MassHunter Mass Profiler Software

Quick Start Guide

What is Mass Profiler?

Agilent MassHunter Mass Profiler software is a stand-alone program specifically designed to investigate features in your LC/MS and GC/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

Mass Profiler helps you:

- Find and extract the molecular features in your sample data.
- Align and normalize features from different samples.
- Review the features in your samples individually and by grouping.
- Investigate statistically meaningful 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.
- Perform data analysis using various workflows including metabolomics, integrated biology, pharmaceutical impurity analysis, forensic analysis, environmental analysis, and pesticide analysis.

Mass Profiler operates on MassHunter LC/MS and GC/MS data files, including raw data acquired from LC/IM-MS instruments, EI-GC/MS data files acquired in both accurate and unit mass modes, and compound exchange files produced by MassHunter Qualitative Analysis and Profinder. Retention time, mass, and abundance LC/MS data can also be imported from any source using comma separated value files.

Feature extraction, combined with alignment across multiple data files using m/z, retention time, and drift time (LC/IM-MS data), is a critical step in differential analysis workflows exhibiting minimal false positive or negative feature differences between samples or sample groups.

Features can be exported by Mass Profiler for statistical data analysis by 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. The profile data may also be exported for data analysis by other database search programs, identification environments, or statistical analysis packages.

What's new in B.08.00?

- An option is available in displaying results to display only the features that have been identified or to display all of the features.

- Features can be exported to a CEF file.

- Select whether or not to report any single-ion feature with a charge state of one (1). This is especially useful for small molecule features.

- Specify a minimum number of charges for identified features. This is especially useful for peptide features.

- Support is added for EI-mode GC/MS data files: TOF (.d), quadrupole profile and unit mass (.d), and AMDIS (.cef).

- Quality scores for the LC/IM-MS feature extraction algorithm (IMFE - retention time, drift time, m/z, and abundance) are now calibrated with those generated by LC/MS molecular feature extraction algorithm (MFE - retention time, m/z, and abundance).

- Improved performance of the IMFE groups all isotopes from a single species, typically $(M+H)^+$, into one feature, but does not group differently adducted species into one compound, such as $(M+H)^+$, $(M+NH_4)^+$, $(M+2H)^{2+}$ and $(2M+H)^+$, since they are typically separated by the ion mobility stage.

- Drift times and CCS values, when available, are exported with features found using IMFE.

- Export a CEF file for each individual sample when you are processing ion mobility data (average mass, RT, DT, and CCS).

- Export feature summary includes data columns that contain the abundance of the feature in each of the samples.

- Select a feature in the Feature Table by directly selecting the feature appearance in the Feature Plot.

- Mass Profiler installs and uses ID Browser B.08.00.

Functionality of Mass Profiler

- Locate and extract the molecular features in your data files, not just chromatographic peaks.

  Note: When you process IM-MS data, features based on isotopic ion clusters are located and extracted instead of molecular features. IM-MS data is processed using isotopic clusters because differently adducted molecular ions may have different ion mobility drift times.

- Visualize statistically meaningful differences between two sample groups, such as healthy versus disease samples.

- Calculate abundance ratios and clearly identify differentially expressed features.

- Quantify a compound by maximum ion intensity (height), maximum ion volume, or feature volume.

- Analyze and compare your data using flexible feature selection.

- 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.

- Perform data analysis using various workflows such as 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, alignment, and differential analysis, and save them to disk as a Mass Profiler Method file (.mpm).

- Review your features and alignment using an intuitive graphical interface.

- Filter and curate your differential analysis 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. As part of an untargeted differential analysis experiment, Mass Profiler may be employed in steps 3, 4, and 5:

1 Prepare for your experiment.

2 Acquire your data using Data Acquisition.

3 Extract spectral features in your data files using Profinder or Mass Profiler.

4 Compare two samples or sample groups using Mass Profiler.

5 Identify features in your data using ID Browser with Mass Profiler.

6 Export features for advanced statistical analysis using MPP.

7 Save your project using Mass Profiler and MPP.
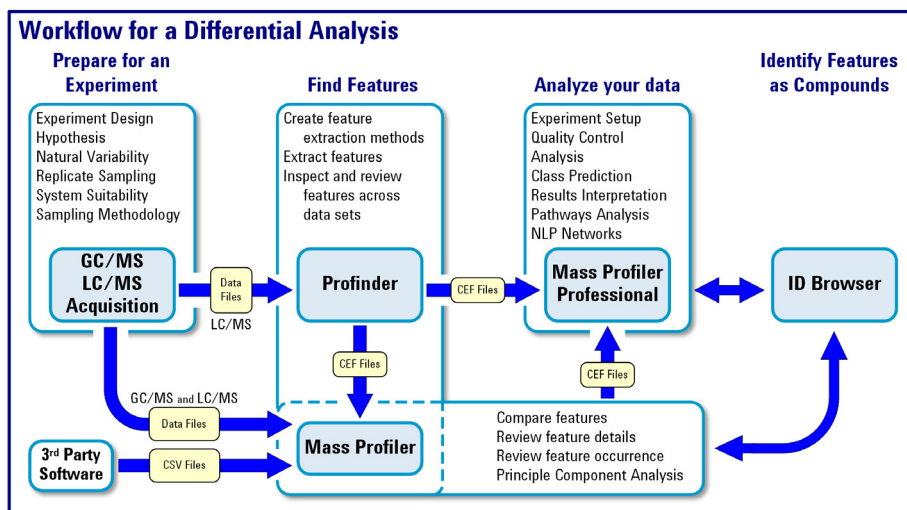
Figure 1   Workflow for a differential analysis

Figure 1 shows the steps and MassHunter tools that are used in an untargeted and targeted differential analysis. The feature finding steps performed by Mass Profiler can alternatively be accomplished in 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 perform a principal component analysis with your samples organized into two groups. Compared to Mass Profiler Professional, Mass Profiler is unique in that you can view compound chromatograms or drift spectra and mass spectra during your analysis.

Feature extraction performed by the feature extraction and finding algorithm in Mass Profiler is similar to the feature extraction and finding algorithm in MassHunter Qualitative Analysis and Profinder; however, Mass Profiler and Profinder add the functionality of recursive grouping across multiple data files which improves isotopic grouping and minimizes false positive and false negative differences.

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 online Help including basic tasks, user interface, and reference information.

Resource App

Install the Mass Profiler Resource App from the Mass Profiler installation guide. This Resource App gives you access to the Mass Profiler Quick Start Guide (this guide) and the Mass Profiler Familiarization Guide.

Install the **MPP Resource App** from the **Mass Profiler Professional Supplemental Disc**. The MPP Resource App gives you access to the Mass Profiler Professional documents and Workflow Guides described in this guide, as well as interactive training guides for Mass Profiler Professional

Documents

Mass Profiler Familiarization Guide   Use this guide to learn how to use Mass Profiler. This guide can be found in the Mass Profiler Resource App and also online at http://www.agilent.com/cs/library/usermanuals/public/G3297-90018_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 / Overview
- Integrated Biology with Agilent Mass Profiler Professional - Workflow Guide / Overview
- Class Prediction with Agilent Mass Profiler Professional - Workflow Guide / Overview
- Agilent Lipidomics Workflow Guide / Overview

Training

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

Training Courses   Visit www.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 50.

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 "Workflow Guides and Overviews" guides (see "Documents" on page 7). 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 samples among the groups in your experiment

6 Review the exercises in the *Familiarization Guide* to learn how to extract and analyze the features in your samples. An example "Basic Mass Profiler Workflow" is illustrated 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. For LC/IM-MS data, ions representing different ion species and charge state are not combined because these ions are typically separated by drift time in the ion mobility stage.

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. Because the these default names can be changed this guide may refer to the Experiment group as <Group 1> and the Control group as <Group 2>.

IMFE   Refers to the algorithm that operates on LC/IM-MS data to group all of the isotopes from the same adducted neutral molecule into a single, reported feature. IMFE is short for IM-MS feature extractor or IM-MS feature extraction.

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.

MFE   Refers to the algorithm that operates on GC/MS and LC/MS data to group all of the isotopes from all of the ion species derived from the same neutral molecule into a single, reported composite compound. MFE is short for molecular feature extractor or molecular feature extraction.

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.

See "Open Project" on page 20 for important information regarding opening project files on different computers.

Sample GC/MS and 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 sequence of steps executed for an analytical task, the type and sequence of which can be documented via a graphical overview. A workflow may cover more than one wizard and may include steps performed by more than one MassHunter software program.

Feature extraction using MFE and IMFE

MFE is a composite compound feature finder applied to non-IM LC/MS data. MFE feature finding and extraction parameters are retention time, m/z, and abundance. MFE creates a single composite compound from all of the isotopes of all of the differently adducted molecular ion species, such as $(M+H)^+$, $(M+NH_4)^+$, $(M+2H)^{2+}$ and $(2M+H)^+$. Differently adducted molecular ions are combined into one feature.

IMFE is an ion cluster feature finder applied to find the features in LC/IM-MS data. IMFE feature finding and extraction parameters are retention time, ion mobility drift time, m/z, and abundance. IMFE finds features based on ion clusters and groups only the isotopes from a single adducted species. IM-MS data is processed using isotopic clusters because differently adducted molecular ions may have different ion mobility drift times.

The feature finding and extraction algorithm treats all of the mass spectral data from the experiment as a large array of values and removes from that array any values that correspond to persistent or slowly-changing background. Then the algorithm searches for ions that have a common elution and drift profile (for example, for m/z values whose abundances reach a local maximum at very nearly the same retention and drift times). Ions are then grouped into one or more features containing related mass or m/z values to form a composite compound or an ion cluster.

Unlike MFE which creates a single composite compound from all of the isotopes of all of the differently adducted molecular ion species, IMFE finds features based on ion clusters and groups only the isotopes from a single adducted species into one feature. Since differently adducted ions of the molecular ion may have different drift times, IMFE does not group different ions from differently adducted species into a single composite compound, such as $(M+H)^+$, $(M+NH_4)^+$, $(M+2H)^{2+}$ and $(2M+H)^+$. The selection of the charge state carriers has been disabled when using IMFE.

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 14 and Figure 4 on page 15).

Use the Feature Table to:

- View all of the feature information for two samples or larger sample sets 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 33 for more information.

Use the Feature Plot to:

- Visualize and investigate features using different plot types, for example: Abundance comparison, Mass difference, RT difference, Abundance vs. mass, Abundance vs. RT, Mass vs. RT.

- Investigate all features, features unique to the Experiment group (<Group 1>), or features unique to the Control group (<Group 2>).

- Investigate features in an individual sample, all samples, or compared to each group.

- Investigate features that are above and below a specified highlighting threshold filter setting.

- Investigate features by abundance, mass, drift time, and retention time across all samples using color to distinguish individual features or to identify features by sample or group.

See "Feature Plot" on page 37 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, drift time, mass, and m/z using extracted ion chromatograms and mass spectra for all of the samples.

See "Feature Details" on page 41 for more information.

Identification

Mass Profiler (Figure 2) works with MassHunter ID Browser to help you identify features via molecular formula generation or an accurate mass database search using a personal compound database (.cdb or .csv formats). 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.
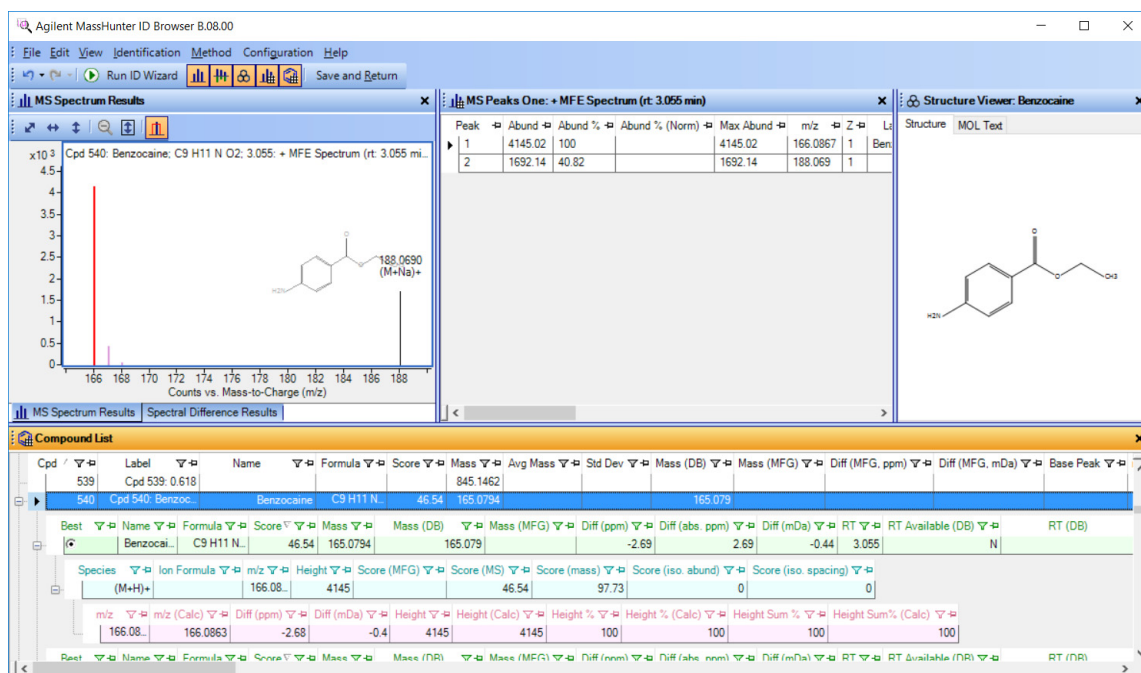

Figure 2 ID Browser after identifying features as compounds

Exporting data

Features found and aligned across samples 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) Menu Bar, (2) Toolbar, and (3) Main Window. The main functional areas are shown in Figure 3.
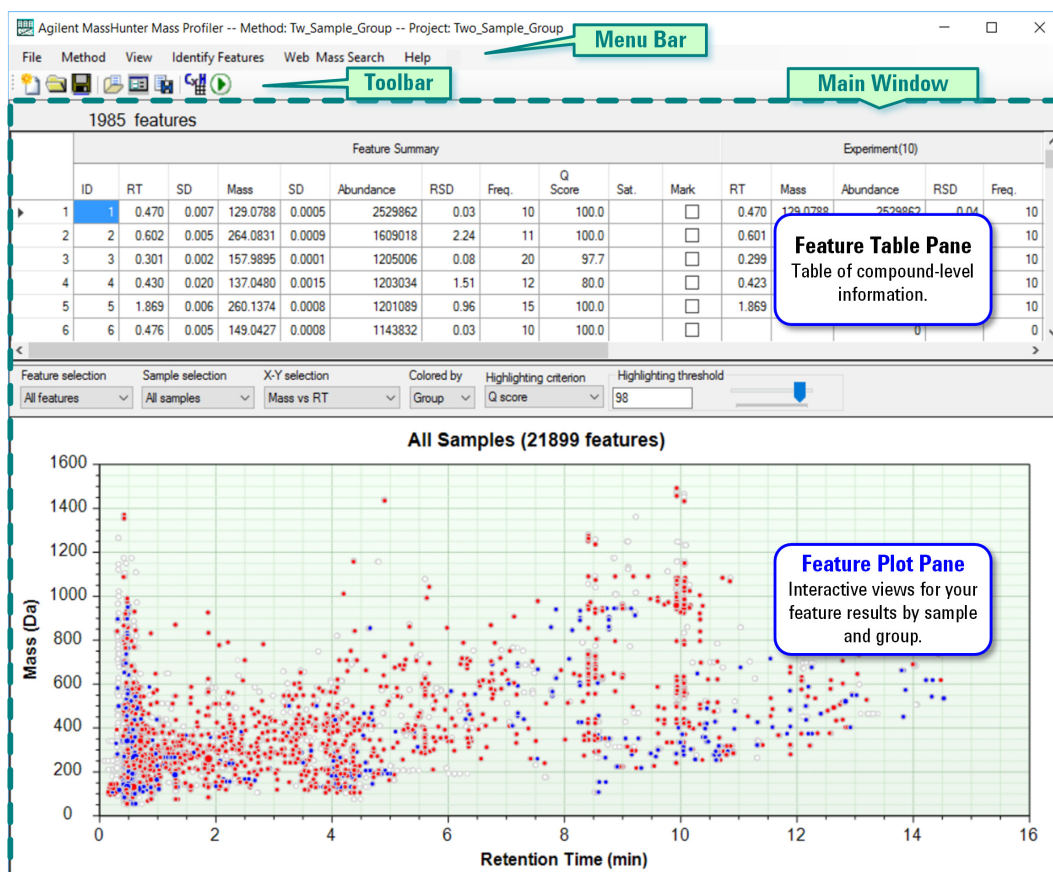

Figure 3   The main functional areas of Mass Profiler after a method is run on a project. If selected in the View menu, the Feature Plot can be replaced with a Features Details view shown in Figure 4 on page 15.
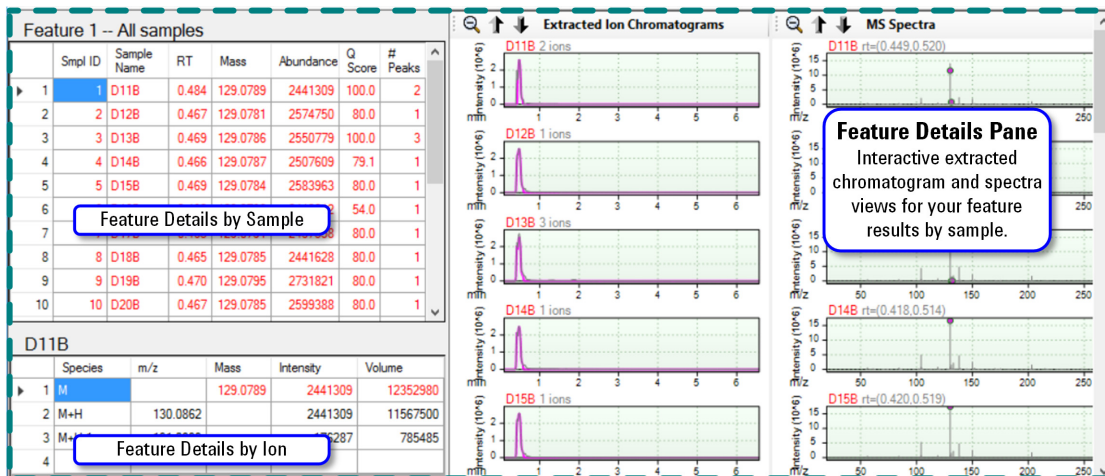
Figure 4 When you click **View > Switch to Feature-Details Mode**, the Feature Details pane (above) replaces the Feature Plot pane in the main window shown in Figure 3 on page 14.

Menu Bar

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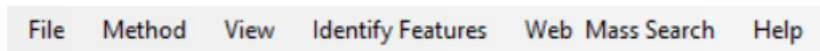
Toolbar

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

Main Window

The main window, see Figure 3 on page 14, is further divided into two areas, the Feature Table and the 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 into sections: (1) Feature Summary, (2) Experiment Group Information, (3) Control Group Information, and (4) Group Comparison Information as shown in Figure 5. Each feature is presented in a row and the feature information is presented in columns. The number of samples in each group is shown in the table heading and is the maximum frequency possible for a feature in the respective group.

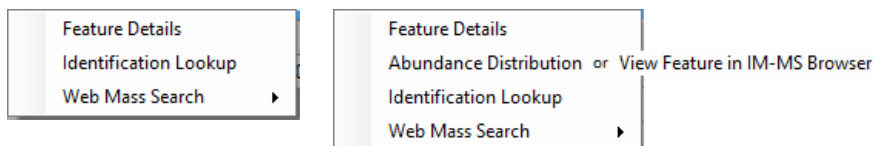**Figure 5**   Data areas within the Feature Table Pane

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, and exporting. The columns in the Group Comparison section report the mass, RT, and DT differences between the experiment 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 commands to review and identify the individual feature.

> **Abundance Distribution** is available when you have a 2-group project and at least one group has two or more samples.

View Feature in IM-MS Browser is available when you have ion mobility data and when the total number of samples (regardless their grouping) is one or two. When you have more than two ion mobility samples in your project, you can launch IM-MS Browser from *Feature Details pane* for each individual sample.

| Feature Details |
| Identification Lookup |
| Web Mass Search ▶ |

| Feature Details |
| Abundance Distribution or View Feature in IM-MS Browser |
| Identification Lookup |
| Web Mass Search ▶ |

Feature Plot   One of the plots presented in the Feature Plot is organized as a set of graphical information data that helps you view the distribution of the features in mass versus retention time (RT) as shown in Figure 6. The relative size of the marker representing a feature is an indication of the average abundance of the feature.

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 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.
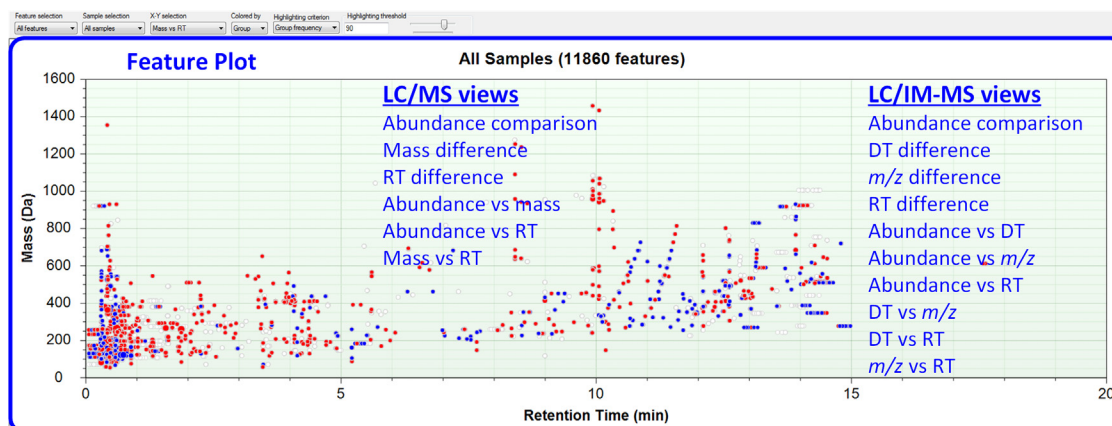

Figure 6   Views available within the Feature Plot pane for LC/MS and LC/IM-MS data
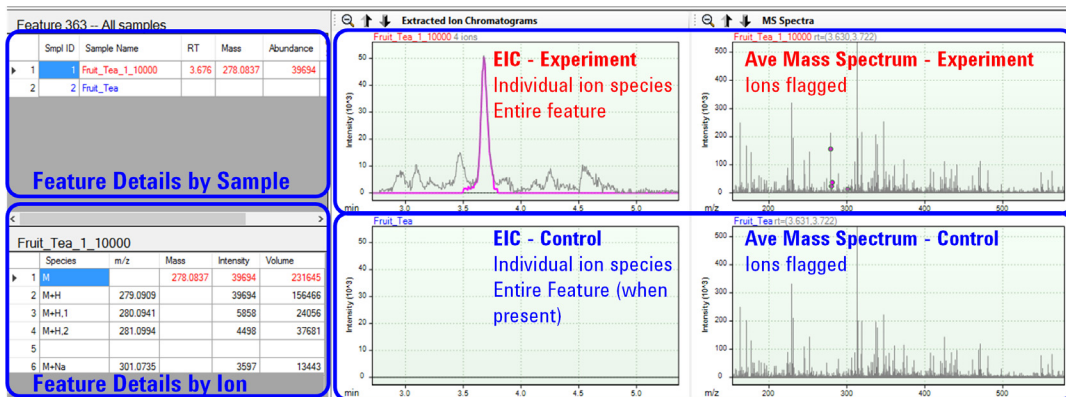
When you click within the Feature Plot, you can zoom in on the axes to see more of the feature detail.

When you right-click within a Feature Plot, you have additional commands 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 commands to unzoom, print, copy and save the graphical information.
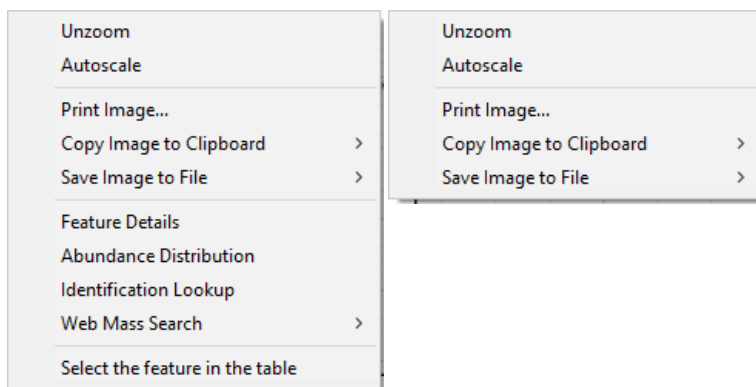
When you move the pointer over a feature in the plot, a data window shows details about the feature, including mass, RT, DT (for LC/IM-MS), and abundance, including absolute and RSD values across a group.

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 (Figure 7 on page 19) or Drift Spectra (Figure 8 on page 19) graphics, and (4) Average Mass Spectrum graphics. EIC's can be summed for all ions or created from individual ions. The chromatogram and spectrum graphics are available if the method was run on raw data files.

Figure 7 Data areas within the Feature Details pane. The EIC is replaced with the Drift Spectra for IM data.
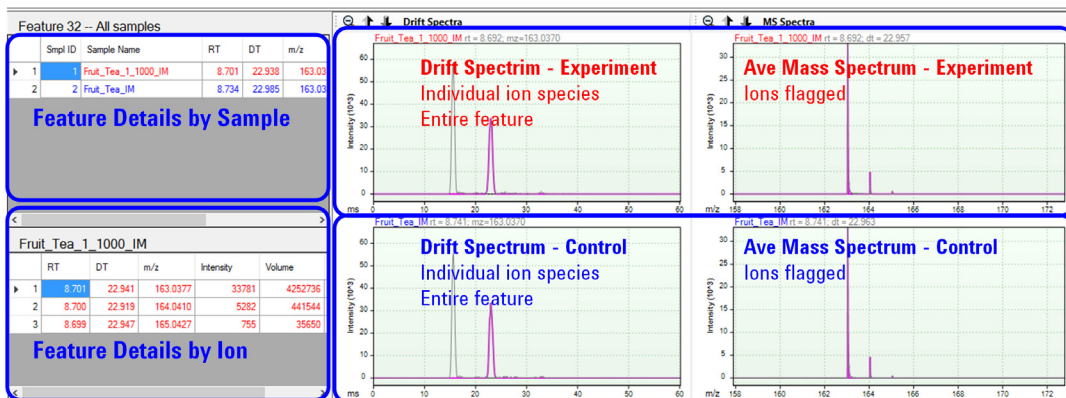
Figure 8 Data areas within the Feature Details pane for LC/IM-MS data.

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 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 commands to print, copy and save the graphical information.

Menu bar

The following menus are available in Mass Profiler:

- "File menu"
- "Method menu" on page 24
- "View menu" on page 25
- "Identify Features menu" on page 28
- "Web Mass Search menu" on page 30
- "Help menu" on page 31

File menu
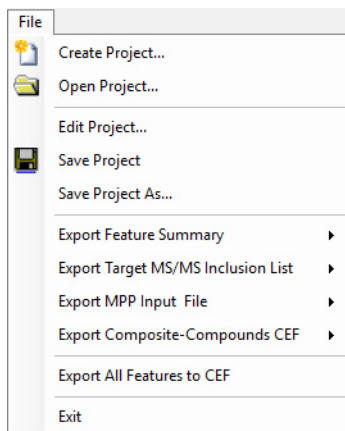
Create Project   Opens the **Create Project** dialog box that you use to begin a new project. See "Create a project" in the *Familiarization Guide*.

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.

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 review the results. However, to access the Feature Details ("Feature Details" on page 18), 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, or .c4d file (for LC/IM-MS data), that is stored in the data file and an error message is displayed. The path where the data files are expected is displayed in the error message.

Reprocessing a project with different method parameters also requires that the data files are available in the path that is saved with the project.

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 the file in Windows Explorer. You can export from the Feature Table using one of three commands:

- **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 commands 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 command 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 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 commands:

- **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 commands 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 command 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 commands:

- **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 commands 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 command 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 commands:

- **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 commands 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 command 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.

Export All Features to CEF   All of the features associated with each sample file in the project are exported to a CEF file of the same name as the sample file. The CEF files are saved in the **Input Data** folder.

Exit   Closes the Mass Profiler window and exits the program.

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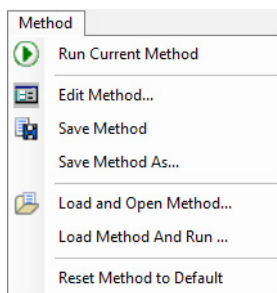
Parameters specified in a method define how to extract, align, normalize, filter, and compare the features found in your sample data files. When the method is run, the results are displayed in the Feature Table and Feature Plot in the main window.

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 clicked (default setting) at the bottom of the dialog box, typical parameters are available to review and edit. When the **All parameters** option is clicked, 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." The following parameters have different default values for LC/IM-MS versus LC/MS data files:

- **Measure of abundance** is *Max Ion Volume* versus *Max Ion Height*
- **Ion Intensity >=** is *100* versus *600*

View menu
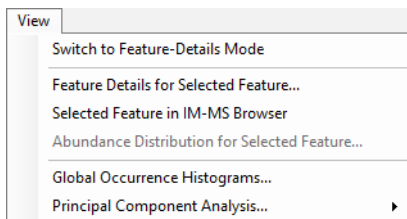
Switch to Feature-Details Mode   If a Feature Plot is displayed in the main window, this command is available instead of **Switch to Feature-Plot Mode**. Replaces the Feature Plot pane in the main window with details of the feature selected in the Feature Table as shown in Figure 4 on page 15.

Switch to Feature-Plot Mode   If Feature Details is displayed in the main window, this command is available instead of **Switch to Feature-Details Mode**. Replaces the Feature Details pane in the main window with the Feature Plot pane as shown in Figure 3 on page 14.

Feature Details for Selected Feature   If a Feature Plot is displayed in the main window, a new window labeled **Feature-Detail View** 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*.

Selected Feature in IM-MS Browser   Available only if the project contains no more than two sample files. Opens one or two IM-MS Browser windows and zooms into the RT, DT, *m/z* space around the feature. With IM-MS Browser you can perform interactive browsing and visualization of the data from a single LC/IM-MS data file. See "View the feature in IM-MS Browser (LC/IM-MS data)" in the *Familiarization Guide*.

Abundance Distribution for Selected Feature   If the project contains two groups and three or more data files, 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. The curves are color-coded for each group and are calculated by using the least-squared fit to a Gaussian shape. See "View the feature abundance distribution" in the *Familiarization Guide*.
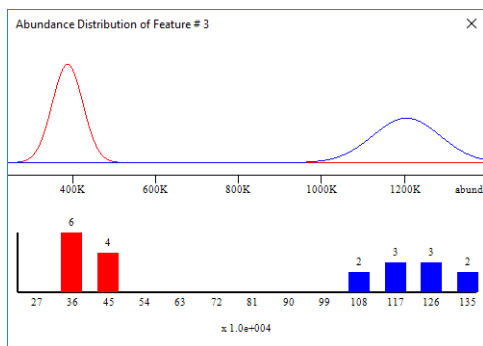
Global Occurrence Histograms   Displays histograms that show how frequently a feature occurs in samples within each group and across the total set of data files. See "View the global occurrence histograms" in the *Familiarization Guide*.
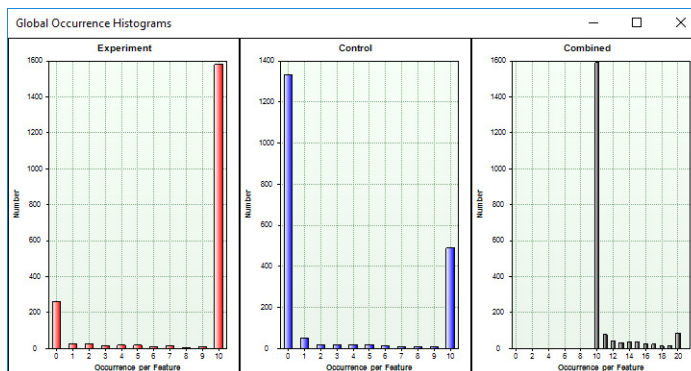
Principal Component Analysis menu   Displays the results of performing a mathematical process by which data containing a number of potentially correlated variables is transformed into a data set in relation to a smaller number of variables called principal components which account for the most variability 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 is 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 these commands:

- **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 previous principal component analysis a *PCA Mark* column is added to the Feature Table and two additional commands are available:

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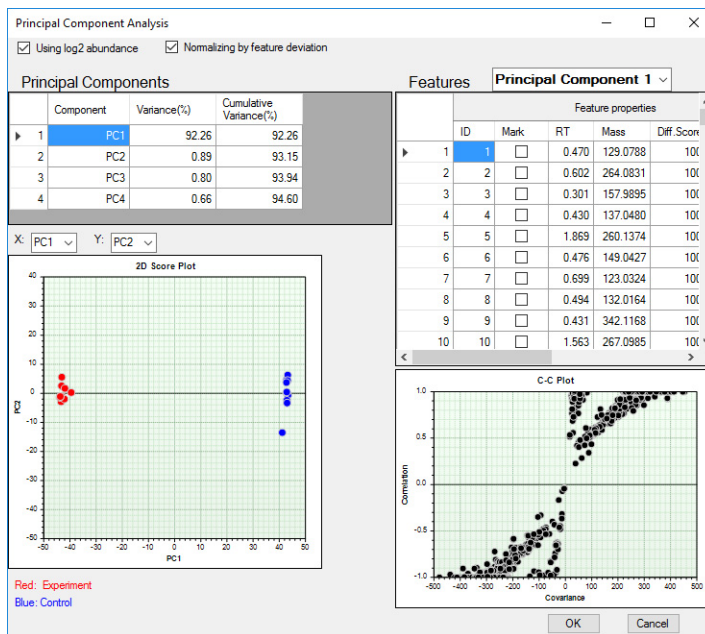
Identify Features menu

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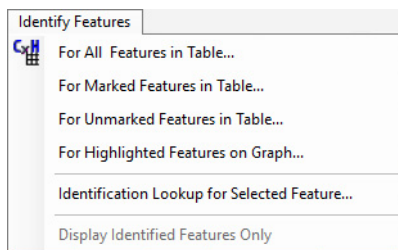
For All Features in Table   All of the features in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.
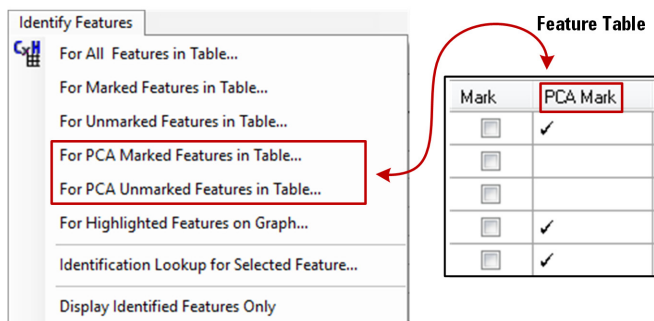
For Marked Features in Table   The features marked in the *Mark* column in the Feature Table are passed to ID Browser for identification. The results saved in ID Browser are automatically added to the Feature Table after you close the ID Browser window.

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.

Identification 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 previous principal component analysis two additional commands 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.

Feature Table

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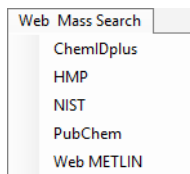
**ChemIDplus**   Mass search is directed to the NIH U.S. National Library of Medicine TOXNET toxicology data network at https://chem.nlm.nih.gov/chemidplus/.

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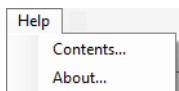
Contents   Opens the online help window for Mass Profiler with the **Contents**
tab displayed.

About   Displays the version information for Mass Profiler.

Toolbar

The following toolbar buttons are available in Mass Profiler.

Button	Equivalent Command
	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 14) 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.

The following main headings and actions are presented:

- "Feature summary"
- "Experiment <Group 1> and Control <Group 2>" on page 35
- "Comparison" on page 36
- "Table shortcut menu" on page 36

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 9 on page 45.

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.

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

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

CAS   If you have identified features with ID Browser and the compound ID includes CAS numbers, this additional column appears.

RT   Average retention time of the feature

(RT) SD   Standard deviation of the retention time of the feature

DT   ion mobility drift time of the feature (LC/IM-MS data)

(DT) SD   Standard deviation of the ion mobility drift time (LC/IM-MS data)

Mass   Average mass of the feature (non-IM data)

(Mass) SD   Standard deviation of the average mass of the feature (non-IM data)

m/z   Mass to charge ratio of a single adducted species of the feature (LC/IM-MS data)

(*m/z***) SD**   Standard deviation of the mass to charge ratio (LC/IM-MS data)

Abundance   Average abundance of the feature across 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 larger average abundance. The value reported can be *Max ion intensity*, *Max ion volume*, or *Feature volume* depending on the setting in **Measure of abundance**.

RSD   Relative standard deviation of the abundance of the feature (more than one sample in a group)

Z   Number of charges for the ion (LC/IM-MS data)

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 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 commands in the **View > Principal Component Analysis** menu (see "Perform a PCA analysis" in the *Familiarization Guide*).

Experiment <Group 1> and Control <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

DT   Average ion mobility drift time of the feature (LC/IM-MS data)

Mass   Average mass of the feature

m/z   Average mass to charge ratio of a single adducted species of the feature (LC/IM-MS data)

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, DT (for LC/IM-MS data), mass or *m/z*, 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>)

DT   The difference in the ion mobility drift time of the feature between the groups (<Group 1> - <Group 2>) (for LC/IM-MS data)

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

m/z   The difference in the mass to charge ratio of a single adducted species of the feature (for LC/IM-MS data)

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

See for commands when you right-click on the leading cell of a feature row.

Feature Plot

The Feature Plot pane shows a graphical result of processing the project with the current method. By adjusting the parameters 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, or abundance in individual samples: the larger the marker, the more abundant the feature.

The following sections and actions are presented:

- "Feature selection"
- "Sample selection" on page 38
- "X-Y selection" on page 38
- "Colored by" on page 39
- "Highlighting criterion and Highlighting threshold" on page 39
- "Plot axes" on page 40
- "Plot mouse actions" on page 40
- "Plot shortcut commands" on page 40

Feature selection

Select the features in the project to process and display in the Feature Plot.

All features   All of the features are displayed based on the selection, color, and highlighting criteria.

Unique to Experiment <Group 1>   The features only associated with <Group 1> are displayed based on the selection, color, and highlighting criteria. This feature selection is not available for a project with a single group.
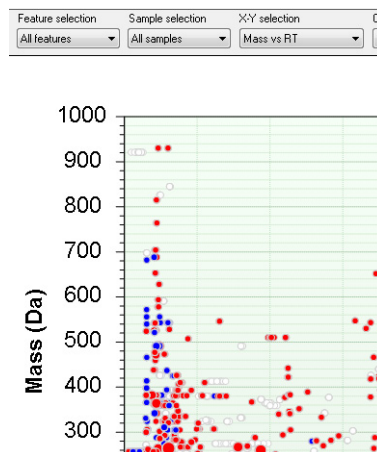
Unique to Control <Group 2>   The features only associated with <Group 2> are displayed based on the selection, color, and highlighting criteria. This feature selection is not available for a project with a single group.

Fully identified   Only the features that have been identified with both formula and name are displayed based on the selection, color, and highlighting criteria. This option is only available when features with both formula and name are present in the table.

Sample selection

When **All features** or **Fully identified** is selected for the Feature selection, you can select from **Group avg.**, **Composite**, **All samples**, or any individual sample file. **Group avg.** is not available for a project with a single group.

When **Unique to <Group 1>** or **Unique to <Group 2>** is selected for the Feature selection you can select from **Composite**, **All samples**, or any individual sample file.

Group avg.   The plot displays comparison and differences of the features that meet *X-Y selection* and the *Highlighting criterion* for all of the samples using monochrome markers.

Composite   The plot displays the average values of the features that meet *X-Y selection* and the *Highlighting criterion* for all of the samples using monochrome markers.

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

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

X-Y selection

When **Group avg.** is selected for the Sample selection, you can select from Abundance comparison, Mass difference, and RT difference for LC/MS data. For LC/IM-MS data you can select from Abundance comparison, DT difference, *m/z* difference, and RT difference. Group avg. is not available for a project with a single group.

When **Composite** is selected for the Sample selection, you can select from Abundance vs mass, Abundance vs RT, and Mass vs RT for LC/MS data. For LC/IM-MS data you can select from Abundance vs DT, Abundance vs m/z, Abundance vs RT, DT vs m/z, DT vs RT, and m/z vs RT.

When **All samples** or an individual sample is selected for the **Sample selection**, you can select from **Abundance comparison**, **Mass difference**, **RT difference**, **Abundance vs mass**, **Abundance vs RT**, and **Mass vs RT** for LC/MS data. For LC/IM-MS data you can select from **Abundance comparison**, **DT difference**, *m/z* **difference**, **RT difference**, **Abundance vs DT**, **Abundance vs** *m/z*, **Abundance vs RT**, **DT vs** *m/z*, **DT vs RT**, and *m/z* **vs RT**.

Colored by

None   Color is not used in the Feature Plot.

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>.

Highlighting criterion and Highlighting 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 have values below the threshold value are plotted as white markers and the features that have values above the threshold value are solid markers.

None   All of the features are plotted using solid markers.

Q score   Display features with a quality score, an algorithmic estimate of how likely the feature is an actual compound, above a specified threshold using solid markers.

Group frequency   Display features that are present at or above the specified percentage in one group of samples using solid markers.

Differential score   Display features with a differential score, a value between 0 and 100 that represents whether the feature is significantly different between the two groups, above the specified threshold using solid markers. Available in a project with more than two samples.

Max abundance-RSD   Display features whose abundance relative standard deviation, within a group, is greater than or equal to the specified threshold using solid markers. Available in a project with more than two samples.

Plot axes

Some of the plots axes use mathematical expressions as defined below.

Abundance vs   Plot of the Log base 2 (Log_2) of the ratio of the average feature abundance versus the selected parameter (DT, mass, *m/z*, RT).

Abundance comparison   Plot of the Log_2 of the average abundance of a feature:

- When **Group avg.** is selected for the Sample selection, the plot axes are Log_2 of the average abundance in <Group 1> versus Log_2 of the average abundance in <Group 2>.
- When an **Individual sample** is selected for the Sample selection, the plot axes are Log_2 of the feature abundance in the single sample versus Log_2 of the average abundance of the feature.

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 commands

See "Feature Plot shortcut menu" on page 46 for commands when you right-click a plot.

Feature Details

The Feature Details (see Figure 4 on page 15) shows the details of the feature selected when you double-click the row number of a feature in the 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.
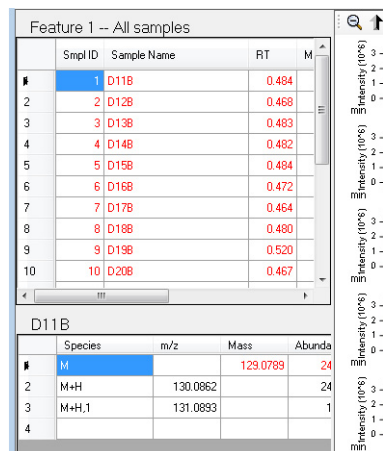
The following sections and actions are presented:

- "Feature details by sample"
- "Feature details by ion" on page 42
- "Extracted Ion Chromatograms (LC/MS data)" on page 43
- "Drift Spectra (LC/IM-MS data)" on page 44
- "Feature Details shortcut actions" on page 45

Feature details by sample

The information in this table is for the feature selected in the "Feature Table" on page 33.

Samples that are part of the Experiment group or <Group 1> are shown in a red color. Samples that are part of the Control group or <Group 2> 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

DT   ion mobility drift time of the feature (LC/IM-MS data)

m/z   Mass to charge ratio of a single adducted species of the feature (LC/IM-MS data)

Abundance   Abundance of the feature in the sample file. The abundance for the respective ion species is reported as either *Max ion intensity*, *Max ion volume*, or *Feature volume* depending on the method setting for **Measure of abundance**.

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 for LC/MS data after selecting Feature Details for a new feature in the Feature Table.

Feature details by ion

The information in this table is for the feature selected in the "Feature Table" on page 33, 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, the maximum ion intensity in the *Intensity* column, and the feature volume in the *Volume* column. 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.

For LC/MS data the table has the following columns:

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.

Intensity   Reports the Max ion intensity in the header row, otherwise the ion intensity.

Volume   Reports the Feature volume in the header row, otherwise the ion volume.

For LC/IM-MS data the table has the following columns:

RT   Average retention time of the feature

DT   ion mobility drift time of the feature

m/z   Mass to charge ratio of a single adducted species of the feature

Intensity   Reports the ion intensity.

Volume   Reports the ion volume.

Q-Score   Quality score of the ion in the sample file.

Extracted Ion Chromatograms (LC/MS data)

This information is not available for CEF and CSV file types. For LC/IM-MS data the extracted ion chromatogram is replaced with drift spectra.

Double-clicking any row number in the **Feature Details by Sample** updates the **Feature Details by Ion**. 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 intensity values of the individual ions. The y-axis does not change when changing the **Measure of abundance** setting in the method.

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 the zoom out and y-axis scale buttons to further adjust the zoom factor on all of the chromatograms.

Drift Spectra (LC/IM-MS data)

The left pane shows a single drift spectrum at the RT and *m/z* with the maximum ion intensity for the largest evidence ion. The height of the drift spectrum peak corresponds to the intensity for the largest evidence ion reported in the **Feature Details by Ion** table.

Mass Spectra

The right 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 volume of the largest evidence ion. 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.

Mass Spectra (LC/IM-MS data)

The right pane shows a single profile mass spectrum at the RT and DT with the maximum ion intensity for the largest evidence ion. The height of the mass spectrum peaks corresponds to values in the Intensity column in the **Feature Details by Ion** table.

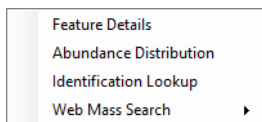### Feature Details shortcut actions

See "Feature Details shortcut menu" on page 47 for commands when you right-click within a chromatogram or spectrum.

Shortcut menu commands
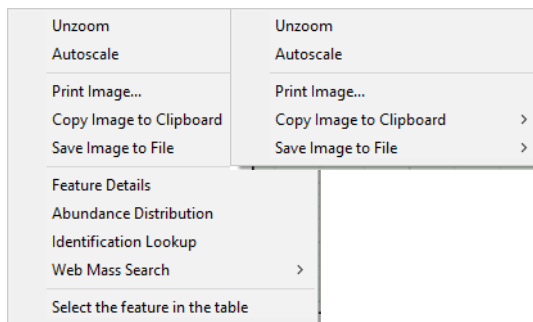
Shortcut menus provide commands (Figure 9 on page 45) applicable to the context where you click and to your current view. The following shortcut menus are presented:

- "Feature Table shortcut menu" on page 45
- "Feature Plot shortcut menu" on page 46
- "Feature Details shortcut menu" on page 47

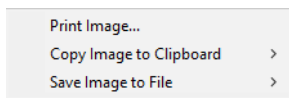**Figure 9**   Some of the shortcut menu commands available within the main window

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 9 on page 45.

Feature Details   Opens the **Feature-Detail View** dialog box 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.

View Feature in IM-MS Browser   Available only if the project contains not more than two sample files. Opens one or two IM-MS Browser windows and zooms into the *m/z*, RT, DT space around the feature. With IM-MS Browser you can perform interactive browsing and visualization of the data from a single LC/IM-MS data file.

Abundance Distribution   If the project contains two groups and at least three data files, 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. Identification results are not be returned to the feature table.

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 commands are **ChemIDplus**, **HMP** (Human Metabolite Project), **NIST**, **PubChem**, and **Web METLIN**.

Feature Plot shortcut menu

These commands are available when you right-click the Feature Plot change with whether your pointer is inside the graph region or along the plot border that contains the mass axes and plot title. The shortcut menus are shown in Figure 9 on page 45.

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** (.jpg or .tif) format.

Feature Details   Opens the **Feature-Detail View** dialog box 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   If the project contains two groups and at least three data files, opens the **Abundance Distribution of Feature #** dialog box 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. Identification results are not returned to the feature table.

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 commands 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 available when you right-click the extracted ion chromatogram, drift spectrum, or mass spectrum plot. The shortcut menus are shown in Figure 9 on page 45.

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** (.jpg or .tif) format.

For LC/IM-MS data files, the following two commands are available when you right-click the to the left of each sample row. This allows you to sequentially open up to four IM-MS Browser windows which automatically zoom into the m/z, RT, DT space around the selected feature. When you try to open a fifth IM-MS Browser window, a message asks you to first close one of the four open IM-MS Browser windows.

View Feature in IM-MS Browser   Available if the feature selected in the main feature table was detected in the selected sample.

View Data in IM-MS Browser   Available if the feature selected in the main feature table was not detected in the selected sample.

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 10 on page 49. 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 displayed 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.
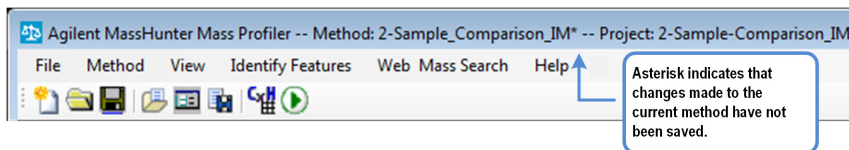

Figure 10   Indication of unsaved changes in your method is by the appearance of an asterisk in the title bar.

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 or RAID which includes stripped disks, such as RAID 1 or 10, 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 files during the process and is recommended for processing LC/IM-MS data.

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.08.00. Figure 11 shows the **Software Setup** dialog box that is displayed when a prior version of Mass Profiler is already installed.
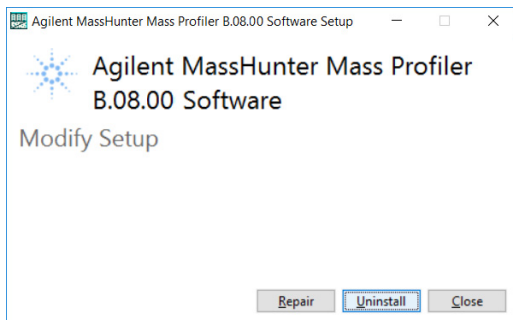
Figure 11 **Software Setup** dialog box indicating Mass Profiler is already installed.

3 Click **Uninstall** to remove the prior installation of Mass Profiler, and then rerun the installation program (**MPSetup.exe**).

This page intentionally left blank

www.agilent.com

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.08.00 revision or higher of MassHunter Mass Profiler Software, until superseded.

Agilent Technologies