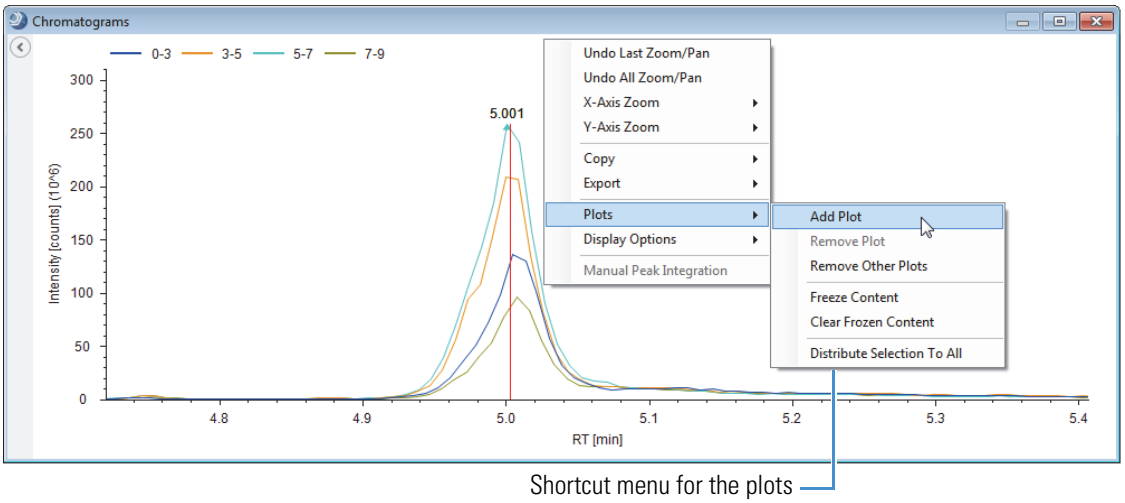
3. To view the chromatograms for the four time points more clearly, right-click the Chromatograms view and choose **Display Options > ✔ Show Detected Peaks** (Figure 28).

Figure 28. Shaded peaks for esomeprazole



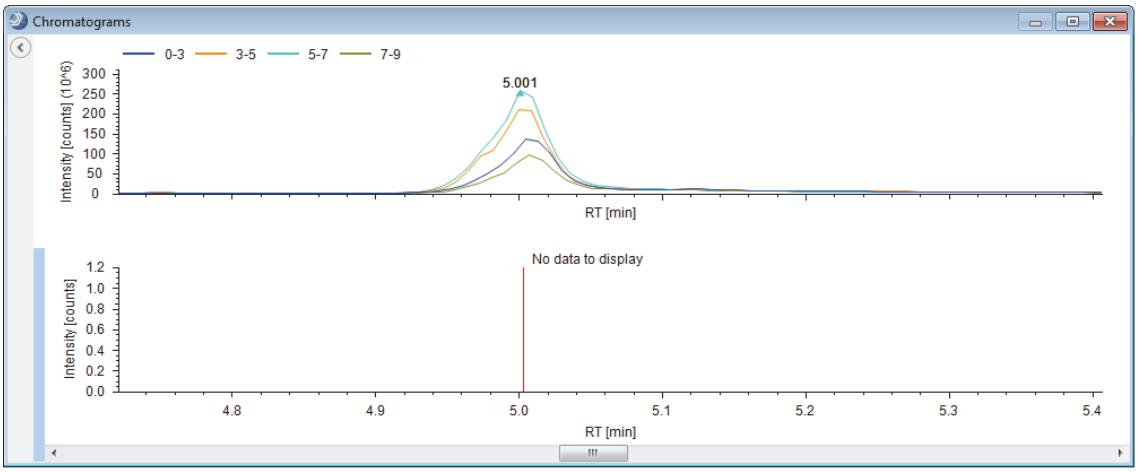
4. To add a plot below the current plot, right-click the Chromatograms view and choose **Plots > Add Plot**.

Figure 29. Unshaded peaks for esomeprazole



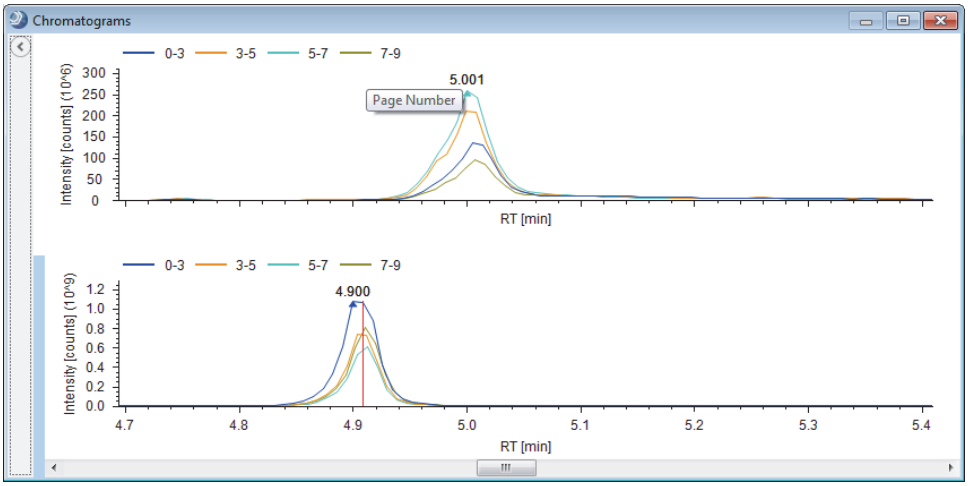
An empty plot appears below the current plot. The blue bar at the left of the plot indicates that the plot is active.

Figure 30. Two plots



5. In the Compounds table, select **row 8** (5-Hydroxyomeprazole). See Figure 27 on page 32.

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



Go to the next topic to "Compare the trend line plots for two compounds."

Compare the trend line plots for two compounds

❖ To compare the trend line plots for 5-hydroxyomeprazole and esomeprazole

1. If you have not already filtered the compounds table by the Area (Max) column and sorted the Compounds table by the Name column in descending order, filter it and sort it now.

   For details about filtering and sorting, see "Filter the Compounds table to reduce the number of compounds to review" on page 29 and "Common layout modifications for the result tables and views" on page 23.

2. From the application menu bar, choose **View > Trend Chart**.

   The Trend Chart view opens to the right of the result tables.

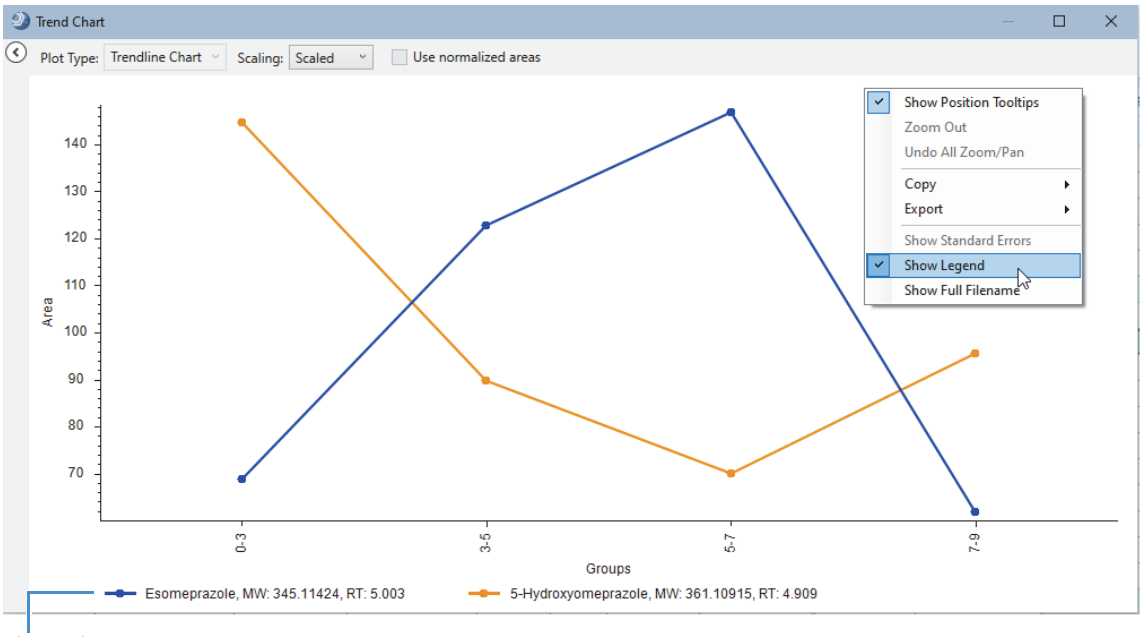
3. Select **row 6** (esomeprazole) in the Compounds table. Then, hold down the CNTRL key and select **row 8** (5-hydroxyomeprazole) in the Compounds table.

   The Plot Type automatically changes to Trendline Chart, and the plot shows the trend lines for the two compounds (Figure 31).

4. Right-click the plot 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 hours show a delay in the oxidation of omeprazole due to enzyme inhibition by its metabolites. After 7 hours, the subject begins to metabolize the current dosage of omeprazole.

Figure 31. Trend Chart view with trend line plots for two compounds



Go to the next topic to "View a mass defect plot."

View 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, 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.

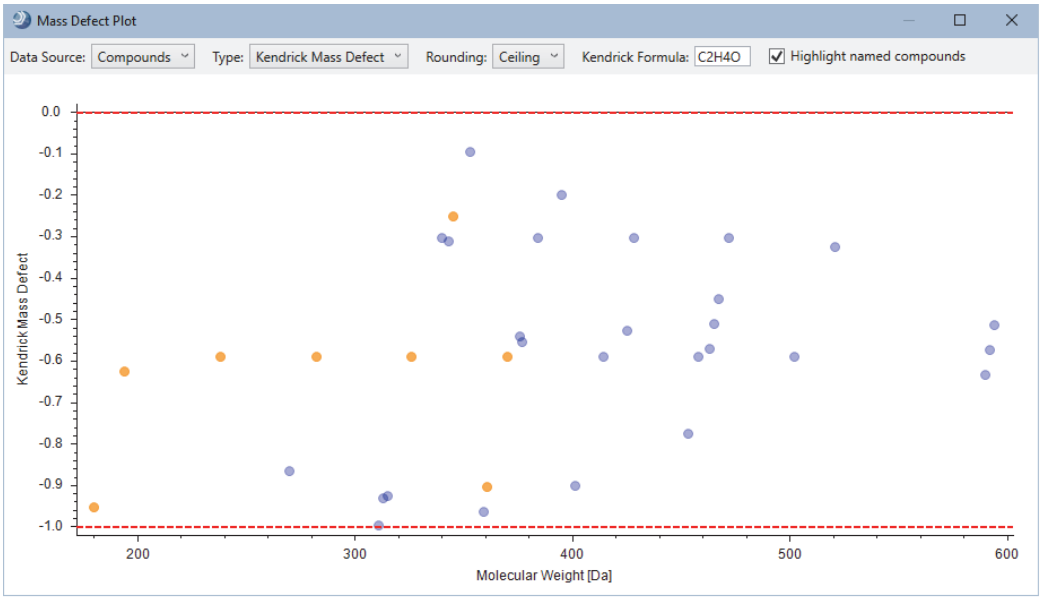
❖ To set up a mass defect plot for a Kendrick formula

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

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

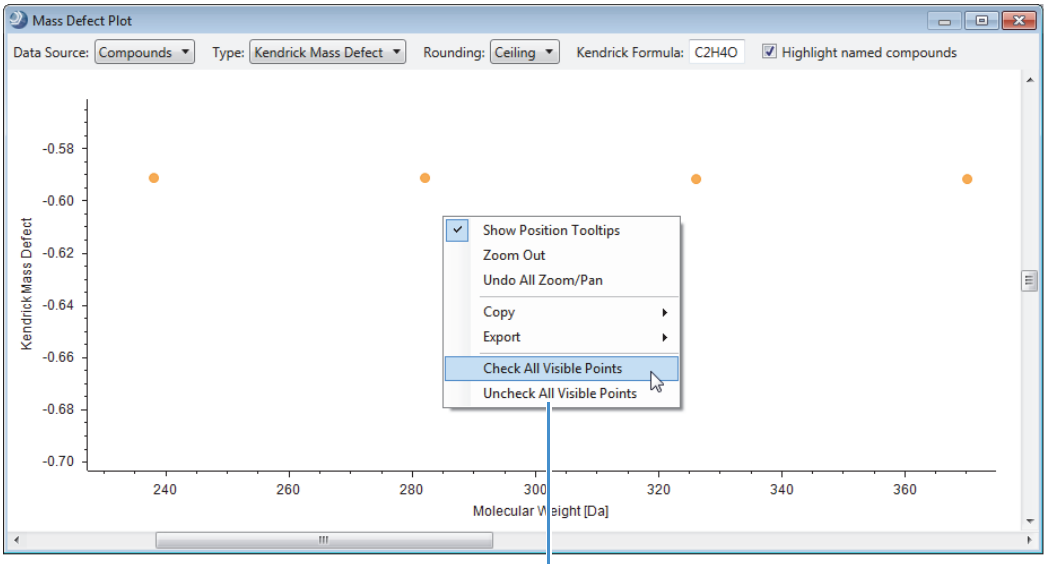
2. In the Kendrick Formula box, type C2H4O.
3. Select the **Highlight Named Compounds** check box.

Figure 32. Mass Defect Plot view with a plot of the Kendrick mass defect versus the molecular weight of the compounds



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

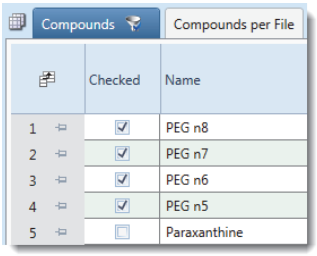
Figure 33. Mass Defect Plot view with shortcut menu



Shortcut menu for the Mass Defect Plot view

6. If the Compounds table is not currently sorted by the Name column in descending order, sort it now.

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



Go to the next topic to "Review the targeted compounds."

Review 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.

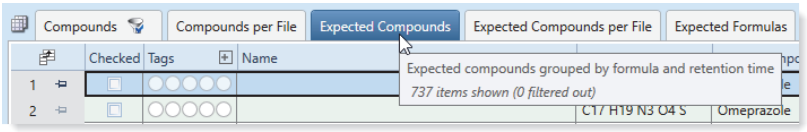
To review the targeted (expected) compounds, do the following:

1. Filter the Expected Compounds table by peak area
2. Sort the Expected Compounds table by the transformations
3. Review the mass spectra for the expected compounds
4. Determine the most probable explanation for each expected compound
5. View the dealkylation product for an expected compound
6. Name and propose structures for the expected compounds

Filter the Expected Compounds table by peak area

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

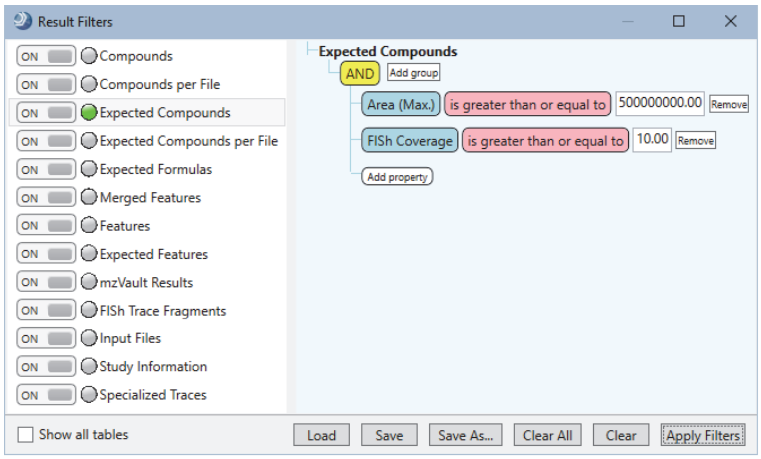
1. Click the **Expected Compounds** tab
2. Check the number of compounds in the Expected Compounds table by pointing to the Expected Compounds tab.



3. Reduce the number of compounds to review by filtering the Expected Compounds table as follows:
   a. From the application menu bar, choose **View > Result Filters**.

      The Result Filters view opens.
   b. In the left pane, select the **Expected Compounds** table.
   c. In the right pane set up the following filters (Figure 34):
      – Area (Max.) is Greater Than or Equal to 5e8
      – FISh Coverage is Greater Than or Equal to 10

Figure 34. Filters for the Expected Compounds table



d. Click **Apply Filters**.

Only 13 compounds are now visible in the Expected Compounds table for the example result file (Figure 35).

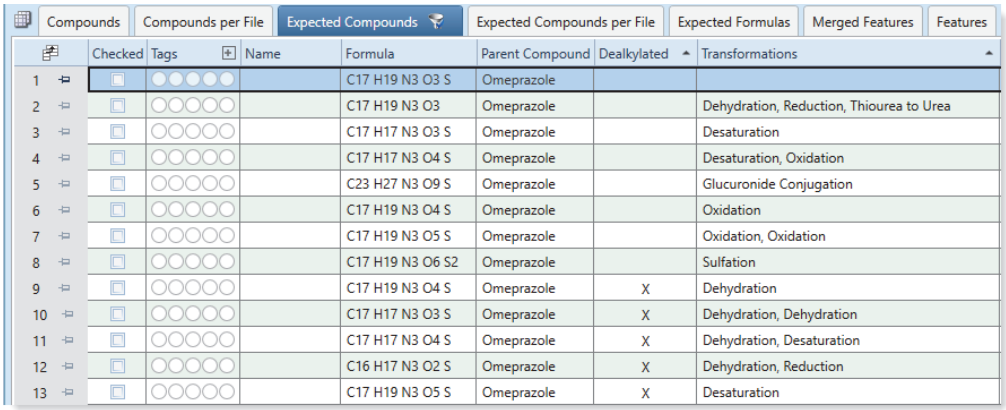
Sort the Expected Compounds table by the transformations

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

1. 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.
2. Hold down the CTRL key and click the **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.

Figure 35. Filtered Expected Compounds table sorted by the Dealkylated and Transformations columns



	Checked	Tags	Name	Formula	Parent Compound	Dealkylated	Transformations
1				C17 H19 N3 O3 S	Omeprazole		
2				C17 H19 N3 O3	Omeprazole		Dehydration, Reduction, Thiourea to Urea
3				C17 H17 N3 O3 S	Omeprazole		Desaturation
4				C17 H17 N3 O4 S	Omeprazole		Desaturation, Oxidation
5				C23 H27 N3 O9 S	Omeprazole		Glucuronide Conjugation
6				C17 H19 N3 O4 S	Omeprazole		Oxidation
7				C17 H19 N3 O5 S	Omeprazole		Oxidation, Oxidation
8				C17 H19 N3 O6 S2	Omeprazole		Sulfation
9				C17 H19 N3 O4 S	Omeprazole	X	Dehydration
10				C17 H17 N3 O3 S	Omeprazole	X	Dehydration, Dehydration
11				C17 H17 N3 O4 S	Omeprazole	X	Dehydration, Desaturation
12				C16 H17 N3 O2 S	Omeprazole	X	Dehydration, Reduction
13				C17 H19 N3 O5 S	Omeprazole	X	Desaturation

Go to the next topic to "Review the mass spectra for the expected compounds."

Review the mass spectra for the expected compounds

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:

- Inspect the isotope pattern in the full MS scan
- View the fragmentation scans
- FISh coverage score

Inspect the isotope pattern in the full MS scan

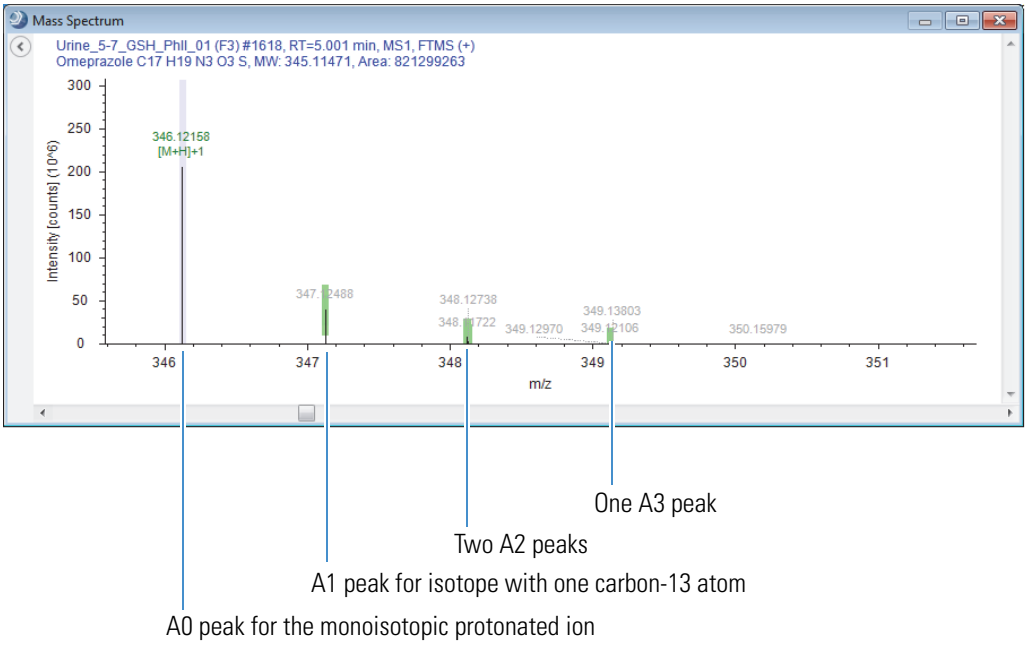
❖ **To inspect the isotope pattern for a selected compound**

1. Select the first row in the sorted Expected Compounds table (with omeprazole sorted to the first row as shown in Figure 35).
2. In the Mass Spectrum view, inspect the compound's isotope pattern in the full MS scan.

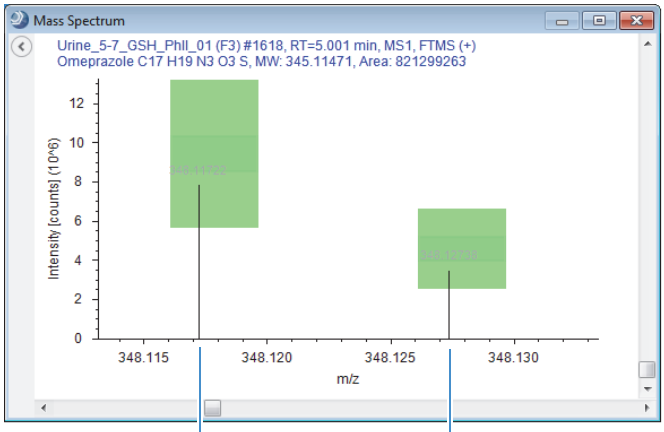
Note This tutorial uses the following terminology for isotope patterns:

- A0—The monoisotopic ion (typically the leftmost spectrum peak)
- 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.



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).

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:

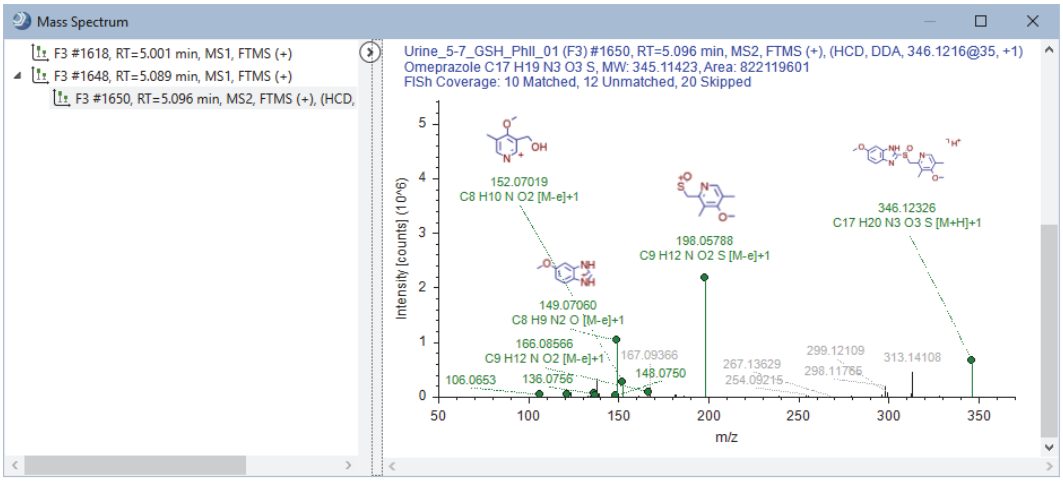
- Direct match—(●) Green
- Shifted match—(●) Blue

❖ 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 36. Annotations for scan #1650 generated by the FISh Scoring node during data processing



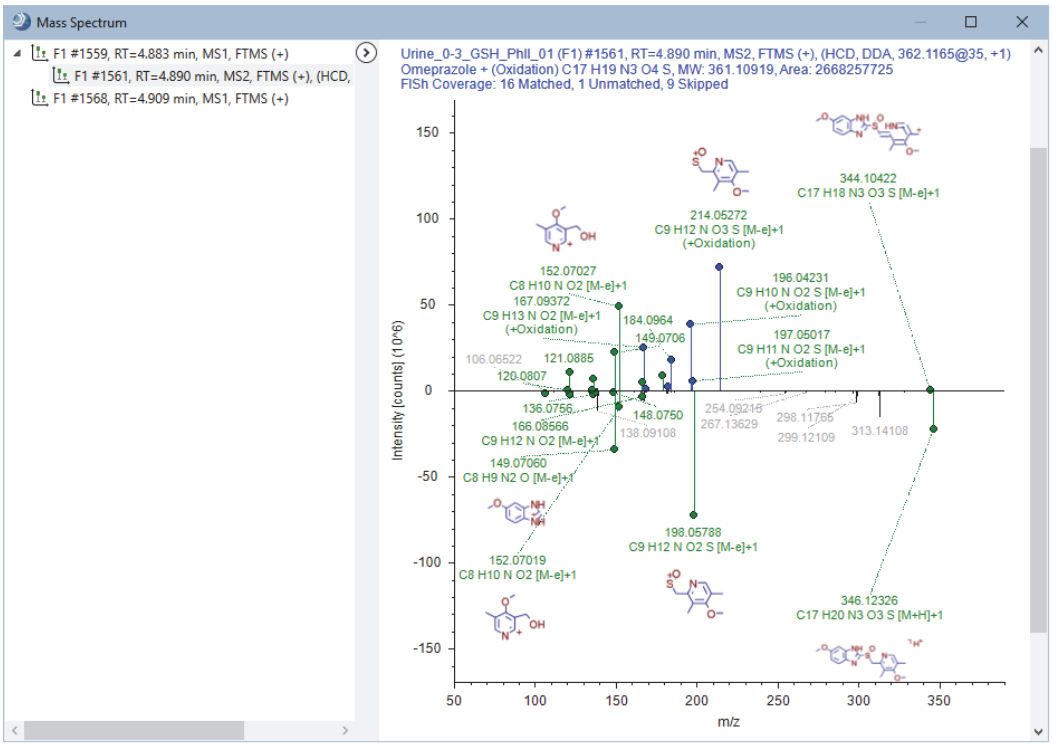
Note The FISh Scoring node cannot calculate a FISh Coverage score for compounds that lack DDA scans.

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 sorted Expected Compounds table (Figure 35), select **row 6** (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.

   Figure 37 shows a mirror plot with the data-dependent fragmentation scan for omeprazole set as the reference.

Figure 37. Mirror plot with omeprazole on the bottom and one of its metabolites on the top



FISh coverage score

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.

> **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.

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:

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

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.

Determine the most probable explanation for each expected compound

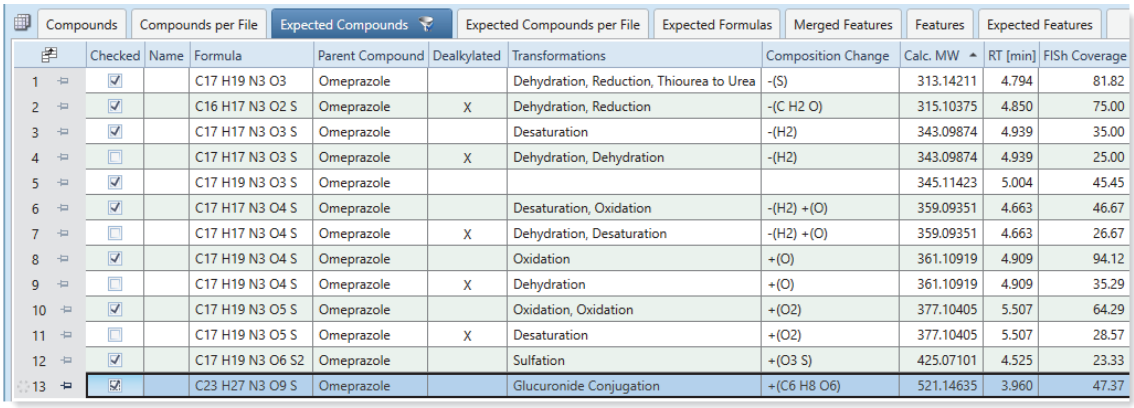
❖ To display only the most probable explanation for each compound

1. If you have not already filtered the Expected Compounds table by the chromatographic peak area [Area (Max.)] and the FISh Coverage, do so now. See step 3 on page 29.
2. Sort the Expected Compounds table by the **Calc. MW** column in ascending 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.

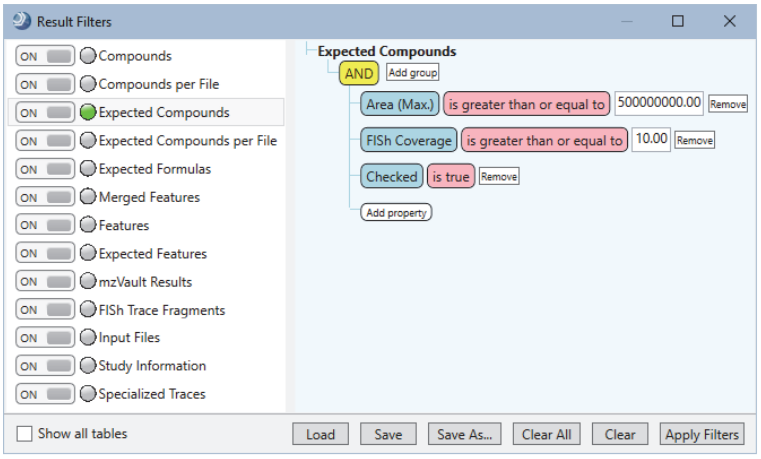
   This figure shows the selection of the explanations with the higher FISh coverage score.



Compounds | Compounds per File | Expected Compounds | Expected Compounds per File | Expected Formulas | Merged Features | Features | Expected Features

	Checked	Name	Formula	Parent Compound	Dealkylated	Transformations	Composition Change	Calc. MW	RT [min]	FISh Coverage
1	☑		C17 H19 N3 O3	Omeprazole		Dehydration, Reduction, Thiourea to Urea	-(S)	313.14211	4.794	81.82
2	☑		C16 H17 N3 O2 S	Omeprazole	X	Dehydration, Reduction	-(C H2 O)	315.10375	4.850	75.00
3	☑		C17 H17 N3 O3 S	Omeprazole		Desaturation	-(H2)	343.09874	4.939	35.00
4	☐		C17 H17 N3 O3 S	Omeprazole	X	Dehydration, Dehydration	-(H2)	343.09874	4.939	25.00
5	☑		C17 H19 N3 O3 S	Omeprazole				345.11423	5.004	45.45
6	☑		C17 H17 N3 O4 S	Omeprazole		Desaturation, Oxidation	-(H2) +(O)	359.09351	4.663	46.67
7	☐		C17 H17 N3 O4 S	Omeprazole	X	Dehydration, Desaturation	-(H2) +(O)	359.09351	4.663	26.67
8	☑		C17 H19 N3 O4 S	Omeprazole		Oxidation	+(O)	361.10919	4.909	94.12
9	☐		C17 H19 N3 O4 S	Omeprazole	X	Dehydration	+(O)	361.10919	4.909	35.29
10	☑		C17 H19 N3 O5 S	Omeprazole		Oxidation, Oxidation	+(O2)	377.10405	5.507	64.29
11	☐		C17 H19 N3 O5 S	Omeprazole	X	Desaturation	+(O2)	377.10405	5.507	28.57
12	☑		C17 H19 N3 O6 S2	Omeprazole		Sulfation	+(O3 S)	425.07101	4.525	23.33
13	☑		C23 H27 N3 O9 S	Omeprazole		Glucuronide Conjugation	+(C6 H8 O6)	521.14635	3.960	47.37

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



5. Click **Apply Filters**.

   The Expected Compounds table now displays only nine compounds.

Go to the next topic to "View the dealkylation product for an expected compound."

View the dealkylation product for an expected compound

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

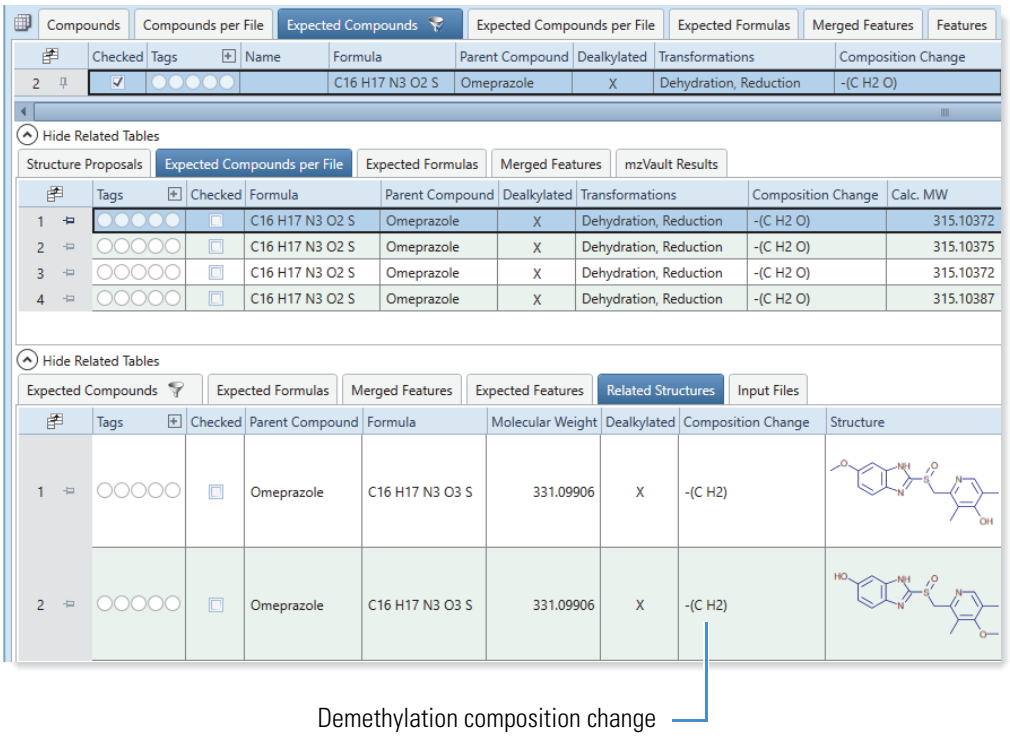
1. In the filtered Expected Compounds table (sorted in ascending order by Calc. MW), select a compound that is the product of a dealkylation reaction and one or more transformations.

   For example, select **row 2** (C16 H17 N3 O2 S).

2. Click **Show Related Tables** at the bottom left of the result page.
3. Click the **Expected Compounds per File** tab. Then, select **row 1** in the Expected Compounds per File table.
4. Click **Show Related Tables** at the bottom left of the result page.
5. Click the **Related Structures** tab.

   The Related Structures table shows the dealkylation products for compounds with additional transformations.

Figure 38. Related structures table for dealkylation product



Go to the next topic, "Name and propose structures for the expected compounds."

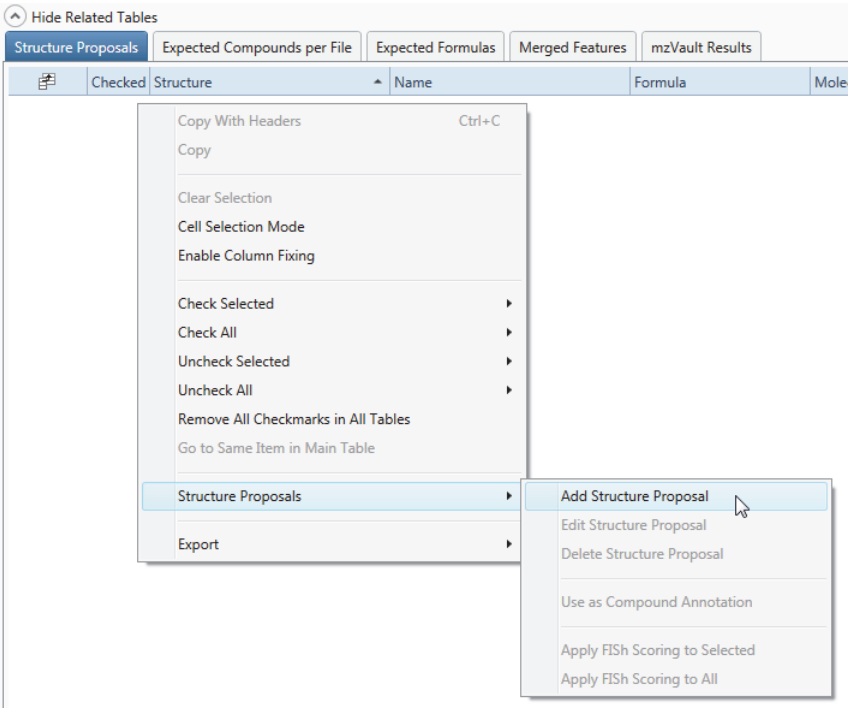
Name and propose structures for the expected compounds

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

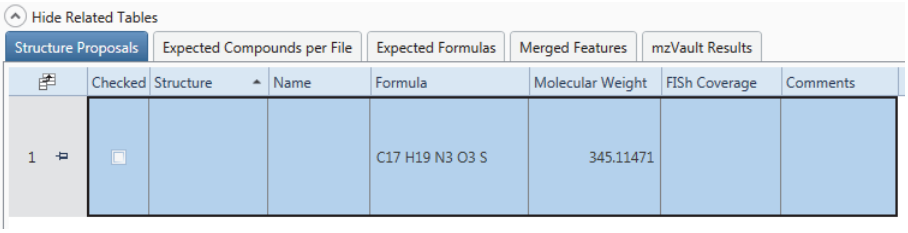
❖ To populate the Name and Structure columns in the Expected Compounds table

1. Sort the filtered Expected Compounds table in descending order by Area (Max.).
2. Select **row 5** (Calc. MW 345.11423).
3. Click S**how Related Tables**. Then, click the **Structure Proposals** tab.
4. Right-click the empty Structure Proposals table and choose **Structure Proposals > Add Structure Proposal**.

Figure 39. Shortcut menu for the Structure Proposal result table



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



5. Double-click the row in the Structure Proposals table.

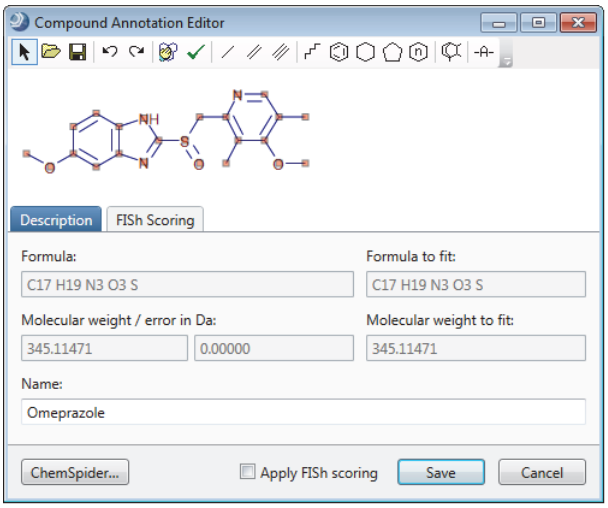
   The Compound Annotation Editor opens to the Description page.

6. 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.

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



8. Run the FISh scoring algorithm as follows:
   a. Select the **Apply FISh Scoring** check box.
   b. 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.

9. 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).

10. To view the structure in the Expected Compounds table, display the Structure column as follows:
    a. Click the **Field Chooser** icon, .
    b. Select the **Structure** check box.

11. 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.

Save or reset the layout of the result file

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:

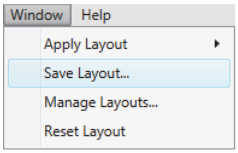
- In the toolbar, click the **Save the Currently Active Item** icon, .

–or–

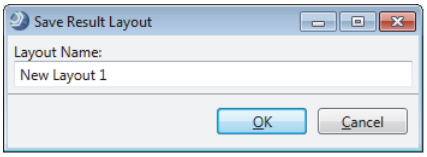
- From the menu bar, choose **File > Save** (save the currently active item).

❖ 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.

Print reports

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 "Determine the most probable explanation for each expected compound." Sort the table by the column of interest, for example, by retention time (see "Common layout modifications for the result tables and views" on page 23).

> **Note** For information about creating and editing your own custom report templates, refer to the *Compound Discoverer 3.2 Reporting Quick Start*, the reporting chapter in the *Compound Discoverer 3.2 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

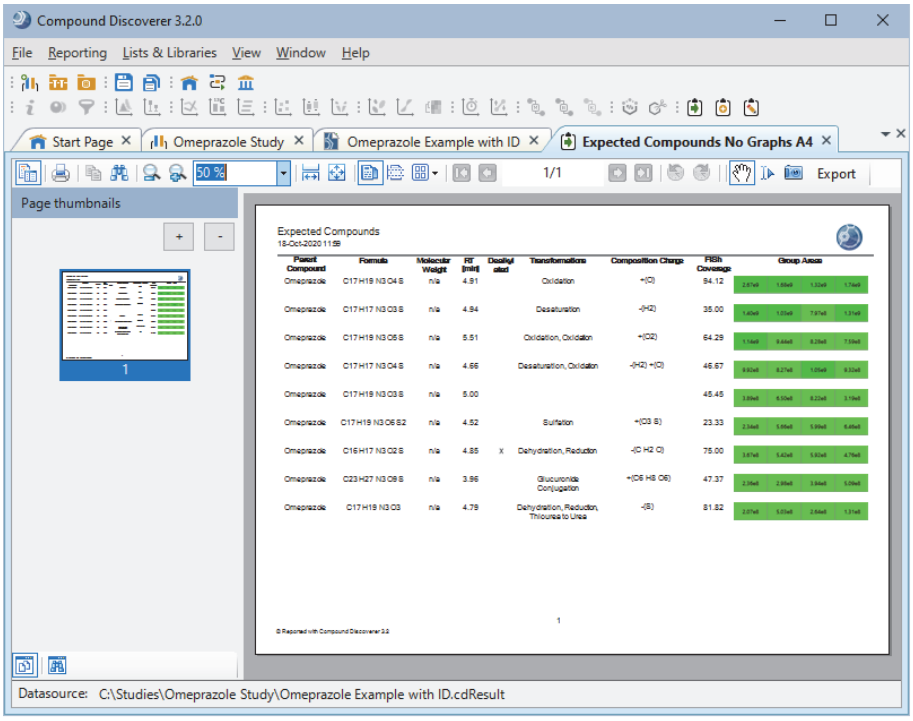
1. On the result file page, click the **Expected Compounds** tab.
2. From the menu bar, choose **Reporting > Create Report**.

   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**.

Trademarks