# **Compound Discoverer 3.1 Tutorial for E & L Studies**

To familiarize yourself with the Thermo Compound Discoverer<sup>m</sup> 3.1 application and its features that address the data analysis requirements of the Extractables & Leachables market, follow the topics in this tutorial to set up a study and an analysis, process a set of example Xcalibur<sup>m</sup> RAW files, review the result file produced by the analysis, and create a report.

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- Setting Up a New Study and a New Analysis
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# **Overview**

To create a practice study, use the example Xcalibur RAW files. These files are provided on the key-shaped USB key in the software media kit. You can find these files in the following folder:

Example Studies\E & L O-ring Study\

Copy the E & L O-ring Study folder to your data processing computer. This figure shows the tutorial's workflow.



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The E & L O-ring Study folder contains the following files.

# Figure 1. Example files for E&L studies

Name	Туре	Name	Туре
Red_H2O_2.raw	RAW File	Brown_H2O_2.raw	RAW File
Red_H2O_1.raw	RAW File	Brown_H2O_1.raw	RAW File
Red_100EtOH_2.raw	RAW File	Brown_100EtOH_2.raw	RAW File
Red_100EtOH_1.raw	RAW File	Brown_100EtOH_1.raw	RAW File
Red_50EtOH_2.raw	RAW File	Brown_50EtOH_2.raw	RAW File
Red_50EtOH_1.raw	RAW File	Brown_50EtOH_1.raw	RAW File
H2O_2.raw	RAW File	100EtOH_2.raw	RAW File
H2O_1.raw	RAW File	100EtOH_1.raw	RAW File
EandL O-ring Example.cdStudy	CDSTUDY File	50EtOH_3.raw	RAW File
EandL Example.cdResultView	CDRESULTVIEW File	50EtOH_2.raw	RAW File
EandL Example.cdResult	CDRESULT File	50EtOH_1.raw	RAW File

# **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.

# Toolbar



# Accessing Help

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

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

- 1. Open the view, tabbed page, or dialog box.
- 2. On the computer keyboard, press the F1 key.

The application also provides a user guide, four tutorials by study field, and a quick start guide as PDF files.

# To access the user documentation

- 1. From the menu bar, choose **Help > Manuals**.
- 2. Select from the following documents.

Compound Discoverer Metabolism Tutorial Compound Discoverer Metabolomics Tutorial Compound Discoverer E&L Tutorial

Compound Discoverer Stable Isotope Labeling Tutorial Compound Discoverer Reporting Quick Start Compound Discoverer User Guide

# Checking the Computer's Access to the External Databases

This tutorial uses a processing workflow that searches the online mzCloud and ChemSpider databases and the local Extractables and Leachables mass list that comes with the application. To run the online searches, your processing computer must have unblocked access to the databases on the Internet.

# ✤ To verify that your computer has access to the online databases

- 1. From the menu bar, choose **Help > Communication Tests**.
- 2. Run the mzCloud communication tests as follows:
  - a. Click the **mzCloud** tab.
  - b. Click **Run Tests**. When the tests are complete, go to the next step.
- 3. Run the ChemSpider communication test as follows:
  - a. Click the **ChemSpider** tab.
  - b. Click **Run Tests**.

If your computer has an Internet connection and these tests fail, leave the Communication Tests dialog box open and press the F1 key to open the Help to the testing communication topic. Then, follow the instructions to troubleshoot the communication failure. If your computer does not have an Internet connection, you can complete this tutorial by modifying the processing workflow (see "To remove the search nodes that require Internet access" on page 15).

Go to the next topic "Setting Up a New Study and a New Analysis."

Make sure to copy the Xcalibur RAW files from the USB flash drive (in the software media kit) to an appropriate folder on your processing computer. See "Overview" on page 1.

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

- 1. Opening the New Study and Analysis Wizard
- 2. Setting Up the Study Folders
- 3. Selecting the Processing Workflow
- 4. Adding the Input Files to the Study
- 5. Defining the Study Variables
- 6. Setting Up the Sample Groups and Ratios
- 7. Closing the Wizard
- 8. Reviewing the Processing Workflow

To set up a new study, use the New Study and Analysis Wizard.

To open the New Study and Analysis Wizard

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

The New Study and Analysis Wizard opens.

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

# Setting Up a New Study and a New Analysis

# Opening the New Study and Analysis Wizard

Use the second page of the New Study and Analysis Wizard to name the study, select the studies folder for storing all or some of your studies, and select a processing workflow for the current analysis.

ي S	New Study and Analysis Wi tudy Name and Processir Specify a unique name for and select a processing v	zard - Step 2 of 6  Workflow  r this study and its folder, select the studies folder for storing all of your study folder: workflow for the current analysis.	= <b>X</b>	
	Study Name and Director	y Structure		
	Study Name: Studies Folder: Study Template File: Description:	New Study       (Optional)		— Initially, the top-level study folde is undefined.
	Processing	(appty workflow)		
(	?	Cancel < Back Next > Fit	inish	

# 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. On the Study Name and Processing Workflow page, in the Study Name box, name the study.
  - For an extractables and leachables study with the o-ring example files, type E and L Example.
  - For a study that includes your own Xcalibur RAW files, type an appropriate name.
- 2. Select the folder where you want to store your Compound Discoverer study folders as follows:
  - a. Click the browse icon, ...., next to the Studies Folder box.
  - b. Browse to your local disk drive or a location on your local network.
  - c. Click New Folder to create a new folder and name the folder Studies.

Study Name:	E and L O-ring Example	
Studies Folder:	C:\Studies	
Study Template File:	(Optional)	
Description:	(Optional)	

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 E and L Example.cdStudy file, stores the study file in the E and L O-ring Example folder, and stores the E and L O-ring Example folder in the Studies folder. When you run an analysis, the application stores the result files (.cdResult) in the E and L O-ring Example folder.



In the Compound Discoverer application, the processing method that interprets the raw data is called a processing workflow (.cdProcessingWF). The application comes with defined processing workflows for several fields of study including the E & L field. This tutorial uses a defined processing workflow that searches the mzCloud and ChemSpider databases, a local mzVault library, and the Extractables and Leachables mass list to identify the compounds detected in a set of input files (Xcalibur RAW files).

# To select the processing workflow

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

Workflow Templates\EandL\E and L w Stats Unknown ID w Online and Local Database Searches

Processing		
Workflow:	WorkflowTemplates \ EandL \ E and L w Stats Unknown ID w Online and Local Database Searches	
Workflow Description:	Untargeted E&L ID workflow with statistics: Detect and identify unknowns with differential analysis. -Performs retention time alignment, unknown compound detection, and compound grouping across all samples. Predicts elemental compositions for all compounds, and hides chemical background (using Blank samples). Identifies compounds using mzCloud (ddMS2 and/or DIA), ChemSpider (exact mass or formula) and local database searches against Mass Lists (exact mass with or withou RT). Performs spectral similarity search against mzCloud for compounds with ddMS2. Applies mzLogic to rank order structures from ChemSpider and mass list search results. Applies spectral distance scoring to mass list and ChemSpider matches. Calculates Kendrick mass defects for specified polymer repeating units. Imports UV, PDA, analog traces into the result file for correlation with MS data. Fill gaps by redetecting peaks for compounds with empty area for differential analysis.	

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

# To add input files to the study

- 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 18 of the Xcalibur RAW files in this folder and click Open.

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

New Study and Analysis Wizard - Step 3 of 6		
Input File Selection Select the input files for this analysis.		
🙀 Add Files 🛛 🗱 Remove Files		
Files		
50EtOH_1 Type: RAW File	Date modified: 6/25/2015 4:50:58 AM Size: 557.59 MB	*
50EtOH_2 Type: RAW File	Date modified: 6/25/2015 5:52:06 AM Size: 557.47 MB	E
100EtOH_1 Type: RAW File	Date modified: 6/26/2015 5:19:10 AM Size: 559.44 MB	•
18 files		
<i>?</i>	Cancel < Back Next	> Finish
<i>?</i>	Cancel < Back Next	> Finish

Number of selected data files

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

# Adding the Input Files to the Study

# Defining the Study Variables

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 the study variables, which include the sample types and the study factor values. In this tutorial, you are comparing the compounds extracted from two types of o-rings by three different solvents. The o-rings are identified by their colors—Red or Brown. The three solvents are water, 50% ethanol, and 100% ethanol. The sample types are Sample and Blank.

Follow these topics:

- Defining and Assigning the Study Factors
- Manually Selecting the Blank Sample Type

This figure shows the Input File Characterization page with the newly imported input files.

variables from each input file, click A	dvanced.	or eac	in input i	ile. Or,	to setup a regular expression that automa	tically extracts the	study
Delimiters: 🔲 Underscore 🔲 Hyph	en 🔳 Com	nma	Space	e 🔳 P	lus 🗌 Other 🛛 🎾 Assign 👔	💿 Reset 🛛 🕿 A	dvanced
Study Factors Paste Copy	Add 🕶 S	Sampl	es				
	E	rror	Samp 🔺	File	Sample Identifier	Sample Type	
	0				•		*
			S1	F1	50EtOH_1	Sample	•
			S10	F10	Brown_H2O_2	Sample	•
			S11	F11	H2O_1	Sample	-
			S12	F12	H2O_2	Sample	•
			S13	F13	Red_50EtOH_1	Sample	-
			S14	F14	Red_50EtOH_2	Sample	-
			S15	F15	Red_100EtOH_1	Sample	*
			S16	F16	Red_100EtOH_2	Sample	•
			S17	F17	Red_H2O_1	Sample	-
			S18	F18	Red_H2O_2	Sample	-
			S2	F2	50EtOH_2	Sample	-
			S3	F3	100EtOH_1	Sample	-
			S4	F4	100EtOH_2	Sample	•
			S5	F5	Brown_50EtOH_1	Sample	•
			S6	F6	Brown_50EtOH_2	Sample	•
			S7	F7	Brown_100EtOH_1	Sample	•
			S8	F8	Brown_100EtOH_2	Sample	
			S9	F9	Brown_H2O_1	Sample	
	6	✓) Sh	ow Asso	ciated I	File		

# **Defining and Assigning the Study Factors**

To define and assign the study factors, follow these procedures:

- 1. To select the delimiters that separate the factors in the file names
- 2. To add a categorical study factor for the solvent
- 3. To add a categorical study factor for the o-ring color
- 4. To assign the study factor values to the samples

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

Select the **Underscore** check box.

Delimiters: 🗹 Underscore 🔲 Hyphen 📄 Comma 💭 Space 💭 Plus 💭 Other

The study factors are o-ring (red or brown) and solvent (water, 50% ethanol, or 100% ethanol).

#### To add a categorical study factor for the solvent

 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.

tudy Factors	Paste Copy Add
[new factor]	Apply Cancel 🗙
Items:	

LText-entry box for study factor items

- 2. Type the factor name: Solvent.
- 3. If the editor closes before you type o-ring click Edit to reopen it. Select [new factor] and type Solvent.

[new factor] Edit ×

4. Add the solvents types to the Items list by entering text in the box next to the Add button:

**Note** When the file names include study factors that are separated by the selected delimiters, the application automatically parses the file names and enters the study factor values in the box next to the Add button as you type.

- a. Begin typing H2O and click **Add**.
- b. Begin typing 50EtOH and click Add.

c. Begin typing 100EtOH and click Add.

5. Click Apply.

# ✤ To add a categorical study factor for the o-ring color

- 1. In the Study Factors area of the Input File Characterization page, choose Add > Categorical Factor.
- 2. Type the factor name: O-ring.
- 3. Add the o-ring colors to the Items list by entering text in the box next to the Add button:

a. Begin typing Red and click Add.

- b. Begin typing Brown and click Add.
- 4. Click Apply.

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

#### To assign the study factor values to the samples

In the command bar next to the delimiters, click Assign.

🎾 Assign 🛛 Reset ा 🕿 Advanced

The application assigns the study factor items to the samples.

This figure shows the study factor assignments.

Delimiters: 📝 Under	score 🔲 Hyphen 🔲 Comm	a 🔳 Sp	oace 🔲 Plus	Other		🌮 Assig	n 🔊 Reset	🐨 Advan
Study Factors	Paste Copy Add •	Samp	les					
Co-ring	Edit ¥	Error	Sample	▲ File	Sample Identifier	Sample Type	Solvent	O-ring
Apromig	Decum		• •	• •	•	• •		• •
	Red		S1	F1	50EtOH_1	Sample	50EtOH •	n/a
			S10	F10	Brown_H2O_2	Sample	H2O •	Brown
Solvent	Edit ×		S11	F11	H2O_1	Sample	H2O •	n/a
	100EtOH		S12	F12	H2O_2	Sample	H2O •	n/a
	50EtOH		S13	F13	Red_50EtOH_1	Sample	50EtOH •	Red
	H20		S14	F14	Red_50EtOH_2	Sample	50EtOH •	Red
			S15	F15	Red_100EtOH_1	Sample	100EtOH -	Red
			S16	F16	Red_100EtOH_2	Sample	100EtOH •	Red
			S17	F17	Red_H2O_1	Sample	H2O •	Red
			S18	F18	Red_H2O_2	Sample	H2O -	Red
			S2	F2	50EtOH_2	Sample	50EtOH -	n/a
			S3	F3	100EtOH_1	Sample	100EtOH •	n/a
			S4	F4	100EtOH_2	Sample	100EtOH •	n/a
			S5	F5	Brown_50EtOH_1	Sample	50EtOH +	Brown
			S6	F6	Brown_50EtOH_2	Sample	50EtOH •	Brown
			S7	F7	Brown_100EtOH_1	Sample	100EtOH •	Brown
			S8	F8	Brown_100EtOH_2	Sample	- 100EtOH -	Brown
			S9	F9	Brown H2O 1	Sample	H2O -	Brown

# Manually Selecting the Blank Sample Type

Because the file names for these samples do not include the text Blank, you must manually select the sample type.

# ✤ To select the sample type for the solvent blanks

- 1. To sort the samples by the O-ring column, click the O-ring column header.
- 2. To select the solvent blanks, use the SHIFT key and select the samples with the n/a assignment in the O-ring column.

Tip To select a table row, click a cell in the row that does not have a drop-down list.

3. Right-click the selection and choose Set Sample Type To > Blank.

This figure shows the selected sample rows that are highlighted in blue and the shortcut menu.

n/a n/a n/a	Copy With Headers Copy	Ctrl+C		
n/a n/a	Cell Selection Mode Enable Row Grouping			
Red	Set Sample Type to	•	Sample	Shortcut menu
Red	Set Solvent to	•	Control	
Red	Set O-ring to	•	Blank	
Red Red	Set as Input File		Quality Control Identification Only Standard Labeled	

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 three differential analyses that compare the compounds extracted from the two o-ring types by the three solvents:

- Red versus Brown in water
- Red versus Brown in 50% ethanol
- Red versus Brown in 100% ethanol

This figure shows the study variables on the left and the ungrouped samples on the right. If you set up the study factors as described in "Defining and Assigning the Study Factors" on page 6, solvent is the primary study factor, and o-ring is the secondary study factor.

Primary study factor	
Secondary study factor	
Wew Study and Analysis Wizar I - Step 5 (16	
Sample Groups and Ratios Select the study variables fo sample g ouping and add ratios for gro	oup comparisons.
Sample Group and Ratio Spr cification	Generated Sample Groups
Study Variables	Blank 50EtOH n/a F1: 50EtOH_1 Blank 50EtOH n/a F2: 50EtOH_2 Blank 100EtOH n/a F3: 100EtOH_1 Blank 100EtOH n/a F4: 100EtOH_2 Sample 50EtOH Brown F5: Brown_50EtOH_1 Sample 100EtOH Brown F7: Brown_100EtOH_2 Sample 100EtOH Brown F8: Brown_100EtOH_2 Sample 100EtOH Brown F9: Brown_100EtOH_2 Sample 100EtOH Brown F9: Brown_100EtOH_2 Sample H2O Brown F10: Brown_H2O_1 Sample H2O Brown F10: Brown_H2O_2 Blank H2O n/a F11: H2O_1
Bulk Ratio Generation	Blank       H2O       n/a)       F12: H2O_2         Sample       50EtOH       Red       F13: Red_50EtOH_1         Sample       50EtOH       Red       F14: Red_50EtOH_2         Sample       100EtOH       Red       F15: Red_100EtOH_1         Sample       100EtOH       Red       F16: Red_100EtOH_1         Sample       100EtOH       Red       F16: Red_100EtOH_2         Sample       H2O       Red       F17: Red_H2O_1         Sample       H2O       Red       F18: Red H2O 2
No sample groups available for creating ratios.	Generated Ratios
<i>\$</i>	Cancel < Back Next > Finish

# To make Solvent the primary study factor

**Note** If Solvent is not the primary study factor, change its hierarchy.

- 1. Place the pointer over the handle ( ) to the left of **Solvent**.
- 2. When the move cursor  $(\clubsuit)$  appears, drag the variable up in the list.



# ✤ To set up the sample groups

In the Study Variables area, select the **Solvent** and **O-ring** check boxes.

The application groups the samples by solvent, o-ring color, and solvent blanks (n/a). Nine sample groups with two samples each appear in the Generated Sample Groups area. Three groups, one for each solvent, appear in the Bulk Ratio Generation area.

2	New Study and Analysis Wizard - Step 5 of 6	
S	Sample Groups and Ratios Select the study variables for sample grouping and add ratios for gr	oup comparisons.
	Sample Group and Ratio Specification	Generated Sample Groups
	Study Variables	50EtOH p/a
	🔲 File	Blank 50EtOH n/a F1: 50EtOH_1
	Solvent	Blank 50EtOH n/a F2: 50EtOH_2
Selected	O-ring	100EtOH n/a
variables	Sample Type	Blank         100EtOH         n/a         F3: 100EtOH_1           Blank         100EtOH         n/a         F4: 100EtOH         2
	Manual Ratio Generation	50EtOH Brown
	Numerator:	Sample 50EtOH Brown F5: Brown_50EtOH_1 Sample 50EtOH Brown F6: Brown 50EtOH 2
	Denominator:	100EtOH Brown
	Bulk Ratio Generation	Sample 100EtOH Brown F7: Brown 100EtOH 1
	Denominators to be used:	
	<ul> <li>Solvent : 50EtOH</li> <li>O-ring : Brown</li> </ul>	Sample H20 Brown F0 Brown H20 1
	O-ring : Red	Sample H2O Brown F10: Brown_H2O_2
<b>-</b>	<ul> <li>Solvent : 100EtOH</li> </ul>	H2O n/a
The solvents are the	O-ring : Brown	Blank H2O n/a F11: H2O_1
for automatic ratio	<ul> <li>Solvent : H2O</li> </ul>	Blank H2O n/a F12: H2O_2
generation.	O-ring : Brown	50EtOH Red
	O-ring : Red	Sample 50EtOH Red F13: Red_50EtOH_1
		Sample SUETOH Ked F14: Ked_SUETOH_2
		Samula 100ErOH Red
		Sample 100EtOH Red F15: Red_100EtOH_1 Sample 100EtOH Red F16: Red_100EtOH_2
		H20 Red
		Sample H2O Red F17: Red_H2O_1
		Sample H2O Red F18: Red_H2O_2

# **\*** To set up the ratios with the Bulk Ratio Generator

- 1. In the Bulk Ratio Generation area, select the brown O-ring as the denominator for each ratio as follows:
  - a. Place the pointer to the left of any of the O-ring color: Brown check boxes.

The Select/Deselect Item in All Groups icon, the appears.

- Bulk Ratio Generation
Denominators to be used:
Solvent : 50EtOH
O-ring : Brown
Select/deselect item in all groups.
Solvent : 100EtOH
O-ring : Brown
O-ring : Red
▲ Solvent : H2O
O-ring : Brown
O-ring : Red

b. Click the **Select Item in All Groups** icon, **H**.

# 2. Click Add Ratios.

The three ratios, one for each extraction solvent, appear in the Generated Ratios area.

**Figure 2.** Ratios for a differential analysis



# Closing the Wizard

# To close the wizard and save the study

Click Finish.

The E and L O-ring Example tab, the two analysis page tabs (Grouping & Ratios and Workflows), and the Analysis pane appear.

The Analysis pane lists the 18 input files. The As Batch check box is not selected, so the analysis will generate one result file. The Workflow box contains the name of the selected processing workflow and the Result File box contains the file name of the first file in the Files for Analysis list.

Default file name

										A	As Batch check box	
<b>^</b>	ft Start Page × / III E and L O-ring Example ×											*>
<b>6</b> ,	Add File	s 💥 Remove File	s 🔍 O	pen Containing Folder 🛛 🌼 New Analysis	🅼 Open Analy	sis T	empla	ate				
Stud	ly Defin	ition Input Files	Samples	Analysis Results Grouping & Ratios	Workflows	A	nalysi	s			🗌 As Batch 💣 Run 📙 Save	×
Error	ID 🔺	Name	File Type	Sample Information								*
		•			•		Proce	ssing	Step (Fully Processing)		Edit	
	F1	50EtOH_1	.raw	Sample Type: [Blank], Solvent: [50EtOH], C	-ring: [n/a]							
	F2	50EtOH_2	.raw	Sample Type: [Blank], Solvent: [50EtOH], C	-ring: [n/a]		Wor	ktiow:	E and L w Stats Unkn	o vn ID w Online and Local	Database Searches	
	F3	100EtOH_1	.raw	Sample Type: [Blank], Solvent: [100EtOH],	O-ring: [n/a]		Kesu	iit File	: DUETOH_1.cdResult -	-		
	F4	100EtOH_2	.raw	Sample Type: [Blank], Solvent: [100EtOH],	0-ring: [n/a]		₩ A	iles fo	r Analysis: (18)		样 Clear All	
	F5	Brown_50EtOH_1	.raw	Sample Type: [Sample], Solvent: [50EtOH],	O-ring: [Brown]		×	F1	50EtOH_1	Sample Type: [Blank], Solv	vent: [50EtOH], O-ring: [n/a]	
	F6	Brown_50EtOH_2	.raw	Sample Type: [Sample], Solvent: [50EtOH],	O-ring: [Brown]		x	F2	50EtOH_2	Sample Type: [Blank], Solv	vent: [50EtOH], O-ring: [n/a]	
	F7	Brown_100EtOH_1	.raw	Sample Type: [Sample], Solvent: [100EtOH	], O-ring: [Brown]		x	F3	100EtOH_1	Sample Type: [Blank], Solv	vent: [100EtOH], O-ring: [n/a]	
	F8	Brown_100EtOH_2	.raw	Sample Type: [Sample], Solvent: [100EtOH	], O-ring: [Brown]		x	F4	100EtOH_2	Sample Type: [Blank], Solv	vent: [100EtOH], O-ring: [n/a]	
	F9	Brown_H2O_1	.raw	Sample Type: [Sample], Solvent: [H2O], O-	ring: [Brown]		x	F5	Brown_50EtOH_1	Sample Type: [Sample], Se	olvent: [50EtOH], O-ring: [Brown]	
	F10	Brown_H2O_2	.raw	Sample Type: [Sample], Solvent: [H2O], O-	ring: [Brown]		х	F6	Brown_50EtOH_2	Sample Type: [Sample], Se	olvent: [50EtOH], O-ring: [Brown]	
	F11	H2O_1	.raw	Sample Type: [Blank], Solvent: [H2O], O-rin	ng: [n/a]		x	F7	Brown_100EtOH_1	Sample Type: [Sample], Se	olvent: [100EtOH], O-ring: [Brown	] ≡
	F12	H2O_2	.raw	Sample Type: [Blank], Solvent: [H2O], O-rin	ng: [n/a]		x	F8	Brown_100EtOH_2	Sample Type: [Sample], Se	olvent: [100EtOH], O-ring: [Brown	1
	F13	Red_50EtOH_1	.raw	Sample Type: [Sample], Solvent: [50EtOH],	O-ring: [Red]		x	F9	Brown_H2O_1	Sample Type: [Sample], Se	olvent: [H2O], O-ring: [Brown]	
	F14	Red_50EtOH_2	.raw	Sample Type: [Sample], Solvent: [50EtOH],	O-ring: [Red]		x	F10	Brown_H2O_2	Sample Type: [Sample], Se	olvent: [H2O], O-ring: [Brown]	
	F15	Red_100EtOH_1	.raw	Sample Type: [Sample], Solvent: [100EtOH	], O-ring: [Red]		×	F11	H2O_1	Sample Type: [Blank], Solv	vent: [H2O], O-ring: [n/a]	
	F16	Red_100EtOH_2	.raw	Sample Type: [Sample], Solvent: [100EtOH	], O-ring: [Red]		×	F12	H2O_2	Sample Type: [Blank], Solv	vent: [H2O], O-ring: [n/a]	
	F17	Red_H2O_1	.raw	Sample Type: [Sample], Solvent: [H2O], O-	ring: [Red]		×	F13	Red_50EtOH_1	Sample Type: [Sample], Se	olvent: [50EtOH], O-ring: [Red]	
	F18	Red_H2O_2	.raw	Sample Type: [Sample], Solvent: [H2O], O-	ring: [Red]		×	F14	Red_50EtOH_2	Sample Type: [Sample], Se	olvent: [50EtOH], O-ring: [Red]	
					×	F15	Red_100EtOH_1	Sample Type: [Sample], Se	olvent: [100EtOH], O-ring: [Red]			
							×	F16	Red_100EtOH_2	Sample Type: [Sample], Se	olvent: [100EtOH], O-ring: [Red]	
							×	F17	Red_H2O_1	Sample Type: [Sample], Se	olvent: [H2O], O-ring: [Red]	
4	_						×	F18	Red_H2O_2	Sample Type: [Sample], Se	olvent: [H2O], O-ring: [Red]	
💌 s	how De	tails					۰ 📃			m	•	-

# Reviewing the Processing Workflow

Before submitting the analysis to the job queue, review the processing workflow and make changes as needed. If your processing computer does not have Internet access, remove the Search mzCloud node and the Search ChemSpider node (see "To remove the search nodes that require Internet access" on page 15).

# To review the processing workflow

- 1. Click the Workflows tab to open the Workflows page.
  - Figure 3. Processing workflow for a metabolomics study



Review the settings for the following workflow nodes, but do not change the settings for this tutorial: Detect Compounds, Group Compounds, Search ChemSpider, Search mzCloud, Calculate Mass Defect, and Search Mass Lists.

To check the settings for a node, select the node in the Workflow Tree area and review its parameter settings on the Parameters page to the left.

2. Select the **Detect Compounds** node and check the parameter settings.

Parameters of 'Detect Compo	arameters of 'Detect Compounds'			
Hide Advanced Parameters		Workflow	F and L w Stats Unkno	
▲ 1. General Settings				
Mass Tolerance [ppm]	5 ppm 💌	Description:	Untargeted E&L ID we	
Intensity Tolerance [%]	30		and compound grou	
S/N Threshold	3			
Min. Peak Intensity	1000000	Workflow Tre	e	
Ions	[2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]		L	
Base Ions	[M+H]+1; [M+NH4]+1; [M-H]-1		•	
Min. Element Counts	СН	հնակ ու		
Max. Element Counts	C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5	Align	Retention Times	
▲ 2. Peak Detection				
Filter Peaks	True		1 I	
Max. Peak Width [min]	0.8	6		
Remove Singlets	False	Dete	ct Ompounds 🛛 🔅	
Min. # Scans per Peak	3			
Min. # Isotopes	1		I	
			V	

For the Detect Compounds node, do the following:

• Check the Min. Peak Intensity setting against the suggested setting for your data set.

The minimum peak intensity setting defines the base peak intensity for the unknown compound detection. For this tutorial, keep the setting of 1 000 000.

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 $^{\text{\tiny TM}}$ , Q Exactive Plus $^{\text{\tiny TM}}$ , Q Exactive HF	500 000 to 1 000 000
Exactive <sup>™</sup> , Exactive Plus <sup>™</sup> , Orbitrap Elite <sup>™</sup> , Orbitrap Velos Pro <sup>™</sup>	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

• Check the Base Ions list. For this tutorial, do not change the selection.

**Tip** Mobile phase additives can have a significant effect on the base ions (adduct ions with the highest intensity) in the full scan data for an LC/MS experiment. To ensure that the application interprets the isotopic ion clusters correctly, select the predominant adduct ions for the chromatographic analysis from the Base Ions list. For example, if the mobile phase contains a significant amount of ammonium acetate, consider adding the ammonium adduct, [M+NH4]+1, to the list. All the processing workflows in the E and L folder include [M+NH4]+1 in the Base Ions list.

3. Select the **Group Compounds** node and review the parameter settings. Check the Preferred Ions list. The list should include the base ions selected in the Detect Compounds node. Do not change the settings for this tutorial.

Par	rameters of 'Group Compo	unds'				
Sh	now Advanced Parameters					
⊿	4 1. Compound Consolidation					
	Mass Tolerance	5 ppm 💌				
	RT Tolerance [min]	0.1				
⊿	2. Fragment Data Select	tion				
	Preferred Ions	[M+H]+1; [M+NH4]+1; [M-H]-1				
L 1						

4. Select the **Search ChemSpider** node and review its parameter settings. For other analyses, select the databases that you want the analysis to search and the maximum number of results per compound to return.

In the selected processing workflow template, the Search ChemSpider node searches 7 out of 363 databases. Do not change the settings for this tutorial.

Par	ameters of 'Search ChemSpider'	
Hi	de Advanced Parameters	
⊿	1. Search Settings	
	Database(s)	Alfa Chemistry; EPA DSSTox; EPA Toxcast; NIST; PurePEG; Sigma-Aldrich; TCI 💽
	Search Mode	By Formula or Mass
	Mass Tolerance	5 ppm
	Max. # of results per compound	10
	Result Order (for Max. # of results per compound)	Order By Reference Count (DESC)
	Max. # of Predicted Compositions to be searched per Compound	3
⊿	2. Predicted Composition Annotation	
	Check All Predicted Compositions	False

5. Select the Search mzCloud node and review its parameter settings.

With the current settings, the node runs an identity search and a similarity search. For this tutorial, do not change the settings.

arameters of 'Search mzCloud'		
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	——— This feature is enabled (set to True) in all t
4 2. DDA Search		processing workflow templates provided v
Identity Search	Cosine	the application
Match Activation Type	True	
Match Activation Energy	Match with Tolerance	
Activation Energy Tolerance	20	
Apply Intensity Threshold	True	
Similarity Search	Similarity Forward	
Match Factor Threshold	60	
4 3. DIA Search		
Use DIA Scans for Search	True	
Max. Isolation Width [Da]	500	
Match Activation Type	True	
Match Activation Energy	Any	
Activation Energy Tolerance	100	
Apply Intensity Threshold	True	
Match Factor Threshold	20	

6. Select the Search Mass Lists node and review its parameter settings.

For this tutorial, do not change the settings.

Pa	rameters of 'Search Mass Lists'					
Sł	Show Advanced Parameters					
⊿	1. Search Settings					
	Input file(s)	\Extractables and Leachables HRAM Compound Database.csv				
	Consider Retention Time	True				
	RT Tolerance	0.1				
	Mass Tolerance	5 ppm				

7. Select the Calculate Mass Defect node and review its parameter settings.

The node is set to calculate the mass defect for polymers with the following repeating units:

- -[C2H4O]- polyethylene glycol
- -[C2H3Cl]-
- -[C2H2F2]-
- -[C3H6]- propyl repeating units
- -[C8H8]- styrene repeating units

For this tutorial, do not change the settings.

Parameters of 'Calculate Mass Defect'							
Hic	le Advanced Parameters						
⊿	▲ 1. Mass Defect						
	Fractional Mass	False 💌					
	Standard Mass Defect	False					
	Relative Mass Defect	False					
	Kendrick Mass Defect	True					
	Nominal Mass Rounding	Floor					
⊿	2. Kendrick Formula						
	Formula 1	C2 H4 O					
	Formula 2	C2 H3 CI					
	Formula 3	C2 H2 F2					
	Formula 4	C3 H6					
	Formula 5	C8 H8					

# To remove the search nodes that require Internet access

**Note** Only follow this procedure if your computer does not have Internet access.

- 1. If the Workflows page is closed, open it.
- 2. In the Workflow Tree area (Figure 3 on page 12), do the following:
  - Right-click the Search mzCloud node and choose Cut.
  - Right-click the Search ChemSpider node and choose Cut.

Submitting<br/>the Analysis<br/>to the Job<br/>QueueIn the E a<br/>workflow<br/>to the Job<br/>to the Job<br/>to ro su

In the E and L w Stats and Unknown ID w Online and Local Database Searches processing workflow, none of the workflow nodes require customization.

# To submit the analysis to the job queue

1. 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.

2. In the Result File box, rename the result file **E and L Example**.

					Kun command
A	nalysis	🗌 As Batch 💕 Run	📙 Save	×	
Γ,				_	
	Processing Step (Fully Processing)		E	dit	
	Workflow: E and L w Stats Unknown ID w Online and Loca	I Database Searches			
	Result File: E and L Example.cdResult				
	▼ Files for Analysis: (18)		样 Clear /	AII	Result file name

- 3. Make sure that the As Batch check box is not selected, and click **Run** to submit the analysis to the job queue. The Job Queue page opens.
- 4. To view the processing messages, click the expand icon, 
  <sup>⊥</sup>, to the left of the job row.

😭 Start Page 🗙 👔	E and L (	O-ring Exan	nple × 🗟 Job	Queue ×		_
👘 Pause 🎲 Resum	e 🇊 A	bort 🗶	Remove 🏾 🎅 R	efresh 🚺 O	pen Results 🔳	Disp
Job Queue:	Job Queue:					
Execution State	Details	Progress	Туре		Name	
E. Running		2 %	Processing	E and L Examp	le	

**Note** During the run, the analysis generates several warning messages, which you can ignore. Warning messages have a yellow background.

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

# Reviewing the Analysis Results

# Follow these topics to review the analysis results:

- Opening the Result File
- Default Layout for a Result Page
- Working with the Result Tables
- Working with the Chromatograms View
- Working with the Mass Spectrum View
- Reviewing the Mass List Search Results
- Reviewing the mzLogic Analysis for a Compound
- Viewing a Mass Defect Plot
- Applying the Statistics Layout
- Working with the Volcano Plot for the Differential Analysis
- Viewing a Hierarchical Cluster Analysis
- Viewing the Principal Component Analysis
- Working with the Partial Least Squares Discriminate Analysis View
- Making Structure Proposals
- Using the Result Filters View

# 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, open the result file provided on the Compound Discoverer 3.1 USB key—**Example Studies & L O-ring Study EandL Example.cdResult**. Then, from the menu bar, choose **Window** > **Reset Layout**.

# ✤ To open the result file from the Job Queue page

- 1. If you closed the Job Queue page, from the application menu bar, choose View > Job Queue.
- 2. On the Job Queue page, double-click the run.

🔄 Job Queue 🗡					
🎲 Pause 🎲 Resun	ne 🎲 Abort	💢 Remove 🧯	🕑 Refresh 🛛 🚺 O	pen Results 📃	Display Verbose Messages
Job Queue:					
Execution State	Details	Progress	Туре	Name	
	Warnings	100 %	Processing	E and L Example	

- Double-click to open the result file.

# Default Layout for a Result Page

- 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 for the compound in the first row of the Compounds table. The view automatically zooms in to the start and end points of the chromatographic peak for the compound.
- A Mass Spectrum view on the top right that is populated with the MS1 scan (for a preferred ion) that is closest to the chromatographic peak apex for the selected compound across the input files. The spectrum tree to the left includes the MS1 scans and the fragmentation scans for the preferred ions that were acquired within the following retention time window:
  - Peak apex (RT) ± the peak's full width at half maximum (FWHM)
  - -or-
  - 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.

DIA scans include all-ion fragmentation scans (AIF) and data-independent scans (DIA) from your Thermo Scientific mass spectrometer.

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

Figure 4 shows the factory default layout for the E and L Example.cdResult file.

Figure 4. Factory default layout for the example result file



Field Chooser icon

Select Table Visibility icon

Table 2 describes the main result tables that the selected processing workflow produces. The Compounds table is the active table and is sorted by the Area (Max.) column. The first row displays the compound with the largest chromatographic peak area (found in one of the sample files). Because the selected processing workflow includes the Mark Background Compounds node, the Compounds tab has a filter icon with a check mark ( $\Im$ ). 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.

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	S
Compounds	Displays all the compounds that the analysis detected grouped by their molecular weight and retention time (MW×RT) dimensions across all the sample files.
Compounds per File	Displays all the compounds that the analysis detected across all the sample files on a per file basis. Does not list compounds that the Fill Gaps node detected by filling a full gap.
Merged Features	Displays all the features (ions with the same $m/z \times RT$ dimensions) across the sample files in descending order by the Max. Area column.
Features	Displays all the features (ions with the same $m/z \times RT$ dimensions) that the analysis detected across all the sample files on a per file basis in descending order by the Area column.

	• • •
Result table	Description
mzCloud Results	Lists the mzCloud search results across all the sample files.
ChemSpider Results	Lists the ChemSpider search results across all the sample files.
Mass List Search Results	Lists the mass list search results across all the sample files in descending order by molecular weight.
Input Files	Describes the input files that the application processed to create the result file.
Specialized Traces	Lists the specialized traces. For this analysis, lists the analog traces that the Create Analog Trace node extracted across the sample files.
Visible tables related to the C	Compounds table
Structure Proposals	Displays your structure proposals for the selected 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. The predicted compositions are based on the molecular weight of the neutral compound.
Merged Features	Displays the features detected across the sample files for the selected compound.
mzCloud Results	Displays the mzCloud results for the selected compound.
ChemSpider Results	Displays the ChemSpider results for the selected compound.
Mass List Search Results	Displays the mass list search results for the selected compound.
Visible tables related to the F	eatures table
Compound per File	Displays the compound detected for the selected feature.
Chromatogram Peaks	Describes the chromatographic peaks for the selected feature.
Visible tables related to the l	nput 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 (when the processing workflow includes the Fill Gaps node). Imputed peak areas improve the statistical analysis results.

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

# Working with the Result Tables

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

- Showing or Hiding Result Tables and Viewing the Related Tables
- Showing or Hiding Table Columns
- Sorting Table Rows

# Showing or Hiding Result Tables and Viewing the Related Tables

The default layout does not include all the results tables that the selected processing workflow produces. By default, the following tables are hidden: the Filled Gaps related table, which is created by the Fill Gaps node, and the Adducts table.

- To show or hide result tables
- 1. Click the **Select Table Visibility** icon, <sup>(IIII)</sup>, 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.
  - For this tutorial, do not change the selections.
- 3. Click **OK**.

# To view the tables related to the Compounds table

With the main Compounds table selected as the active table, click **Show Related Tables** at the bottom of the window to display the tables related to the Compounds table.

Field Chooser

# ChemSpider Results

# mzCloud Results# Similarity Results

Annotation Source

🗸 Adj. P-value

🖌 Area (Max.)

□ Background✓ Checked

FISh Coverage

Group Areas

Group CV [%]

✓ Mass Defect

MS2

✓ Name✓ P-value

✓ Ratio

RT [min]

RT Tolerance [min]

1

Mass List MatchesMolecular Weight

✓ mzCloud Best Match

mzCloud Best Sim, Match

mzCloud Library Matches

FormulaGap Status

Area

# **Showing or Hiding Table Columns**

# To display or hide a table column

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

In the Compounds table, the following columns are hidden, by default:

- #Adducts
- #Similarity Results
- Area (for compound in each sample file)
- Background
- Gap Status
- RT Tolerance [min]
- Structure
- 2. Do one of the following:
  - To display a column, select its check box.
  - To hide a column, clear its check box.

# ✤ To show the Area column in the Compounds table

- 1. Click the **Field Chooser** icon, **F**.
- 2. Select the Area check box.

The Area column appears in the Compounds table to the left of the Group Areas column. The Area column contains 18 subcolumns, one for each input file (samples and blanks).

														Structure			
														E	kpand ic	on —	
Area																	+
4.68e9	4.88e9	4.95e5	8.58e5	1.25e9	1.18e9	7.65e9	8.08e9	5.51e5	5.45e5	1.31e9	1.24e9	4.63e9	4.46e9	1.54e6	5.51e5	4.32e7	4.22e7

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

4. To view the sample names for the area columns, click the expand icon, *∃*, to the right of the column name.

The names of the input files appear vertically above the individual area columns. The name of the first input file, 50EtOH\_1.raw (F1), appears in bold font and has an asterisk indicating that it is the selected area column.

Г	- Select	ed colu	mn											Collap	se icon		
Brown_100EtOH_1.raw (F7) & ea	Brown_100EtOH_2.raw (F8)	100EtOH_1.raw (F3)	100EtOH_2.raw (F4)	Red_100EtOH_1.raw (F15)	Red_100EtOH_2.raw (F16)	Brown_50EtOH_1.raw (F5)	Brown_S0EtOH_2.raw (F6)	50EtOH_1.raw (F1)	50EtOH_2.raw (F2)	Red_50EtOH_1.raw (F13)	Red_50EtOH_2.raw (F14)	Brown_H2O_1.raw (F9)	Brown_H2O_2.raw (F10)	H2O_1.raw (F11)	H2O_2.raw (F12)	Red_H2O_Lraw (F17)	Red_H2O_2.raw (F18)
4.68e9	9 4.88e9	4.95e5	8.58e5	1.25e9	1.18e9	7.65e9	8.08e9	5.51e5	5.45e5	1.31e9	1.24e9	4.63e9	4.46e9	1.54e6	5.51e5	4.32e7	4.22e7

- 5. Click the collapse icon,  $\square$ , to hide the sample names.
- 6. To hide the Area column, open the Field Chooser box and clear the Area check box.

# **Sorting Table Rows**

#### \* To sort the table rows based on the contents 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.

# To view a mass chromatogram

- In the Compounds table, click the Area (Max.) column heading to sort the table by Area (Max.) in descending order (▼).
- 2. To display the overlaid traces for the compound with the largest peak area, select **row 1 (TriPhenylphosphine oxide)** (Figure 4 on page 17).

By default the display options for the Chromatograms view are set to Show ToolTips, Show Detected Peaks, Show Legend, and Zoom to Detected Peaks. The *x*-axis zoom is set to the width of the detected peaks, and the *y*-axis zoom is set to auto scale.

This figure displays the overlaid traces for the input files that contain the selected compound and the legend and shortcut menu for the Chromatograms view. (The Mass Spectrum view is closed.)



Shortcut menu -

Working with the Chromatograms View 3. Under Filter By, expand the all the filters by clicking their expand icons, 4.



- 4. Review the information in the collapsible left pane:
  - Under Group By, the counts for the O-ring and Solvent study factors are 3 out of 3 (3/3), the count for Sample Type is 2 out of 2 (2/2), and the count for File is 18 out of 18 (18/18). These counts mean that the analysis detected the selected compound in all the input files.

**Tip** Because the processing workflow included the Fill Gaps node, the analysis detected the selected compound in all the input files, including the solvent blanks.

- Under Filter By, all the check boxes are selected. This means that the XIC traces for all the files are displayed in the Chromatograms view.
- 5. To view the traces for the solvent blanks, under Filter By > Sample Type, clear the **Sample** check box.

This figure shows the traces for the solvent blanks.



- 6. To view the chromatographic traces without the shading that can mask underlying peaks, right-click the Chromatograms view and choose **Display Options > Show Detected Peaks**.
- 7. To view the traces for the o-ring samples, under Filter By > Sample Type, select the **Sample** check box and clear the **Blank** check box.

This figure shows the overlaid traces for the 12 samples that you are comparing. The application displays the six sample groups in different colors. The legend shows the display colors for the groups.



Working with the Mass Spectrum View

- Follow these procedures to review the mass spectra for a compound.
- To view the MS1 spectrum
- To inspect the isotopic cluster for the most intense adduct ion
- To view the results of an mzCloud search for a compound and display a mirror plot
- To view the MS1 spectrum
- 1. Sort the Compounds table by the Area (Max.) column in descending order (▼).
- 2. Select row 1: Triphenylphosphine oxide (Figure 4 on page 17).
- 3. Close the Chromatograms view by clicking the **Close** icon on its title bar.
- 4. To expand the Mass Spectrum view to the full screen width, drag the view by its title bar to a second monitor.

This figure shows the Mass Spectrum view as a floating window. The spectrum tree on the left lists the MS1 scans and the fragmentation scans for the preferred ions that are within the search window.

The *x* axis expands to display the detected adduct ions: [M+H]+1, [2M+H]+1, [M+Na]+1, and [2M+Na]+1. The analysis detected these adduct ions across the input files. In input file F6, the [2M + Na]+1 ion is from a gap-filled chromatographic peak.



- To inspect the isotopic cluster for the most intense adduct ion
- 1. In the Compounds table, select row 1: Triphenylphosphine oxide (Figure 4 on page 17).
- 2. To zoom in on the isotopic cluster for the [M+1]+1 adduct ion, drag the pointer horizontally across the mass spectrum from m/z 279 to m/z 282.

Because the selected compound has a predicted formula, the MS1 spectrum includes color-coded centroids.



The centroids in the MS1 scan are color-coded as follows:

ī

- Lavender bars indicate the A0 peak for the monoisotopic ion. The *x*-axis position and the width of the bar reflect the expected *m/z* value of the centroid and the user-specified mass tolerance, respectively.
- 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 view the results of an mzCloud search for a compound and display a mirror plot
- 1. Sort the Compounds table by the **mzCloud Best Match** column in descending order ( $\checkmark$ ).
- 2. In the Compounds table, select row 1(Triethyleneglycol dimethyl ether).
- 3. To display the related tables below the main tables, click Show Related Tables.

4. Click the mzCloud Results tab and select row 1(Triethyleneglycol dimethyl ether).

	Corr	npou	nds 💡	Compounds pe	r File Merged Featu	ires Features	mzCloud Results	ChemSpider Resu	Its Mass List Sea	irch Result	s Input Files
	Ē	(	Checked	Name		Formula	Annotation Source	+ FISh Coverage	Molecular Weight	t RT [min]	Area (Max.)
	1 🖻	· [		Triethyleneglycol di	methyl ether	C8 H18 O4			178.12014	5.393	1650012524
	2 👳			PEG n6		C12 H26 O7			282.16689	6.384	187767429
4											
0	Hide	e Rela	ated Tabl	25							
	Structu	ire Pi	roposals	Compounds per F	File Predicted Comp	oositions Me	rged Features mzClo	ud Results Che	mSpider Results	Mass List	Search Results
	Ē		Checked	Compound Match	Structure		Name		For	mula	
	1 +	Û			~ <sup>0</sup> ~~_0~~	.0	Triethyleneglycol dime	thyl ether	C8	H18 O4	

An annotated mirror plot appears in the Mass Spectrum view with scan #734 from input file F17 on top and the mzCloud reference spectrum on the bottom.

The centroids for the matching fragments are displayed as green sticks with a green circle at the end. Red circles on the *x*-axis indicate the m/z values of the missing fragments.



# Reviewing the Mass List Search Results

# **\*** To review the mass list search results for the analysis

- 1. In the main tables, click the Mass List Search Results tab.
- 2. To display a tooltip that lists the number of compounds found across the input files, point to the Mass List Search Results tab.

This figure shows that the mass list search found 181 matching entries.

	1	Compo	unds 💡	Compounds	per File	Merged Features	Features	mzCloud Results	ChemSpider Results	Mass I	List Search Results	Input Files
		<b>#</b>	Structure		Name			Formula	Molecular Weight 🔻	Refer Re	sults of a Mass List	Search
	1	ά	×		Tris[2,4-	bis(2-methyl-2-prop	anyl)phenyl] j	C42 H63 O4 P	662.44640	1 Extractat	81 items shown (0 fil les and Leachables	tered out) 95906-11-9
	2	<b>4</b>			3,9-Bis[	2,4-bis(2-methyl-2-pr	ropanyl)phen	C33 H50 O6 P2	604.30826	Extractat	ies and Leachables	26741-53-7
0	)	Show R	elated Tables	5								

— Tooltip

- 3. In the main tables, click the **Compounds** tab.
- Sort the Compounds table in descending order (▼) by the Mass List Matches column. Then, hold down the CTRL key and sort the table in descending order (▼) by the Area (Max) column.

The color-coded rectangles indicate whether the mass list search found matching compounds.

- ( ) Red—Multiple matches found
- (**D**) Green—Single match found
- ( ) Gray—No matches found

5. In the sorted Compounds table, select **row 8** (3,6,7,12,15,18-Hexaoxaicosane-1,20-diol).

Figure 5. Compounds table sorted by the Mass List Matches column and the Area (Max) column in descending order

•	Compo	ounds 🌹	Compounds per File	Merged Features	Features	mzCloud Results	ChemSpider Res	ults M	lass List Search R	esult	
É		Checked	Name		Formula	Annotation 🛨	Molecular Weight	RT [min]	Area (Max.) 🔻		Mass List Matches 🔻 🛨
1	-12		Bis(2-butoxyethyl) adipate		C18 H34 O6		346.23461	31.968	5073684440	99.1	
2	-12		Bis[2-(2-butoxyethoxy)eth	yl] adipate	C22 H42 O8		434.28701	31.429	4613052498	0.0	
3	-12		Dipropylene glycol dimeth	yl ether	C8 H18 O3		162.12533	12.876	4362142212	0.02	
4	-12		Tris(2-ethylhexyl) trimellita	ite	C33 H54 O6		546.39047	50.532	3640992512		
5	-12		Triethyleneglycol bis(2-eth	ylhexanoate)	C22 H42 O6		402.29680	41.179	1130003719	99.1	
6	-12		1-[3-(2-Hydroxy-2-propan	yl)phenyl]ethanone	C11 H14 O2		178.09913	14.163	802875603	0.9	
7	-12		Tris(2-ethylhexyl) trimellita	ite	C33 H54 O6		546.39464	34.481	750822902	0.06	
8.02	-12		3,6,9,12,15,18-Hexaoxaico	sane-1,20-diol	C14 H30 O8		326.19314	9.720	586780214		R
9	-12		diethylmaleate		C8 H12 O4		172.07323	31.965	394819547		Multiple matches found
10	-12		Dipropylene glycol dimeth	yl ether	C8 H18 O3		162.12530	13.298	322650493	0.0	
11	-12		NN-Dimethyllauramide		C14 H29 N 0		227.22444	33.966	244289421	0.05	
4						iii iii					
ି <b>ଚ</b>	how R	elated Tal	bles								

- 6. Open the related tables and click the Mass List Search Results tab.
- 7. Review the mass list search results for the selected compound.

This figure shows the mass list search results for 3,6,7,12,15,18-Hexaoxaicosane-1,20-diol. In the Mass List Matches column of the Compounds table, the red status ( $\blacksquare$ ) indicates that the analysis found multiple matches. The related Mass List Search Results table describes the three matches. The first compound is a full match ( $\blacksquare$ ) and has an mzLogic score of 83.7. The formulas of the second and third compounds do not match the formula of the selected compound ( $\blacksquare$  indicates No match). Each of these compounds has an mzLogic score of 4.1.

Figure 6. Mass list search results for 3,6,7,12,15,18-Hexaoxaicosane-1,20-diol



mzLogic scores for the — three structure candidates

Reviewing the mzLogic Analysis for a Compound When an mzCloud search yields no identity matches for an unknown compound, use the mzLogic<sup>™</sup> scores for the search hits to determine the best explanation for a compound.

An mzLogic analysis requires candidate structures from the ChemSpider database, a Metabolika pathway, or a mass list with structures. It also requires spectral similarity hits from an mzCloud similarity search. The processing workflow for this E&L tutorial (Figure 3 on page 12) includes two nodes that provide structures—the Search ChemSpider node and the Search Mass Lists node.

# \* To review the results of an mzLogic analysis for structure candidates

- 1. From the application menu bar, choose **Window > Reset Layout**.
- 2. Sort the Compounds table in descending order by Area (Max.).
- 3. Open the Compound table's Field Chooser dialog box (see "To display or hide a table column" on page 19) and select the **#Similarity Results** check box.
- 4. In the Compounds table, expand the Annotation Sources column.

For cholestane-3,6,7,8,15,16,26-heptol (row 4), the analysis found no matching structures from the mzCloud identity search. But it did find six ChemSpider hits and three structures from the mzCloud similarity search.



ChemSpider hits ——— #Similarity results

- 5. In the Compounds table, select row 4 (Cholestane-3,6,7,8,15,16,26-heptol).
- 6. From the application menu bar, choose **View > mzLogic Analysis**.

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



7. Below the Candidates pane, click Similar Structures from mzCloud.

The application automatically selects the highest ranking candidate in the Candidates pane and highlights the matching substructures in blue in the Similar Structures from mzCloud pane. If you select another candidate, the application determines the structural coverage for that candidate.



# Viewing a Mass Defect Plot

You can view a mass defect plot for the compounds in the Compounds table. Follow this procedure to set up a mass defect plot that highlights polyethylene glycols (PEG).

- To set up a mass defect plot for a Kendrick formula
- 1. From the application menu bar, choose View > Mass Defect Plot.

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

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



4. Zoom in on named compounds that form a horizontal line.

9	Mass Defe	ect Plot									×
Da	ata Source:	Compounds 🔻	Type: Kendrick	Mass Defect 🔻	Rounding	: Ceiling 🔻	Kendrick Form	nula: C2H4C	þ		
V	Highlight	named compound	ls								
+	-0.180 ·	1									*
Defec	5 -0.185 ·	-									
Mace	ĝ0.190 ·	•				MD	0 180			•	
ndrick	-0.195 -					PEG r	14 28 H58 O15				
K	-0.200 ·					MW: RT: 1	651.40293 Da 3.952 min				
		600 61	0 620	630	640	650	660	670	680	690	
					Molecul	ar Weight [Da]					-
٠											F

- 5. Right-click the plot and choose Check All Visible Points.
- 6. Sort the Compounds table by the Name column in descending order.
- 7. Notice that the three PEG compounds are checked. Clear these check boxes.

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, and then choose Window > Apply Layout > Statistics.

For the example result file, the Statistics layout does the following (Figure 7):

- Moves the result tables to the top of the page and hides most of the main tables, except for the Compounds table Merged Features table, Specialized Traces 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 Log<sub>2</sub> 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.

# Applying the Statistics Layout



# Figure 7. Statistics layout for a result file

Working with the Volcano Plot for the Differential Analysis

The processing workflow for this tutorial included the Differential Analysis post-processing node. This node runs a differential analysis on the ratios that you set up and displays the results of the analysis as a volcano plot when you open the Differential Analysis view.

To familiarize yourself with viewing and modifying a differential analysis, follow these procedures:

- To view the volcano plot for the current sample comparison
- To change the p-value for the analysis
- To change the log 2 fold change value for the analysis
- To view a different comparison
- To automatically check the up-regulated or down-regulated compounds in the Compounds table
- To uncheck selected compounds
- To view information about a specific data point (compound) in the other open views

# To view the volcano plot for the current sample comparison

1. Apply the Statistics layout (see "Applying the Statistics Layout" on page 28).

The Differential Analysis view displays a volcano plot—a plot of the p-value, the result of a significance test, on the y axis versus the log<sub>2</sub> 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.

The shaded regions of the volcano plot contain compounds where the relative difference between the comparison groups was statistically significant for the specified p-value and outside the specified upper and lower fold change thresholds:

- The pink region contains up-regulated compounds; that is, compounds where the amount extracted from the red o-rings was significantly different from the brown o-rings and greater than the upper fold-change threshold.
- The green region contains down-regulated compounds; that is, compounds where the amount extracted from the red o-rings was significantly different from the brown o-rings and less than the lower fold-change threshold.
- 2. To change the pane on the bottom left to a floating window, drag it away from the application window.
- 3. To display the Differential Analysis view's legend, right-click the view and choose **Show Legend** (Figure 8 on page 30).

The legend appears at the bottom of the page with details about the color-coded data points.

4. To display information about a data point, point to it.

The ToolTip displays the coordinates, MW, RT, maximum area, and number of adducts for the selected compound.

Note You might need to click the view to activate the tooltips.

Figure 8 shows the differential analysis for the comparison between the relative amount of each compound (by chromatographic peak area) extracted from the red o-rings versus the brown o-rings with a solution of 50:50 ethanol/water.

Figure 8. Differential analysis for the compounds extracted from red o-rings or brown o-rings with 50% ethanol/water



#### To change the p-value for the analysis

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

# $\checkmark$ To change the log $_2$ fold change value for the analysis

Move the Log<sub>2</sub> Fold change slider to the left or right. Or, type a value in the box next to the slider.

This figure shows a volcano plot for a p-value setting of 0.05 and a fold change of 4 ( $\log_2 4 = 2$ ).



# To view a different comparison

In the Comparison list, select another ratio.

#### To automatically check the up-regulated or down-regulated compounds in the Compounds table

Do one or both of the following:

- Right-click the view and choose Check All Up-Regulated Points.
- Right-click the view and choose Check All Down-Regulated Points.

The color of the checked points changes to blue and the application selects the check boxes for the selected compounds in the Compounds table. The application also selects the same data points in other open statistical views.

This figure shows the selection of the up-regulated data points.



# To view information about a specific data point (compound) in the other open views

Double-click the compound in the volcano plot to view it in the Compounds table and other open views.

Double-clicking a data point highlights the row for the corresponding compound in the Compounds table; it does not select the corresponding check box.

# To uncheck selected compounds

- To uncheck a single data point, right-click it and choose Uncheck Point.
- To uncheck all the selected data points, right-click the view and choose Uncheck All Visible Points.

# Viewing a Hierarchical Cluster 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 28).
- 2. To change the bottom right pane to a floating window, drag it away from the application window.

④ Hierarchical Cluster Analysis	5		
<ul> <li>▲ Color By:</li> <li>Solvent (0/0)</li> <li>○ O-ring (0/0)</li> </ul>	Data Source: Compounds	Scale Values: Scale Before Clustering	Use normalized areas
Sample Type (0/0) File (0/0) Filer By:			
ON Solvent      On Solvent      On Sample Type			
ON      File  Principal Component Analysis	The second	Hierarchical Cluster Analysis	

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

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

4. In the left pane, under Color By, select the Solvent and O-ring check boxes.

Color bars appear above the heat map to visually differentiate the samples by their study factors variables.

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



- 5. To zoom in on a specific area of the heat map, drag the pointer across the rectangular area of interest.
- 6. 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 lower right corner of the heat map and their corresponding dendograms.



Go to the next topic "Viewing the Principal Component Analysis."

Viewing the Principal Component Analysis 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 28).
- 2. Click the Principal Component Analysis tab.

The Principal Component Analysis view opens to the Scores Plot page (Figure 9 on page 35).

Use the scores plot to interpret the relationship among the sample groups. Sample groups that are near each other are similar.

The data for the brown o-rings is clustered in the lower-right quadrant, which means that the choice of extraction solvent made little difference.

The data for the red o-rings is divided into three separate quadrants, which means that the choice of extraction solvent did make a difference.

Figure 9. Principal component analysis of compounds extracted from two types of o-rings with three different solvents



Go to the next topic "Working with the Partial Least Squares Discriminate Analysis View."

Working with the Partial Least Squares Discriminate 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.

To familiarize yourself with running and reviewing a partial least squares discriminant analysis, follow these procedures:

- To make the Partial Least Squares Discriminant Analysis view active
- To rerun the analysis on a different 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 28).
- 2. Click the Partial Least Squares Discriminant Analysis tab.
- 3. If the graph includes checked data points, right-click the view and choose Uncheck All Visible Points.

Figure 10 shows the Partial Least Squares Discriminant Analysis view with the default settings for the Statistics layout. The orange circles represent the five discriminating compounds.



Figure 10. Default settings for the PLS-DA view

 To zoom in on the points of interest, drag the pointer across the region with the orange circles. This figure shows the five orange circles that represent the discriminating compounds. To display a ToolTip,



# To rerun the analysis on a different 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.

# \* 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. If the Differential Analysis view is open, the same compounds 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.

You can add your own custom structure proposals to the result file.

# To add a structure proposal for a compound

- 1. To reset the result file's layout, from the menu bar, choose Window > Reset layout.
- 2. With the default layout, the Compounds table is sorted in descending order by the Area (Max.) column.
- 3. Select row 5 (Bis[2-(2-butoxyethoxy)ethyl] adipate).
- 4. Click Show Related Tables below the main tables.
- 5. Click the **ChemSpider Results** tab.

Making

Structure

Proposals

The ChemSpider Results table for the selected compound lists five structures.

6. In the ChemSpider Results table, select the first two rows. Then, right click the table and choose Add to Structure Proposals and Apply FISh Scoring.

**Tip** To submit multiple entries to the FISh Scoring Queue, hold down the CTRL key, select the structures of interest, right-click and choose **Add to Structure Proposals and Apply FISh Scoring**.

Figure 11. ChemSpider Results table for Bis[2-(2-butoxyethoxy)ethyl] adipate

) Hide Re	lated Table	25									
Structure I	Proposals	Compounds per l	File	Predicted Compositions	Me	rged Features	mzCloud Results	ChemSpider Result	Mass List Searc	th Results	
Ē	Checked	Compound Match	Stru	cture		Name			Formula	Molecular Wei	ght
1 🗢		•	**		***	AU8420000			C22 H42 O8	434.287	97
2 🖶						(1S,2S,4R,5R)-	2,4-Diamino-5-[(2,3- With Headers	diamino-2,3-dideoxy	C18 H38 N6 O6 Ctrl+C	434.285	28
3 🗢						Clear S Clear S Cell Se Enable	Selection election Mode e Column Fixing			434.287	97
4 ⇔			~	~~~ <sup>0</sup> y <sup>a</sup> ~~ <sup>0</sup> ~ <sup>0</sup> ~ <sup>0</sup>	$\sim$	Check 1, Check Unche	Selected All ck Selected		> >	434.287	97
5 ⇔			~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~°~	Bi Go to	ve All Checkmarks in Same Item in Main	All Tables Fable		434.287	97
		1	<u> </u>			Add to Add to Export	o Structure Proposal	s s and Apply FISh Scori	ng 🍃		

The Specify FISh Scoring Settings dialog box opens. You can change the settings. For example, you can increase the depth of the reaction pathways to a maximum of 20. However, increasing the maximum depth also increases the processing time. If you want the scoring algorithm to ignore small fragments, raise the S/N threshold to 20.

Specify FISh Scoring Settings		×
Annotate full spectrum tree	High accuracy mass tolerance:	mmu
Use general rules		ining -
Allow aromatic cleavage	0.5	Da 🔻
5 - Max. Depth	S/N threshold:	
	<u>O</u> K	<u>C</u> ancel

# 7. Click **OK**.

The FISh Scoring Queue opens to the right of the result tables.

④ FISh Scoring Queue	
1918 1918	X
Na MW [[	me: AU8420000 Dal: 434,28797
Processing Sir	nce: 40 s here Processing
	*
MW [Da]	: (15,25,4K,5K)-2,4-Diamino-5-[(2,3-diamino-2,3-dideoxy-alpha-D-glucopyranosyl]oxy/cyclohexyl 2,6-diamino-2,3,4,6-tetradeoxy-alpha-D-erythro-hexopyranoside : 434.28528
Queued Since	: 40 s
State	: woating

When the processing is complete, a custom structure proposal and a FISh coverage score appear in the Structure Proposals table for the selected compound.

# To use a custom annotation for the compound

- 1. With row 5 (Bis[2-(2-butoxyethoxy)ethyl] adipate) selected in the Compounds table, click the **Structure Proposals** tab in the related tables.
- 2. In the Structure Proposals table for Bis[2-(2-butoxyethoxy)ethyl] adipate, right-click **row 1** and choose **Structure Proposals > Use as Compound Annotation**.

This figure shows the shortcut menu for the Structure Proposals table.

tructure P	roposals	Compounds per File	Predicted Compositions	Merged Features	mzCloud	Results	ChemSpide	er Results	Mass I	List Search Results	
Ē	Checked	Structure		Nar	me	Formula	3	Molecular	Weight	FISh Coverage 🔻	Comments
1 🕈		****		Copy With Hea	8420000 Iders	C22 H4	2 08 Ctrl+	434 C	4.28797	81.58	
2 🗢				Copy Clear Selection Cell Selection M Enable Column	Mode 1 Fixing			434	4.28528	7.89	
				Check Selected Check All Uncheck Select Uncheck All	l ted			> > >			
				Remove All Ch Go to Same Ite	eckmarks in A em in Main Ta	<b>II Tables</b>			Add Stru Edit Stru Delete S	ucture Proposal ucture Proposal tructure Proposal	
				Structure Prop	osals			<b>`</b>	Use as C	ompound Annotat	ion
			L			_			Apply FI Apply FI	Sh Scoring to Selec Sh Scoring to All	ted

The FISh Coverage column appears in the Compounds table. The custom annotation includes the new name, structure, formula, and FISh coverage score for the compound.

3. To view display the Structure column, open the Field Chooser dialog box and select the Structure check box.

•	Compo	unds 💎	Compou	inds per File	Merged Features	Features	mz(	Cloud Results	ChemSpider Results	Mass List Searc	h Results Input F	iles Sp	ecialized Traces
	Ē	Checked	Structure			Name		Formula	Annotation Source 🛨	FISh Coverage	Molecular Weight	RT [min]	Area (Max.) 🔻
5	; -¤		****	*	<sup>1</sup> ******	AU8420000		C22 H42 O8		81.58	434.28701	31.429	4613052498

# Using the Result Filters View

The analysis found a total of 1133 compounds, including 137 background compounds. So the Compounds table displays 996 compounds. To reduce the number of compounds to review, filter the table.

	c	ompo	unds 💡	Compounds per File	Merged Features	Features	mzClou
	Ē		Checked	Compounds grouped by molecular weight and retention tim			
1	1 🗢 🗌			996 of 1133 items shown (	137 filtered out)		
2	2	<b>-</b> Þ		Bis(2-butoxyethyl) adipate	C18 H34 O6		

# To reduce the number of Compounds to review by the Area (Max.) column

- 1. Click the **Compounds** tab in the set of main tables.
- 2. From the menu bar, choose **View > Result Filters**.

The Result Filters view opens as a floating window. 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.

② Result Filters		- • •
ON       Compounds         ON       Compounds per File         ON       Merged Features         ON       Features         ON       ChemSpider Results         ON       Mass List Search Results         ON       Input Files         ON       Specialized Traces	Compounds AND Add aroup Background is false Remove (Add property)	
Show all tables	ad Save Save As Clear All	Clear Apply Filters

3. Click Add Property and select Area (Max.).



4. In the relation list, select Is Greater Than or Equal To.

Result Filters		
ON       Compounds         ON       Compounds per File         ON       Features         ON       Features         ON       Compounds per File         ON       Features         ON       ChemSpider Results         ON       Mass List Search Results         ON       Input Files         ON       Specialized Traces	Compounds AND Add aroup Background is false Remove Area (Max) is equal to is equal to is not equal to is less than is less than is greater than or equal is between by	
Show all tables	d Save Save A has no value	Apply Filters

5. In the value box, type 4e9.

Result Filters		
ON Compounds	Compounds	
ON Compounds per File	AND Add aroup	Value bey
ON Merged Features	Background is false Remove	
ON Features		
ON Cloud Results	Area (Max.) is greater than or equal to 8e7 Remove	
ON Common Commo	(Add property)	
ON ChemSpider Results		
ON Mass List Search Results		
ON DInput Files		
ON Specialized Traces		
Show all tables	Save Save As Clear All Clear Apply Filters	Applies the filters.

6. Click Apply Filters.

The Compounds table now displays only seven compounds.

- To display only the checked compounds
- 1. Click the **Compounds** tab in the set of main tables.
- 2. From the menu bar, choose **View > Result Filters**.
- 3. Click Add Property and select Checked.
- 4. Click Apply Filters.

Before exporting the results to a spreadsheet, filter the results table or select the check boxes for the compounds of interest (see "Using the Result Filters View" on page 39).

# To check the number of table rows

Point to the Compounds tab.

A tooltip appears with the number of displayed compounds.

# ✤ To select the columns that you want to export

- 1. Click the **Field Chooser** icon, **F**, for the Compounds table.
- 2. For this tutorial, clear all the check boxes, except for the following:
  - Area (Max.)
  - Formula
  - Mass List Matches
  - MW
  - Name
  - RT

**Exporting the** 

- ✤ To export the filtered and sorted results to an Excel spreadsheet
- 1. Right-click the Compounds table and choose **Export > As Excel**.

17		
Сору		
Clear Selection		
Cell Selection Mode		
Enable Column Fixing		
Collapse All Column Headers		
Expand All Column Headers		
Check Selected		
Check All		
Uncheck Selected		
Uncheck All		
Remove All Checkmarks in All Tables		
Edit Compound Annotation		
Clear Compound Annotation		
Apply FISh Scoring	4	As Plain Text
	4	As Excel
Export •	4	As Xcalibur Inclusion/Exclusion List
	4	As TraceFinder List
	4	As mzVault Library
	A	Add Compound to Existing mzVault Library
	4	As Mass List
	ļ	Add Selected Compounds to Existing Mass List
	_	

The Export to Excel dialog box opens.

- 2. Check the file name and location in the Path box. Change the file name and folder as appropriate.
- 3. In the Options area, select the **Open File After Export** check box.

Export to Excel	×
Path: C:\Users\Public\Documents\E and L O-ring Example.xlsx Items and related tables to be exported Level 1: Compounds Level 2: Level 3:	Options Checked items only Open file after export
	<u>Export</u> <u>Cancel</u>

4. Click Export.

The Excel spreadsheet opens.

🔣   🚽 🤊 🔹 🔍 🖃 E and L O-ring Example.xlsx - Microsoft Excel														23	
	File Home Insert Page Layout Formulas Data Review View Nitro Pro Acrobat Team										0	• 🕜		F 23	
A1 • ( Jr Name													* *		
	A B C D E F G H I									I.		J			
1	Name	Formula	Molecular V	Veight	RT [min]	Area (Max.)	Mass Lis	t Match: E	xtractables	and Leach	ables HRA	M Compou	ind D	ataba	ise
2	Triphenyl	C18 H15 O P	27	8.08558	21.853	8084284133	Single n	natch foun	d						=
3	Bis(2-buto	C18 H34 O6	34	6.23461	31.968	5073684440	Multipl	e matches	found						
4	Sudan III	C22 H16 N4 O	35	2.13707	20.057	4982336781	No mat	ches found	l						
5	Cholestan	C27 H48 O7	48	4.33856	34.549	4749677345	No mat	ches found	l						
6	AU842000	C22 H42 O8	43	4.28701	31.429	4613052498	Multipl	e matches	found						
7	20-(4-Non	C29 H52 O8	5	28.3645	34.48	4451926524	No mat	ches found	1						
8	Dipropyle	C8 H18 O3	16	2.12533	12.876	4362142212	Multipl	e matches	found						-
14	A b b Co	mpounds 🥂	]/												•
Re	ady										1009	6 <del>-</del>	-0		+ .;;

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