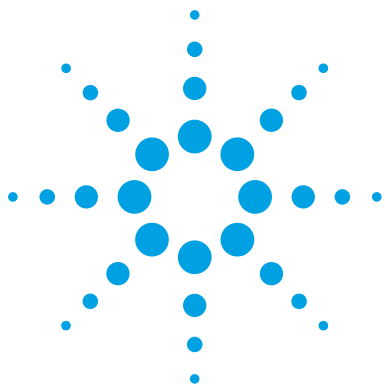




MassHunter Mass Profiler Software

Quick Start Guide

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What is Mass Profiler?

Agilent MassHunter Mass Profiler software is a stand-alone program specifically designed to investigate features in your LC/MS sample data files and compare feature similarities and differences in your data between two samples, within a single sample group, or between two samples groups. The feature investigation capabilities are well suited for the comparison of two samples from different environmental locations (for example, two locations in a river or a ground water well) and different batches in a food or chemical synthesis process. Mass Profiler can also be used for expression profiling applications such as biomarker discovery. Mass Profiler uses a unique feature extraction and correlation algorithm that locates and aligns all the components in even the most complex sample mixtures.



Agilent Technologies

What is Mass Profiler?

Mass Profiler helps you:

- Find and extract the features in your sample data.
- Align and normalize features from different samples.
- Review the features in your samples individually and by grouping.
- Investigate similarities and differences in features across two samples, a single sample, or two sample groups.
- Conduct a sample classification in pairs using Principal Component Analysis.
- Identify features via a molecular formula generator or accurate mass database search using MassHunter ID Browser.

Mass Profiler operates on raw data files and compound exchange files produced by MassHunter Qualitative Analysis and Profinder on LC/MS data files. Retention time, mass, and abundance LC/MS data can also be imported from any source using comma separated value files. GC/MS data files currently are not supported

Feature extraction, combined with chromatographic alignment across multiple data files, is a critical step in the peak finding and data reduction workflow that minimizes the appearance of both false positive and false negative features by “binning” the features in the chromatographic time domain.

When used to export features, Mass Profiler reduces your acquired data size and complexity, yielding a smaller data set that is more easily processed without any compromise in the information quality.

The feature files generated by Mass Profiler can be imported into Mass Profiler Professional (MPP), a powerful chemometrics platform used in any MS-based differential analysis to determine relationships among two or more sample groups and variables.

What's new in B.07.00?

- Projects can be created from raw MassHunter data files and directly extract features using Find Compounds by Molecular Feature (also referred to as Molecular Feature Extractor, MFE), allowing an entire profiling workflow to be conducted in Mass Profiler.
- MFE employs recursive grouping of ions across multiple data files to minimize false positive and false negative features.
- Added flexibility added for specifying how MFE finds related ions with different charge carriers and which charge carrier to assign for single-species ions.
- Post-alignment filtering is available based on quality score (Q-Score) or abundance. Q-Score is a new attribute that when used for filtering features provides an advantage over abundance filtering when features have small abundances.
- Feature Details view provides the ability to view extracted ion chromatograms (EIC) from all or individual ions associated with a feature and to view the averaged mass spectra across a feature elution.
- Streamlined Method Editor dialog box with two editing modes: only commonly changed parameters and all method parameters.
- Saving a project automatically includes an embedded copy of the method used to create the last results. You can also save methods separately. Additionally, Mass Profiler internally retains the current method parameters on exiting, whether or not the method was saved to disk.
- Principal Component Analysis (PCA) is available.
- Identify features using ID Browser B.07.00.

Features of Mass Profiler

- Unique feature extraction and correlation algorithm locates the molecular features in your data files, not just chromatographic peaks.
- Visualization tools help you identify statistically meaningful differences between two sample groups, such as healthy versus disease samples.
- Calculate abundance ratios and clearly identifies differentially expressed features.
- Flexible feature selection to selectively analyze and compare your data.
- Export compound information for import into database search programs, identification environments, non-MassHunter statistical analysis packages, and advanced statistical data analysis by Mass Profiler Professional software.
- Supports data analysis workflows including metabolomics, integrated biology, pharmaceutical impurity analysis, forensic analysis, environmental analysis, and pesticide analysis.
- Open all or some of the files associated with your Agilent data set (sample files) in a single selection process.
- Create methods for your feature extraction, chromatographic alignment, and differential analysis, and save them to disk as a Mass Profiler Method file (.mpm).
- Review your features and chromatographic alignment using an intuitive graphical interface.
- Delete false positive features from your results.
- Save your feature extraction and feature review progress as a Mass Profiler project file (.pjt) and open the project at a later time to review, adjust, and reprocess your data files.

Where is Mass Profiler used in your experiment?

Mass Profiler is used to extract and/or review the features from LC/MS data files you acquired from your experimental samples. An untargeted differential analysis experiment may include the steps as shown below.

- 1 Prepare for your experiment
- 2 Acquire your data - Data Acquisition
- 3 Extract spectral features in your data files - Mass Profiler, Profinder, or Qualitative Analysis/DA Reprocessor
- 4 Compare two samples or sample groups - Mass Profiler
- 5 Identify features in your data - ID Browser in Mass Profiler
- 6 Export features for advanced statistical analysis - MPP
- 7 Save your project - Mass Profiler and MPP

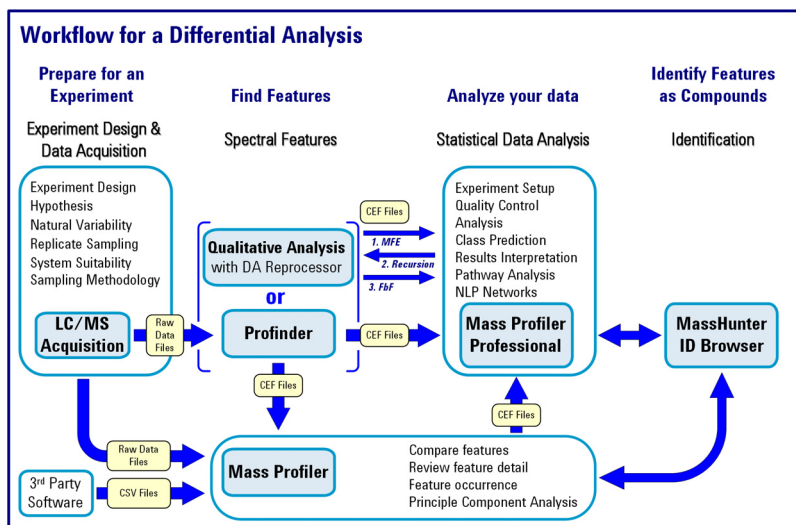


Figure 1 Feature extraction performed by the MFE algorithm in Mass Profiler is similar to the MFE algorithm in MassHunter Qualitative Analysis/DA Reprocessor or Profinder; however, Mass Profiler and Profinder add the functionality of recursive grouping across multiple data files.

Where to find more information

Figure 1 shows the steps and MassHunter tools that are used in an untargeted and targeted (Profinder and Qualitative Analysis) differential analysis. The feature finding steps performed by Mass Profiler can alternatively be accomplished in Qualitative Analysis/DA Reprocessor or Profinder (TOF and Q-TOF data) as described in the *Class Prediction with Agilent Mass Profiler Professional - Workflow Guide*.

Mass Profiler provides easy to use data analysis capabilities to help you review features, compare features between two samples or sample groups, and a perform a principal component analysis with your samples organized into two groups. Compared to MPP, Mass Profiler is unique in that you can view compound chromatograms and spectra during your analysis.

Where to find more information

Online Help

Press F1 To get more information about a pane, window, or dialog box, place the cursor on the pane, window, or dialog box of interest and press **F1**.

Help Menu Click **Help > Contents** to access the contents of the Mass Profiler Help including basic tasks, user interface, and reference information.

Documents

Mass Profiler Familiarization Guide Use this guide to learn how to use Mass Profiler. This guide can be found on the installation disc and also online at http://www.chem.agilent.com/Library/usermanuals/Public/G3297-90012_MassProfiler_Familiarization.pdf.



Mass Profiler Professional

- Agilent G3835AA MassHunter Mass Profiler Professional Software - Quick Start Guide
- Agilent G3835AA MassHunter Mass Profiler Professional Software - Familiarization Guide
- Agilent G3835AA MassHunter Mass Profiler Professional Software - Application Guide

- Agilent MassHunter Mass Profiler Professional User Manual

Workflow Guides and Overviews

- Agilent Metabolomics Workflow - Discovery Workflow Guide (p/n 5990-7067EN)
- Agilent Metabolomics Workflow - Discovery Workflow Overview (p/n 5990-7068EN)
- Integrated Biology with Agilent Mass Profiler Professional - Workflow Guide (p/n 5991-1909EN)
- Integrated Biology with Agilent Mass Profiler Professional - Workflow Guide Overview (p/n 5991-1910EN)
- Class Prediction with Agilent Mass Profiler Professional Workflow Guide (p/n 5991-1911EN)
- Class Prediction with Agilent Mass Profiler Professional Workflow Overview (p/n 5991-1912EN)

MassHunter Qualitative and Quantitative Analysis

- Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide for LC/MS
- Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide for GC/MS
- Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide

Training

Road Show Slide Presentation Metabolome Analysis - From Sample Prep Through Data Analysis (Metabolomics Road Show, March 2011)

Training Courses Visit www.chem.agilent.com to view a listing of training courses for Mass Profiler.

Getting Started

How do I get started?

This *Quick Start Guide* helps you install Mass Profiler, become familiar with the Mass Profiler user interface, perform feature extraction, and perform a two sample or two sample group comparison using your data.

- 1 Install Mass Profiler. Follow the instructions in “[Mass Profiler Installation](#)” on page 44.
- 2 Start Mass Profiler. Double-click the **Mass Profinder** icon  located on your desktop or in the **Agilent MassHunter Workstation** desktop folder, or click **Start > All Programs > Agilent > MassHunter Workstation > Mass Profiler > Mass Profiler**.
- 3 Review this *Quick Start Guide* to learn about:
 - [Getting Started](#)
 - [User Interface](#)
- 4 Review the *Familiarization Guide* to learn how to:
 - Process Data
 - View Results
 - Identify Features
 - Export Data
- 5 Review the “[MassHunter Qualitative and Quantitative Analysis](#)” guides (see “[Documents](#)” on page 6). These guides help you improve your results by covering topics that help you:
 - Prepare for an experiment
 - Explain the parameters related to finding features
 - Compare two samples or sample groups
- 6 Review the *Familiarization Guide* to learn how to extract the features in your samples. Follow the example Basic Mass Profiler Workflow in “[Exercise B: 2-Sample-Group-Comparison](#)”.

Terminology

- Algorithm** An algorithm is a set of automated, sequential mathematical tasks performed to find, filter, align, extract, compare, and identify features from your chromatographic/ mass spectral data sets.
- Feature** A feature is synonymous with compound. A feature is referred to interchangeably with compound, descriptor, element, entity, metabolite, or molecular feature during the various steps of analysis using MassHunter software. A feature can consist of one or multiple related ions, including isotopes, and different ion species (charge carrier, multimers) and charge states.
- Group** Samples that have a common relationship within the definition of the experiment design, for example, samples from a group of healthy versus diseased specimen. Related samples are grouped together for analysis. For a project with two groups, Mass Profiler initially assigns “Experiment” and “Control” for the group names. These default names can be changed.
- Method** A method is a set of all the parameters used for processing sample files in a Mass Profiler project, including feature finding, aligning, normalizing, and filtering. Methods can be saved using unique file names.
- Project** Two samples, or one or two sample groups, and the associated method that form your feature analysis and investigation. A project can be saved and opened at a later time to continue your analysis.

NOTE

The data file path is saved with the project. If you move a project to a different computer, you can open the project and see the results. But to access the Feature Details, the data files on the target computer must be in the same path as on the source computer. If they are not, Mass Profiler cannot find the *.rmc file for the first data file it tries to access, and you get an error message. The *.rmc files are stored in the data file. The error message indicates the path where the data files are expected to be found.

Reprocessing the project with different method parameters also fails if the data files are not found in the path that is saved with the project.

- Sample** LC/MS data acquired from a specimen and understood to be representative of the larger specimen or population. Individual samples are imported into Mass Profiler in the form of raw data files, CEF files, or CSV files (see [Figure 1](#) on page 5).

Workflow A workflow is a document or a graphical overview that captures a sequence of steps to guide you through an analytical task. A workflow may cover more than one wizard and may include steps performed by more than one MassHunter software program.

Processing and viewing results

The results of processing samples appear in the Feature Table, Feature Plot and Feature Details in the main window. In the main window you select whether to show the Feature Plot or the Feature Details to appear with the Feature Table in the main window (see [Figure 3](#) on page 13 and [Figure 4](#) on page 14).

- Use the Feature Table to:**
- View all of the feature information for two samples or represented statistically across one or two groups.
 - View feature information in four sections showing information across all samples, each of the two sample groups, and comparison of two sample groups.
 - Mark and unmark features for identification and PCA analysis.

See [“Feature Table”](#) on page 30 for more information.

- Use the Feature Plot to:**
- Visualize and investigate features using different plot types: Mass vs. Retention Time, Log₂ Ratio vs. Retention Time, Log/Log Abundance, Unique to <Group 1>, and Unique to <Group 2>, the latter two are available with projects containing two groups.
 - Investigate features in individual or multiple samples.
 - Investigate features that exceed a specified visibility threshold filter setting.
 - Investigate features by mass and retention time across all samples or use color to distinguish individual features or to identify features by sample or group.

See [“Feature Plot”](#) on page 33 for more information.

- Use the Feature Details to:**
- Visualize and investigate specific individual feature details such as appearance in each sample file, appearance in each group, and the ion species within the feature.
 - Compare the feature’s appearance across all of the samples.

- Investigate features by retention time, mass, and m/z using extracted ion chromatograms and mass spectra for all of the samples.

See “Feature Details” on page 36 for more information.

Identification

Mass Profiler (Figure 2.) helps you identify features via Molecular Formula Generation or an accurate mass database search (a .cdb or .csv formatted database) using MassHunter ID Browser. Features can be identified individually or in a batch. Additional mass-based identification methods are supported using publicly available database searches. Spectral library search is not supported in ID Browser for LC/MS data.

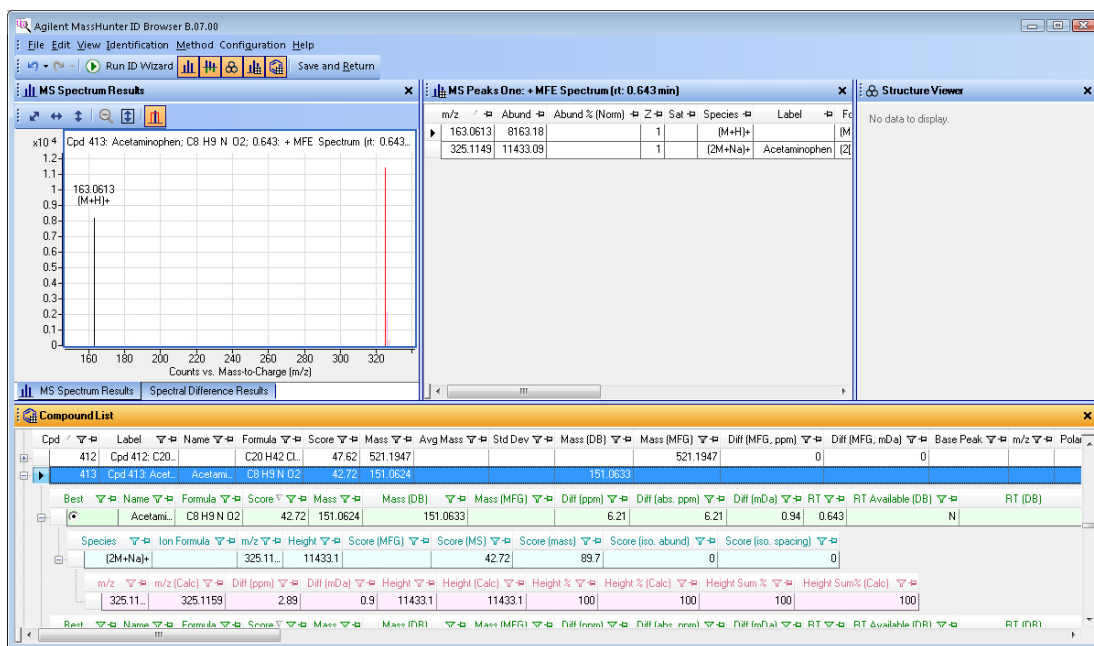


Figure 2 ID Browser after identifying features as compounds

Exporting data

Features found and aligned across samples form “composite features” and can be exported in several formats for different purposes. You can export features in the following formats:

- .cef - Export file format that contains a list of the found features in each sample with the algorithm used to find the features, and includes MS ions (only precursor ions) with mass, abundance, RT, charge state, and charge carrier. A compound exchange format (CEF) file is created to exchange data between Agilent software. CEF files are typically used for import into MPP for advanced statistical analysis.
- .csv - Averaged ion data (m/z and RT of representative ions) for each feature for use in data acquisition in targeted MS/MS experiments. An ASCII text comma separated value (CSV) file is created containing specific feature information that can be readily imported into acquisition software as an MS/MS inclusion list or imported into text editors and spreadsheets.
- .txt - Feature data for use in Mass Profiler Professional. A tab delimited ASCII text (TXT) file is created containing the feature information for each sample data file that is readily imported into MPP, other data analysis software, text editors, and spreadsheets.
- .xls - Feature Summary data for use in Excel. This format is the same tab delimited ASCII text file as the .txt format, but the .xls extension allows the data to be readily imported into Microsoft Excel. The file can also be imported into text editors.

User Interface

Main functional areas

The main Mass Profiler window consists of three parts: (1) the Menu Bar, (2) the Toolbar, and (3) the Main Window. The main functional areas are shown in Figure 3.

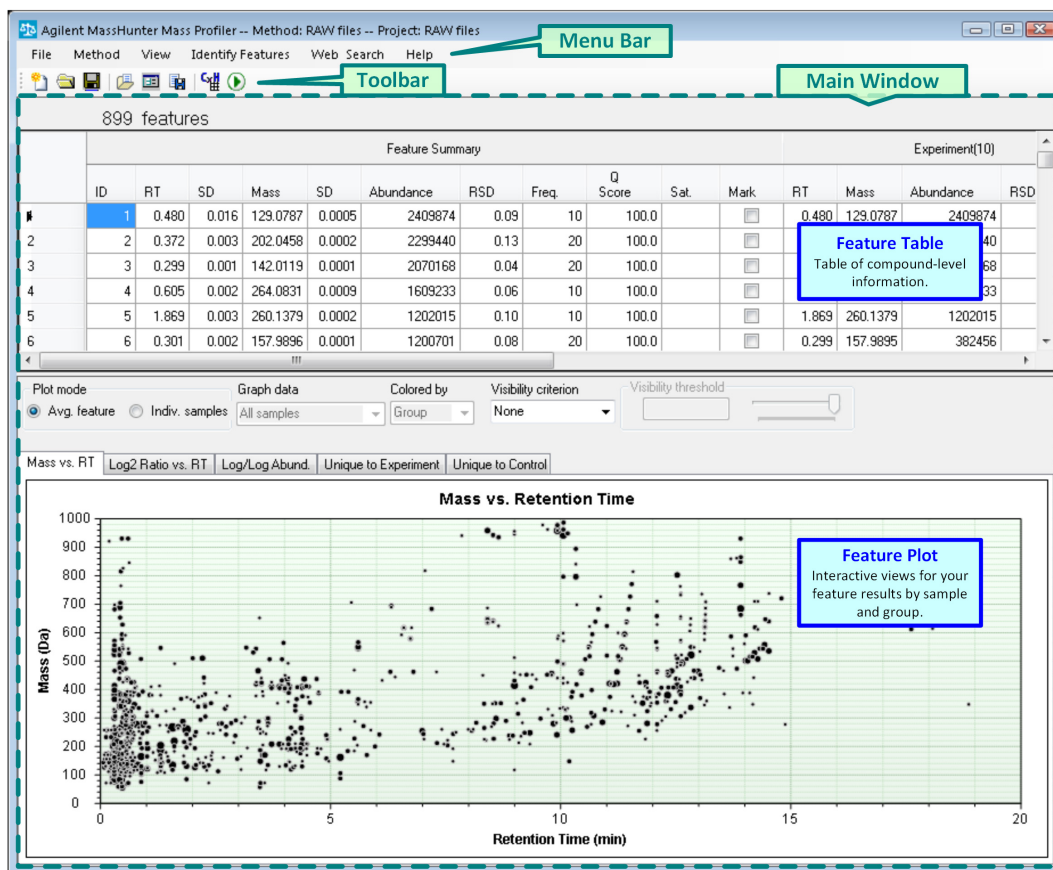


Figure 3 The main functional areas of Mass Profiler after a method is run on a project. If selected in the View menu, the Feature Plot can be replaced with a Features Details view shown in Figure 4 on page 14.

User Interface

Main functional areas

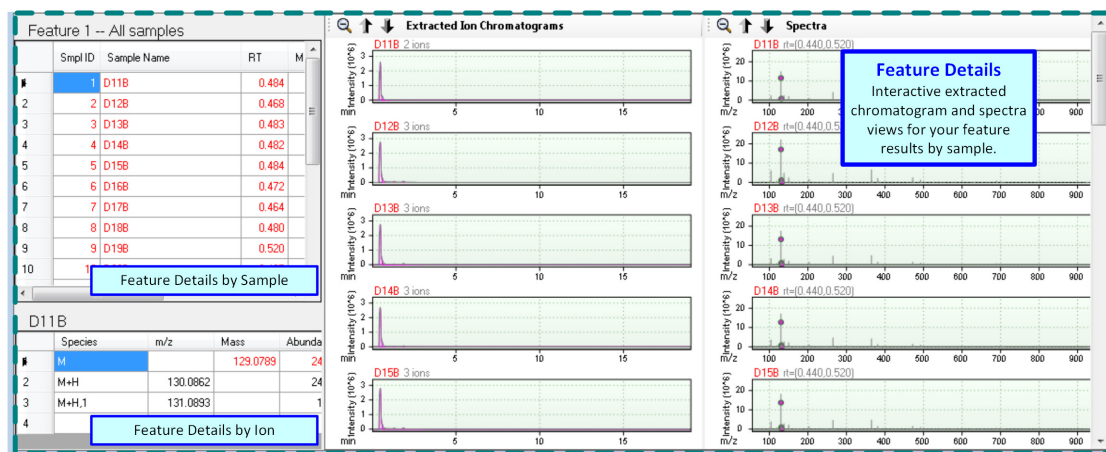


Figure 4 When you click **View > Show Feature Details** from the menu, the Feature Details view replaces the Feature Plot view in the main window shown in Figure 3 on page 13.

1. Menu Bar

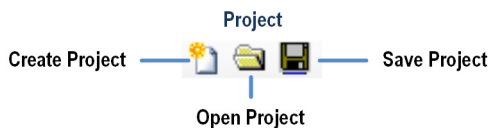
The menu bar provides actions that are used for managing your projects, methods, display, and identifying features.

File Method View Identify Features Web Search Help

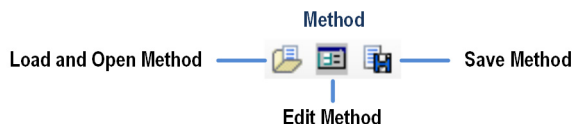
2. Toolbar

The toolbar is located below the menu bar and contains three groups of buttons for commonly used tasks:

Project: Create Project, Open Project, and Save project



Method: Load and Open Method, Edit Method, and Save Method.



ID & Run: Identify All Features (opens and runs ID Browser) and Run Current Method.



3. Main Window

The main window, see [Figure 3](#) on page 13, is further divided into two areas – Feature Table and Feature Plot that are used to review the results from applying the feature extraction, alignment, filtering, and comparison defined in the method to your data set.

Feature Table The data presented in Feature Table is organized by four sections: (1) Feature Summary with ID, (2) Experiment (group) information, (3) Control (group) information, and (4) Comparison information as shown in [Figure 5](#). Each feature is presented in a row and information related to each section is presented in a column.

9 features																														
Feature summary														Experiment()					Control()					Comparison						
#	ID	Name	Formula	CAS	RT	SD	Mass	SD	Abundance	RSD	Freq	Q	Score	Meta	RT	Mass	Abundance	RSD	Freq	RT	Mass	Abundance	RSD	Freq	F	Mass	Log2(A1/A2)	Expression	Diff Score	
2	2	Sulfamethoxazole	C12H14N4O2 S	51,681	3.680	270.0536	39094	1	100.0	0	1	100.0	0	3.680	270.0536	39094	1	1	1.024	553.0770	2830	0	0	0	16.00	up				
3	3	Sulfamethoxazole	C12H14N4O2 S	52,112	4.973	310.0737	26078	1	100.0	0	1	100.0	0	4.973	310.0737	26078	1	1	1.024	553.0770	2830	0	0	0	16.00	up				
4	4																													
6	6	Sulfamethoxazole	C9H10N4O2 S2	58,652	3.676	270.0247	7911	1	100.0	0	1	100.0	0	3.676	270.0247	7911	1	1	1.024	553.0770	2830	0	0	0	16.00	up				
7	7	Sulfamethoxazole	C10H12N4O2 S	60,300	3.987	284.0139	5560	1	100.0	0	1	100.0	0	3.987	284.0139	5560	1	1	1.024	553.0770	2830	0	0	0	16.00	up				
8	8																													
9	9																													

Feature Summary with ID

Experiment (group) info

Control (group) info

Comparison

Figure 5 Data areas within the Feature Table

Feature information is presented as the average across all of the samples in a single group project. When your project contains two groups the *Feature Summary* columns report the average mass and RT, but the higher (averaged) abundance and Q-Score across both groups, as well as standard deviations and relative standard deviations. One column indicates if there was saturation in

the compound spectrum, and another column allows you to mark features for additional tasks such as identification, PCA calculation, or exporting. The columns in the Comparison section report the mass and RT differences between groups, the intensity ratio, up- or down-regulation, and differential score.

When you right-click the row number within the Feature Table you have additional options to review and identify the individual feature.

Feature Plot The data presented in Feature Plot is organized as a set of graphical data that help you view the distribution of the features in mass versus retention time (RT) axes (see [Figure 6](#)). The relative size of the marker representing a feature is an indication of the average abundance of the feature.

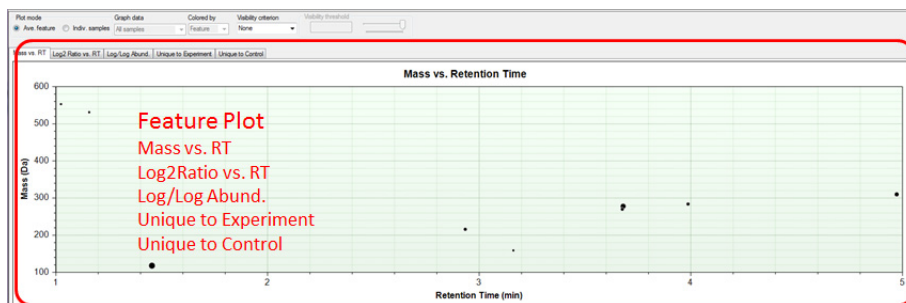


Figure 6 Views available within the Feature Plot

The two additional graphs designed to compare abundance ratios between groups are not informative with a single group project. However, when your project contains two groups the additional to graphs help you visualize the abundance ratios with respect to retention time and mass and help you visualize the features with respect to group membership.

When you click within the Feature Plot you can zoom in on the mass and retention time axes to see more of the feature detail.

When you right-click within a Feature Plot you have additional options to unzoom, review and identify the feature, and print, copy and save the graphical information. When you right-click along the axes or border of a Feature Plot you have options to unzoom, print, copy and save the graphical information.

When you move the pointer over a feature in the plot, a data window shows details about the feature, including mass, RT, and abundance, including absolute and relative standard deviation values across a group.

Feature Details The data presented in Feature Details is organized in four panes: (1) Feature Details by Sample - a table of the feature appearance in each sample data file, (2) Feature Details by Ion, (3) Extracted Ion Chromatogram graphics, and (4) Average Mass Spectrum graphics. The graphics can be arranged by group as shown in [Figure 7](#), or by sample. The chromatogram and spectrum graphics are available if the method was run on raw data files.

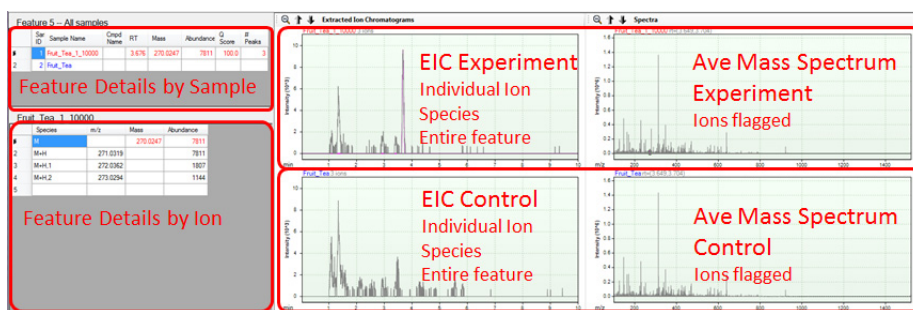


Figure 7 Data areas within the Feature Details

Information available in the Feature Details is limited for file types other than raw data files. For CEF files extracted ion chromatograms are not available. For CSV files Feature Details are not available.

When you double click on any row number the information displayed updates to reflect to the current feature. When you click within any chromatogram or spectrum you can zoom in on the axes of the entire data set to see more of the feature detail.

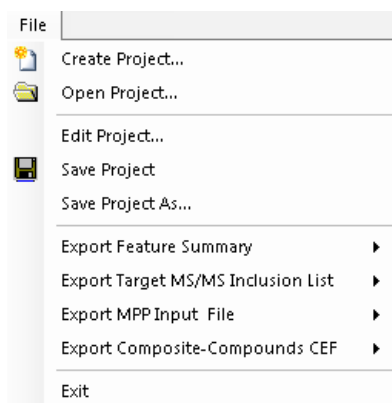
When you right-click within any chromatogram or spectrum you have additional options to print, copy and save the graphical information.

Menu bar

The following menu items are available in Mass Profiler:

- “File menu”
- “Method menu” on page 21
- “View menu” on page 23
- “Identify Features menu” on page 25
- “Web Search menu” on page 27
- “Help menu” on page 28

File menu



Create Project Opens the **Create Project** dialog box that you use to begin new project. See “[Create a project](#)” in the *Familiarization Guide*.



Open Project Loads a previously saved project into the main window. Because **Open Project** also loads the method used to create the results saved in the project, if you have unsaved changes in your current method a dialog box prompts you to save your current method before the **Open** dialog box is opened.

Edit Project Opens the **Edit Project** dialog box where you can edit the current project. See “[Edit a project](#)” in the *Familiarization Guide*.



Save Project Saves changes to the current project using the **Project name** previously entered in the **Create Project** dialog box. The default folder is **\MassHunter\MassProfiler\Projects**.

Save Project As Opens the **Save Project As** dialog box so you can save the current project using a different name. You can also specify a different folder to save the project.

Export Feature Summary > From Table... Opens the **Save As** dialog box to export the feature summary information from the Feature Table for use in a spreadsheet. The export file format is tab-separated value (TSV) and can be opened in a text editor, but it is saved as an XLS file so that Excel opens the file automatically when you double-click on the file in Windows Explorer. You can export from the Feature Table using one of three options:

- **From Table - All Features**
- **From Table - Marked Features**
- **From Table - Unmarked Features**

If you have marked features from a principal component analysis a *PCA Mark* column is added to the Feature Table and two additional options are available:

- **From Table - PCA Marked Features**
- **From Table - PCA Unmarked Features**

Export Feature Summary > From Graphed Features The features that meet the parameters you select and specify in the **Plot mode** are displayed in the Feature Plot. This menu item opens the **Save As** dialog box to export the features in the current Feature Plot for use in a spreadsheet. The export file format is TSV and can be opened in a text editor, but it is saved as an XLS file so that Excel opens the file automatically when you double-click on the file in Windows Explorer.

Export Target MS/MS Inclusion List > From Table... Opens the **Export Inclusion List Options** dialog box where you specify filter parameters for exporting the averaged ion data (m/z , z , RT, delta RT) for each feature in the Feature Table for use in data acquisition in targeted MS/MS experiments. The export file format is CSV. You can export from the Feature Table using one of three options:

- **From Table - All Features**
- **From Table - Marked Features**

- **From Table - Unmarked Features**

If you have marked features from a principal component analysis a *PCA Mark* column is added to the Feature Table and two additional options are available:

- **From Table - PCA Marked Features**
- **From Table - PCA Unmarked Features**

Export Target MS/MS Inclusion List > From Graphed Features

The features that meet the parameters you select and specify in the **Plot mode** are displayed in the Feature Plot. This menu item opens the **Export Inclusion List Options** dialog box where you specify filter parameters for exporting the averaged ion data (*m/z*, *z*, RT, delta RT) for each feature in the Feature Table for use in data acquisition in targeted MS/MS experiments. The export file format is CSV.

Export MPP Input File > From Table... Opens the **Save As** dialog box to export the feature summary information from the Feature Table for use with MPP. The export file format is a tab-delimited text file. You have a choice to export with a .txt extension for opening in a text editor, or an .xls extension for direct opening in Excel. You can export from the Feature Table using one of three options:

- **From Table - All Features**
- **From Table - Marked Features**
- **From Table - Unmarked Features**

If you have marked features from a principal component analysis a *PCA Mark* column is added to the Feature Table and two additional options are available:

- **From Table - PCA Marked Features**
- **From Table - PCA Unmarked Features**

Export MPP Input File > From Graphed Features The features that meet the parameters you select and specify in the **Plot mode** are displayed in the Feature Plot. This menu item opens the **Save As** dialog box to export the features in the current Feature Plot for use in MPP. The export file format is a tab-delimited text file. You have a choice to export with a .txt extension for opening in a text editor, or an .xls extension for direct opening in Excel.

Export Composite-Compounds CEF > From Table... Opens the **Save As** dialog box to export the feature summary information from the Feature Table for use with MPP for advanced statistical analysis. The export file format is a CEF file. The features within a group are summed to form a composite compound. You can export from the Feature Table using one of three options:

- **From Table - All Features**
- **From Table - Marked Features**
- **From Table - Unmarked Features**

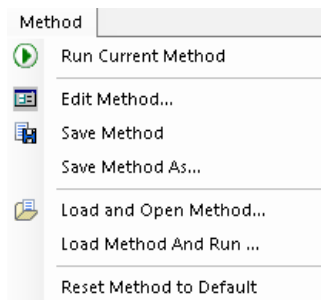
If you have marked features from a principal component analysis a *PCA Mark* column is added to the Feature Table and two additional options are available:

- **From Table - PCA Marked Features**
- **From Table - PCA Unmarked Features**

Export Composite-Compounds CEF > From Graphed Features The features that meet the parameters you select and specify in the **Plot mode** are displayed in the Feature Plot. This menu item opens the **Save As** dialog box to export the features in the current Feature Plot for use with MPP for advanced statistical analysis. The export file format is a CEF file. The features within a group are summed to form a composite compound.

Exit Closes the Mass Profiler window and exits the program.

Method menu



Parameters specified in a method define how to extract, align, normalize, filter, and compare the features found in your sample data files. When the method is run the results are displayed in the Feature Table and Feature Plot in the main window.



Run Current Method Applies the current method parameters to your project. Prior results are replaced and the new results are displayed in the main window.



Edit Method Opens the **Method Parameters** dialog box. When the **Commonly changed parameters** option is selected (default setting), located at the bottom of the dialog box, typical parameters are available to review and edit. When the **All parameters** option is selected all of the method parameters are edited within three tabs: Feature Finding/Loading, Filters, Alignment & Normalization, and Statistics & Filters. See “[Edit a method](#)” in the *Familiarization Guide*. When you change parameters in a method, the method name in the window title indicates an unsaved change using an asterisk (*).



Save Method Saves changes to the current method.

Save Method As Opens the **Save Method As** dialog box saves the current method parameters in the folder you select and using a name you type for the **File name** with the extension .mpm.

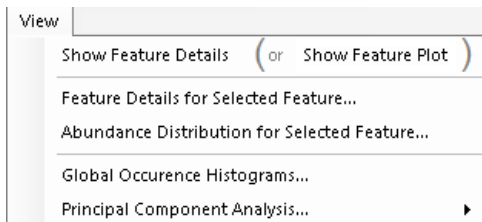


Load and Open Method Opens the **Open** dialog box. After you select and open a saved method the **Method Parameters** dialog box is automatically opened for you to review and edit the method parameters. See “[Apply a different method](#)” in the *Familiarization Guide*.

Load Method and Run Opens the **Open** dialog box. After you select and open a saved method the method is automatically applied to your current project. See “[Apply a different method](#)” in the *Familiarization Guide*.

Reset Method to Default Immediately restores all method parameters to default values. If your method has unsaved changes a **Mass Profiler** dialog box allows you to save your current method or cancel the reset action. The window title displays “Method: Default Method.”

View menu

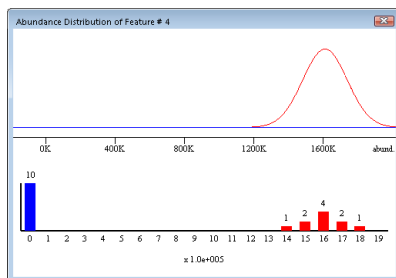


Show Feature Details If a Feature Plot is displayed in the main window, this menu item is available instead of **Show Feature Plot**. Replaces the Feature Plot view in the main window with details of the feature selected in the Feature Table as shown in [Figure 4](#) on page 14.

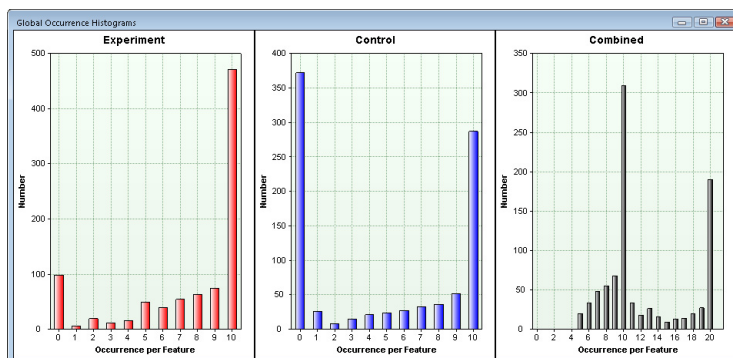
Show Feature Plot If Feature Details is displayed in the main window, this menu item is available instead of **Show Feature Details**. Replaces the Feature Details view in the main window with the Feature Plot as shown in [Figure 3](#) on page 13.

Feature Details for Selected Feature If a Feature Plot is displayed in the main window, a new window labeled **FeatureDetailFrame** opens and displays details of the feature selected in the Feature Table. If Feature Details is displayed in the main window, the Feature Details information is updated to show the details of the feature selected in the Feature Table. See “[View results in the Feature Details](#)” in the *Familiarization Guide*.

Abundance Distribution for Selected Feature Opens the **Abundance Distribution of Feature #** window and displays the abundance profile of the feature and a histogram of abundances for the feature within each group. For two groups the curves are color-coded for the group. The curves are calculated by using the least-squared fit to a Gaussian shape. See “[View the feature abundance distribution](#)” in the *Familiarization Guide*.



Global Occurrence Histograms Displays histograms that show how frequently a feature occurs in samples within each group and across the total set of data files. See “[View the global occurrence histograms](#)” in the *Familiarization Guide*.



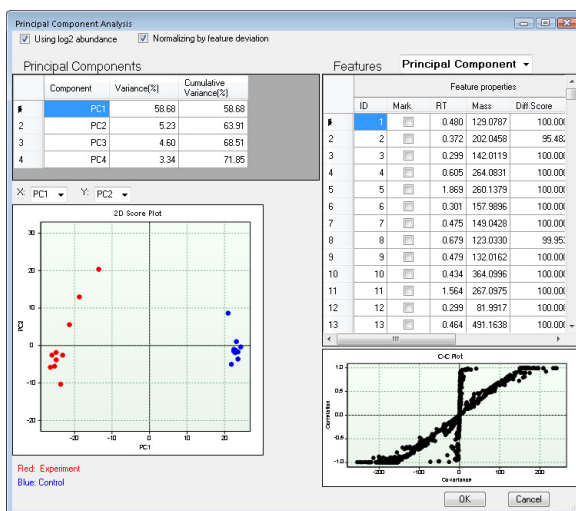
Principal Component Analysis > ... Displays the results of performing a mathematical process by which data containing a number of potentially correlated variables is transformed into a data set in relation to a smaller number of variables called principal components which account for the most variability in the data. The result of the data transformation leads to the identification of the best explanation of the variance in the data. For a project with two groups the objective to identify whether variations among the features separate the samples by group. For a project with a single group the analysis can identify samples that may be outliers.

You can export from the Feature Table using one of options:

- **For All Features in Table**
- **For Marked Features in Table**
- **For Unmarked Features in Table**
- **For Highlighted Features on Graph** - The features that meet the parameters you select and specify in the **Plot mode** as displayed in the Feature Plot.

If you have marked features from a principal component analysis a *PCA Mark* column is added to the Feature Table and two additional options are available:

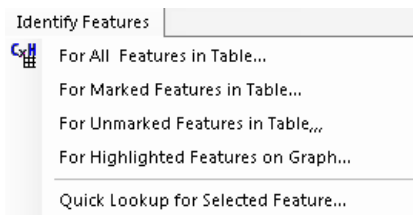
- **From Table - PCA Marked Features**
- **From Table - PCA Unmarked Features**



Identify Features menu

For each of the menu selections ID Browser is opened with the selected feature(s) for identification. See “[Identify Features](#)” in the *Familiarization Guide* for more information.

Note: Identify Features does not apply to data imported from a CSV file or from a CEF file with LMFE results.



For All Features in Table All of the features in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

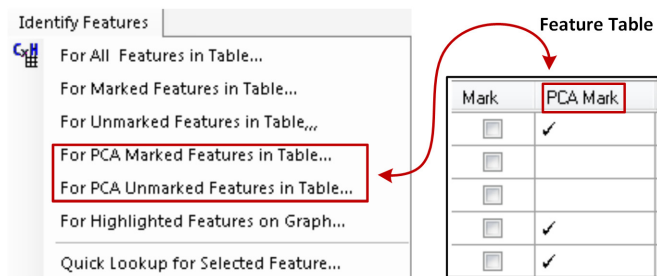
For Marked Features in Table The features marked in the *Mark* column in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

For Unmarked Features in Table The features that are not marked in the *Mark* column in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

For Highlighted Features on Graph The features that meet the parameters you select and specify in the **Plot mode** are displayed in the Feature Plot and are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

Quick Lookup for Selected Feature The feature currently selected in the Feature Table (the feature with a ► in the row header indicating the table row number) is passed to ID Browser for identification. The results viewed in ID Browser are **not** added to the Feature Table after you close the ID Browser window. See “[Lookup the identity of a single feature](#)” in the *Familiarization Guide*.

If you have marked features from a principal component analysis two additional options are available in the **Identify Features** menu. The *PCA Mark* column is only visible in the Feature Table if compounds have been marked in the PCA window.

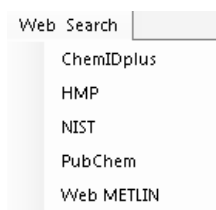


For PCA Marked Features in Table The features marked in the *PCA Mark* column in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

For PCA Unmarked Features in Table The features that are not marked in the *PCA Mark* column in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

Web Search menu

Performs a mass search via one of several popular online structure databases for the feature currently selected in the Feature Plot or Feature Table. The results are displayed in your Internet browser window.



ChemIDplus Mass search is directed to the NIH U.S. National Library of Medicine TOXNET toxicology data network at <http://chem.sis.nlm.nih.gov/chemidplus>.

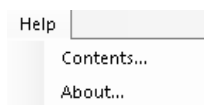
HMP Mass search is directed to the Human Metabolome Project (HMP) Human Metabolome Database at <http://www.hmdb.ca/structures/search/metabolites/mass>.

NIST Mass search is directed to the NIST Chemistry WebBook at <http://webbook.nist.gov/chemistry/mw-ser.html>.

PubChem Mass search is directed to the National Center for Biotechnology Information (NCBI) at <http://www.ncbi.nlm.nih.gov/pccompound?TabCmd=Limits>.

Web METLIN Mass search is directed to the Scripps Center for Metabolomics METLIN metabolite and tandem MS data base at http://metlin.scripps.edu/metabo_advanced.php.

Help menu



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

About Displays the version information for Mass Profiler.

Toolbar

The following toolbar options are available in Mass Profiler.

Button	Equivalent menu item
	File > Create Project
	File > Open Project
	File > Save Project
	Method > Load and Open Method
	Method > Edit Method
	Method > Save Method
	Identify Features > For All Features in Table
	Method > Run Current Method

Feature Table

The Feature Table (see [Figure 3](#) on page 13) shows the results of processing the sample files selected in your project with the current method. Features are automatically displayed when you run the current method and when you open a project that has been previously processed. The table can be sorted by the values in any column by clicking on the corresponding column heading.

899 features							
	Feature Summary						
	ID	RT	SD	Mass	SD	Abundance	RS
1	1	0.480	0.016	129.0787	0.0005	2409874	
2	2	0.372	0.003	202.0458	0.0002	2299440	
3	3	0.299	0.001	142.0119	0.0001	2070168	
4	4	0.605	0.002	264.0831	0.0009	1609233	
5	5	1.863	0.003	260.1379	0.0002	1202015	
6	6	0.301	0.002	157.9896	0.0001	1200701	
7	7	0.475	0.002	149.0428	0.0007	1144013	
8	8	0.670	0.010	123.0220	0.0006	1071040	

The following main headings and actions are presented:

- “Feature summary”
- “<Group 1> and <Group 2>” on page 32
- “Comparison” on page 32
- “Table shortcut actions” on page 33

Feature summary

Unless otherwise noted, the values reported for each column are the average of the feature across *all of the samples*.

(Row Number) The leftmost column, without a column heading, contains the row number for the features. The row number order does not change when the features are sorted by any of the data columns. A right-click in this column brings up the shortcut menu shown in [Figure 8](#) on page 40.

Formula If you have identified features with ID Browser this additional column appears (see “[Identify Features](#)” in the *Familiarization Guide*).

Name If you have identified features with ID Browser this additional column appears (see “[Identify Features](#)” in the *Familiarization Guide*).

ID An identification (ID) number is assigned to each feature based in descending order of the values in the **Abundance** column. The Feature Table is initially sorted by the ID value, and therefore by composite feature abundance, but the sort order can be changed by clicking on the heading of any other column.

RT Average retention time of the feature

RT SD Standard deviation of the retention time of the feature

Mass Average mass of the feature

Mass SD Standard deviation of the average mass of the feature

Abundance Average abundance of the feature in all of the samples for in a single group. For a project with two groups the abundance value reported in the Feature Summary is the average across all of the samples in the group with the greatest average abundance.

RSD Relative standard deviation of the abundance of the feature

Freq. The number of samples that contain this feature

Q-Score The Quality Score is an algorithmic estimate of how likely the feature is an actual compound. Q-Score filtering provides an advantage over abundance filtering when the features have small abundances.

Sat. Feature abundance saturation is indicated in this column. Saturation is an indication that the relative abundance of the feature with respect to other features or between groups may not be reliable and the mass of the feature could be slightly shifted.

Mark The check boxes in this column are used to **Mark** one or more features of special interest to your project. The mark 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 the check box again to clear the mark.

PCA Mark If you have marked features from a principal component analysis this additional column appears. You can only mark and clear features in this column from within the **Principal Component Analysis** dialog box, click one of the options under **View > Principal Component Analysis** (see “[Perform a PCA analysis](#)” in the *Familiarization Guide*).

<Group 1> and <Group 2>

The values reported for each column are averages for the feature across the samples *within the individual group only*.

RT Average retention time of the feature

Mass Average mass of the feature

Abundance Average abundance of the feature

RSD Relative standard deviation of the abundance of the feature

Freq. The number of samples that contain this feature

Comparison

The values reported for each column are calculated between the average values of RT, mass and, abundance in <Group 1> and in <Group 2>.

RT The difference in the retention time of the feature between the groups (<Group 1> - <Group 2>)

Mass The difference in the mass of the feature between the groups (<Group 1> - <Group 2>)

$\log_2(A1/A2)$ The log base 2 of the ratio of the average feature abundance where $A1 = \text{<Group 1>}$ and $A2 = \text{<Group 2>}$

Expression Indication of whether the average abundance of the feature in <Group 1> is greater (up) or lower (down) with respect to the average abundance of the feature in <Group 2>. When the equation $\log_2(A1/A2)$ is greater than 0 the expression is reported as up-regulated. When the equation $\log_2(A1/A2)$ is less than or equal to 0 the expression is reported down-regulated.

Diff. Score The differential score is a value between 0 and 100 that represents whether the data groups are significantly different. The score is calculated using the Student's t-test. A larger value indicates a higher confidence that the data sets in the two groups are different.

Table shortcut actions

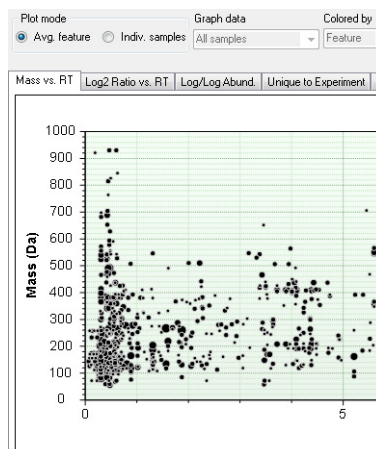
See “Feature Table shortcut menu” on page 40 for options when you right-click on a plot.

Feature Plot

The Feature Plot shows a graphical result of processing the project with the current method. By adjusting the parameters above the tabs corresponding to the available plots you can adjust the plot to visualize the feature differences by sample and group. The Feature Plot is displayed automatically when you open a project or run a method. The relative size of the plot markers representing a feature is an indication of the average abundance of the feature: the larger the marker, the more abundant the feature.

The following sections and actions are presented:

- “Plot mode”
- “Graph data” on page 34
- “Colored by” on page 34
- “Visibility criterion and Visibility threshold” on page 34
- “Feature Plot tabs” on page 35
- “Plot mouse actions” on page 35
- “Plot shortcut actions” on page 36



Plot mode

Select whether to display the filtered features for all samples in monochrome or for a specific selection of sample files in color.

Avg. feature The plot displays the average abundance of the features that meet the *Visibility criterion* for all of the samples using monochrome markers.

Indiv. samples The plot displays the average abundance of the features specified by the *Graph data*, highlighted by the *Colored by* and that meet the *Visibility criterion* using color. markers.

Graph data

When **Indiv. samples** is selected for the Plot mode you can select from any single sample or *All samples* to display in the plot.

Colored by

Feature Adjusts the plot so each feature is represented by a different color. When more features exist than can be plotted in different colors, features in the same area of the plot are plotted in different colors to help you distinguish the features.

Sample Adjusts the plot so features in the same sample are represented by the same color.

Group Adjusts the plot so features in the same group are represented by the same color: red represents <Group 1> and blue represents <Group 2>.

Visibility criterion and Visibility threshold

Allows you to filter the features based on criteria, whose threshold value determines which features appear as solid color markers in the plot. Features that exceed the threshold value are plotted as unfilled markers.

None All of the features are plotted using solid markers.

Max RSD abundance Features whose abundance relative standard deviation, within a group, is less than or equal to the entered threshold are plotted using solid markers.

SD RT Features whose retention time standard deviation, within a group, is less than or equal to the entered threshold are plotted using solid markers.

SD Mass Features whose mass standard deviation, within a group, is less than or equal to the entered threshold are plotted using solid markers.

Differential Score Features whose differential score is less than or equal to the entered threshold are plotted using solid markers.

Feature Plot tabs

You can click any plot tab above Feature Plot to view a different plot type.

Mass vs. RT Plot of Mass versus retention time for each feature.

Log2 Ratio vs. RT Plot of the log base 2 of the ratio of the average feature abundance versus retention time.

- When **Avg. feature** is selected for the Plot mode, the abundance ratio is the average abundance for <Group 1> over the average abundance for <Group 2>.
- When **Indiv. samples** is selected for the Plot mode, the feature abundance ratio is the abundance for the feature in a single sample over the average abundance of that feature in a group.

Log/Log Abund. Plot of the $\log_2$ of the average abundance of a feature:

- When **Avg. feature** is selected for the Plot mode, the plot axes are $\log_2$ <Group 1> versus $\log_2$ <Group 2>.
- When **Indiv. samples** is selected for the Plot mode, the plot axes are $\log_2$ of the feature in a single sample versus $\log_2$ of the average of that feature in a group.

Unique to <Group 1> Plot of mass versus retention time for each feature associated with <Group 1>. This plot type is not available for a project with a single group.

Unique to <Group 2> Plot of mass versus retention time for each feature associated with <Group 2>. This plot type is not available for a project with a single group.

Plot mouse actions

Zoom: Click and drag the pointer within the graph to define an area to enlarge. The area expands when you release the mouse button.

Marker information: Pass the pointer over a feature to view information about that feature.

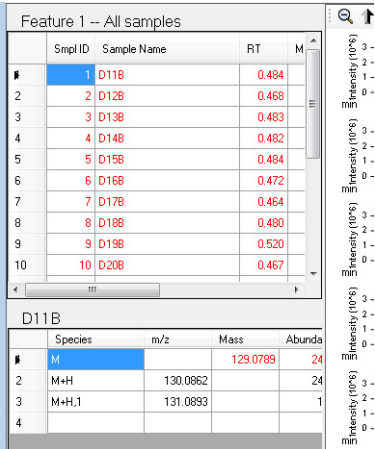
Plot shortcut actions

See “Feature Plot shortcut menu” on page 41 for options when you right-click on a plot.

Feature Details

The Feature Details (see [Figure 4](#) on page 14) shows the details of the feature selected when you double click on the row number of a feature in Feature Table. The data presented in Feature Details is organized in four panes: a table of the feature appearance in each sample data file, a table of the feature ions in the selected sample data file, and, if available for the imported data type, extracted ion chromatograms and spectra of the feature for each sample data file.

Information available in the Feature Details is limited for file types other than raw data files. For CEF files extracted ion chromatograms are not available. For CSV files only the Feature by all samples pane is available.



The following sections and actions are presented:

- “Feature details by sample pane”
- “Feature details by ion pane” on page 37
- “Extracted ion chromatograms pane” on page 38
- “Spectra pane” on page 39
- “Feature Details shortcut actions” on page 39

Feature details by sample pane

The information in this table is for the feature selected in the “Feature Table” on page 30.

Samples that are part of the Experiment group are shown in a red color. Samples that are part of the Control group are shown in a blue color.

(Row Number) The leftmost column, without a column heading, contains the row number for the features. The row number order does not change when the features are sorted by any of the data columns.

Smpl ID An identification (ID) number is assigned to each sample in the project ascending alphabetical order of the file name. The Feature Table is initially sorted by the Smpl ID value, but the sort order can be changed by clicking on the heading of any other column.

Sample Name The file name of the sample data file

RT Retention time of the feature in the sample file

Mass Mass of the feature in the sample file

Abundance Abundance of the feature in the sample file

Q-Score Quality score of the feature in the sample file. The quality score is an algorithmic estimate of how likely the feature is an actual compound. Q-Score filtering provides an advantage over abundance filtering when the features have small abundances.

Peaks The number of ions associated with the feature in each sample. The number of ions is also annotated in the chromatogram titles after selecting Feature Details for a new feature in the Feature Table.

Feature details by ion pane

The information in this table is for the feature selected in the “[Feature Table](#)” on page 30, and for the sample selected in the **Feature Details by Sample** table above. This information is not available for the CSV file type.

The top row functions as a *header row* and reports the calculated neutral mass of the feature, and either reports the abundance of the most intense ion, if the abundances are reported as *Height*, or reports the sum of all ion abundances, if the abundances are reported as *Volume*. The header row is shown in a red color if the values for the ions are from a sample in the Experiment group. The header row is shown in a blue color if the values for the ions are from a sample in the Control group.

Species The different molecular ion species associated with the feature in the selected sample file.

m/z The mass to charge ratio for each ion species.

Mass The neutral mass calculated for the feature based on the associated ion species.

Abundance The MFE abundance for the respective ion species reported as either *Height* or *Volume*, depending on the **Report abundance as** setting in the method.

Extracted ion chromatograms pane

This information is not available for CEF and CSV file types.

Double-clicking any row number in the **Feature Details by Sample** table, except the red or blue header row, updates the **Feature Details by Ion** table. By default all chromatograms are extracted using all ions associated with the selected feature. The number of ions is annotated in the chromatogram titles.

Double-clicking any row number in the **Feature Details by Ion** table updates the EICs to extract only the selected ion species and annotate its m/z value in the chromatogram titles.

The height of the chromatographic peak for all ions of a feature is similar, but not identical, to the sum of the *Height* abundances of the respective ions. The y-axis does not change when selecting **Report abundance as** *Volume*.

The gray line represents the EIC of the selected ions across the entire retention time range, the magenta colored line represents the elution profile of the feature as determined by MFE.

When you click within any chromatogram you can zoom in on the axes of the entire data set to see more of the feature detail. You can click on the zoom out and y-axis scale buttons to further adjust the zoom factor on all of the chromatograms.

Spectra pane

This information is not available for CEF and CSV file types.

The spectra pane shows the averaged raw centroid mass spectra across the common elution time range for the selected feature in all samples. The ions associated for the selected feature in each sample is marked by a red dot. The y-position of the red dot corresponds to the abundance of that ion in the species table if **Report abundance as** is set to *Volume*. The absolute height of the signals in the spectrum is arbitrarily scaled so they are slightly higher than the indicating dot. However, the relative difference of signals within a spectrum, or between spectra of the different samples, is maintained.

When you click within any spectrum you can zoom in on the axes of the entire data set to see more of the feature detail. You can click on the zoom out and y-axis scale buttons to further adjust the zoom factor on all of the spectra.

Feature Details shortcut actions

See “[Feature Details shortcut menu](#)” on page 42 for options when you right-click within a chromatogram or spectrum.

Shortcut menu commands

Shortcut menus provide options ([Figure 8](#) on page 40) applicable to the context where you click and to your current view. The following shortcut menus are presented:

- “[Feature Table shortcut menu](#)” on page 40
- “[Feature Plot shortcut menu](#)” on page 41
- “[Feature Details shortcut menu](#)” on page 42

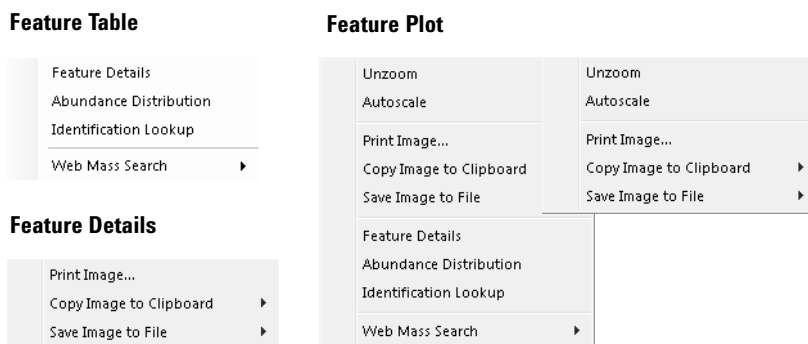


Figure 8 Shortcut menu commands available within the main window

Feature Table shortcut menu

Available when you right-click along the left-most row column in the Feature Table. The shortcut menu is shown in [Figure 8](#) on page 40.

Feature Details Opens the **FeatureDetailFrame** window in which you can review the feature details by sample and by ion, the extracted ion chromatogram, and the mass spectrum for the feature as it appears in each sample file if the Feature Plot is currently shown in the lower part of the main window. If the Feature Details are shown in the lower part of the window, the information is updated with the data from the selected feature.

Abundance Distribution Opens the **Abundance Distribution of Feature #** window and displays the abundance profile of the feature and a histogram of abundances for the feature within each group.

Identification Lookup Opens ID Browser with the selected feature for identification.

Web Mass Search Opens your default Internet browser and performs a web search for the selected feature to propose an identification based on the feature mass. The available web search options are ChemIDplus, HMP (Human Metabolite Project), NIST, PubChem, and Web METLIN.

Feature Plot shortcut menu

The menu commands available when you right-click in the Feature Plot change with whether your pointer is inside the graph region or along the plot border containing the axes and plot title. The shortcut menus are shown in Figure 8 on page 40.

Unzoom Restores the previous zoom level of the Feature Plot.

Autoscale Resets the axes of the Feature Plot to show all of the features.

Print Image Prints the current Feature Plot image based on your current printer settings.

Copy Image to Clipboard Copies the current Feature Plot image to the Clipboard to paste into other applications as either an **Enhanced Meta file** (a group of objects that form the image) or a **Bitmap** format (a single image object).

Save Image to File Saves the current Feature Plot image as either an **Enhanced Meta file** (.emf) or **Bitmap** (.bmp) format.

Feature Details Opens the **FeatureDetailFrame** window in which you can review the feature details by sample and by ion, the extracted ion chromatogram, and the mass spectrum for the feature as it appears in each sample file.

Abundance Distribution Opens the **Abundance Distribution of Feature #** window and displays the abundance profile of the feature and a histogram of abundances for the feature within each group.

Identification Lookup Opens ID Browser with the selected feature for identification.

Web Mass Search Opens your default Internet browser and performs a web search for the selected feature to propose an identification based on the feature mass. The available web search options are ChemIDplus, HMP (Human Metabolite Project), NIST, PubChem, and Web METLIN.

Feature Details shortcut menu

If available for the imported file type in your project, menu commands are only available when you right-click in the extracted ion chromatogram or spectrum plots. The shortcut menus are shown in Figure 8 on page 40.

Print Image Prints the current Feature Plot image based on your current printer settings.

Copy Image to Clipboard Copies the current Feature Plot image to the Clipboard to paste into other applications as either an **Enhanced Meta file** (a group of objects that form the image) or a **Bitmap** format (a single image object).

Save Image to File Saves the current Feature Plot image as either an **Enhanced Meta file** (.emf) or **Bitmap** (.bmp) format.

Unsaved parameter changes

When you make a change to your method in Mass Profiler, the software automatically places an asterisk “*” after the method name in the Mass Profiler title bar as shown in [Figure 9](#) on page 43. The asterisk indication helps you remember that the changes you have made to the method have not been saved to a method file on disk.

Methods are saved as follows:

- 1 You can save the method parameters as an .mpm file. Saving a method file is optional.
- 2 Method parameters used to process or reprocess a project are automatically saved when you save the project. The method parameters are embedded with the project. When a project is reloaded the parameters used to obtain the saved results are loaded into memory and the method name is the name of the project, without an asterisk.
- 3 When you exit Mass Profiler the current method parameters are automatically saved internally and reloaded the next time Mass Profiler is launched, regardless of whether the method parameters are changed or unchanged.

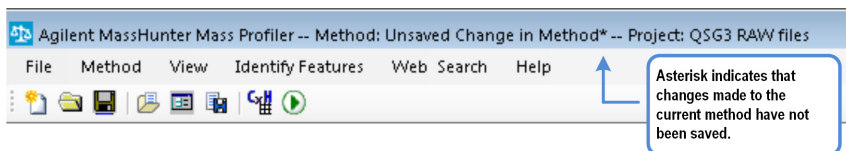


Figure 9 Indication of unsaved changes in your method is by the appearance of an asterisk in the title bar.

Mass Profiler Installation

- 1 Install Mass Profiler on the highest performing computer you have available to reduce the time it takes to extract and view the features in your sample files. Mass Profiler requires a computer running Windows 7 (64-bit) with at least 8GB of RAM.

Note: For better processing performance choose a higher frequency CPU over a larger number of cores. A solid-state hard drive can improve performance specifically when extracting features on multiple data files. Depending on the size of a project, and the number of features extracted per data file, additional RAM reduces processing time by minimizing the use of the page file used during the process.

- 2 If an earlier version of Mass Profiler is already installed on your computer, remove the earlier version of the software before installing Mass Profiler B.07.00.
- 3 Refer to the README file that can be found on the installation disc for additional information.

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In this guide

*The Agilent G3297AA
MassHunter Mass Profiler
Software - Quick Start Guide*
presents an overview of the
MassHunter Mass Profiler
Software.

This guide is valid for the
B.07.00 revision or higher of
MassHunter Mass Profiler
Software, until superseded.

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Revision A, November 2014



G3297-90008



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