Compound Discoverer 3.2 Stable Isotope Labeling Tutorial

To familiarize yourself with using the Thermo Compound Discoverer™ 3.2 application to detect compounds labeled with a stable isotope such as carbon-13, follow the topics in this tutorial to set up a study and an analysis, process a set of example Xcalibur™ RAW files, review the result file produced by the analysis, and export the results to a Microsoft™ Excel™ spreadsheet.

Note For isotopic labeling experiments, you must use a high resolution accurate mass (HRAM) Thermo Scientific mass spectrometer coupled with a liquid chromatography (LC) inlet to acquire the raw data.

Contents

Overview

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

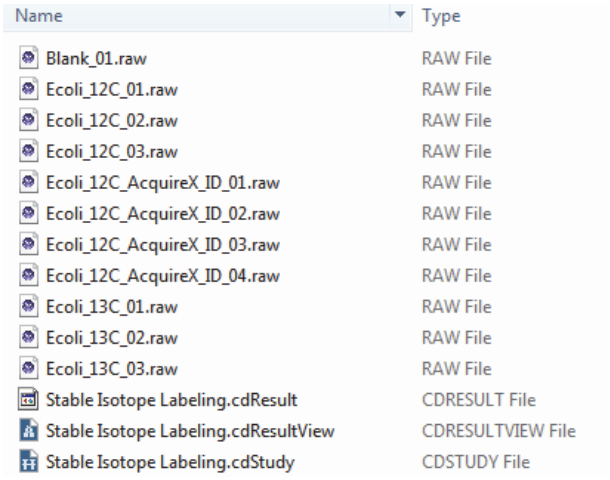
- Location of the example files
- Tutorial workflow
- The Help system

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\Stable Isotope Labeling

Copy the Stable Isotope Labeling Study folder to your data processing computer.



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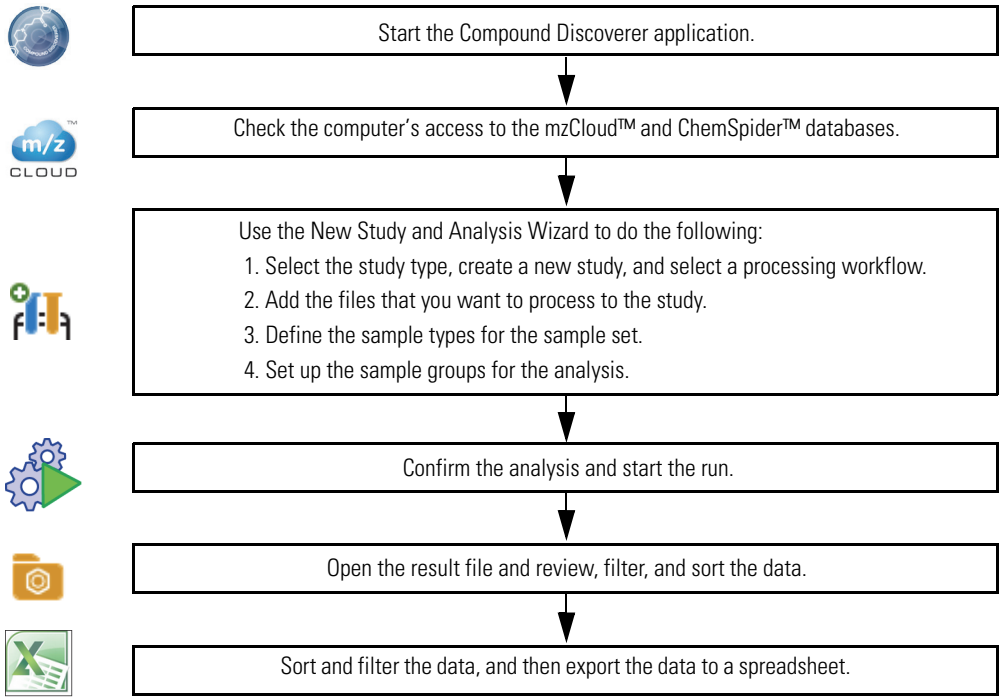
Tutorial workflow

This figure shows the typical workflow for a stable isotope labeling analysis.



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. On the computer keyboard, press the F1 key.

Start the application

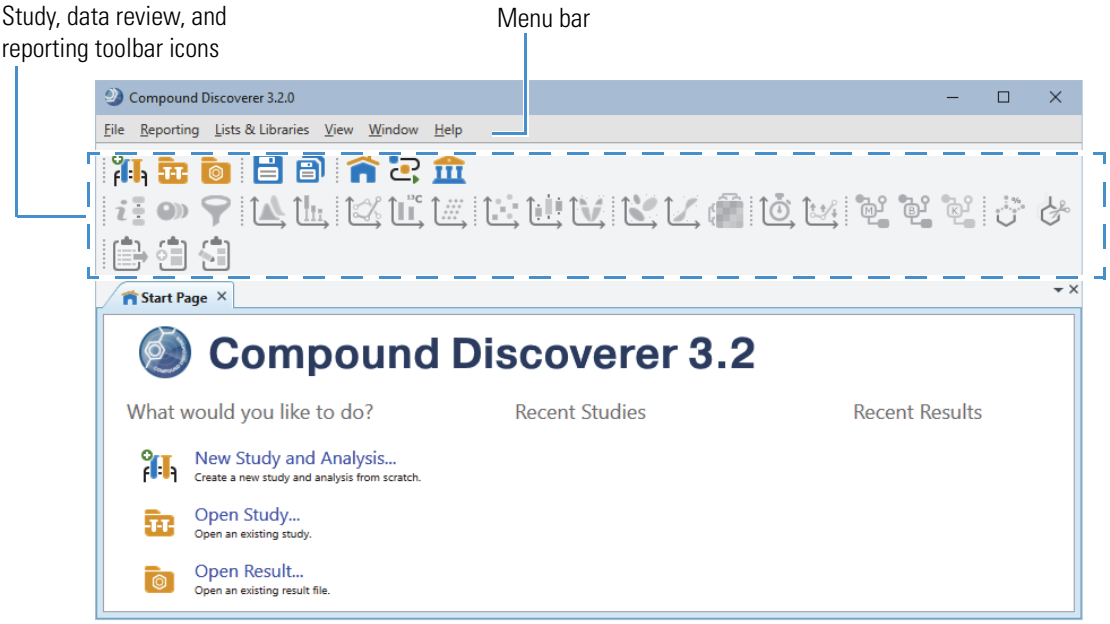
❖ To start the application

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

–or–

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

The application opens to the Start Page.



Check whether the processing computer can access the external databases

To use any of the processing workflows that use the online databases, such as mzCloud™ and ChemSpider™, your processing computer must have unblocked access to these databases on the Internet.

❖ **To verify that your computer has access to the external mass spectral databases**

1. From the menu bar, choose **Help > Communication Tests**.
2. Click the **mzCloud** tab and click **Run Tests**. When the tests are complete, go to the next step.
3. Click the **ChemSpider** tab and click **Run Tests**.

If your computer has an Internet connection, but these tests fail, leave the Communication Test dialog box open and press the F1 key to open the Help. Then, follow the instructions to troubleshoot the communication failure.

Go to the next topic to "Set up a new study and a new analysis."

Set up a new study and a new analysis

Make sure to copy the Xcalibur RAW files to an appropriate folder on your processing computer. See "Overview" on page 1.

Follow these steps to create a new study and a new analysis:

1. Open the New Study and Analysis Wizard
2. Select the study type, specify the directory folder, and name the new study
3. Select the processing workflow
4. Add the input files to the study
5. Specify the sample types
6. Set up the sample groups
7. Customize the processing workflow

Open the New Study and Analysis Wizard

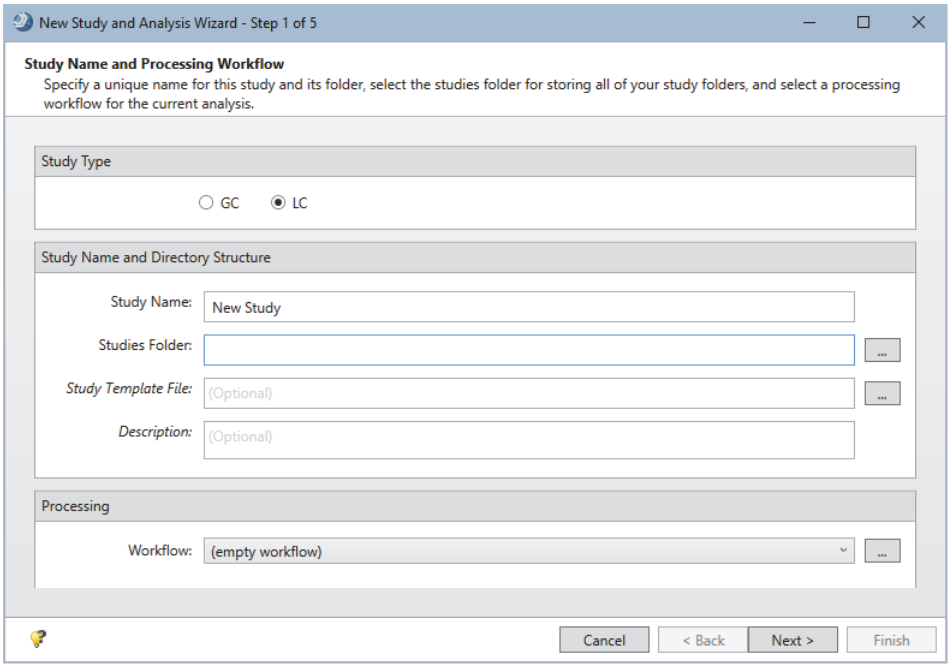
❖ **To open the New Study and Analysis Wizard**

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 and Analysis from Scratch** 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. The first time you create a new study, the (top-level) studies folder is undefined.

Figure 1. Study Name and Processing Workflow page of the wizard



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

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

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

   The application stores this selection until you change it.

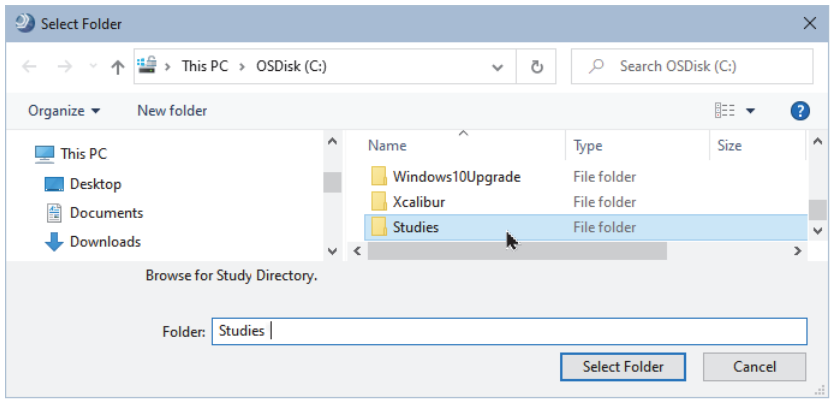
   Note There are two types of studies: GC for gas chromatography-mass spectrometry data and LC for liquid chromatography-mass spectrometry data.

2. In the Study Name and Directory Structure area, select the studies directory folder as follows:
   a. Click the **browse** icon, ..., next to the Studies Folder box.

      The Select Folder dialog box opens.

   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**.

   Figure 2. Select Folder dialog box



   Note The first time you create a new study, you must specify the directory (Studies Folder) where you want to store your studies. Thereafter, you can use the same studies folder or create additional studies folders.

3. In the Study Name and Directory Structure area, in the Study Name box, name the new study.

   For example, type **Stable Isotope Labeling** 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 task to "Select the processing workflow."

Select the processing workflow

In the Compound Discoverer application, the processing method that interprets the raw data is called a processing workflow (.cdProcessingWF). The application provides defined processing workflows for several applications including stable isotope labeling experiments.

This tutorial uses a defined processing workflow that searches the mzCloud and ChemSpider databases to identify the unlabeled compounds detected in the sample files. It uses the Analyze Labeled Compounds node to detect the isotopologues of these compounds. This workflow also maps compounds to their biological pathways by using the local Metabolika pathway files.

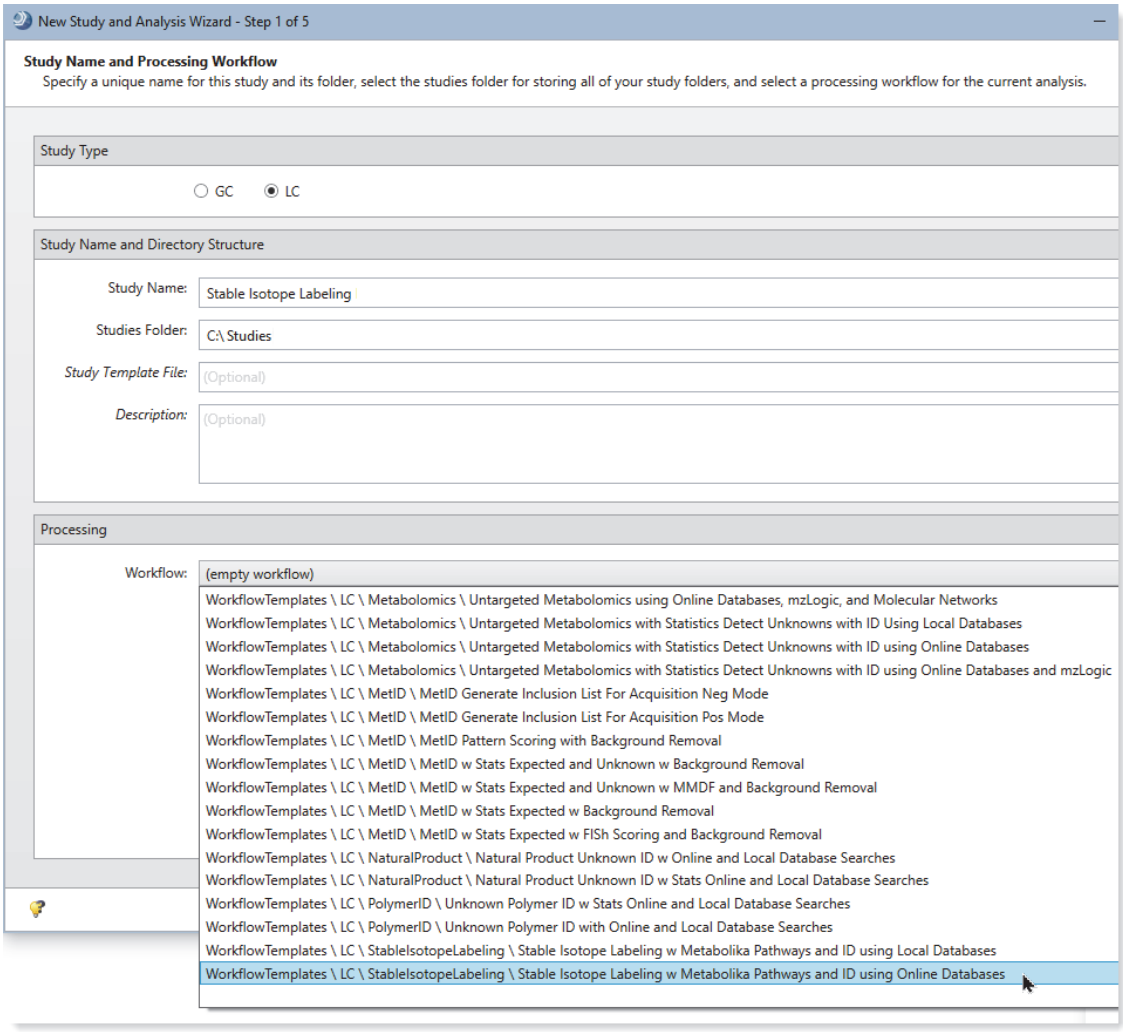
Note If your processing computer does not have Internet access, select the following processing workflow: Stable Isotope Labeling w Metabolika Pathways and ID using Offline Databases**.**

❖ To select the processing workflow

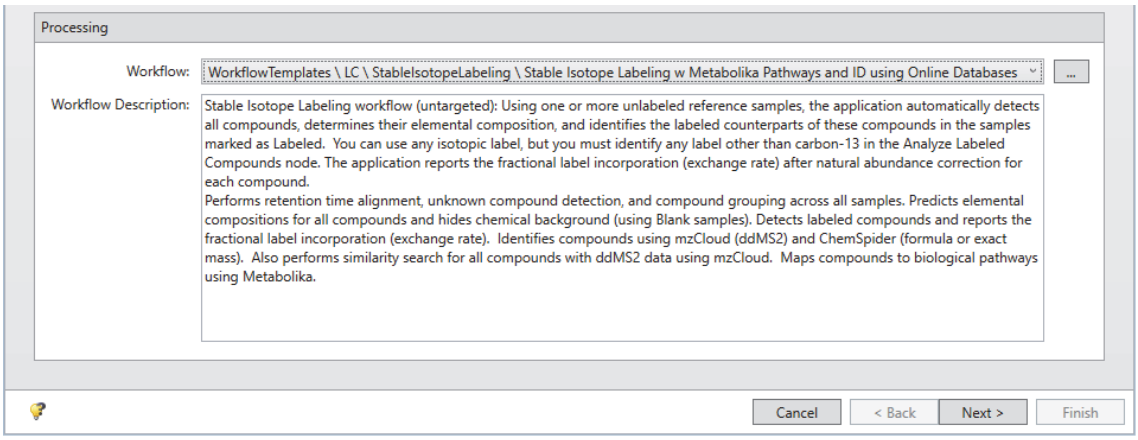
1. In the Processing area on the Study Name and Processing Workflow page of the wizard, select the following processing workflow from the Workflow list:

   Workflow Templates \LC\ Stable Isotope Labeling\Stable Isotope Labeling w Metabolika Pathways and ID using Online Databases

Figure 3. Selecting the processing workflow template from the Workflows list



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



2. Read the description.

Note When you complete the wizard, the application creates the Stable Isotope Labeling Examplel.cdStudy file, stores the study file in the Stable Isotope Labeling Example folder, and stores the Stable Isotope Labeling Example folder in the Studies folder.

Drive:\Stable Isotope Labeling Tutorial\ Stable Isotope Labeling Example\Stable Isotope Labeling Example.cdStudy file

When you run the analysis in this tutorial, the application stores the result file (.cdResult) in the Stable Isotope Labeling Tutorial folder.

Click **Next** to go to the Input File Selection page of the wizard, and then go to the next topic to "Add the input files to the study."

Add the input files to the study

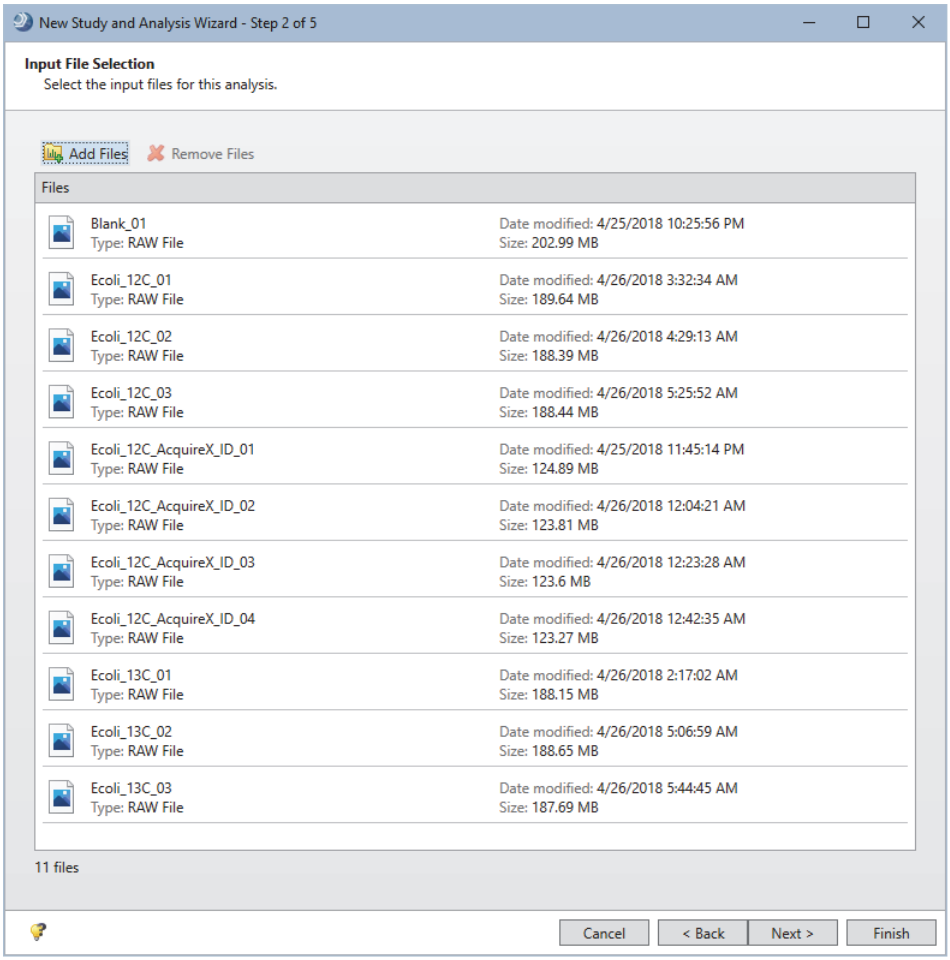
❖ To add input files to the study

1. On the Input File Selection page, click **Add Files**.
2. In the Add Files dialog box, browse to the folder where you copied the Xcalibur RAW files.

Tip The application assigns a file number to each input file in the order you import them (see Figure 5). The file numbers are useful for tracking the input files in the result file tables.

3. Select all the Xcalibur RAW files in this folder and click **Open**.

Figure 4. Imported example files



Click **Next** to open the Input File Characterization page of the wizard, and then go to the next topic to "Specify the sample types."

Specify the sample types

This figure shows the newly added samples in the Samples area. By default, the application assigns Sample as the Sample Type to new samples.

Figure 5. Imported files with assigned file numbers

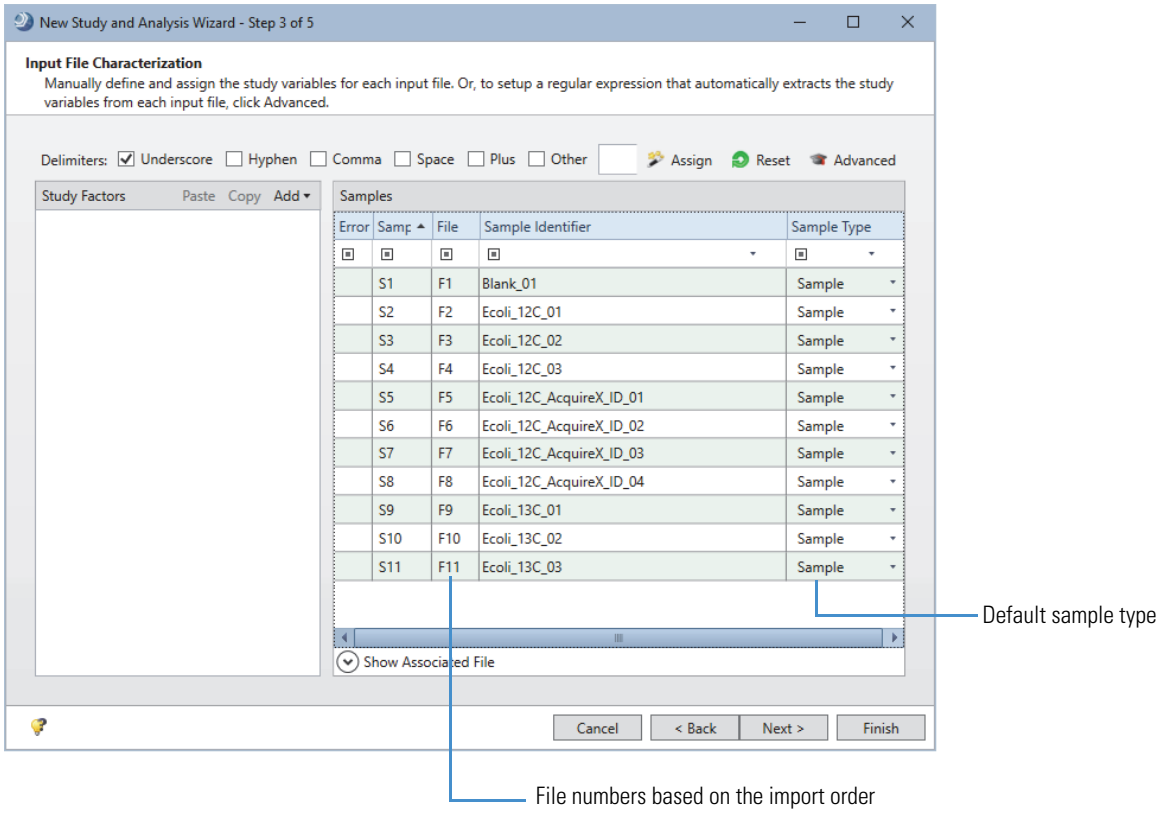


Table 1 describes the sample types for the Stable Isotope Labeling data set.

Table 1. Sample types

Sample type	Application use
Sample	Detects the unlabeled compounds in the sample.
Blank	Marks the background compounds.
Identification Only	Does not report the chromatographic peak areas for the compounds. Uses the sample's fragmentation scans for component identification.
Labeled	Determines the isotopic label incorporation.

To specify the sample types, do the following:

- Automatically assign the blank sample type
- Specify the compound identification samples
- Specify the labeled samples

Automatically assign the blank sample type

❖ **To assign the Blank sample type**

Note When you select the appropriate delimiters, the application assigns the Blank sample type to files named Blank or files with Blank in the file name.

In the command bar, click **Assign**.

The application assigns the Blank sample type to the Blank.raw file.

Specify the compound identification samples

Compound identification samples must have fragmentation scans.

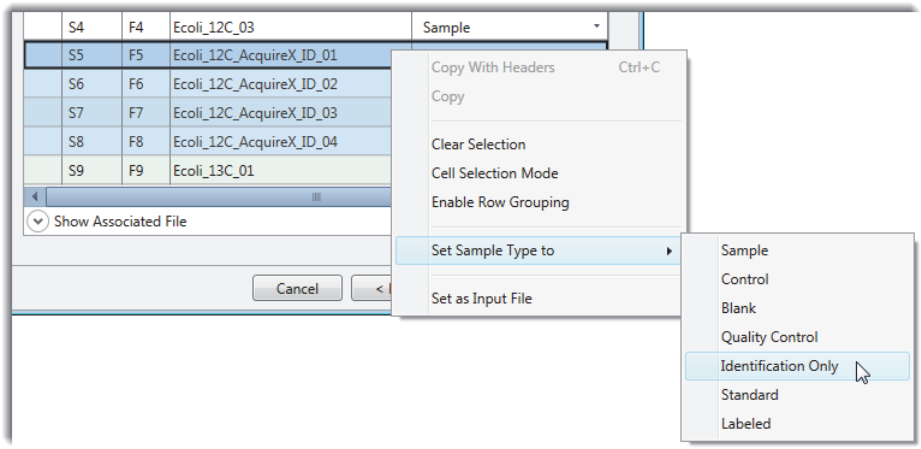
❖ **To specify the samples to use for compound identification**

Use the SHIFT key to select the four Acquire_X_ID files. Then, right-click the selected rows and choose **Set Sample Type To > Identification Only** (Figure 6).

Tip To select a row, you can click any column but the Sample Type column.

Note The Acquire_X_ID.raw files contain the data-dependent fragmentation scans for the input file set.

Figure 6. Defining the samples to be used for Identification Only

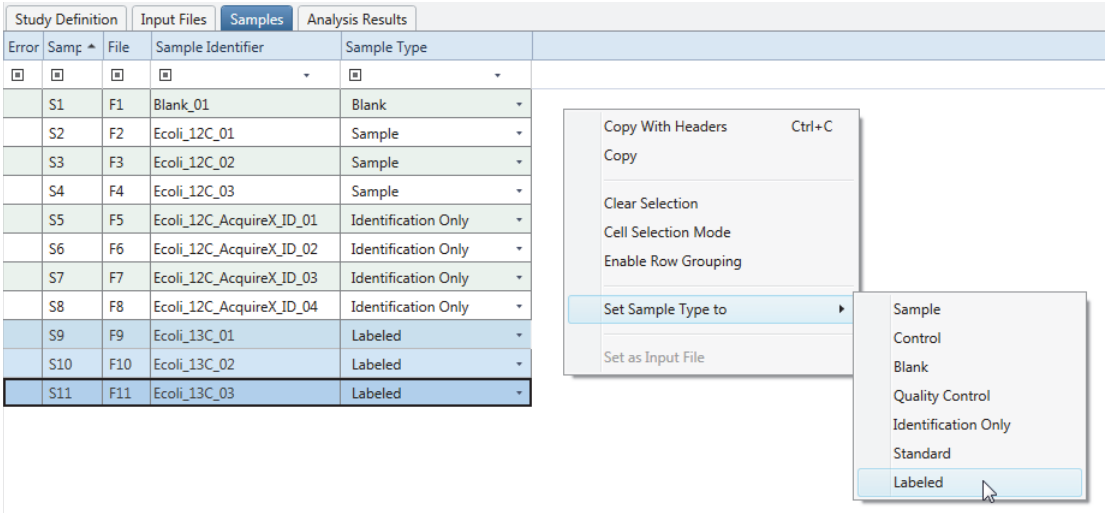


Specify the labeled samples

❖ **To specify the labeled samples for the detection of labeled compounds**

Use the CTRL key to select the files with 13C in their file name. Then, right-click the selected rows and choose **Set Sample Type To > Labeled** (see Figure 7 on page 8).

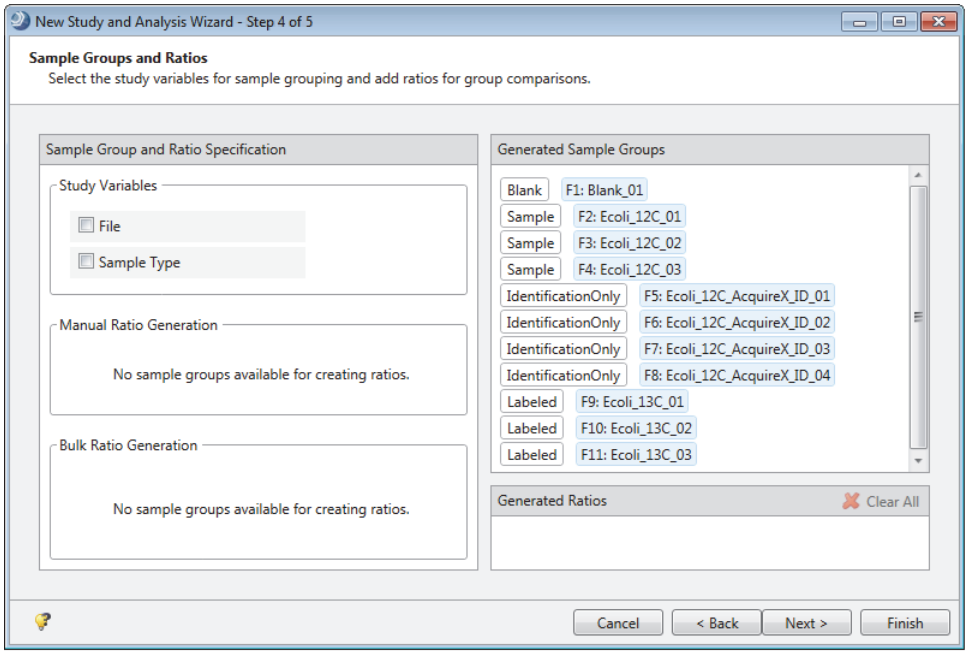
Figure 7. Defining the labeled samples



Click **Next** to open the Sample Groups and Ratios page of the wizard, and then go to the next topic to "Set up the sample groups."

Set up the sample groups

Use the Sample Groups and Ratios page to set up the sample groups and ratios for a differential analysis.



Tip The example data for this tutorial does not include time as a study variable. To set up the study factors for a metabolic flux experiment, follow the embedded wizard Help or press the F1 key to access the Help system.

❖ To set up the sample groups

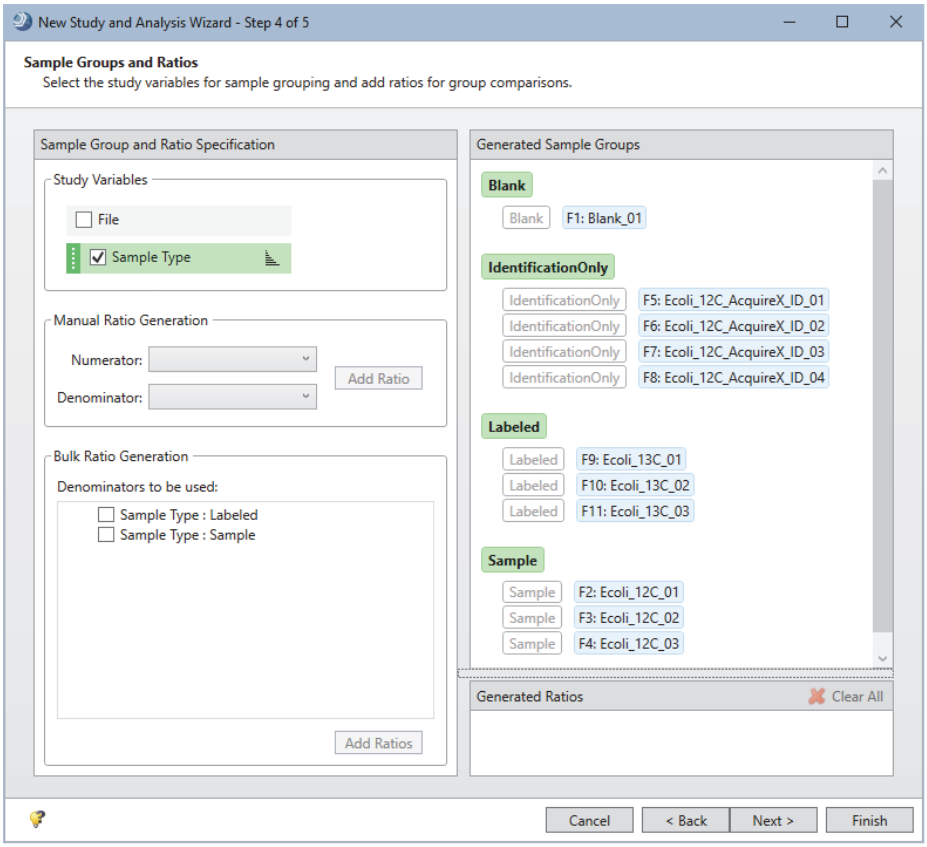
In the Study Variables area, select the **Sample Type** check box.

The sample groups—Blank, Sample, Identification Only, and Labeled—appear in the Generated Sample Groups area (see Figure 8 on page 10).

Note For the example data set, grouping the samples by sample type makes reviewing the data in the result tables easier.

Tip If you are setting up a metabolic flux study for your own data set, use the Sample Groups and Ratio page to set up ratios for a differential analysis.

Figure 8. Samples grouped by sample type



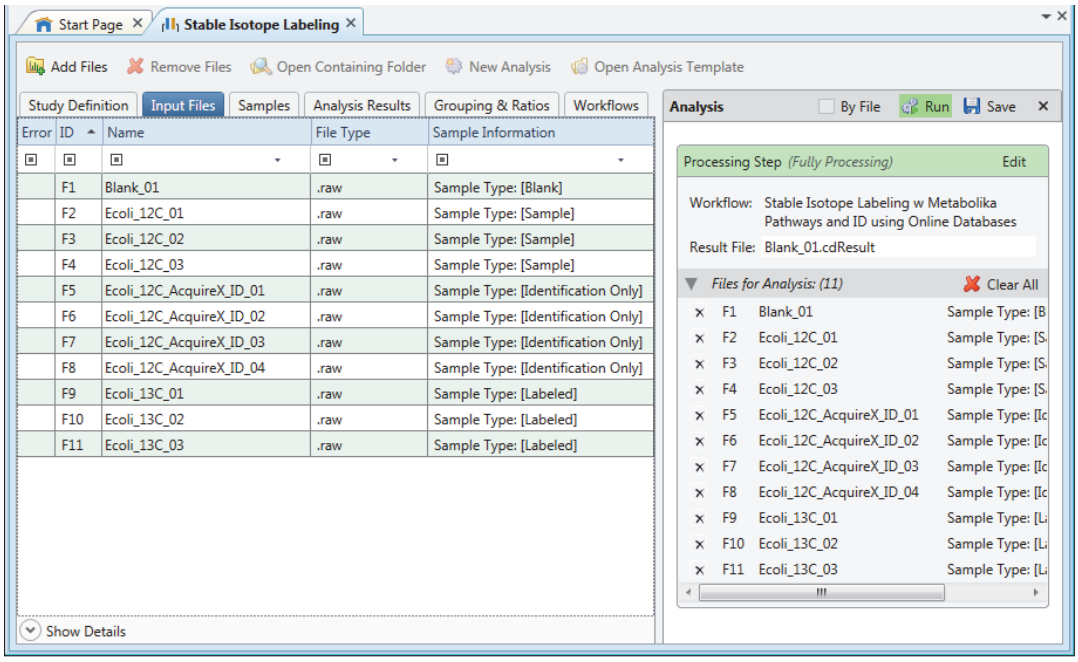
4. Click **Finish** to save the study and close the wizard.

   The tabbed study page and the analysis that you set up with the wizard open.

   The Analysis pane lists the 11 input files in the example data set. The analysis is set up to combine the processed results from these files into one result file—that is, the By File check box is clear and the file name for the result file is available for editing.

   This figure shows the Analysis pane with a list of files for analysis.

Figure 9. Study with an analysis that is ready for processing



Customize the processing workflow

Before submitting the analysis to the job queue, review the processing workflow and make changes as needed.

❖ To review and customize the processing workflow for this tutorial

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

   The Workflows page displays the processing workflow that you selected with the wizard.

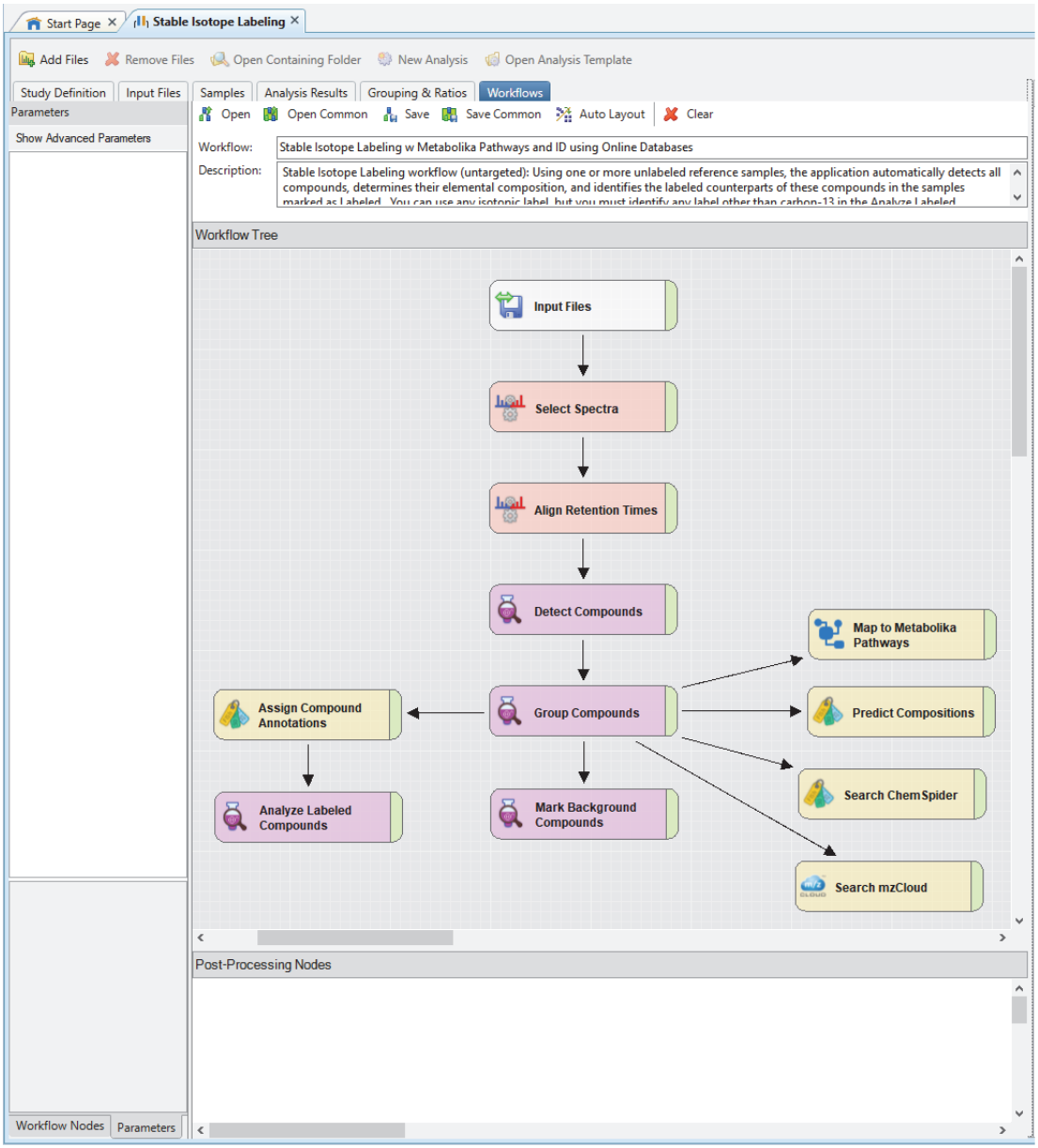
2. To review the parameter settings for a workflow node, select the node in the Workflow Tree pane.

   The Parameters pane for the selected node appears on the left.

   Figure 10 shows the processing workflow in the Workflow Tree pane. Because none of the nodes is selected, the Parameters pane on the left is empty.

IMPORTANT For this tutorial, you change the minimum peak intensity for the Detect Compounds node. See

Figure 10. Processing workflow template for a stable isotope labeling experiment



3. Open the parameter settings for the Detect Compounds node. If you are not using the example data set, check the Min. Peak Intensity setting against the suggested setting for your data set (Table 2).

   The minimum peak intensity setting defines the base peak intensity for compound detection. For this tutorial, change the setting to 100 000 (1e5), as the raw data files were acquired with an Orbitrap ID-X™ mass spectrometer (see Table 2).

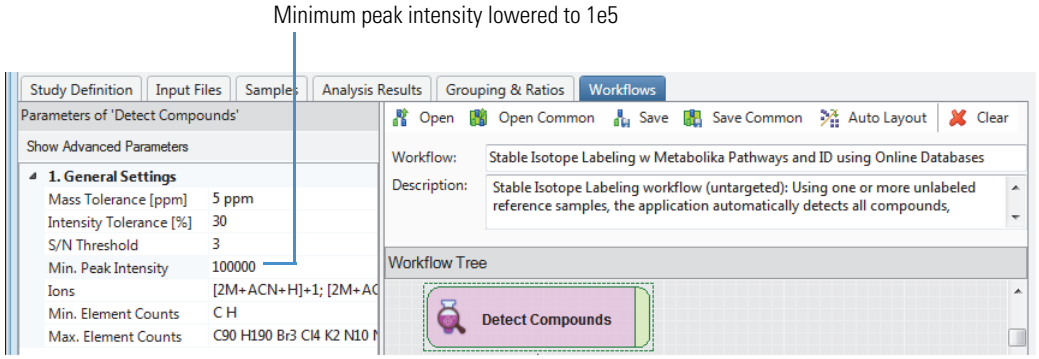


Table 2 lists the recommended range for the Min. Peak Intensity parameter. The optimal setting depends on the sensitivity of the mass spectrometer.

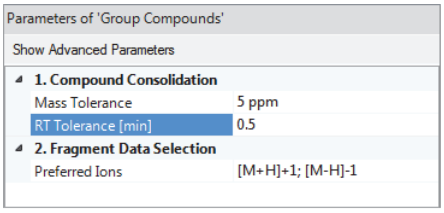
Table 2. Recommended minimum peak intensity range

Mass spectrometer	Minimum peak intensity (chromatographic peak height)
Q Exactive™, Q Exactive Plus™, Q Exactive HF	500 000 to 1 000 000
Exactive™, Exactive Plus™, Orbitrap Elite™, Orbitrap Velos Pro™	100 000 to 500 000
Orbitrap Fusion™, Orbitrap Lumos, Orbitrap ID-X	50 000 to 100 000
LTQ Orbitrap XL™, LTQ Orbitrap Velos™	25 000 to 100 000

4. Open the parameter settings for the Group Compounds node.

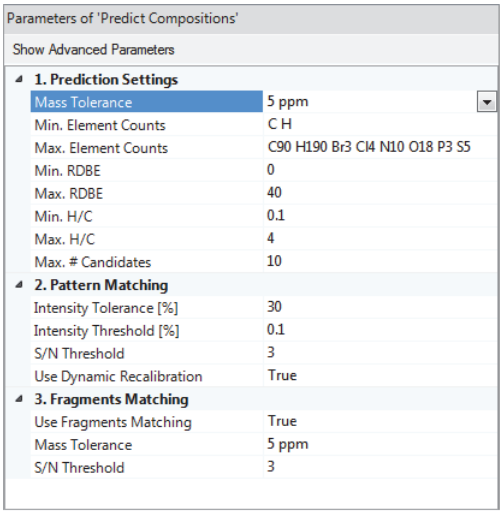
   In the selected processing workflow template, the RT Tolerance has been set to 0.5 min. This setting is slightly wider than the default setting of 0.2 min. For this tutorial, do not change the node settings.

   The Group Compounds node creates the MSn tree that is saved to the result file and used by the search nodes and the Predict Compositions node.



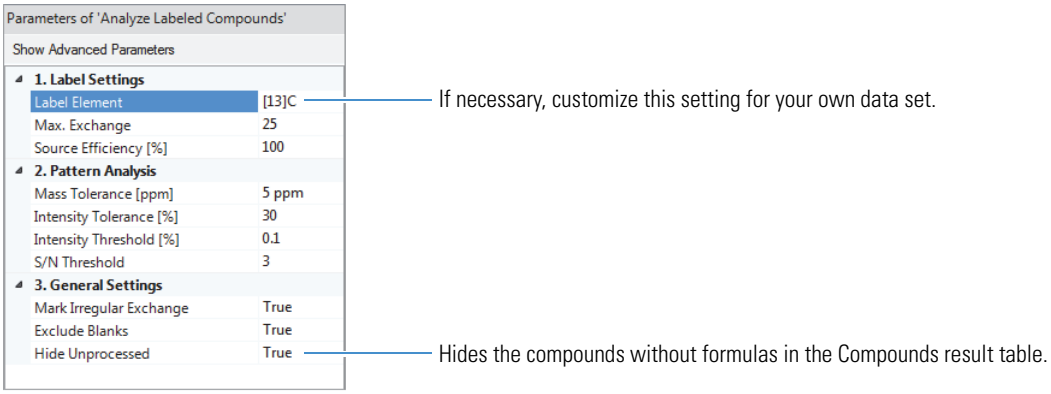
5. Open the parameter settings for the Predict Compositions node. For the example data set, keep the template settings.

   The Predict Compositions node predicts the elemental compositions for compounds without hits from the search nodes.



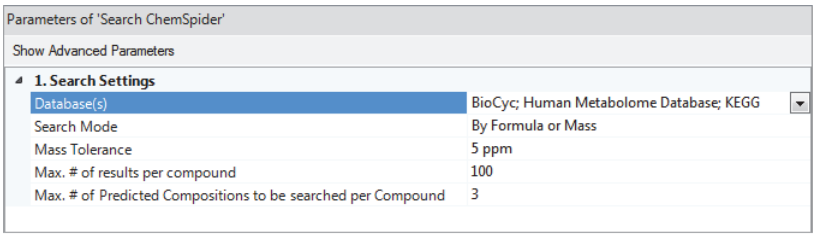
6. Open the parameter settings for the Analyze Labeled Compounds node.

   For the example data set, keep the default settings. For a different data set, enter the appropriate isotope for the Label Element parameter.



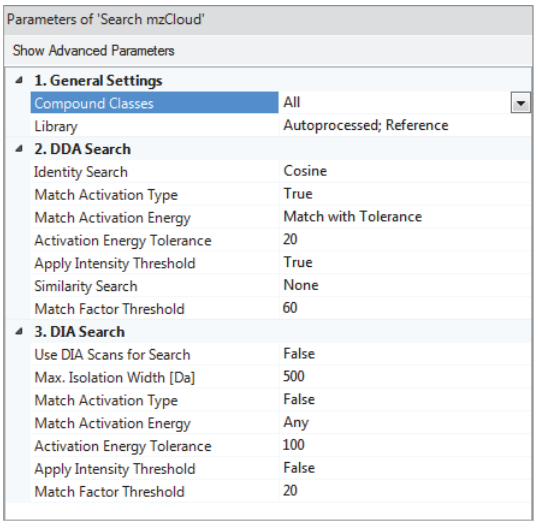
7. Open the parameter settings for the Search ChemSpider node.

   In the selected template, 3 out of 275 databases are selected. For this tutorial, do not change the selection.



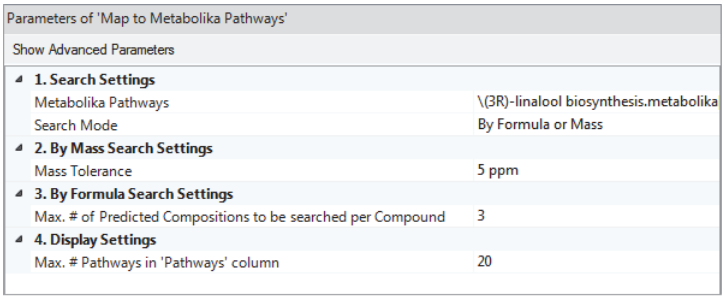
8. Open the parameter settings for the Search mzCloud node.

   The Search mzCloud node is set up to search the entire mzCloud database and run an Identity search. For this tutorial, do not change the settings.



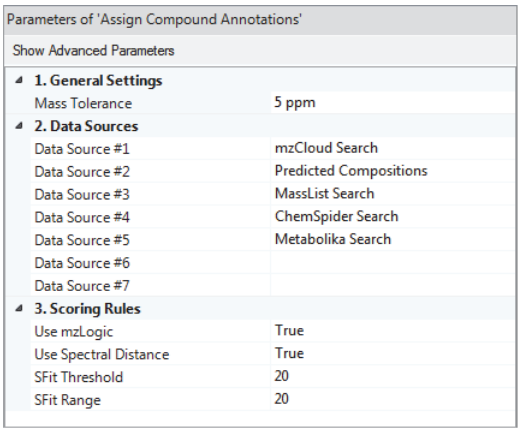
9. Open the parameter settings for the Map to Metabolika Pathways node.

   For this tutorial, do not change the settings.



10. Open the parameter settings for the Assign Compound Annotations node.

    For the example data set, do not change the settings.



Tip If you are working with your own data set and the analysis does not identify the correct isotopologues, consider changing Data Source #1 to a custom mass list for your analytes and reprocessing the analysis.

11. (Optional) Save the modified processing workflow with a new name and location.

Submit the analysis to the job queue

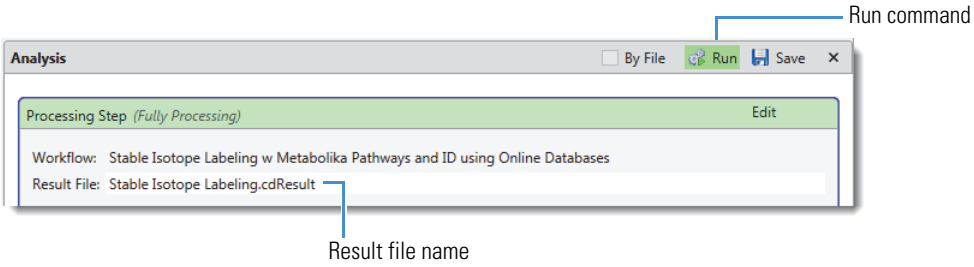
For the example data set and analysis, you are ready to start the processing run.

Tip If you modified the analysis and the Run button is unavailable, remedy the issues listed in the Current Workflow Issues pane on the Workflows page. If the Caution symbol remains, point to it and remedy other analysis errors, for example, no input files in the Files for Analysis area of the Analysis pane.

❖ To submit the analysis to the job queue

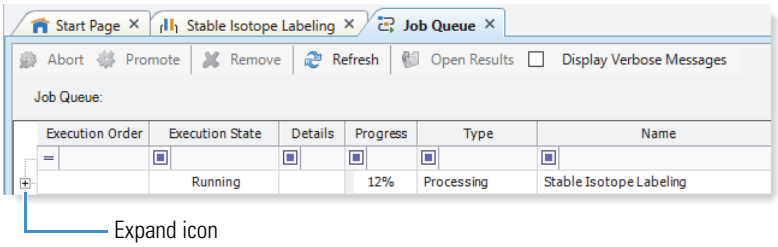
1. To create one result file for the input file set, leave the **By File** check box clear.

   By default, the application uses the name of the first input file for the file name of the result file.
2. In the Result File box, rename the result file **Stable Isotope Labeling**.



3. Click **Run**.

   The Job Queue page opens.
4. To view the processing messages, click the expand icon, ⊞, to the left of the job row.



Note During the run, the Search ChemSpider node generates warning messages, which you can ignore. Warning messages have a yellow background.

5. Leave the Job Queue page open and go to "Review the analysis results."

Review the analysis results

Follow these topics to review the analysis results.

- Open the Result File
- Review the default layout for the result page
- Apply the Stable Isotope Labeling layout
- Review the exchange rates
- Review the labeling status
- View a trend chart for a single compound or a set of trend lines for multiple compounds
- View the distribution of the isotopologues for each compound
- View the Metabolika pathways for a compound

For more information about a specific result table or view, select the table or view to make it active, and then press the F1 key. The Compound Discoverer application provides F1 Help for all the views that you access from the View menu and all the result tables.

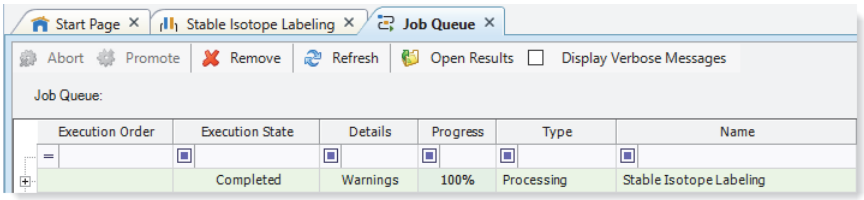
Open the Result File

You can open a result file from multiple locations: the Job Queue page, the Analysis Results page of a study, the Compound Discoverer Start Page, or the menu bar.

Note For this tutorial, you can create a result file by setting up and running an analysis with the example data set. Or, you can open the result file—Stable Isotope Labeling—in the same folder where you found the example data set.

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

When the run is completed, double-click the run on the Job Queue page.



Review the default layout for the result page

The result file opens as a tabbed document (see Figure 11 on page 17). By default, the Chromatograms view opens in the upper left, the Mass Spectrum view opens in the upper right, and a set of tabbed result tables opens in the bottom half of the page. The Compounds table is the active table. The detected compounds are listed in descending order of the maximum chromatographic peak area [Area (Max.)] across the input files. The Chromatograms and Mass Spectrum views are populated with data for the first compound in the table.

The Mass Spectrum view displays the MS1 scan in the spectrum tree that is closest to the apex of the compound's chromatographic peak. 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)

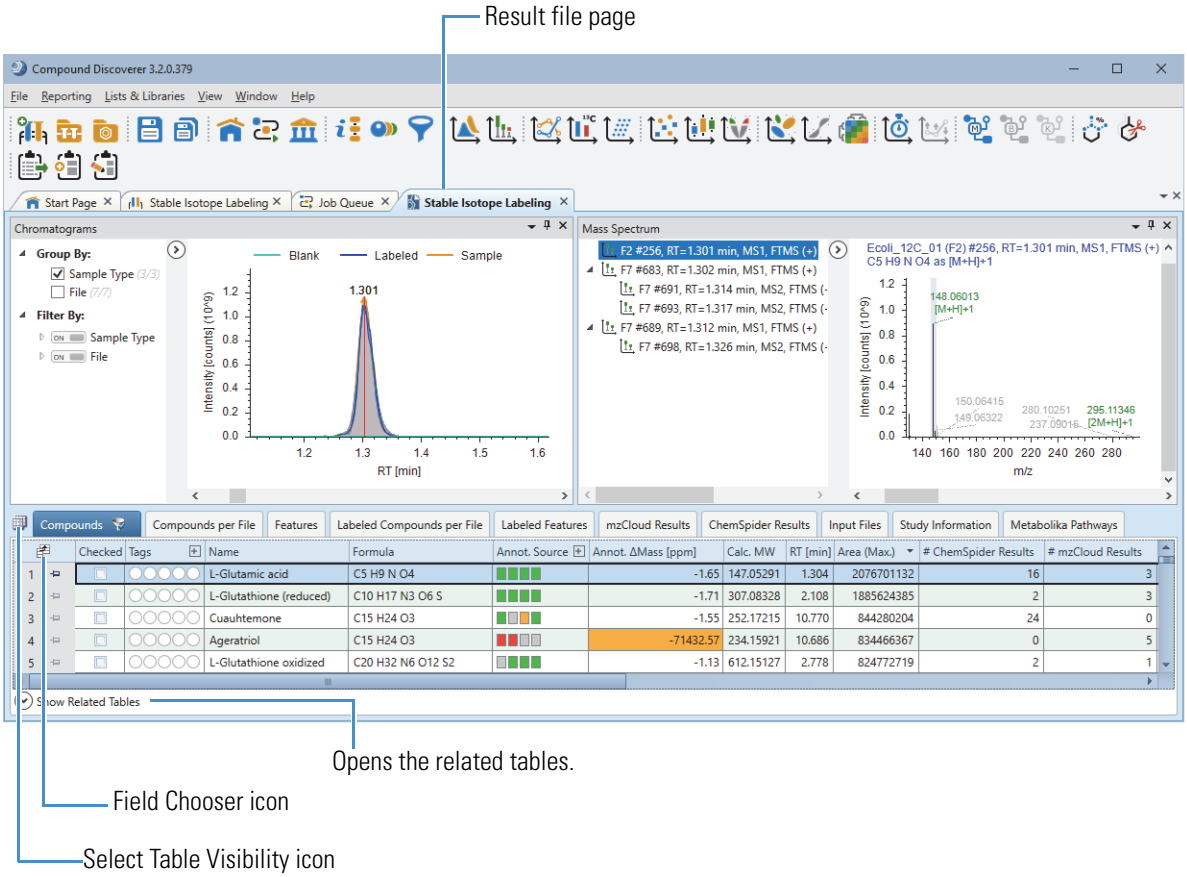
–or–

- The Start and end points of the chromatographic peak, as determined by the peak detection algorithm

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

Figure 11 shows the factory default layout for the Stable Isotope Labeling.cdResult file.

Figure 11. Default result file layout



Tip You can hide or display any of the columns in a result table or any of the result tables in a result file.

To hide or display columns for a result table, click the table's field chooser icon ( ). Then, in the Field Chooser dialog box, select or clear the check boxes for the columns that you want to display or hide, respectively.

To hide or display result tables, click the Select Table Visibility icon ( ). Then, in the Select Visible Tables dialog box, select or clear the check boxes for the tables that you want to display or hide, respectively.

The screen captures in this tutorial do not display all the tables and columns that are visible with the default layout.

Table 3 describes the main result tables that the selected processing workflow produces. In the active Compounds table, which is sorted by the Area (Max.) column, the first row displays the compound with the largest chromatographic peak area (found in one of the input files).

Because the selected processing workflow includes the Mark Background Compounds node and the Analyze Labeled Compounds node, the 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. In addition, the compounds without a formula are hidden from the table.

Table 3. Main tables and some of the related tables for the selected processing workflow

Result table	Description
Visible main (top-level) tables	
Compounds	Lists all the compounds that the analysis detected, grouped by their molecular weight and retention time (MW×RT) dimensions, across all the sample input files[a]. This table also displays the background compounds and the compounds without a formula when you turn off the filter.
Compounds per File	Lists all the compounds that the analysis detected across all the "sample input files" on a per file basis. Does not list compounds that the Fill Gaps node detected by filling a full gap.
Features	Lists all the features (ions with the same mass-to-charge values and retention time) that the analysis detected across all the "sample" input files on a per file basis.
Labeled Compounds per File	Lists the labeled compounds, by input file, that the analysis detected across all the "labeled input files[b]."
Labeled Features	Lists the ions that the analysis detected across all the "labeled" input files on a per file basis. The m/z column displays the *m/z* value of the unlabeled parent ion.
mzCloud Results	Lists the mzCloud search results for the detected compounds.
ChemSpider Results	Lists the ChemSpider search results for the detected compounds.
Input Files	Describes the input files that the application processed to create the result file.
Study Information	Displays the file name, sample type, and study factor values for each input file. Also displays the ratios that include the input file.
Metabolika Pathways	Lists the Metabolika pathways that contain at least one of the detected compounds.
Visible tables related to the Compounds table (tables for individual compounds)	
Structure Proposals	Displays your structure proposals for the compound selected in the Compounds table. Initially, this table is empty.
Compounds per File	Displays information about the selected compound on a per file basis.
Predicted Compositions	Displays the predicted compositions for the selected compound.
Labeled Compounds per File	Displays information about the isotopologues of the selected compound on a per file basis.
Metabolika Results	Displays the search results from the mapped Metabolika pathways for the selected compound.
mzCloud Results	Displays the mzCloud results for the selected compound.
ChemSpider Results	Displays the ChemSpider results for the selected compound.
Metabolika Pathways	Displays the mapped Metabolika pathways for the selected compound.
Visible table related to the Features table and the Labeled Features tables	
Chromatogram Peaks	Describes the chromatographic peak for the selected feature.
Visible table related to the Input Files table	
File Alignments	Describes the alignment for the input file selected in the Input File table.

[a] Sample input files refers to input files that have been assigned the Sample, Control, or Standard sample type.
[b] Labeled input files refers to input files that have been assigned the Labeled sample type.

Apply the Stable Isotope Labeling layout

The application comes with the factory default layout and four named layouts: Identification, Quantification, Statistics and Stable Isotope Labeling Layout. When reviewing the results of a stable isotope labeling analysis, apply the Stable Isotope Labeling layout.

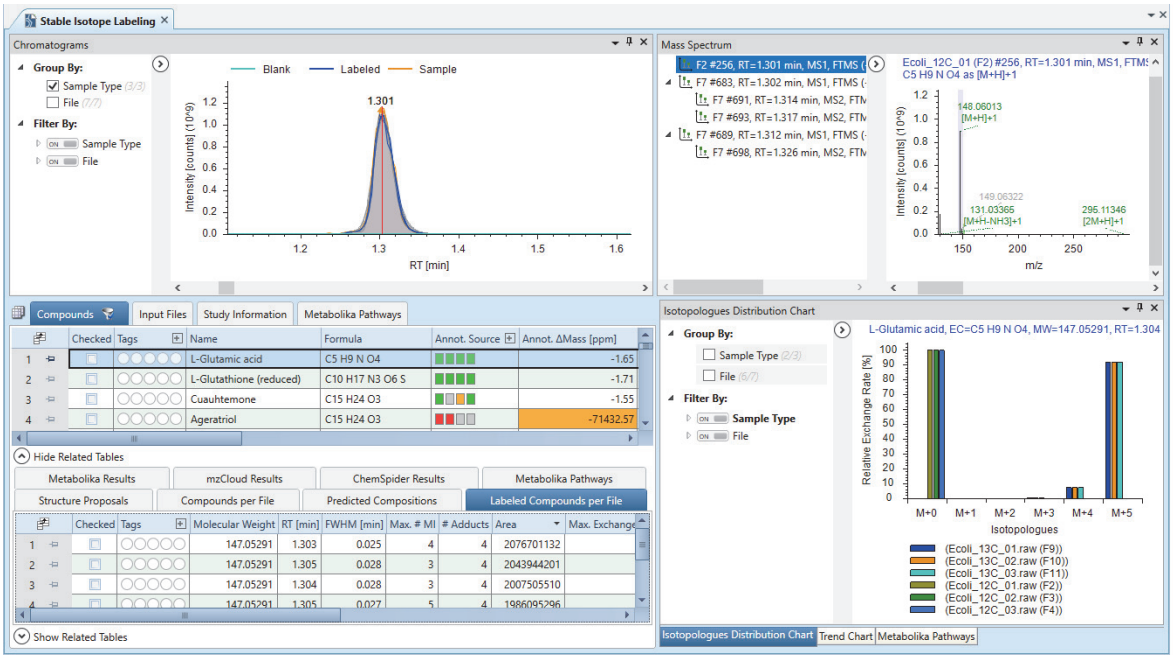
❖ To apply the Stable Isotope Labeling layout to the current result file

From the application menu bar, choose **Window > Apply Layout > Stable Isotope Labeling**.

Applying the Stable Isotope Labeling layout does the following to the example result file:

- Hides the following main tables:
  - Compounds per File
  - Features
  - ChemSpider Results
  - Labeled Compounds per File
  - Labeled Features
  - mzCloud Results
- Hides the following columns in the Compounds table:
  - # Adducts
  - #Metabolika Pathways
  - Annot. Δ Mass [Da]
  - Annotation MW
  - Avg. Exchange
  - Background
  - FISh Coverage
  - Gap Status
  - Metabolika Pathways
  - MS Depth
  - mzCloud Library Matches
  - RT Tolerance [min]
  - Structure
- Opens the Related Tables pane to the Labeled Compounds per File table for the first compound in the Compounds table.
- Opens the Isotopologues Distribution Chart, Trend Chart, and Metabolika Pathways views as tabbed views on the bottom right of the page.
- Selects the Rel. Exchange data property for the Trend Chart view.

Figure 12. Default layout after applying the Stable Isotope Labeling layout



Review the exchange rates

To review the details about the detected compounds in the result tables, follow these procedures:

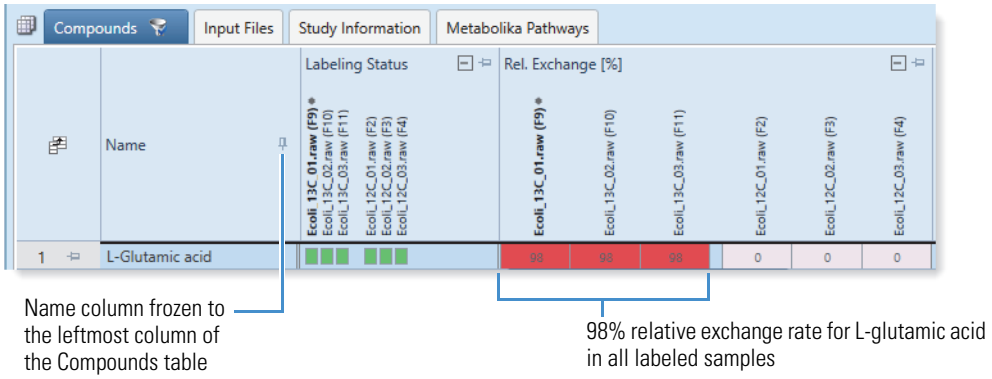
- To review the relative exchange rates for a compound across the input file set
- To view the exchange rate for each isotopologue of a compound
- To view the exchange rates for the adducts of a compound in a specific input file

❖ To review the relative exchange rates for a compound across the input file set

1. In the Compounds table, freeze the Name column as follows:
   a. Right-click the Compounds table and choose **Enable Column Fixing**.
   b. Click the pin icon to the right of the Name column heading (pinned, , and unpinned,).
2. Select the compound of interest.
3. Scroll right to the Rel. Exchange [%] column.
4. To view the input file names, click the expand icon (⊞) next to the column heading. Or, right-click the Compounds table and choose **Expand All Column Headers** to expand all the table column headers.

   This figure shows the relative exchange rate for L-glutamic acid in each input file. The relative exchange rate for the labeled samples is 98%.

Figure 13. Relative exchange rate for L-glutamic acid (For the example data, L-glutamic acid sorts to row 1 when the table is sorted by Area (Max.) in descending order.)



❖ To view the exchange rate for each isotopologue of a compound

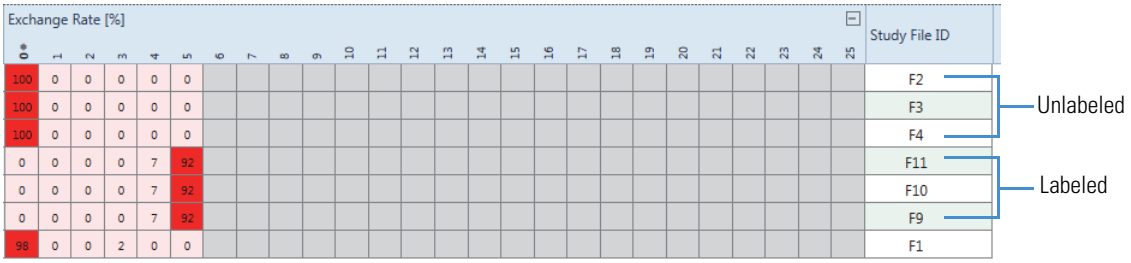
1. In the main Compounds table, select the compound of interest.

   If you applied the Stable Isotope Labeling layout, the related tables pane is visible and the Labeled Compounds per File table is the active table. In addition, the Compounds table is sorted by Area (Max.) in descending order.
2. In the related Labeled Compounds per File table, scroll to the Exchange Rate [%] column.
3. To view the isotopologues, click the expand icon (⊞) next to the Exchange Rate [%] column heading.

   Figure 14 shows the exchange rates in the labeled (F9, F10, and F11) and unlabeled (F2, F3, and F4) samples. The exchange rates for the labeled samples are 92% for the $^{13}C_5H_9NO_4$ isotopologue and 7% for the $^{13}C_4CH_9NO_4$ isotopologue of L-glutamic acid.

   The Exchange Rate [%] column contains 25 subcolumns because the analysis specified a maximum exchange rate of 25 for any of the detected compounds. Irrelevant subcolumns for unprocessed elemental compositions have a gray background. Subcolumns for isotopologues have a pink to red background that turns darker as the exchange rate increases.

Figure 14. Exchange Rate column in the Labeled Compounds per File table for L-glutamic acid

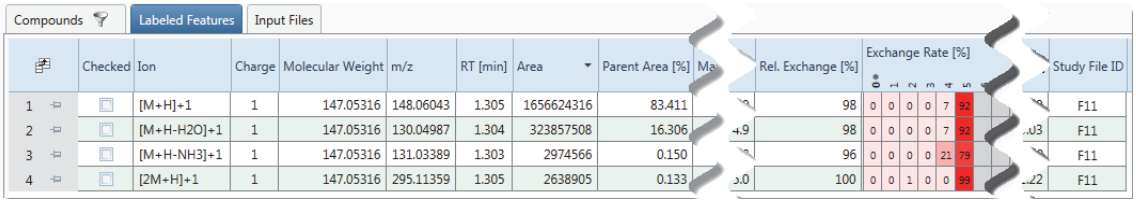


❖ To view the exchange rates for the adducts of a compound in a specific input file

1. In the related Labeled Compounds per File table, select the input file of interest.
2. In the related tables pane, click the **Labeled Features** tab to make it the active table.

   This figure shows the relative amounts (by chromatographic peak area) of the labeled adduct ions that the analysis detected for L-glutamic acid in study file F11 (a labeled sample).

Figure 15. Labeled features for L-glutamic acid in study file F11



Review the labeling status

The Labeling Status column in the Compounds table and the Status column in the Labeled Compounds per File table provide information about the quality of the analysis.

(■) Red—Indicates a contaminating mass in an unlabeled sample.

(■) Blue—Indicates an irregular exchange rate for a labeled sample.

(■) Orange—Indicates a low fit between the measured and fitted isotope patterns.

(■) Gray—Indicates the absence of isotopologues for the detected compound.

A contaminating mass in an unlabeled sample is more problematic than an irregular exchange rate for a labeled sample.

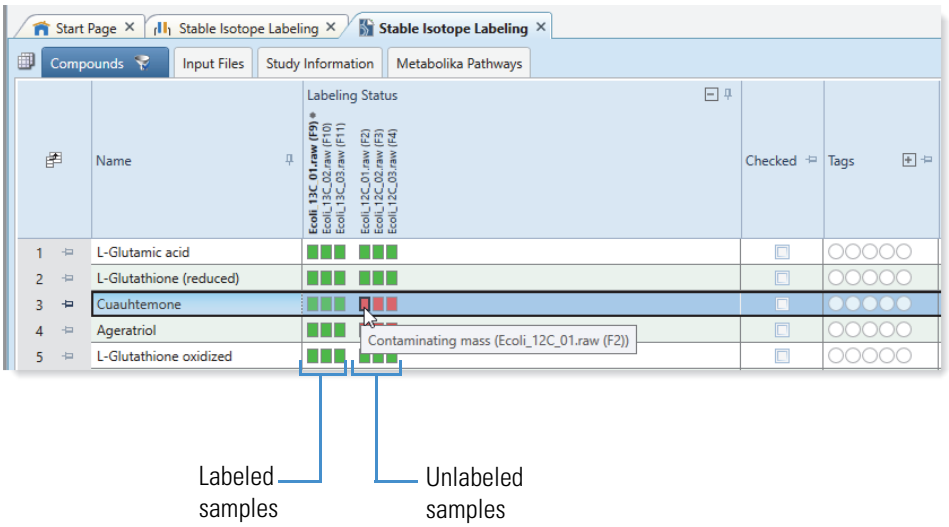
❖ To investigate a contaminating mass in an unlabeled sample

1. In the Compounds table for the example result file, sort the compounds in descending order by Area (Max.).
2. Select **row 3** (Cuauhtemone).
3. Click the expand icon, ⊞, for the Labeling Status column.

   Because you grouped the samples by sample type (Figure 8), the samples are also grouped by sample type in the Labeling Status column.

   The red status for cuauhtemone (row 3) in the unlabeled samples indicates the presence of a contaminating mass in these samples.

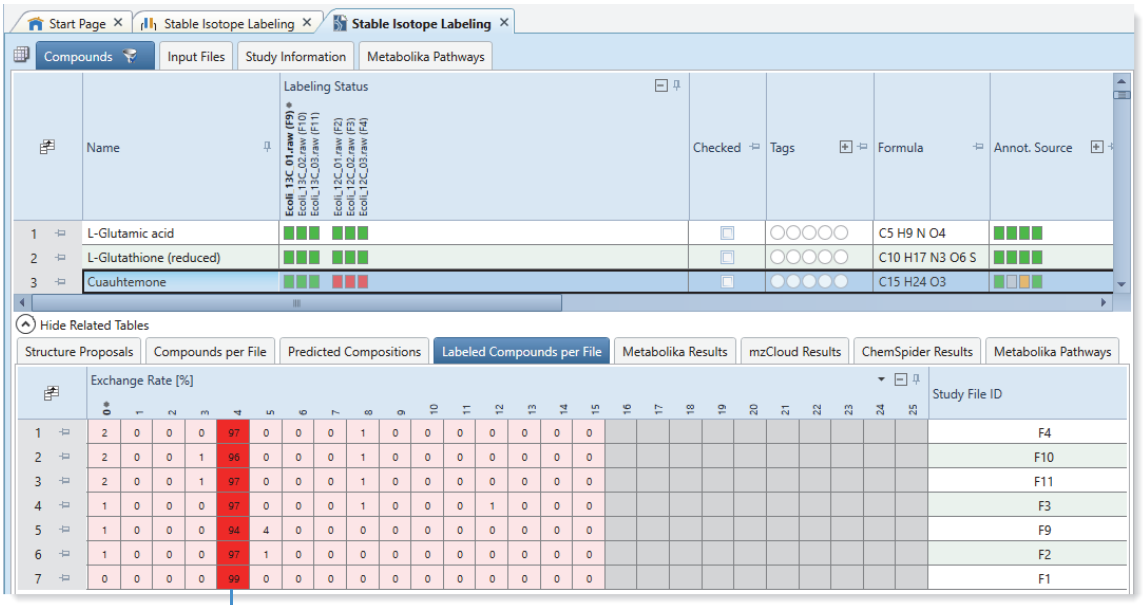
Figure 16. Cuauhtemone selected in the Compounds table (with the Name and Labeling Status columns frozen at the left)



4. In the related Labeled Compounds per File table for cuauhtemone, click the expand icon, ⊞, for the Exchange Rate [%] column.

This figure shows the exchange rate for cuauhtemone in its related Labeled Compounds per File table. The Exchange Rate [%] column shows that the contaminating mass is possibly a compound with a mass of M+4.

Figure 17. Labeled Compounds per File table for cuauhtemone



The false exchange rate of four carbon-13 atoms for an unlabeled compound was probably caused by a contaminating mass of M + 4.

View a trend chart for a single compound or a set of trend lines for multiple compounds

When you apply the Stable Isotope Labeling layout, the Trend Chart view opens as a hidden view below the Isotopologues Distribution Chart view.

Use the Trend Chart view to compare the relative exchange rate [%] for each compound by input file, sample group, or study variable (for example, the time points in a metabolic flux study). When you select a single compound in the Compounds table, you can view its distribution as a box-and-whiskers plot or as a trend line plot. When you select multiple compounds in the Compounds table, the application automatically displays the distribution for each compound as a trend line plot.

Note The example data set does not include metabolic flux samples.

Follow these procedures:

- To view a trend line plot for a compound
- To view a box-and-whiskers plot for a compound

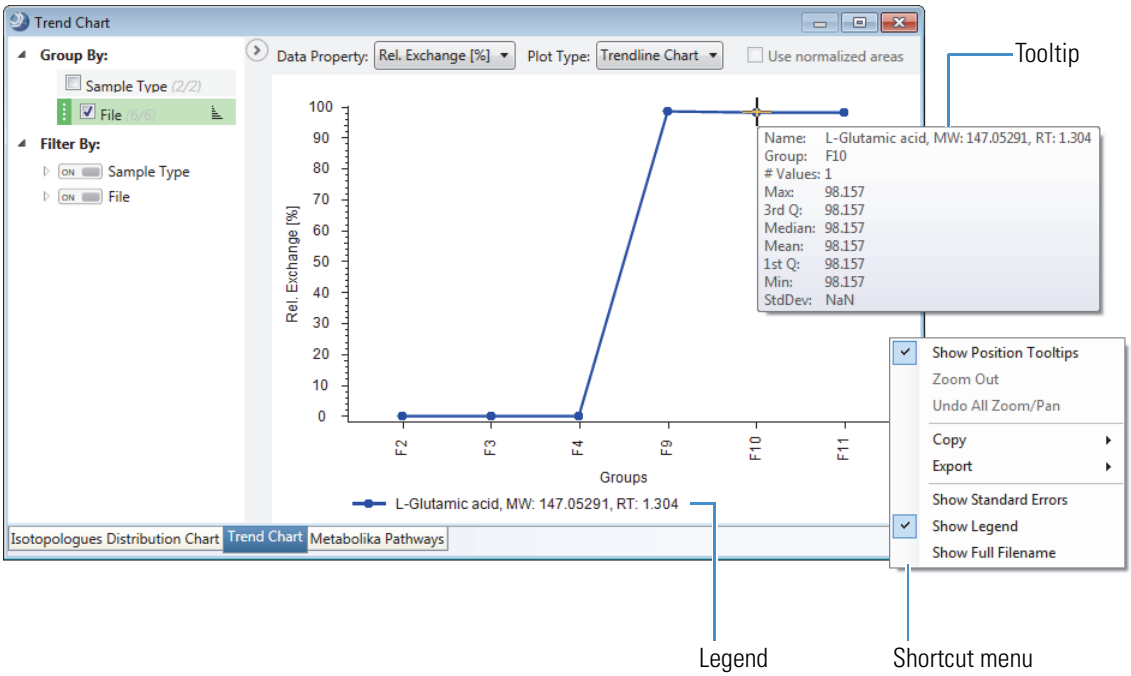
❖ To view a trend line plot for a compound

1. To sort the main Compounds table in descending order by area, click the Area (Max.) column heading.

   For the example data set, L-glutamic acid sorts to the top of the table.
2. In the Compounds table, select **L-Glutamic Acid** (row 1).
3. In the set of tabbed views to the right of the result table, click the **Trend Chart** tab.

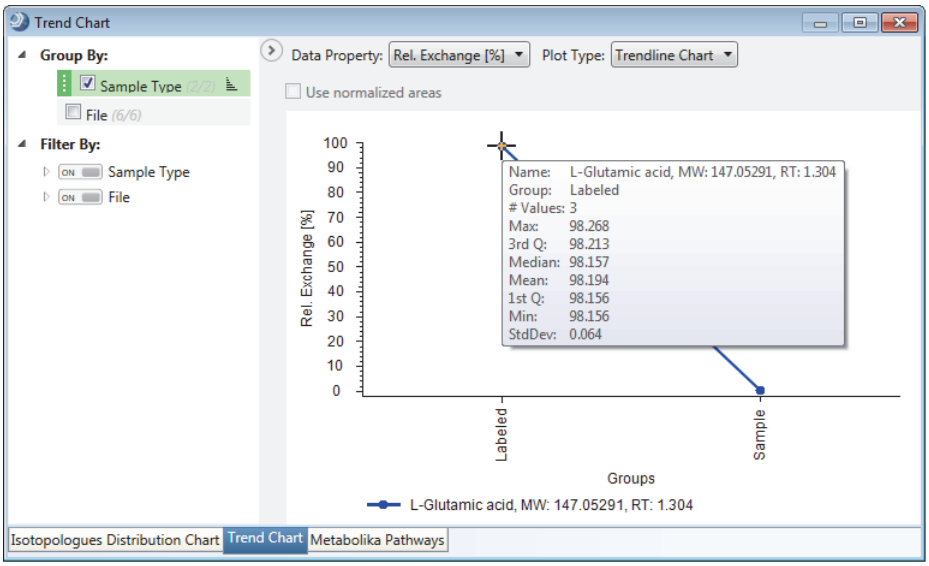
   The Trend Chart view displays a trend line plot for the relative exchange rate per input file.
4. Right-click the chart and choose **Show Legend**.
5. To display a tooltip with descriptive statistics, point to a data point.

This figure shows the trend line plot for L-glutamic acid with the samples grouped by input file.



6. To view the samples grouped by sample type, in the left pane, under Group By, clear the **File** check box and select the **Sample Type** check box.

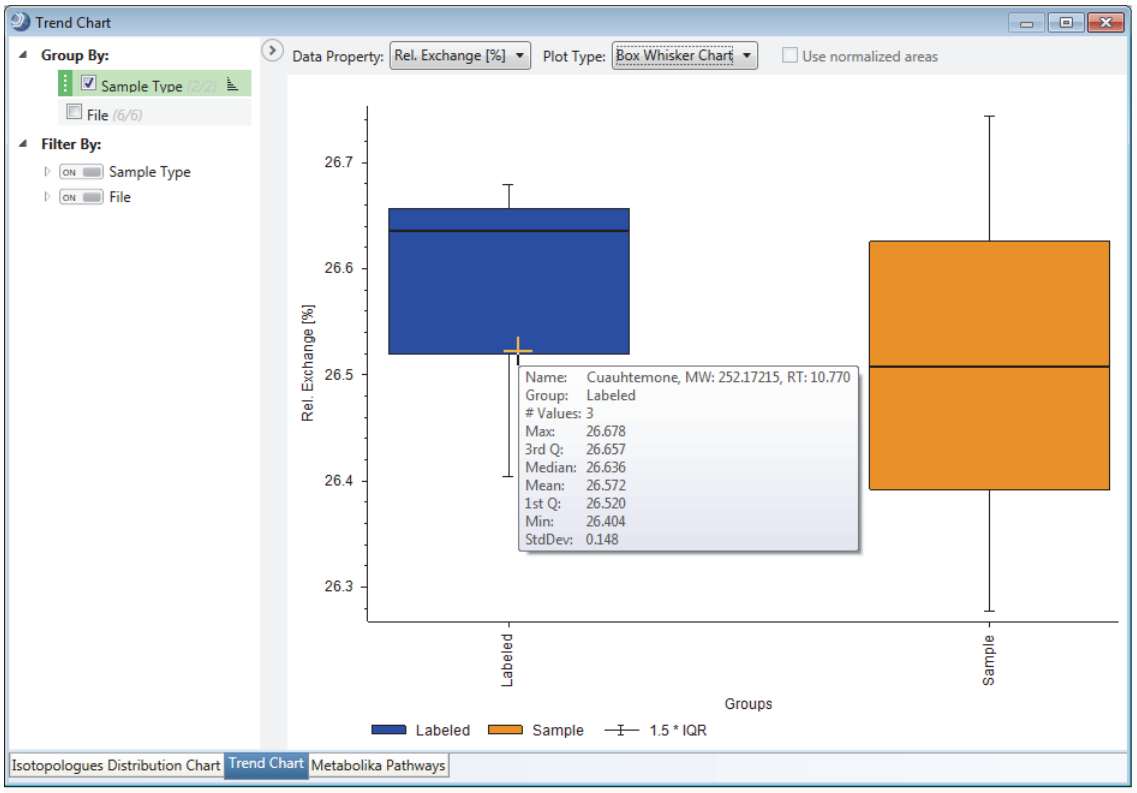
   This figure shows the trend line plot for L-glutamic acid with the samples grouped by sample type (labeled versus unlabeled).



❖ To view a box-and-whiskers plot for a compound

1. In the Compounds table (sorted in descending order by Area (Max.)), select **row 3** (cuauhtemone).
2. To view the samples grouped by sample type, in the left pane, under Group By, clear the **File** check box and select the **Sample Type** check box.
3. In the Plot Type list, select **Box Whiskers** chart.
4. Right-click the chart and choose **Show Legend**.
5. To display a tooltip with descriptive statistics, point to a whisker.

This figure shows the box-and-whiskers plot for cuauhtemone with the samples grouped by sample type.



View the distribution of the isotopologues for each compound

Use the Isotopologues Distribution Chart view to visualize the distribution of the isotopologues for a compound.

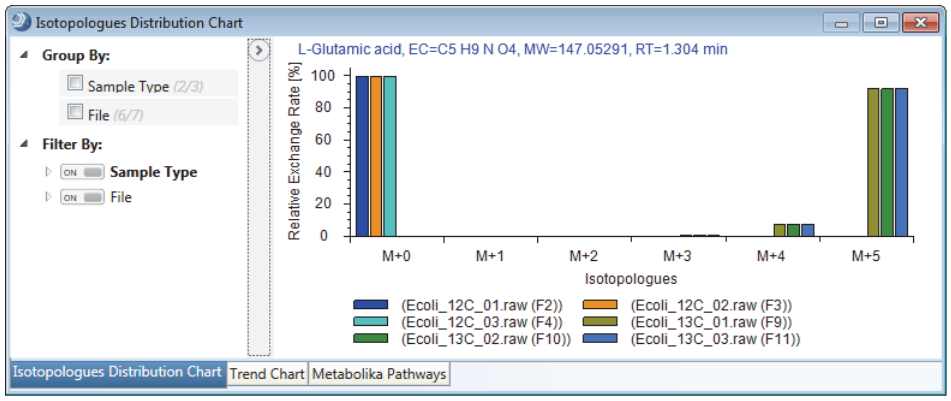
❖ To view the distribution of the isotopologues for a compound

1. Apply the Stable Isotope Labeling layout to the result file (see "Apply the Stable Isotope Labeling layout" on page 19).

   The Isotopologues Distribution Chart view opens to the right of the result tables.

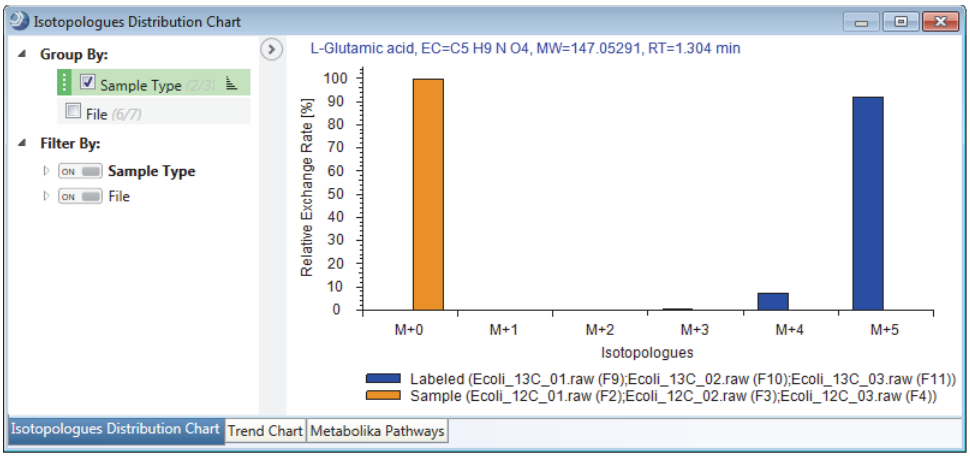
2. In the Compounds table, select a compound of interest.

   This figure shows the distribution for L-glutamic acid with no sample grouping.



3. To group the samples by Sample Type, under Group By, select the **Sample Type** check box.

This figure shows the isotopologue distribution for L-glutamic acid with the samples grouped by sample type.



View the Metabolika pathways for a compound

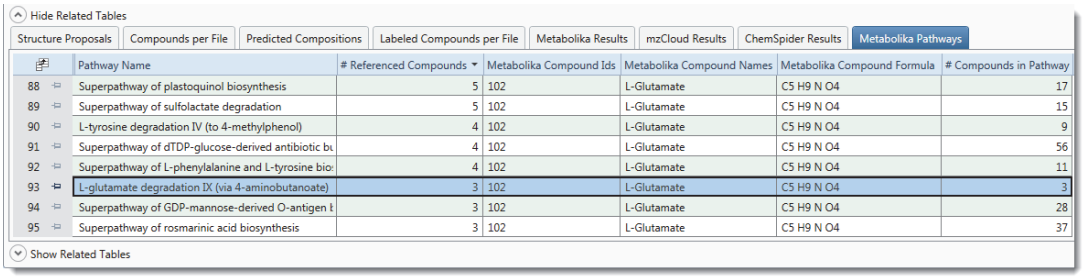
The Map to Metabolika Pathways node (in the selected processing workflow) returns a set of mapped pathways for each detected compound.

❖ To view the Metabolika pathways that include a selected compound

1. Apply the Stable Isotope Labeling layout to the result file (see "Apply the Stable Isotope Labeling layout" on page 19).

   The Isotopologues Distribution Chart, Trend Chart, and Metabolika Pathways views open as tabbed views on the bottom right of the page. The related tables pane opens below the main tables pane.
2. In the example result file, sort the Compounds table by the Area (Max.) column in descending order.
3. Select **row 1** (L-glutamic acid).
4. To view a Metabolika pathway that includes L-glutamic acid, do the following:
   a. In the related tables pane, click the **Metabolika Pathways** tab to make it the active table.
   b. For this tutorial, scroll down to **row 93**—the **L-glutamate degradation IX (via 4-aminobutanoate)** pathway and select it.

      This figure shows the selected Metabolika pathways file for L-glutamic acid.



	Pathway Name	# Referenced Compounds	Metabolika Compound Ids	Metabolika Compound Names	Metabolika Compound Formula	# Compounds in Pathway
88	Superpathway of plastoquinol biosynthesis	5	102	L-Glutamate	C5 H9 N O4	17
89	Superpathway of sulfolactate degradation	5	102	L-Glutamate	C5 H9 N O4	15
90	L-tyrosine degradation IV (to 4-methylphenol)	4	102	L-Glutamate	C5 H9 N O4	9
91	Superpathway of dTDP-glucose-derived antibiotic bu	4	102	L-Glutamate	C5 H9 N O4	56
92	Superpathway of L-phenylalanine and L-tyrosine bio	4	102	L-Glutamate	C5 H9 N O4	11
93	L-glutamate degradation IX (via 4-aminobutanoate)	3	102	L-Glutamate	C5 H9 N O4	3
94	Superpathway of GDP-mannose-derived O-antigen b	3	102	L-Glutamate	C5 H9 N O4	28
95	Superpathway of rosmarinic acid biosynthesis	3	102	L-Glutamate	C5 H9 N O4	37

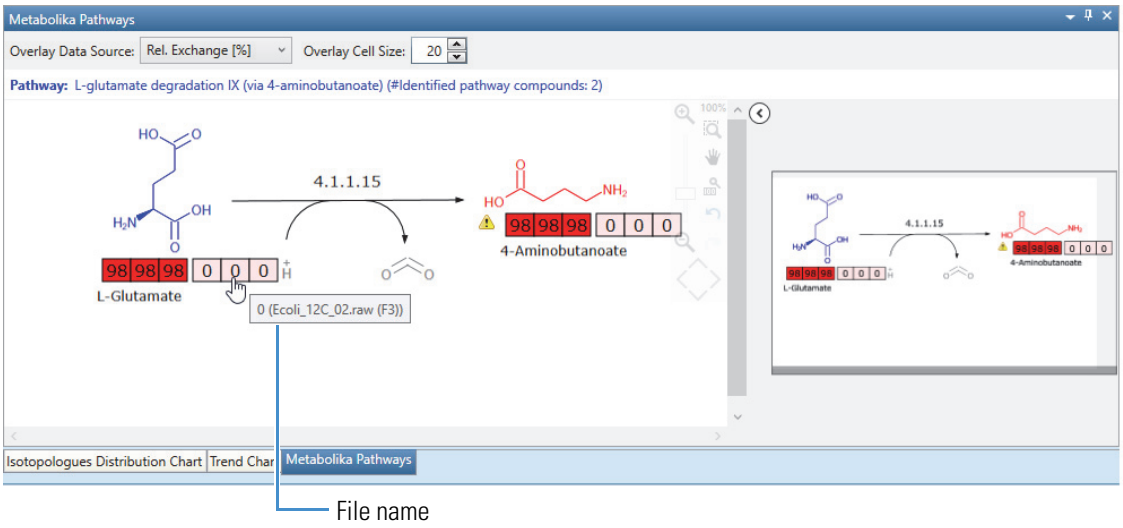
   c. In the tabbed views to the right, click the **Metabolika Pathways** tab.

      The mapped pathway appears in the Metabolika Pathways view. The Stable Isotope Labeling layout automatically selects the Tags column as the overlay data source with an overlay cell size of 10 pixels.

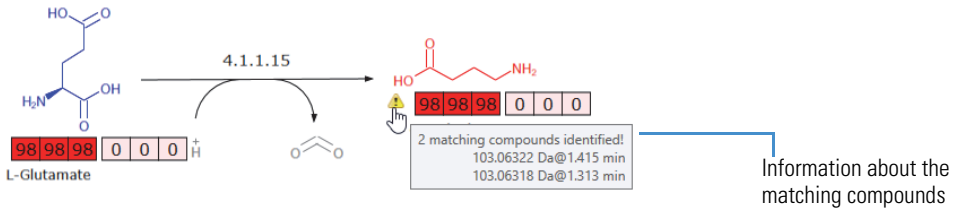
      The structure for the compound that you selected in the Compounds table is blue, the structures for other detected compounds are red, and the structures for undetected compounds in the pathway are black.
5. In the Overlay Data Source list, select **Rel. Exchange [%]**.
6. To enlarge the overlaid data, increase the value in the Overlay Cell Size box (Range: 6 to 30 pixels in width).

7. To view the file name for a specific value, point to the value.

   This figure shows the selected Metabolika pathway with an overlay of the relative exchange [%] data for the selected compound—L-glutamate. The overlay cell size has been increased to 20 pixels. A Caution symbol next to a compound indicates that the analysis found multiple matches.

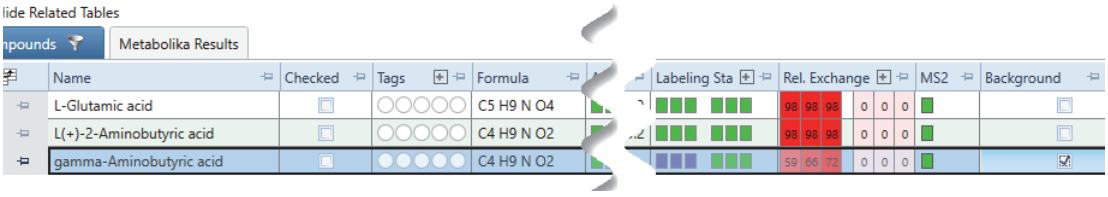


8. To view information about the matching compounds for a structure with multiple matches, point to the Caution symbol.

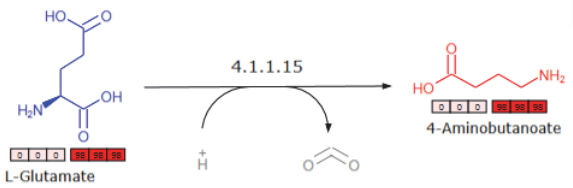


9. To keep only the appropriate explanation for the structure, mark the incorrect explanation as a background compound as follows:
   a. In the second pane of related tables, open the related Compounds table for the selected Metabolika pathway.
   b. Open the Field Chooser dialog box for the related Compounds table and select the check box for the Background column.

      The Background column appears in the related Compounds table.
   c. To mark a compound as a background compound, select its check box in the Background column.



   In the Metabolika pathways view, the Caution symbol below the structure disappears (and the structure remains red).



Export the analysis results

To create a report for your records, filter the compounds table to display only the compounds of interest, and then export the results using the appropriate format.

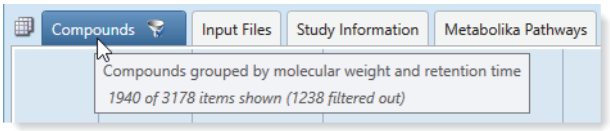
Follow these procedures to filter the Compounds table and export the results:

1. Use the Result Filters view to select compounds of interest
2. Export the results to a spreadsheet

Use the Result Filters view to select compounds of interest

The analysis detected a total of 3180 compounds, including 1241 hidden compounds that were marked as background compounds or compounds without a formula. To reduce the number of compounds to export, filter the table or select the check boxes for the compounds of interest.

Note Pointing to the Compounds tab or the scroll bar for the Compounds table displays a tooltip.



Follow either of these procedures:

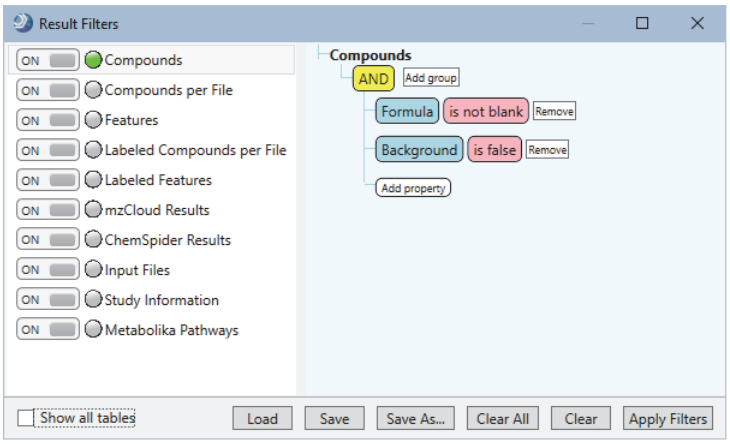
- To filter the compounds by the relative exchange rate
- To filter the Compounds table by the checked compounds

❖ To filter the compounds by the relative exchange rate

1. Click the **Compounds** tab to make it the active table.
2. From the application menu bar, choose **View > Result Filters**.

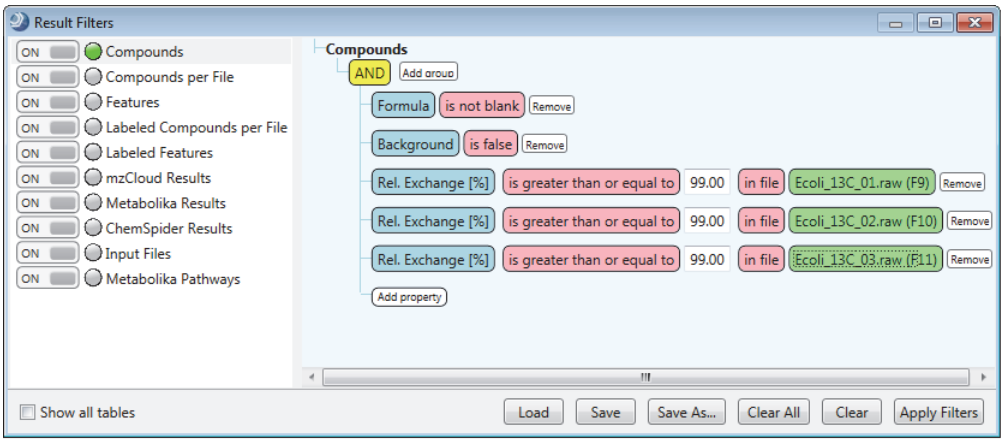
   The Result Filters view opens as a floating window. Because the processing workflow included the Mark Background Compounds node and the Analyze Labeled Compounds node, the filter for the Compounds table already includes a filter for background compounds and a filter for components without a formula.

   This figure shows the default filters for the example result file.



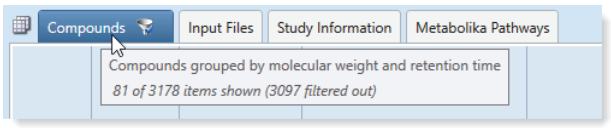
3. On the right side of the Result Filters view, set up filters for the relative exchange rate as follows:
   a. Click **Add Property**, and then select **Rel. Exchange [%]** from the list.
   b. In the pink relation list, select **Is Greater Than or Equal To**.
   c. In the value box next to the relation list, type **99**.
   d. In the pink condition list, select **In File**.
   e. In the Green sample list, select one of the labeled input files.
   f. Repeat steps step 3a through step 3e to add a filter for all three labeled input files.

This figure shows the filter set.



4. Click **Apply Filters**.

   The applied filter set reduces the number of displayed rows in the Compounds table to 81.



5. To undo the relative exchange filters without removing the background and formulas filters, click **Remove** to their right. Then, click **Apply Filters** again.

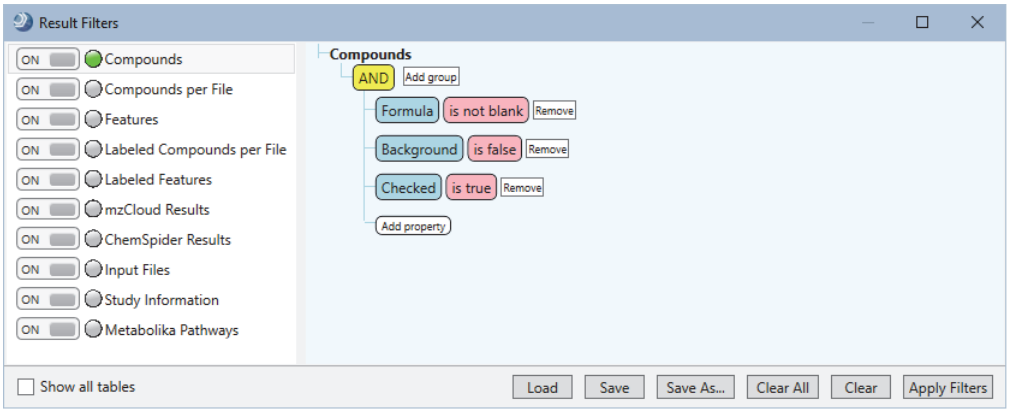
   The Compounds table contains the original set of compounds.

❖ To filter the Compounds table by the checked compounds

1. If the Compounds table is not the active table, click its tab to make it active.
2. Manually select the check boxes for the compounds of interest.
3. From the menu bar, choose **View > Result Filters**.

   Because the processing workflow included the Mark Background Compounds node and the Analyze Labeled Compounds node, the Compounds table is currently filtered by two properties—Background and Formula.
4. Click **Add Property** and select **Checked**.

   This figure shows the filter set.



5. Click **Apply Filters**.

   The Compounds table displays only the selected compounds.
6. To undo the Checked filter, click **Remove** to its right. Then, click **Apply Filters** again.

   The Compounds table contains the original set of compounds.

Export the results to a spreadsheet

Before exporting the results to a spreadsheet, filter the results table as described in "Use the Result Filters view to select compounds of interest" on page 27 or select the check boxes for the compounds of interest.

To create a report, follow these procedures as needed:

- To check the number of table rows
- To display the table columns that you want to export
- To sort the rows
- To export the filtered and sorted results to an Excel™ spreadsheet

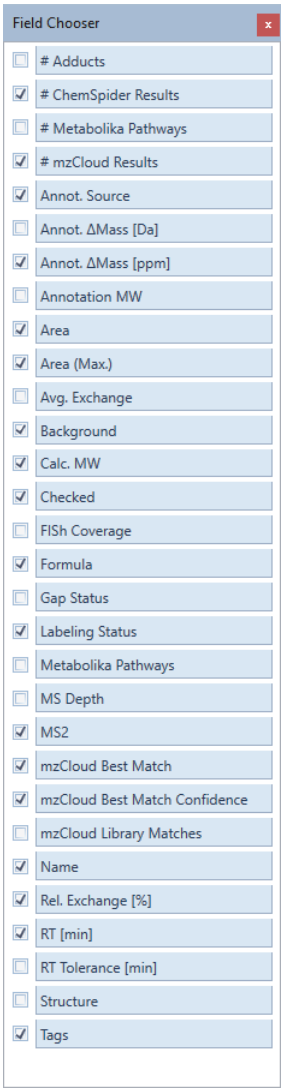
❖ To check the number of table rows

Point to the vertical scroll bar to the right of the compounds table.

A tooltip appears with the row count.

❖ To display the table columns that you want to export

Open the Field Chooser box and select the check boxes for the columns of interest and clear the other check boxes.



❖ To sort the rows

Click the column heading that you want to sort by.

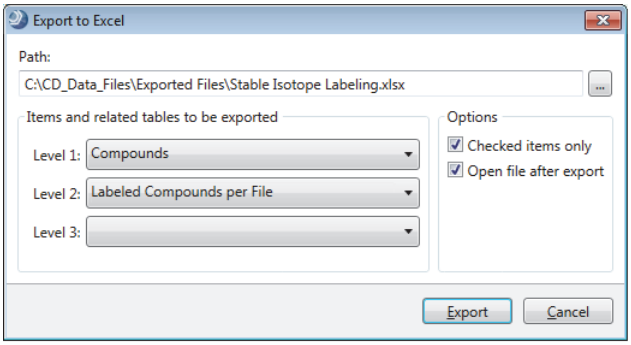
❖ To export the filtered and sorted results to an Excel™ spreadsheet

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

   The Export to Excel dialog box opens.

2. Check the file name and location in the Path box. Then, change the file name and location as appropriate.

3. In the Options area, select the **Checked Items Only** and **Open File After Export** check boxes.

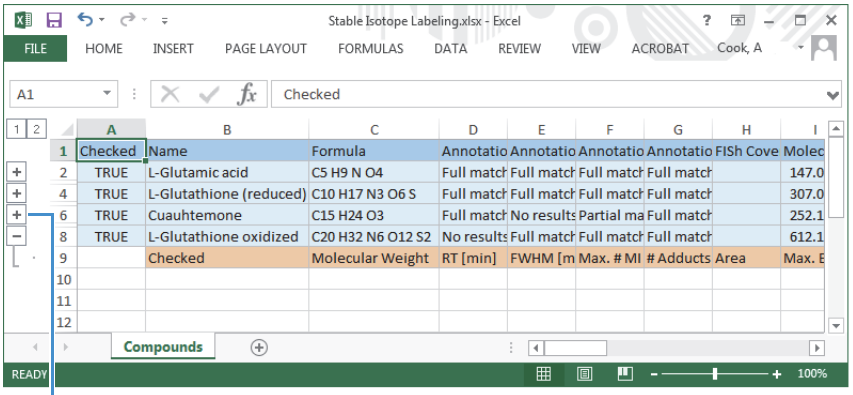


4. Click **Export**.

   The Excel spreadsheet opens (Figure 18).

   This spreadsheet shows the exported Compounds table. Clicking the expand icon to the left of a row opens the exported Labeled Compounds per File table for the compound.

Figure 18. Exported compounds



Trademarks