

Compound Discoverer 3.1 Metabolomics Tutorial

To familiarize yourself with the Thermo Compound Discoverer™ 3.1 application, 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.

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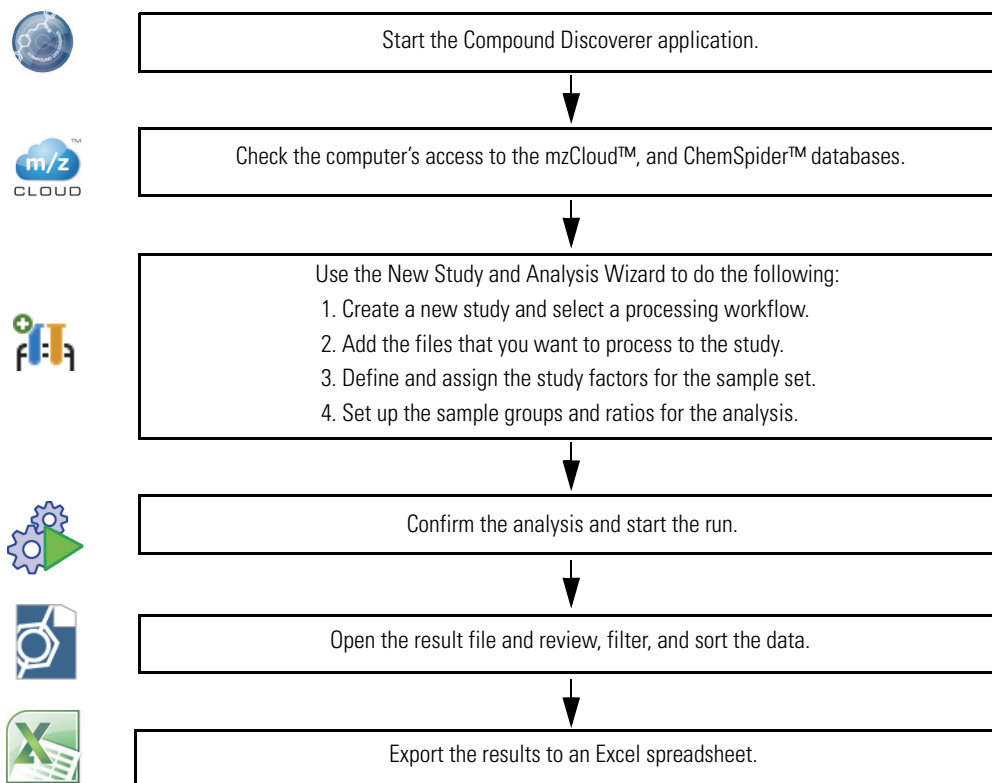
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Overview

These files are provided on the key-shaped USB key in the software media kit. You can find these files in the following folder:

Example Studies\Metabolomics\ZDF\

Copy the ZDF folder to your data processing computer. This figure shows the tutorial's workflow.



Starting the Application

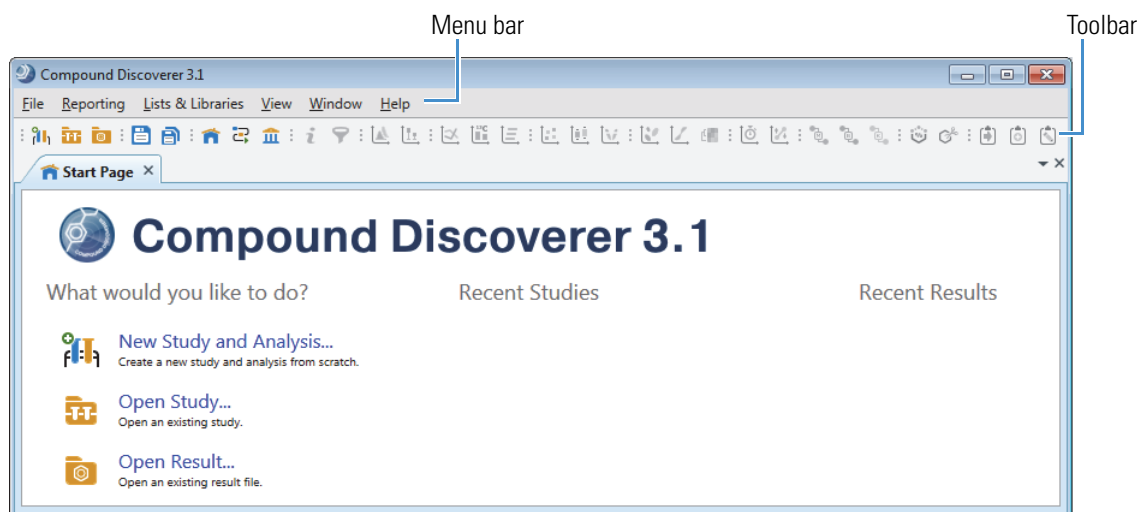
❖ To start the application

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

–or–

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

The application opens to the Start page.



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.



The Help opens to the selected topic.

This tutorial uses a processing workflow that uses online mass spectral databases to identify the unknown compounds. To use any of the processing workflows that use 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 window open and press the F1 key to open the Help. Then, follow the instructions to troubleshoot the communication failure.

Go to the next topic [“Setting Up a New Study and a New Analysis.”](#)

Make sure to copy the example data files to an appropriate folder on your processing computer (see [“Overview”](#) on page 1).

To create a new study and a new analysis, follow these topics:

1. [Setting Up the Study Folders](#)
2. [Selecting the Processing Workflow](#)
3. [Adding the Input Files to the Study](#)
4. [Defining the Study Factors](#)
5. [Setting Up the Sample Groups and Ratios](#)
6. [Modifying the Processing Workflow](#)

Checking the Computer's Access to the External Databases

Setting Up a New Study and a New Analysis

Setting Up the Study Folders

Each time 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. When you first install the Compound Discoverer application, you must set up a top-level folder for the study folders.

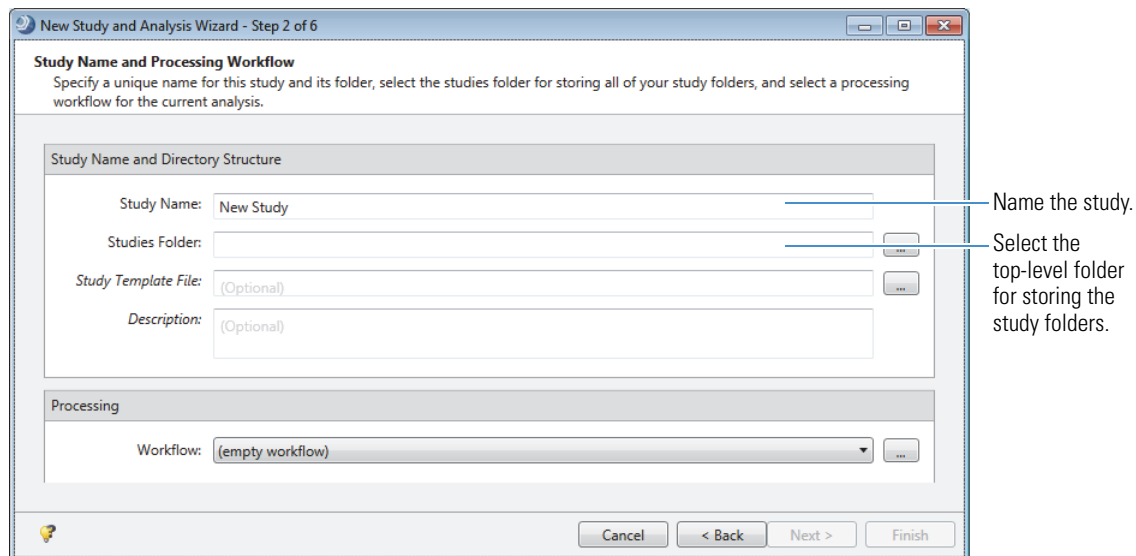
❖ To name the new study and set up the top-level folder

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

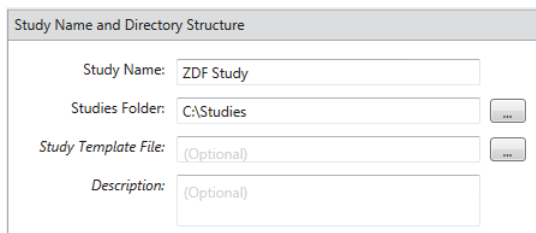
The New Study and Analysis Wizard opens.

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 the study folders is undefined.



3. In the Study Name box, name the study.
 - For a metabolomics study with the ZDF (rat) example files, type **ZDF 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. Browse to your local disk drive or a location on your local network.
 - b. Click **New Folder** to create a new folder and name the folder **Studies**.



After you select or create a top-level folder, stay on this wizard page and go to the next topic [“Selecting the Processing Workflow.”](#)

When you complete the wizard, the application creates the ZDF Study.cdStudy file, stores the study file in the ZDF Study folder, and stores the ZDF Study folder in the Studies folder.

Drive:\Studies\ZDF Study folder

When you run the analysis in this tutorial, the application stores the result file (.cdResult) in the ZDF Study folder.

Selecting the Processing Workflow

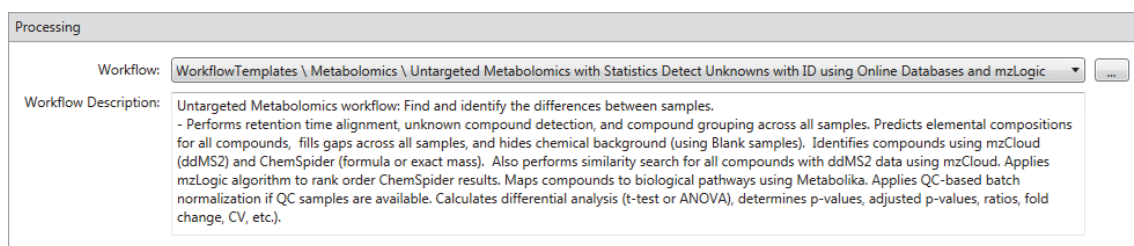
In the Compound Discoverer application, the processing method that interprets the raw data is called a processing workflow (.cdProcessingWF). The application provides defined processing workflows for several fields of study including the metabolomics field. This tutorial uses a defined processing workflow that searches the mzCloud and ChemSpider databases to identify the compounds detected in the input files (Xcalibur RAW files). This workflow also maps compounds to their biological pathways by using the local Metabolika pathway files.

❖ To select the processing workflow

1. Under Processing, select the following processing workflow from the Workflow list:

Workflow Templates\Metabolomics\Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Online Databases and mzLogic

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



2. Read the description.
3. Click **Next** to open the Input File Selection page of the wizard.

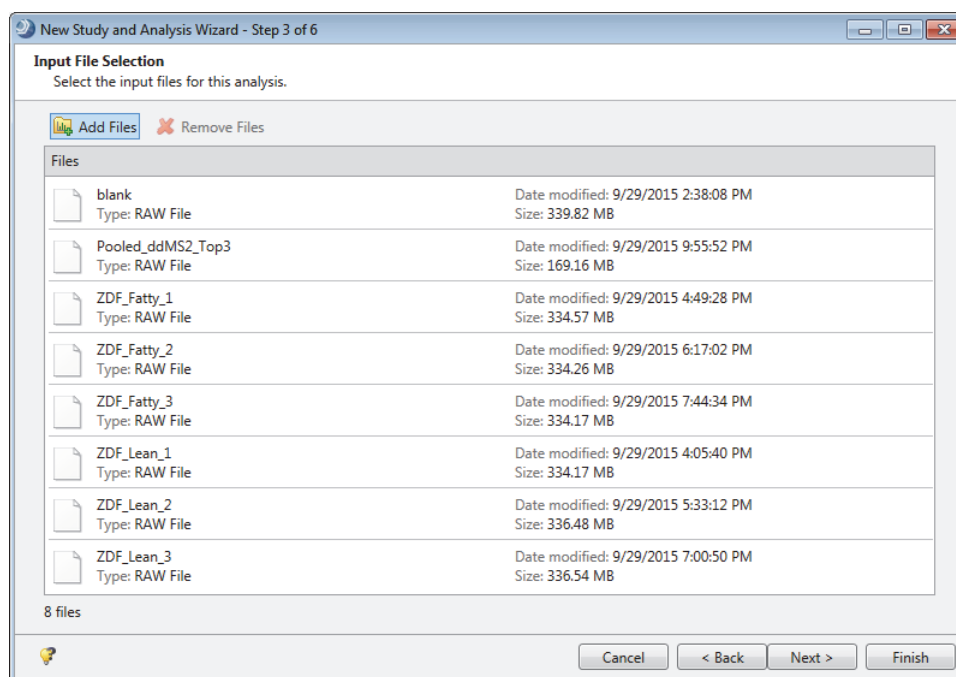
❖ To add input files to the study

1. On the Input File Selection page of the wizard, click **Add Files** in the command bar.

The Add Files dialog box opens.

2. Browse to the folder where you copied the Xcalibur RAW files.
3. Select all the Xcalibur RAW files in this folder and click **Open**.

The names of the selected files appear in the Files box on the Input File Selection page.

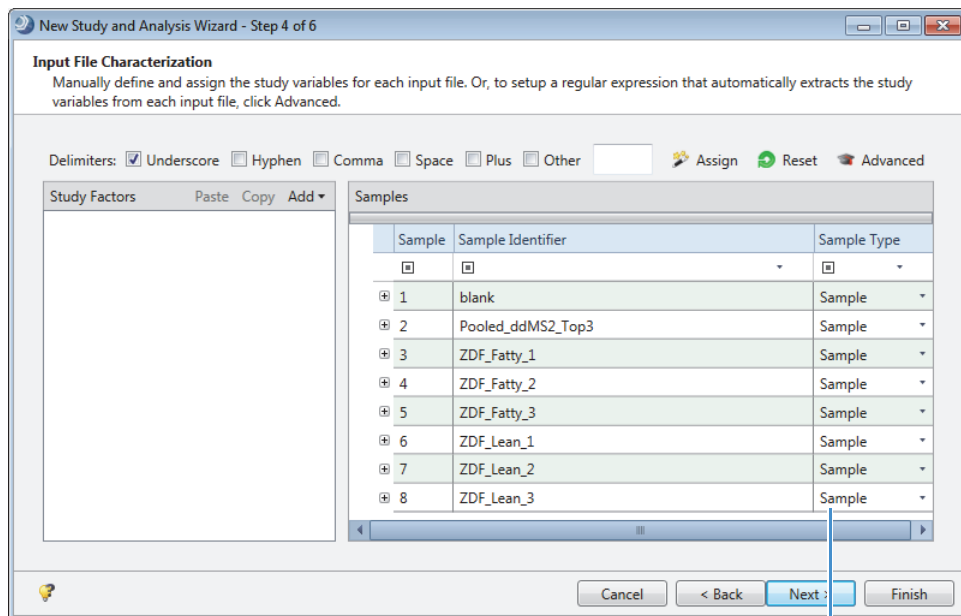


4. Click **Next** to open the Input File Characterization page of the wizard.

Adding the Input Files to the Study

Defining the Study Factors

This figure shows the newly added samples in the Samples pane of the Input File Characterization page. By default, the application assigns Sample as the Sample Type to new samples. To group the samples according to your experimental design, you must define their study factors. In this tutorial, you are comparing the compounds in the plasma from two phenotypes of ZDF rats—Fatty and Lean.



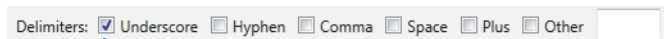
Default Sample Type

To define and assign the study factors, follow these topics in order:

1. [Selecting the Delimiters for Parsing the File Names](#)
2. [Defining Phenotype as a Categorical Study Factor](#)
3. [Assigning the Phenotypes and the Sample Types to the Samples](#)

❖ To select the delimiters that separate the factors in the file names

1. Select the **Underscore** check box.



Select this check box.

In the example files, the underscore character is the delimiter that separates the study factors from the other parts of the file name.

ZDF_Fatty_1	ZDF_Fatty_2	ZDF_Fatty_3	Blank
ZDF_Lean_1	ZDF_Lean_2	ZDF_Lean_3	Pooled_ddMS2

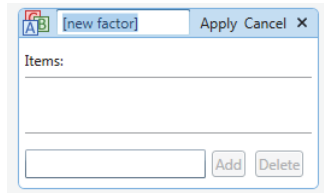
The study factor is Phenotype (Lean or Fatty).

Selecting the Delimiters for Parsing the File Names

Defining Phenotype as a Categorical Study Factor

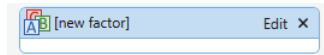
❖ To set up Phenotype as a categorical study factor

- In the Study Factors area of the Input File Characterization page, choose **Add > Categorical Factor**.
The categorical study factor editor opens in the Study Factors area with the [new factor] box selected.

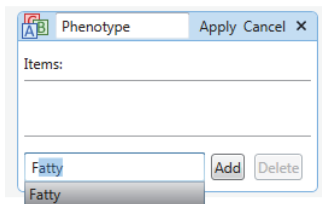


- Type the factor name: **Phenotype**.

If the editor closes before you type Phenotype, click **Edit** to reopen it. Select [new factor] and type **Phenotype**.



- To add the Fatty and Lean phenotypes to the Items list, do the following:
 - In the box next to the Add button, type **F**.



The application parses the file names in the Sample Identifier column and Fatty appears in the box.

- Click **Add**.

Fatty appears in the list.

- Type **L** in the box.

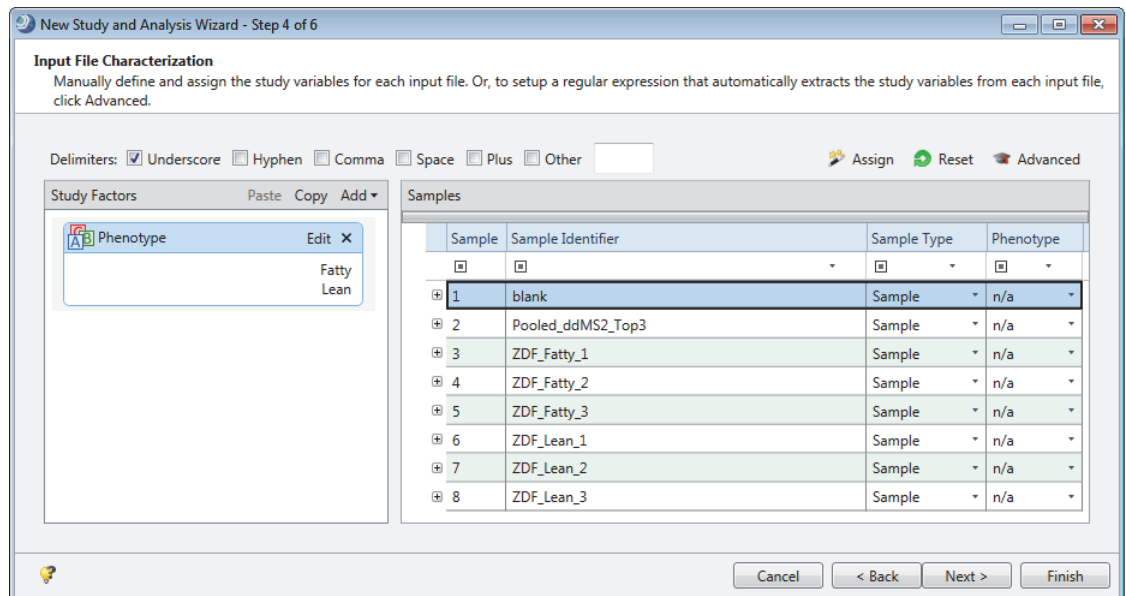
Lean appears in the box.

- Click **Add**.

Lean appears in the list.

- Click **Apply**.

The editor closes and the Edit button replaces the Apply and Cancel buttons.



Assigning the Phenotypes and the Sample Types to the Samples

❖ To assign the phenotypes and the sample types

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



The application does the following:

- Assigns the study factor items for the Phenotype study factor, Fatty or Lean, to the samples.
- Assigns the Blank sample type to the Blank.raw file.

This figure shows the study variable assignments. Study variables include the study factor and the sample type assignments for the samples.

Sample	Sample Identifier	Sample Type	Phenotype
1	blank	Blank	n/a
2	Pooled_ddMS2_Top3	Sample	n/a
3	ZDF_Fatty_1	Sample	Fatty
4	ZDF_Fatty_2	Sample	Fatty
5	ZDF_Fatty_3	Sample	Fatty
6	ZDF_Lean_1	Sample	Lean
7	ZDF_Lean_2	Sample	Lean
8	ZDF_Lean_3	Sample	Lean

Because the file names for the Pooled_ddMS2_Top3 sample and the Blank sample do not match the defined pattern, *ZDF_categorical_factor_*, the application assigns these samples a value of n/a for the Phenotype study factor.

IMPORTANT The assignment of n/a for a study factor is appropriate for the Blank and Identification Only sample types, as the application does not use these sample types in statistical analyses.

Note The Pooled_ddMS2_Top3 sample is a blend of the six non-blank samples. The Pooled_ddMS2_Top3.raw file acquired from this sample contains the data-dependent MS2 scans for the input file set. The full scan data for this sample was acquired at a lower resolution than that for the other samples. The Blank sample is a matrix blank and only contains the background compounds.

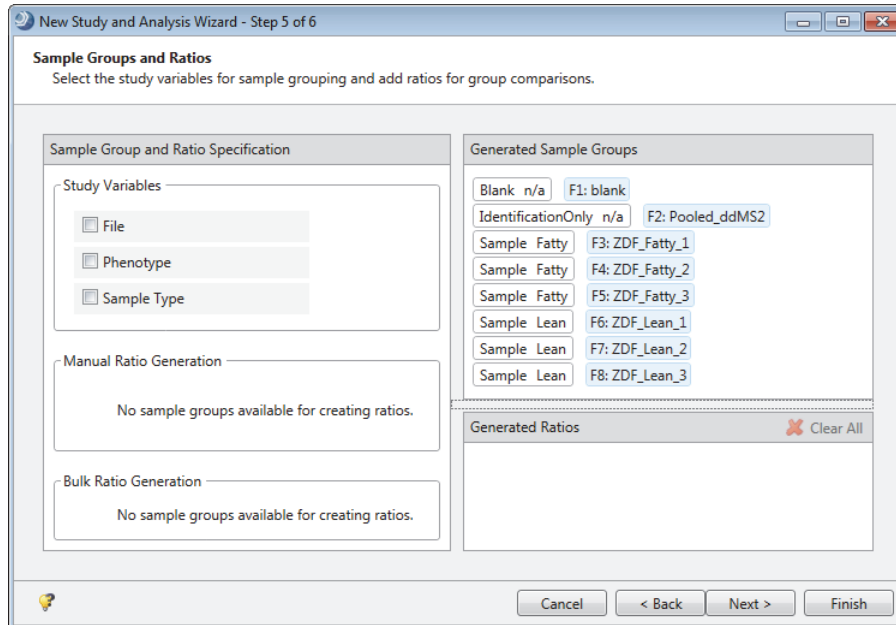
2. For the Pooled_ddMS2_Top3 sample, select **Identification Only** in the Sample Type list.

Error	Sampl	File	Sample Identifier	Sample Type	Phenotype
	S1	F1	blank	Blank	n/a
	S2	F2	Pooled_ddMS2	Sample	n/a
	S3	F3	ZDF_Fatty_1	Sample	Fatty
	S4	F4	ZDF_Fatty_2	Control	Fatty
	S5	F5	ZDF_Fatty_3	Blank	Fatty
	S6	F6	ZDF_Lean_1	Quality Control	Lean
	S7	F7	ZDF_Lean_2	Identification Only	Lean
	S8	F8	ZDF_Lean_3	Standard	Lean

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

Setting Up the Sample Groups and Ratios

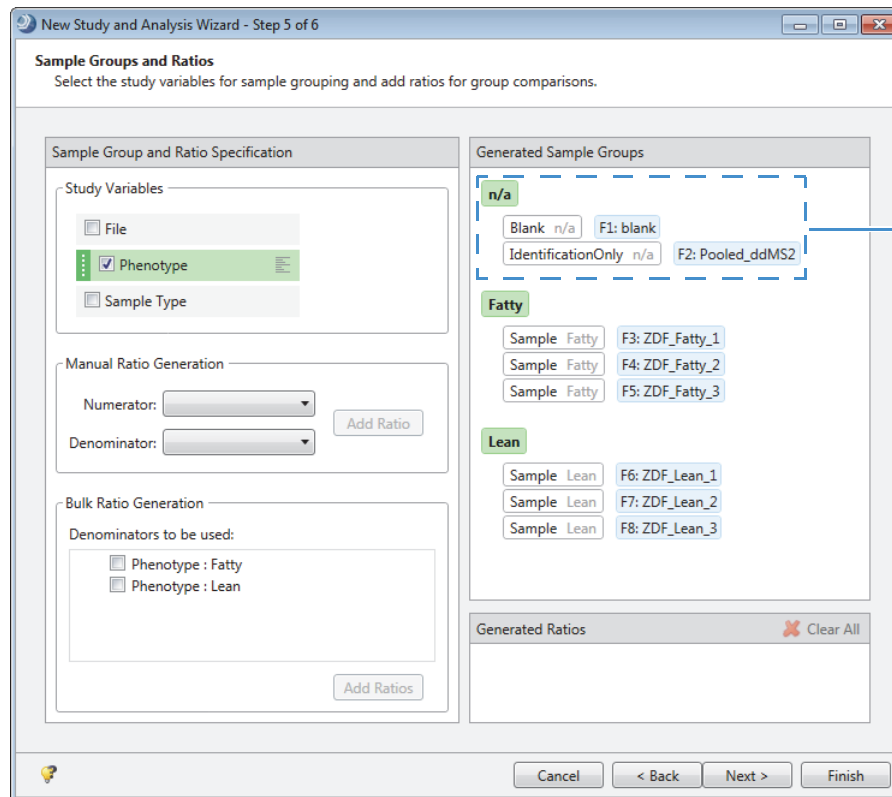
Use the Sample Groups and Ratios page of the wizard to set up a differential analysis for the two phenotypes: Lean and Fatty.



❖ To set up the sample groups and ratios

1. In the Study Variables area, select the **Phenotype** check box.

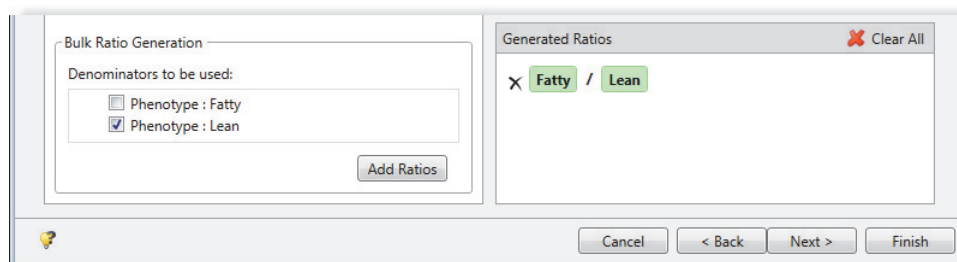
The sample groups—n/a, Fatty, and Lean—appear in the Generated Sample Groups area.



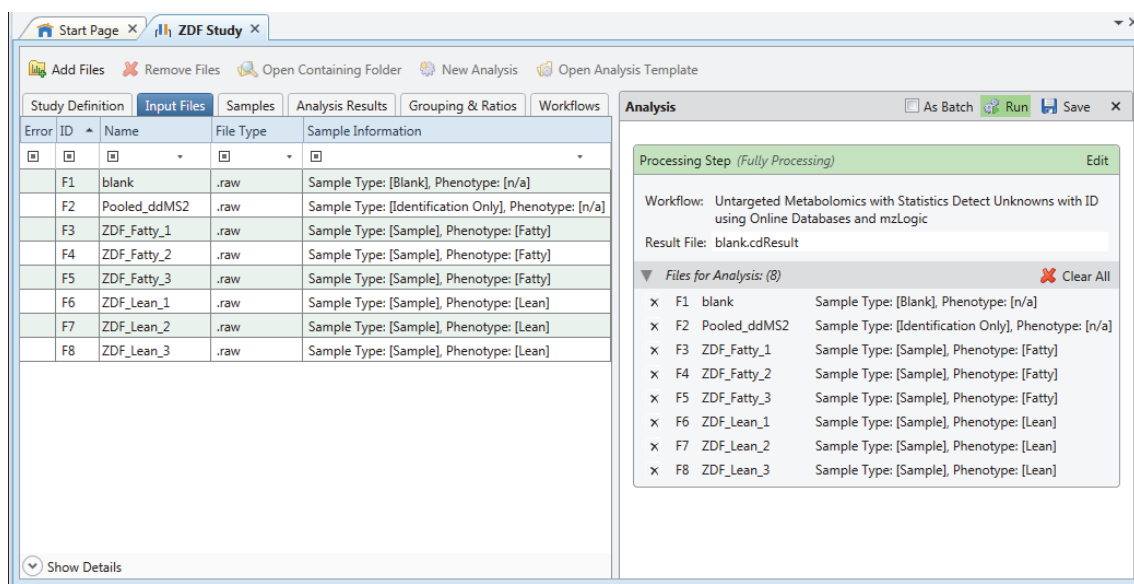
The n/a group includes only these sample types:

- Blank
- Identification Only

2. If an error message (❗) appears in the Generated Sample Groups area, do the following:
 - a. Return to the Input File Characterization page and verify the following sample type assignments:
 - Blank is assigned to the Blank sample.
 - Identification Only is assigned to the Pooled_ddMS2 sample.
 - b. Return to the Sample Groups and Ratios page and verify that you have corrected the sample type assignment error.
3. In the Bulk Ratio Generation area, select the **Phenotype: Lean** check box to make the Lean group the control group.
The Add Ratios button becomes available.
4. Click **Add Ratios**.
The ratio, Fatty/Lean, appears in the Generated Ratios area.
The analysis is set up to compare the two sample groups: Fatty versus Lean.



5. Click **Finish** to save the study and close the wizard.
The ZDF Study tab, the two analysis page tabs (Grouping & Ratios and Workflows), and the Analysis pane appear.
The Analysis pane lists the eight input files. The analysis is set up to combine the mass spectral data in these files into one result file—that is, the As Batch check box is clear and the Result File name is available for editing.



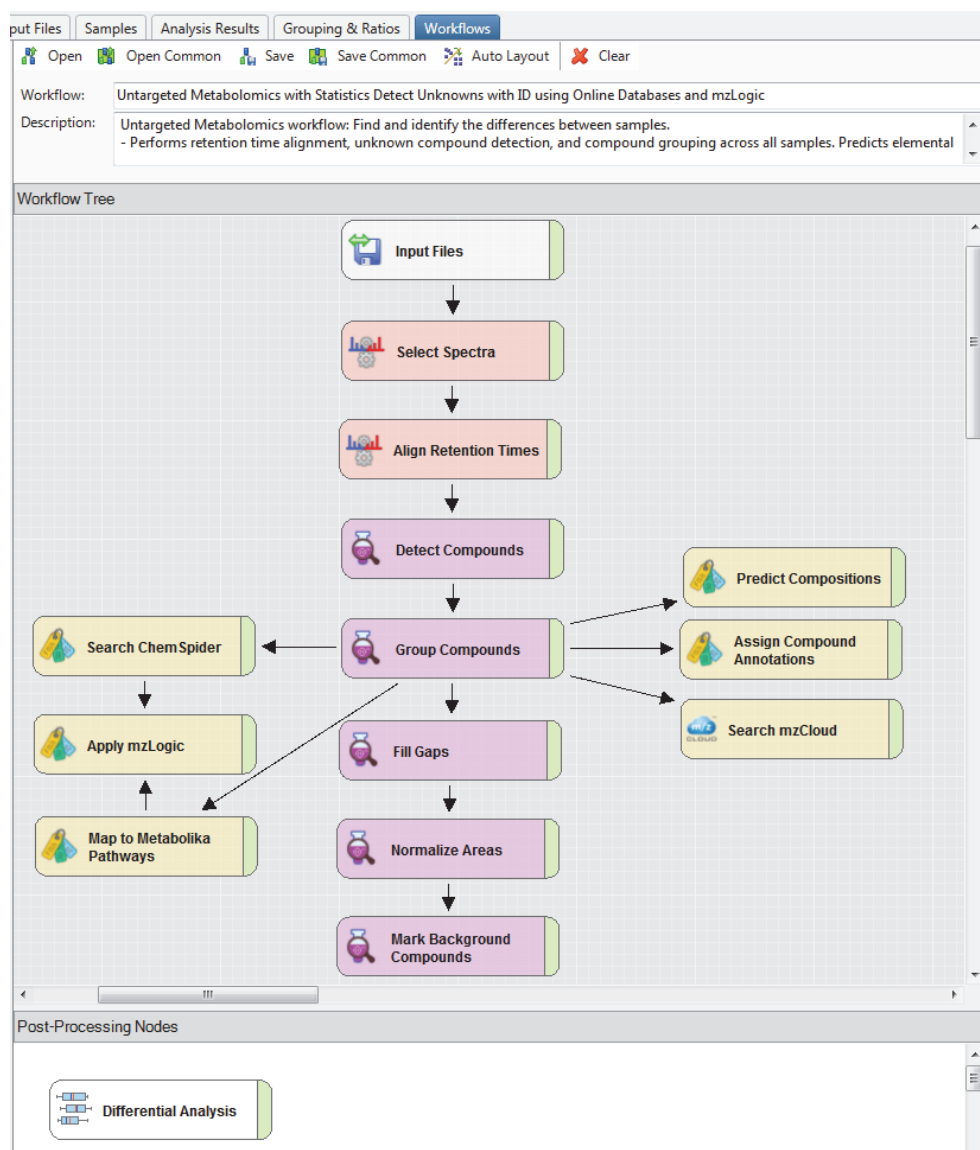
Modifying the Processing Workflow

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

❖ To review the processing workflow

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

Figure 1. Processing workflow for this tutorial



- Open the parameter settings for the Detect Compounds node and check the Min. Peak Intensity setting against the suggested setting for your data set (Table 1).

The minimum peak intensity setting defines the base peak intensity for the unknown compound detection. For the example ZDF data files, keep the setting of 1 000 000.

Tip To open the parameter settings for a workflow node, click the node in the Workflow Tree pane. The Parameters page for the node opens to the left.

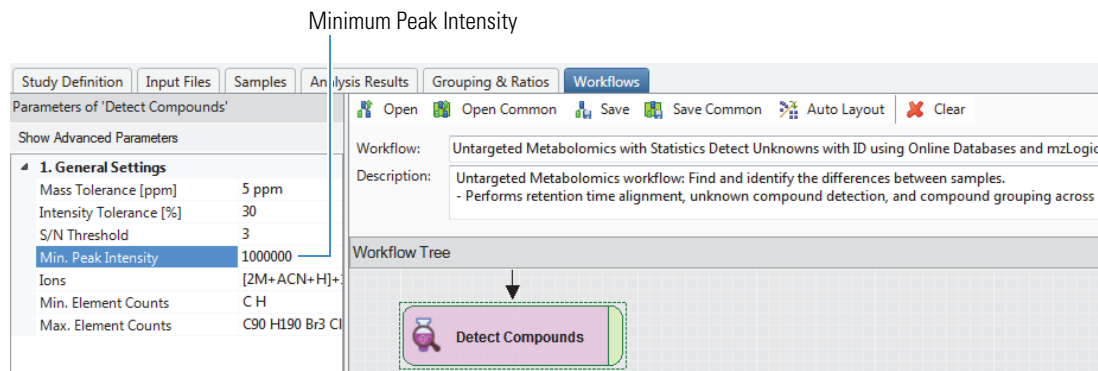


Table 1 lists the recommended range for the minimum peak intensity parameter. The optimal setting depends on the sensitivity of the mass spectrometer.

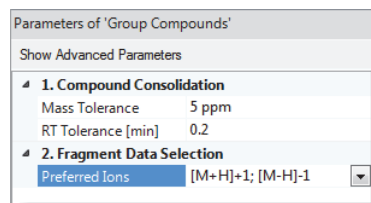
Table 1. Recommended minimum peak intensity range

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

- Open the parameter settings for the Group Compounds node.

The node is set to the default settings. For this tutorial, do not change the selection. For other analyses, select the appropriate ions from the Preferred Ions list, making sure that the selected ions include the ion selected for the Base Ions parameter in the Detect Compounds node.

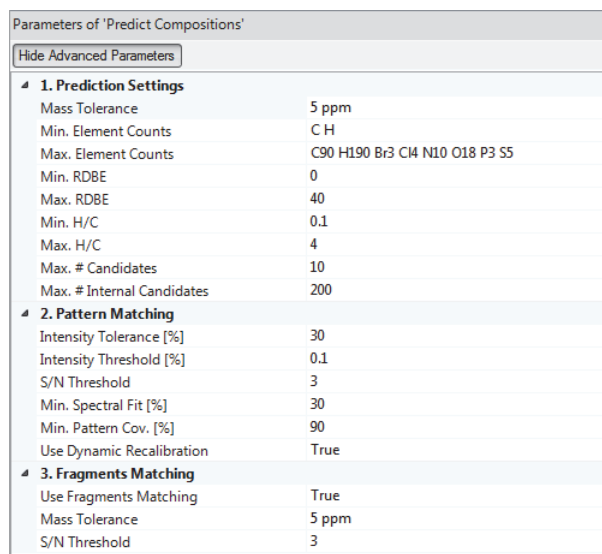
For the preferred adduct ions of each detected compound, the node sends all the data-dependent MS2 scans to the search nodes and the best MS1 scan and best MS2 scan across the raw data file set to the Predict Compositions node.



IMPORTANT When setting up the node parameters, make sure that the list of preferred ions specified in the Group Compounds node includes the list of base ions specified in the Detect Compounds node.

4. Open the parameter settings for the Predict Compositions node.

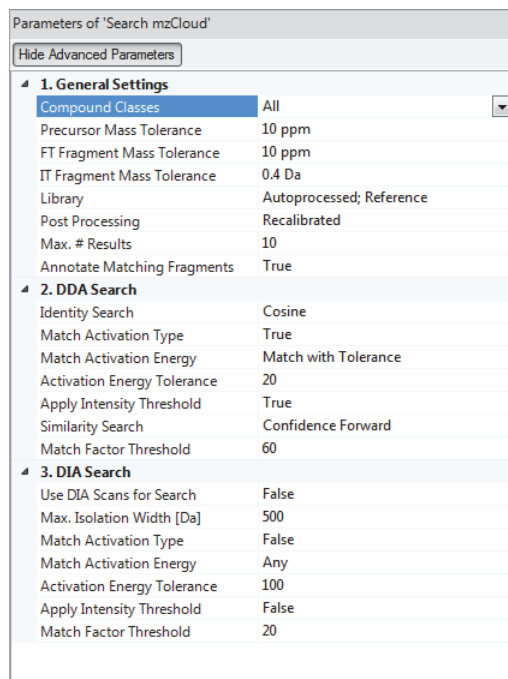
The node is set to the default settings. For each detected compound, the Pattern Matching algorithm uses the best MS1 scan and the Fragments Matching algorithm uses the best MS2 scan that the analysis finds across the raw data file set.



Parameters of 'Predict Compositions'	
<input type="button" value="Hide Advanced Parameters"/>	
1. Prediction Settings	
Mass Tolerance	5 ppm
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 N10 O18 P3 S5
Min. RDBE	0
Max. RDBE	40
Min. H/C	0.1
Max. H/C	4
Max. # Candidates	10
Max. # Internal Candidates	200
2. Pattern Matching	
Intensity Tolerance [%]	30
Intensity Threshold [%]	0.1
S/N Threshold	3
Min. Spectral Fit [%]	30
Min. Pattern Cov. [%]	90
Use Dynamic Recalibration	True
3. Fragments Matching	
Use Fragments Matching	True
Mass Tolerance	5 ppm
S/N Threshold	3

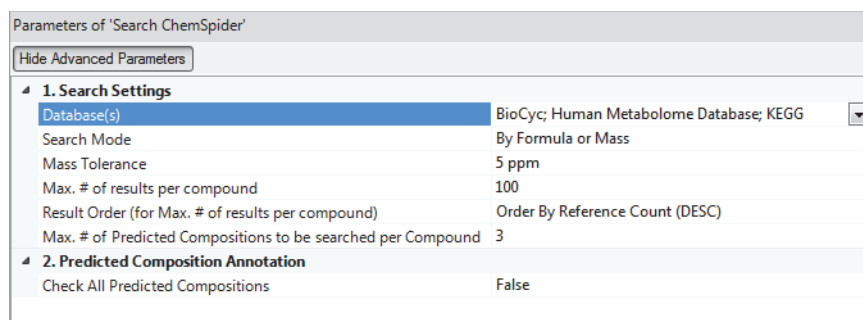
5. Open the parameter settings for the **Search mzCloud** node.

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

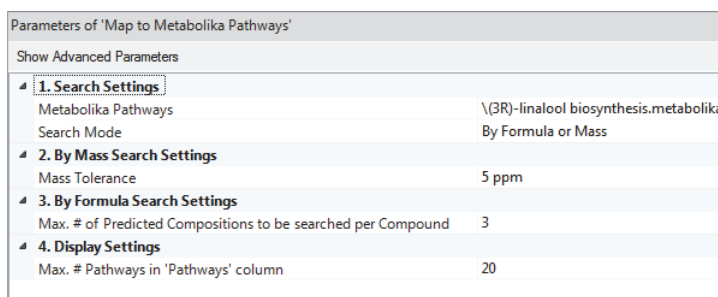


Parameters of 'Search mzCloud'	
<input type="button" value="Hide Advanced Parameters"/>	
1. General Settings	
Compound Classes	All
Precursor Mass Tolerance	10 ppm
FT Fragment Mass Tolerance	10 ppm
IT Fragment Mass Tolerance	0.4 Da
Library	Autoprocessed; Reference
Post Processing	Recalibrated
Max. # Results	10
Annotate Matching Fragments	True
2. DDA Search	
Identity Search	Cosine
Match Activation Type	True
Match Activation Energy	Match with Tolerance
Activation Energy Tolerance	20
Apply Intensity Threshold	True
Similarity Search	Confidence Forward
Match Factor Threshold	60
3. DIA Search	
Use DIA Scans for Search	False
Max. Isolation Width [Da]	500
Match Activation Type	False
Match Activation Energy	Any
Activation Energy Tolerance	100
Apply Intensity Threshold	False
Match Factor Threshold	20

- Open the parameter settings for the Search ChemSpider node.
Three out of 363 databases are selected. For this tutorial, do not change the selection.



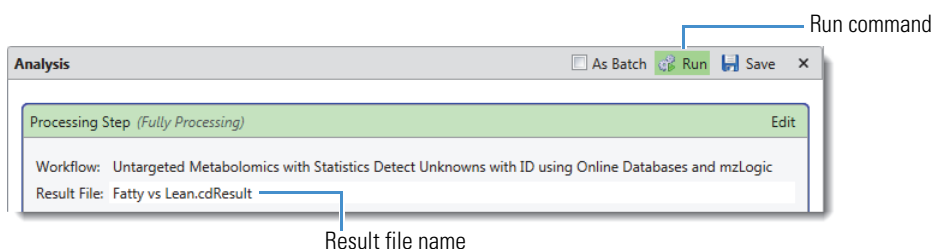
- Open the parameter settings for the Map to Metabolika Pathways node.
The node is set to search all the Metabolika pathways. For this tutorial, do not change the settings.



When the Run command in the Analysis pane becomes available, you can start the processing run.

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

- To create one result file for the input file 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.
- In the Result File box, rename the result file **Fatty vs Lean**.




- To submit the analysis to the job queue, click **Run**.

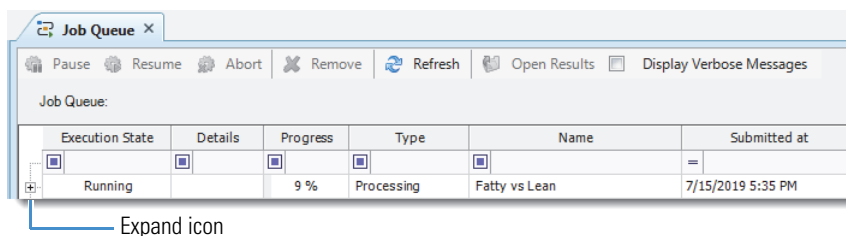
Tip The processing workflow for this tutorial includes the Differential Analysis node. If no groups or ratios are set up on the Grouping & Ratios page of the analysis, a warning message appears. If you are not interested in grouping or comparing the samples, you can ignore this warning.

- If a warning message appears, for this tutorial, do the following:
 - Click **Abort**.
 - Open the **Grouping & Ratios** page.
 - In the Study Variables area, select the **Phenotype** check box if it is not already selected.
 - In the Bulk Ratio Generation area, select the **Lean** check box if it is not already selected and click **Add Ratios**.
 - To restart the analysis, click **Run** in the Analysis pane.

The Job Queue page opens.

Submitting
the Analysis
to the Job
Queue

5. To view the processing messages, click the expand icon, , to the left of the job row.



Note During the run, the Map to Metabolika node generates a warning message, which you can ignore. Warning messages have a yellow background.

6:39 PM (34): Map to Metabolika Pa... Warning 8 pathway compounds with invalid elemental composition not persisted.

Reviewing the Analysis Results

To review the analysis results, follow these topics:

- Opening the Result File
- Default Result Page Layout
- Modifying the Layout of the Result Tables
- Reviewing the Chromatograms for Each Compound
- Reviewing the Full Scan MS1 Spectra
- Reviewing the Fragmentation Spectra
- Applying the Statistics Layout
- Working with the Trend Chart View
- Working with the Volcano Plot in the Differential Analysis View
- Viewing the Principal Component Analysis
- Working with the Partial Least Squares Discriminant Analysis View
- Running a Hierarchical Clustering Analysis
- Viewing the Metabolika Pathways for a Compound
- Reviewing the Results of the mzLogic Analysis

Opening the Result File

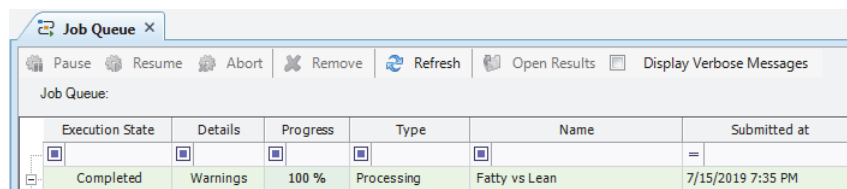
You can open a result file from multiple locations: the Job Queue page, the Analysis Results page of a study, the Compound Discoverer Start Page, or the menu bar.

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

1. Open the result file provided on the Compound Discoverer 3.1 USB key:
Example Studies\Metabolomics Study\ZDF Study\ZDF.cdResult
2. From the menu bar, choose **Window > Reset Layout**.
3. Sort the Compounds table in descending order by the Area (Max) column.

❖ To open the result file generated by the analysis

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



Tip If the Job Queue page is closed, from the menu bar, choose **View > Job Queue** to open it.

Default Result Page Layout

The factory default layout for a result file includes the following items:

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

Note If the data set does not include data-dependent MS2 scans within the retention time window but does include DIA scans within this window, the spectrum tree includes the DIA scans. Your Thermo Scientific data-visualization application displays DIA or AIF in the scan headers of data-independent scans. The Compound Discoverer application displays DIA for both of these scan types.

- A set of tabbed main tables below the two graphical views. For a metabolomics analysis, the Compounds table is the active table and is sorted by the Area (Max.) column—that is, the first row displays the compound with the largest chromatographic peak area (found in one of the input files).

Note Because the selected processing workflow includes the Mark Background 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.

- A collapsed area for the related tables below the main tables.

Figure 2 shows the factory default layout for the Fatty vs Lean.cdResult file (or the ZDF.cdResult file).

Note In Compound Discoverer 3.1, the chromatographic peak area for compounds and features is reported in counts \times seconds. The chromatographic peak area for a compound in each input file is the combined peak area for all its detected preferred ions (features).

Figure 2. Default result file layout

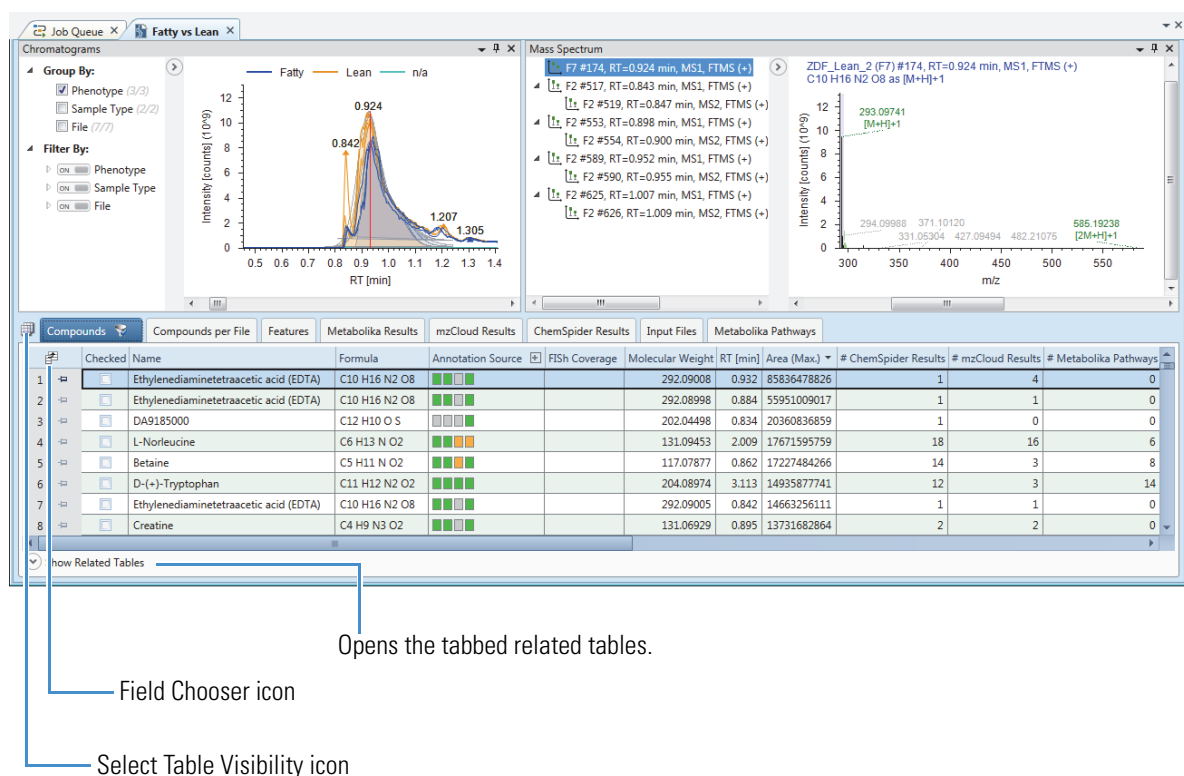


Table 2 describes the main result tables that the selected processing workflow produces.

Table 2. Main tables and some of the related tables for the selected processing workflow (Sheet 1 of 2)

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 input files. The RT of a compound is the weighted average (by area) of the compound across all the input files (see the related Compounds per File table)
Compounds per File	Lists all the compounds that the analysis detected across all the 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 input files on a per file basis.
Metabolika Results	Lists the mapped compounds across all the input files.
mzCloud Results	Lists the mzCloud search results across all the input files.
ChemSpider Results	Lists the ChemSpider search results across all the input files.
Input Files	Describes the input files that the application processed to create the result file.
Metabolika Pathways	Lists the Metabolika Pathways that contain at least one of the detected compounds across the input files.
Visible tables related to the Compounds table	
Structure Proposals	Displays your structure proposals for the selected compound. Initially, this table is empty.
Compound per File	Displays information about the selected compound on a per file basis.
Predicted Compositions	Displays the predicted compositions for the selected compound. The predicted compositions are based on the molecular weight of the neutral compound.

Table 2. Main tables and some of the related tables for the selected processing workflow (Sheet 2 of 2)

Result table	Description
Metabolika Result	Displays information about the matched and partially matched structures 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	Lists the pathways that include the selected compound.
Visible table related to the Features table	
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.
Hidden tables	
Adducts (main table)	Lists the adducts in the Adducts library.
Filled Gaps (related table)	Provides information about the imputed chromatographic peak areas.

Modifying the Layout of the Result Tables


The following topics describe how to modify the layout of the result tables:

- [Selecting the Visibility of the Result Tables](#)
- [Showing the Related Tables](#)
- [Showing or Hiding Table Columns](#)
- [Displaying the Input File Names for the Subcolumns](#)
- [Sorting Table Rows](#)

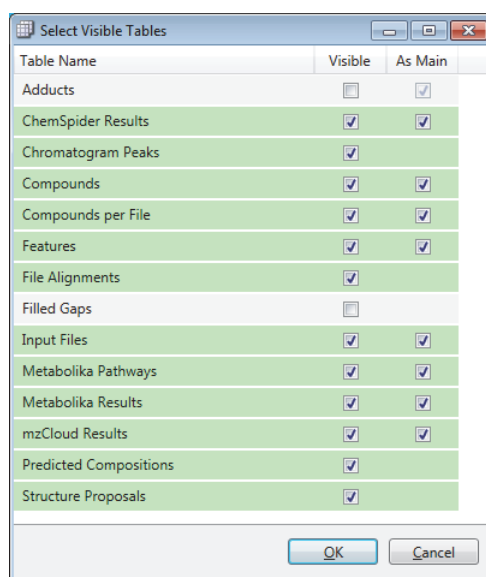
Selecting the Visibility of the Result Tables

The default layout does not include all the results tables that the selected processing workflow produces. By default, the Filled Gaps related table produced by the Fill Gaps node is a hidden table in the first set of related tables.

❖ To show or hide result tables

1. Click the **Select Table Visibility** icon, , to the left of the result table tabs.

The Select Visible Tables dialog box opens.



2. To display a table, select its check box. To hide a table, clear its check box.
By default, the Adducts and Filled Gaps tables are hidden.
For this tutorial, do not change the selections.
3. Click **OK**.

Showing the Related Tables

The main tables provide information about each compound across the input file set. The tables related to a main table provide details about the item selected in the main table. For example, the Compounds per File table for a compound in the main Compounds table provides information about the compound in each input file where it is detected.

❖ To view the tables related to the Compounds table


Click **Show Related Tables** at the bottom of the page to display the tables related to the Compounds table.

Tip To view the information for a specific compound in the main Compounds table, do the following:

1. Select the compound in the main table.
2. Click **Show Related Tables**.
3. In the related tables pane, click the tab of interest.

Showing or Hiding Table Columns

❖ To display or hide a table column

1. Click the **Field Chooser** icon, , to open the Field Chooser box.

In the Compounds table, the following columns are hidden:

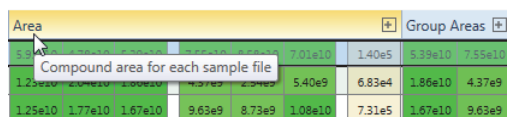
- #Adducts
- Area (per input file)
- Background
- Gap Status
- Normalized Area
- RT Tolerance [min]
- #Similarity Results
- Structure

2. Do one of the following:

- To display a column, select its check box.


For example, do display the Area column in the Compounds table, select the Area check box.

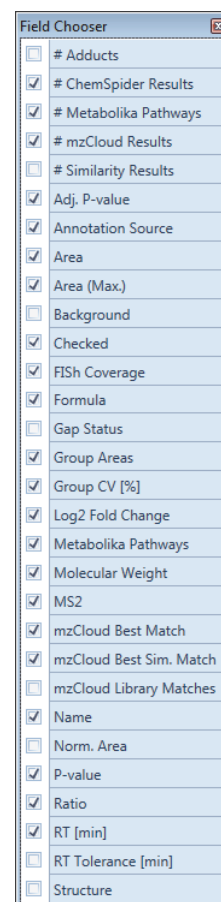
The Area column appears in the Compounds table to the left of the Group Areas column.



Area	Group Areas
1.25e10	1.40e5
1.77e10	5.40e9
1.67e10	6.83e4
9.63e9	1.86e10
8.73e9	4.37e9
1.08e10	7.31e5
7.31e5	1.67e10
1.67e10	9.63e9

- To hide a column, clear its check box.


3. To close the Field Chooser box, click its close icon, .



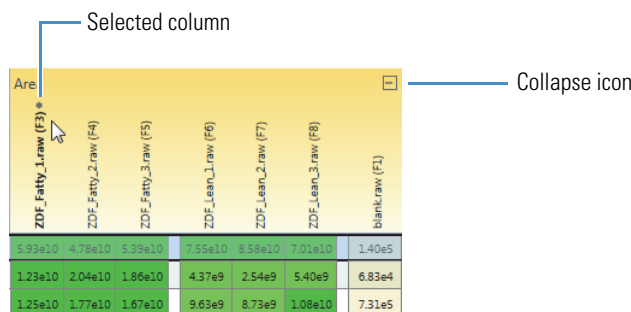
Displaying the Input File Names for the Subcolumns

In the Compounds table, the following columns contain a subcolumn for each input file in the processed data set: Metabolika Pathways, Areas (hidden by default), Group Area, Group CV [%], Ratio, Log₂ Fold Change, P-Value, and Adj. P-Value.

❖ To view the input file names for the area subcolumns

1. 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.raw (F1), appears in bold font and has an asterisk indicating that it is the selected area column.



Selected column

Collapse icon

Area							
ZDF_Fatty_1.raw (F3)*	ZDF_Faty_2.raw (F4)	ZDF_Faty_3.raw (F5)	ZDF_Lean_1.raw (F6)	ZDF_Lean_2.raw (F7)	ZDF_Lean_3.raw (F8)	blank.raw (F1)	
5.99e10	4.78e10	5.39e10	7.55e10	5.59e10	7.01e10	1.40e5	
1.23e10	2.04e10	1.96e10	4.37e9	2.54e9	5.40e9	6.83e4	
1.25e10	1.77e10	1.67e10	9.63e9	8.73e9	1.08e10	7.31e5	

2. Click the collapse icon, , to hide the sample names.

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

Sorting Table Rows

❖ To sort the table rows by the content of the table columns

1. Click a column header to sort the rows between ascending order (A, B, C ...) and descending order (Z, Y, X ...), based on the contents of the column.

Note The application treats formulas the same as text strings and sorts them by the order of the characters in the formula string, not by the actual number of elements in the formula.

2. To sort the data by a second column, hold down the CTRL key and click the second column heading.

❖ To sort the table rows by a column that contains a distribution map

1. Click the expand icon to display the vertical headings of the subordinate columns.
2. Select the heading of the subordinate column that you want to sort by.

The selected subordinate column heading appears in bold text.

3. Click the column heading to sort the table rows.

Follow these topics to review the chromatographic data. With the factory default layout, the Chromatograms view displays the overlaid traces for the compound that you select in the Compounds table. The integrated area of each chromatographic peak is shaded.

Follow these topics to select specific traces to view and to modify the display options.

- [Modifying the Display Options](#)
- [Filtering the Chromatographic Traces](#)

Reviewing the Chromatograms for Each Compound

Modifying the Display Options

❖ To view the chromatographic traces without the shading that can mask underlying peaks

1. Sort the Compounds table by Area (Max.) in descending order.
2. Select **row 8** to display the overlaid XIC traces for Creatine.

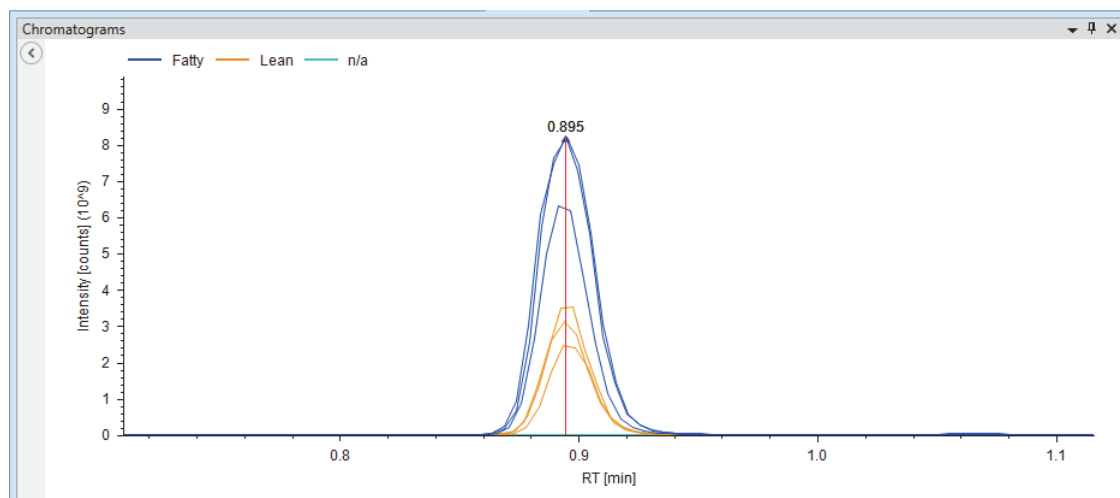
This figure displays the overlaid traces for the input files that contain the selected compound as well as the shortcut menu for the Chromatograms view. By default, the display options for the Chromatograms view are set to Show ToolTips, Show Detected Peaks, and Show Legend.



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

This figure shows the overlaid traces for all the samples.

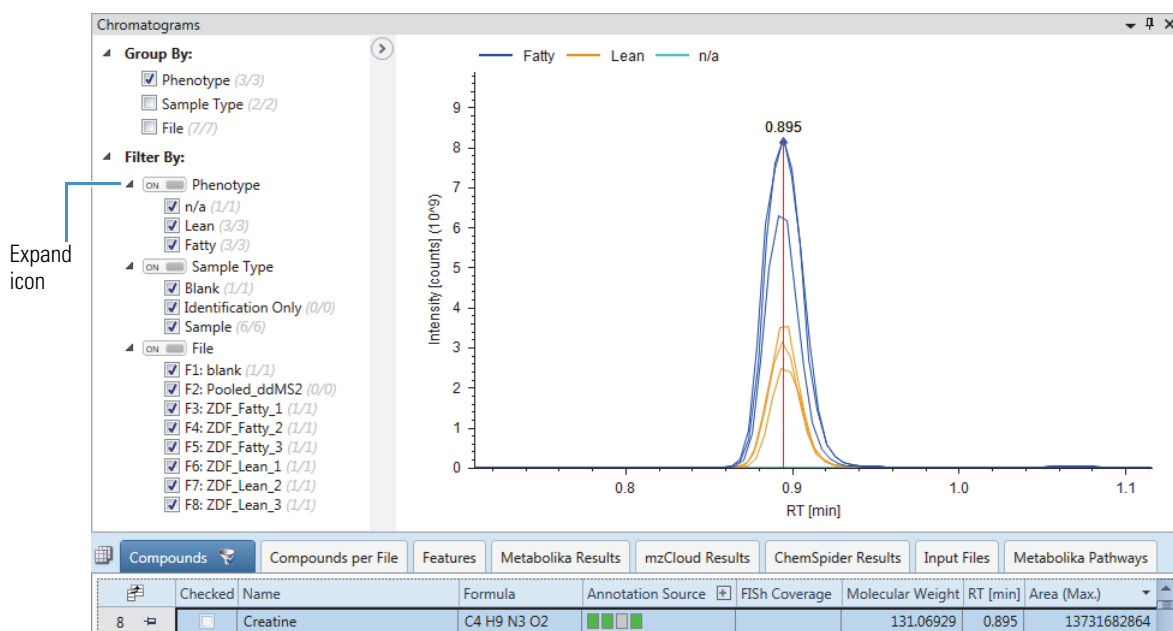
The application displays the three sample groups in different colors. The legend shows the display colors for the groups—blue for Fatty, and orange for Lean, and turquoise for N/A (the blank sample).



Filtering the Chromatographic Traces

❖ To review the traces for creatine by phenotype, sample type, or individual files

1. Under Filter By, expand Phenotype, Sample Type, and File by clicking their expand icons, ▲.



Note Under Group By, the count for Phenotype is 3 out of 3 (3/3). Under Filter By, Phenotype lists three groups, and all three groups (Lean, Fatty, and N/A) are selected. The N/A group consists of the Blank sample.

Under Filter By > File, the count for F2: Pooled_ddMS2 is (0/0). The application does not report compounds for the Identification Only sample type. It uses the fragmentation scans in this sample type for the mzCloud and mzVault searches and the Predict Composition node's Fragments matching algorithm.

Under Filter By > File, the count for the Blank is (1/1). When the processing workflow includes the Fill Gaps node, the application detects the compound (calculates a nonzero area value) in all the input files if it detects the compound in any of the input files.

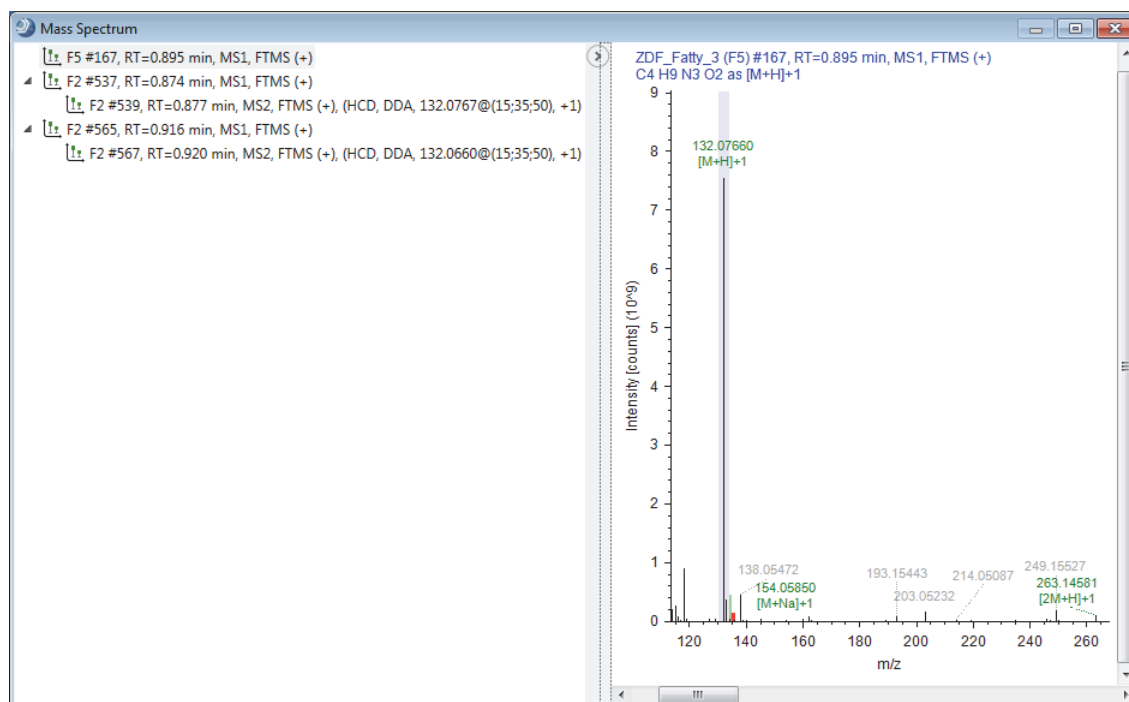
Reviewing the Full Scan MS1 Spectra

- Do any of the following under Filter By:
 - To view only the overlaid traces for the Lean phenotype, under Phenotype, click the **N/A** and **Fatty** check boxes.
 - To view only the overlaid traces for the Fatty phenotype, clear the **N/A** and **Lean** check boxes.
 - To view only the trace for the blank sample, under Sample Type, clear the **Identification** and **Sample** check boxes. Or, clear the Lean and Fatty check boxes under Phenotype.
 - To view only the trace for a specific file, under File, clear all the check boxes except for the check box for the file you want to view.





❖ To view a mass spectrum with color-coded isotopes for a compound with a predicted formula

- With the Compounds table sorted in descending order by the Area (Max.) column, select **row 8** (Creatine, C₄H₉N₃O₂).
- Expand the Mass Spectrum view to the full screen width by closing the Chromatograms view or by dragging the Mass Spectrum view by its title bar to a second monitor.

To close the Chromatograms view, click its **Close** icon.

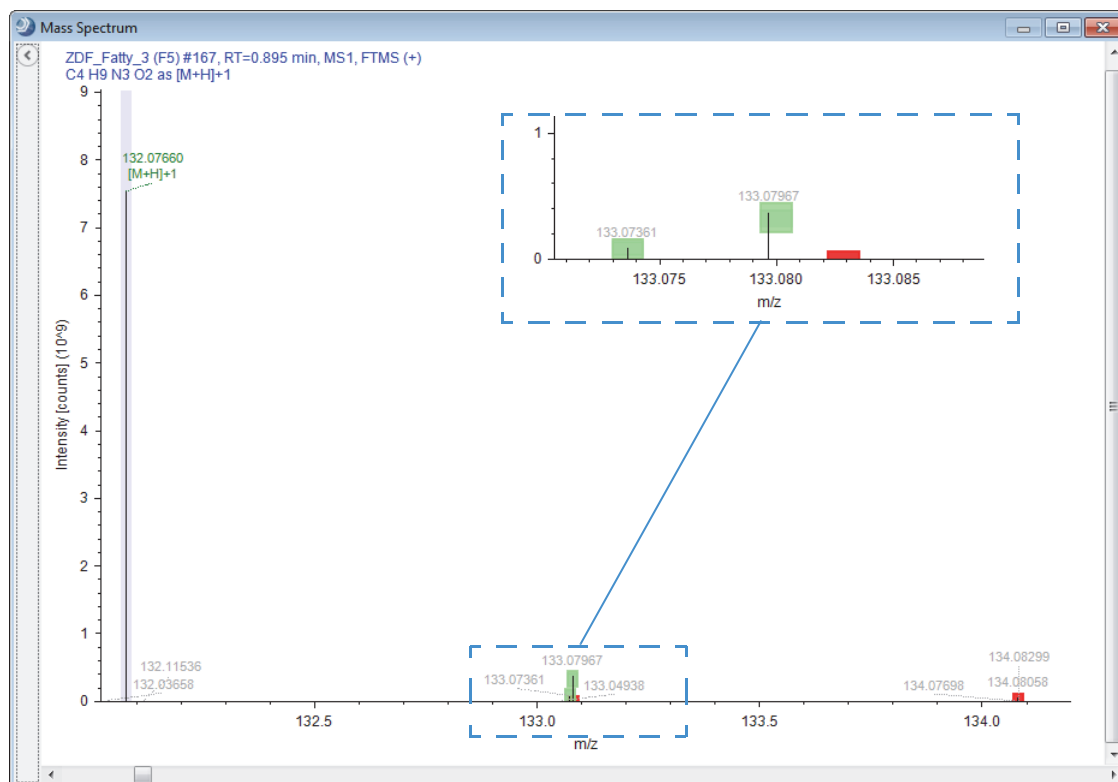


The mass spectral peaks in the full MS scan are color-coded as follows:

-  Lavender bars indicate centroids for A₀ (monoisotopic) ions. 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.
-  Green rectangles indicate matching centroids for isotopic ions. 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 rectangles indicate centroids that are missing from the expected isotopic pattern.
-  Blue rectangles indicate centroids that are missing from the expected isotopic pattern but that are also expected to have an intensity below the measured baseline noise (determined by the Fourier transform mass spectrometry (FTMS) mass analyzer).

- To zoom in on the isotopes detected for the $[M+H]^+$ adduct ion, drag the mouse pointer across the x axis from m/z 132 to m/z 135.

This figure shows three matching isotopes and two missing isotopes for the $[M+H]^+$ adduct ion.



- For more information about the matching and missing isotopes, click **Show Related Tables** below the Compounds table. Then, click the **Predicted Compositions** tab.

This figure shows that the Predict Compositions node matched 3 out of 5 isotopes to the theoretical isotope pattern for the calculated formula.

Hide Related Tables

Structure Proposals Compounds per File **Predicted Compositions** Metabolika Results mzCloud Results ChemSpider Results Metabolika Pathways

	Checked	Compound Match	Formula	Molecular Weight	Δ Mass [Da]	Δ Mass [ppm]	RDBE	H/C	Rank	# Matched Iso.	# Missed Iso.	# Matched Frag.	SFit [%]
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	C4 H9 N3 O2	131.06948	-0.00018	-1.40	2.0	2.2	1	3	2	8	34

Reviewing the Fragmentation Spectra

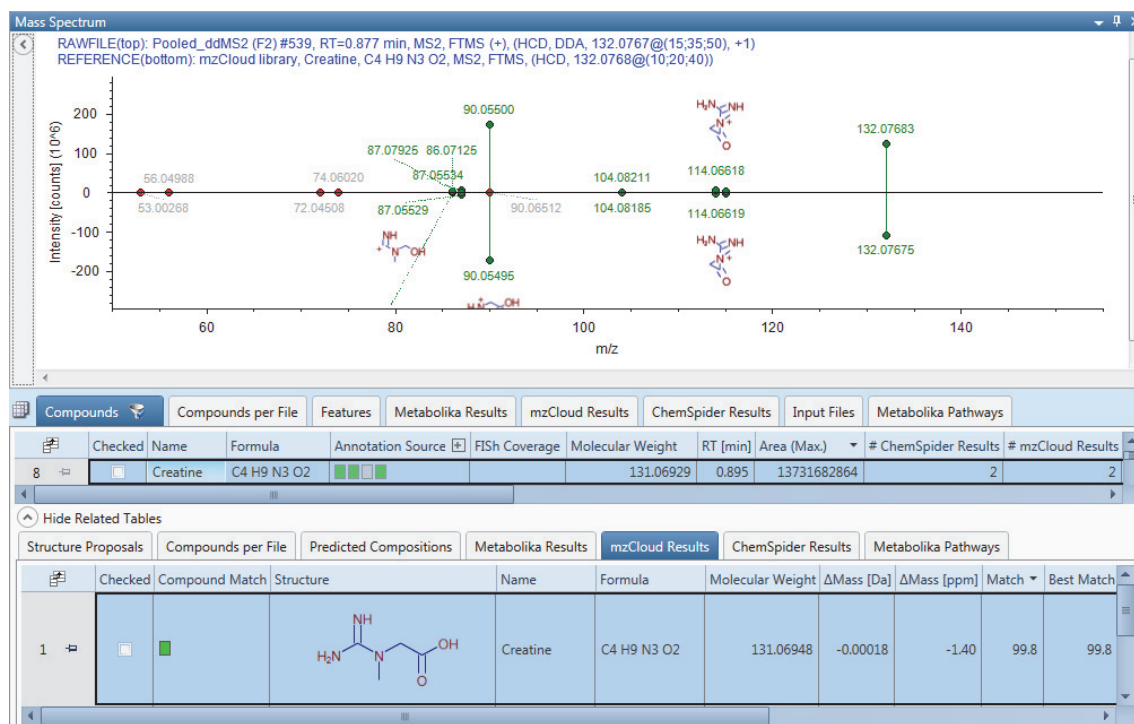
❖ To view the results of an mzCloud search for a compound and display a mirror plot

- With the Compounds table sorted in descending order by the Area (Max.) column, select **row 8** (Creatine, C4 H9 N3 O2).
- Click **Show Related Tables** to display the related tables below the main tables.
- Click the **mzCloud Results** tab to display the mzCloud Results table.
- Select the **row 1** in the mzCloud Results related table.

An annotated mirror plot appears in the Mass Spectrum view with the mass spectrum from the Pooled_ddMS2 sample on the top and the mzCloud reference spectrum on the bottom (Figure 3 on page 24).

A red circle on the m/z axis indicates an unmatched fragment. The green circles at the ends of centroids indicate matched fragments.

Figure 3. Annotated mirror plot for creatine



Applying the Statistics Layout

The application comes with the factory default layout and four named layouts: Identification, Quantification, Stable Isotope Labeling, and Statistics. When running statistical analyses, for ease of use, apply the Statistics layout.

❖ To apply the Statistics layout

1. If the result file is not the active page in the application window, click the result file tab.
2. From the menu bar, choose **Window > Reset Layout**. Then, choose **Window > Apply Layout > Statistics**.

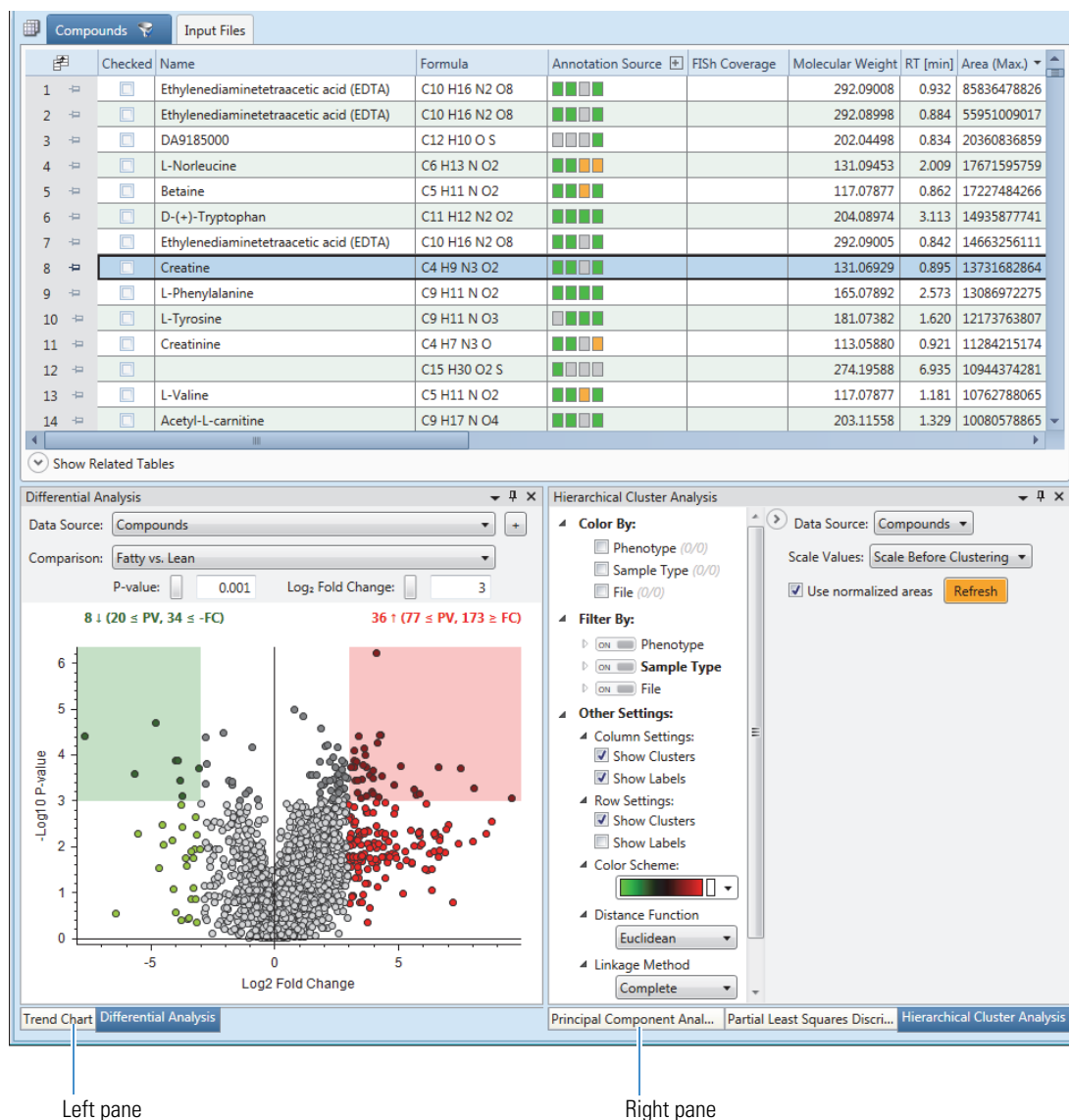
For the example result file, the Statistics layout does the following (Figure 4):

- Closes the Chromatogram view and the Mass Spectrum view. Moves the result tables to the top of the page and hides most of the main tables, except for the Compounds table and Input Files table.
- Opens the Differential Analysis and Trend Chart views as tabbed views on the bottom left of the page with the Differential Analysis view selected as the active tab. For the differential analysis, sets the P-value parameter to 0.001 and the Log₂ Fold Change parameter to 3.
- Opens the Principal Component Analysis, Partial Least Squares Discriminant Analysis, and Hierarchical Cluster Analysis views as tabbed views on the bottom right of the page.

Note The Compounds table, the Partial Least Squares Discriminant Analysis view, the Differential Analysis view, the Hierarchical Cluster Analysis view, and the Loadings Plot page of the Principal Component Analysis view are interactive—that is, checking the points in any of these views checks the points in all of these views.

In the Hierarchical Cluster Analysis view, a segmented bar—red for checked and gray for unchecked—to the right of the heat map indicates whether a compound is checked. In the other views, checked data points turn blue.

Figure 4. Statistics layout for the example result file



Go to the next topic “Working with the Trend Chart View.”

Working with the Trend Chart View

Use the Trend Chart view to compare the distribution of the chromatographic peak areas for each compound by sample group. When you select a single compound in the Compounds table, you can view the 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.

Follow these procedures in order:

1. To view a box-and-whiskers plot for a single compound
2. To view trend line plots for multiple compounds

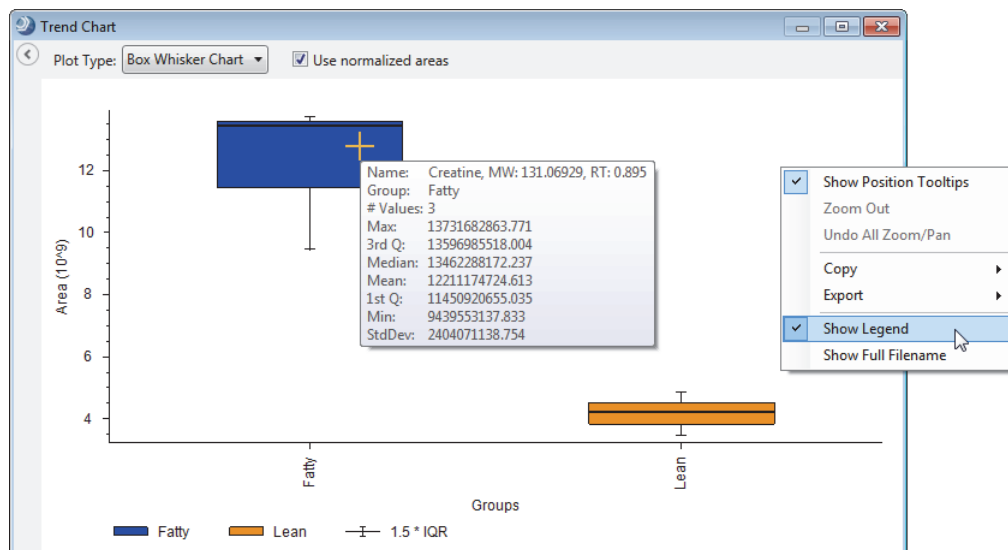
❖ To view a box-and-whiskers plot for a single compound

1. Apply the Statistics layout (see “Applying the Statistics Layout” on page 24).

Note Applying the Statistics Layout changes the sort order of the Compounds table.

2. Sort the Compounds table in descending order by Area (Max.).
3. Click the **Trend Chart** tab in the left pane.
4. Select **row 8** (Creatine, C₄H₉N₃O₂).

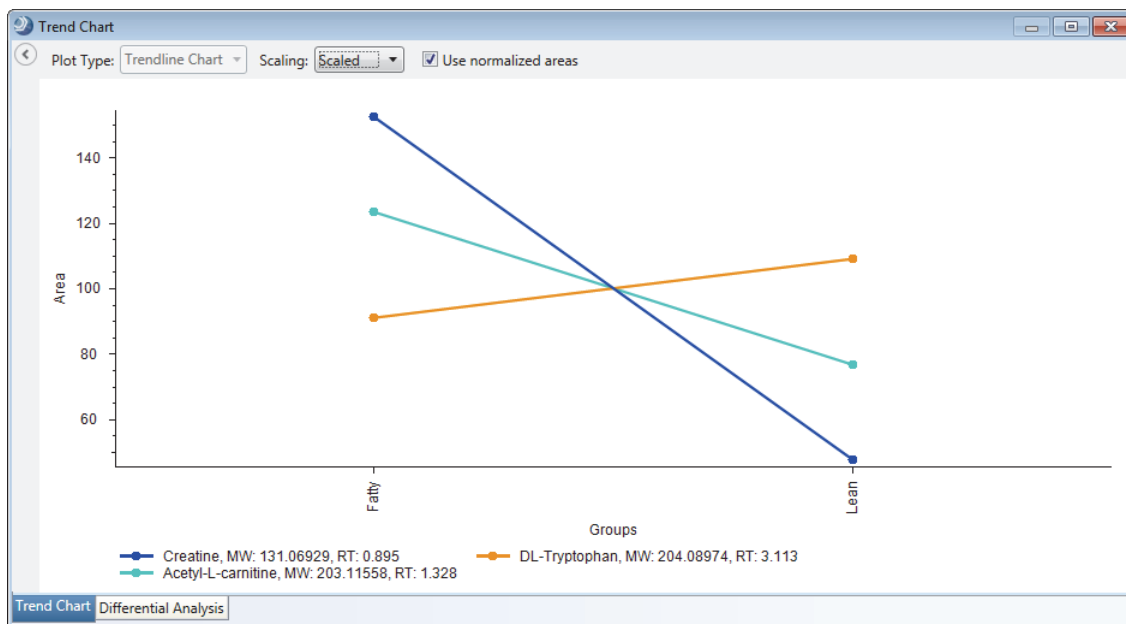
5. Right-click the chart and choose **Show Legend**.
6. Place the cross-hair cursor anywhere on a box or whisker to display a ToolTip with descriptive statistics. This figure shows the box-and-whiskers plot for creatine and the ToolTip for the Fatty sample group.



❖ **To view trend line plots for multiple compounds**

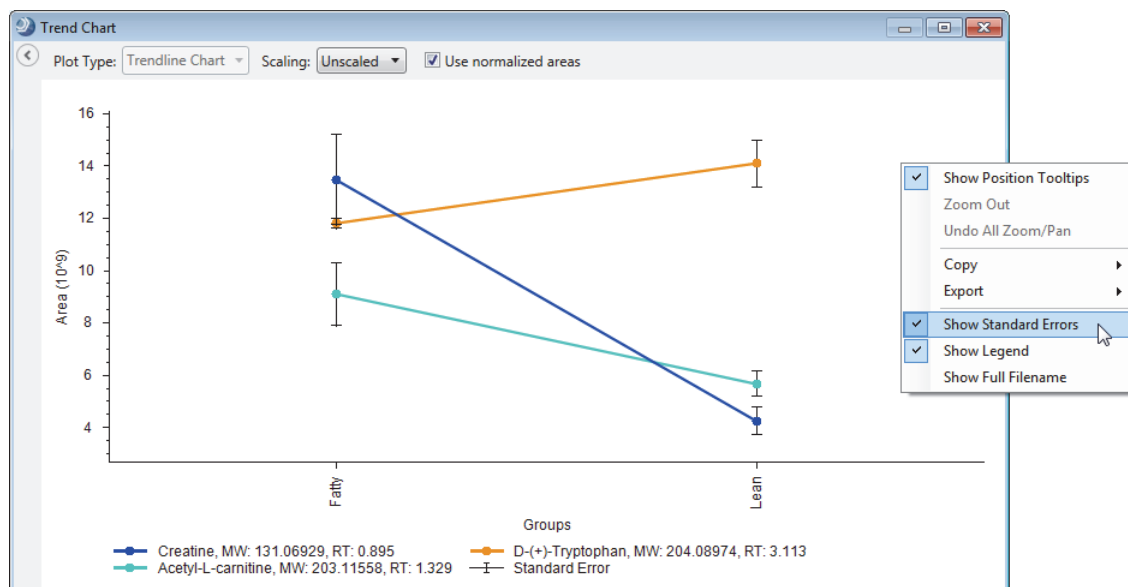
Hold down the CTRL key and select the following rows in the Compounds table: **row 8** (creatine), **row 6** (D-(+)-tryptophan), and **row 14** (acetyl-L-carnitine).

This figure shows the trendline plots for the selected compounds in the Trend Chart view.



7. To display the error bars for the trend line plots, do the following:
 - a. In the Scaling list, select **Unscaled**.
 - b. Right-click the plot and choose **Show Standard Errors**.

This figure shows trend line plots with error bars for the standard error.



Working with the Volcano Plot in the Differential Analysis View

Use the volcano plot in the Differential Analysis view to select the significant compounds for the comparison groups, which in this case are the Fatty and Lean ZDF rats. The volcano plot is a plot of the p-value, the result of a significance test, on the y axis versus the \log_2 fold change between two sample groups on the x axis. The y -axis scale is the $-\log_{10}$ of the p-value. As the p-value increases from 0 to 1, the $-\log_{10}$ of the p-value decreases from infinity to 0 ($-\log_{10} 0.05$ equals 1.3). The compounds in the shaded regions are statistically significant for the specified p-value and outside the specified upper and lower fold change thresholds.

If you have not already applied the Statistics Layout, apply it now (see “Applying the Statistics Layout” on page 24). Then, sort the Compounds table by the Area (Max) column.

To familiarize yourself with the Differential Analysis view, follow these procedures:

- To display the Differential Analysis view
- To display the legend for the volcano plot
- To change the p-value
- To change the \log_2 fold change value
- To select the up-regulated compounds or the down-regulated compounds
- To view information about a compound in the Compounds table and the graphical views
- To copy a compounds list with structures to the clipboard

❖ To display the Differential Analysis view

Click the **Differential Analysis** tab.

❖ To display the legend for the volcano plot

Right-click the view and choose **Show Legend**.

The legend appears at the bottom of the page. Each data point represents a compound. Placing the crosshair cursor over a data point displays a ToolTip with the XY coordinates, MW, RT, maximum area, and number of adducts.

This figure shows the default Statistics layout for the Differential Analysis view, the added legend, and the ToolTip for the data points.



❖ **To change the p-value**

Move the P-value slider to the left or right. Or, type a value in the box next to the slider.

❖ **To change the log₂ fold change value**

Move the Log₂ Fold Change slider to the left or right. Or, type a value in the box next to the slider.

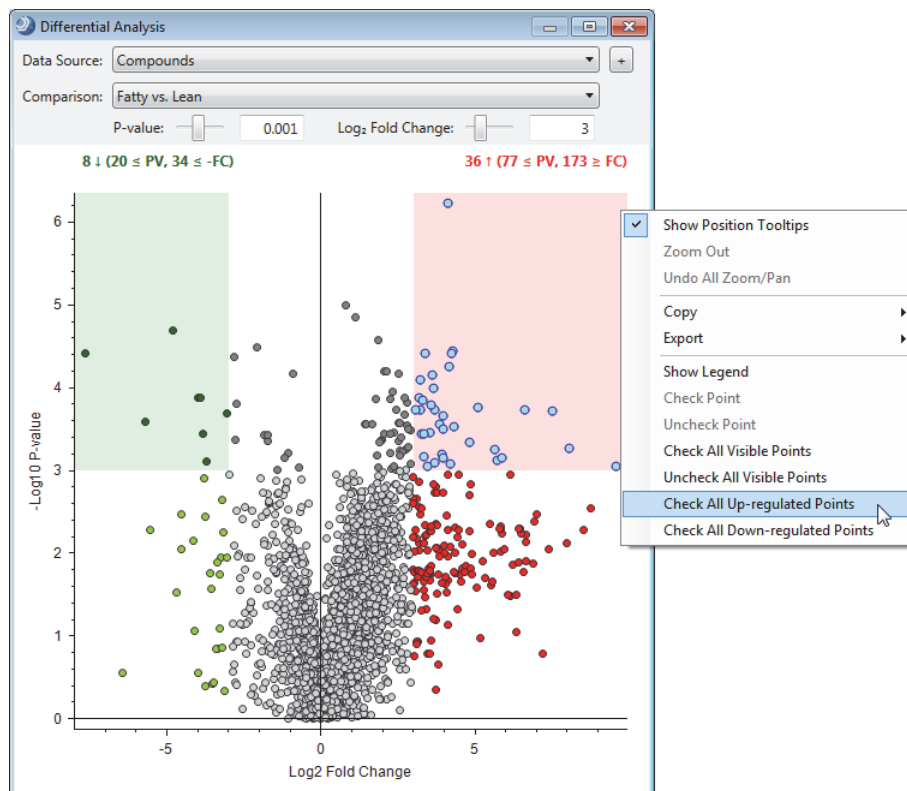
❖ **To select the up-regulated compounds or the down-regulated compounds**

- Right-click the plot and choose **Check All Up-regulated Points**.

–or–

- Right-click the plot and choose **Check All Down-regulated Points**.

The checked data points turn blue.



❖ **To view information about a compound in the Compounds table and the graphical views**

In the Differential Analysis view, double-click the compound or right-click the compound and choose **Check Point**.

The Compounds table automatically scrolls to the selected compound. If the Chromatograms view is open, it displays the overlaid XIC traces for the compound. If the Mass Spectrum view is open, it displays the MS1 scan for the compound.

❖ **To copy a compounds list with structures to the clipboard**

Right-click the Differential Analysis view and choose **Copy > Point Details**.

Use the Principal Component Analysis view to display the results of the principal component analysis. The principal component analysis reduces the dimensionality of the data set to a set of principal components, PC1, PC2, and so on, where PC1 is the principal component with the most variance.

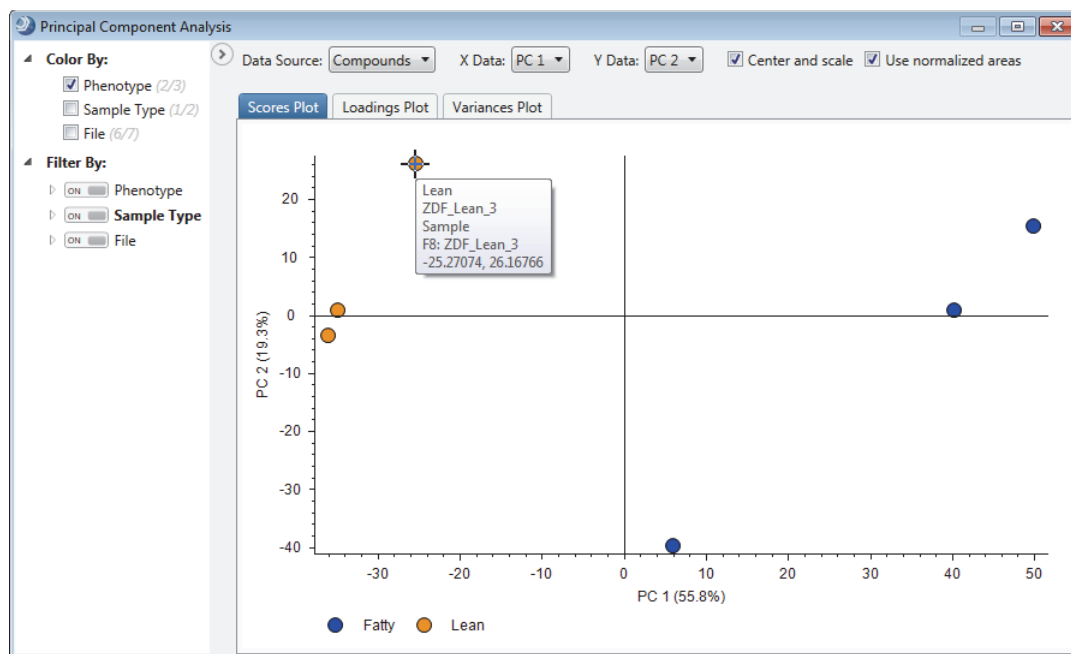
❖ **To view the Principal Component Analysis**

1. Apply the Statistics layout (see [“Applying the Statistics Layout”](#) on page 24).
2. Click the **Principal Component Analysis** tab in the right pane.

Use the Scores Plot page to interpret the relationship among the sample groups. Sample groups that are near each other are similar.

**Viewing the
Principal
Component
Analysis**

This figure shows the Scores Plot page for the Fatty and Lean sample groups.



Working with the Partial Least Squares Discriminant Analysis View

Use the Partial Least Squares Discriminant Analysis view to find a set of compounds that you can use to discriminate between sample groups.

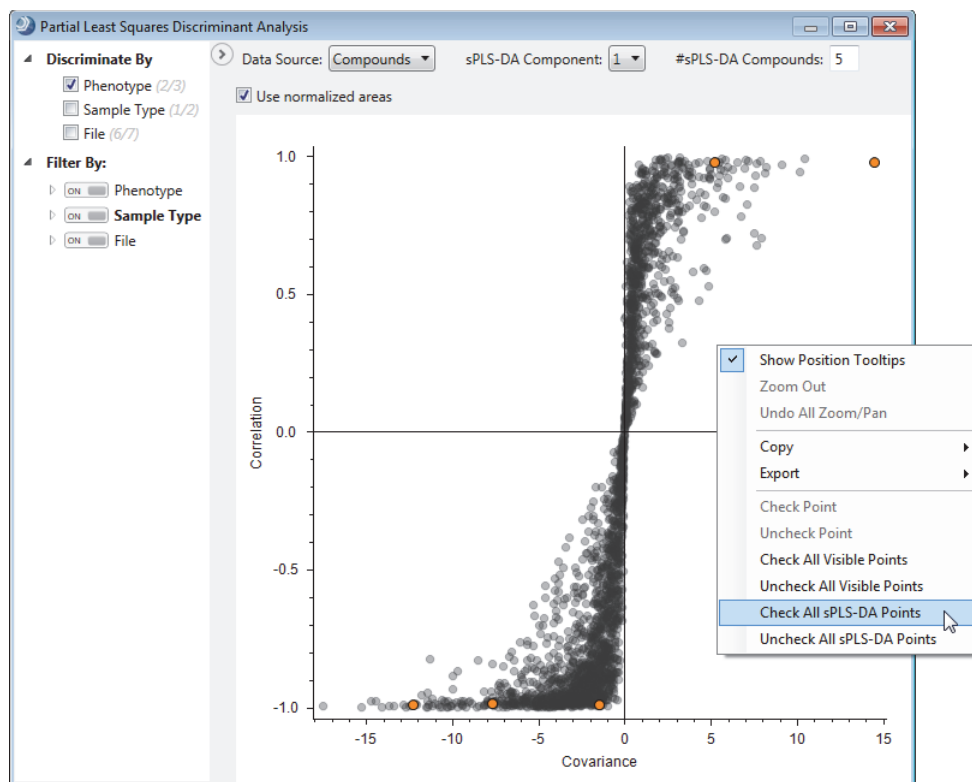
Follow these procedures:

- To make the Partial Least Squares Discriminant Analysis view active
- To change the number of discriminating compounds
- To automatically select the check boxes for the discriminating compounds in the Compounds table

❖ To make the Partial Least Squares Discriminant Analysis view active

1. Apply the Statistics layout (see “Applying the Statistics Layout” on page 24).
2. Click the **Partial Least Squares Discriminant Analysis** tab in the right pane.
3. If the graph includes checked data points (highlighted in blue), right-click the view and choose **Uncheck All Visible Points**.

This figure shows the Partial Least Squares Discriminant Analysis view with the default settings for the Statistics layout. The orange circles represent the five discriminating compounds.



❖ **To change the number of discriminating compounds**

1. Type the number of discriminating compounds in the **#sPLS-DA Compounds** box.
2. To start the analysis, click the view.

Orange circles represent the discriminating compounds. Place the crosshair cursor over a circle to display a ToolTip.

❖ **To automatically select the check boxes for the discriminating compounds in the Compounds table**

Right-click the view and choose **Check All sPLS-DA Points**.

The color of the circles changes to blue and the check boxes for the associated compounds in the Compounds table are selected.

Note If the discriminating compounds are checked, right-click the view and choose **Uncheck All sPLS-DA Points** before changing the number of discriminating compounds.

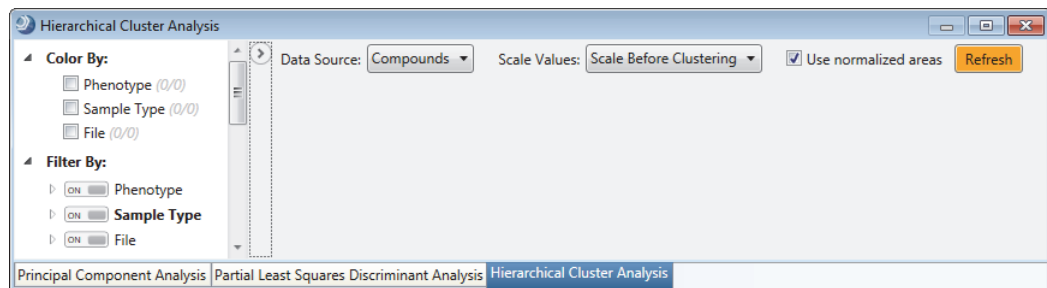
To display only the checked compounds in the Compounds table, see “[To filter the Compounds table by the checked compounds](#)” on page 38.

Running a Hierarchical Clustering Analysis

Use the Hierarchical Cluster Analysis view to visualize the correlation between detected compounds and selected samples in a two-dimensional array of color-coded rectangles (heat map) where each rectangle represents the relative amount (by area) of a specific compound in a specific sample.

❖ To view a hierarchical cluster analysis for the compounds in the Compounds table

1. Apply the Statistics layout (see “Applying the Statistics Layout” on page 24).
2. Click the **Hierarchical Cluster Analysis** tab in the right pane.



3. In the Hierarchical Cluster Analysis view, click **Refresh**.

A heat map and two dendrograms for the cluster analysis appear.

4. In the left pane, under Color By, select the **Phenotype** check box.

A color bar appears above the heat map to visually differentiate the samples by the two phenotypes—Fatty and Lean.

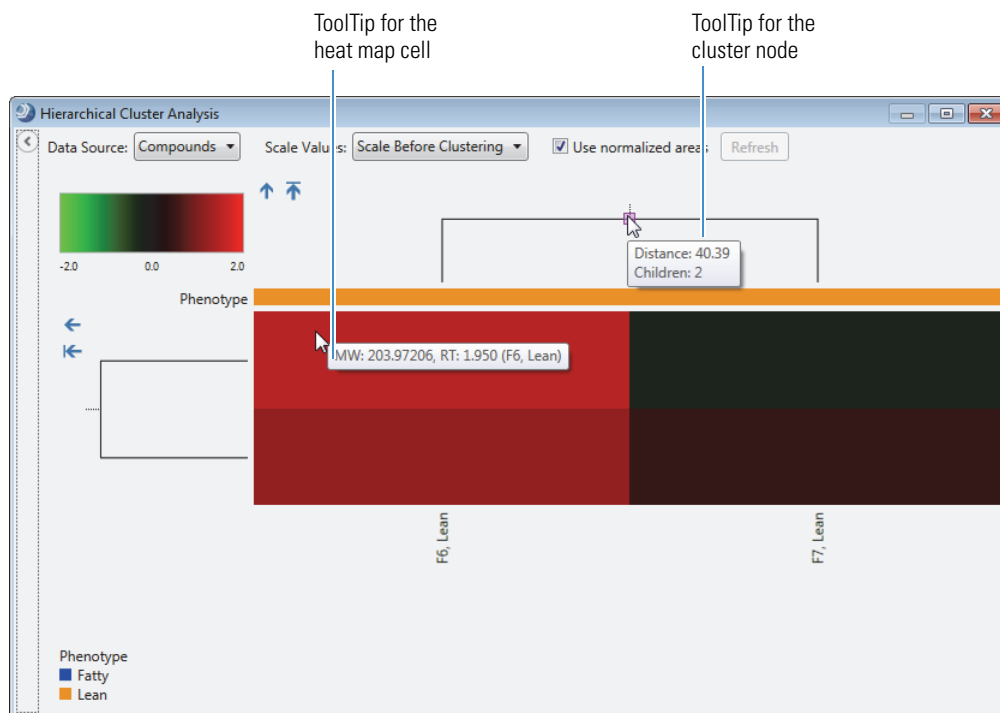
This figure shows a hierarchical cluster analysis for the compounds in the Compounds table. Pointing to a cluster's node displays a ToolTip.



- To zoom in on a specific area of the heat map, drag the pointer across the rectangular area of interest.
- To view information for a cell in the heat map, point to it.

The ToolTip displays the row and column coordinates. The row coordinates are the compound's name, MW, and RT. The column coordinates are the file ID and study factor values.

This figure shows a magnified view of four cells in the upper right corner of the heat map and their corresponding dendrograms.



Viewing the Metabolika Pathways for a Compound

The Map to Metabolika Pathways node searches for compounds that match the detected compounds in the selected Metabolika Pathway files.

❖ To view a Metabolika pathway for a detected compound

- From the menu bar, choose **Window > Reset Layout**.
- Sort the Compounds table by the Area (Max.) column in descending order.
- Select **row 10** (L-Tyrosine).
- Below the Compounds table, click **Show Related Tables**.
- Click the **Metabolika Pathways** tab.
- In the Metabolika Pathways table, select **row 5** (L-tyrosine degradation IV (to 4-methylphenol)).

Checked	Name	Formula	Annotation Source	FISH Coverage	Molecular Weight	RT [min]	Area (Max.)	# ChemSpider Results	# mzCloud Results	# Metabolika Pathways	Metabolika Pathways
<input checked="" type="checkbox"/>	L-Tyrosine	C9 H11 N O3			181.07382	1.620	12173763807	18	3	11	
<input checked="" type="checkbox"/>	Creatinine	C4 H7 N3 O			113.05880	0.921	11284215174	2	1	0	

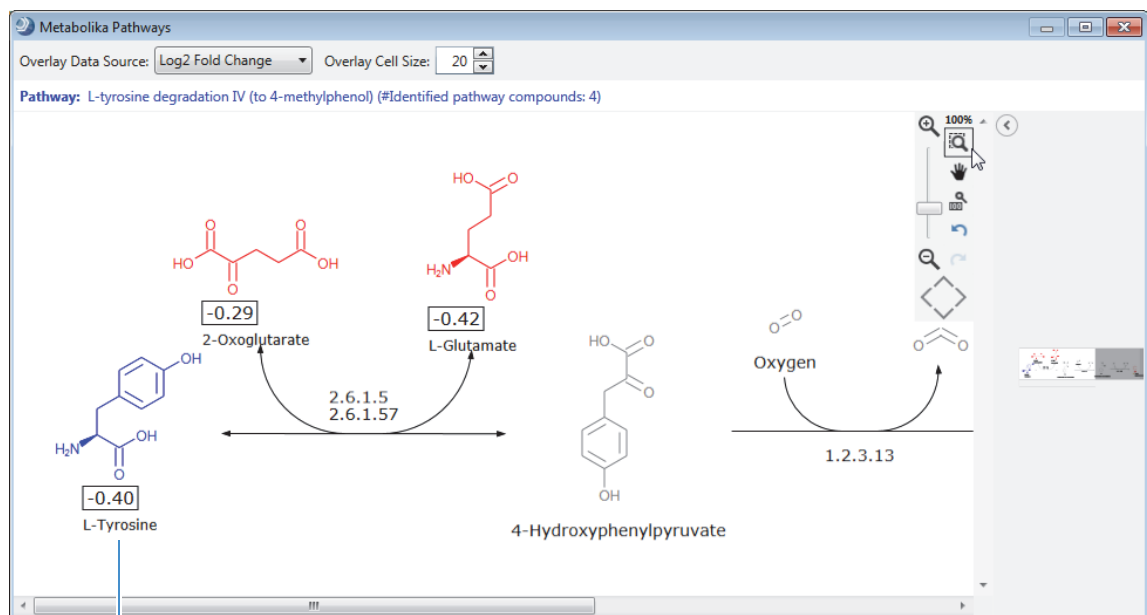
Checked	Pathway Name	# Referenced Compounds	Metabolika Compound Ids	Metabolika Compound Names	Metabolika Compound Formula	# Compounds in Pathway
<input type="checkbox"/>	Superpathway of chorismate metabolism	17	692	L-Tyrosine	C9 H11 N O3	152
<input type="checkbox"/>	Superpathway of aromatic amino acid biosynthesis	15	692	L-Tyrosine	C9 H11 N O3	47
<input type="checkbox"/>	Superpathway of thiamine diphosphate biosynthesis I	11	692	L-Tyrosine	C9 H11 N O3	44
<input type="checkbox"/>	Superpathway of betalain biosynthesis	10	692	L-Tyrosine	C9 H11 N O3	59
<input checked="" type="checkbox"/>	L-tyrosine degradation IV (to 4-methylphenol)	9	692	L-Tyrosine	C9 H11 N O3	9
<input type="checkbox"/>	Superpathway of 4-hydroxybenzoate biosynthesis (yeast)	8	692	L-Tyrosine	C9 H11 N O3	22
<input type="checkbox"/>	Superpathway of L-tyrosine biosynthesis	7	692	L-Tyrosine	C9 H11 N O3	28
<input type="checkbox"/>	Superpathway of rosmarinic acid biosynthesis	7	692	L-Tyrosine	C9 H11 N O3	37
<input type="checkbox"/>	Novobiocin biosynthesis	6	692	L-Tyrosine	C9 H11 N O3	60
<input type="checkbox"/>	Superpathway of plastoquinol biosynthesis	6	692	L-Tyrosine	C9 H11 N O3	17
<input type="checkbox"/>	Superpathway of L-phenylalanine and L-tyrosine biosynt	5	692	L-Tyrosine	C9 H11 N O3	11

- From the application menu bar, choose **View > Metabolika Pathways**.

The view opens to the right of the result tables and displays the selected pathway. The structure for the selected compound is blue, the structures for other compounds in the Compounds table are red, and the structures for other compounds in the pathway that are not in the Compounds table are black.

- In the Overlay Data Source list, select **Log2 Fold Change**.
- In the Overlay Cell Size list, increase the value to **20**.

This figure shows the selected pathway, data source, and overlay cell size.



Compound selected in the Compounds table

Reviewing the Results of the mzLogic Analysis

An mzLogic analysis requires candidate structures from the ChemSpider database, a Metabolika pathway, or a mass list with structures. It also requires matching structures from an mzCloud similarity search. The processing workflow for this metabolomics tutorial (Figure 1 on page 10) includes the two nodes that provide structures—the Search ChemSpider node and the Map to Metabolika Pathways node.

❖ To review the results of an mzLogic analysis for structure candidates

- In the Compounds table (sorted in descending order by the Area (Max.) column, select **row 8** (Creatine).
- Open the Compound table's Field Chooser dialog box ("To display or hide a table column" on page 18) and select the **#Similarity Results** check box.
- In the Compounds table, review the number of ChemSpider results and the number of similarity results for the selected compound.

For Creatine, the both the ChemSpider search and the mzCloud similarity search found two matches.

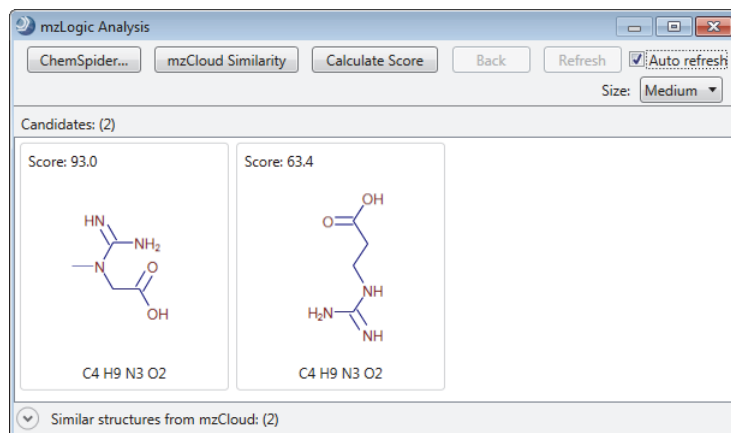
Checked	Name	Formula	Annotation Source	# ChemSpider Results	# mzCloud Results	# Similarity Results	# Metabolika Pathways
8	Creatine	C4 H9 N3 O2	Metabolika Results	2	2	2	0

Number of ChemSpider results

Number of mzCloud similarity results

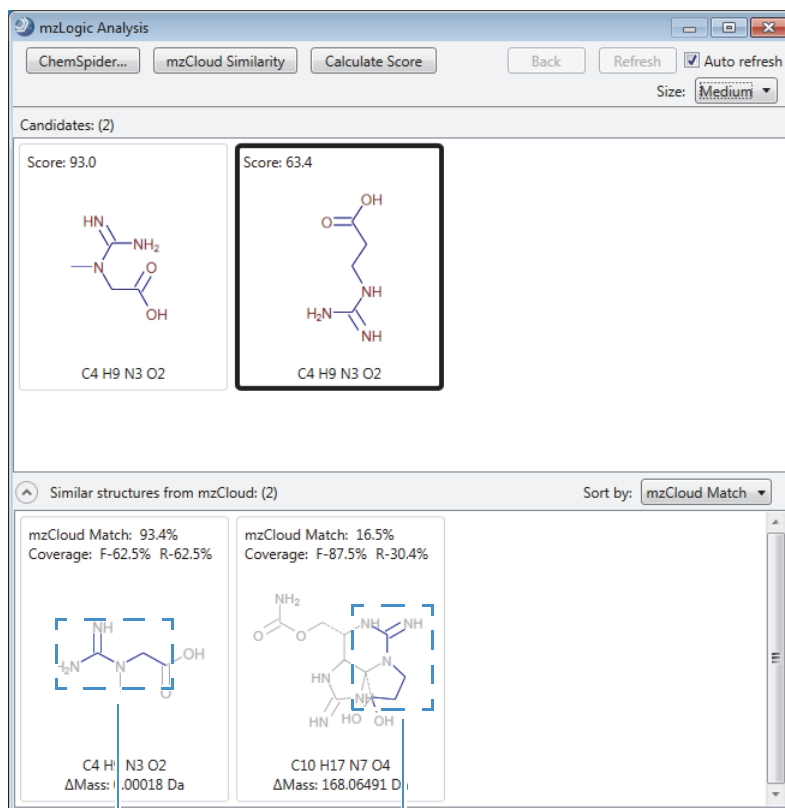
- From the application menu bar, choose **View > mzLogic Analysis**.

The mzLogic Analysis view opens to the right of the result tables.



- In the Candidates pane, select the structure for the lower ranking hit with a score of 63.4.
- Below the Candidates pane, click **Similar Structures from mzCloud**.

This figure shows the matching substructure in blue for the lower ranking compound in the related ChemSpider Results table for creatine.

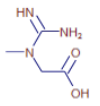



Matching substructure

Figure 5 shows the two structures in the ChemSpider Results table for creatine.

Figure 5. ChemSpider Results table for creatine

Comounds											
Checked	Name	Formula	Annotation So	FISH Coverage	Molecular Weight	RT [min]	Area (Max.)	# ChemSpider Results	# mzCloud Results	# Similarity Results	
8	Creatine	C4 H9 N3 O2			131.06929	0.895	13731682864	2	2	2	

Hide Related Tables											
ChemSpider Results											
Checked	Compound Match	Structure	Name	Formula	Molecular Weight	Δ Mass [Da]	Δ Mass [ppm]	CSID	# References	mzLogic Score	
1	<input checked="" type="checkbox"/>		Creatine	C4 H9 N3 O2	131.06948	-0.00018	-1.40	566	17637	93.0	
2	<input type="checkbox"/>		1705262	C4 H9 N3 O2	131.06948	-0.00018	-1.40	61020	230	63.4	

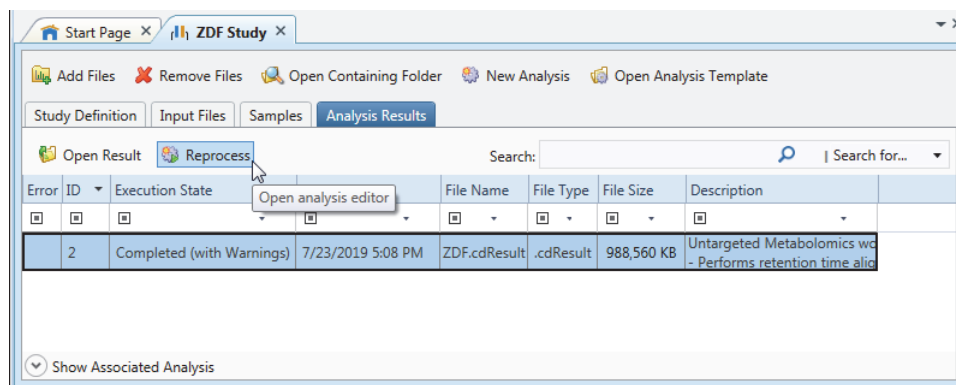
Reprocessing an Analysis

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

Note In the processing workflow for this tutorial, the Input Files, Select Spectra, Align Retention Times, Detect Compounds, Group Compounds, and Fill Gaps nodes are core nodes.

❖ To reprocess the same set of input files with a modified processing workflow

1. Open the Analysis Results page of the study.
2. Select the analysis that you want to reprocess.



3. Click **Reprocess**.

The Grouping & Ratios and Workflows tabs appear, and a Caution symbol appears in the Analysis pane. Pointing to the Caution symbol displays the following message:

Unchanged workflow. No node chosen for processing.

4. Click the **Workflows** tab.
All the nodes have lost their distinguishing colors, and the right sides of all the nodes are gray.
5. Right-click the **Input Files** node and choose **Reprocess**.
6. To reprocess the data, click **Run**.

Exporting the Analysis Results

Using the Result Filters to Select the Compounds of Interest

To create a report for your records or a file for external use by the TraceFinder™ application or the Xcalibur data system, 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. [Using the Result Filters to Select the Compounds of Interest](#)
2. [Exporting the Results to a Spreadsheet, Text, or CSV File](#)

The analysis found a total of 4086 compounds, including 1473 background compounds. By default, background compounds are hidden, so the Compounds table lists 2613 compounds. To reduce the number of compounds to review—for example, to display only compounds that are statistically significant, filter the table by p-value. To reduce the number of compounds to the discriminating compounds for the comparison groups or to the compounds of interest for other reasons, check the discriminating compounds or the compounds of interest to you and filter the table by the checked property.

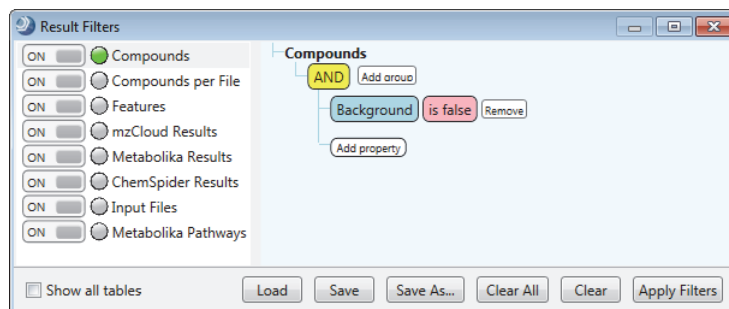
Note Pointing to the Compounds tab displays a ToolTip with the number of compounds in the table.

Checked	Compounds grouped by molecular weight and retention time	mzCloud Results
2	2613 of 4086 items shown (1473 filtered out)	6 N2 O8
3	DA9185000	C12 H10 O 5

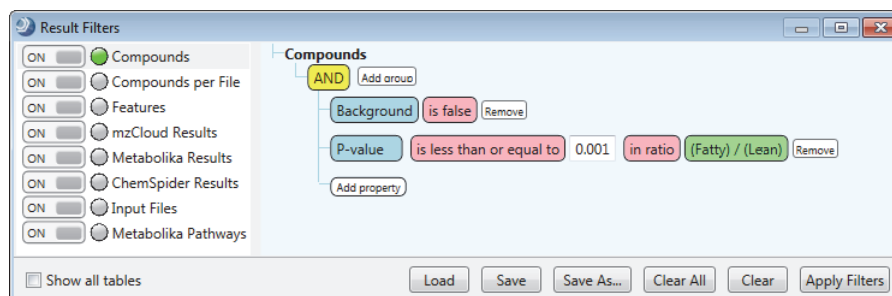
❖ To reduce the number of Compounds to review by using the p-value as a filter

1. In the main tables, click the **Compounds** tab.
2. From the 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, the filter for the Compounds table already includes a Background filter and the table does not display the background compounds.



3. In the right pane of the Result Filters view, set up a filter for the p-value as follows:
 - a. Click **Add Property** and select **P-Value** from the list.
 - b. In the pink relation list, select **Is Less Than or Equal To**.
 - c. In the value box next to the relation list, type **0.001**.
 - d. In the pink condition list, select **In Ratio**.
 - e. In the Green sample list, select **Fatty/Lean**.



4. Click **Apply Filters**.

The filter reduces the number of displayed rows in the Compounds table to 134.

❖ **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. Do one of the following:

- In the Partial Least Squares Discriminant Analysis view, select the compounds of interest as described in “To automatically select the check boxes for the discriminating compounds in the Compounds table” on page 31.
- In the Differential Analysis view, select the compounds of interest as described in “To select the up-regulated compounds or the down-regulated compounds” on page 29.
- 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, the Compounds table is currently filtered by one property—Background.

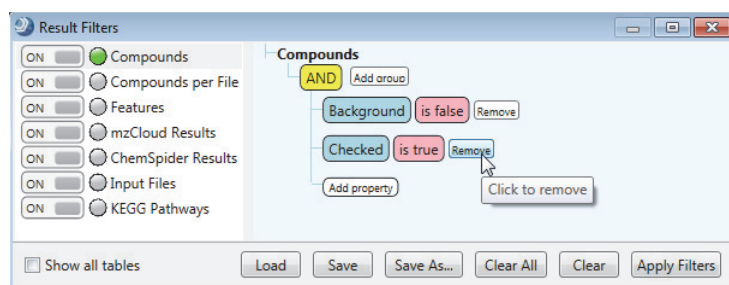
4. If there are more filter properties, remove them by clicking **Remove** to the right of each filter.

5. Click **Add Property** and select **Checked**.

6. Click **Apply Filters**.

The Compounds table displays only the selected compounds.

This figure shows the filter tree for the Compounds table.



7. To undo the Checked filter, click **Remove** to its right. Then, click **Apply Filters**. The Compounds table contains the original set of compounds.

Before exporting the results to a spreadsheet, an Xcalibur™ inclusion/exclusion list, a TraceFinder™-compatible CSV file, or a new mzVault library, filter the results table as described in “Using the Result Filters to Select the Compounds of Interest” on page 37.

Follow these topics as needed:

- [Creating a Report](#)
- [Creating an Xcalibur Inclusion/Exclusion List](#)
- [Creating a File to Import into the TraceFinder Compounds Database](#)
- [Exporting a Compound to an Existing mzVault Library](#)
- [Exporting Compounds to a New Library](#)

To create a report, follow these procedures as needed:

- [To check the number of table rows](#)
- [To sort the rows](#)
- [To display the table columns that you want to export](#)
- [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 sort the rows**

Click the column heading that you want to sort by.

❖ **To display the table columns that you want to export**

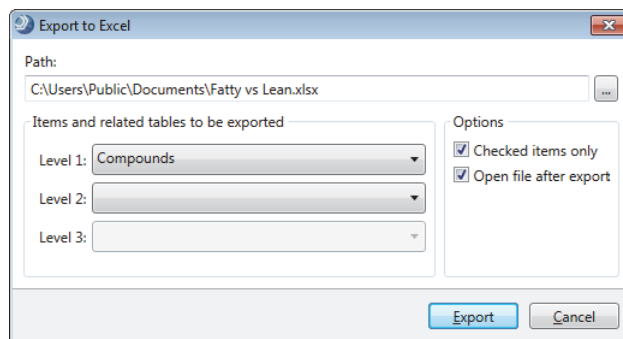
Open the Field Chooser box and select the check boxes for the columns of interest and clear the other check boxes (see “To display or hide a table column” on page 18). To view the areas for each input file, select the **Areas** check box.

❖ **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.

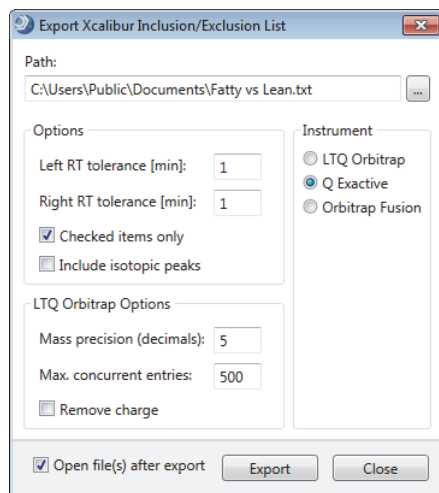
2. Check the file name and location in the Path box. Change the file name and location as appropriate.
3. In the Options area, select the appropriate options.



4. Click **Export**.
5. At the prompt, click **OK**.

❖ **To export the results to an Xcalibur Inclusion/Exclusion list**

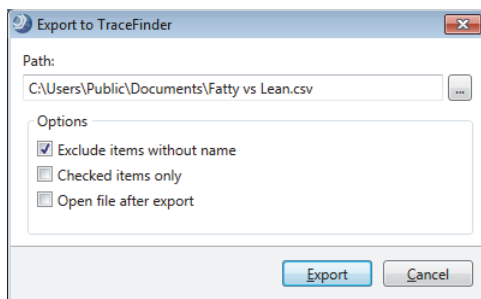
1. Right-click the Compounds table and choose **Export > As Xcalibur Inclusion/Exclusion List**.



2. In the Instrument area, select the instrument format for the exported masses.
3. (Optional) To export only the selected compounds, select the **Checked Items Only** check box.
4. (Optional) To automatically open the file in a spreadsheet application, select the **Open File(s) after Export** check box.
5. Click **Export**.
6. At the prompt, click **OK**.

❖ To export the results to a file that is compatible with the TraceFinder Compounds Database

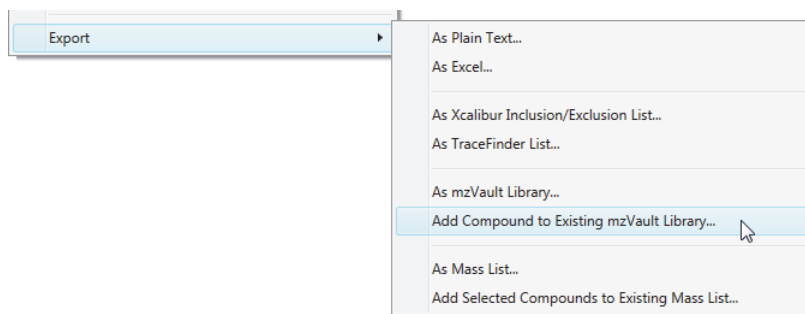
1. Right-click the Compounds table and choose **Export > As TraceFinder List**.
2. Select one or more of the options.



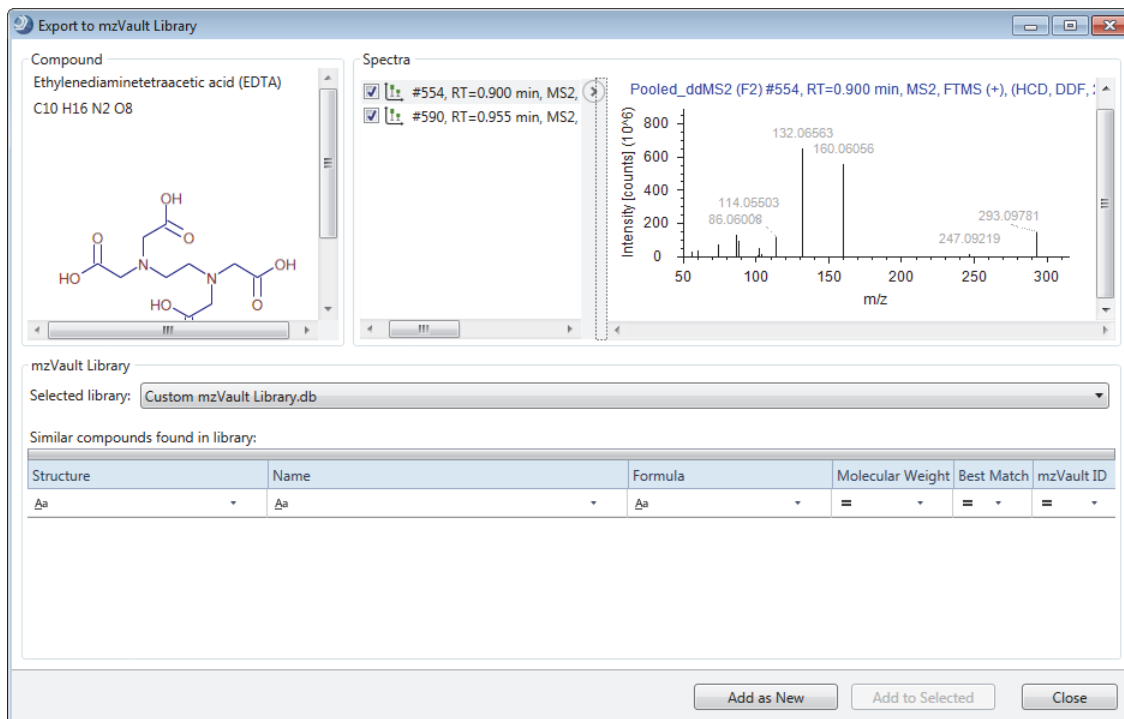
3. Click **Export**.
4. At the prompt, click **OK**.

❖ To export a compound to an existing mzVault Library

1. Right-click the compound in the Compounds table and choose **Export > Add Compound to Existing mzVault Library**.



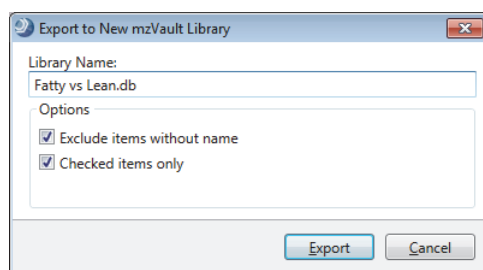
The Export to mzVault Library dialog box opens. Its Spectra view displays the available fragmentation spectra for the selected compound.



2. Do the following:
 - In the spectrum tree, select the check boxes for the spectra that you want to add to the compound entry.
 - In the mzVault Library area, select the existing mzVault library from the Selected Library list.
3. Do one of the following:
 - To add the spectra to an existing compound entry in the library, select the entry in the Similar Compounds Found in Library list. Then, click **Add to Selected**.
 - To add the compound and the selected spectra as a new compound entry, click **Add as New**.
4. Click **Close**.

❖ **To export the checked compounds to a new mzVault Library**

1. Right-click the Compounds table and choose **Export > As mzVault Library**.
The Export to New mzVault Library dialog box opens. By default, the Exclude Items Without Name check box is selected.
2. Select the **Checked Items Only** check box.



3. Click **Export**.
4. At the prompt, click **OK**.

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