Agilent

MassHunter Explorer 2.0

Introduction Workbook

Notices

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Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

Software Revision

This guide is valid for the MassHunter Explorer 2.0 program or higher and compatible MassHunter Explorer 2.0 programs, until superseded.

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CAUTION

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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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Contents

1 Introduction

About this Workbook

This workbook provides instructions on the MassHunter Explorer 2.0 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 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 MassHunter Explorer 2.0.

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**). If you have a question or get stuck, find your local sales and support contact by visiting 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

Before completing the chapters in this training workbook, ensure that the most recent release of MassHunter Explorer is installed on your system. 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.

2 User Interface and General Navigation

MassHunter Explorer Overview

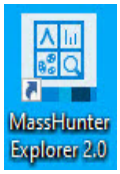
MassHunter Explorer is designed to investigate compounds in your Agilent LC/TOF LC/Q-TOF data files by comparing similarities and differences between samples or groups of samples. Software features are well-suited for the comparison samples from different environmental locations (for example, two locations 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 defines 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.

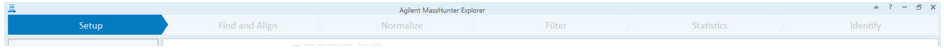
Launching MassHunter Explorer

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



2 The software splash screen displays the software initialization.

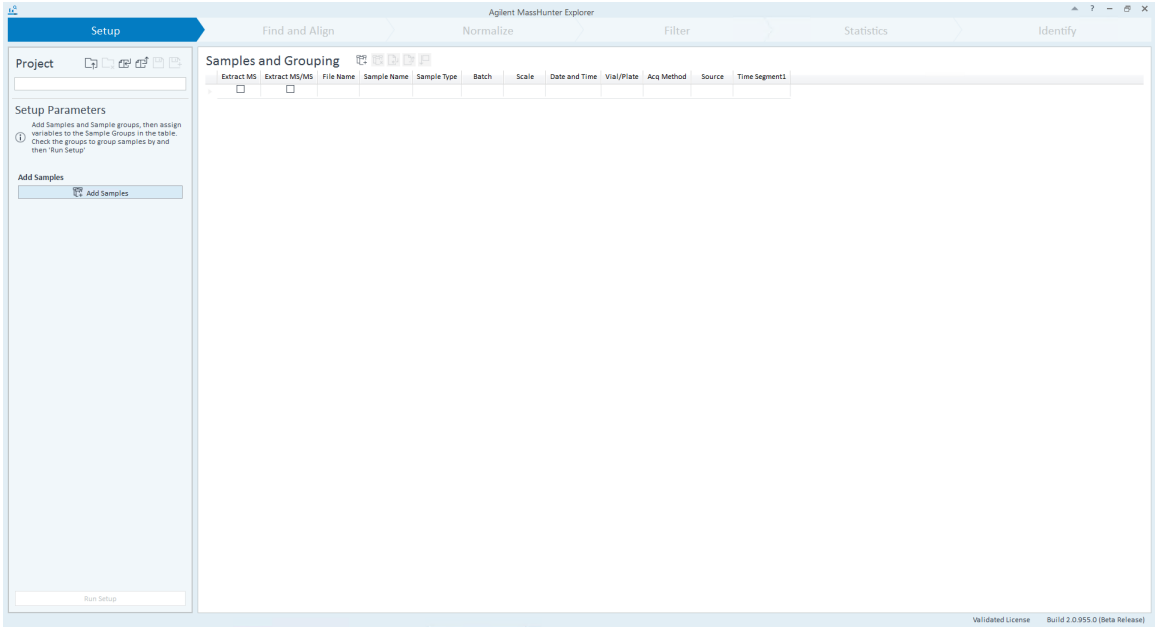
3 Upon launch, the first step in the workflow, Setup, is active on the ribbon.



Step Definitions

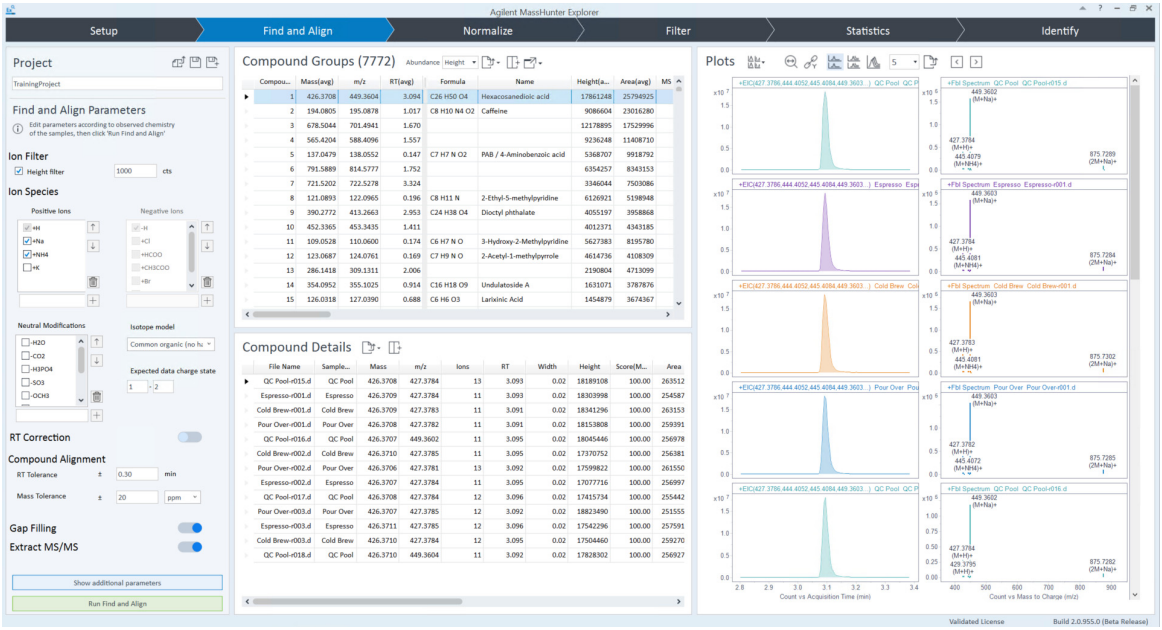
Setup Step

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.



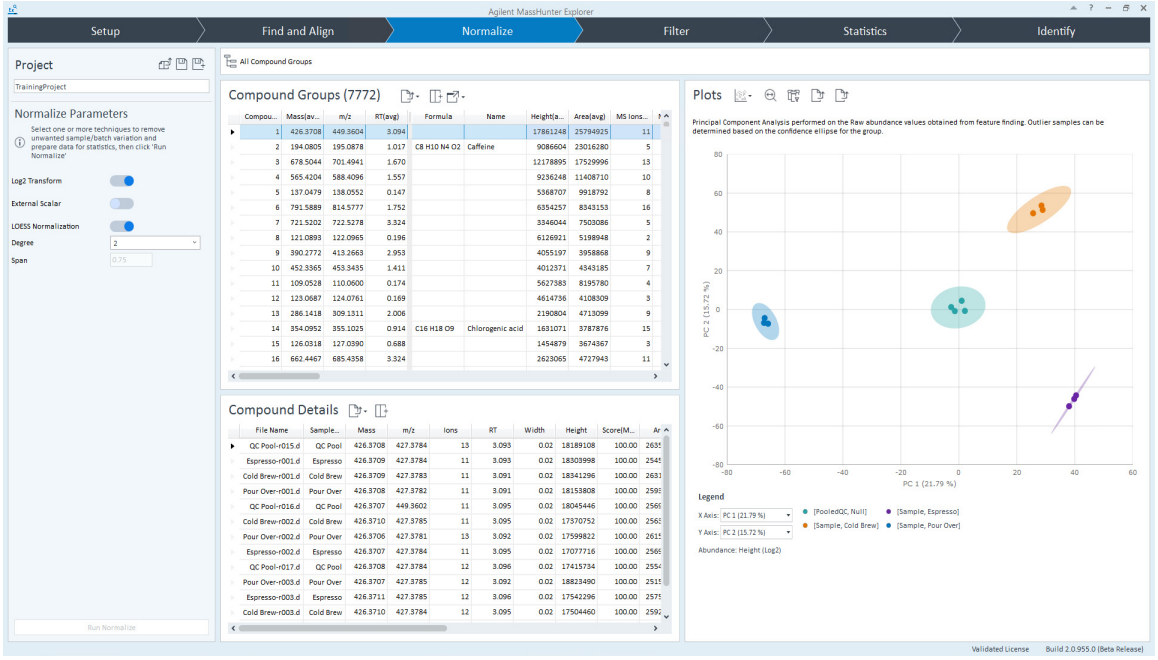
Find and Align Step

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 ions into Compounds, and align Compounds across samples into a Compound Group.



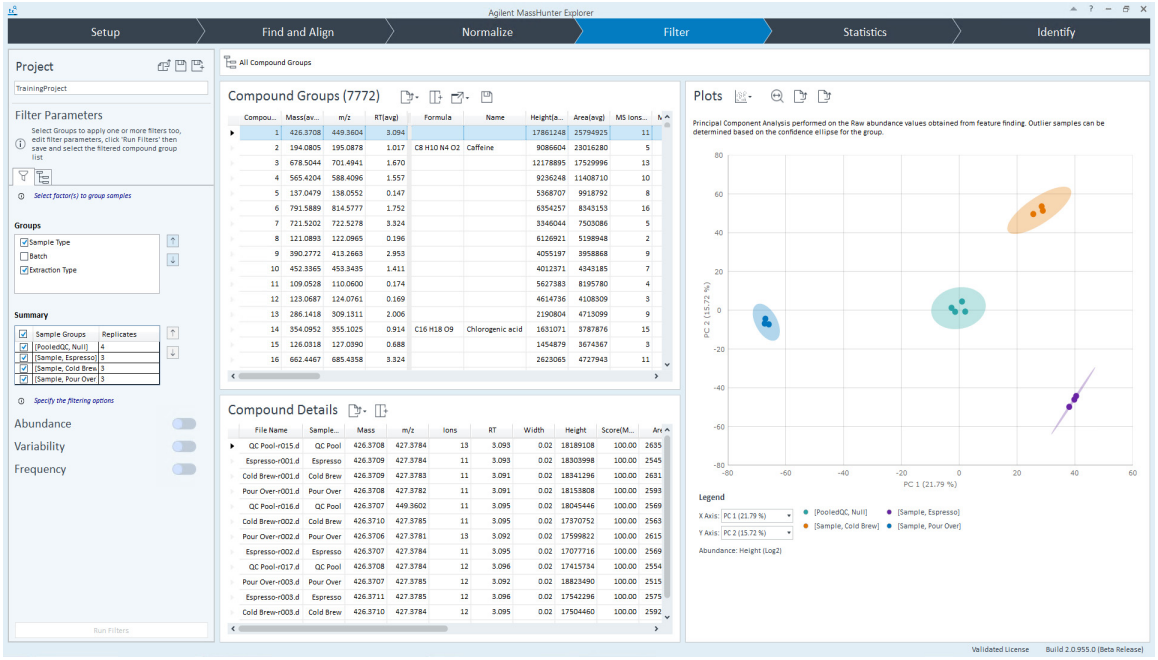
Normalize Step

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.



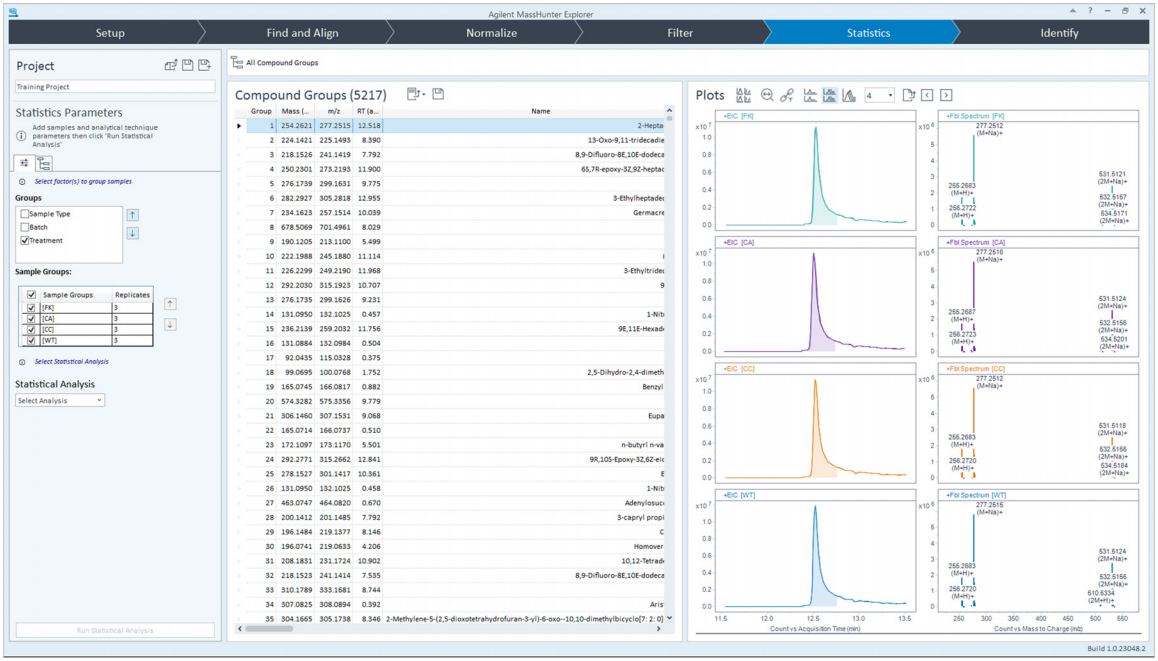
Filter Step

The Filter step allows filtering of data based on abundance, variability, or frequency to look for differences between sample groups. During 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.



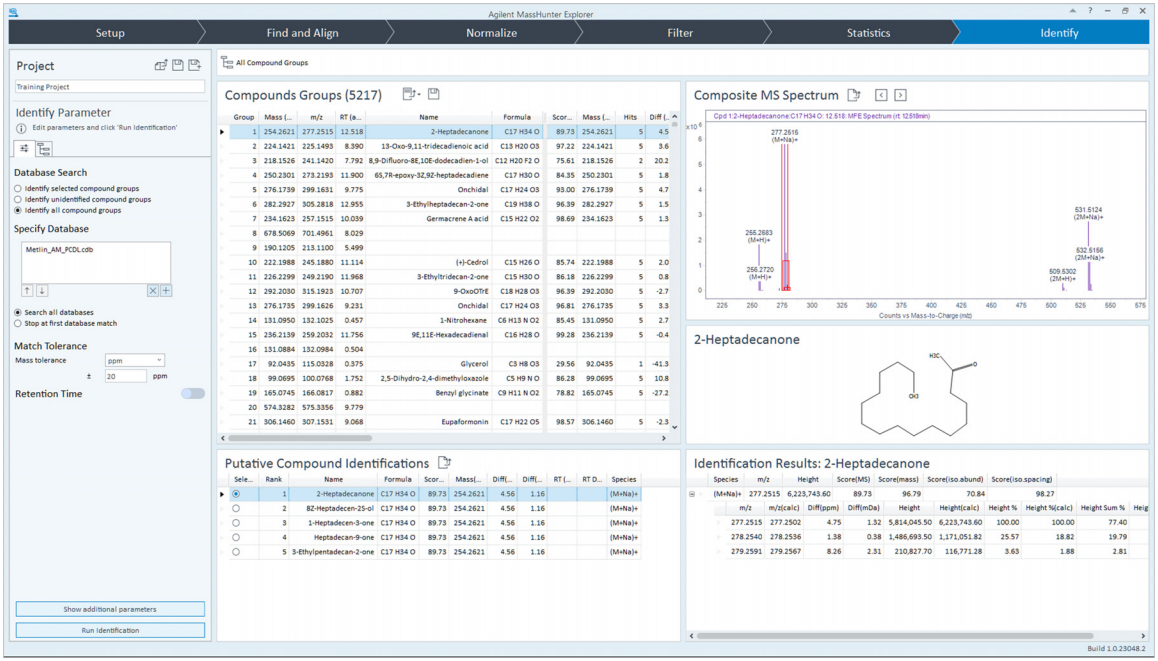
Statistics Step

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.



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 and 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.



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3 Setup

Set Up Overview

The Setup step allows you 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

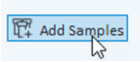
For the first step in the training experiment, add samples and save a new project file.

Set Up Training Task Overview:

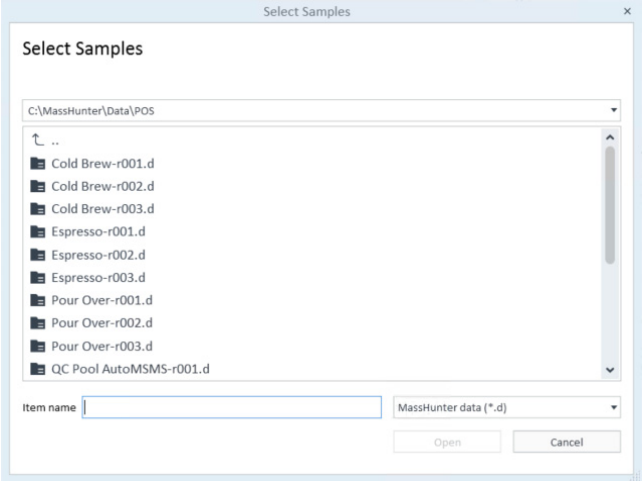
1. Add samples to a project.
2. Assign groups to samples, using fill down.
3. Save a new project file.
4. Extract the total ion chromatogram.

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.

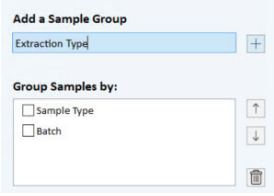
Coffee Extraction Methods & Sample Preparation

Coffee samples were prepared using the same batch of beans and extracted via espresso, pour over, and cold brew. Each method influences flavor differently. This experiment explores which chemical compounds drive those flavor differences.

To standardize dilution, espresso and pour over samples were adjusted to match the cold brew's water-to-coffee ratio. All samples were collected in a single batch. All samples were analyzed in triplicate, alongside pooled QC samples and iterative MS/MS experiments on a Revident LC/Q-TOF MS system. File names in the dataset help identify each sample's extraction method and grouping.

Assign Groups

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

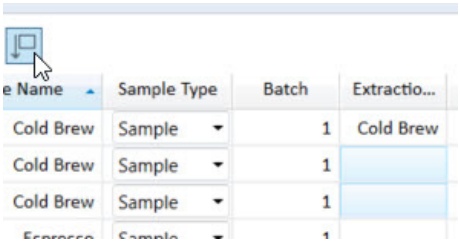


2 Sort the samples by clicking on the column header **Sample Name**, or any other column header if needed, then review the data for anomalies.

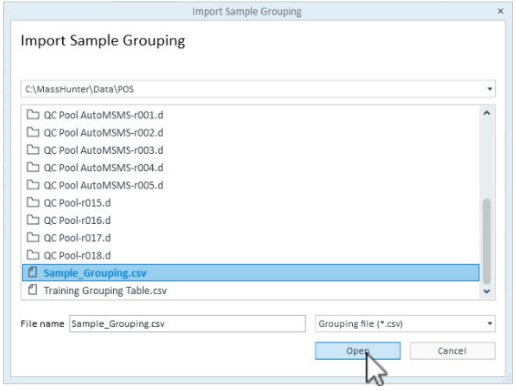
3 Select the first cell under Cold Brew to highlight, then add the information from the table below in the following format to sort samples into Sample Groups:

Sample Name	Extraction Type
Cold Brew-r001	Cold Brew
Expresso-r001	Expresso
Pour Over-r001	Pour Over

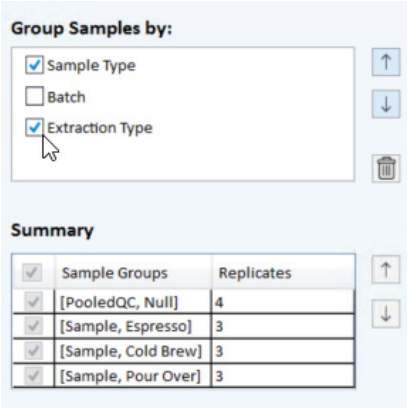
4 Enter *Cold Brew* in the selected cell, then click to select the cell again. Holding the Shift key down, click to select the bottom most cell in the Sample name series for this designation. Click **Fill Down** to automatically fill in the selected cells.



5 Import the sample grouping file provided to assign groups to the remaining samples. Click , select **Sample_ Grouping.csv** from the dialog box**,** then click **Open.**

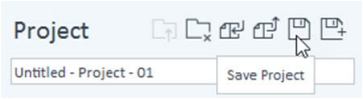


6 In Groups, select the **Extraction Type** check box.Verify the replicates are listed in Sample Groups as expected, in this example four groups of three.



Save Project

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

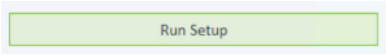


2 Enter *TrainingProject* in the File name field and click **Save**.

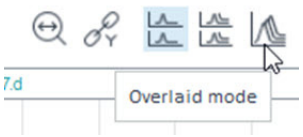
3 Click **OK** to confirm.

Run Setup

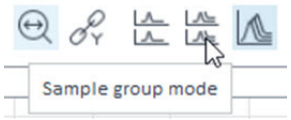
1 Click **Run Setup**.



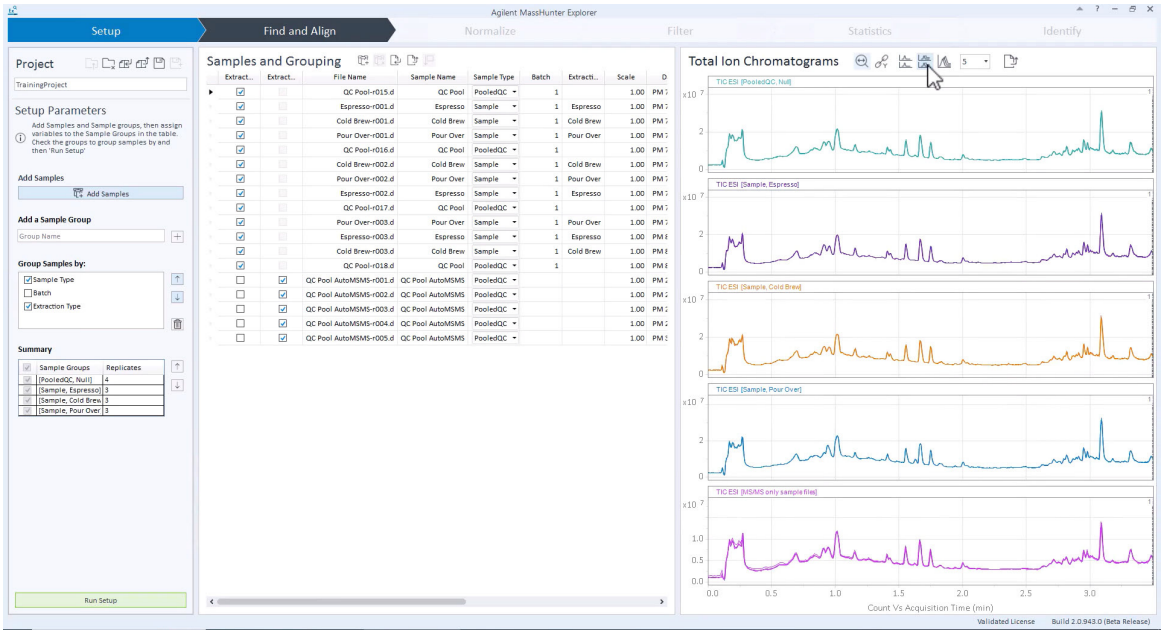
2 The Total Ion Chromatograms load on the right pane of the window. Visually inspect the data by clicking **Overlaid mode**.



3 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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4 Find and Align

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 Plots.

Find and Align Training Task Overview:

1. Adjust Parameters.
2. Run Feature Finding.
3. Review Plots using Sample Group modes.

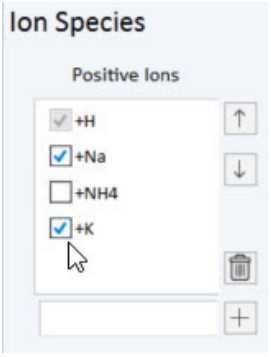
Coffee Sample Preparation

Find and Align parameters vary depending on the sample chemistry, preparation process, and chromatography conditions. Part of the coffee sample was diluted with one part methanol and vortex mixed. After centrifugation, the supernatant was transferred to a vial for injection. The LC solvents were water and methanol, both modified with 0.1% formic acid. It is expected to see sodium and potassium adducts as well as neutral water loss in positive ion mode.

Find and Align Parameters

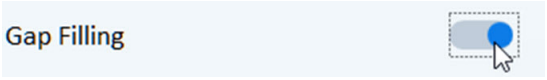
1 Click **Find and Align** on the Ribbon to move to the next step.

2 Under Ion Species in the Positive Ions section, deselect **+NH4** and select **+K**.

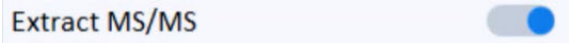


3 Under Compound Alignment, adjust the RT Tolerance to **0.03** min.

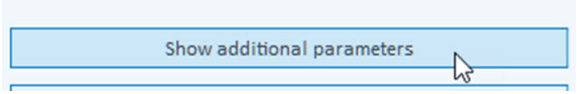
4 Activate **Gap Filling.**



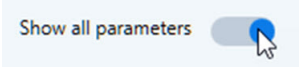
5 Activate Extract MS/MS.



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

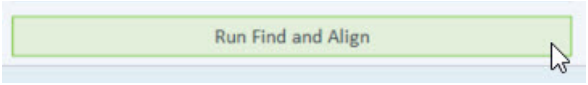


7 Activate **Show all Parameters** and expand the window if needed. All Find and Align Parameters display.

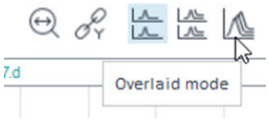


8 Click **OK** to close the Parameters for Find and Align Features window.

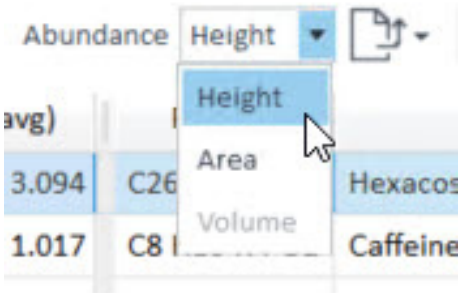
9 Click **Run Find and Align.** When complete, the Find and Align screen displays the Compound Groups, Compound Details, and Plots sample results.



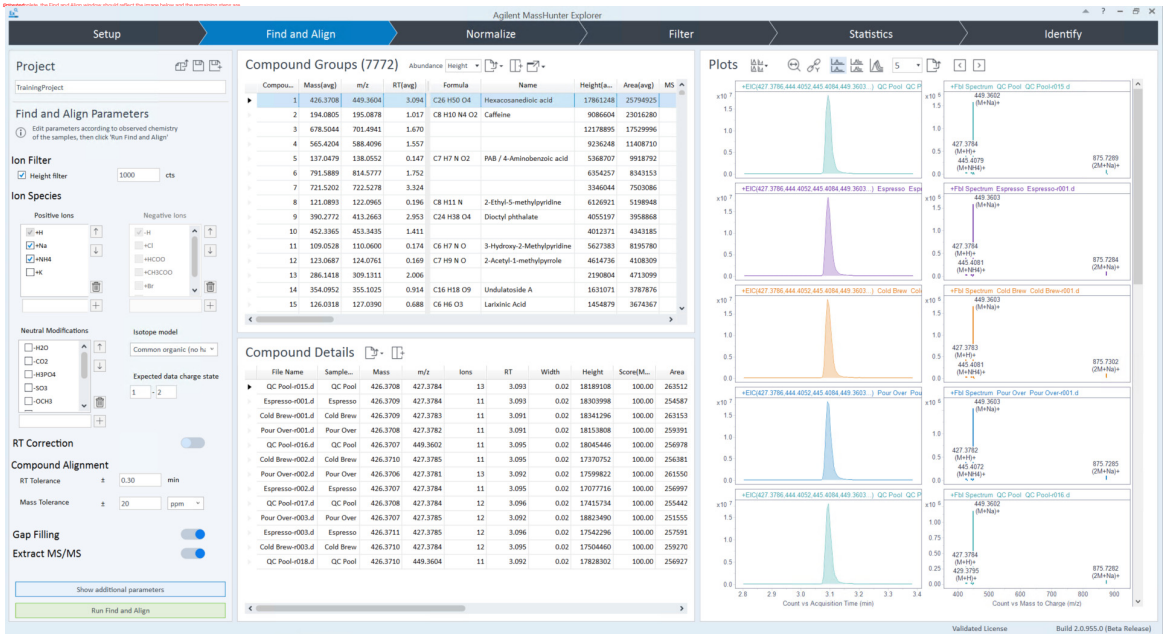
10 Check the reproducibility of the compounds by clicking **Overlaid mode** with all samples shown.



11 In the Compound Groups tile, click the Abundance drop down and select **Height.**



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



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5 Normalize

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, batch-to-batch variation, or to 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:

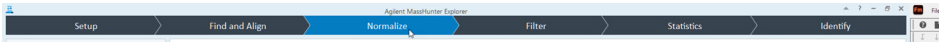
- Normalize data
- View PCA plots
- Add or remove samples from the PCA plot

Normalize Training Task Overview:

1 Adjust Normalize parameters.
2 Run Normalization.

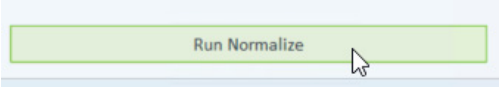
Normalize Settings

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

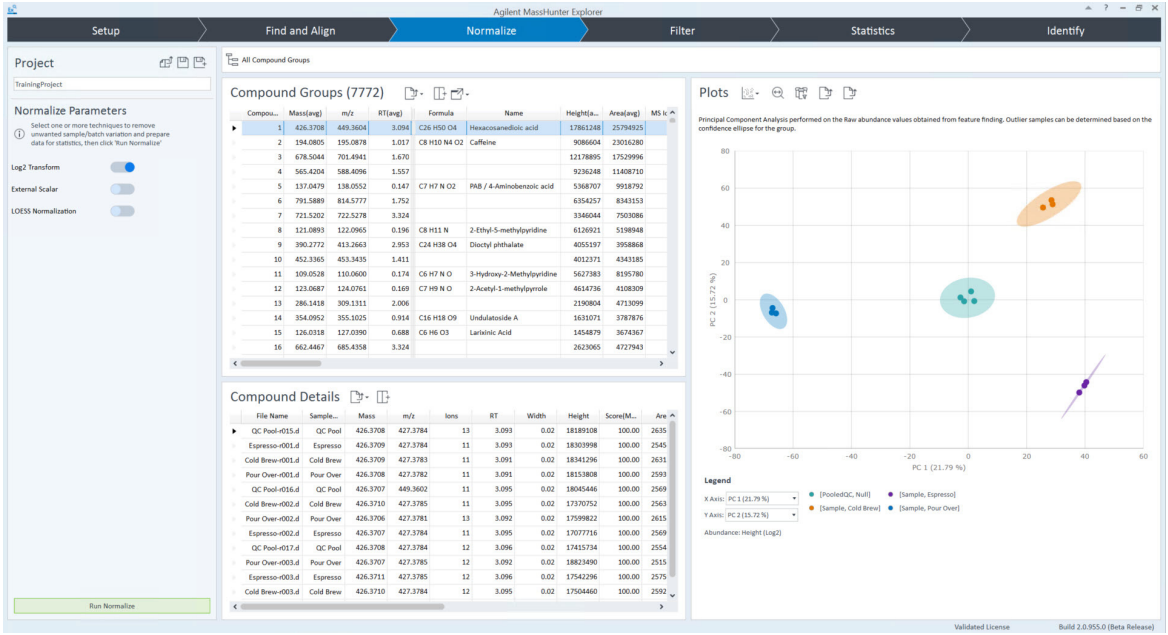


2 Adjust Normalize Parameters as needed. Click to activate **Log2Transform** for the example sample set.

3 Click **Run Normalize**.



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



6 Filter

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.

Perform the following tasks in the Filter step:

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

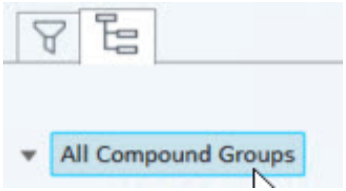
Filter Training Task Overview:

- Adjust Filter parameters

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.

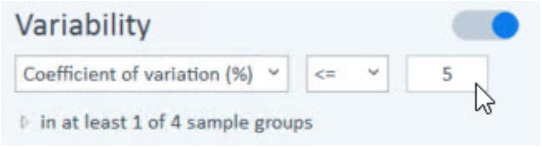
2 In the Compound Groups tab, select **All Compound Groups.**



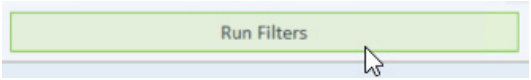
3 In Groups, deselect **Extraction Type**.

4 In Summary, deselect all options except for **(PooledQC, Null)**.

5 Activate **Variability** and review the settings. Change the Coefficient of Variation value to **5**.

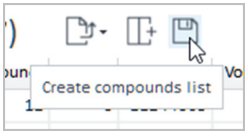


6 Click **Run Filters**.



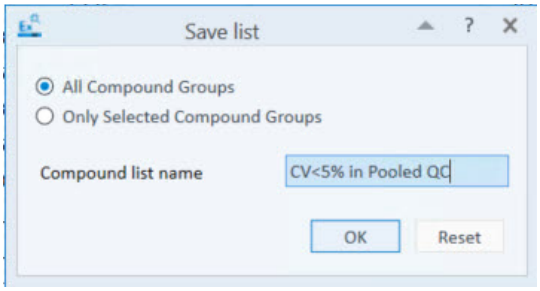
Save Compound List

1 Click **Create compounds list**.

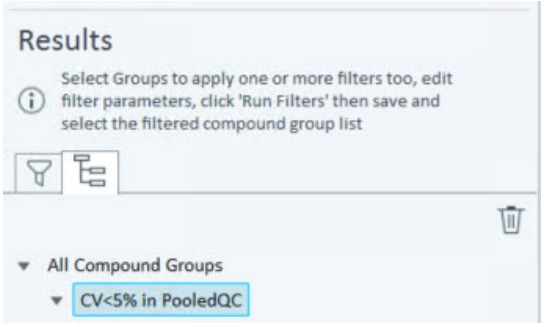


2 Select **All Compound Groups**.

3 Enter a Compound List Name, in this example *CV<5% in Pooled QC* and click **OK**.



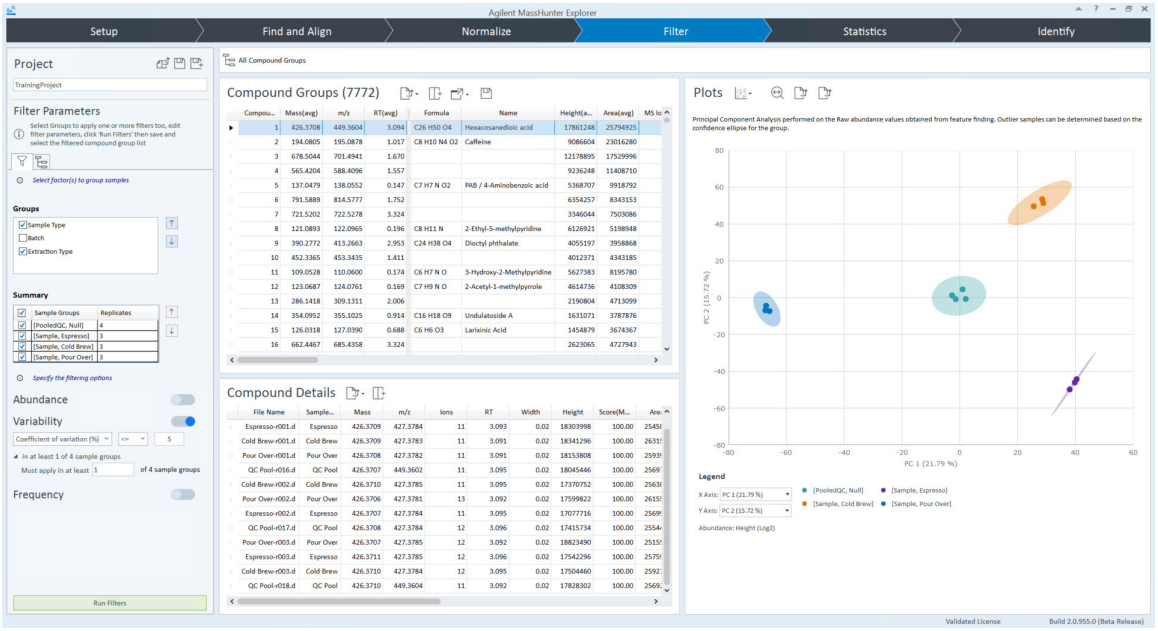
4 To view the list, click the **Compound Groups** tab.



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.



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7 Statistics

Statistics Overview

MassHunter Explorer provides a selection of statistical analysis and visualization techniques that aim to find and focus on significant compounds. Apply statistical techniques to mass spectrometry data to 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:

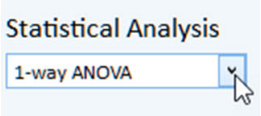
1 Adjust Statistic Settings
2 Run Statistical Analysis
3 Create a Compound Groups list

Statistics Settings

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

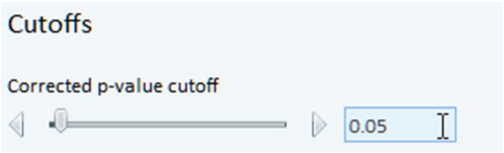
2 Select the CV<5% in Pooled QC compound list.
3 In the Statistics Parameters, set the Group and Sample Group options to “Extraction Type” and deselect “Null.”

4 Click to select **Statistical Analysis**, then select *1-way ANOVA*.

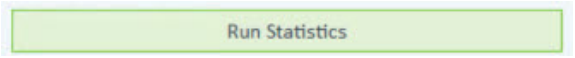


5 Under Fold Change Pairing, for **Pairing** select *Pair Groups*

6 Under Cutoffs, adjust the value to **0.05**.



7 Click **Run Statistics**.



Review Results

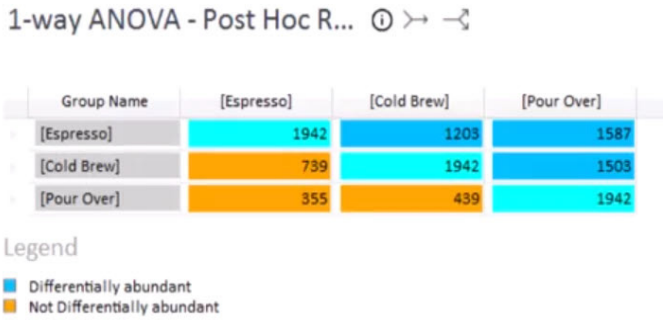
1 In the Plots tile, select **Link Y-axis** to observe any significant differences in the instrument data.

2 Select the Sample Group Mode in the Plots tile so that the significant differences can be observed in the instrument data.

3 Save the data of interest, click **Create compound groups list**.

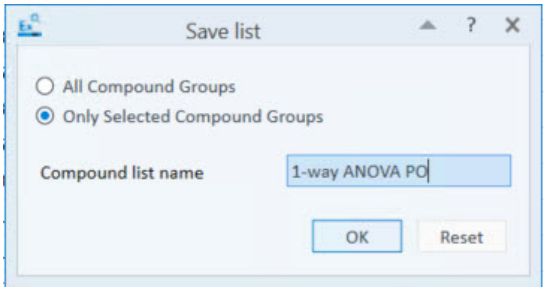
4 Enter a Compound Groups List name, in this example *1-way ANOVA, p<=0.05* and click **OK**

5 In the Post Hoc Results Pane, click any of the compounds to view the specific compound groups in the Compound Groups pane, for example, a pairwise comparison of the Cold Brew row and the Pour Over column.



6 Click **Create compound groups list** and select **Only Selected Compound Groups**.

7 Enter a name and Click **OK**.



8 Click to view the Compound Group List and select the **1-way ANOVA, p<=0.05** list.

9 Click the Statistical Analysis drop-down, then select **Fold Change**.

10 Under Fold Change Pairing, click the Pairing drop-down and select **Pairing**.

11 Under Cutoffs, change Fold-Change to *10*.

12 Click **Run Statistics**.

13 Click **Create compound groups list** and select **All Compound Groups**.

14 Enter a Compound Groups List name, in this example FC >= 10. and click **OK.**

15 Click to view the Compound Group List and select the **FC >= 10** list.

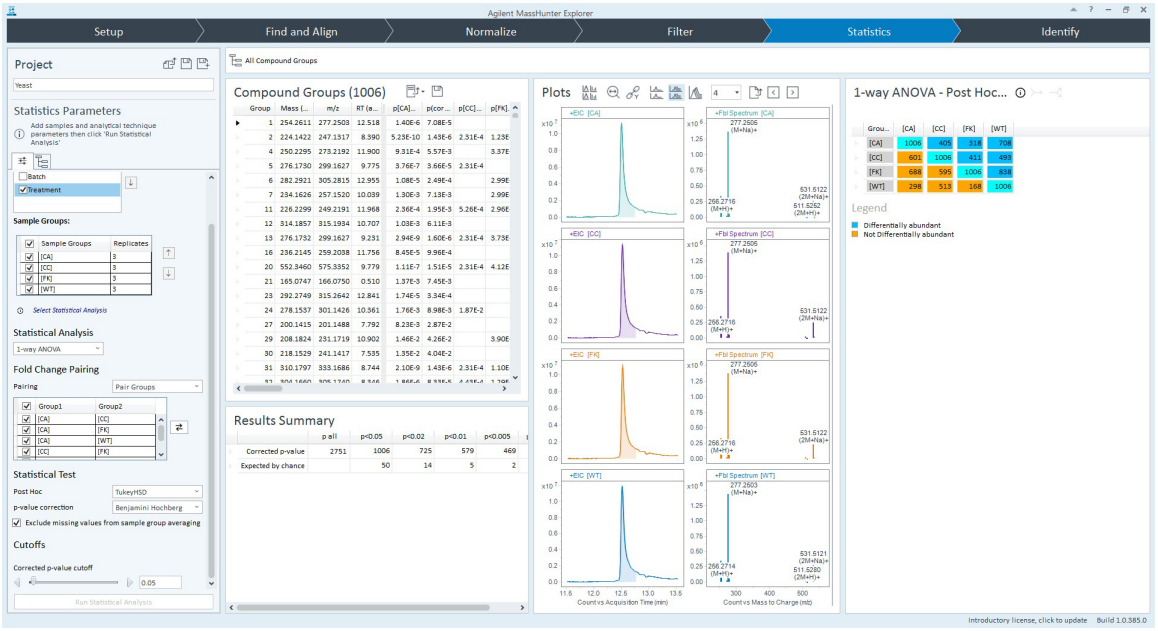
16 Click the Statistical Analysis drop-down, then select **Hierarchical Clustering**.

17 Click **Run Statistics**.

18 In the Compound Groups table, select multiple compound groups to observe changes in the heat map.

19 Press CTRL and drag a box in the heat map to select compound groups in the table.

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



8 Identify

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 with the compound, along with their assigned charge states and adduct annotation. Additionally, match the measured isotope pattern to the theoretical isotope pattern expected from the compound's elemental formula.

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

Perform the following tasks in the Identify step:

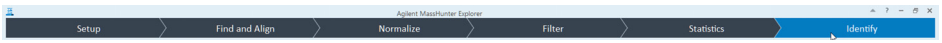
- Edit Identify parameters
- Run Identification
- Save or select a list of compounds

Identify Training Task Overview:

- Adjust Identify Settings
- Export data.

Identify Settings

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



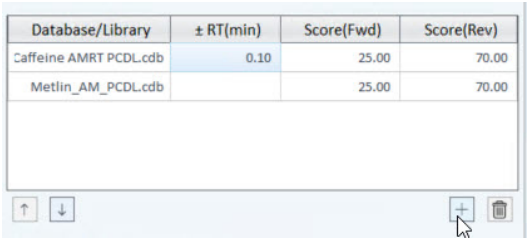
2 Select the **All Compound Groups** list.

3 Click **+** to Specify Database.

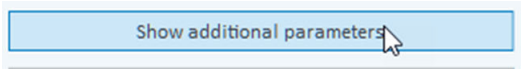
4 Browse to C:\MassHunter\Data and select **Caffeine AMRT PCDL.cbd,** then click **OK**.

5 Adjust the RT tolerance column to **0.1** min.

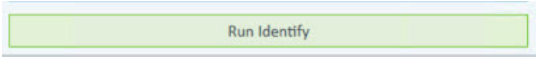
6 Browse to C:\MassHunter\Data and select **Metlin_AM_PCDL.cdb,** then click **OK**.



7 Click **Show Additional Parameters** to review. Click **OK** to close.



8 Click **Run Identify**.



Sorting Data

1 To sort results, in the Compound Groups table, click the **ID Source** column header.

2 To sort within those results, while holding CTRL click a different column header. For this example, click the **Score** column to sort scores within each ID Source.

Interpreting Identification Results

- To change the selected identification and update all results tiles, select **a radio button** in the Putative Compound Identifications tile.
- For detailed score calculations, click the **Show/Hide Detail Identification Results** icon in the Putative Compound Identifications tile toolbar to open the Identification Results tile.
- If a compound group has multiple MS/MS hits, selecting a different hit updates the mirror plot.
- The Composite MS Spectrum tile displays a combined MS spectrum with an overlay of the expected isotope pattern when MS evidence supports the identification. When MS/MS evidence is available, click **Toggle to Show/Hide Mirror Plots** to compare the expected spectrum.

Send to NIST MS or SIRIUS

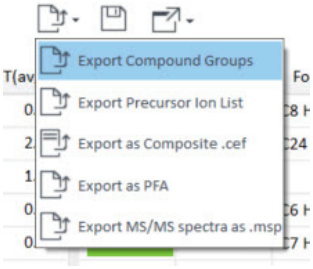
1. After selecting a compound group, select the Send button in the Compound Groups tile and select Send to NIST MS or Send to SIRIUS.
2. In the dialog box, choose:
   - **Selected** - sends only the selected compound groups
   - **All** - sends all displayed compound groups.
   - **Cancel** - cancels the action.

NOTE

MassHunter Explorer supports integration with the NIST MS Search and SIRIUS software for compound identification. See the *MassHunter Explorer Installation Guide* for more information.

Exporting Results

1 In the Compound Groups Pane, click **Export** to expand the options. Select **an export type from the list of options.**



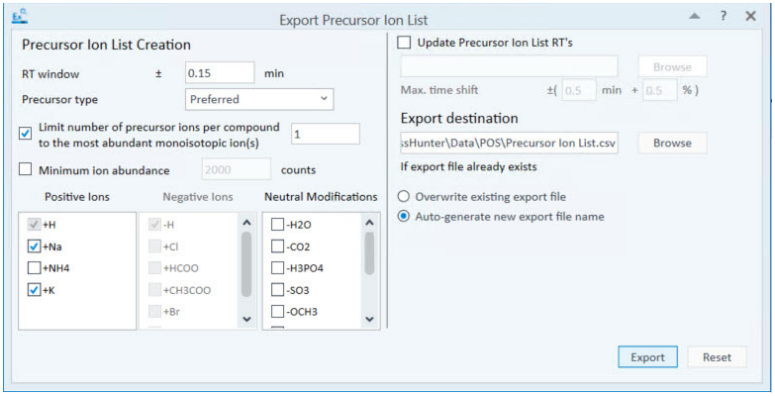
2 Select File Destination Options and click **Save**.

3 Click **OK**.

Export Precursor Ion List

1 In the Compound Groups Pane, click Export to expand the options. Select **Export Precursor Ion List**

2 Adjust settings as needed.



3 Click **Export**.

4 Click **OK**.

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