



MassHunter Explorer 1.0

Introduction Workbook

Notices

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Software Revision

This guide is valid for the MassHunter Explorer 1.0 program or higher and compatible MassHunter Explorer 1.0 programs, until superseded.

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Video Support

This Introduction Workbook has supplemental video support available. Each chapter uses step by step instructions supported by on line videos to view and review the material as needed. Scan the code below or use this link to access the videos (<https://aglt.co/ExplorerIntro>).



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Introduction

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This workbook provides instructions on the MassHunter Explorer 1.0 workflow.

For additional information on the software and detailed instructions on the workflow not covered in this workbook, refer to the Online Help.

Use the following exercises to experience how to utilize MassHunter Explorer to determine relationships among sample groups and variables, then export to desired formats. Example data is provided with the installation of the software to introduce these steps.

This workbook is your introductory guide for the set-up and execution of basic procedures with the MassHunter Explorer. This workbook is divided into chapters, each building upon the last, so we recommend that each chapter is completed in succession. During each chapter, lessons are guided by video support.

By completing this learning event, you will have an introductory level of experience in the use of MassHunter Explorer.

How to use this Workbook

This learning experience introduces basic concepts in a learning-by-doing, guided manner. Each chapter uses step-by-step instructions.

Task steps look like this:

1 Tasks or items needed to complete tasks look like this.

If you are expected to enter any information or if something is important, it is set in italicized type like this:

Type *Blank One* in the field.

If you are expected to press a key on the keyboard or button on the software screen, the key is displayed in bold like this:

Press Enter.

Cross references appear in blue:

(For example, [Link](#))

Notes and Alerts

NOTE

The Note text appears here.

CAUTION

The Caution text appears here.

WARNING

The Warning text appears here.

What this Workbook covers



In this learning experience, the goal is to get up and running using the software as quickly as possible. After completing this learning MassHunter Explorer event with your Agilent consultant, you will have an introductory level of experience in the use of Mass Hunter Explorer 1.0.

This learning experience introduces basic concepts in a learning-by-doing, guided manner. Each chapter uses step by step instructions and is supported by on line videos to view and review the material as needed. Scan the code to the right or use this link to access the videos (<https://aglt.co/ExplorerIntro>). At any time if you have a question or get stuck, to find your local sales and support contact, visit the following page using the link below.

- <https://www.agilent.com/en/contact-us/page>

For technical support, visit the following page:

- <https://www.agilent.com/en/support>

The following exercises are designed to support the execution of the MassHunter Explorer workflow along with video support. The videos are intended to provide visual support for working with the software. We recommend reviewing the video demonstrations first, then attempting the workflow processes, using each exercise as a guide. If at any time you have questions or run into an event that is not in alignment with this workbook, please reach out to your Agilent Consultant.

Requirements

To complete the chapters in this training workbook, you need to have installed the software to the most recent release recommended. The software is found on the install media provided in the install kit, along with specially prepared data sets to load onto the software system before the start of this learning exercise.

Please refer to the Online Help or Installation Guide for further information. As always, feel free to contact Agilent Support for additional assistance.



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User Interface and General Navigation

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MassHunter Explorer Overview

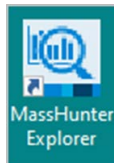
MassHunter Explorer is designed to investigate compounds in your Agilent LC/TOF LC/Q-TOF data files by comparing similarities and differences between samples or groups of samples. Software features are well-suited for the comparison samples from different environmental locations (for example, two locations in a river) as well as different batches in a food or chemical synthesis process. MassHunter Explorer uses a feature extraction and alignment algorithms that find and aligns all the compounds across samples, even very complex mixtures.

The following list define the steps in the MassHunter Explorer workflow:

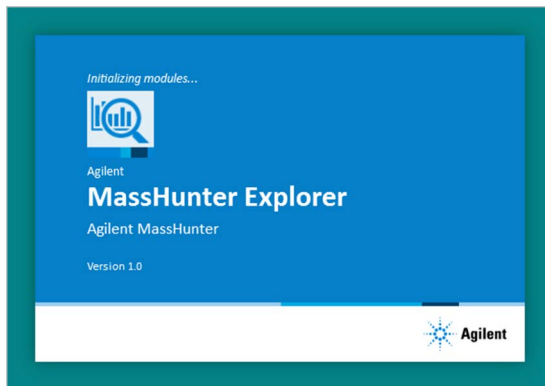
- **Setup:** Add or edit samples loaded in a new project or an existing project and group samples by adding Sample Group information according to the experiment's design.
- **Find and Align:** Extract ion features from data, group related ion into Compounds, and align Compounds across samples into a Compound Group.
- **Normalize:** Reduce unwanted systematic error due to sample preparation or instrument process.
- **Filter:** Create a subset list of compounds that are measured reliably within or across the sample groups or pass abundance criteria.
- **Statistics:** Focus on significant compounds with statistical analysis and visualization tools for data.
- **Identify:** Use mass spectrometry data and compounds databases to identify measured compounds.

Launching MassHunter Explorer

- 1 Double click on the MassHunter Explorer Icon to launch the software.



- 2 The software splash screen displays the software initialization.



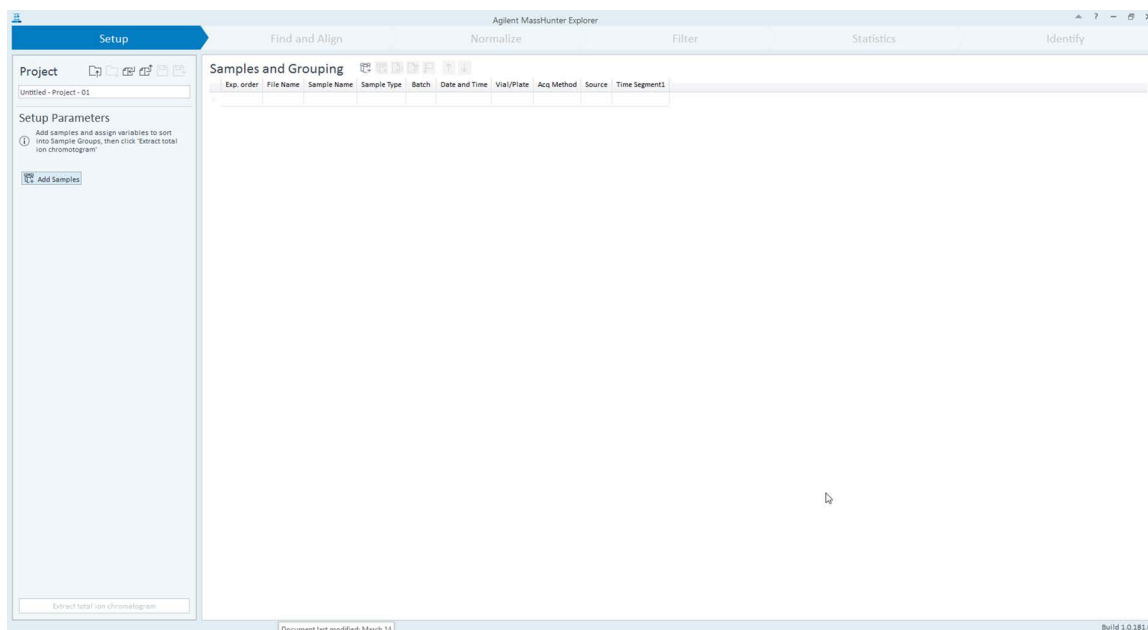
- 3 Upon launch, the first step in the workflow, Setup, is active on the ribbon.



Step Definitions

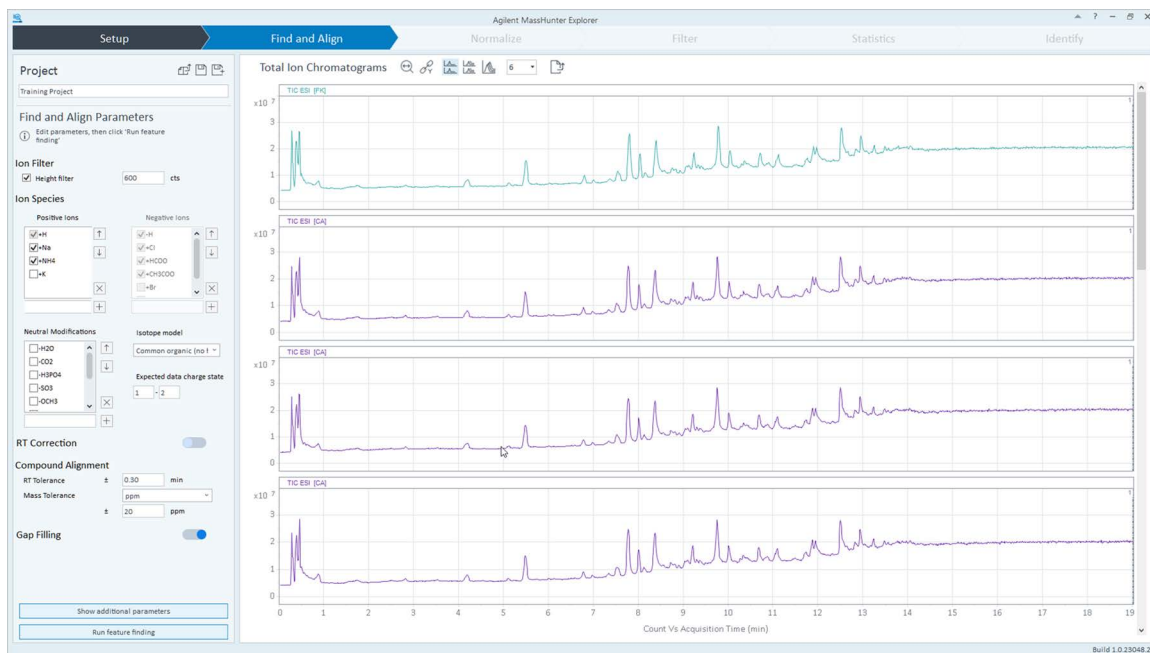
Setup Step

The Setup step is where new projects are created that use mass spectrometry data to find significant chemical differences between sample groups. In this step, add or edit samples loaded in a new project or an existing project, then group samples by adding Sample Group information according to the experiment's design.



Find and Align Step

The Find and align step is where features that statistically differ in abundance between two or more sets of experimental conditions are found. In this step extract ion features from your data, group related ion into Compounds, and align Compounds across samples into a Compound Group.



Normalize Step

The Normalize step is where data is organized in ways that eliminate redundancy and inconsistent dependencies. Use these functions to reduce unwanted systematic error due to sample preparation or instrument process.

The screenshot displays the Agilent MassHunter Explorer software interface during the 'Normalize' step. The top navigation bar includes 'Setup', 'Find and Align', 'Normalize', 'Filter', 'Statistics', and 'Identify'. The main window is divided into several panels:

- Project:** Training Project
- Normalize Parameters:**
 - Add samples and assign variables to sort into Sample Groups
 - Log2 Transform:
 - LOESS Normalization:
- Compound Groups (5217):** A table listing compound groups with columns for Group, Mass, m/z, RT, Name, Formula, RSD, Found, Miss, and Height. The table is sorted by m/z.
- Compound Details:** A table listing individual compounds with columns for File Name, Sample Name, Mass, Sat., m/z, Ions, RT, Width, Height, Score, Area, and Diff/Tgt. The table is sorted by Sample Name.
- Plots:** A Principal Component Analysis (PCA) plot showing Abundance: Area (Raw) versus PC1 (20.54%) and PC2 (11.31%). The plot displays several clusters of data points, each enclosed in a colored ellipse (purple, red, green, blue).

Filter Step

The Filter step allows filtering of data based on abundance, variability, or frequency to look for differences between sample groups. At this step you are able to create subset lists of compounds that are measured reliably within or across the sample groups or pass abundance criteria.

Project
Training Project

Filter Parameters
Edit sample groups and filters parameters then, then click "Run Filter"

Select factor(s) to group samples

Groups
 Sample Type
 Batch
 Treatment

Sample Groups:

Sample Groups	Replicates
<input checked="" type="checkbox"/> (PK)	3
<input checked="" type="checkbox"/> (CA)	3
<input checked="" type="checkbox"/> (CC)	3
<input checked="" type="checkbox"/> (FK)	3
<input checked="" type="checkbox"/> (WT)	3

Specify the filtering options

Abundance
 Value Percentile
 Minimum: 7.5 Maximum: 20.5
 100% of files in at least one sample group

Variability
 Coefficient of variation (%): 25
 100% of files in at least 1 of 4 sample groups

Frequency
 100% of files in at least one sample group

Run Filter

Compound Groups (5217)

Group	Mass (m/z)	RT (min)	Name	Formula	RSD	Found	Missed	Height
1	254.2621	277.2515	2-Heptadecanone	C17 H34 O	0.64	12	0	1124406
2	224.1421	325.1493	13-Oxo-9,11-tridecadienoic acid	C13 H20 O3	1.35	12	0	948544
3	218.1526	241.1419	8,9-Difluoro-8E,10E-dodecadien-1-ol	C12 H20 F2 O	0.67	12	0	935542
4	250.2301	275.2193	65,78-epoxy-32,92-heptadecadiene	C17 H30 O	0.90	12	0	594777
5	276.1759	299.1651	Onchidial	C17 H40 O3	0.80	12	0	658727
6	282.2927	305.2818	3-Ethylheptadecan-3-one	C19 H38 O	1.27	12	0	668052
7	234.1623	257.1514	Germacrene A acid	C15 H22 O2	0.53	12	0	524357
8	478.5069	701.4961			0.60	12	0	871329
9	180.1205	213.1100			0.69	12	0	490332
10	222.1988	245.1880	(+)-Cedrol	C15 H26 O	0.80	12	0	396649
11	226.2299	249.1928	3-Ethyltridecan-3-one	C15 H30 O	0.82	12	0	449301
12	292.2030	315.1923	9-OxoOHE	C18 H28 O3	0.46	12	0	364511
13	276.1735	299.1626	Onchidial	C17 H24 O3	0.73	12	0	500626
14	131.0950	132.1025	1-Nitrohexane	C6 H13 N O2	1.91	12	0	764797
15	236.2139	259.2032	9E,11E-Heptadecadienal	C16 H28 O	0.97	12	0	212257
16	131.0884	132.0984	Glycerol	C3 H8 O3	19.97	12	0	116059
17	92.0435	115.0318	Glycerol	C3 H8 O3	13.72	12	0	410973

Compound Details

File Name	Sample Name	Mass	Sat	m/z	Ions	RT	Width	Height	Score	Area	Diff(%)
FK_RP_pos_8_d	FK_RP_pos_8	232.2789	False	255.2683	15	12.528	0.07	10881218	100.00	63758419	-21.982
CA_RP_pos_7_d	CA_RP_pos_7	508.5230	False	531.5124	14	12.504	0.06	11254502	100.00	63644557	254.261
CA_RP_pos_8_d	CA_RP_pos_8	508.5230	False	531.5123	14	12.504	0.06	11257543	100.00	64122953	254.261
CA_RP_pos_9_d	CA_RP_pos_9	232.2791	False	255.2684	15	12.503	0.06	11233629	100.00	64329389	-21.982
CC_RP_pos_7_d	CC_RP_pos_7	232.2790	False	255.2683	14	12.517	0.07	11181847	100.00	65578152	-21.982
CC_RP_pos_8_d	CC_RP_pos_8	232.2789	False	255.2683	14	12.524	0.06	11481876	100.00	65055786	-21.982
CC_RP_pos_9_d	CC_RP_pos_9	232.2790	False	255.2684	14	12.517	0.06	11183237	100.00	65133845	-21.982
FK_RP_pos_7_d	FK_RP_pos_7	232.2791	False	255.2685	14	12.524	0.06	10978756	100.00	63681152	-21.982
FK_RP_pos_8_d	FK_RP_pos_8	232.2787	False	255.2681	14	12.524	0.06	11208935	100.00	63549779	-21.982
WT_RP_pos_7_d	WT_RP_pos_7	232.2787	False	255.2681	14	12.524	0.06	11759272	100.00	68037305	-21.982
WT_RP_pos_8_d	WT_RP_pos_8	232.2793	False	255.2686	14	12.520	0.06	11947594	100.00	68088188	-21.982
WT_RP_pos_9_d	WT_RP_pos_9	232.2790	False	255.2685	14	12.520	0.06	11905304	100.00	67866240	-21.982

Plots
Abundance: Area (Raw)
Principal Component Analysis performed on the Raw abundance values obtained from feature finding. Outlier samples can be determined based on the confidence ellipse for the group.

PC2 (11.31%)

PC1 (20.54%)

X Axis: PC1 (20.54%) Y Axis: PC2 (11.31%)

Build 1.0.230482

Statistics Step

The Statistics step is used to find statistically valid differences between sample groups. This step enables you to focus on significant compounds with statistical analysis and visualization tools for your data.

The screenshot displays the Agilent MassHunter Explorer interface during the Statistics step. The main window is titled "Agilent MassHunter Explorer" and features a navigation bar with tabs for Setup, Find and Align, Normalize, Filter, Statistics (the active tab), and Identify. On the left side, there is a "Project" panel showing "Training Project" and "Statistics Parameters" with options to add samples and run statistical analysis. Below this, there are sections for "Groups" (Sample Type, Batch, Treatment) and "Sample Groups" (Sample Groups, Replicates) with checkboxes for [PG], [CA], [CC], and [WT]. The "Statistical Analysis" section allows selecting an analysis method. The central area shows a table of "Compound Groups (5217)" with columns for Group, Mass (m/z), RT (min), and Name. The table lists various compounds such as 2-Hepta, 13-Oxo-9,11-tridecadiene, 8,9-Difluoro-8E,10E-dodecane, 3-Ethylhexadecane, Gemmacr, 3-Ethyltridecane, 1-Nitro, 9E,11E-hexadecane, 2,5-Dihydro-2,4-dimethyl-Benzyl, Eupa, n-butyl n-va, 9R,10S-Epoxy-3Z,6Z-elic, 1-Nitro, Adenofosol, 3-capryl propyl, C, Homover, 10,12-Tetradecane, and Aris. On the right side, the "Plots" panel displays five chromatograms and mass spectra for selected compounds: 2-Hepta (m/z 277.2612), 8,9-Difluoro-8E,10E-dodecane (m/z 266.2663), 3-Ethylhexadecane (m/z 277.2618), 1-Nitro (m/z 277.2612), and 2,5-Dihydro-2,4-dimethyl-Benzyl (m/z 277.2616). Each plot includes a title, y-axis scale, x-axis label, and a legend for the data series.

Identify Step

The last step is Identify, where compounds of interest are putatively identified, and results can be exported in various formats. This allows for communication of the results, further acquisition and extraction of MS/MS spectra to aid compound identification. This step uses mass spectrometry data and compounds databases to identify your measured compounds.

The screenshot displays the Agilent MassHunter Explorer software interface during the Identify step. The main window is titled "All Compound Groups" and shows a list of 21 compounds with their respective mass, m/z, retention time (RT), name, formula, score, mass, hits, and difference (Diff.). The top compound listed is 2-Heptadecanone (C17H34O) with a score of 89.73 and a mass of 254.2621. Below the list, there are sections for "Putative Compound Identifications" and "Identification Results: 2-Heptadecanone". The identification results table shows the following data:

Species	m/z	Height	Score(MS)	Score(mass)	Score(iso.abund)	Score(iso.spacing)
(M+Na) ⁺	277.2515	6,223,743.60	89.73	96.79	70.84	98.27
	277.2515	277.2502	4.75	1.32	5,814,045.50	6,223,743.60
	278.2540	278.2536	1.38	0.38	1,486,693.50	1,371,051.82
	279.2591	279.2567	8.26	2.31	210,827.70	116,771.28

The interface also includes a "Composite MS Spectrum" plot showing relative intensity versus m/z, and a chemical structure of 2-Heptadecanone with a methyl ester group (MeCO) highlighted.



3

Setup

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Set Up Overview

The Setup step allows the ability to add or edit samples in a new or existing project, to group samples by adding Sample Group information, as well as importing or exporting information for an experiment's design.

The ability to perform the following tasks is available in the Setup step:

- Add or remove samples from a project
- Assign groups to samples
- Import or export a method
- Import or export sample groups
- Fill down sample groups
- Extract the total ion chromatogram

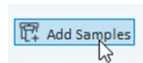
For the first step in the training experiment, we add samples and save a new project file.

Set Up Training Task Overview:

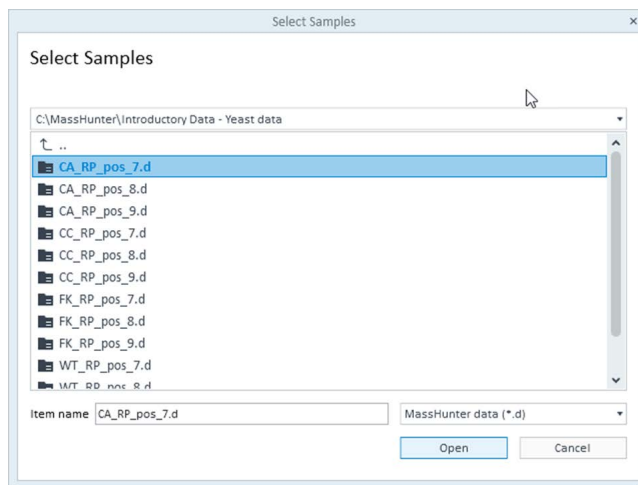
- 1 Add samples to a project.
- 2 Assign groups to samples, using fill down.
- 3 Save a new project file.
- 4 Extract the total ion chromatogram.

Add Samples

- 1 Click **Add Samples** to load the Select Samples Dialog box.



- 2 Browse to locate the files of interest or to an alternate directory.

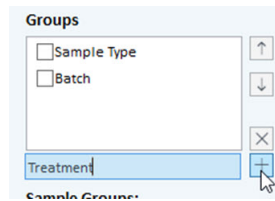


- 3 Click to select the desired files individually or press **CTRL+A** on the keyboard to select all samples in the list, then click **Open** to load them into Explorer.

The samples load into the Samples and Groupings pane.

Assign Groups

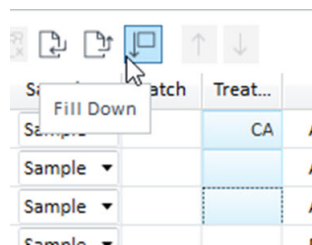
- 1 Add a custom group by entering a name into the Groups field, in this example *Treatment*, then click + to add to the Groups list.



- 2 Sort the samples by clicking on the column header **Sample Name**, or any other column header if needed, then review the data for anomalies.
- 3 Select the first cell under Treatment to select, then add the information from the table below in the following format to sort samples into Sample Groups:

Sample Name	Treatment
CA_RP_pos_X-r001	CA
CC_RP_pos_X-r001	CC
FK_RP_pos_X-r001	FK
WT_RP_pos_X-r001	WT

- 4 Enter *CA* in the selected cell, then click to select the cell again. Holding the Shift key down, click to select the bottom most cell in the Sample name series for this designation. Click **Fill Down** to automatically fill in the selected cells.

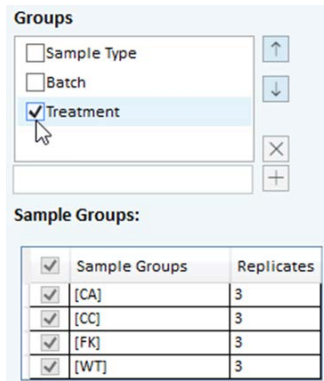


Repeat this action with the remaining Treatment groups for each corresponding Sample name.

Setup

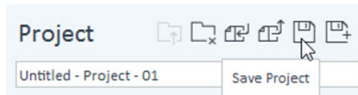
Save Project

- In Groups, select the **Treatment** check box. Verify the replicates are listed in Sample Groups as expected, in this example four groups of three.



Save Project

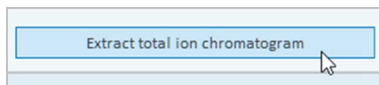
- Click **Save Project** to save the imported project sample files as an Explorer Project.



- Enter *Training Project* in the File name field and click **Save**.
- Click **OK** to confirm.

Extract Total Ion Chromatograms

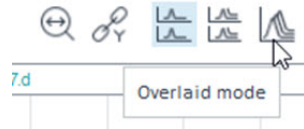
- Click **Extract Total Ion Chromatograms**.



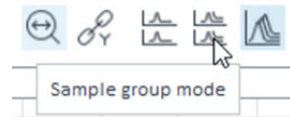
Setup

Extract Total Ion Chromatograms

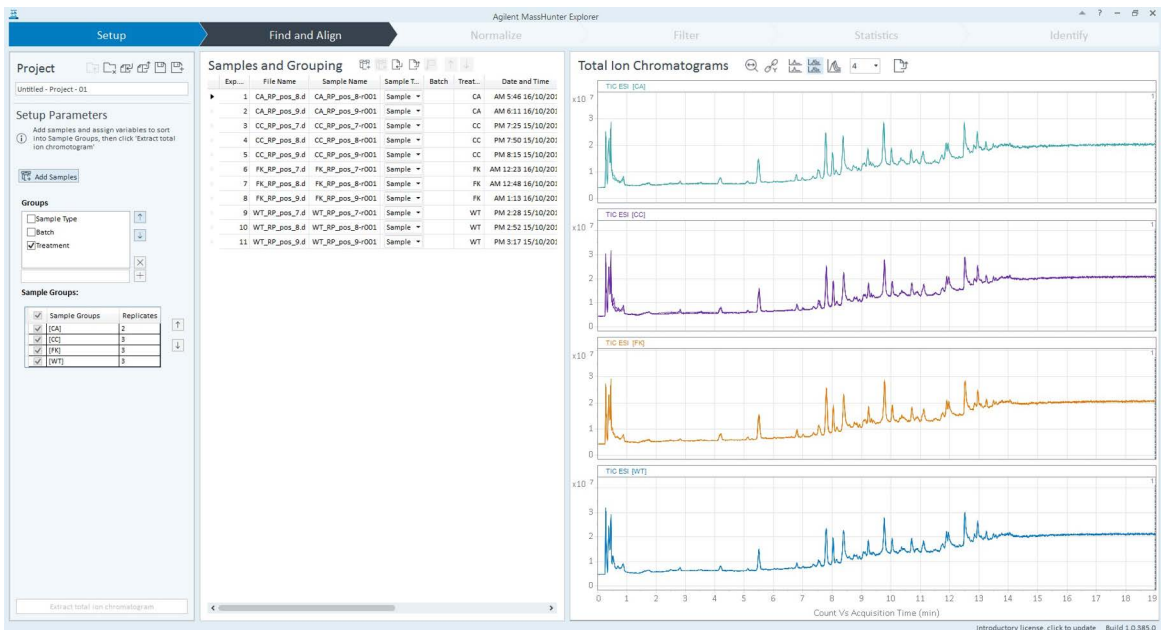
- The Total Ion Chromatograms load on the right pane of the window. Visually inspect the data by overlaying the Total Ion Chromatograms clicking **Overlaid mode**.



- Group the replicates together by clicking **Sample group mode** to observe the reproducibility.



Once complete, the Setup window should reflect the image below and the Find and Align step is activated.





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Find and Align

Find and Align Overview 28

Find and Align Training Task Overview: 28

Find and Align Parameters 28

Find and Align Overview

In the Find and Align step, the software extracts and groups ion features from raw data that represent a compound, then aligns compounds between data files to create compound groups, so that statistical analyses can be conducted.

The ability to perform the following tasks is available in the Find and Align step:

- Edit Find and Align parameters
- Run feature finding

For the second step in the training experiment, we adjust parameters, run Feature Finding and review Total Ion Chromatograms.

Find and Align Training Task Overview:

- 1 Adjust Parameters.
- 2 Run Feature Finding.
- 3 Review total ion chromatogram using Sample Group modes.

Find and Align Parameters

- 1 Click Find and Align on the Ribbon to move to the next step.
- 2 Adjust the parameters depending on what type of chemistry is used during sample preparation and acquisition. For the example sample set, activate Gap Filling.

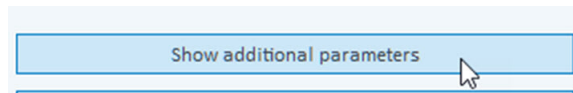
Gap Filling



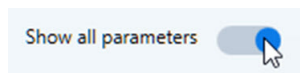
Find and Align

Find and Align Parameters

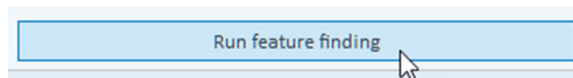
- 3 Click **Show Additional Parameters** to review the settings in depth.



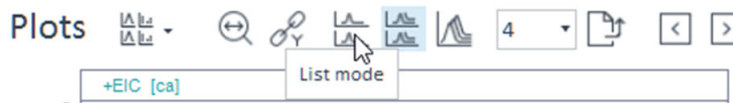
- 4 Activate **Show all Parameters** and expand the window, if needed. All Find and Align Parameters display.



- 5 Click **OK** to close the Parameters for Find and Align Features window.
- 6 Click **Run feature finding**. When complete, the Find and Align screen displays the Compound Groups, Compound Details, and Plots sample results.



- 7 Check the reproducibility of the compounds by clicking **List mode** with all samples shown.



- 8 Regroup the Total Ion Chromatograms by clicking **Sample group mode**.

Find and Align

Find and Align Parameters

Once complete, the Find and Align window should reflect the image below and the remaining steps are activated.

The screenshot displays the Agilent MassHunter Explorer software interface during the 'Find and Align' step. The interface is organized into several functional panels:

- Project:** Shows the current project name 'Yesst'.
- Find and Align Parameters:** Contains settings for ion filtering (Height filter: 1000 cts), ion species selection (Positive ions: -NH, -NH₂, -NH₃, -NH₄; Negative ions: -Cl, -CO, -HCOO, -HSO₃CO, -H₂P, -H₂P), neutral modifications (H₂O, CO₂, H₃PO₄, SO₃, OCH₃), RT correction, compound alignment (RT Tolerance: 0.30 min, Mass Tolerance: 20 ppm), and gap filling.
- Compound Groups (2751):** A table listing 18 groups with columns for Group, Mass, m/z, RT, RSD, Found, Missed, Height, and Vol. The first group is highlighted.
- Compound Details:** A table showing file names, sample names, mass, m/z, ions, RT, width, and height for various peaks.
- Plots:** Displays five mass spectra: EIC [CA], EIC [CC], EIC [FQ], EIC [VT], and F+I Spectrum [CA]. Each plot shows a peak at m/z 277.2506.
- Statistics:** Shows the F+I Spectrum [CA] with peaks at m/z 277.2506, 266.2696, 266.2716, 531.6122, and 532.6102.
- Identify:** Shows the F+I Spectrum [CA] with peaks at m/z 277.2506, 266.2696, 266.2716, 531.6121, 532.6105, and 532.6102.

The bottom right corner of the interface indicates 'Introductory license, click to update' and 'Build 1.0.383.0'.



5

Normalize

Normalize Overview 32

Normalize Training Task Overview: 32

Normalize Settings 32

Normalize Overview

In the Normalize step, data quality is reviewed with a Principal Component Analysis (PCA) when four or more samples are loaded. If needed, treat the data to reduce unwanted systemic error due to sample preparation error, instrument error, or batch-to-batch variation, or organize the data to eliminate redundancy and inconsistent dependency. Normalization is a type of smoothing that enables feature optimization and is helpful in seeing trends and choosing appropriate downstream statistics.

The ability to perform the following tasks is available in the Normalize step:

- Normalize data
- View PCA plots
- Add or remove samples from the PCA plot

Normalize Training Task Overview:

- 1 Adjust Normalize parameters.
- 2 Run Normalization.

Normalize Settings

- 1 Click Normalize on the Ribbon to move to the next step.



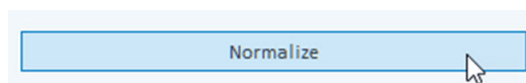
Normalize

Normalize Settings

- 2 Adjust Normalize Parameters as needed. Click to activate Log2Transform for the example sample set.



- 3 Click **Normalize**.



Once complete, the Normalize window should reflect the image below.

The screenshot displays the Agilent MassHunter Explorer software interface, specifically the 'Normalize' tab. The interface is divided into several sections:

- Project Panel (Left):** Contains 'Normalize Parameters' with options for 'Log3 Transform' (checked) and 'LOESS Normalization' (unchecked).
- Compound Groups (2751) Table (Center):** A table listing various compounds with columns for Group, Mass (m/z), RT (min), Found, Missed, Height (L), Volu., %RS, Scor., Area (me...), and %RS. The table shows 17 rows of data.
- Compound Details Table (Below):** A table providing detailed information for each compound, including File Name, Sample Name, Mass, m/z, Ions, RT, Width, Height, Scor., Area, Diff(L), and Diff. It lists multiple entries for different sample groups like CA, CC, FK, and WT.
- Plots Panel (Right):** Displays a Principal Component Analysis (PCA) plot. The Y-axis is labeled 'PC-2 (13.60%)' and the X-axis is 'PC-1 (37.82%)'. The plot shows several distinct clusters of data points, each enclosed in a colored ellipse (purple, orange, blue, green). A legend indicates 'Abundance: Area (Log2)'. Below the plot, there are dropdown menus for 'X Axis: PC1 (37.82%)' and 'Y Axis: PC2 (13.60%)'.



6

Filter

Filter Overview 36

Filter Training Task Overview: 36

Filter Parameters 36

Save Compound List 39

Filter Overview

In the Filter step, select compounds that are measured reliably within or across the sample groups for the downstream statistical analysis. Use the Principal Component Analysis (PCA) performed on this filtered list of compounds to decide which samples pass the quality criteria.

The ability to perform the following tasks is available in the Filter step:

- Run a filter
- Save or select a list of compounds

Filter Training Task Overview:

- Adjust Filter parameters
- Assign groups to samples, using fill down.
- Save a new project file.
- Extract the total ion chromatogram.

Filter Parameters

- 1 Click **Filter** on the Ribbon to move to the next step. **Review Filter Parameters Groups and Sample Groups** and double-check they are correctly defined.



Filter

Filter Parameters

- 2 Activate **Abundance** and review the settings. Choose either the Value or Percentile option, then adjust the minimum/maximum range. No changes are needed for this data set.

The screenshot shows the 'Abundance' filter settings. At the top, the word 'Abundance' is displayed next to a blue toggle switch that is turned on. Below this, there are two radio buttons: 'Value' (which is selected) and 'Percentile'. A horizontal slider is positioned below the radio buttons, with tick marks at 7.5, 14, and 20.5. Below the slider, there are two input fields: 'Minimum' with the value '1' and 'Maximum' with the value '27'. At the bottom of the panel, there is a status indicator that reads '100 % of files in at least one sample group'.

Filter

Filter Parameters

3 Expand the settings to review.

100 % of files in at least one sample group

Must apply in at least

100 % of files

across all sample files
 in at least one sample group
 in each sample group

4 Activate Variability.

Variability

Coefficient of variation (%) <= 25

in at least 1 of 4 sample groups

5 Expand the settings to review.

in at least 1 of 4 sample groups

Must apply in at least 1 of 4 sample groups

6 Activate Frequency and expand the settings.

Frequency

100 % of files in at least one sample group

Must apply in at least

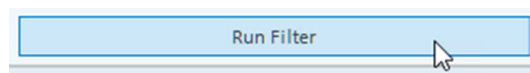
100 % of files

in all sample files
 in only one sample group
 in at least one sample group
 in each sample group

Filter

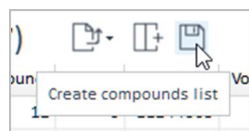
Save Compound List

- 7 Click **Run filter**.

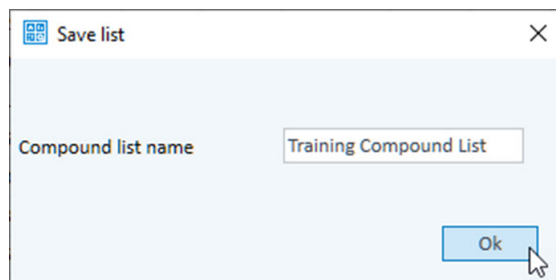


Save Compound List

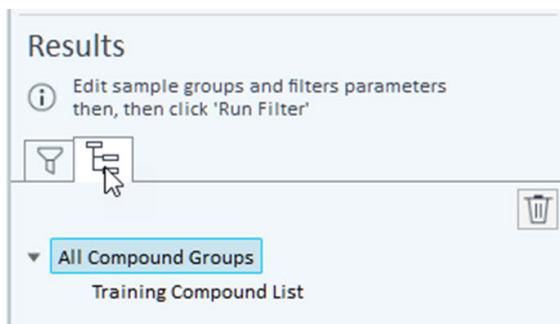
- 1 Click **Create compounds list**.



- 2 Enter a Compound List Name for the list, in this example *Training Compound List* and click **OK**.



- 3 To view the list, click the **Compound Groups** tab.



Filter

Save Compound List

NOTE

To use a specific filtered list for downstream statistical analysis, select the list before moving onto the Statistics step. It is not selected automatically.

Once complete, the Filter window should reflect the image below.

The screenshot displays the Agilent MassHunter Explorer software interface during the 'Filter' step. The main window is titled 'All Compound Groups' and shows a list of 2750 groups. The 'Filter Parameters' panel on the left allows for selecting factors to group samples, with 'Treatment' selected. The 'Compound Details' panel at the bottom provides a detailed view of the selected compounds, including their mass, m/z, and other properties. The 'Plots' panel on the right shows a Principal Component Analysis (PCA) plot with PC1 (37.83%) on the X-axis and PC2 (13.60%) on the Y-axis, displaying several clusters of data points.

Group	Mass (m/z)	RT (min)	RSD (%)	Found	Miss	Height	Volu.	NRS	Scor.	Area (me.)	NRS	
1	254.2611	277.2503	12.518	0.23	12	0	11287179	0	0.0	100.00	66513033	0.2
2	224.1422	247.1317	8.390	1.01	12	0	9607958	0	0.0	100.00	50933998	0.2
3	218.1520	241.1418	7.792	0.59	12	0	9425634	0	0.0	100.00	46798626	0.4
4	230.2195	273.2192	11.900	0.41	12	0	6156169	0	0.0	100.00	42101639	1.2
5	218.1700	299.1627	9.775	0.35	12	0	6513012	0	0.0	100.00	40479090	0.1
6	282.2021	305.2815	12.955	0.60	12	0	6724006	0	0.0	100.00	27816046	0.3
7	234.1626	257.1520	10.039	0.40	12	0	5261106	0	0.0	100.00	29100427	0.4
8	678.5068	701.4962	8.029	0.48	12	0	8712938	0	0.0	100.00	27100860	0.2
9	190.1210	213.1105	5.499	0.49	12	0	4954793	0	0.0	100.00	28346315	0.2
10	222.1986	245.1882	11.114	0.27	12	0	4202101	0	0.0	100.00	34431474	0.1
11	226.2299	249.2191	11.968	0.27	12	0	4515998	0	0.0	100.00	24723241	0.1
12	314.1857	315.1954	10.707	0.19	12	0	3645111	0	0.0	100.00	22740812	0.2
13	276.1732	299.1627	9.231	0.63	12	0	5026148	0	0.0	100.00	20908139	0.2
14	131.0933	132.1011	0.457	5.44	12	0	7691289	0	14.2	76.97	14716546	15.0
15	284.1834	307.1528	9.066	0.70	12	0	2339982	0	0.0	100.00	19224076	0.7
16	236.2145	259.2028	11.756	0.18	12	0	2615095	0	8.9	100.00	17613774	0.3
17	67.1470	116.0847	0.374	7.74	12	0	2139311	0	19.4	100.00	14311684	4.4

File Name	Sample Name	Mass	m/z	Ions	RT	Width	Height	Scor.	Area	Dirf.	D
CA_RP_pos_7_d	CA_RP_pos_7-r001	254.2613	277.2506	13	12.504	0.06	11278300	100.00	65894397	-0.03	
CA_RP_pos_8_d	CA_RP_pos_8-r001	254.2612	277.2505	13	12.504	0.06	11279717	100.00	66514129	-0.12	
CA_RP_pos_9_d	CA_RP_pos_9-r001	254.2611	277.2505	13	12.508	0.06	11256056	100.00	65766394	-0.18	
CC_RP_pos_7_d	CC_RP_pos_7-r001	254.2612	277.2505	13	12.517	0.07	11216253	100.00	67081945	-0.13	
CC_RP_pos_8_d	CC_RP_pos_8-r001	254.2612	277.2505	13	12.524	0.06	11442994	100.00	66711938	-0.12	
CC_RP_pos_9_d	CC_RP_pos_9-r001	254.2612	277.2505	13	12.517	0.07	11207463	100.00	66812968	-0.12	
FK_RP_pos_7_d	FK_RP_pos_7-r001	254.2612	277.2505	13	12.524	0.06	11001726	100.00	66817674	-0.13	
FK_RP_pos_8_d	FK_RP_pos_8-r001	254.2612	277.2505	13	12.528	0.07	10904908	100.00	65220215	-0.09	
FK_RP_pos_9_d	FK_RP_pos_9-r001	254.2611	277.2505	12	12.528	0.06	11232990	100.00	65817073	-0.14	
WT_RP_pos_7_d	WT_RP_pos_7-r001	254.2610	277.2503	12	12.524	0.06	11782824	100.00	69547003	-0.27	
WT_RP_pos_8_d	WT_RP_pos_8-r001	254.2611	277.2504	12	12.520	0.06	11971758	100.00	70422876	-0.17	
WT_RP_pos_9_d	WT_RP_pos_9-r001	254.2611	277.2504	12	12.520	0.06	11929784	100.00	70198356	-0.19	

7

Statistics

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Statistics Training Task Overview: 42

Statistics Settings 42

Review Post HOC Results 44

Statistics Overview

MassHunter Explorer provides a selection of statistical analysis and visualization techniques that aim to provide find and focus on significant compounds. By applying statistical techniques to mass spectrometry data, uncover hidden patterns, identify significant features, and derive meaningful insights. These statistical techniques enhance the reliability, reproducibility, and interpretability of mass spectrometry experiments.

Perform the following tasks in the Statistics step:

- Run a statistical analysis
- Save or select a list of compounds

Statistics Training Task Overview:

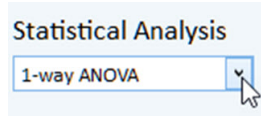
- 1 Adjust Statistic Settings
- 2 Run Statistical Analysis
- 3 Create compound groups list.

Statistics Settings

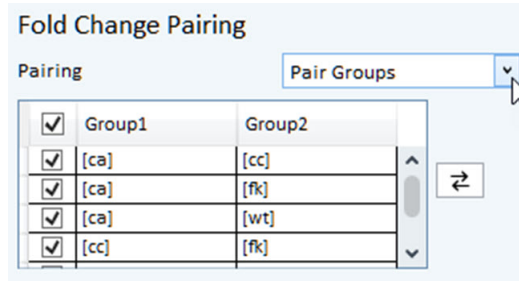
- 1 Click **Statistics** on the Ribbon to move to the next step.



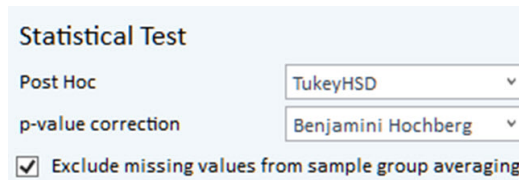
- 2 Under Statistics Parameters, adjust **Group** and **Sample Group** options if needed.
- 3 Click to select **Statistical Analysis**, then select *1-way Anova*.



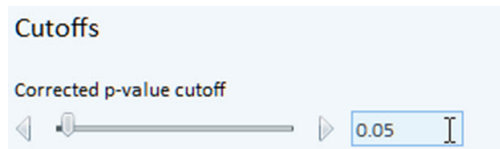
- 4 Under Fold Change Pairing, for Pairing select *Pair Groups*



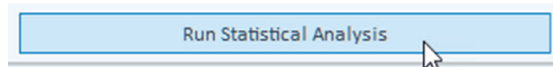
- 5 Under Statistical Test, for Post Hoc select *TukeyHSD*.
- 6 For p-value correction, select *Benjamini Hochberg*.
- 7 Select Exclude missing data values from sample group average.



- 8 Under Cutoffs, adjust the value to 0.05.



- 9 Click Run Statistical Analysis to view the results.



Review Post HOC Results

- 1 In the Post HOC Results Pane, click any of the compounds to view the specific Compound Groups in the Compound Groups pane.

ANOVA - Post Hoc Results ⓘ > ↗

Grou...	[ca]	[cc]	[fk]	[wt]
▶ [ca]	1111	455	392	801
▶ [cc]	656	1111	423	567
▶ [fk]	719	688	1111	920
▶ [wt]	310	544	191	1111

Legend

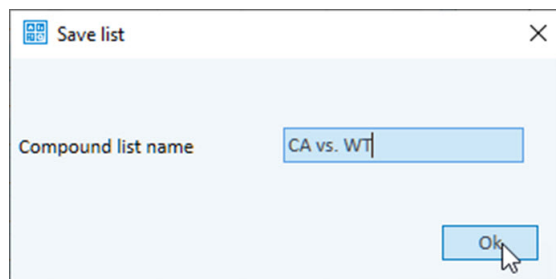
- Differentially abundant
- Not Differentially abundant

- 2 Save the data of interest, click Create compound groups list.

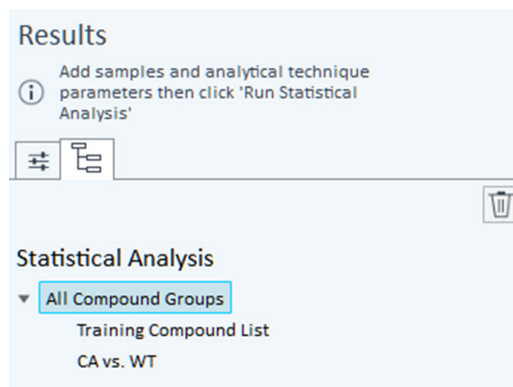
Compound Groups (1111) ⓘ ↗

Group	Mass [...]	m/z	Create compound groups list ...	p[cc]...	p[fk]...		
▶ 1	254.2611	277.2504	12.510	1.24E-6	1.13E-4	6.42E-3	1.00E0
▶ 2	224.1422	247.1317	8.390	1.43E-10	6.57E-7	2.31E-4	5.22E0
▶ 4	250.2293	273.2192	11.900	1.69E-3	1.24E-2	1.00E0	2.28E0
▶ 5	282.2920	305.2815	12.955	1.42E-5	4.76E-4	1.00E0	3.66E0
▶ 6	276.1730	299.1626	9.774	2.10E-6	1.48E-4	2.43E-4	1.00E0
▶ 7	234.1626	257.1520	10.039	1.30E-3	1.01E-2	1.00E0	2.99E0
▶ 11	226.2299	249.2191	11.968	1.96E-4	2.68E-3	4.67E-4	2.36E0
▶ 12	314.1857	315.1934	10.707	1.02E-3	8.51E-3	1.00E0	1.00E0
▶ 13	276.1732	299.1628	9.231	3.18E-9	3.64E-6	2.31E-4	2.55E0

3 Enter a Compound Groups List name, in this example *CA vs. WT* and click OK.



4 Click to view the Compound Group List.



Once complete, the Statistics window should reflect the image below.

The screenshot displays the Statistics window in Agilent MassHunter Explorer, showing the results of a 1-way ANOVA - Post Hoc analysis for Compound Groups (1006). The window is divided into several panels:

- Project:** Shows the current project name and sample groups.
- Statistics Parameters:** Includes options for adding samples and analytical techniques, and a list of sample groups (Treatment).
- Sample Groups:** Lists the sample groups and their replicates: [CA] (3), [CC] (3), [FK] (3), and [WT] (3).
- Statistical Analysis:** Shows the selected statistical analysis (1-way ANOVA).
- Fold Change Pairing:** Shows the selected pairing method (Pair Groups).
- Statistical Test:** Shows the selected post hoc test (TukeyHSD) and p-value correction (Benjamini Hochberg).
- Cutoffs:** Shows the corrected p-value cutoff (0.05).
- Compound Groups (1006):** A table listing the compound groups with their mass, m/z, retention time, and other parameters.
- Plots:** Displays five chromatograms (EIC) and mass spectra for the compound groups: [CA], [CC], [FK], and [WT].
- 1-way ANOVA - Post Hoc...:** A table showing the results of the post hoc analysis, including p-values for comparisons between groups [CA], [CC], [FK], and [WT].
- Results Summary:** A table showing the results of the statistical analysis, including p-values for comparisons between groups [CA], [CC], [FK], and [WT].

Results Summary Table:

	p<0.05	p<0.02	p<0.01	p<0.005
Corrected p-value	2751	1006	725	579
Expected by chance	50	14	5	2

1-way ANOVA - Post Hoc... Table:

Group	[CA]	[CC]	[FK]	[WT]
[CA]	1006	400	531	700
[CC]	501	1006	531	483
[FK]	688	395	1006	831
[WT]	288	531	148	1006

Legend:

- Differentially abundant
- Not Differentially abundant



8

Identify

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Identify Training Task Overview: 48

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Exporting Data 50

Identify Overview

In the Identify step, use compound databases to putatively identify compounds and match the measured Neutral mass of a compound extracted from the data to the theoretical Neutral mass of a compound stored in a compound database. The measured Neutral mass is determined from the evidence ions associated to the compound, along with their assigned charge states and adduct annotation.

Matching the measured isotope pattern to the theoretical isotope pattern expected from the compound's elemental formula.

Optionally, matching the measured retention time (RT) of the compound (when known and stored in the compound database) with the expected RT.

The ability to perform the following tasks is available in the Identify step:

- Edit Identify parameters
- Run Identification
- Save or select a list of compounds

Identify Training Task Overview:

- Adjust Identify Settings
- Export data.

Identify Settings

- 1 Click **Identify** on the Ribbon to move to the next step.



Identify

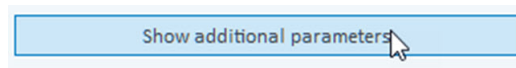
Identify Settings

- 2 Click + to Specify Database.

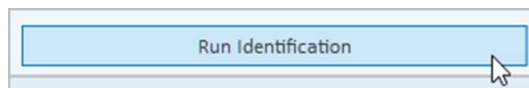


- 3 Browse to D:\MassHunter\Data and select Metabolite.cbd, then click OK.

- 4 Click Show Additional Parameters to review. Click OK to close.



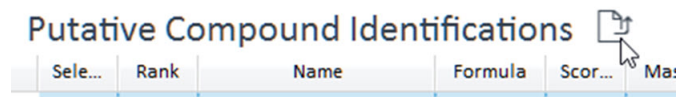
- 5 Click Run Identification.



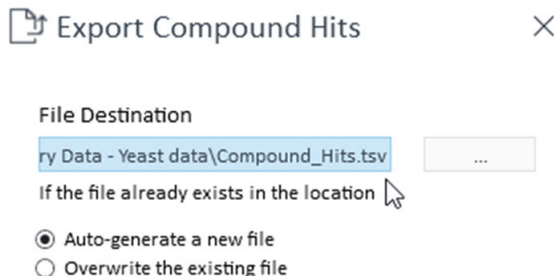
Exporting Data

Exporting Putative Compound Identifications

- 1 In the Putative Compound Identifications Pane, click Export Compound Hits.



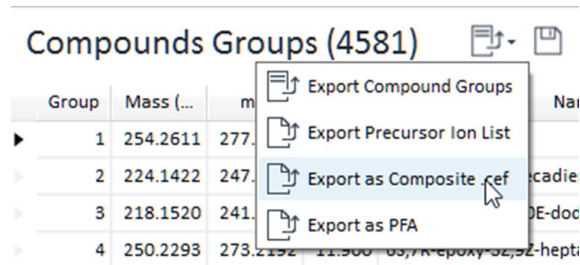
- 2 Select a file destination option and click Save.



- 3 Click OK.

Export Compound Groups

- 1 In the Compound Groups Pane, click Export to expand the options. Select Export as Composite .cef



- 2 Select File Destination Options and click Save.

3 Click OK.

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