Compound Discoverer 3.0 Metabolism Tutorial

If you are unfamiliar with the Thermo Compound Discoverer™ 3.0 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 result file produced by the analysis, and print a report.
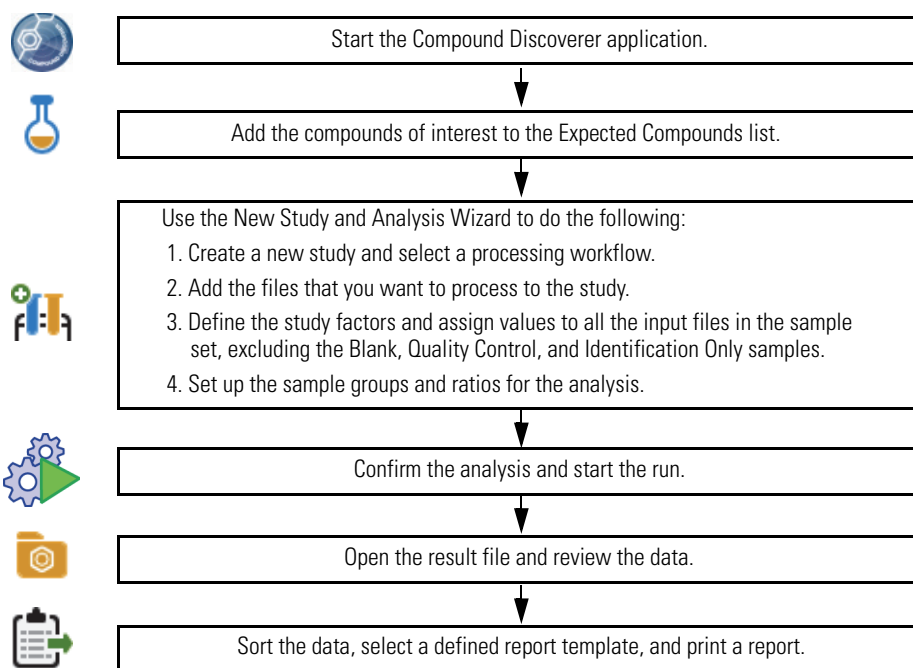
Contents

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 on the key-shaped USB key in the software media kit in the following folder:

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.

	Start the Compound Discoverer application.
	Add the compounds of interest to the Expected Compounds list.
	Use the New Study and Analysis Wizard to do the following: 1. Create a new study and select a processing workflow. 2. Add the files that you want to process to the study. 3. Define the study factors and assign values to all the input files in the sample set, excluding the Blank, Quality Control, and Identification Only samples. 4. Set up the sample groups and ratios for the analysis.
	Confirm the analysis and start the run.
	Open the result file and review the data.
	Sort the data, select a defined report template, and print a report.

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Starting the Application

❖ **To start the application**

- From the taskbar, choose **Start > All Programs** (or **Programs**) **> Thermo Compound Discoverer 3.0**.

–or–

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

The application opens to the Start Page.

Menu bar   Toolbar
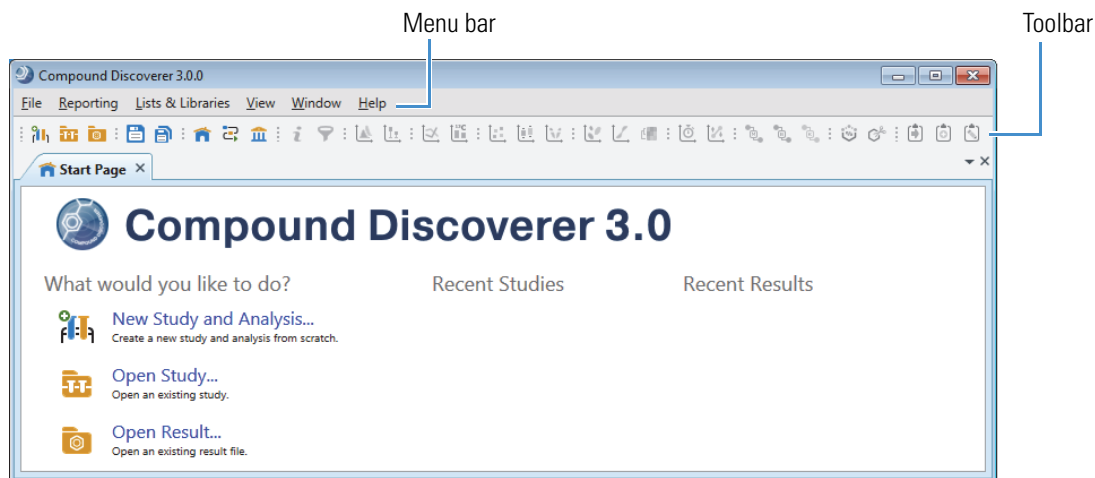
Accessing Help

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. On the computer keyboard, press the F1 key.

Adding Compounds to the Expected Compounds List

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

> **Tip** If you are using the omeprazole example data set for this tutorial, skip this topic, as the default Expected Compounds list already contains omeprazole.

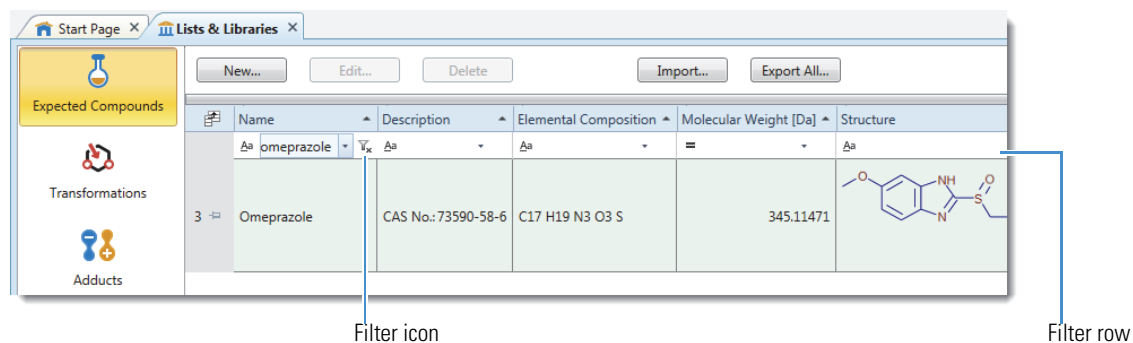Follow these procedures to set up your Expected Compounds list:

- To check for the compound of interest in the Expected Compounds list
- To add a compound to the Expected Compounds list

❖ **To check for the compound of interest in the Expected Compounds list**

1. From the menu bar, choose **Lists & Libraries > Expected Compounds**.

   The Expected Compounds view opens.

2. If your list contains a large number of compounds, type **Omeprazole** in the Name column of the filter row to display only the omeprazole entry.

Filter icon   Filter row

3. Click the filter icon, ▽×, to remove the filter and display the entire compounds list.

If your Expected Compounds list does not include omeprazole, add it as described in the next procedure.

❖ **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.
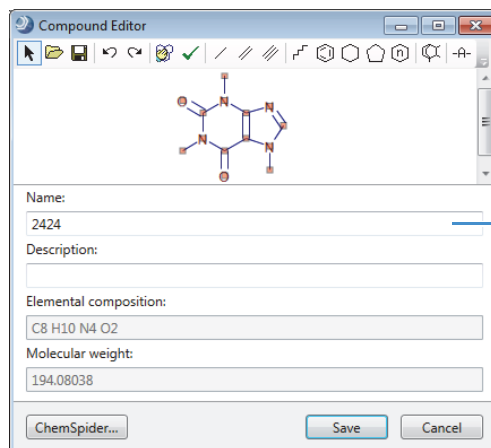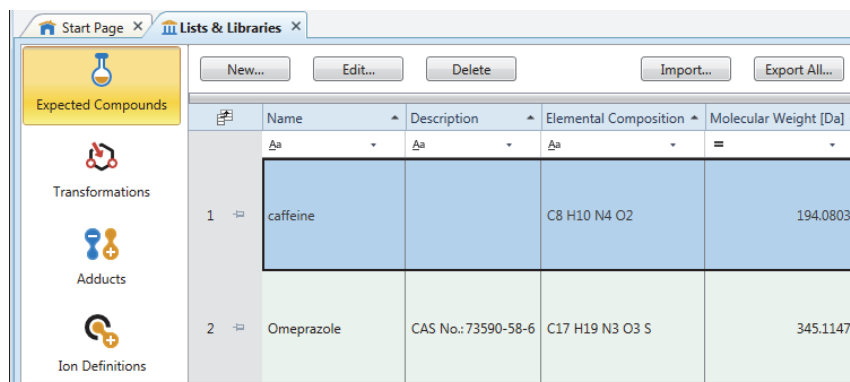

Replace the ChemSpider ID number with the compound name.

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

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 One or More Study Variables
6. Setting Up Ratios for Group Comparisons
7. Troubleshooting the Analysis

IMPORTANT To analyze a data set of your own, make sure that the compound of interest is in the Expected Compounds list before you create the new study and analysis (see "Adding Compounds to the Expected Compounds List" on page 2).

Naming the Study and Selecting Its Storage Location

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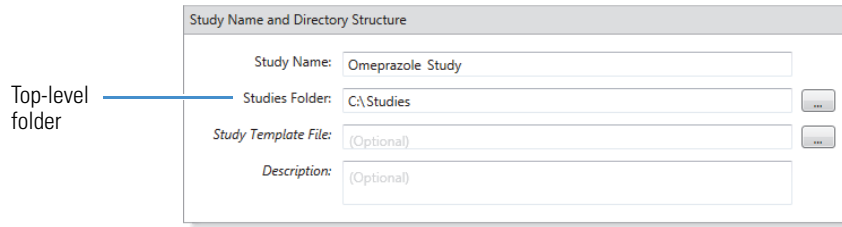
❖ **To name the new study and select the top-level folder for studies of this type**

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 study folders 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.
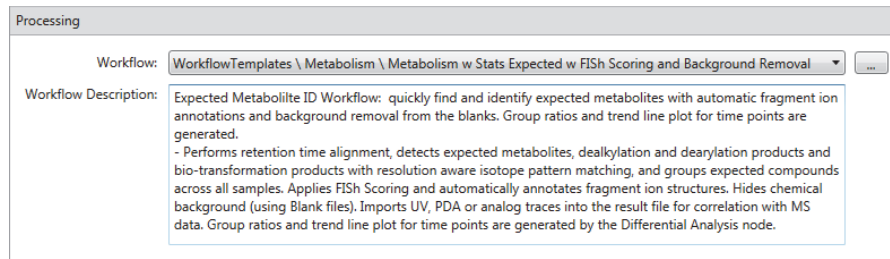   c. Click **New Folder** and name the new folder **Studies**.

Study Name and Directory Structure

Study Name:	Omeprazole Study
Top-level folder —— Studies Folder:	C:\Studies
Study Template File:	(Optional)
Description:	(Optional)

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\Metabolism\Metabolism w Stats Expected w FISh Scoring and Background Removal

Processing

Workflow: WorkflowTemplates \ Metabolism \ Metabolism w Stats Expected w FISh Scoring and Background Removal

Workflow Description: Expected Metabolite ID Workflow: quickly find and identify expected metabolites with automatic fragment ion annotations and background removal from the blanks. Group ratios and trend line plot for time points are generated.
- Performs retention time alignment, detects expected metabolites, dealkylation and dearylation products and bio-transformation products with resolution aware isotope pattern matching, and groups expected compounds across all samples. Applies FISh Scoring and automatically annotates fragment ion structures. Hides chemical background (using Blank files). Imports UV, PDA or analog traces into the result file for correlation with MS data. Group ratios and trend line plot for time points are generated by the Differential Analysis node.

2. Read the description of the processing workflow.

> **Note** The node-based workflows in the Common Workflows folder have been optimized by vertical market.
>
> The processing workflow for the example data set includes these changes to the default node settings:
> - For the Find Expected Compounds node, the Minimum Peak Intensity parameter is preset to 100 000.
> - For the Generate Expected Compounds node, all the Phase I and Phase II transformations are selected.

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

❖ **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 Xcalibur RAW files.
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.
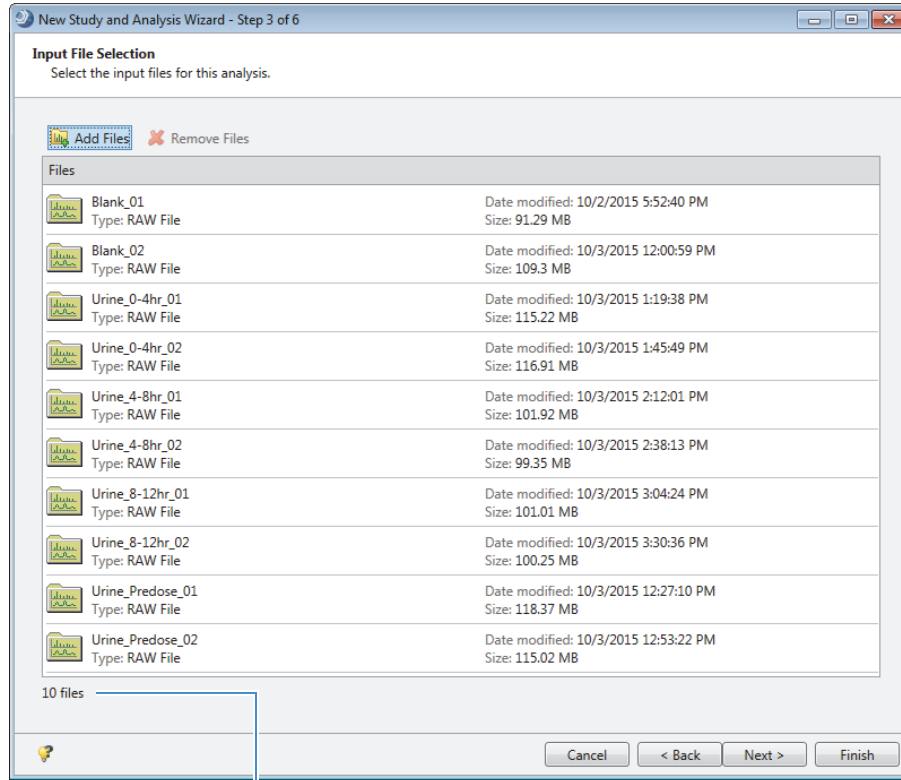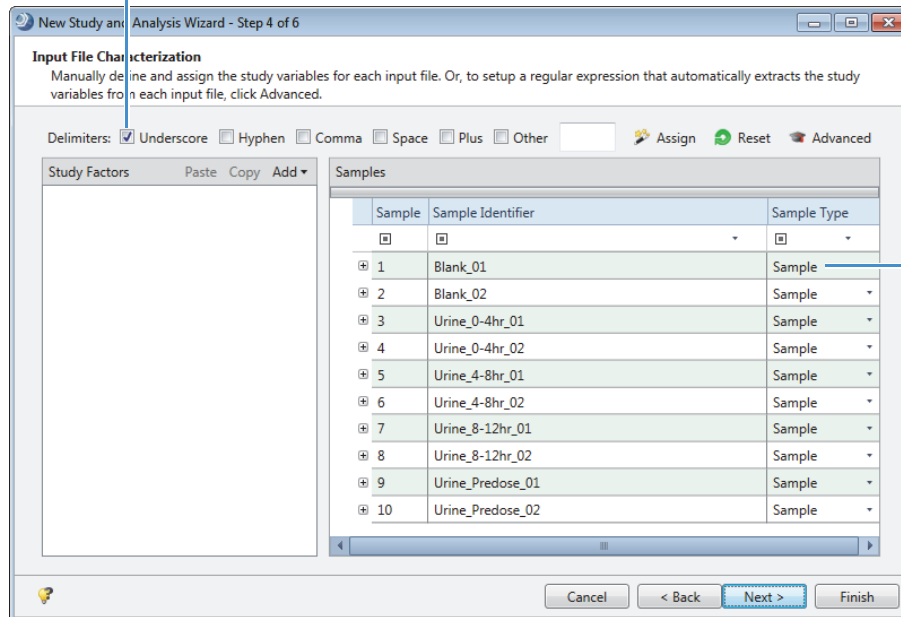

Number of selected files

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

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

Default file name delimiter

Default sample type

To define the study factors, go to the next topic.

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 samples collected at different time points from a subject dosed with the drug omeprazole. The time points begin with a predose sample and end 12 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, omeprazole sulfide, 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, keep the default selection of **Underscore** 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 from the other text in the file name (Urine_*0-4hrs*_01).

2. In the Study Factors area, choose **Add > Categorical Factor**.
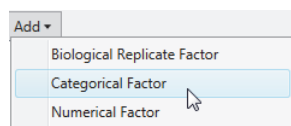
   Tip If you are not working with the omeprazole data set, select the study factor type as follows:
   - To define study factors that include alphanumeric items, use the categorical study factor editor.
   - To define study factors that include only numbers, use the numerical study factor editor.
   - 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.
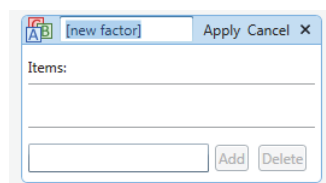

3. To replace the [New Factor] text, type the factor name, **Time Points**.

4. For each item that you want to add to the Items list, do the following:
   a. In the Items box (next to the Add button), begin typing a factor item, for example, **0-4hr**.

      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.

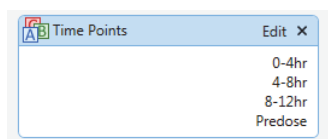   Finish typing the appropriate alphanumeric text or select an item from the list.
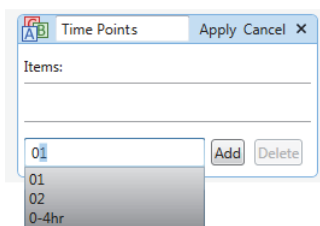
      The Add button becomes available.

5. Click **Add**.

   The current item appears in the Items list.

6. 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.


7. Click **Assign**.

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).
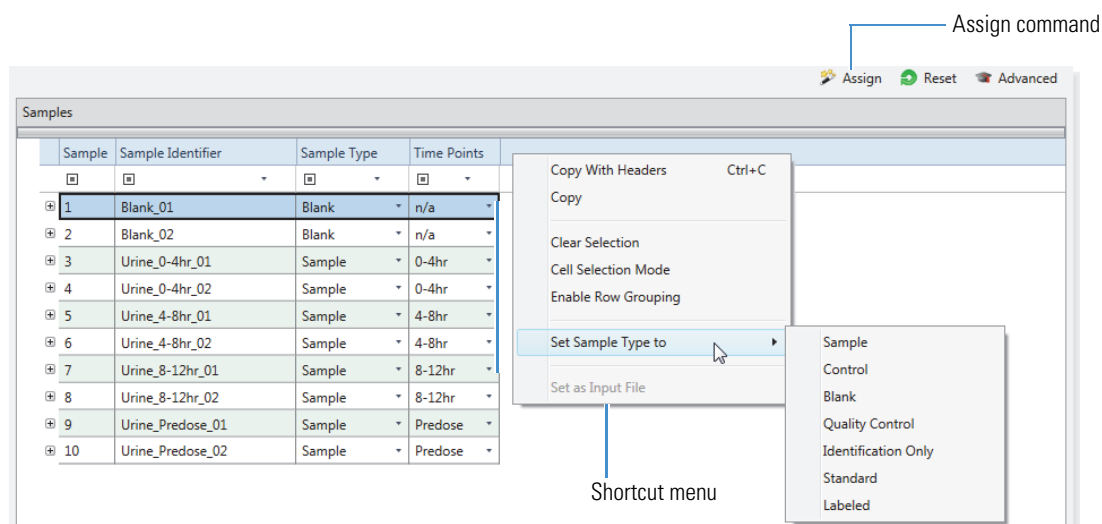
8. For the example data set, keep the default assignments of **Blank** and **Sample**.

For other data sets, select the sample types as needed:

- To select the sample type for a single sample, select the sample type from the list.
- To select the same sample type for a consecutive sample range, use the SHIFT key in the Sample Identifier column to 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, use the CTRL key to select the samples of interest. Then, right-click and choose **Set Sample Type To** > *Sample Type*.
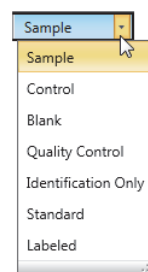
The sample type affects the processing actions.

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 and the processing workflow include grouping, the application reports the group areas for these sample types by study factor.
	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 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.

Table 1. Sample types (Sheet 2 of 2)

Sample type	Processing actions
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	Detects the components in the sample. When the processing workflow includes the Normalize Areas node, the application does the following: • 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 (DDF) fragmentation scans for component identification when the processing workflow includes the Group Unknown Compounds node.
Labeled	Not used for targeted analyses.

9. 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.

Grouping the Samples By One or More Study Variables

Follow these procedures to set up the sample groups:

1. To set up the sample groups for the example data set
2. To fix an assignment error caused by an n/a sample group (if necessary)

❖ **To set up the sample groups for the example 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, this group only includes Blank samples.
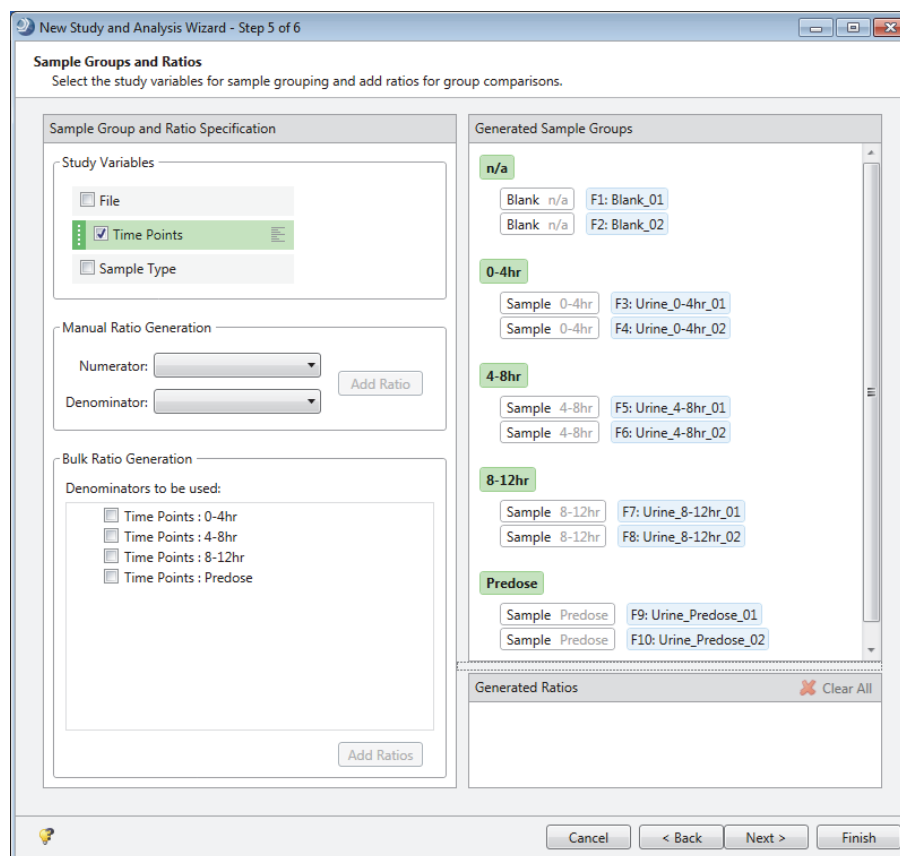
2. Do one of the following:

- If no error messages appear, go to the next topic "Setting Up Ratios for Group Comparisons."

- 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 the next procedure "To fix an assignment error caused by an n/a sample group."

> **IMPORTANT** 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.

❖ **To fix an assignment error caused by an n/a sample group**

> **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.

1. In the Generated Sample Groups pane on the Samples Groups and Ratios page, check whether any of these sample types are part of an n/a sample group:

- Sample

- Standard

- Control

2. To assign values to the Sample, Standard, or Control samples, do the following:

   a. Click **Back** to return to the Input File Characterization page.

   b. Select study factor values for all the samples except for the Blank and Identification Only samples.

   For example, if you assigned the Control sample type to the predose samples and did not add Predose as a value to the Time Point study factor, add the predose value to the Time point study factor, and then assign the Predose value to the Control samples.

3. Return to the Sample Groups and Ratios page.

4. In the Study Variables area, select the study variables of interest.

5. In the Generated Samples Groups pane, make sure that the n/a group only includes the Blank samples.

Go to the next topic "Setting Up Ratios for Group Comparisons."

Setting Up Ratios for Group Comparisons

For the example data, set up group ratios to compare the samples for the three time points, 0-4hr, 4-8hr, and 8-12hr, to the Predose samples. The subject collected the predose samples before ingesting omeprazole (to control acid reflux). After ingesting the drug, the subject collected additional urine samples at 4, 8, and 12 hours.

❖ **To set up the ratios**

1. In the Bulk Ratio Generation area, select the **Time Points: Predose** check box.

   The Add Ratios button becomes available.

2. Click **Add Ratios**.

   The sample comparison ratios appear in the Generated Ratios pane.

3. Click **Finish** to save the study and close the wizard.

The application creates the Omeprazole Study.cdStudy file, stores the study file in the Omeprazole Study folder, and stores the Omeprazole Study folder in the Studies folder.

📁 Computer > OSDisk (C:) > Studies > Omeprazole Study > Omeprazole Study.cdStudy

The study opens as a tabbed document in the application window. The subtabs for the four study pages and the two tabs for the analysis pages appear on the left. The Analysis pane appears on the right. The Run command is unavailable, and Processing Step in the Analysis pane has a Caution symbol (⚠). This symbol indicates that the analysis has one or more errors that you must correct before submitting the run.
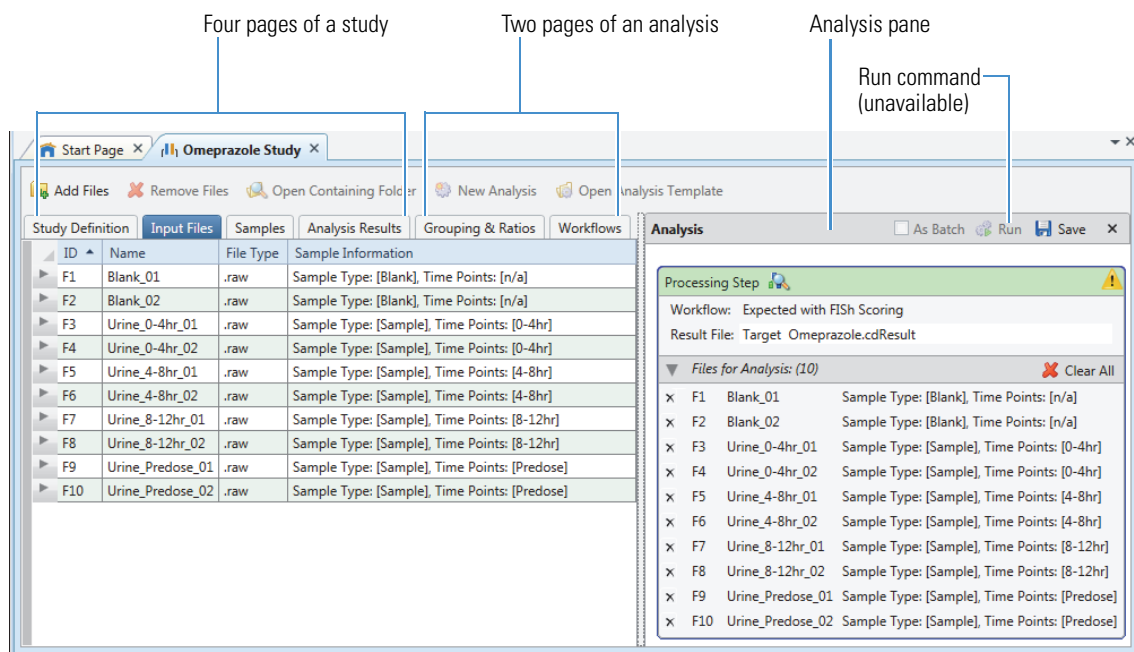
Go to the next topic to "Troubleshooting the Analysis."

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, review the messages in the Current Workflow Issues pane at the bottom of the Workflows page.

The application displays an explanation for the analysis error.
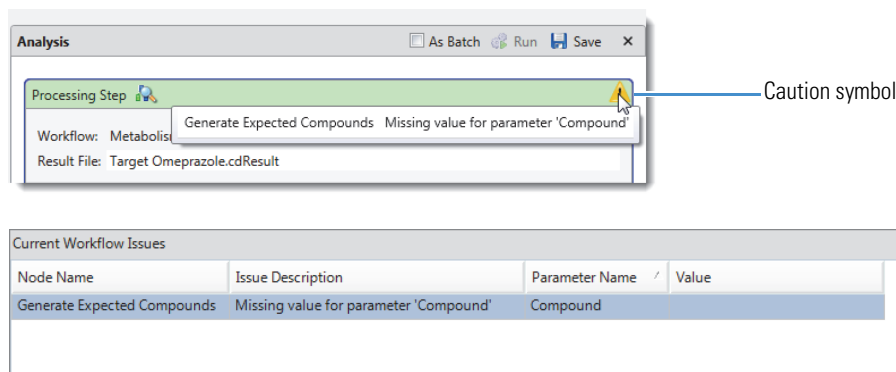
2. To fix the analysis errors, go to "Customizing a Targeted Processing Workflow."

Customizing a Targeted 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.

To customize the processing workflow, follow these topics:

- Specifying the Target Compounds and Adduct Ions (required)
- Saving the Processing Workflow as a Template (optional)
- Commonly Customized Parameters for a Targeted Workflow

Specifying 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, close the Analysis pane by clicking its close icon (☒), add the target compounds to the Expected Compounds list (see "Adding Compounds to the Expected Compounds List" on page 2), and then start a new analysis (see "Starting a New Analysis from an Existing Study" on page 16).
>
> Closing the Analysis pane erases the analysis settings, which include the input file list, the processing workflow, and the sample groups and ratios.

Follow the appropriate procedure for your data set:

- To specify omeprazole as the target compound for the example data set
- To specify the ions for the example data set
- To specify the target compounds for a different data set

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

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

This figure show the Workflows page with the selected processing workflow. The exclamation mark in the upper right corner of the Generate Expected Compounds node indicates that the node is missing a parameter value, and the Current Workflow Issues pane at the bottom describes the missing parameter value for this node.

Select the compounds of interest. — Workflows tab — Indicates a missing parameter value.

Parameters page — Describes the workflow issues.

3. In the Compound box, type **O**.

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.

Keep the Parameters page for the Generate Expected Compounds node open and go to the next procedure "To specify the ions for the example data set."

❖ **To specify the ions for the example data set**

On the Parameters page for the Generate Expected Compounds node, open the Ions list and select the check boxes for the sodium and potassium adducts.

Additional adduct ions

Note For most LC/MS experiments, the default selection of the protonated ([M+H]+1) and deprotonated ([M–H]–1) adduct ions is appropriate.

To start the analysis, go to

❖ **To specify the target compounds for a different data set**

1. If you have added the target compounds to the Expected Compounds list, click the **Workflows** tab to open the Workflows page. Otherwise, follow the instructions in the Important box on .

2. Determine whether you need to add more Generate Expected Compounds nodes to the processing workflow.

> **Note** The processing workflow for the example data set includes one Generate Expected Compounds node.

- To target multiple compounds with the same set of transformation rules, use one Generate Expected Compounds node.
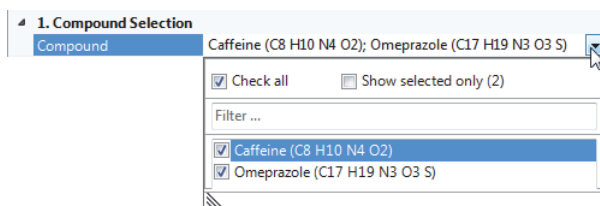
- To target multiple compounds with a different set of transformation rules for each compound, add an additional Generate Expected Compounds node for each set of transformation rules.
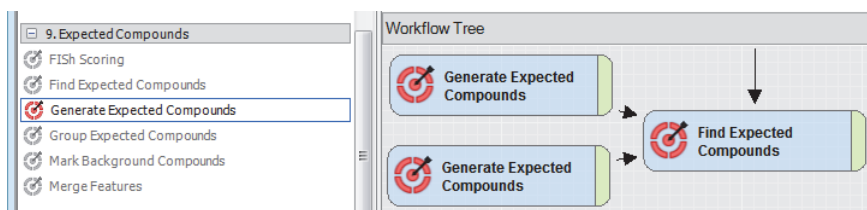
3. Add the necessary number of Generate Expected Compounds nodes to the processing workflow as follows:
   a. Drag the nodes from the Workflow Nodes pane to the Workflow Tree pane.
   b. Connect the nodes to the **Find Expected Compounds** node.

4. Select the compounds for each Generate Expected Compounds node in the workflow as follows:
   a. In the Workflow Tree pane, select a **Generate Expected Compounds** node.

   The Parameters pane for the Generate Expected Compounds node opens to the left.
   b. Select the target compounds from the Compound list.

5. For information about customizing other parameters in a Generate Expected Compounds node, open the Help, and type **"Generate Expected Compounds Node"** (quotation marks included) in the search box.

To save the modified processing workflow, go to the next topic "Saving the Processing Workflow as a Template."

Saving the Processing Workflow as a Template

If you save the processing workflow as a template, you can reuse it in another study.

❖ **To save the processing workflow as a template**

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

2. In the Workflow box, type a name for the processing workflow template.

3. In the Description box, type a general description of the processing workflow.

   This description appears in the Description column on the Job Queue and Analysis Results pages.

4. In the Workflows command bar, click **Save**.

   The Save Workflow dialog box opens.

5. Name the template, select the folder where you want to store the template, and click **Save**.

When customizing a targeted processing workflow, consider doing the following:

- Basing the Ions list in the Generate Expected Compounds node on the sample matrix or other experimental factors.
- Changing the Min. Peak Intensity setting in the Find Expected Compounds node.
- Modifying the Preferred Ions list in the Group Expected Compounds node.
- Changing the Sample/Blank ratio threshold in the Mark Background Compounds node.
- Adding the Create FISh Trace node. When you add the Create FISh Trace node to a processing workflow, you must check the following parameter settings in the node: Scan Polarity (positive or negative) and Fragmentation Mode (All-Ion or Data-Dependent).

If you erased your original analysis by closing the Analysis pane, set up a new analysis.

> **Tip** If you are using the omeprazole example data set for this tutorial, skip this topic unless you closed the application before submitting the run to the job queue.

❖ **To start a new analysis from an existing study**

1. If you closed the study, choose **File > Open Study**. Then, locate the study and click **Open**.

   The study opens to the Study Definition page.

2. Click **New Analysis** in the study's command bar.

   Starting a new analysis clears the Analysis pane, the Workflows page, and the Sample Groups and Ratios page.
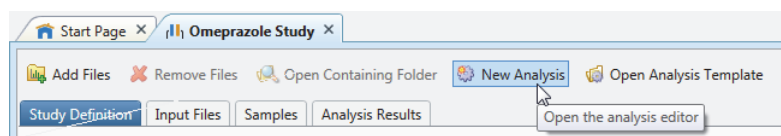
   The Analysis pane opens, and the Grouping and Ratios tab and Workflows tab appear to the right of the Analysis Results tab.

3. Set up the processing workflow as follows:

   a. Click the **Workflows** tab to open the Workflows page of the analysis.

   b. In the Workflows command bar, click **Open Common**.

   c. In the Open Workflow dialog box, select the **Workflow Templates** folder.

   d. Select the **Metabolism** folder.

   e. Select **Metabolism w Stats Expected w FISh Scoring and Background Removal.cdProcessingWF**.
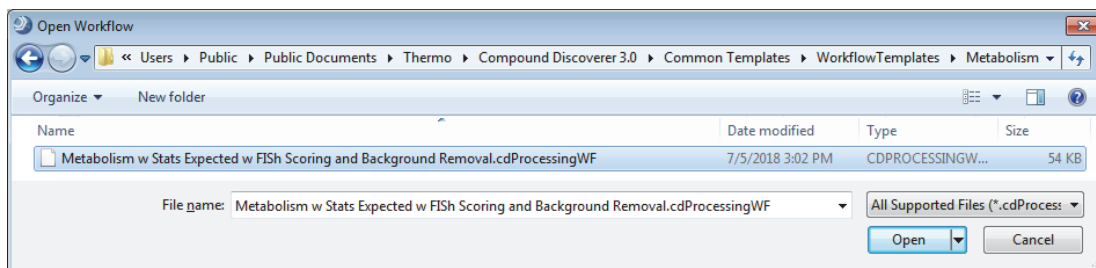
   f. Click **Open**.

   The processing workflow appears in the workspace.

   g. Add the targeted compounds to the workflow (see "Specifying the Target Compounds and Adduct Ions" on page 12).

4. Add the input files to the analysis as follows:
   a. Open the study's Input Files page or Samples page.
   b. Use the SHIFT key to select all the input files or samples.
   c. Right-click the page and choose **Set As Input File** to automatically add the files to the Files for Analysis area of the Analysis pane or drag the selection to this area.
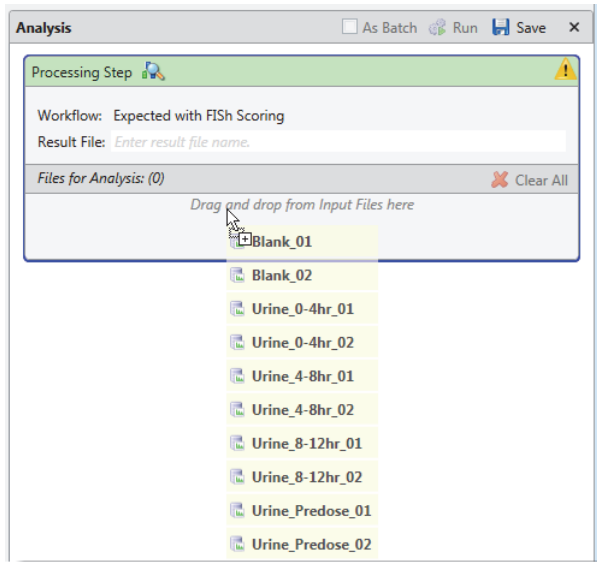
5. Set up the sample groups and ratios as follows:
   a. Click the **Grouping & Ratios** tab.
   b. In the Study Variables area, select the variables that define the sample groups (see "Grouping the Samples By One or More Study Variables" on page 9).
   c. In the Bulk Ratio Generation area, select the denominators to be used (see "Setting Up Ratios for Group Comparisons" on page 10).
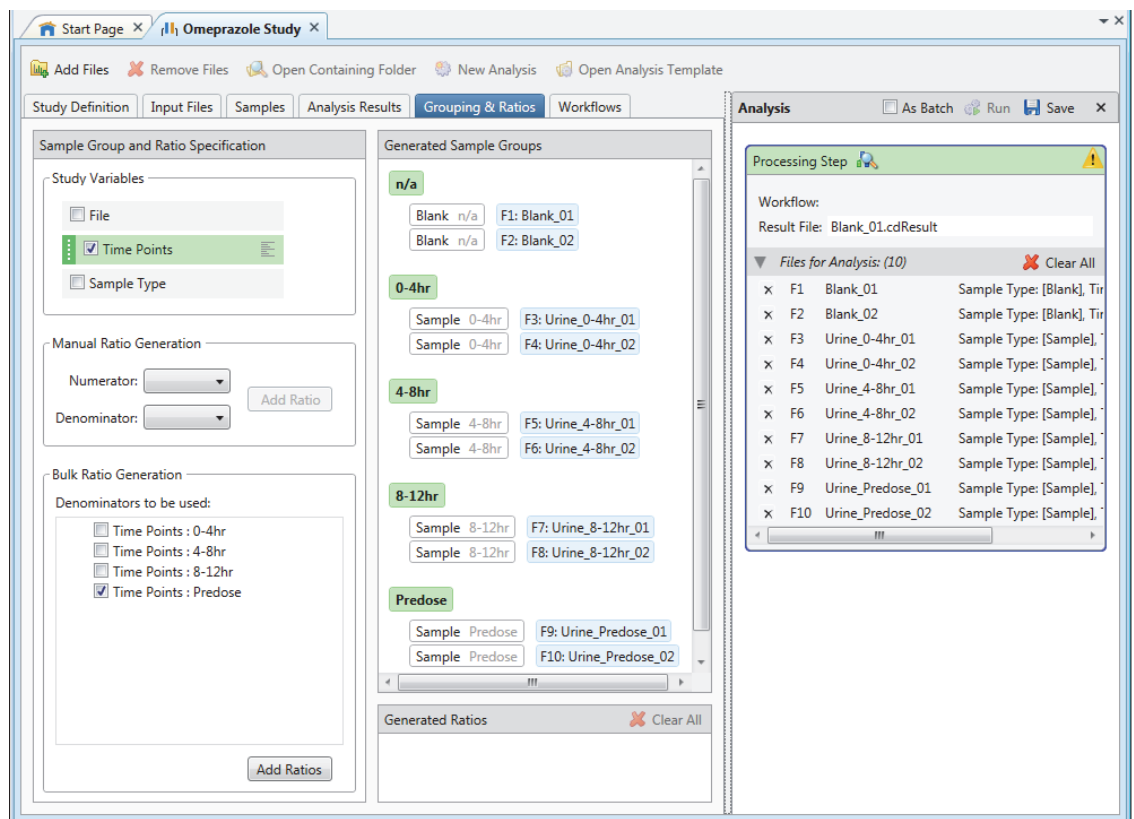
For the omeprazole study, set up the analysis as described on the previous pages of this tutorial. If you are working with a different study, and the Run command is not available in the Analysis pane, see this topic "Troubleshooting the Analysis" on page 11.

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

1. 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.

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

Analysis	☐ As Batch	Run	Save	×

Processing Step
Workflow: Expected with FISh Scoring
Result File: Target Omeprazole.cdResult

—————— Renamed result file
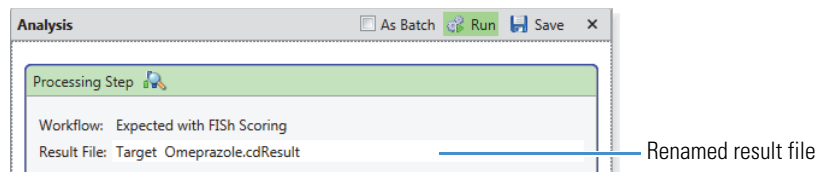
> **Note** When you run two or more input files as a batch run by selecting the As Batch check box, the application uses the input file name for each result file; you cannot change the file name in the Result File box. During a batch run, the application creates one result file for each input file.

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

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

🏠 Start Page ×	📊 Omeprazole Study ×	🔁 Job Queue ×

🗐 Pause	🔄 Resume	🗑 Abort	✖ Remove	🔄 Refresh	📂 Open Results	☐ Display Verbose Messages

Job Queue:

Execution State	Details	Progress	Type	Name	Submitted at	Study
▣	▣	▣	▣	▣	▣	▣
⊞ Running		86 %	Processing	Target Omeprazole	3/31/2017 4:53 PM	Omeprazole Study
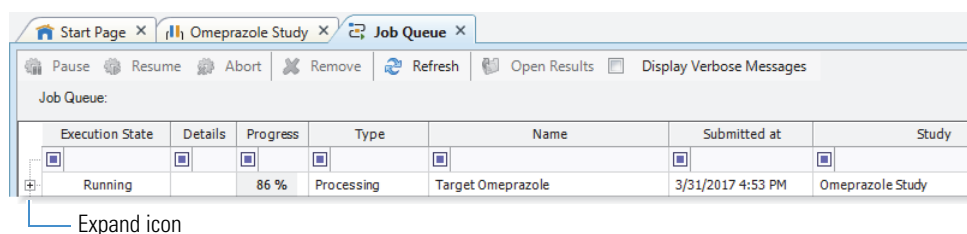
└—— Expand icon

4. 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 following nodes generate warning messages that you can ignore:
> • The Spectrum Selector node generates warning messages for invalid spectra.
> • The Align Retention Times node generates warning messages for alignment failures caused by insufficient landmark features.
> • The Merge Features node generates a warning message when it attempts to compare the features found by the Detect Unknown Compounds node to the features found by the Find Expected Compounds node because the selected processing workflow does not include the Detect Unknown Compounds node.

Leave the Job Queue page open and go to the next topic "Reviewing the Analysis Results."

After the run is completed, follow these topics to review the analysis results:

• Opening the Result File

• Default Layout for a Result Page

• Reviewing the Expected Compounds Found

• Reviewing the Chromatograms for the Compounds

• Reviewing the Mass Spectra for the Compounds

• Viewing a Trendline Plot of the Metabolism Rate

• Displaying Only the Compounds of Interest

• Naming and Proposing Structures for Compounds

• Saving or Resetting the Result File's Layout
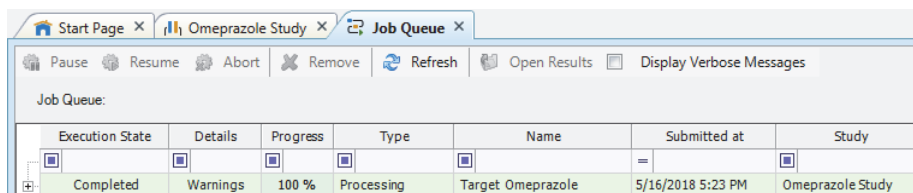
Opening the Result File

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 1). For information about all the ways you can open a result file, refer to the Help.

> **Tip** For this tutorial, you can open your result file if you processed the example data set or you can open the result file—Omeprazole Example.cdResult—provided with the example data set.

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

Do one of the following:

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

- If you closed the study after the run completed, under Recent Results on the Start Page, click **Target Omeprazole** (hypertext).

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

Opens the Target Omeprazole result file.
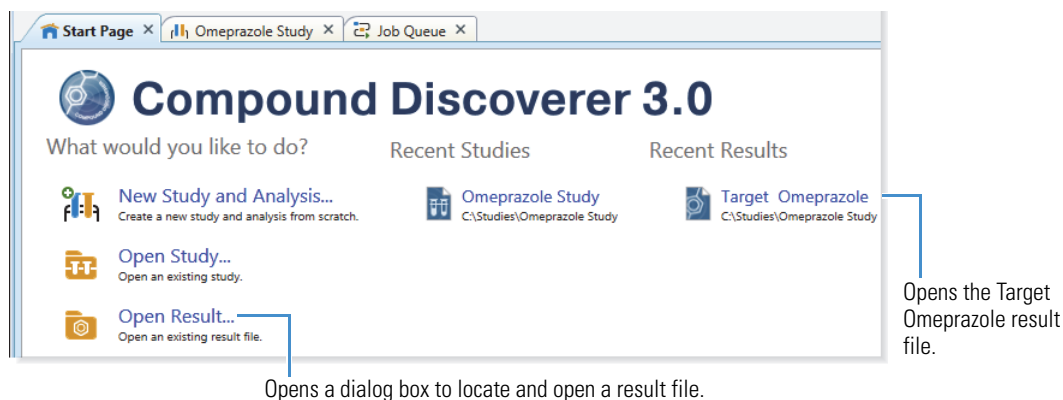
–or–

- If you did not reprocess the example data set, open the result file provided on the Compound Discoverer 3.0 USB key:

  Example Studies\Omeprazole Metabolism Study\Omeprazole Example.cdResult

Default Layout for a Result Page

The factory default layout for a result file includes the following items (Figure 1):

- 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.
- 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:

  chromatographic peak apex for the selected compound ± peak width at half maximum (FWHM)

> **Note** If the data set does not include data-dependent MS2 scans within the retention time window but does include AIF scans within this window, the spectrum tree includes the AIF scans.

- A set of tabbed main tables below the two graphical views.
- A collapsed area for the related tables below the main tables.

Figure 1. Default layout for the result file generated by the processing workflow

Closest MS1 scan to the chromatographic peak apex for the selected compound

Shows the related tables.
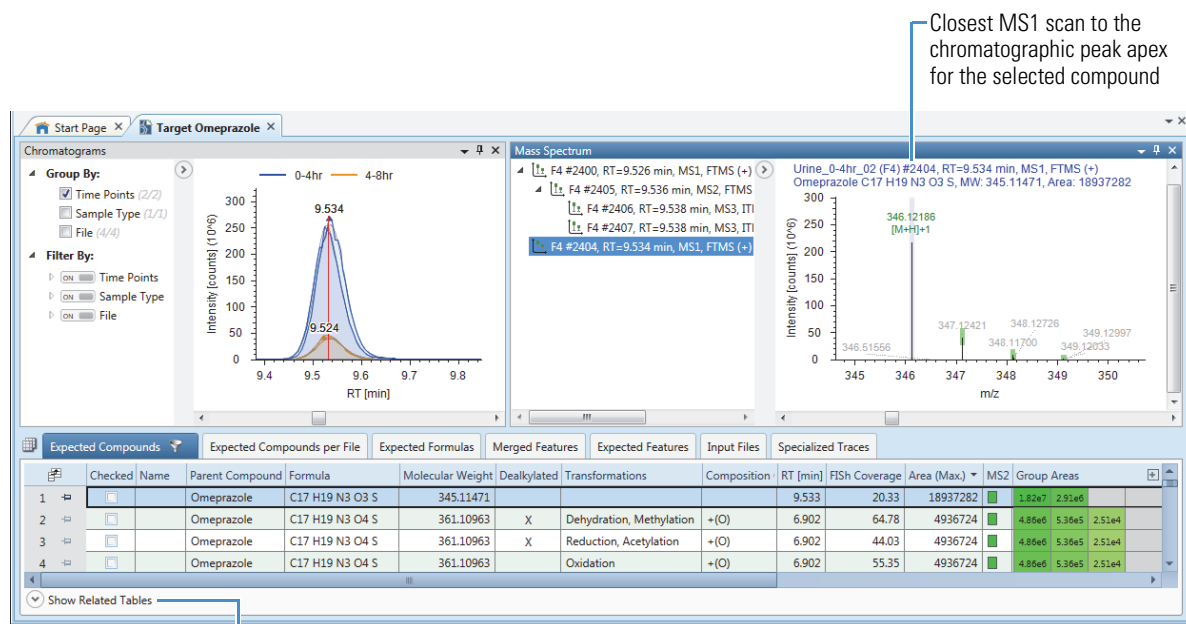
The selected processing workflow creates the following main tables:

- Expected Compounds — Lists all the expected compounds that the analysis found in one or more of the input files, and groups the compounds by molecular weight and retention time. The Group Expected Compounds node creates this table.

- Expected Compounds per File — Lists all the expected compounds that the analysis found in each input file (Xcalibur RAW file) and groups the compounds by molecular weight and retention time. The Find Expected Compounds node creates this table.

- Expected Formulas — Lists the expected formulas that the analysis searched against. The expected formulas list is the output from the Generate Expected Compounds node.

- 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.

- Expected Features — Lists the features found across all the input files.

- Input Files — Lists the input files for the analysis.

- Specialized Traces — Lists the traces extracted by the Create Analog Trace node (for this processing workflow). This table also lists the FISh traces, pattern traces, and mass traces when the processing workflow includes the Create FISh Trace, Create Pattern Trace, and Create Mass Trace nodes, respectively.

Reviewing the Expected Compounds Found

When you open a result file (with the default layout) for a targeted analysis (that does not include the Detect Unknown Compounds node), the Expected Compounds table is the active table and is sorted by the Area (Max.) column, with the first row displaying the compound with the largest chromatographic peak area.

Because the processing workflow that you selected for this tutorial includes the Mark Background Compounds node, the Expected Compounds tab has a filter icon with a check mark ( ). The compounds that the analysis identified as background compounds are marked as background compounds in both the blank and non-blank samples and are hidden from the table.

By default, these columns are hidden in the Expected Compounds table:

- #Adducts — Displays the adduct definitions in the Adducts list.
- Area — Displays the area for a selected compound in each input file as subcolumns.
- Background — Displays a check box that indicates whether the selected compound was found in a blank sample above the user-specified threshold. A selected check box marks the compound as a background compound.
- RT Tolerance [min] — Displays the retention time tolerance that the processing workflow specified for compound grouping across the input files.

Follow these procedures as needed:

- To view the total number of compounds in the Expected Compounds table
- To view information about each column
- To view the results for the sample groups and ratios
- To view the peak areas for each compound in each sample
- To sort the table columns
- To sort a column with multiple sub-columns
- To review the information in the tables related to the Expected Compounds table

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

Point to the vertical scroll bar on the right.

For this analysis, the Expected Compounds table contains 152 entries.

❖ **To view information about each column**

To display a ToolTip for each column, drag the mouse pointer across the column headings.

> **Note** The Help system includes a separate topic for each result table. To view the Help topic for a specific table, make the table active by clicking its tab, and then press the F1 key.

❖ **To view the results for the sample groups and ratios**
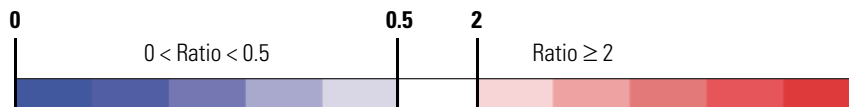
Review the Group Areas, Group CV(%), Ratio, and Log2 Fold Change columns. These columns are further subdivided into subcolumns for each group. The background colors of the table cells provide visual information about the numeric values in these columns (see Table 2).
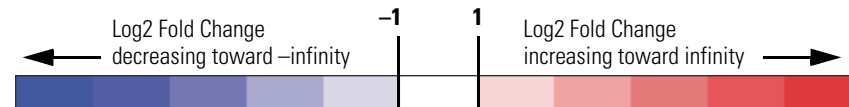
Table 2. Meaning of the background colors for the grouping and ratios columns

Column	Meaning
Group Areas	Gray background: The compound is not found. As the value of the median group area increases, the background color changes from pale yellow to green.
Group CV(%)	Red background: The CV% is ≥ 20.
Ratio	• Dark purple background: The ratio equals 0 (0/*X*) (0.000). • Orange background: The ratio is undefined (*X*/0), labeled as Infinity (Infinity) . • Blue background: The ratio is between 0 and 0.5. Ratios between 0 and 0.5 are divided into five equal bins. The table cells turn darker blue as the ratio approaches zero. • Red background: The ratio is greater than 2. Ratios > 2 are divided into five equal bins. The table cells turn darker red as the ratio increases. 0 0.5 2 0 < Ratio < 0.5 Ratio ≥ 2
Log2 Fold Change	• Orange background: The $\log_2$ fold change is infinity (Infinity). • Dark purple background: The $\log_2$ fold change is –infinity (ratio 0/X). • Blue background: The $\log_2$ fold change is less than –1.00. Values < –1.00 are divided into five equal bins. The table cells turn darker blue as the negative value decreases (approaches –infinity). • Red background: The $\log_2$ fold change is greater than 1.00. Values > 1.00 are divided into five equal bins. The table cells turn darker red as the positive value increases (approaches infinity). −1 1 Log2 Fold Change decreasing toward –infinity Log2 Fold Change increasing toward infinity

❖ **To view the peak areas for each compound in each sample**

1. Display the hidden Area column as follows:

   a. Click the **Field Chooser** icon, ⊞.
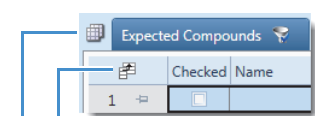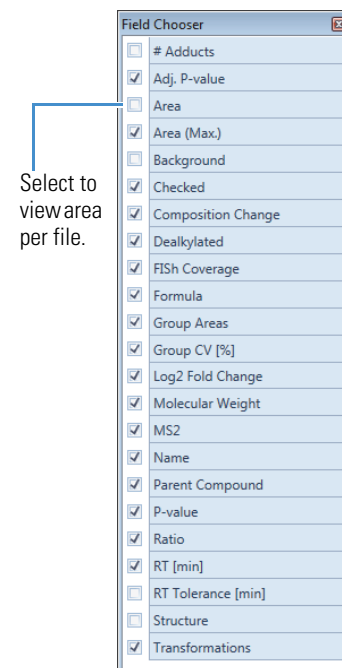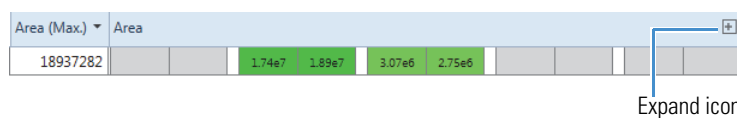
   Opens the Field Chooser box.
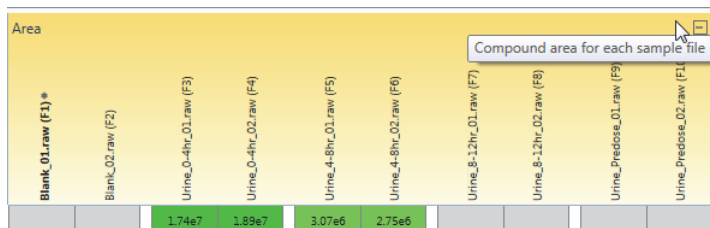
   Opens the Select Visible Tables dialog box.

   The Field Chooser box for the Expected Compounds table opens.

   Select to view area per file.

   b. Select the **Area** check box.

   The Area column appears in the Expected Compounds table to the right of the Area (Max.) column.

   Expand icon

2. To view the sample names for the area columns, click the expand icon, ⊞, to the right of the column name.

The names of the input files appear vertically above the individual area columns. The name of the first input file, Blank_01.raw (F1), appears in bold font and has an asterisk to indicate that it is the selected area column.

Collapse icon

3. Click the collapse icon, ▭, to hide the sample names.

4. To hide the Area column for the remaining portion of this tutorial, open the Field Chooser box and clear the **Area** check box.

❖ **To sort the table columns**

- To sort a column, click the column header. Click the column header a second time to reverse the sort order.

- To sort multiple columns, use the CTRL key and click the column headers of the first column and any additional columns that you want to sort by.

❖ **To sort a column with multiple sub-columns**

1. Click the expand icon 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.

❖ **To review the information in the tables related to the Expected Compounds table**

1. Sort the Expected Compounds table by **Area (Max.)** in descending order.

2. Select the fourth row (the oxidation product of omeprazole).

3. Click **Show Related Tables** (see Figure 1 on page 20).

4. Click the **Expected Compounds per File** tab.

The related Expected Compounds per File table lists the study files (input files) that contain the selected compound in the Expected Compounds table. The blank samples (F1 and F2) and one of the predose samples (F9) do not contain the selected compound (omeprazole). Study file F3 contains the largest amount (by peak area) of the selected compound. The peak area for study file F3 matches the Area (Max.) value in the Expected Compounds table.

5. Select the first row in the Expected Compounds per File table.

6. Click **Show Related Tables** below the Expected Compounds per File table to open the secondary set of related tables.

7. Click the **Expected Features** tab to display the Expected Features table.

Because the modified processing workflow (see "To specify the ions for the example data set" on page 14) generates a list of these adduct ions, [M+H]+1, [M+Na]+1, [M+K]+1, the Expected Features table lists these ion species (features).

In input file F3, the oxidation product of omeprazole had three adduct ions (features).

Seven input files contained the oxidation product of omeprazole.

Reviewing the Chromatograms for the Compounds

When you select a row in the Expected Compounds table, overlaid traces for the selected compound appear in the Chromatograms view.

To explore the Chromatograms view, follow these procedures:

- To view the overlaid traces of the parent compound
- To turn off the shading of the integrated peak areas
- To work with the collapsible left pane

❖ **To view the overlaid traces of the parent compound**

1. In the Expected Compounds table, sort the columns to bring the parent compound (no dealkylation or transformation reactions) to the first row as follows:

   a. Click the **Dealkylated** column heading to sort the rows by the occurrence of a dealkylation reaction.

   b. Use the CTRL key and click the **Transformations** column heading.

   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.


2. Select the first row.

   Overlaid traces for the selected compound appear in the Chromatograms view. The collapsible left pane lists the grouping and filtering options.

   By default the display options for the Chromatograms view are set to Show Tooltips, Show Detected Peaks, and Show Legend. The *x*-axis zoom is set to Detected Peaks (Active Plots) and the *y*-axis zoom is set to Auto Scale Y-axis.

This figure displays the overlaid traces for the input files that contain the selected compound. By default, the sample grouping matches the sample grouping that you set up on the Sample Groups and Ratios page (or Grouping & Ratios page) of the analysis. In this case, the samples are grouped by the Time Points study factor, which means that the view displays the time point replicates (two per group) in the same color. The legend above the chromatographic peak shows the display colors for the two time point groups where the analysis found the parent compound, omeprazole. The integrated peak area is shaded.

Samples are grouped by the Time Points study factor.

Shortcut menu
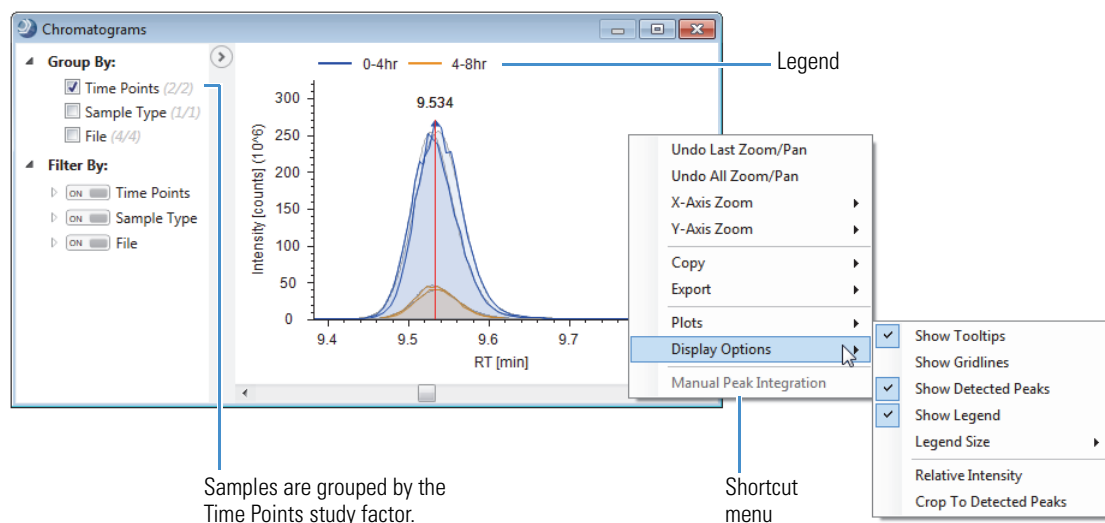
The peak shading can sometimes hide other traces.

❖ **To turn off the shading of the integrated peak areas**

Right-click the Chromatograms view and choose **Display Options > Show Detected Peaks**.

The shading disappears as shown in the next figure.

❖ **To work with the collapsible left pane**

> **Note** A collapsible pane with grouping and filtering options appears to the left of these views: Chromatograms, Trend Chart, Principal Component Analysis, Descriptive Statistics, and Differential Analysis.

Under Filter By, expand the **File**, **Sample Type,** and **Time Points** lists.

This figure shows the expanded lists in the collapsible pane. The Chromatograms plot displays the overlaid traces without any shading of the integrated peak areas.
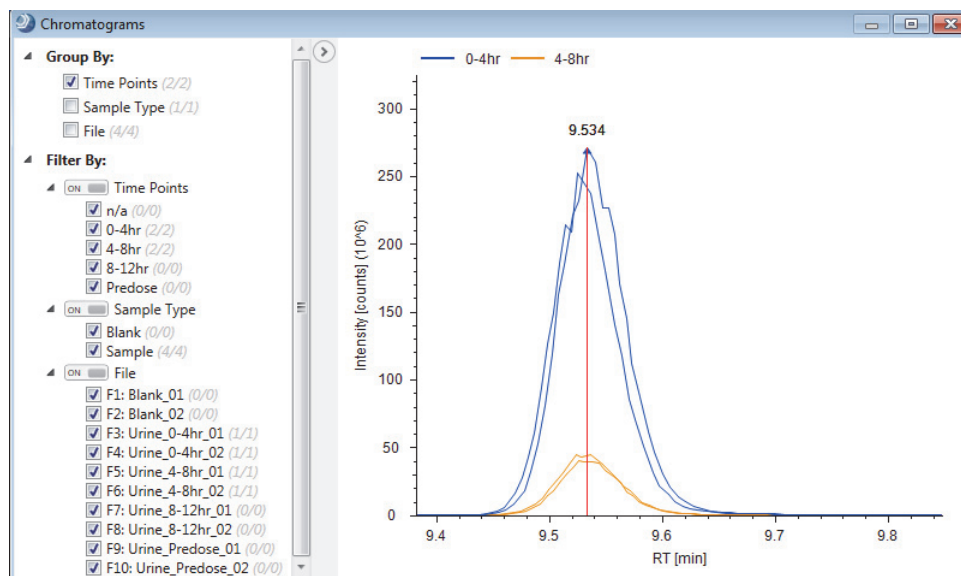
Review the expanded lists:

- Under File, all the check boxes are selected by default. The blanks, the 8–13 hr, and the predose samples have counts of 0 out of 0 (0/0). The analysis did not find the selected compound in these samples. It did find omeprazole in the samples from the 0–4 and 4–8 hr time points.

- Under Sample Type, the Blank sample type has a count of 0 out of 0 (0/0), and the Sample sample type has a count of 4 out of 4 (4/4).

Reviewing the Mass Spectra for the 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:

- Inspecting the Isotope Pattern in the Full MS Scan

- Viewing the Fragmentation Scans

- FISh Coverage Score

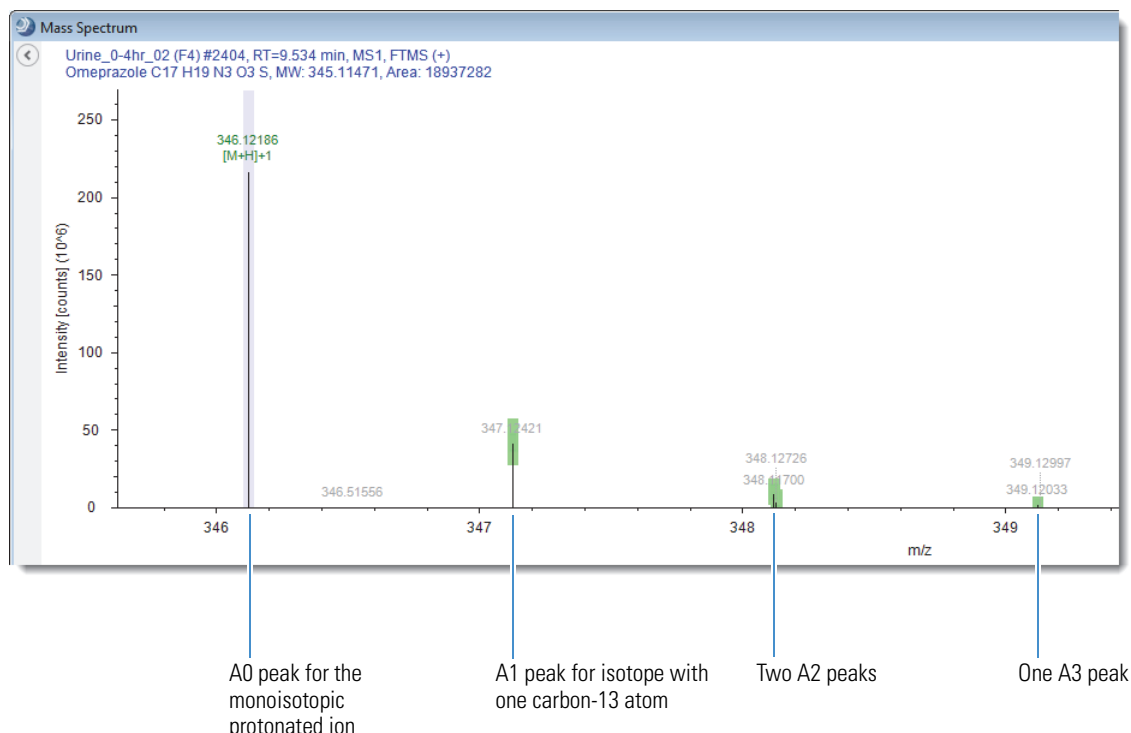**Inspecting the Isotope Pattern in the Full MS Scan**

❖ **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).

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, two ^{13}C atoms replace two ^{12}C atoms, and so on.
> - 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 and the A3 isotope at *m/z* 349.1.
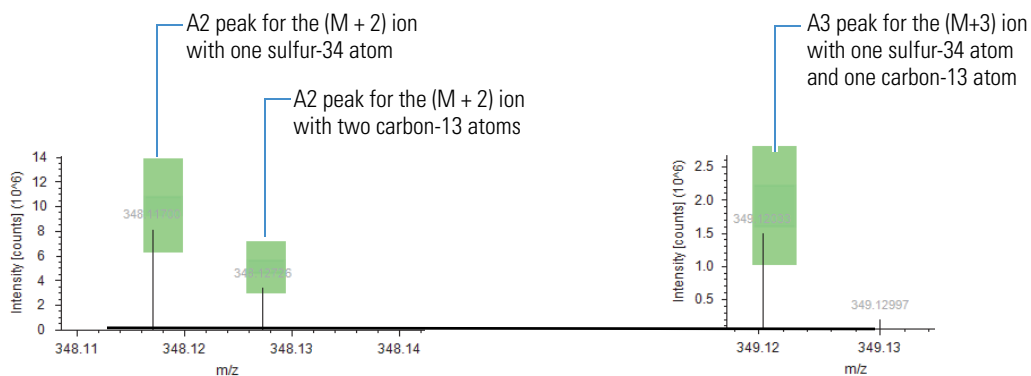
The full MS scan shows the isotope pattern for the detected compound. Colored rectangles highlight the mass spectrum peaks (centroids) that match the theoretical isotope pattern.

Table 3. 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, DDF scan, for example, scan #**2405**.

The annotated fragmentation scan appears in the right pane.

Figure 2. Annotations for scan #2405 generated by the FISh Scoring node during data processing
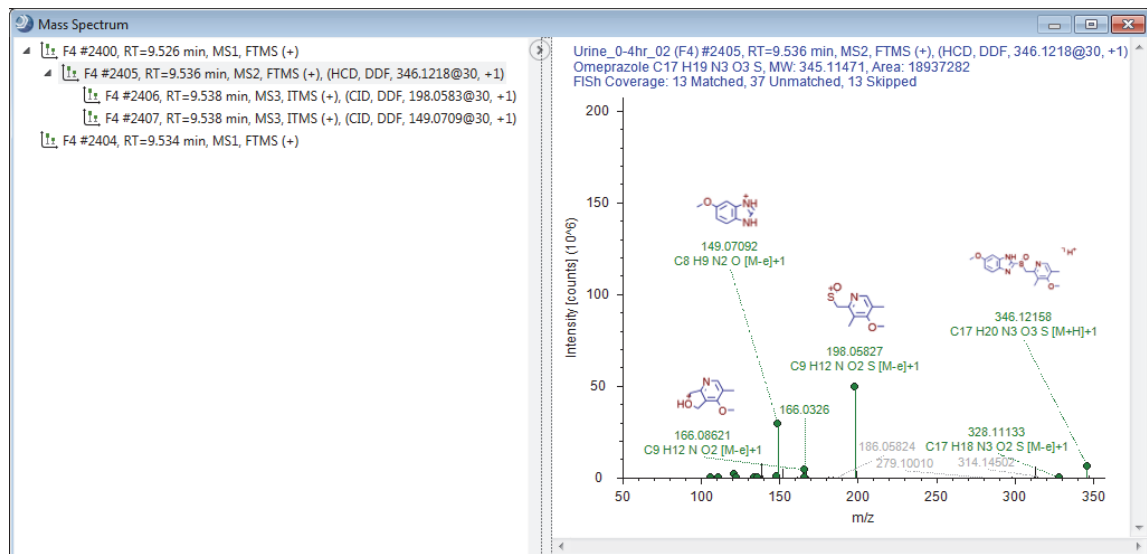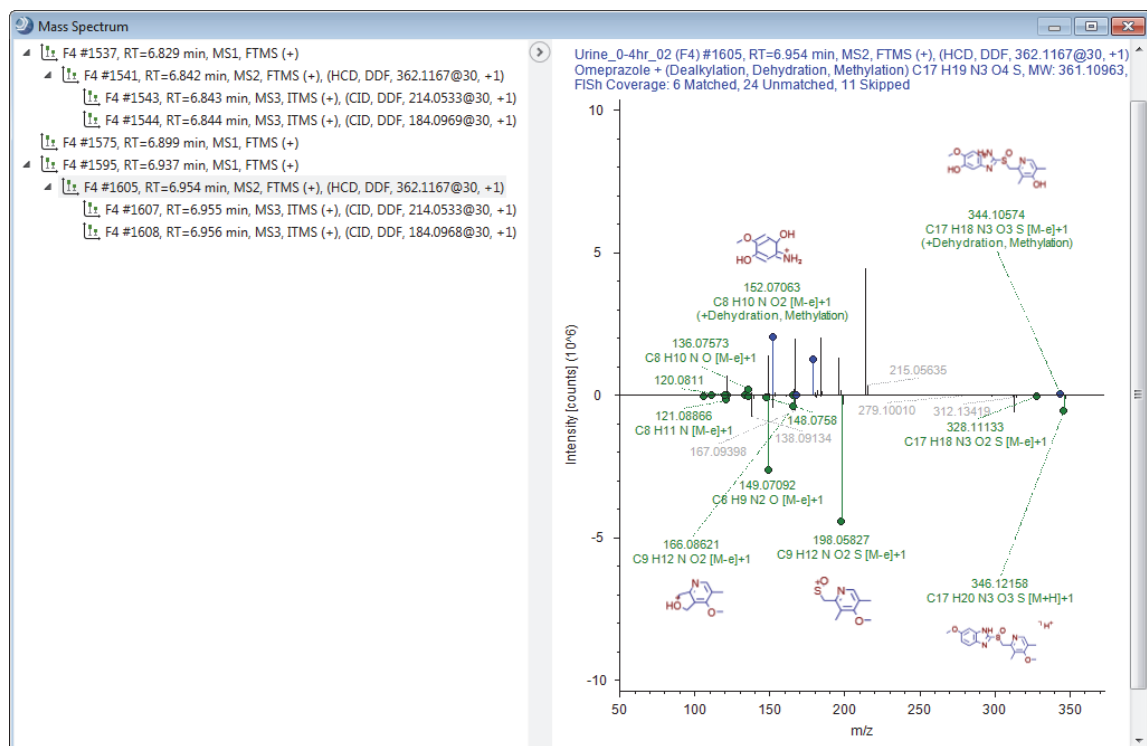

> **Note** The FISh Scoring node cannot calculate a FISh Coverage score for compounds that lack DDF scans. This means that the FISh Scoring node cannot calculate FISh Coverage scores for data acquired with a Thermo Scientific™ Exactive™ mass spectrometer or chromatographic peaks that contain only all-ion fragmentation (AIF) 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. Click the row for a metabolite of interest, for example, the compound with the next largest Area (Max.) value.

   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.

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:

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

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 DDF scans in the Expected Compounds table.

> **Note** In the current version of the application, the FISh scoring algorithm uses all the fragmentation scans (in the spectrum tree for a compound), compared to earlier versions of the application that only used MS2 scans. Therefore, if you reprocess data sets that you already processed with an earlier version of the application, and these data sets include MSn scans (where n > 2), the FISh coverage scores might be lower.
>
> The Orbitrap Fusion™ and Orbitrap hybrid mass spectrometers do not provide signal-to-noise (S/N) data for ITMS3 and ITMS4 scans; therefore, the FISh scoring algorithm uses all the spectral peaks in the ITMS3 and ITMS4 scans to calculate the FISh coverage score.

Viewing a Trendline Plot of the Metabolism Rate

Use the Trend Chart view to view the rate of metabolism for a compound as a trendline plot.

❖ **To view the rate of metabolism as a trendline plot**

1. Sort the Expected Compounds table by **Area (Max.)** in descending order.
2. Select the first row in the sorted Expected Compounds table.
3. From the menu bar, choose **View > Trend Chart**.

   The Trend Chart view opens in the lower right corner of the active result page.
4. In the left pane, under Filter By, expand the Time Points list and clear the **n/a** check box.

   The blank samples make up the n/a sample group.
5. In the Plot Type list, select **Trendline Chart**.

   A trendline plot of the four time points appears.
6. Hold down the CTRL key and select the second row in the sorted table.
7. Right-click the Trend Chart view and choose **Show Legend**.

This figure shows the Trend Chart view with trend line plots for two compounds. The legend at the bottom of the chart describes the two plots.
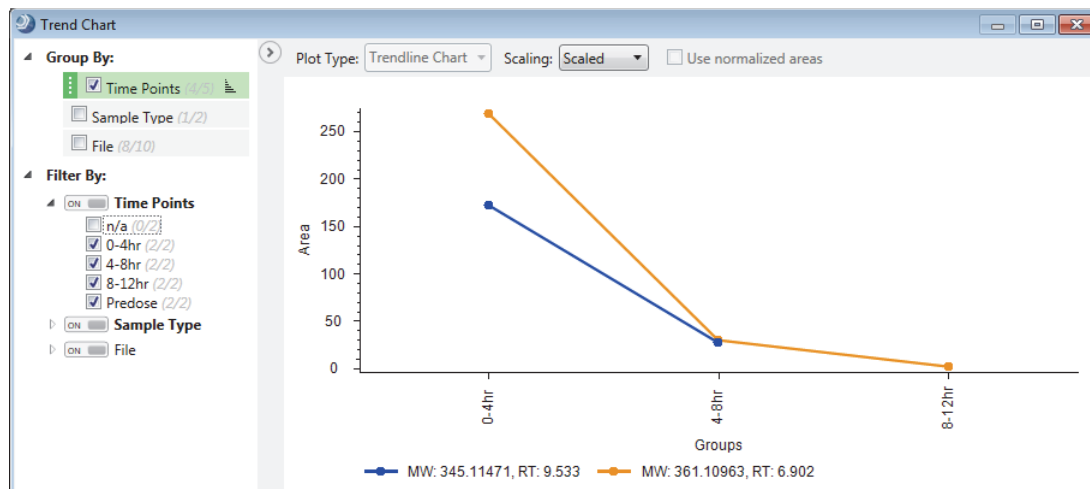
**Displaying Only the Compounds of Interest**

To learn how to use the result filters to filter out compounds that you do not want to include in your reports, follow these procedures:

- To reduce the number of expected compounds in the Expected Compounds table
- To display only the most probable explanation for each compound
- To save the current filter settings for reuse

❖ **To reduce the number of expected compounds in the Expected Compounds table**

1. Click the **Expected Compounds** tab in the set of main tables.

2. From the menu bar, choose **View > Result Filters**.

   The Result Filters view opens as a floating window. The Expected Compounds table is selected in the table list on the left. Because the processing workflow included the Mark Background Compounds table, the Expected Compounds table is currently filtered by background and the table's filter indicator is green. The expected compounds found in the blanks are hidden for all the samples.
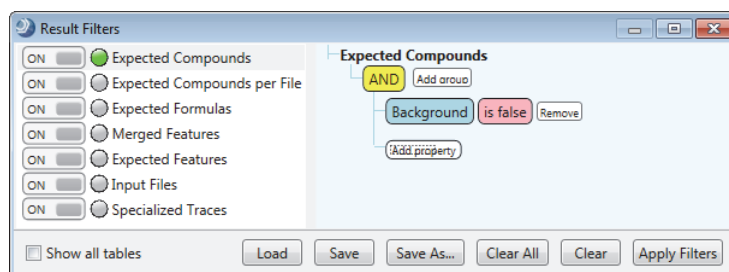
3. In the right pane, add a filter that hides compounds with maximum peak areas below 1 000 000 counts as follows:

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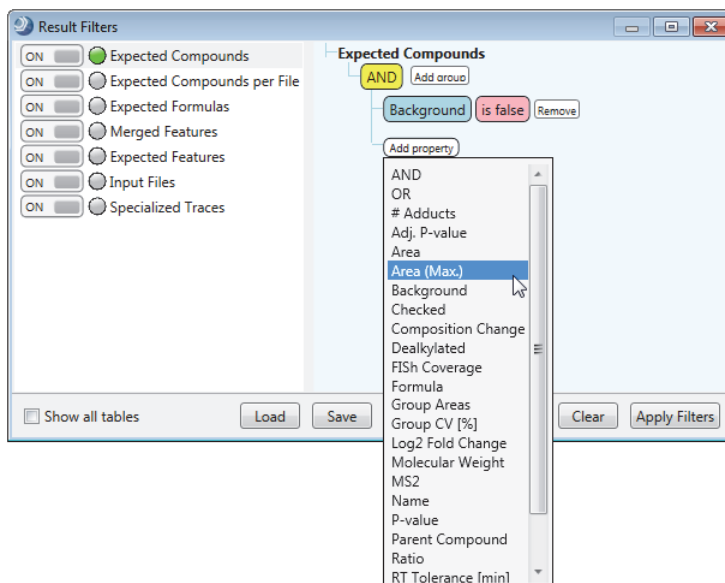
   b. Click the (pink) relation list and select **Is Greater Than or Equal To**.

   c. Type **1e6 (**or **1 000 000)** in the box next to the relation box.
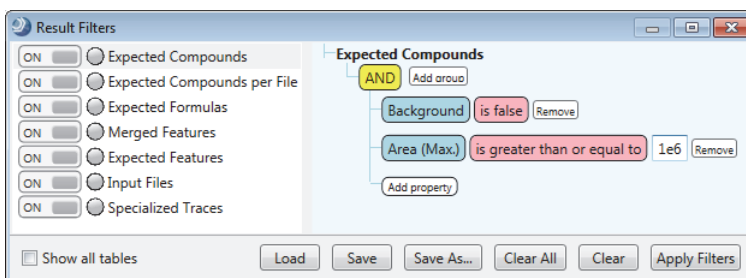
   d. Click **Apply Filters**.

   The Expected Compounds table now displays only 11 entries.

Keep the Result Filters view open and go to the next procedure "To display only the most probable explanation for each compound."

❖ **To display only the most probable explanation for each compound**

1. 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 reaction pathway for each explanation. The correct explanation is often the one with the fewest steps.

   • Compare the FISh Coverage score for each explanation. The probability that an explanation is correct increases as the FISh Coverage score increases.

   > **Note** Because the example data was acquired on an Orbitrap Fusion MS and includes ITMS3 scans (without S/N data), the FISh coverage scores are not as relevant as the number of steps in the reaction pathway for determining the most probable explanation for each compound.

   For example, select row 2 for the component at 6.902 minutes, row 7 for the component at 4.435 minutes, and row 10 for the component at 5.596 minutes.

		Checked	Name	Parent Compound	Formula	Molecular Weight	Dealkylated	Transformations	Composition Change	RT [min]	FISh Coverage
1		✓		Omeprazole	C17 H19 N3 O3 S	345.11471				9.533	20.33
2		✓		Omeprazole	C17 H19 N3 O4 S	361.10963		Oxidation	+(O)	6.902	55.35
3		☐		Omeprazole	C17 H19 N3 O4 S	361.10963	X	Dehydration	+(O)	6.902	47.17
4		☐		Omeprazole	C17 H19 N3 O4 S	361.10963	X	Reduction, Acetylation	+(O)	6.902	44.03
5		☐		Omeprazole	C17 H19 N3 O4 S	361.10963	X	Dehydration, Methylation	+(O)	6.902	64.78
6		✓		Omeprazole	C16 H17 N3 O2 S	315.10415	X	Dehydration, Reduction	-(C H2 O)	10.290	63.08
7		✓		Omeprazole	C17 H17 N3 O4 S	359.09398		Desaturation, Oxidation	-(H2) +(O)	4.435	41.28
8		☐		Omeprazole	C17 H17 N3 O4 S	359.09398	X	Dehydration, Desaturation	-(H2) +(O)	4.435	44.04
9		☐		Omeprazole	C17 H17 N3 O4 S	359.09398	X	Acetylation	-(H2) +(O)	4.435	20.18
10		✓		Omeprazole	C16 H17 N3 O3 S	331.09906	X		-(C H2)	5.596	37.19
11		☐		Omeprazole	C16 H17 N3 O3 S	331.09906	X	Methylation	-(C H2)	5.596	29.75

Fewest reaction steps for 331.09906 Da × 5.596 minutes

Fewest reaction steps for 359.09398 Da × 4.435 minutes

Fewest reaction steps for 361.10963 Da × 6.902 minutes

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

3. Click the (pink) relation list and select **Is True**.
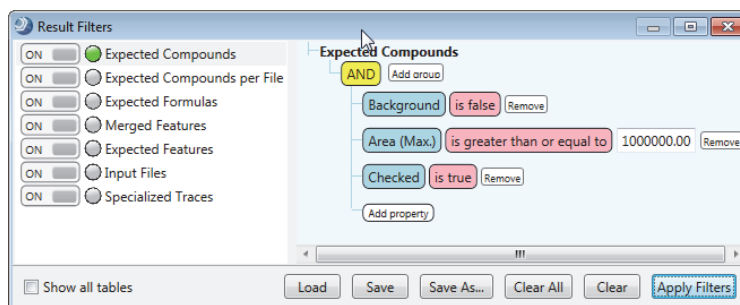
4. Click **Apply Filters**.

   The Expected Compounds table now displays only five compounds.

❖ **To save the current filter settings for reuse**

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

2. Select the directory folder where you want to save the filter set file.

3. Name the filter set and click **Save**.

Naming and Proposing Structures for Compounds

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

❖ **To populate the Name and Structure columns**

1. In the main Expected Compounds table, right-click the row for omeprazole—row 1 when sorted by Area (Max.)—and choose **Edit Compound Annotation**. Or, double-click the row for omeprazole.

   The Compound Annotation Editor opens to the Description page.
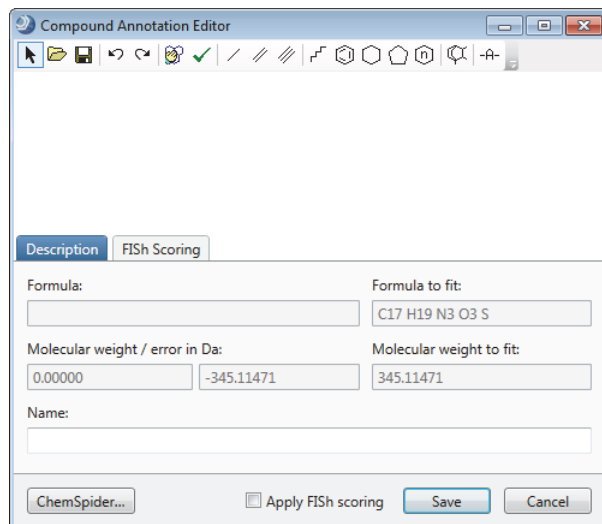
2. To assign a name to this custom annotation, type a name in the Name box.

3. 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.

4. Rerun the FISh scoring algorithm as follows:

   a. Click the **FISh Scoring** tab.

   b. In the Max. Depth list, select **20**; that is, increase the depth of the theoretical fragmentation pathway from 5 (default) to 20.

   c. At the bottom, select the **Apply FISh Scoring** check box.
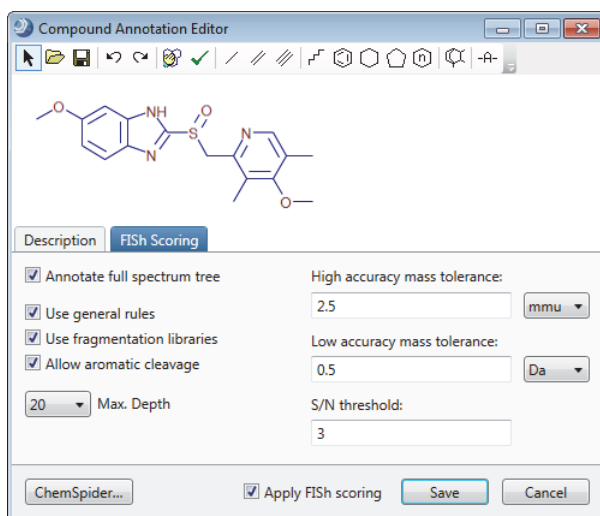
   d. Click **Save**.

   The FISh Scoring Queue opens. The application recalculates the FISh Coverage score and modifies the fragmentation spectra annotations by using the proposed structure.

5. 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.

This figure shows the Structure and Name columns in the Expected Compounds table, the recalculated FISh Coverage score, and the new annotations for fragmentation scan #2405. Compare

Figure 3. FISh coverage score generated by changing the depth to 20
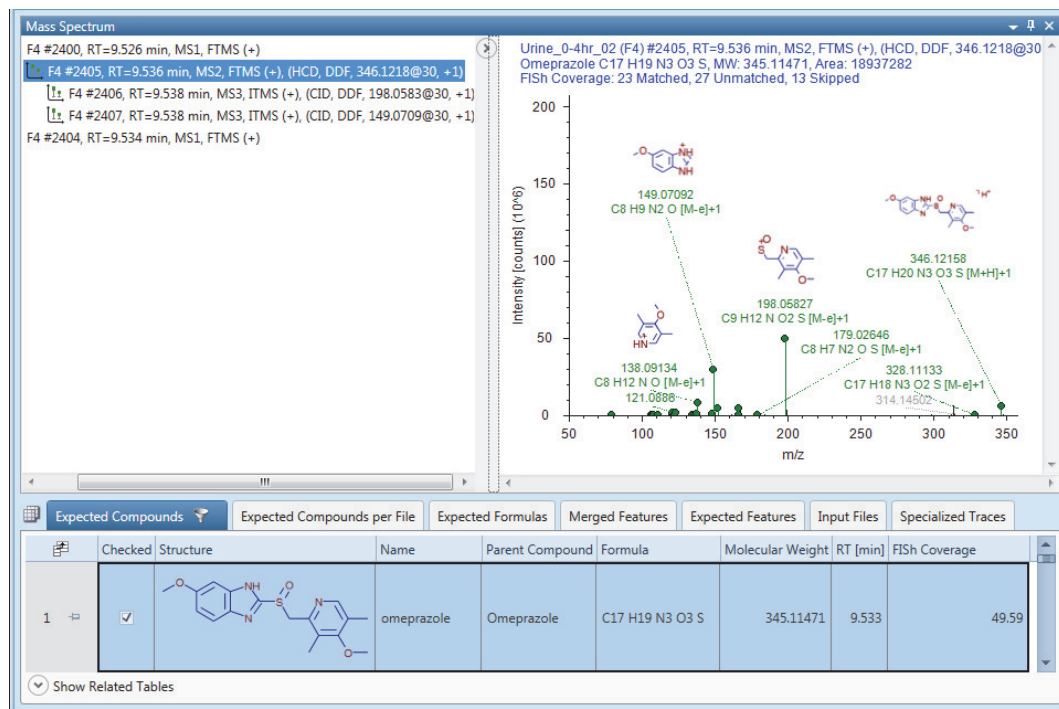
6. If necessary, to restore the original processing results, right-click the newly annotated row and choose **Reset Compound Annotation**.

   The application removes the structure and name annotations and restores the original FISh Coverage score.

Saving or Resetting the Result File's Layout

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**

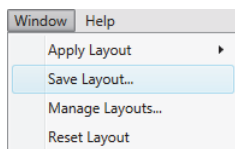   With the result file selected as the active page, do one of the following:

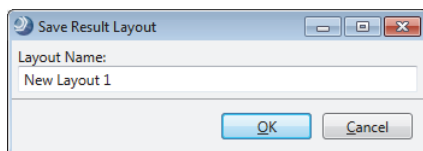   • In the toolbar, click the **Save the Currently Active Item** icon, 💾.

   –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.

Printing 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 "Displaying Only the Compounds of Interest." Sort the table by the column of interest, for example, by retention time (see "To sort the table columns" on page 23).

> **Note** For information about creating and editing your own custom report templates, refer to the *Compound Discoverer 3.0 Reporting Quick Start*, the reporting chapter in the *Compound Discoverer 3.0 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 procedures:

- To generate a list of formulas for the targeted compounds and their transformation products
- To export the formulas to a spreadsheet
- To convert the spreadsheet to an inclusion list for data acquisition

❖ **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 an existing study (see "Starting a New Analysis from an Existing Study" on page 16).
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 **Metabolism** folder.
   d. Select the following processing workflow:

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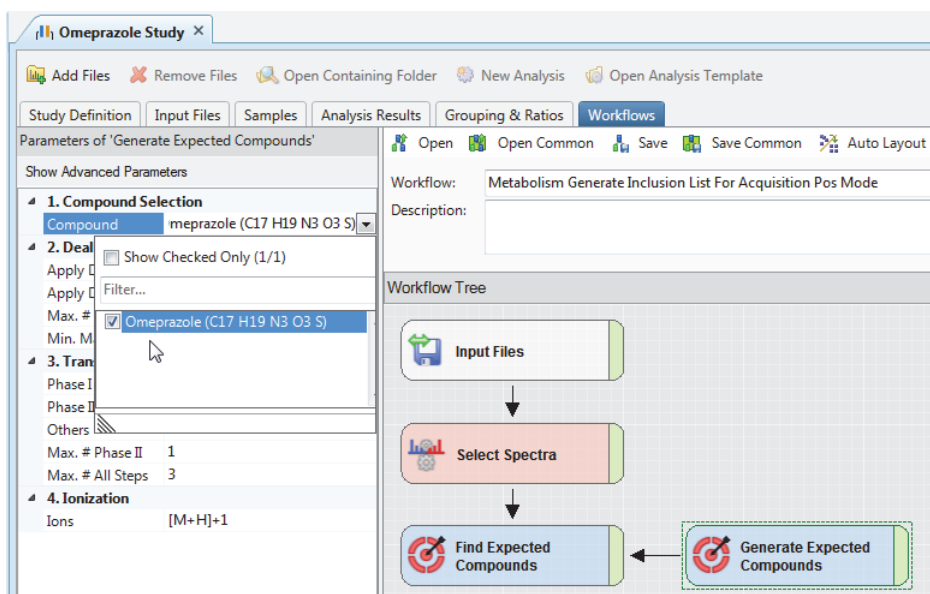
6. Drag any input file from the Input Files page to the Files for Analysis area of the Analysis pane.

   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.

Rename the result file.
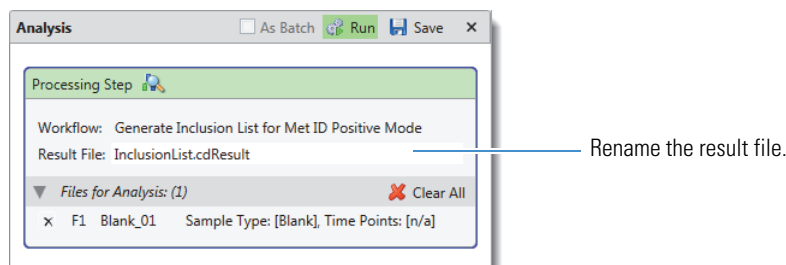
8. Click **Run**.

   The Job Queue page opens.

❖ **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.

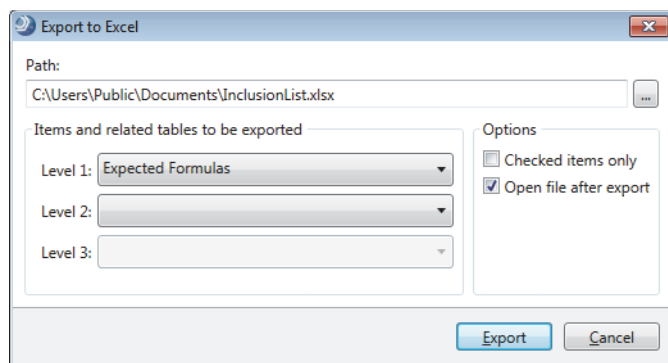2. On the result file page, click the **Expected Formulas** tab.

3. 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.
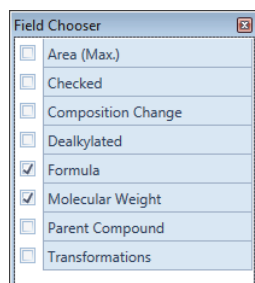
4. Right-click the table and choose **Export > As Excel**.
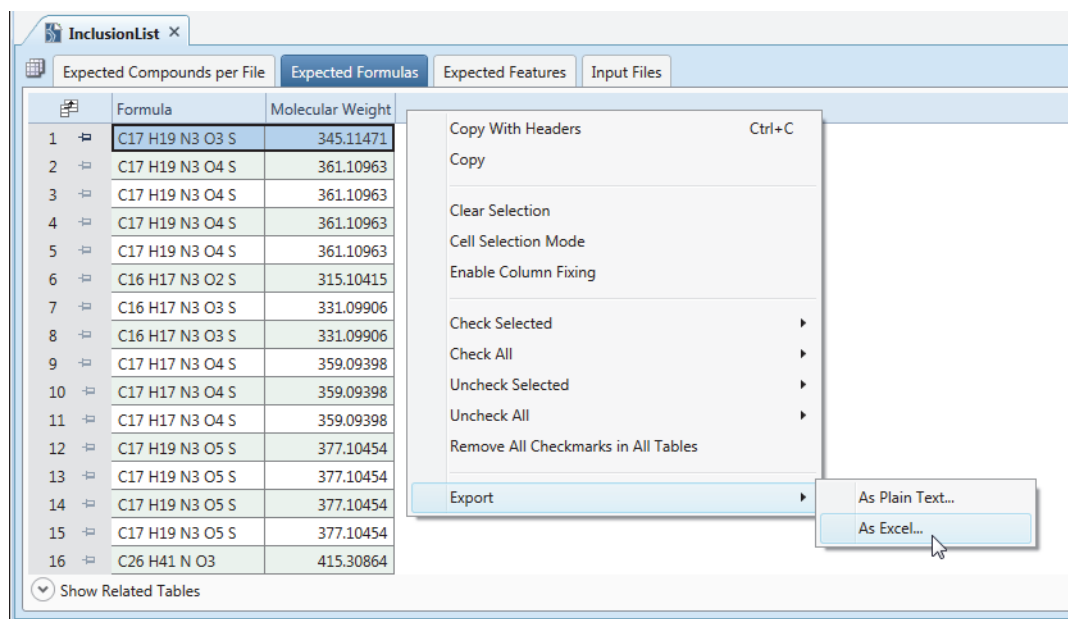
5. In the Export To Excel dialog box, select a folder, name the file, and click **Export**.

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

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

1. Open the exported spreadsheet in a spreadsheet application.

   The Molecular Weight column displays the molecular weight of the neutral compound.

2. Name the third column **m/z**.

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

4. Check the cell format and verify that the number of decimal places is appropriate for your MS.

5. Save the modified spreadsheet.

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