



# Agilent MassHunter Mass Profiler Software

## Quick Start Guide

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This guide describes how to install and use MassHunter Mass Profiler Software.

## Where to find more information

Use the online help for in-depth information not given in this *Quick Start Guide*. Display online help in either of the following ways:

- Click **Contents** on the MassHunter Mass Profiler Software Help menu.
- Press the **F1** key to get more information about a window, tab, or dialog box.



**Agilent Technologies**

## What is MassHunter Mass Profiler software?

MassHunter Mass Profiler software operates on the compound data of different samples stored in **.cef** (compound exchange file) format files that are produced by MassHunter Qualitative Analysis software. It lets you:

- Investigate similarities and differences in features across multiple analyses and samples.
- Align and normalize features from different samples.
- Identify features using the MassHunter ID Browser program.

### Terminology

**Features** Features are discrete molecular entities defined by a particular retention time and mass.

**Samples** Content imported from **.cef** or **.csv** files represent individual sample analyses and are called *samples* in MassHunter Mass Profiler software.

**Groups** Related samples can be grouped together for analysis. By default, the two groups are named “Experiment” and “Control”, but these can be changed.

**Project** A project is one or two sample groups considered together for analysis.

**Method** A method is all the parameters used for filtering, aligning, normalizing, and processing samples in MassHunter Mass Profiler software.

### Processing and Viewing Results

The results of processing samples appear in the MassHunter Mass Profiler software main window Feature Plot and Feature Table.

#### Use the Feature Plot to:

- Investigate features using five different plot types: Mass vs. Retention Time, Log2 Ratio vs. Retention Time, Log/Log plot, Unique to <Group 1> and Unique to <Group 2>.
- Investigate features in individual or multiple samples
- Investigate features that exceed a certain visibility threshold
- Investigate features by mass and RT across all samples or use color to distinguish individual features or to identify features by sample or group.

See [“Feature Plot”](#) on page 11 for more information.

**Use the Feature  
Table to:**

- View all the features for their summary, group statistics and differences between the two groups.
- View individual feature details, including species clusters found in a selected sample.

See [“Feature Table”](#) on page 15 for more information.

## Identification

MassHunter Mass Profiler software lets you identify feature composition using the MassHunter ID Browser program. Features can be identified individually or in a batch. The identification methods available are Database search (CSV, PCD/METLIN) and MFG (molecular formula generation). *Spectral library search is not currently supported in ID Browser for LC/MS data.*

See [“Identifying Features”](#) on page 36 for more information.

## Exporting data

You can export data generated by MassHunter Mass Profiler software that is displayed in the Feature Table or Feature Plot in the following formats:

- Feature Summary data for use in Excel.
- Averaged ion data (m/z and RT of representative ions) for each feature in the table for use in data acquisition in targeted MS/MS experiments.
- Feature data for use in Mass Profiler Professional.
- Feature data from different samples are summed up to form a composite compound. This export file format can be used with the Find by Formula feature in MassHunter Workstation Qualitative Analysis software or as input to standalone ID Browser program.

See [“Exporting Mass Profiler Data”](#) on page 40 for more information.

## What's New in Version B.03.00 SP1?

- Compatible with Windows 7 (64-bit)
- Improved retention time alignment
- Support for ID Browser B.03.01 SP4 (required for Windows 7)

## Installation

If MassHunter Mass Profiler Software B.03.00 or earlier is already installed on your system, remove it first. See [“To remove the software”](#) below.

- 1 Insert the disk labeled Agilent G3297AA into the drive.
- 2 From the installation disk, double-click **Setup.exe**.

If the operating system is Windows 7, right-click **Setup.exe**, then select **Run as administrator**.

- 3 Follow the instructions on the screen to install the software.

You are prompted to enter a folder where data will be stored. The default is **D:\MassHunter**. A **MassProfiler** folder is created in the location you specify, with the following subdirectories:

- **Input data**
- **Methods**
- **Projects**
- **Results**

### To remove the software

Use the **Add/Remove** window of the Control Panel.

## Startup

To start the MassHunter Mass Profiler Software:

- Double-click the **Mass Profiler** icon  on the desktop, *or*
- Select **Programs> Agilent> MassHunter Workstation> Mass Profiler** from the Windows Start menu.

## Main Window

The MassHunter Mass Profiler Software main window has the following areas and controls.

- “[Menus](#)” (below)
- “[Toolbar](#)” on page 10
- “[Feature Plot](#)” on page 11
- “[Feature Table](#)” on page 15

### Menus

The following menu options appear in MassHunter Mass Profiler Software:

#### File menu



**Create New Project** Opens the New Project dialog box, which lets you set up a new project for use in MassHunter Mass Profiler Software. See “[To create a project](#)” on page 19.



**Open Project** Lets you open a project. (\*.pjt file). The results from the previous analysis are loaded to the Feature Table and Feature Plots. The method used to process the project previously is also loaded automatically.

**Edit Project** Opens the Edit Project dialog box, which lets you edit the current project. See “[To edit a project](#)” on page 26.



**Save Project** Saves changes to the current project.

**Save Project As** Saves the current project under a new name.

**Export Feature Summary > From Graphed Data** Exports the features highlighted in the current plot for use in Excel. The export file format is tab-separated value, but it is saved with a **.xls** extension so that Excel will open automatically when you double-click on the file in Windows Explorer.

**Export Feature Summary > From Feature Table** Exports the feature summary information from the Feature Table for use in Excel. The export file format is tab-separated value, but it is saved with a **.xls** extension so that Excel will open automatically when you double-click on the file in Windows Explorer. You can export **All Features**, **Marked Features**, or **Unmarked Features**.

**Export Targeted MS/MS Inclusion List > From Graphed Data** Opens the Export Inclusion List Options dialog box, which lets you specify parameters for exporting the averaged ion data ( $m/z$  and RT of representative ions) for each feature that is highlighted in the plot (in **.csv** format), for use in data acquisition in targeted MS/MS experiments.

**Export Targeted MS/MS Inclusion List > From Feature Table** Opens the Export Inclusion List Options dialog box, which lets you specify parameters for exporting the averaged ion data ( $m/z$  and RT of representative ions) for each feature in the table (in **.csv** format), for use in data acquisition in targeted MS/MS experiments. You can export **All Features**, **Marked Features**, or **Unmarked Features**.

**Export MPP Input File > From Graphed Data** Exports the features currently highlighted in the Feature Plot for use in Mass Profiler Professional (**.txt** format).

**Export MPP Input File > From Feature Table** Exports the feature table for use in Mass Profiler Professional (**.txt** format). You can export either **All Features**, **Marked Features**, or **Unmarked Features**.

**Export Composite-Compounds CEF > From Graphed Data** Exports the features currently highlighted in the Feature Plot for use with other programs (**.cef** format).

**Export Composite-Compounds CEF > From Feature Table** Exports the feature table for use with other programs (**.cef** format). You can export **All Features**, **Marked Features**, or **Unmarked Features**. The export file contains a list of compounds. Each compound is based on one row of the Feature Table. The data from different samples are summed up to form a composite compound. This export file format can be used with the Find by Formula feature in MassHunter Workstation Qualitative Analysis software or as input to standalone ID Browser program.

**Exit** Closes the MassHunter Mass Profiler Software window and exits the program.

## Method menu

Method parameters define and restrict the features that appear in the Feature Plot and Feature Table.



**Open and Apply Method** Lets you open a method and automatically process the current project with the parameters in the method you open. See [“To apply a different method”](#) on page 26.



**Edit Method** Opens the Method Parameters dialog box, which lets you edit the current method. Method parameters appear on three tabs: Pre-Analysis Filters, Alignment & Normalization, and Result Filters. See [“To edit a method”](#) on page 21.



**Save Method** Saves changes to the current method.

**Save Method As** Saves the current method parameters as a new method.

**Reset Method to Default** Restores all method parameters to default values.

## View menu

**Show Corrected Times/Show Raw Times** If an RT correction is performed as part of the method, then a “corrected time” is calculated for each feature and each sample. Selecting **Show Corrected Times** displays the values of these corrected retention times. Selecting **Show Raw Times** displays the raw (original) retention times from the input files (*without* correction).

**Feature Details for Selected Feature** Opens the Feature Information window for the feature selected in the Feature Table. See [“To view feature details”](#) on page 31. *Not available for data imported from .CSV file.*

**Abundance Distribution for Selected Feature** Displays plots that show the distribution of abundance for the samples in the two groups. The abundance distribution of each sample for the feature is presented as a histogram and as curves that are color-coded for the group. The curves are calculated by using the least-squared fit to a Gaussian shape. See [“To view the abundance distribution graph for a feature”](#) on page 33.

**Global Occurrence Histograms** Displays histograms that show how frequently a feature occurs in samples within each group and across both groups. See [“To view global occurrence histograms”](#) on page 34.

## Web menu

Performs a mass search for the feature currently selected in the Feature Plot or Feature Table and displays the results in your internet browser window.

You can search the following databases:

- ChemIDplus
- HMP
- NIST
- PubChem
- Web METLIN

## Image menu

**Print Image** Prints the current Feature Plot image.

**Copy Image to Clipboard** Copies the current Feature Plot image to the Clipboard to paste into other applications as either a **Metafile** (.emf format) or **Bitmap** (.bmp format).

**Save Image to File** Saves the current Feature Plot image as either a **Metafile** (.emf format) or **Bitmap** (.bmp format).



## Identify Features menu

*These items do not apply to data imported from .CSV file or LMFE input data.*

**For Highlighted Features on Graph** Lets you identify compounds for the features highlighted in the selected tab of the Feature Plot, using the Agilent MassHunter ID Browser program. Results you save in ID Browser are available for review in Mass Profiler when you close the ID Browser window.



**For All Features in Table** Lets you identify compounds for all features displayed in the Feature Table, using the Agilent MassHunter ID Browser program. Results you save in ID Browser are available for review in Mass Profiler when you close the ID Browser window.

**For Marked Features in Table** Lets you identify compounds for the features marked in the Feature Table, using the Agilent MassHunter ID Browser program. Results you save in ID Browser are available for review in Mass Profiler when you close the ID Browser window.

**For Unmarked Features in Table** Lets you identify compounds for the features that are unmarked in the Feature Table, using the Agilent MassHunter ID Browser program. Results you save in ID Browser are available for review in Mass Profiler when you close the ID Browser window.

**Quick Lookup for Selected Feature** Opens the Agilent MassHunter ID Browser program with the feature currently selected in Mass Profiler displayed. In this mode, *results from ID Browser are not available in Mass Profiler when you close the ID Browser window.* See [“To identify a single feature”](#) on page 36.

See [“Identifying Features”](#) on page 36 for more information.








## Help menu

**Contents** Opens the online help window for MassHunter Mass Profiler software with the **Contents** tab displayed.

**About** Displays version information for MassHunter Mass Profiler software.

## Toolbar

The following items are available on the MassHunter Mass Profiler Software toolbar:

Button	Equivalent menu item
	File > Create New Project
	File > Open Project
	File > Save Project
	Method > Open and Apply Method
	Method > Edit Method
	Method > Save Method
	Identify Features > For All Features in Table

## Feature Plot

This area of the MassHunter Mass Profiler Software window shows the results of processing a project with the current method and lets you see differences between features in two groups of samples. It displays processed data automatically when you create a new project, edit a project, open and apply a method, or edit a method. The size of a point on the graph indicates the abundance of a feature: the larger the dot, the more abundant the feature.

You can change the plot style and limit the data that appears in the Feature Plot using the options described below. See [“To view results in the Feature Plot”](#) on page 27.

### Graph data

Lets you select between displaying all samples or a single sample. This is set initially to display **All samples**. When the **Plot style** is set to Average Feature, the Graph data is always set to All samples, even if you select a single sample.

### Plot style

Lets you select the style of plot to display from the following:

**Average Feature** Uses the group averages as the feature properties (Mass, RT, and Abundance).

**Colored by feature** Plots each distinct feature in a different color. When more features exist than can be plotted in different colors, then features in the same area of the plot are graphed in different colors.

**Colored by sample** Plots the features in the same sample in the same color (e.g., if there are 10 samples, then there are 10 colors).

**Colored by group** Two colors, blue and red, represent the features in the two groups of samples.

## Visibility criterion

Lets you select one of the following criteria, whose threshold determines which features will be highlighted (i.e. appear as *solid dots*) in the plot. Features that don't meet the threshold value are plotted as dimmed open circles.

**Max RSD abundance** The max of relative standard deviations of the two groups, that is, any feature whose max group abundance-RSD is larger than the threshold is filtered out (plotted as an open circle).

**SD RT** Standard deviation for the retention time

**SD Mass** Standard deviation for the mass

**Differential Score** Relative score from 0 to 100 that indicates if the difference in abundance of a feature in the two groups is significant. Two methods are available for calculating the differential score, **Student t-test** or **SVM sensitivity**, set on the Result Filters tab of the Method Parameters dialog box as described in [“To perform a differential analysis and set result filters”](#) on page 25.

**None** (the default) No visibility criterion is applied, so all features are highlighted.

## Visibility threshold

Sets the threshold for the selected **Visibility Criterion**. Features that don't meet the threshold for the selected Visibility Criterion appear as dimmed open circles in the plot.

## Feature Plot tabs

The tabs at the top of the Feature Plot let you view different plot types:

**Mass vs. RT** Plots mass vs. retention time (RT) for each feature.

**Log2 Ratio vs. RT** Plots the log2 of the ratio of abundance vs. retention time.

- For **Average feature** plot style, the abundance ratio is average abundance for <Group 1> over the average abundance for <Group 2>.

- When Graph data is set to a single sample, the abundance ratio is the abundance for a single feature over the average abundance of that feature in its group.

**Log/Log Plot** Plots the log2 of the average abundance of a feature:

- in <Group 1> vs. log2 of its average abundance in <Group 2> for **Average feature** plot style.
- in a single sample vs. the log2 of the average of its group When Graph data is set to a single sample.

**Unique to <Group 1>** Plots Mass vs. RT for those features that are present in the *first* group of samples, but not in the second group of samples.

**Unique to <Group 2>** Plots Mass vs. RT for those features that are present in the *second* group of samples, but not in the first group of samples.

## Expression

Shows only the features that have a larger average abundance in one group as follows, or **Both**, which displays *all* features in the table.

**Up** Shows only features that have a larger average abundance in <Group 1> than in <Group 2> (called *up-regulated*).

**Down** Shows only features that have a larger average abundance in <Group 2> than in <Group 1> (called *down-regulated*).

## Plot mouse actions

- Zoom—Drag the mouse to define an area of interest. The area expands when you release the mouse button.
- Pass the cursor over a feature to view information about that feature.
- Click in the plot to select the feature that is closest to that location and to highlight the row in the Feature Table that corresponds to that feature.

## Plot shortcut menu

The following options appear when you right-click anywhere on a plot. Note that right-clicking in the plot also changes the selection in the table to the nearest data point.

**Unzoom** Returns the plot to the previous zoom level.

**Autoscale** Returns the plot to the original display.

**Print Image** Sends the plot image to a printer.

**Copy Image to Clipboard** Copies the current plot image to the Clipboard to paste into other applications as either a Metafile or Bitmap.

**Save Image to File** Saves the current plot image in either **.emf** or **.bmp** format.

**Feature Details** Opens the Feature Information window for the feature that is closest to that location on the plot.

**Abundance Distribution** Opens the Single Feature Abundance Distribution window, which displays the plots that show the distribution of abundance for the samples in the two groups.

**Identification Lookup** Opens the ID Browser program with the feature currently selected in Mass Profiler displayed. In this mode, results from ID Browser are *not* available in Mass Profiler when you close the ID Browser window.

**Web** Performs a mass search for the feature currently selected in the plot and displays the results in your internet browser window. You can search the following databases: ChemIDplus, HMP, NIST, PubChem, and Web METLIN.

## Feature Table

This area of the MassHunter Mass Profiler Software window shows the results of processing a project with the current method. It displays processed data automatically when you create a new project, open a project that has been processed before, edit a project, open and apply a method, or edit a method. It lets you see the primary feature values, including the average values for each feature across both groups and then each group separately. The table can be sorted by the values in any column by clicking on the column heading of interest. Results for Internal standards are displayed in green in the table.

### Expression

Shows only the features that have a larger average abundance in one group as follows:

**Up** Filters the table to show only features that have a larger average abundance in <Group 1> than in <Group 2> (called *up-regulated*).

**Down** Filters the table to show only features that have a larger average abundance in <Group 2> than in <Group 1> (called *down-regulated*).

**Both** Shows all features in the table.

### Feature summary

These values are averages for the feature *across all samples*.

**ID (row number)** Feature identification number, assigned based on the values in the **Abundance** column. The table is initially sorted by these values, but the sort order can be changed by clicking on the heading of any other column.

**RT** Average retention time of the feature in all samples

**RT SD** Standard deviation of the retention time of the feature in all samples

**Mass** Average mass of the feature in all samples

**Mass SD** Standard deviation of the average mass of the feature in all samples

**Abundance** Average abundance of the feature across the samples in both groups

**RSD** Relative standard deviation of the abundance of the feature across the samples in both groups

**Freq** The number of samples in both groups that contain this feature

**Mark** Click the check box in the **Mark** cell for a feature of special interest. This annotation is exported along with the other data in the table, so that the feature can still be distinguished when viewed in other programs. If you select a feature in error, click again to *clear* the mark.

### <Group 1>, <Group 2>

The values in these parts of the table are averages for the feature across the samples *within the individual groups only*.

**RT** Average retention time of the feature within the group

**Mass** Average mass of the feature within the group

**Abundance** Abundance of the feature within the group

**RSD** Relative standard deviation of the abundance of the feature in the group

**Freq** The number of samples in the group that contain this feature

### Comparison

The values in this part of the table are average differences for the feature *between samples in both groups*.

**RT** The difference in the retention time of the feature between the groups

**Mass** The difference in the mass of the feature between the groups

**log2(A1/A2)** The log of the ratio of the average feature abundance for all samples in <Group 1> over the feature abundance for all samples in <Group 2>.

**Expression** The absolute value of the above expression.



**Diff. Score** The differential score, which is a number between 0 and 100 that represents the relative confidence that the difference in intensity of a feature between the two groups is real. Two methods are available for calculating the differential score, **Student t-test** or **SVM sensitivity**, set on the Result Filters tab of the Method Parameters dialog box as described in [“To perform a differential analysis and set result filters”](#) on page 25.

### Feature Table shortcut menu

The following options appear when you right-click in any row number cell on the left side of the Feature Table:

**Feature Details** Displays the Feature Information window for the selected feature. *Not available for data imported from .CSV file.*

**Abundance Distribution** Opens the Single Feature Abundance Distribution window, which displays the plots that show the distribution of abundance for the selected feature in the samples in the two groups.

**Identification Lookup** Opens the ID Browser program with the feature currently selected in Mass Profiler displayed. In this mode, results from ID Browser are not available in Mass Profiler when you close the ID Browser window. *Does not apply to data imported from .CSV file or LMFE input data.*

**Web** Performs a mass search for the feature currently selected in the table and displays the results in your internet browser window. You can search the following databases: ChemIDplus, HMP, NIST, PubChem, and Web METLIN. *Does not apply to LMFE input data.*

## Getting Started

The following topics will help you get started with Agilent MassHunter Mass Profiler Software. This guide and the online help explain these activities.

### Processing Data

- [“To create a project”](#) on page 19
- [“To edit a method”](#) on page 21
- [“To filter features”](#) on page 21
- [“To align features”](#) on page 23
- [“To normalize features”](#) on page 24
- [“To perform a differential analysis and set result filters”](#) on page 25
- [“To apply a different method”](#) on page 26
- [“To edit a project”](#) on page 26

### Viewing Results

- [“To view results in the Feature Plot”](#) on page 27
- [“To set Feature Plot filters and colors”](#) on page 28
- [“To select Feature Plot view”](#) on page 29
- [“To view results in the Feature Table”](#) on page 30
- [“To view feature details”](#) on page 31
- [“To print, save, or copy the Feature Plot image”](#) on page 32
- [“To view more information from database websites”](#) on page 32
- [“To view the abundance distribution graph for a feature”](#) on page 33
- [“To view global occurrence histograms”](#) on page 34

### Identifying Features

- [“To identify a single feature”](#) on page 36
- [“To identify a batch of features”](#) on page 37
- [“To identify features with ID Browser”](#) on page 38
- [“To review results in the Compound List Window”](#) on page 39

### Exporting Mass Profiler Data

- [“To mark features for annotation”](#) on page 40
- [“To export data from the Feature Table”](#) on page 40
- [“To export data from the Feature Plot”](#) on page 41

## Processing Data

To process data in MassHunter Mass Profiler Software, samples (.cef files output from MassHunter Qualitative Analysis software or your own generic .csv files) are first organized into groups in a project. Processing parameters are set in methods and the current method is automatically applied to the current project (samples). You can edit the method and set various filters for the data you want to consider.

**Format for .csv files** A generic (.csv) file must begin with a header row (RT, mass, abundance), followed by three columns of information for each sample row, for use as Mass Profiler input.

### To create a project

Use this procedure to set up a project that contains the samples (.cef files) that you want to investigate.



- 1** Click **Create New Project** on the File menu, or click the **Create New Project** button on the toolbar.
- 2** On the New Project dialog box:
  - a** Select the Input data type from one of the following: **Small molecules (LC)**, **Large molecules**, or **Generic (csv)**.
  - b** Type in a **Name** for the project.
  - c** Specify whether you want **1** or **2** groups in the project.
- 3** Set up the first group of samples on the left side of the screen as follows:
  - a** Type in a name for the **Group**, or accept the default name **Experiment**.
  - b** Click the Samples **Add** button to open the Select Files dialog box.
  - c** Select the .cef or .csv files to add to the group. Use **Shift+click** or **Ctrl+click** to select multiple files.
  - d** Click the **Open** button on the Select Files dialog box. The files you selected are listed for the first group on the left side of the New Project dialog box.
  - e** Repeat steps a - d to add additional samples if desired, but each file can be added to the project only once.

### NOTE

To remove an unwanted sample from the list, click in the first (blank) column of the sample row of interest to select the row, then click the **Delete** button.

- 4 If you selected **2 groups** for the project in Step 2, set up the second group of samples on the right side of the screen as follows:
  - a Type in a name for the group, or accept the default name **Control**.
  - b Click the Samples **Add** button to open the Select Files dialog box.
  - c Select the **.cef** or **.csv** files to add to the group. Use **Shift+click** or **Ctrl+click** to select multiple files.
  - d Click the **Open** button on the Select Files dialog box. The files you selected are listed for the second group on the right side of the New Project dialog box.
  - e Repeat steps a - d to add additional samples if desired, but each file can be added to the project only once.
- 5 (*Optional*) Enter a value in the **Scalar** column for a sample to multiply feature abundance values by a set amount in that sample.
- 6 Click **OK** on the New Project dialog box. The samples are automatically processed using the current method and the results are displayed in the Feature Plot and Feature Table in the Mass Profiler main window.
- 7 To obtain different results, apply a different method to the project as described on [page 26](#), or change parameters in the current method as described in [“To edit a method”](#) on page 21.
- 8 (*Optional*) To save the changes to the current project, click **Save Project** on the File menu or click the **Save Project** button on the toolbar.
- 9 (*Optional*) To save the project under a new name for later use, click **Save Project As** on the File menu, enter a name for the project (**.pjt** file), and click the **Save** button on the Save As dialog box.



Projects can be modified later as described in [“To edit a project”](#) on page 26.

## To edit a method

Use this procedure to edit method parameters for processing the samples in your project. You can save methods for later use.



- 1 Click **Edit Method** on the Method menu, or click the **Edit Method** button on the toolbar.
- 2 Set parameters on the following tabs of the Method Parameters dialog box:
  - **Pre-Analysis Filters:** see “[To filter features](#)” below.
  - **Alignment & Normalization:** see “[To align features](#)” on page 23 and “[To normalize features](#)” on page 24
  - **Result Filters:** see “[To perform a differential analysis and set result filters](#)” on page 25
- 3 Click **OK**. The samples in the current project are automatically processed using the current method and the results are displayed in the Feature Plot and Feature Table in the Mass Profiler main window.



- 4 (Optional) To save the changes to the current method, click **Save Method** on the Method menu or click the **Save Method** button on the toolbar.
- 5 (Optional) To save the method under a new name for later use, click **Save Method As** on the Method menu, enter a name for the method (.mpm file), and click the **Save** button on the Save As dialog box.

## To filter features

Use the following procedure to set limits on the raw features that are loaded into MassHunter Mass Profiler Software from the incoming .cef files, which contain all the extracted features from Mass Hunter Qualitative Analysis software.



- 1 Click **Edit Method** on the Method menu, or click the **Edit Method** button on the toolbar to open the Method Parameters dialog box.
- 2 Set filter parameters on the **Pre-Analysis Filters** tab:

**Feature position** – To filter based on retention time and or mass. *The RT position filter is ignored for MFE infusion data.*

**Isotope pattern** – To filter based on isotope distribution pattern. *This filter is ignored for data imported from CSV file and LMFE input data.*

**Charge state** – To filter by charge state. *This filter is ignored for data imported from CSV file and LMFE input data.*

**Special masses** – To exclude or limit features with certain masses.

**Mass defect (Da)** – To set a range for the acceptable mass defect. *This filter is ignored for LMFE input data*

**Number of ions** – A large number of ions detected for a feature usually indicates robustness of the feature detection. Specifying a minimum

**Number of ions** will filter out features that may be less reliable. *This filter is ignored for data imported from CSV file and LMFE input data*

**Neutral losses** – You can load features according to the neutral losses they have as follows. *This filter is ignored for data imported from CSV file and LMFE input data.*

- **Ignored**— ignores neutral-loss information and loads all the features
- **Any**— loads only features with any neutral losses
- **Specified**— lets you type in a list of formulas for neutral losses. In this case, only features that have any neutral loss specified in the list are loaded. For example, the formulas  $\text{H}_2\text{O}$  and  $\text{H}_3\text{PO}_4$  may have been specified for neutral-loss search in Agilent MassHunter Qualitative Analysis Software. So the resulting \*.cef files may contain some features with  $\text{H}_2\text{O}$  neutral loss, some with  $\text{H}_3\text{PO}_4$ , some with both, and some with no neutral losses.

**Abundance** – To specify feature abundance parameters.


- 3 Click **OK** to initiate the analysis, using the values entered in Step 2 above, along with current values from the Alignment & Normalization tab and the Result Filters tab.
- 4 View the results in the Feature Plot and Feature Table, as described in “[Viewing Results](#)” on page 27.
- 5 (Optional) To save the changes to the current method, click **Save Method** on the Method menu or click the **Save Method** button on the toolbar.
- 6 (Optional) To save the method under a new name for later use, click **Save Method As** on the Method menu, enter a name for the method (.mpm file), and click the **Save** button on the Save As dialog box.



## To align features

Use the following procedure to set parameters to adjust for retention time shifts in the raw data and correlate features across samples based on mass and retention time.



- 1 Click **Edit Method** on the Method menu, or click the **Edit Method** button on the toolbar to open the Method Parameters dialog box.
  - 2 Set the following **Alignment parameters** on the **Alignment & Normalization** tab.
    - **Mass tolerance** (Intercept and Slope)
    - **RT tolerance For RT correction** (Intercept and Slope)
    - **RT correction method** (Without standards, With standards, or None)
    - **RT tolerance For feature binning** (Intercept and Slope)
  - 3 If you selected **With standards** for the **RT correction method** in Step 2, set the following additional options on the left side of the screen:
    - a **No. of Internal Standards** (at least *two* need to be defined)
    - b For each internal standard:
      - The **RT** and **Mass**
      - *Mark* the **Algn** (alignment) check box to use this standard for retention time correction.
      - *Clear* the **Norm** (normalization) check box, unless you plan to use the current standard for normalization as described in “[To normalize features](#)” below.
  - 4 Click **OK** to initiate the analysis, using the values entered in Steps 2 and 3 above, along with current values from the Pre-Analysis Filters tab and the Result Filters tab.
  - 5 View the results in the Feature Plot and Feature Table, as described in “[Viewing Results](#)” on page 27.
- 
- 6 (*Optional*) To save the changes to the current method, click **Save Method** on the Method menu or click the **Save Method** button on the toolbar.
  - 7 (*Optional*) To save the method under a new name for later use, click **Save Method As** on the Method menu, enter a name for the method (.mpm file), and click the **Save** button on the Save As dialog box.

## To normalize features

Use this procedure to normalize abundance values across all the files.



- 1 Click **Edit Method** on the Method menu, or click the **Edit Method** button on the toolbar to open the Method Parameters dialog box.
- 2 Select one of the **Normalization** options (Without standards or With standards) on the right side of the **Alignment & Normalization** tab.
- 3 If you selected **With standards** in Step 2, set the following additional options on the left side of the screen:
  - a **No. of Internal Standards** (at least *two* need to be defined)
  - b For each internal standard:
    - The **RT** and **Mass**
    - *Mark* the **Norm** (normalization) check box to use a particular standard for normalization.
    - *Clear* the **Align** (alignment) check box, unless you plan to use this standard for alignment as described in “[To align features](#)” on page 23.
- 4 Click **OK** to initiate the analysis, using the values entered in Steps 2 and 3 above, along with current values from the Pre-Analysis Filters tab and the Result Filters tab.
- 5 View the results in the Feature Plot and Feature Table, as described in “[Viewing Results](#)” on page 27.



- 6 (*Optional*) To save the changes to the current method, click **Save Method** on the Method menu or click the **Save Method** button on the toolbar.
- 7 (*Optional*) To save the method under a new name for later use, click **Save Method As** on the Method menu, enter a name for the method (.mpm file), and click the **Save** button on the Save As dialog box.



## To perform a differential analysis and set result filters



1 Click **Edit Method** on the Method menu, or click the **Edit Method** button on the toolbar to open the Method Parameters dialog box.

2 Set the following **Analysis preferences** on the **Result Filters** tab:

- **Missing-info treatment**—0 abundance or excluded. Note that you can't select **Excluded** if you select **SVM sensitivity** as the Differential scoring method.
- **Differential scoring**—one of the following:

**Student t-test**—assesses if the abundance distribution for a given feature is statistically different between the two groups

**SVM sensitivity**—measures the degree to which the abundance of a given feature contributes to the location and separating power of a hyperplane identified in multidimensional feature-abundance space that maximally separates the features in two groups (SVM=Support Vector Machine)

3 Set the following **Result filters**:

- **Min |log2 (abund1/abund2)|**
- **Min differential score**
- **Expression**—Both, Up (*up-regulated*), or Down (*down-regulated*)
- **Min relative frequency** and *one* of the following options: And unique to a single group, in at least one group, in all groups, or of total
- **Saturated features**—Lets you exclude features that were marked as saturated by MassHunter Qualitative Analysis software.
- **Max number of features**—Unlimited or Most abundant <n> features

4 Click **OK** to perform the analysis, using the values entered in Steps 2 and 3 above, along with current values from the Pre-Analysis Filters tab and the Alignment & Normalization tab.

5 View the results in the Feature Plot and Feature Table, as described in “[Viewing Results](#)” on page 27.



6 (*Optional*) To save the changes to the current method, click **Save Method** on the Method menu or click the **Save Method** button on the toolbar.

7 (*Optional*) To save the method under a new name for later use, click **Save Method As** on the Method menu, enter a name for the method (.mpm file), and click the **Save** button on the Save As dialog box.

## To apply a different method



- 1 Click **Open and Apply Method** on the Method menu, or click the **Open and Apply Method** button on the toolbar.
- 2 Select a method in the Open dialog box and click the **Open** button.

The parameters in the selected method are applied to the current project and the results are displayed in the Feature Plot and Feature Table.

## To edit a project

Use this procedure to change the properties of an existing project, such as name, group names, sample composition, and sample scalar values.

- 1 Click **Edit Project** on the File menu to open the Edit Project dialog box.
- 2 Make the desired changes to the project, such as:
  - Add samples using the **Add** button
  - Delete samples using the **Delete** button
  - Change project name
  - Change group names
  - Change scalar values for samples

### NOTE

Note that you can *not* change the Input data type or the number of groups when you edit a project.



- 3 Click **OK** on the Edit Project dialog box. The samples are automatically processed using the current method and the results are displayed in the Feature Plot and Feature Table in the Mass Profiler main window.
- 4 (*Optional*) To save the changes to the current project, click **Save Project** on the File menu or click the **Save Project** button on the toolbar.
- 5 (*Optional*) To save the project under a new name for later use, click **Save Project As** on the File menu, enter a name for the project (**.pjt** file), and click the **Save** button on the Save As dialog box.

## Viewing Results

Results are shown automatically in the Feature Plot and Feature Table areas of the MassHunter Mass Profiler Software window when a project is processed with the current method parameters. Processing is performed automatically after the following activities:

- Creating or editing a project
- Applying a different method
- Editing a method

Results are also shown when you open a project, although no new processing is done unless you do one of the above activities

**See Also**    [“To view results in the Feature Plot”](#) below  
              [“To view results in the Feature Table”](#) on page 30  
              [“To view feature details”](#) on page 31

### To view results in the Feature Plot

A plot of Mass vs Retention Time for each feature, averaged across all the samples, is automatically displayed in the Feature Plot (**Mass vs. RT** tab).

- 1 Pass the cursor over a data point (feature) of interest on the plot to display a brief description of that feature. Note that the size of a point on the graph indicates the abundance of a feature: the larger the dot, the more abundant the feature.
- 2 Zoom in by dragging the mouse around the area of interest. The area is expanded when you release the mouse button.
- 3 You can change how features are displayed in the Feature Plot as described in [“To set Feature Plot filters and colors”](#) on page 28.
- 4 Click on a different tab above the graph to view a different plot, as described in [“To select Feature Plot view”](#) on page 29.
- 5 Click on a data point in the plot to highlight the corresponding row for that feature in the Feature Table. See [“To view results in the Feature Table”](#) on page 30.
- 6 Right-click in the plot to access the shortcut menu options described on [page 14](#).

**See Also**    [“To view feature details”](#) on page 31  
              [“To print, save, or copy the Feature Plot image”](#) on page 32  
              [“To export data from the Feature Plot”](#) on page 41

## To set Feature Plot filters and colors

Use the following procedure to limit the features that are shown in the plot and to use color to identify features individually, by sample, or by group. These steps can be selected in any order. The plot settings are as follows when the results are first displayed after processing:

- **Graph data = All samples**
  - **Plot style = Average feature**, which shows data points in black, representing the average mass and RT of features across all samples)
  - **Visibility criterion = None** (highlights all features)
  - **Expression = Both** (shows both up- and down-regulated features)
- 1 To see the feature data from a single sample only, select the sample name (input file name) of interest from the **Graph data** list. Note that when Plot style is set as **Average feature**, you may select a single sample but the plot still shows the Average feature (averaged cross all samples).
  - 2 To set a different **Plot style**, select one of the following settings, which changes the color of the data points to distinguish features in various ways:
    - **Colored by feature**—To distinguish each feature with a different color. The data points for the same feature in each sample appear (no longer averaged across all samples). For example, if a feature is present in all ten samples, the black data point for an average feature becomes ten green data points (because of slightly different RTs). The system has a limit of 10 colors, so the same color will be used for multiple features if you have more than 10 features.
    - **Colored by sample**—To distinguish features in the same sample. All features in the same sample appear in the same color. So if there are ten samples, then data points appear in ten colors. The system has a limit of 10 colors, so the same color will be used for multiple samples if you have more than 10 samples.
    - **Colored by group**—To distinguish features in a group. (Red for features in <Group 1>, Blue for features in <Group 2>).
  - 3 To filter features using a visibility criterion and threshold:
    - a Select one of the following from the **Visibility criterion** list: **Max RSD abundance**, **SD RT**, **SD Mass**, or **Differential Score**.
    - b Adjust the **Visibility threshold** to the desired level.

- 4 To show only features that have a larger average abundance in one group:
  - a Select **Up** for **Expression** (below the Feature Plot) to plot only features that have a larger average abundance in <Group 1> than in <Group 2> (called *up-regulated*).
  - b Select **Down** for **Expression** (below the Feature Plot) to plot only features that have a larger average abundance in <Group 2> than in <Group 1> (called *down-regulated*).

## To select Feature Plot view

Use this procedure to select a different plot view for the results of analysis in the Feature Plot. The **Mass vs. RT** plot is presented by default when samples are analyzed. These steps can be done in any order.

- 1 Click the **Log2 Ratio vs RT** tab to view a plot of the  $\log_2(A1/A2)$  value for a feature versus the retention time.
  - For **Average feature** plot style, A1 is the abundance of <Group 1> and A2 is the abundance of <Group 2>.
  - When **Graph data** is set to a single sample, A1 is the abundance of the sample and A2 is the averaged abundance of the group to which the sample belongs.
- 2 Click the **Log/Log Plot** tab to view a plot of the  $\log_2$  of the average abundance of a feature:
  - in <Group 1> vs  $\log_2$  of its average abundance in <Group 2> for **Average feature** plot style
  - When **Graph data** is set to a single sample, the plot shows the  $\log_2$  abundance of a sample versus the  $\log_2$  averaged abundance of the feature cross the group to which the sample belongs.
- 3 Click the **Unique to <Group 1>** tab to plot only those features that are present in the first group of samples, but not in the second.
- 4 Click the **Unique to <Group 2>** tab to view a plot of only those features that are present in the second group of samples, but not in the first.
- 5 Click the **Mass vs. RT** tab to view the original plot, i.e. mass vs. retention time (RT) for each feature.

## To view results in the Feature Table

The Feature Table contains average values and standard deviations for each feature across all samples (Feature summary) and each group separately, including retention time (RT), Mass, Abundance, Freq (frequency), SD (standard deviation) for RT and Mass, and RSD (relative standard deviation) for Abundance values.

Other columns are added to the table to show the results of feature identification from the ID Browser program. See [“Identifying Features”](#) on page 36 for more information.

- 1** If not already visible, scroll horizontally to view **Comparison** data on the right of the table. Green values are internal standards, if used.
- 2** To show only the features that have a larger average abundance in one group:
  - a** Select **Up** for **Expression** (above the Feature Table) to show only up-regulated features.
  - b** Select **Down** for **Expression** (above the Feature Table) to show only down-regulated features.
- 3** By default, the table is ordered from the highest average **Abundance** value to the lowest. Click on a different column heading to sort the table by the values in that column, such as **Mass** or **RT**.
- 4** Right-click in any row number cell on the left side of the Feature Table to access the shortcut menu options, such as Feature Details, Abundance Distribution, Identification Lookup, and Web search. See [page 17](#) for more information.
- 5** To copy the contents of the table for use in another program, drag the mouse to select the cells of interest, then press the **C** key while holding the **Ctrl** key down. The copied contents can then be pasted into other applications, such as Microsoft Excel.

**See Also** [“To view feature details”](#) on the next page  
[“To export data from the Feature Table”](#) on page 40

## To view feature details

Use this procedure to view more information about a selected feature. *This procedure is not available for data imported from .CSV file.*

- 1 Open the Feature Information window for a selected feature in any of the following ways:

### From the Feature Table

- Click to select the feature of interest in the Feature Table, then click **Feature Details for Selected Feature** on the View menu, *or*
- Right-click the row number for the feature in the Feature Table, and select **Feature Details** from the shortcut menu.

### From the Feature Plot

- Right-click on the feature of interest in the Feature Plot, and select **Feature Details** from the shortcut menu.
- 2 Review the information for the feature in all the samples in the **All samples** table in the top left area of the screen, such as File ID number, File name, RT, Mass, and Abundance. Samples in <Group 1> appear in red; samples in <Group 2> appear in blue. Click on a column heading to sort the table by the values in that column, such as **Abundance** or **Mass**. Note the selected feature may not be present in every sample in the project.
  - 3 Click to select a row (sample) in the **All samples** table and view the information for the feature in the selected sample, such as **Species**, **RT**, **m/z**, **Mass**, **Abundance**, and **Width** that is displayed in the Selected sample table below the All samples table. The columns of information that are displayed depend on the input data type of the selected sample.
  - 4 On the right side of the screen, view the feature's species cluster plots for each sample. Zoom in by dragging the mouse around the area of interest. The area is expanded when you release the mouse button.
  - 5 Right-click on a species cluster plot to access the following options:
    - **Unzoom**– Returns the plot to the previous zoom level.
    - **Autoscale**– Returns the plot to the original display.
    - **Print Image**– Sends the image to a printer.
    - **Copy Image to Clipboard**– Copies the current image to the Clipboard to paste into other applications as either a Metafile or Bitmap.
    - **Save Image to File**– Saves the current image as either a Metafile (.emf format) or Bitmap (.bmp format).

## To print, save, or copy the Feature Plot image

- 1 Click the appropriate item on the Image menu:
  - **Print Image**
  - **Copy Image to Clipboard**
  - **Save Image to File**
- 2 If you selected either **Copy Image to Clipboard** or **Save Image to File**, select the file format (either **Metafile** or **Bitmap**).

**Alternate method** These same menu options are available by right-clicking in the Feature Plot.

## To view more information from database websites

Use this procedure to access more information about selected features in any of the following online databases: ChemIDplus, HMP, NIST, PubChem, or Web METLIN.

### From the Feature Table

#### Method 1:

- 1 Click to select the feature of interest in the Feature Table.
- 2 Select which database to search from the **Web** menu.

#### Method 2:

- 1 Right-click on the row number for the feature of interest in the Feature Table to display the shortcut menu.
- 2 Select which database to search from the **Web** submenu.

### From the Feature Plot

#### Method 1:

- 1 Click to select the feature of interest in the Feature Plot. (Note that the selected feature is highlighted in the Feature Table.)
- 2 Select which database to search from the **Web** menu.

#### Method 2:

- 1 Right-click on or near the feature of interest in the Feature Plot to display the shortcut menu.
- 2 Select which database to search from the **Web** submenu.

In each case above, a mass search is performed for the selected feature in the selected database and the results are displayed in your internet browser window.



## To view the abundance distribution graph for a feature

- 1 Open the Abundance Distribution window for a selected feature in any of the following ways:

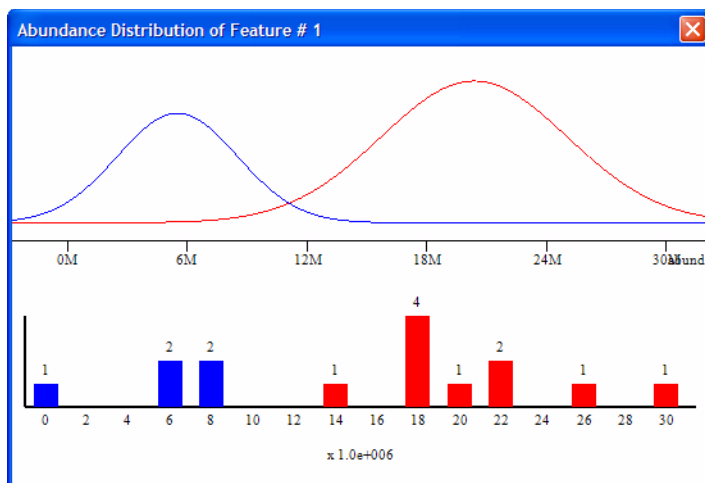
### From the Feature Table

- Click to select the feature of interest in the Feature Table, then click **Abundance Distribution for Selected Feature** on the View menu, *or*
- Right-click the row number for the feature in the Feature Table, and select **Abundance Distribution** from the shortcut menu.

### From the Feature Plot

- Right-click on the feature of interest in the Feature Plot, and select **Abundance Distribution** from the shortcut menu.

- 2 Review the data presented in the Abundance Distribution window, which is presented as both a histogram and as curves color-coded by group (red for <Group 1> and blue for <Group 2>), as shown in the following example.



The lower plot is a histogram, with Abundance values on the x-axis. The height of the bars are determined by the number of samples whose abundance falls into that abundance bin.

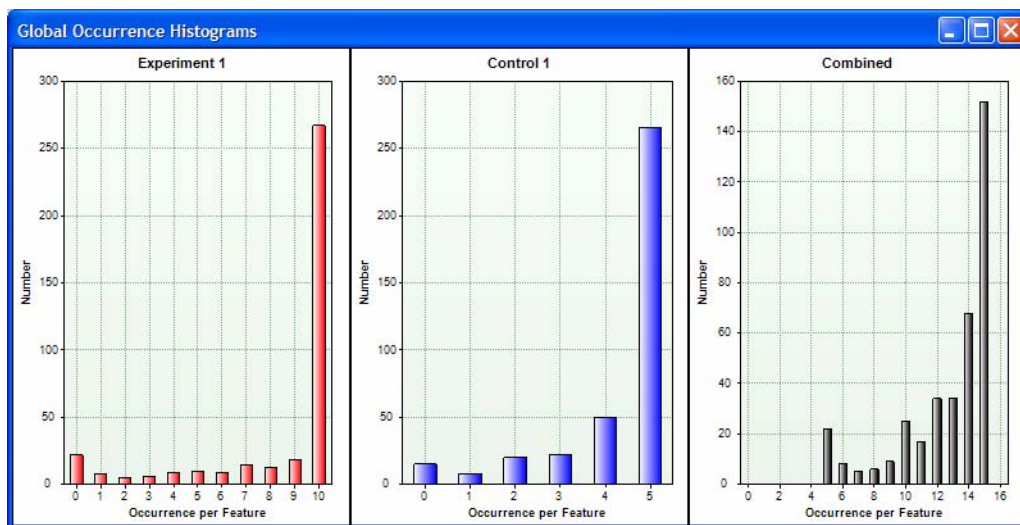
The upper plot has the same x-axis. The distribution curves are calculated using the least-squared fit to a Gaussian shape of the bars in the lower plot.

## To view global occurrence histograms

Use this procedure to view global occurrence histograms, which show the relationship between the number of features and the number of samples.

- 1 Click **Global Occurrence Histograms** on the View menu.
- 2 Review the information presented in the Global Occurrence Histograms window, which displays a histogram for each group and a combined histogram of both groups. Each histogram shows the frequency of feature occurrence in the samples within a group or within both groups. The x-axis is the number of samples that a particular feature occurs in (i.e. 0, 1, 2, 3, 4, up to the number of samples in the group). The y-axis is the number of features that occur in that number of samples (i.e. from 0 up to the number of features in the project).

### Example Global Occurrence Histograms window:



The center histogram (**Control 1** group) in the example above shows that:

- ~20 features do not occur in *any* sample in this group.
- ~10 features occur in 1 sample in this group.
- ~22 features occur in 2 samples in this group.
- ~25 features occur in 3 samples in this group.
- 50 features occur in 4 samples in this group.
- ~270 features occur in *all* 5 samples in this group.

The histogram on the right (**Combined** group) shows that:

- Every feature occurs in at least 5 samples in the project.
- ~21 features occur in 5 of 15 samples in the project.
- ~9 features occur in 6 of 15 samples in the project.
- ~6 features occur in 7 of 15 samples in the project.
- ~7 features occur in 8 of 15 samples in the project.
- ~10 features occur in 9 of 15 samples in the project.
- ~26 features occur in 10 of 15 samples in the project.
- ~17 features occur in 11 of 15 samples in the project.
- ~33 features occur in 12 of 15 samples in the project.
- ~33 features occur in 13 of 15 samples in the project.
- ~68 features occur in 14 of 15 samples in the project.
- ~152 features occur in *all* 15 samples in the project.

## Identifying Features

The following activities allow you to identify features using the MassHunter ID Browser program. *These procedures are not available for data imported from .CSV file or LMFE input data.*

- “To identify a single feature” on page 36
- “To identify a batch of features” on page 37
- “To identify features with ID Browser” on page 38
- “To review results in the Compound List Window” on page 39

### To identify a single feature

*This procedure is not available for data imported from .CSV file or LMFE input data.*

- 1 Select the feature of interest and initiate the search in any of the following ways:
  - a From the Feature Table:
    - Click to select a row (feature) in the Feature Table, then click **Quick Lookup for Selected Feature** on the Identify Features menu.
    - Right-click the row number for the feature of interest in the Feature Table, then select **Identification Lookup** from the shortcut menu.
  - b From the Feature Plot:
    - Right-click on or near the feature of interest on the Feature Plot, then select **Identification Lookup** from the shortcut menu.
- 2 Identify features with ID Browser as described on [page 38](#). The identification methods available are Database search (CSV, PCD/METLIN) and MFG (molecular formula generation). *Spectral library search is not currently supported in ID Browser for LC/MS data.*
- 3 Review results in the Compound List Window as described on [page 39](#).
- 4 Click the **Save and Return** button to close the ID Browser window and return to Mass Profiler.

Results of identification lookup for a single feature are *not* available when you return to Mass Profiler.

## To identify a batch of features

*This procedure is not available for data imported from .CSV file or LMFE input data.*

**1** Select the features and initiate the search in any of the following ways:

**a** From the Feature Table:



- To identify the features currently displayed in the Feature Table, click **For All Features in Table** on the Identify Features menu or click the **Identify All Features in Table** button on the toolbar.
- To identify only the marked features in the Feature Table, click **For Marked Features in Table** on the Identify Features menu.
- To identify only the unmarked features in the Feature Table, click **For Unmarked Features in Table** on the Identify Features menu.

**b** From the Feature Plot:

- Click **For Highlighted Features on Graph** on the Identify Features menu.

**2** Identify features with ID Browser as described on [page 38](#). The identification methods available are Database search (CSV, PCD/METLIN) and MFG (molecular formula generation). *Spectral library search is not currently supported in ID Browser for LC/MS data.*

**3** Review results in the Compound List Window as described on [page 39](#).

**4** Click the **Save and Return** button to close the ID Browser window and return to Mass Profiler.

**5** Review the search results in the Feature Table as described on [page 30](#).

## To identify features with ID Browser

*This procedure is not available for data imported from .CSV file or LMFE input data.*

- 1 Click one of the following items on the Identify Features menu in Mass Profiler.

- For Highlighted Features on Graph
- For All Features in Table
- For Marked Features in Table
- For Unmarked Features in Table
- Quick Lookup for Selected Feature

The Agilent MassHunter ID Browser window opens with the Compound Identification Wizard dialog box displayed.

- 2 Mark one or more of the methods to use to identify compounds:
  - Database search (CSV, PCD/METLIN)
  - MFG (molecular formula generator)

### NOTE

Spectral library searching for LC/MS compounds is not supported in this release.

- 3 If you marked the MFG check box in Step 2, select whether to generate formulas for all compounds or only for compounds that have not been identified yet.
- 4 Click the **Next >>** button. The Compound Identification Wizard Identification Method dialog box opens.
- 5 You can optionally perform the following steps to open a different method or change method parameters.
  - Load a new method.
  - Modify the method parameters.
  - Save the method.
- 6 Click the **Finish** button to start the identification process.
- 7 Review the results in the Compound List window as described on the following page.

## To review results in the Compound List Window

This topic tells how to view compound results in the Compound List Window of the ID Browser.

- 1** View the compounds in the table in the Compound List Window.
  - Each row in the table shows a different compound.
  - Each column describes a different piece of information about each compound. For a list of the information that can be displayed for a compound, see the ID Browser online help.
- 2** Click the + button to the left of a compound to display the second level of the compound table for the selected compound.
  - The second level of the table shows a merged identification list that contains results from Generating Formulas and from Database and Library Search.
  - Second level rows have a green background.
- 3** Click the + button next to a second level search result to display the third level of the compound table for the selected result.
  - The third level of the table shows the species information, if the second level has a formula assigned from either generate formulas or database search.
  - Third level rows have a white background.
- 4** Click the + button next to an entry in the third level of the table to display the fourth level of the compound table.
  - The fourth level of this table shows the peak list. The peak list will show the best hit information even when the highlight changes.
  - Fourth level rows have a blue background.
- 5** Click the - button to close a level that is open. Closing a compound row automatically closes the 2nd, 3rd and 4th level rows for that compound.

There is no limit to how many levels can be open for as many compounds as you want at the same time.

## Exporting Mass Profiler Data

This section describes how to export data from MassHunter Mass Profiler software for use with other programs, such as Excel and GeneSpring MS.

- [“To mark features for annotation”](#) below
- [“To export data from the Feature Table”](#) below
- [“To export data from the Feature Plot”](#) on page 41

### To mark features for annotation

Use this procedure to mark any features of special interest. These marks will be included when you export data from the Feature Table or the Feature Plot.

- Click in the column labeled **Mark** in the rows for the features of interest in the Feature Table.
- To *deselect* a feature that is currently marked, click again in the column labeled **Mark** in the row for the feature you want to clear.

### To export data from the Feature Table

- 1 (Optional) Mark any features that are of special interest as described in [“To mark features for annotation”](#) above.
- 2 Click one of the following items on the **File** menu, depending on the format you want to use to export the data:
  - **Export Feature Summary > From Feature Table** to export summary data for use in Excel.
  - **Export Targeted MS/MS Inclusion List > From Feature Table** to export inclusion ion list for targeted MS/MS experiment.
  - **Export MPP Input File > From Feature Table** to export feature data for use in Mass Profiler Professional.
  - **Export Composite-Compounds CEF > From Feature Table** to export feature data for use with other programs.

For each of the above options, you can select whether to export data for **All Features**, **Marked Features**, or **Unmarked Features**.



- 3 If you selected **Export Targeted MS/MS Inclusion List > From Feature Table** in Step 2, the Export Inclusion List Options dialog box appears. Set the following parameters for selecting representative ions for each feature.
  - a Mark **Most abundant** to use the most abundant ion for each feature.
  - b Enter an **RT tolerance** in minutes or accept the default value of 0.10 minutes. This parameter is included in the file for use by data acquisition software.
  - c Click **OK**.

## NOTE

For a description of other parameters on this dialog box, see the *Export Inclusion List Options Dialog Box* topic in online help.

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- 4 Enter a location and name for the output file and click the **Save** button.

## To export data from the Feature Plot

- 1 Display the features of interest in the Feature Plot as described in [“To set Feature Plot filters and colors”](#) on page 28 and [“To select Feature Plot view”](#) on page 29. Keep the following caveats in mind:
  - When you export graphed features, only the features shown as solid dots are exported. Circles are filled or not based on the current Visibility criterion. If the Visibility criterion is set to **None**, then the result is equivalent to the table-based reports (for Mass vs. RT and Log2 Ratio vs. RT plots).
  - The ratio and comparison plots can only include features that are present in both groups. You may get a different list of features depending on which graph you are exporting. The generated files include annotations to document what graph and visibility criterion were used to generate the export.
  - The supported export formats are based on data averaged over one or more groups. Even if you choose a **Plot style** that displays individual per sample features, the export will still report the averaged feature data.
- 2 Click one of the following items on the **File** menu, depending on the format you want to use to export the data:
  - **Export Feature Summary > From Graphed Data** to export summary data for use in Excel.
  - **Export Targeted MS/MS Inclusion List > From Graphed Data** to export an inclusion ion list for targeted MS/MS experiment.

- **Export MPP Input File > From Graphed Data** to export feature data for use in Mass Profiler Professional.
  - **Export Composite-Compounds CEF > From Graphed Data** to export feature data for use with other programs.
- 3** If you selected **Export Inclusion List > From Graphed Data** in Step 2, the Export Inclusion List Options dialog box appears. Set the following parameters for selecting ions for the inclusion list.
- a** Mark **Most abundant** to use the most abundant ions.
  - b** Enter an **RT tolerance** in minutes or accept the default value of 0.10 minutes. This parameter is included in the file for use by data acquisition software.
  - c** Click **OK**.

## NOTE

For a description of other parameters on this dialog box, see the *Export Inclusion List Options Dialog Box* topic in online help.

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- 4** Enter a location and name for the output file and click the **Save** button.



## **In this guide**

This Quick Start Guide describes how to install and use MassHunter Mass Profiler Software.

This guide is valid for the B.03.00 SP1 revision or higher of MassHunter Mass Profiler Software, until superseded.

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