MassHunter Mass Profiler Software

Familiarization Guide

Agilent Technologies

Process Data

Refer to the *Quick Start Guide* for an overview of the MassHunter Mass Profiler Software.

Processing data in Mass Profiler begins with creating a project that contains sample files that are arranged in one or two groups. A method is then created to set feature finding, alignment, normalization, and filtering parameters. You can create and save multiple methods and projects to widen and narrow the set of features to consider for further analysis or for emphasizing during the acquisition of new data.

The following tasks are presented in this section:

- "Create a project" on page 3
- "Edit a method" on page 6
- "Perform a PCA analysis" on page 13
- "Apply a different method" on page 18
- "Edit a project" on page 18

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.

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.

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.

Create a project

1. Click **File > Create Project**, or click the **Create Project** button on the toolbar.
2. Enter general project information in the **Create Project** dialog box (Figure 1 on page 4):
   a. Select **1** or **2** sample groups for the **Number of groups**.
   b. Type a **Project name**. This project name is automatically used as the project file name when you save your project.

c Select the **Input data type.**

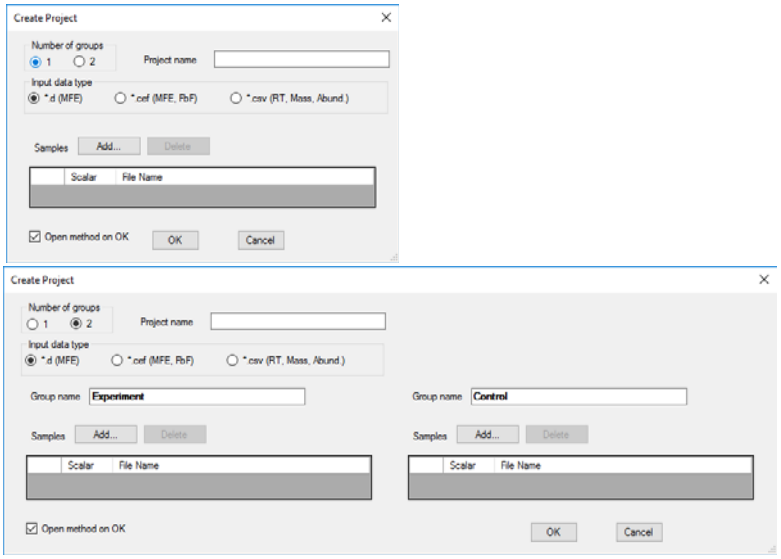


Figure 1 **Create Project** dialog boxes for one and two group projects.

Input data types

***.d (MFE)** - MassHunter data file - A raw data file from MassHunter Data Acquisition software. For this data type Mass Profiler finds the compounds (using MFE or IMFE, depending on the instrument used to acquire the data) and analyzes the results using all of the available method parameters. If more than one sample is part of a project, feature extraction automatically runs the recursive grouping of ions.

***.cef (MFE, FbF)** - CEF file - A compound exchange format file from another MassHunter software program. For this data type the features have already been found in the original data file using Find by Molecular Formula (MFE) or Find by Formula (FbF); Mass Profiler analyzes the results using the method parameters except those related to finding features (*Input filters* and *Sample chemistry and ionization*). If the CEF files contain identifications for features (name and/or formula), the features are forced to align. Otherwise alignment is based on mass and RT values.

***.csv (RT, Mass, Abund.)** - CSV file - A generic data file in which the features have already been found. The file must begin with a header row that contains the text `RT, Mass, Abundance` (in any order).

Subsequent rows contain the numerical information in the same order, separated by commas, for each feature and with one feature per row. Because of the limited feature information available, Mass Profiler analyzes the results using only the parameters available in the *Alignment & Normalization* tab and a portion of the parameters available in the *Statistics & Filters* tab.

3 Set up the first, or only, group of samples as follows:

   a Type a name for the **Group name**, or accept the default name **Experiment**.

   b Click **Add** to open the **Folder Selector** dialog box (for .d files) or the **Select Files** dialog box (for .cef and .csv files).

   c Select the sample files to add to the group. Press and hold **SHIFT** or **CTRL** and click the mouse button to select multiple files.

   d Click **OK** or **Open**. The files you selected are listed under the first group in the **Create Project** dialog box.

   e Repeat steps b through d to add additional samples if desired, but each file can be added to the project only once.

   NOTE: To remove a sample from a group, click in the leftmost column of the sample row, the column without header text, to select the row, then click **Delete**.

4 Set up the second group of samples, if applicable, as follows:

   a Type a name for the **Group name**, or accept the default name **Control**.

   b Repeat steps b through e in step 3 to add samples to the group.

5 Mark **Open method on OK** to immediately open and edit the method parameters after you click **OK** in the **Create Project** dialog box (default setting).

6 (*Optional*) Enter a value in the **Scalar** column for any sample to multiply the feature abundance values in that sample by the value entered.

7 Click **OK**. If **Open method on OK** is marked the **Method Parameters** dialog box is opened, otherwise you are returned to the Mass Profiler main window. The Mass Profiler main window only displays results after you run a method.

   NOTE: A warning is displayed after you click **OK** if a file appears in the project more than once or one group does not contain any data files.

Edit a method

1 Click **Method > Edit Method**, or click the **Edit Method** button on the toolbar.

2 Enter parameters in the **Method Parameters** dialog box.

Edit the parameters in the **Method Parameters** dialog box by selecting one of two editing modes at the bottom of the dialog box as shown in Figure 2. The default editing mode, **Commonly changed parameters**, presents the method parameters in a simplified view within the dialog box.

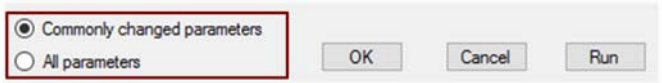


Figure 2 Method parameter editing modes

The method parameters for the **Commonly changed parameters** mode for two different two-group projects is shown in Figure 3.

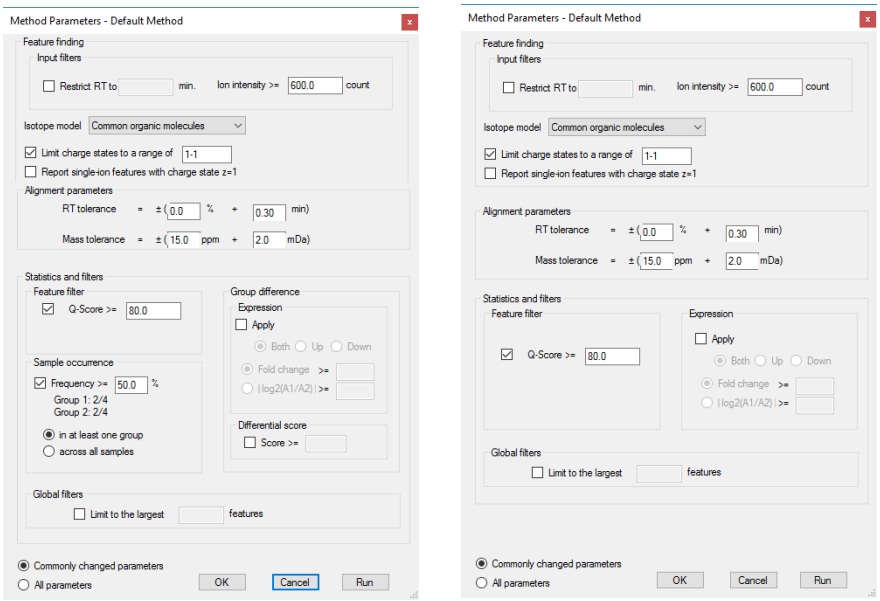
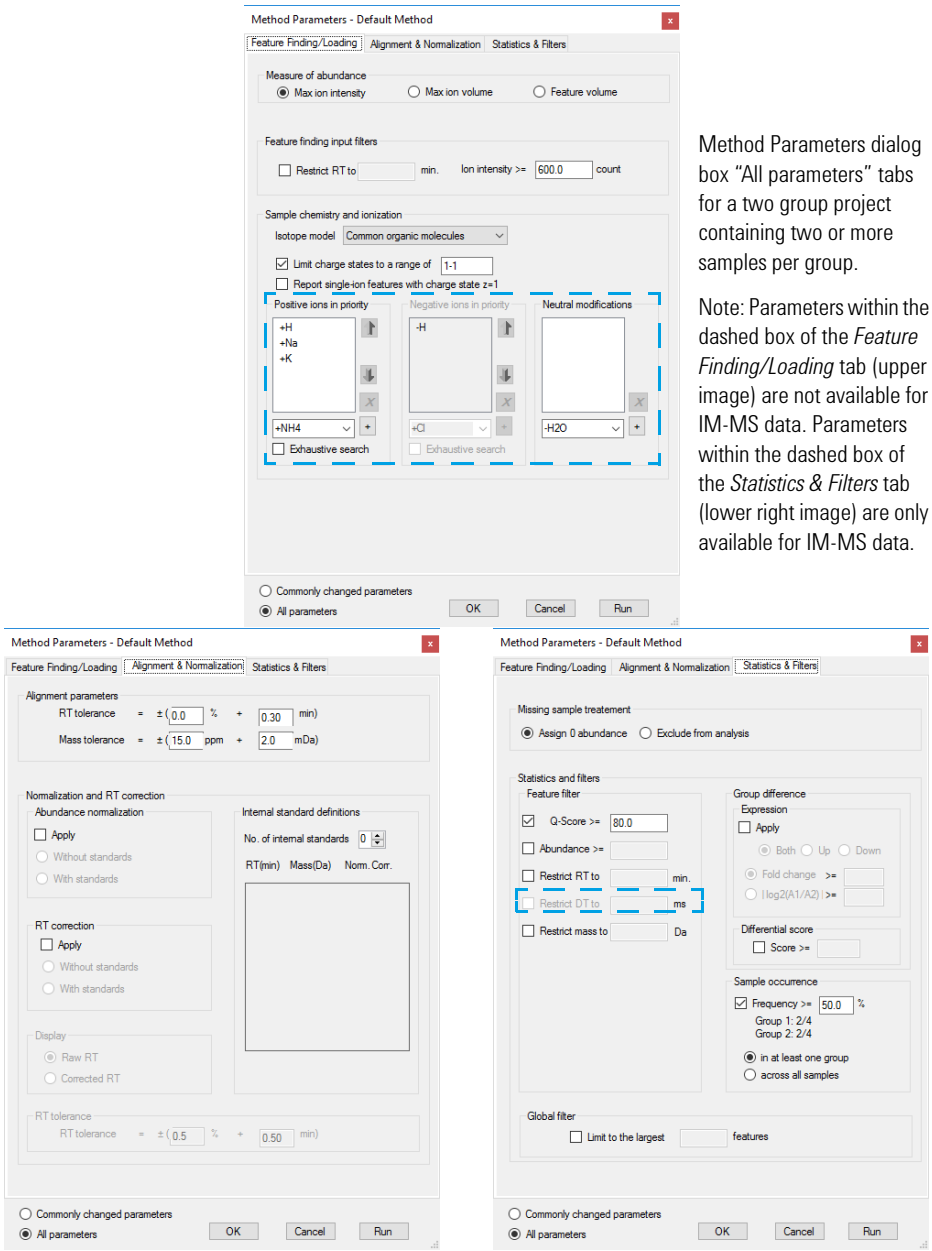


Figure 3 **Method Parameters** dialog box when you have a two group project: (left) two or more samples per group and (right) one sample per group.



Method Parameters dialog box "All parameters" tabs for a two group project containing two or more samples per group.

Note: Parameters within the dashed box of the *Feature Finding/Loading* tab (upper image) are not available for IM-MS data. Parameters within the dashed box of the *Statistics & Filters* tab (lower right image) are only available for IM-MS data.

Figure 4 **Method Parameters** dialog box with default values for **All parameters** mode

The method parameters for the **All parameters** mode, shown in Figure 4 on page 7, are edited using a sequence of three tabs according the workflow:

- Feature Finding/Loading: see "Find features" on page 10
- Alignment & Normalization: see "Align features" on page 11 and "Normalize abundance and correct RT of features" on page 11
- Statistics & Filters: see "Report statistics and feature filters" on page 12

3 Exit the method editor when you have reviewed the method parameters:

a Click **OK** to save the method changes in memory and exit the method editor.

b Click **Cancel** to discard the method changes and exit the method editor.

c Click **Run** to save the method changes in memory, exit the method editor, and process the samples in the current project using the current method parameters. The results are displayed in the Feature Plot and Feature Table in the Mass Profiler main window as shown in Figure 5 on page 9.

To quickly obtain results using a different method, click **Method > Load Method and Run**.

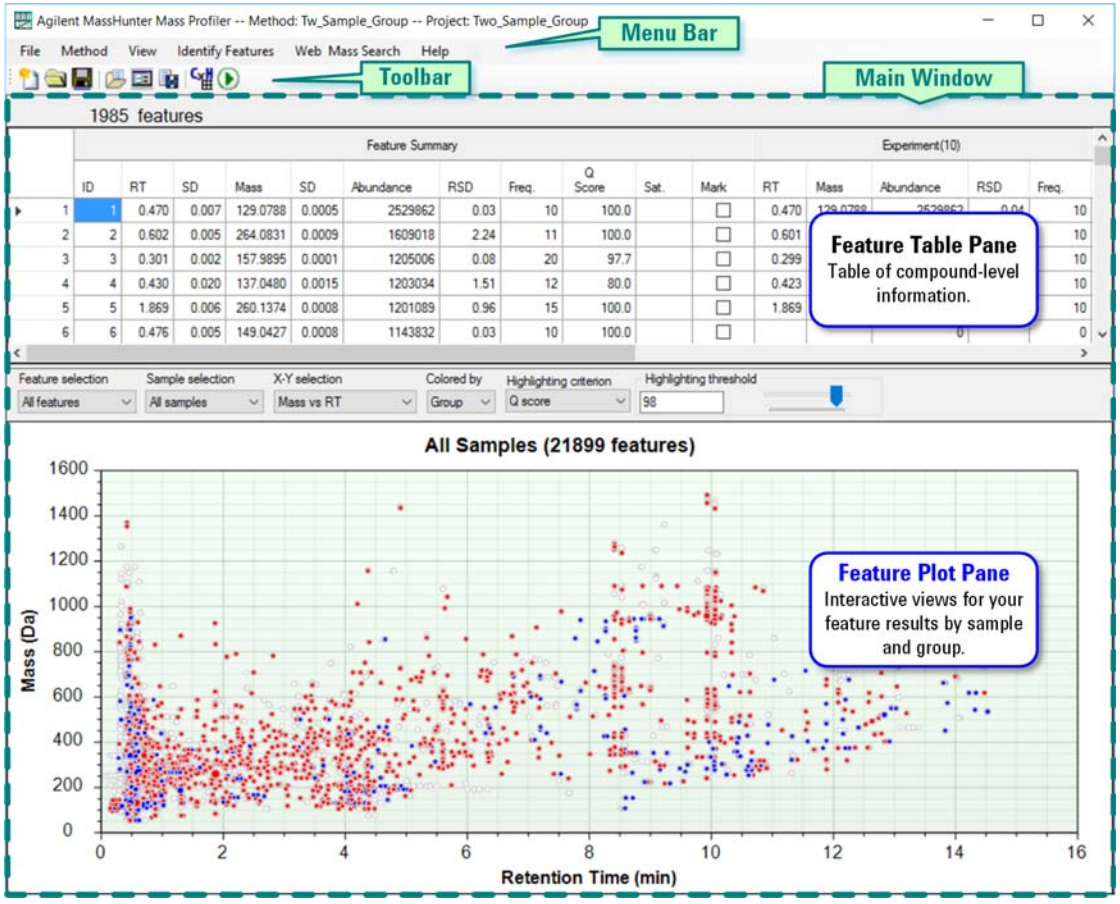


Figure 5 The main functional areas of Mass Profiler after a method is run on a project. If selected in the View menu, the Feature Plot can be replaced with a Features Details pane shown in Figure 6 on page 10.

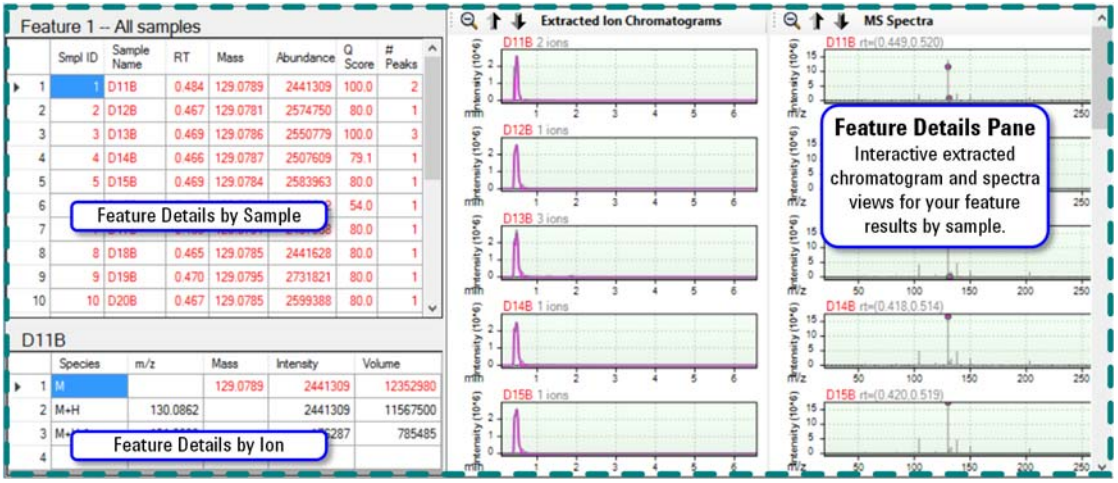


Figure 6 When you click **View > Switch to Feature-Details Mode** from the menu, the Feature Details pane replaces the Feature Plot pane in the main window shown in Figure 5 on page 9.

Save your method

4 Save your method to disk as an .mpm file using one of the following options:

- **a** Click **Method > Save Method**, or click the **Save Method** button on the toolbar to save the method using the current name.
- **b** Click **Method > Save Method As**, to save the method using a new name using the **Save Method As** dialog box.

Save your project

5 Save your project using one of the following options (a copy of the method parameters used to create the results is embedded with the project):

- **a** Click **File > Save Project**, or click the **Save Project** button on the toolbar to save the changes to the current project.
- **b** Click **File > Save Project As** to save the changes to the current project using a new name using the **Save Project As** dialog box.

Find features

The find feature parameters are only available for MassHunter sample data files described in "Input data types" on page 4. Figure 7 on page 11 shows the available feature finding parameters for the two editing modes.

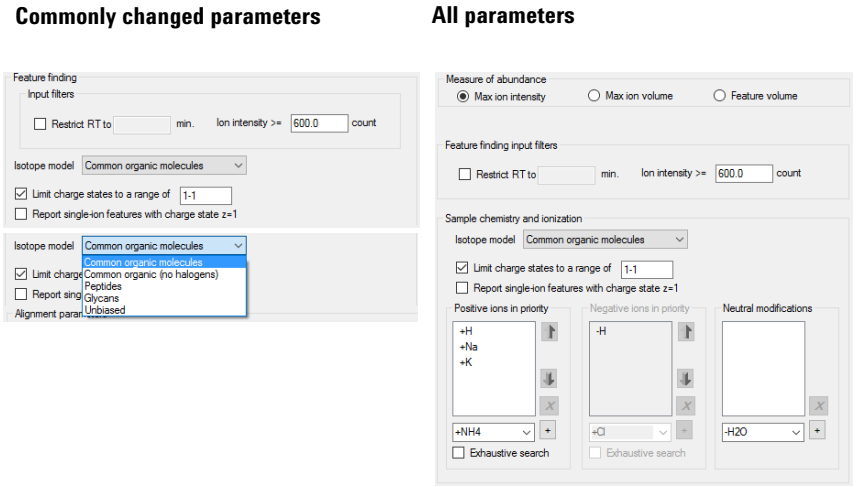


Figure 7 Default feature finding parameters in the **Method Parameters** dialog box for LC/MS data files using both editing modes

Align features

The alignment parameters are available for all of the sample data files described in "Input data types" on page 4. Features are aligned based on tolerance values entered for the retention time and mass.

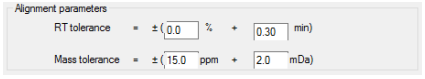


Figure 8 Default feature alignment parameters in the **Method Parameters** dialog box are identical for both editing modes

Normalize abundance and correct RT of features

The normalization and retention time correction parameters are available for all of the sample data files described in "Input data types" on page 4. By default samples are not abundance normalized or retention time corrected and these parameters are only available for editing in the **All parameters** mode.

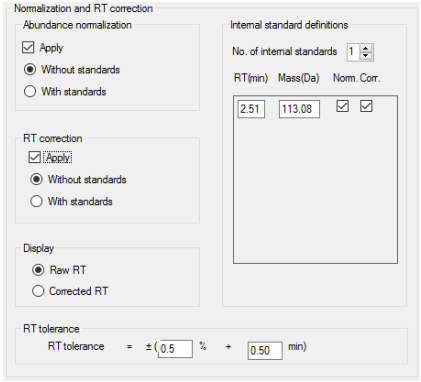


Figure 9 Example feature abundance normalization and RT correction parameters with an example internal standard in the **Method Parameters** dialog box in the **All parameters** mode

Abundance normalization and RT correction are applied to the samples in the project by marking **Apply** and selecting the mode **Without standards** or **With standards**, respectively.

Without standards mode: Mass Profiler applies a maximum RT correction using the **RT tolerance**. This value should be higher than the RT tolerance for alignment, and be set based on the RT shifts expected between the data files. This alignment is used for a preliminary alignment during feature finding so that the Mass Profiler can statistically determine and correct the time shifts between the samples.

With standards mode: Mass Profiler uses the internal standards as defined under the *Internal standard definitions* heading. The number of internals standards is entered and the RT and neutral mass for each internal standard is specified. Mark the respective internal standards to define whether to use the internal standard for abundance normalization (Norm.), RT correction (Corr.), or both.

Report statistics and feature filters

Reporting statistics and feature filtering parameters are available for all of the sample data files described in “Input data types” on page 4. *Group* statistics are only available for projects that contain two groups.

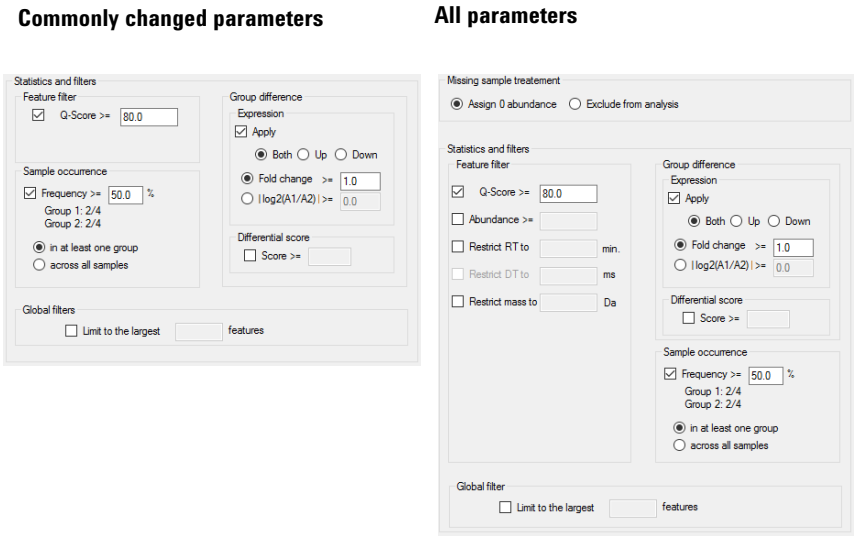


Figure 10 Example statistics reporting and feature filter parameters in the **Method Parameters** dialog box for both editing modes. The *Sample occurrence* filter is only enabled for projects with two groups where each group contains two or more samples.

Perform a PCA analysis

Mass Profiler includes a statistical tool referred to as Principal Component Analysis (PCA) that employs a mathematical process by which the features found within your data are transformed into a new set of data in relation to variables called principal components. Each principle component is optimized to account for the maximum variability in the features among the samples with your project. For a project with a single group the PCA can help you identify samples that are more closely related. For a project with two groups the PCA helps you identify whether variations among the features separate the samples by group.

Using PCA you can easily identify and mark the features that account for greatest similarity, or difference, among your samples and return the marks to your Feature Table summary.

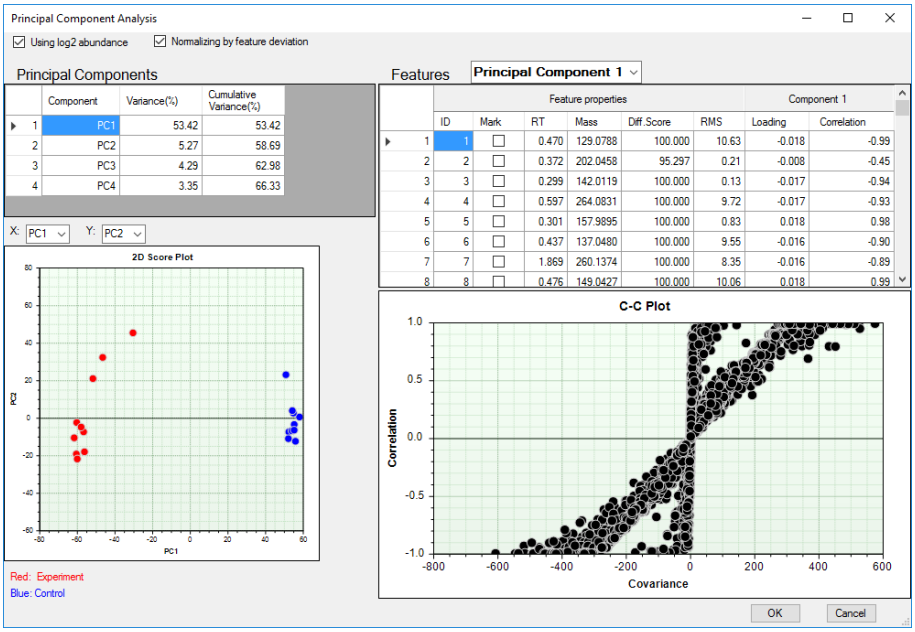


Figure 11 **Principal Component Analysis** dialog box for a project that contains two groups and 10 samples per group

Shortcut menus within the PCA plots provide options applicable to the context where you click and to your current view (Figure 12).

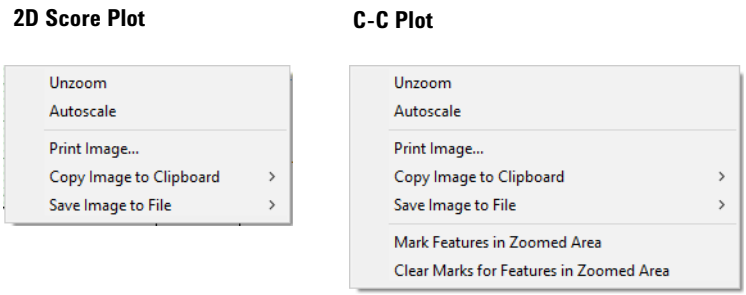


Figure 12 The shortcut menu commands available within the **Principal Component Analysis** dialog box

1 Click **View > Principal Component Analysis > For All Features in Table** to become familiar with the capabilities of PCA. Other options for starting

PCA are noted in "Principal Component Analysis menu" in the *Quick Start Guide*.

2 Mark **Using log_2 abundance** to transform the abundance values of the features to log base 2 for the PCA calculations. This is marked by default.

3 Mark **Normalizing by feature deviation** to normalize the abundance values of the features by their standard deviation for the PCA calculations. This is marked by default.

4 Review the Variance(%) and Cumulative Variance (%) in the **Principal Components** table in the top-left of the **Principal Component Analysis** dialog box (Figure 11 on page 14). If the variance of the first principal component is large (a value that is closer to 100%), a clear separation between sample groups is possible and is usually reflected by a visual separation on the *2D Score Plot*. If the variance of the first principal component is small (a value that is closer to zero), there is a degree of visual overlap between sample groups on the *2D Score Plot*.

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

Component Each principal component is labeled with a number corresponding to the component's share of the PCA variance. The lower number principal components account for more of the variance in the group or feature difference. The *Principal Component Table* is initially sorted by the component *Variance(%)* value, but the sort order can be changed by clicking on any column heading.

Variance (%) The amount of variance that a single principal component is able to account for out of the total possible group or feature variance.

Cumulative Variance (%) The sum of the variance that successive principal components are able to account for out of the total possible group or feature variance.

5 Review the separation, or similarity, of your samples in the **2D Score Plot** in the lower-left of the **Principal Component Analysis** dialog box (Figure 11 on page 14). You can select any of the principal components to plot against each other to view how each principal component separates your samples. Available shortcut menu options are shown in Figure 12 on page 14.

6 Review the features and their principal component attributes **Features** table in the top-right of the **Principal Component Analysis** dialog box (Figure 11 on page 14). You can view the attributes for any of the principal

components using the drop-down list box above the table. When you select a different principal component the table and the C-C Plot automatically update.

(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.

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

Mark The check boxes in this column are used to **Mark** one or more features of special interest to your project. You can mark the features manually or based on features that appear in the C-C Plot. The mark annotations are transferred to the Feature Table when you click **OK**. If you select a feature in error, click the check box again or use the C-C Plot to clear the mark.

Formula If you have identified features with ID Browser this additional column appears and shows the molecular formula for identified features (see "Identify Features" on page 29).

Name If you have identified features with ID Browser this additional column appears and shows the name for the identified features (see "Identify Features" on page 29).

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.

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

Mass Average neutral mass of the feature.

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. The project must have more than one sample per group to display this attribute.

RMS The calculated root mean square (RMS) error computed for each feature during the PCA modeling.

Loading A calculated value during the PCA modeling that can help identify which features account for the greatest variation in the PCA. Entities with a larger loading are more significant to the sample differentiation. The sum of

the squared loadings of all the features of a given principal component is equal to one. The positive or negative sign of a particular principal component is arbitrary.

Correlation A reliability value for the feature. Numerical values closer to ±1 are more reliable and more significant to the sample differentiation.

7 Review the correlation and covariance relationship of the features in the **C-C Plot** in the lower-right of the **Principal Component Analysis** dialog box (Figure 11 on page 14). The C-C Plot automatically updates when you select a different principal component and when you change the abundance transformation.

The C-C Plot shows the covariance and correlation coefficients resulting from the PCA model in a scatter plot. In this plot both magnitude (covariance) and reliability (correlation) are visualized.

The covariance (Cov) and correlation coefficient (Cor) are calculated by the following equations:

$Cov = Sum(p_s * f_s)/(N-1)$

$Cor = Sum(p_s * f_s)/Sqrt(Sum(p_s * p_s) * Sum(f_s * f_s))$;

where,
p_s is the principal component value measured by sample s,
f_s is the feature value measured by sample s,
Sum are value summations over all of the samples, and
N is the number of samples.

Available shortcut menu options are shown in Figure 12 on page 14. In particular, using the zoom functionality and the shortcut menu you can easily mark and clear feature that meet your analysis objectives.

8 Click **OK** when you have completed your review and selection (marking) of the features that meet your analysis objectives and you want to return to the main Mass Profiler window. The marked features appear with a mark in the new column *PCA Mark* in the Feature Table.

9 Save your project using one of the following options (a copy of your method is embedded with the project):

a Click **File > Save Project**, or click the **Save Project** button on the toolbar to save the changes to the current project.

b Click **File > Save Project As** to save the changes to the current project using a new name using the **Save Project As** dialog box.

Apply a different method

This procedure helps you quickly process a project using different methods.

1 Click **Method > Load Method and Run** to open the **Open** dialog box.

2 Select a method in the **Open** dialog box.

3 Click **Open** to load and automatically apply the method to your current project. The parameters in the selected method are applied to the current project and the results are immediately displayed in the Feature Table and Feature Plot.

4 Save your project using one of the following options (a copy of your method is embedded with the project):

   a Click **File > Save Project**, or click the **Save Project** button on the toolbar to save the changes to the current project.

   b Click **File > Save Project As** to save the changes to the current project using a new name in the **Save Project As** dialog box.

Edit a project

This procedure helps you change the properties of an existing project, such as project name, group names, sample composition, and sample scalar values. To change the **Number of groups** or the **Input data type** you must create a new project.

1 Click **File > Edit Project** to open the **Edit Project** dialog box.

2 Make the desired changes to the project, in the same fashion described in "Create a project" on page 3.

3 Exit the **Edit Project** dialog box by:

   a Click **OK** on the **Create Project** dialog box to open the **Method Parameters** dialog box, if **Open method on OK** is marked. If **Open method on OK** is cleared your changes to the project are retained and the **Create Project** dialog box is closed.

   b Click **Cancel** to return to disregard any changes you have made to the current project and return to the main window.

   c Click **Run** to automatically process the project samples using the current method and update the results displayed in the Feature Table and Feature Plot in the main window.

4 Save your project using one of the following options (a copy of your method is embedded with the project):

   a Click **File > Save Project**, or click the **Save Project** button on the toolbar to save the changes to the current project.

   b Click **File > Save Project As** to save the changes to the current project using a new name using the **Save Project As** dialog box.

View Results

When a project is processed with the current method parameters the results are shown automatically in the Feature Table and Feature Plot areas of the Mass Profiler main window. Processing is performed and the main window is updated after the following actions:

- Click **Run** in the **Method Parameters** dialog box
- Click **Method > Load Method and Run**
- Click **Method > Run Current Method**
- Click the **Run Current Method** button

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

The following view tasks are presented:

View results in the Feature Table

The Feature Table (see Figure 5 on page 9) shows the results of processing the sample files selected in your project with the current method. The table can be sorted by the values in any column by clicking on the corresponding column heading. See "Feature Table" in the *Quick Start Guide* for an overview of the information you can view in the Feature Table.

Other columns are added to the table to show the results of performing a PCA ("Perform a PCA analysis" on page 13) and identifying features using the ID Browser program ("Identify Features" on page 29).

For a project with two groups, the results of the comparison of the groups is viewed as follows:

1. Scroll horizontally to view the feature details under the *Comparison* heading located on the right of the table.
2. Click the *Expression* heading to sort the features by up-regulation and down-regulation. Regulation indicates features that have an increased (up) or decreased (down) average abundance in <Group 1> relative to <Group 2>.
3. Right-click on any row number cell on the left side of the Feature Table to access the shortcut menu options: View Feature Details in IM-MS Browser (for LC/IM-MS data files only), Feature Details, Abundance Distribution (only for projects with 2 sample groups and at least 3 sample files), Identification Lookup, and Web mass search.
4. To copy the contents of the table for use in another program, drag the mouse to select the cells of interest (or click on any one or more row number) then press **CTRL-C** to copy the data to the clipboard. The copied contents can then be pasted into other applications, such as a text editor or a spreadsheet.

View results in the Feature Plot

The Feature Plot shows a graphical result of processing the project with the current method. By adjusting the parameters you can adjust any plot to visualize the feature differences by sample and group. The relative size of the plot markers representing a feature is an indication of the average abundance of the feature. See "Feature Plot" in the *Quick Start Guide* for an overview of the information you can view in the Feature Plot.

Inspection of the features in the Mass vs. RT plot is as follows:

1. Move the pointer over a data point (feature) on the plot to display a brief description of that feature (Figure 13 on page 22). The size of a marker indicates the abundance of a feature: the larger the marker, the more abundant the feature.

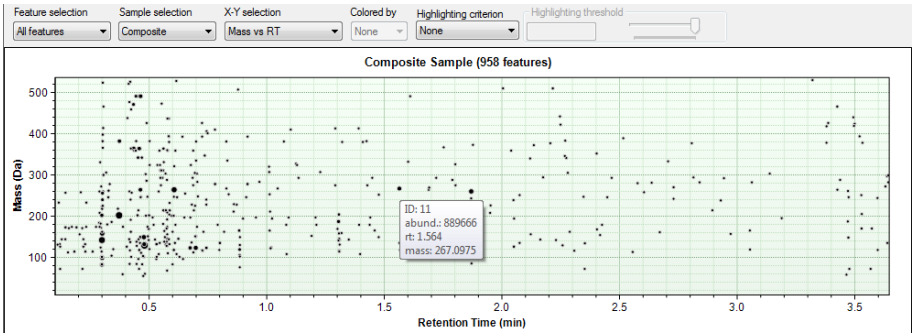


Figure 13 Feature information in the Feature Plot

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

3 Change how and which features are displayed in the Feature Plot as described in "Feature Plot" in the *Quick Start Guide*.

4 Right-click in any Feature Plot graph plot to access the shortcut menu options described on "Plot shortcut commands" in the *Quick Start Guide*.

View results in the Feature Details

The Feature Details (see Figure 6 on page 10) 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 with the **Feature Details by Sample**, a table with the **Feature Details by Ion**, and, if available for the imported data type, extracted ion chromatograms (for LC/MS data), drift spectra (for LC/IM-MS data), and mass spectra of the feature for each sample data file. See "Feature Details" in the *Quick Start Guide* for an overview of the information you can view in the Feature Details.

Two data view modes, *Feature-Details mode* and *Feature-Plot mode*, are available to help you view your results. You can switch between the two modes using the menu item **View - Switch to Feature-Details Mode**, which then changes to **View - Switch to Feature-Plot Mode** allowing you to switch back.

- If **Feature Details** are requested in the *Feature-Plot mode*, the details show in the **Feature Detail View** dialog box.

- If **Feature Details** are requested in *Feature-Details mode* the details show in the lower half of the main window by updating the *Feature Details pane*.

Use this procedure to view more information about a selected feature. The available information is limited for CEF and CSV file types.

1 Open the Feature Details for a selected feature in any of the following ways:

From the Feature Table

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

From the Feature Plot

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

2 Review the information for the feature in all the samples in the **Feature Details by Sample** table in the top left pane as described in "Feature details by sample" in the *Quick Start Guide*. Note the selected feature may not be present in every sample in the project.

3 Double-click a row (sample) in the **Feature Details by Sample** table to view the information for the feature in the **Feature ions** pane as described in "Feature details by ion" in the *Quick Start Guide*. The information displayed depends on the input data type of the sample.

4 Double-click a row (ion species) in the **Feature Details by Ion** table to view a pane that contains the extracted ion chromatogram (for LC/MS data) or the drift spectra (for LC/IM-MS data). These panes are described in "Extracted Ion Chromatograms (LC/MS data)" in the *Quick Start Guide* and "Drift Spectra (LC/IM-MS data)" in the *Quick Start Guide*.

5 Experiment with the shortcut menu options are described in "Feature Details shortcut menu" in the *Quick Start Guide*.

View the feature in IM-MS Browser (LC/IM-MS data)

The MassHunter IM-MS Browser is an application that supports interactive browsing and visualization of data from single LC/IM-MS data files, extraction of various 2D and 3D subsets of that data, finding of features, exporting of extracted data in a variety of formats, and collision cross section calculations.

Launching IM-MS Browser

If your project contains only one or two samples, a feature can be viewed in the IM-MS Browser. Select the row of the desired feature and click **View > Selected Feature in IM-MS Browser** or **View feature in IM-MS Browser** from the context sensitive shortcut menu. One or two IM-MS Browser windows open, automatically extract frames across the elution time of the feature, and zoom the frame viewer into the feature using reasonable drift time and *m/z* ranges, whether the feature is present in both data files or only one.

When you select another row (feature) and view the feature in IM-MS Browser, when IM-MS Browser is already open, the two open IM-MS browser windows refresh accordingly. The previous feature(s) stay in the feature list in the respective IM-MS Browser window so you can easily adjust the view among recently viewed features.

Maximum number of M-MS Browser windows

A maximum of four (4) IM-MS Browser windows can be open at the same time. If your project contains more than two samples, a feature can be viewed from up to four samples (IM-MS Browser windows) by selecting one sample at a time from the feature details by sample pane in **Feature Details**. Click the right mouse button and select **View feature in IM-MS Browser**. If you attempt to view the feature detail from a fifth sample a message dialog box prompts you to close one or more IM-MS Browser windows first. When the Mass Profiler software is closed, all IM-MS Browser windows launched from Mass Profiler are automatically closed.

For information on using IM-MS Browser see the *MassHunter IM-MS Browser Software - Quick Start Guide*, available on the Agilent Literature Library at www.chem.agilent.com.

Print, save, or copy the Feature Plot image

The following options are available in many graphics in Mass Profiler.

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

Copy Image to Clipboard Copies the current 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 plot image as either an **Enhanced Meta file** (.emf) or **Bitmap** (.bmp) format.

Find more feature information from database websites

Use this procedure to conduct a search by neutral mass and access more information about selected features in any of the following online databases: ChemIDplus, HMP, NIST, PubChem, or Web METLIN.

From the Feature Table

Method 1:

1. Click the row number to select the feature of interest in the Feature Table.
2. Click **Web Mass Search** and the database to search from the menu bar.

Method 2:

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

From the Feature Plot

Method 3:

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

In each case above, your default Internet browser is opened to perform the selected mass search as described in "Web Mass Search menu" in the *Quick Start Guide*.

View the feature abundance distribution

This feature is only available if the project contains two sample groups and at least three samples.

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

From the Feature Table

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

From the Feature Plot

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

2 Review the abundance distribution of the samples for the selected feature in the **Abundance Distribution of Feature #** window. The abundance distribution consists of a histogram and a curve color-coded by group (red for <Group 1> and blue for <Group 2>), as shown in Figure 14.

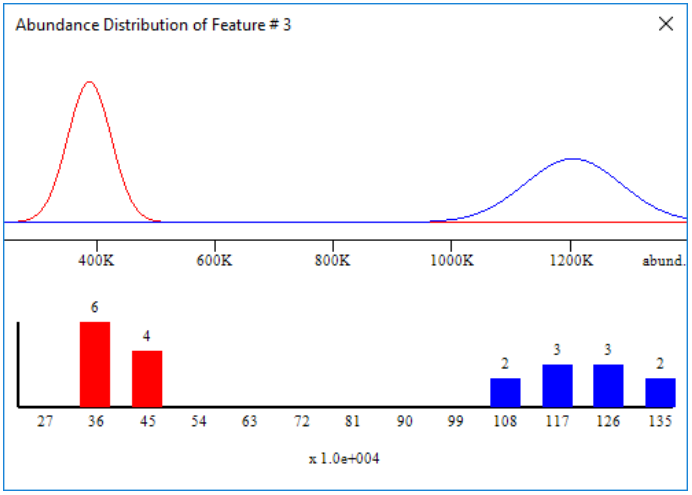


Figure 14 Abundance Distribution of Feature window

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

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

View the global occurrence histograms

1 Click **View > Global Occurrence Histogram**.

2 Review the information presented in the **Global Occurrence Histograms** window. Each histogram shows the frequency of feature occurrence in the samples within each group and or within both groups in your project. The x-axis is the number of samples that a particular feature occurs in, from zero up to the number of samples in the group. The y-axis is the number of features that occur in that number of samples from zero up to the number of features in the project.

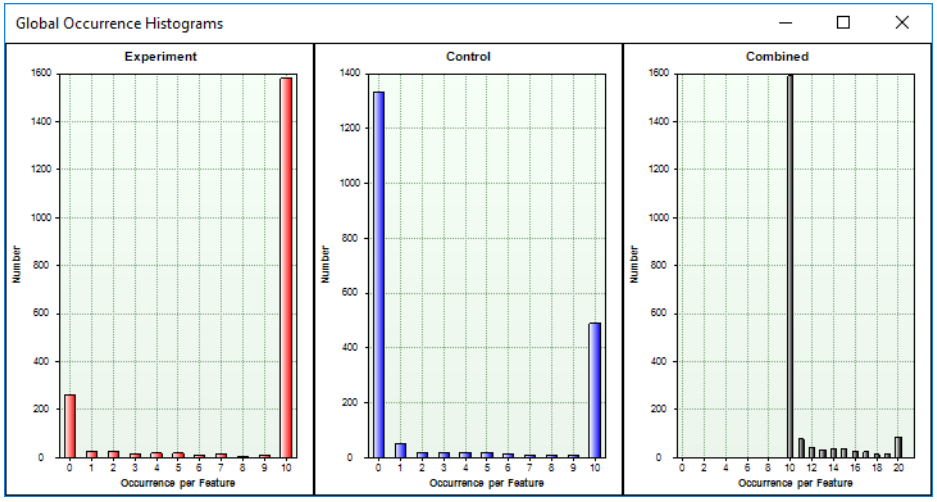


Figure 15 **Global Occurrence Histogram** window

Global Occurrence Histogram shortcut menu

The menu commands available when you right-click in the Global Occurrence Histogram are shown in Figure 16 on page 28.

Copy Copies the histograms in the window graphic to the Clipboard to paste into other applications.

Save Image As Saves the histograms in the window graphic as one of five image file formats: .png, .gif, .jpg, .tif, or .bmp.

Show Point Values Annotate the number of features found in each sample when the pointer is moved over each bar graph.

Unzoom Restores the previous zoom level of the histogram.

Set Scale to default Resets the axes of the histogram to show the full data.

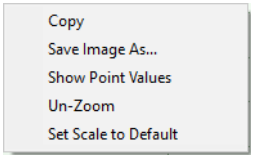


Figure 16 The shortcut menu commands available within the **Global Occurrence Histogram** window

Identify Features

The following procedures help you to identify features using MassHunter ID Browser. Identify features is not available for the CSV file type.

Lookup the identity of a single feature

Results of an identification lookup for a single feature are not returned back to Mass Profiler. The lookup functionality is designed to help you identify features of interest without committing to adding the results to your project. To return feature identification to your project use "Identify multiple features" on page 30.

1 Select the feature of interest and initiate identification in any of the following ways:

From the Feature Table

- Click the row number of the feature of interest in the Feature Table, then click **Identify Features > Identification Lookup for Selected Feature**.
- Right-click the row number for the feature in the Feature Table, and select **Identification Lookup** from the shortcut menu.

From the Feature Plot

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

2 Review and adjust the identification method parameters in the **Compound Identification Wizard**.

3 Click **Next** and **Back** to review all of the method parameters.

4 Click **Finish** to exit the wizard and automatically run the method on the feature.

5 Review results in the Compound List Window as described in "Review identification results" on page 31.

6 Click **Save and Return** in the toolbar to close the ID Browser window and return to Mass Profiler. Your ID Browser results are not returned to Mass Profiler.

Identify multiple features

Results of identifying multiple features are returned back to Mass Profiler.

1 Click **Identify Features** and select one of the following options:

- **For All Features in Table**: All of the features in the Feature Table are passed to ID Browser for identification.
- **For Marked Features in Table**: The features marked in the *Mark* column in the Feature Table are passed to ID Browser for identification.
- **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.
- **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.
- **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.
- **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.

2 Review and adjust the identification method parameters in the **Compound Identification Wizard**.

3 Click **Next** and **Back** to review all of the method parameters.

4 Click **Finish** to exit the wizard and automatically run identification on the selected features.

5 Review results in the Compound List Window as described in "Review identification results" on page 31.

6 Click **Save and Return** in the toolbar to close the ID Browser window and return to Mass Profiler. Your ID Browser results are returned to Mass Profiler.

Review identification results

This section helps you view compound identification results in the Compound List Window of the ID Browser as shown in Figure 17 on page 32.

1 View the compounds in the table in the Compound List Window.

- Each row in the table shows the best compound identification match for each feature.
- Each column contains additional information about each compound. For a list of the information that can be displayed for a compound, see the ID Browser online help for more information.

2 Click the + button to the left of a compound to display the second level of the compound table - the list of compounds that were matched to the original feature based on the formula generation and database and library search.

- Second level rows have a green background.

3 Click the + button next to a second level search result to display the third level of the compound table - species information, if the second level has a formula assigned from either generate formulas or database search.

- Third level rows have a light blue background.

4 Click the + button next to an entry in the third level of the table to display the fourth level of the compound table - the *m/z* peak list.

- Fourth level rows have a light red background.

5 Click the - button to close a level that is open. Closing a compound row automatically closes the 2^{nd}, 3^{rd} and 4^{th} level rows for that compound.

Any number of feature levels can be open for as many compounds as you want at the same time.

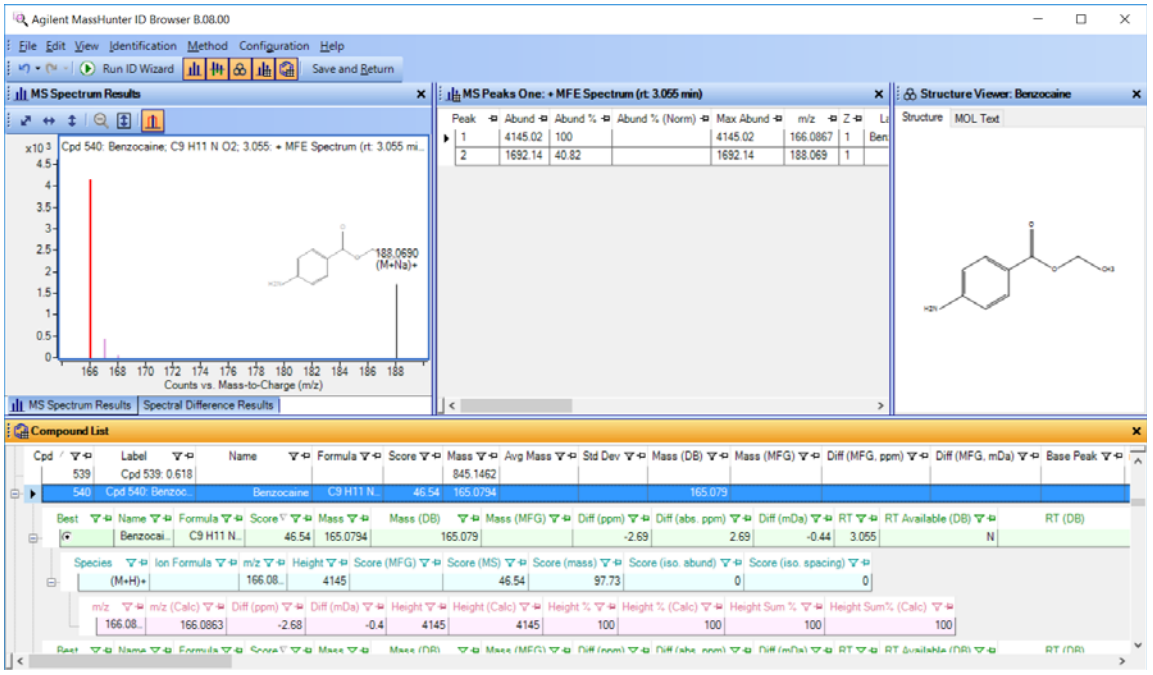


Figure 17 ID Browser after identifying features as compounds

Export Mass Profiler Data

This section describes how to export data from Mass Profiler for use with other programs, such as text editors, spreadsheet, and MassHunter programs Qualitative Analysis, Acquisition, and Mass Profiler Professional. You can export features in the formats described in "Exporting data" in the *Quick Start Guide*:

- "Mark features for Export" below
- "Export data from the Feature Table" below
- "Export data from the Feature Plot" on page 36

Mark features for Export

Use this procedure to mark any features of special interest. These marks are included when you export data from the Feature Table or the Feature Plot as a Feature Summary. The mark notation is not included in the other export formats.

- Mark a feature in the column labeled **Mark** in the rows for the features of interest in the Feature Table.
- Clear a feature that is marked, click again in the column labeled **Mark** in the row for the feature.
- Click **View > Principal Component Analysis** to add PCA Mark features. See "Perform a PCA analysis" on page 13.

Export data from the Feature Table

1. *(Optional)* Mark any features that are of special interest as described in "Mark features for Export" above.
2. Click one of the following items in the **File** menu, depending on the format you want to use to export the data:

- **Export Feature Summary > From Table...:** Export the feature summary information from the Feature Table for use in a spreadsheet such as Excel. The export file format is a tab-delimited text file and can be opened in a text editor, but it is saved with an .XLS extension 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 two additional options are available:
  From Table - PCA Marked Features
  From Table - PCA Unmarked Features

- **Export Target MS/MS Inclusion List > From Table...**: Export 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 a comma-separated value text file with a .CSV extension. 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 two additional options are available:
  From Table - PCA Marked Features
  From Table - PCA Unmarked Features

- **Export MPP Input File > From Table...**: Export the feature summary information from the Feature Table for use with Mass Profiler Professional. 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 two

additional options are available:
From Table - PCA Marked Features
From Table - PCA Unmarked Features

- **Export Composite-Compounds CEF > From Table...**: Export the list of features for import into Mass Profiler Professional for advanced statistical analysis. The export file format is a compound exchange file with a .CEF extension. The features within a group are summed to form a composite compound. This export feature is not available for LC/IM-MS data files. 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 two additional options are available:
  From Table - PCA Marked Features
  From Table - PCA Unmarked Features

3 If you selected **Export Targeted MS/MS Inclusion List > From Table...** in Step 2, the **Export Inclusion List Options** dialog box (Figure 18) appears. Set the following parameters for selecting representative ions for each feature.

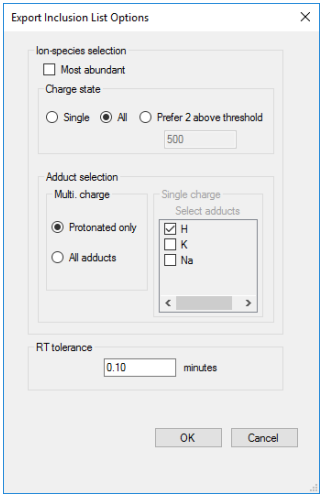


Figure 18 **Export Inclusion List Options** dialog box

a Mark **Most abundant** to use the most abundant ion for each feature.

b Select the parameters for *Charge state*.

c Select the parameters for *Adduct selection*.

d Enter a **RT tolerance** in minutes or accept the default value of 0.10 minutes.

e Click **OK**.

4 Enter a folder and name for the output file and click **Save**.

Export data from the Feature Plot

1 Display the features of interest in the Feature Plot as described in "Feature Plot" in the *Quick Start Guide*. Keep the following caveats in mind:

- When you export graphed features, only the features shown as solid dots are exported. Circles are filled or not based on the current Highlighting criterion. If the Highlighting criterion is set to **None**, then the result is equivalent to the table-based reports.
- The ratio and comparison plots can only include features that are present in both groups. You may get a different list of features depending on which graph you are exporting. The generated files include annotations to document what graph and highlighting criterion were used to generate the export.
- The supported export formats are based on data averaged over one or more groups. Even if you choose a **Plot style** that displays individual per sample features, the exported data report the averaged feature data.

2 Click one of the following items on the **File** menu, depending on the format you want to use to export the data:

- **Export Feature Summary > From Graphed Features**: Export the features in the current Feature Plot for use in a spreadsheet. The export file format is a tab-separated text file with an .XLS extension for direct opening in Excel.
- **Export Target MS/MS Inclusion List > From Graphed Features**: Export 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 a comma-separated value text file with a .CSV extension.

- **Export MPP Input File > From Graphed Features**: 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 Graphed Features**: Export the features in the current Feature Plot for import into Mass Profiler Professional for advanced statistical analysis. The export file format is a compound exchange file with a .CEF extension. The features within a group are summed to form a composite compound. This export feature is not available for LC/IM-MS data files.

3 If you selected **Export Targeted MS/MS Inclusion List > From Table...** in Step 2, the **Export Inclusion List Options** dialog box (Figure 18 on page 35) appears. Set the following parameters for selecting representative ions for each feature.

   a Mark **Most abundant** to use the most abundant ion for each feature.

   b Select the parameters for *Charge state*.

   c Select the parameters for *Adduct selection*.

   d Enter a **RT tolerance** in minutes or accept the default value of 0.10 minutes.

   e Click **OK**.

4 Enter a folder and name for the output file and click **Save**.

Familiarization Exercises

The Mass Profiler installation disc contains files for three sets of data files which you can use to become familiar with the main workflows of Mass Profiler: *2-Sample-Comparison*, *2-Sample-Comparison with LC/IM-MS data*, and 2-*Sample-Group-Comparison*.

Example A: 2-Sample-Comparison

The goal of this exercise is to use untargeted feature finding for the comparison of two samples and find and identify the compound differences between the samples. Four exercises are in this example:

- "Exercise 1. Create a project and run method" on page 38
- "Exercise 2. Edit post-alignment filtering parameters" on page 41
- "Exercise 3. Identify features" on page 43
- "Exercise 4. View feature details" on page 45

The folder **2-Sample-Comparison** contains the following files for this example:

- Method file **2-Sample_Comparison.mpm**
- **Fruit_Tea.d** (a raw data file from a fruit tea extract)
- **Fruit_Tea_1_10000.d** (a raw data file from the fruit tea extract spiked with a low concentration of four sulfa drugs)

Exercise 1. Create a project and run method

In this exercise you create a new project to view the example **2-Sample-Comparison** data files.

1. Copy the example files and folders to the appropriate MassHunter folders on your computer. By default, the **\MassHunter** folder is on the **D:** drive on acquisition systems, and on the **C:** drive on offline systems.
   a. Copy the method file **2-Sample_Comparison.mpm** in the **2-Sample-Comparison** folder to **\MassHunter\MassProfiler\Methods**.
   b. Create a folder named **2-Sample-Comparison** within the folder **\MassHunter\MassProfiler\Input Data**.

c Copy the **Fruit_Tea.d** and **Fruit_Tea_1_10000.d** data folders in the **2-Sample-Comparison** folder to **\MassHunter\MassProfiler\Input Data\2-Sample-Comparison**.

2 Double-click the **Mass Profiler** icon located on your desktop.

3 Create the example project.

a Click **File > Create Project** or the **Create Project** button. The **Create Project** dialog box is opened. The next steps populate the dialog box as shown in Figure 19.

b Verify that the **Number of groups** is set to **2**.

c Type `2-Sample-Comparison` for the **Project Name**.

d Select ***.d (MFE)** for the **Input data type**

e Click **Add** in the *Experiment* group section on the left.

f Navigate to the folder **\MassHunter\MassProfiler\Input Data\2-Sample-Comparison** in the **Folder Selector** dialog box.

g Select the data file **Fruit_Tea_1_10000.d** in the **Folder Selector** dialog box.

h Click **OK** to load the data file into the project.

i Click **Add** in the *Control* group section on the right.

j Select the data file **Fruit_Tea.d** in the **Folder Selector** dialog box.

k Click **OK** to load the data file into the project.

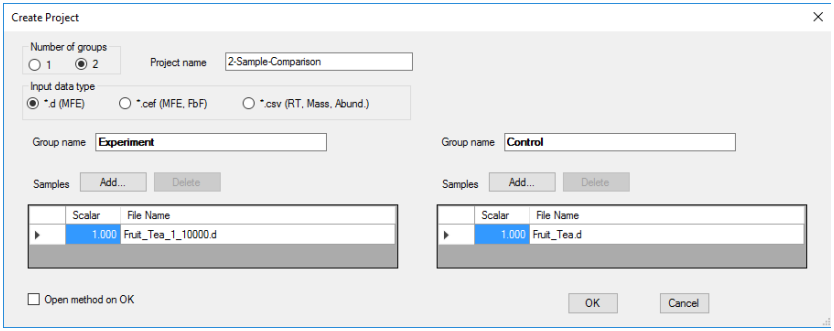


Figure 19 **Create Project** dialog box for the 2-Sample-Comparison project

l Clear **Open method on OK**.

m Click **OK** in the **Create Project** dialog box.

4 Click **Method > Load and Open Method** or the **Load and Open Method** button.

5 Select **2-Sample_Comparison.mpm** in the **Open** dialog box.

6 Click **Open**. The method is immediately loaded and opened for editing in the **Method Parameters - <method file name>** dialog box. The parameters are set as shown in Figure 20.

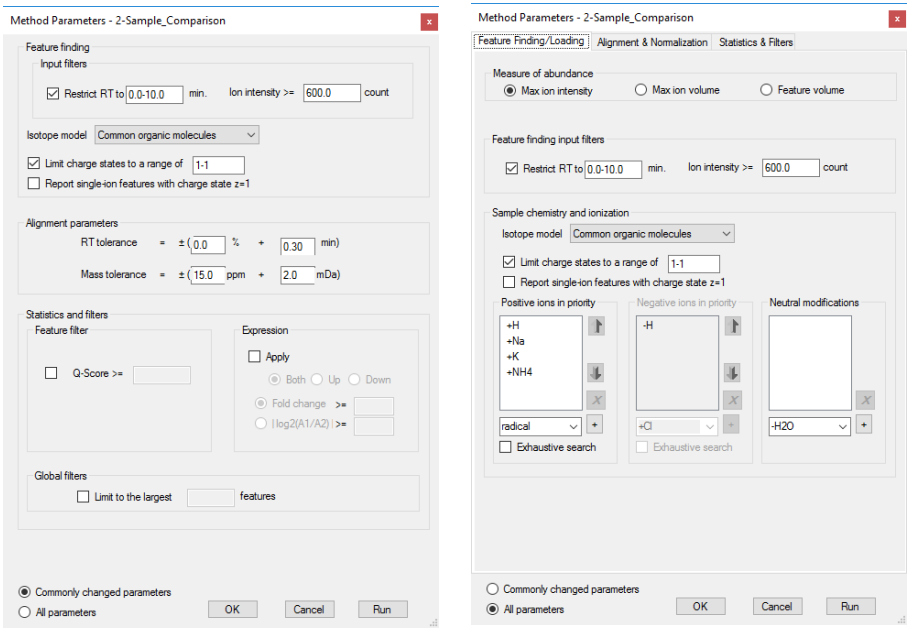


Figure 20 **Method Parameters** dialog box for 2-Sample_Comparison.mpm

7 Click **All parameters** and verify the following parameter settings in the *Feature Finding/Loading* tab:

- **a** *Measure of abundance* is set for **Max ion intensity**
- **b** *Feature finding input filters*, **Restrict RT to** is marked and set to `0.0-10.0` min. and **Ion intensity >=** is set to `600` counts.
- **c** *Sample chemistry and ionization* **Isotope model** is “Common organic molecules” and **Limit charge states to a range of** is marked and set to `1-1`.

8 Click **Run**.

Mass Profiler processes the project with the method parameters and updates the Feature Table and Feature Plot in the main window. The method finds 5,991 features, a large number of features, since no post-alignment filtering is applied.

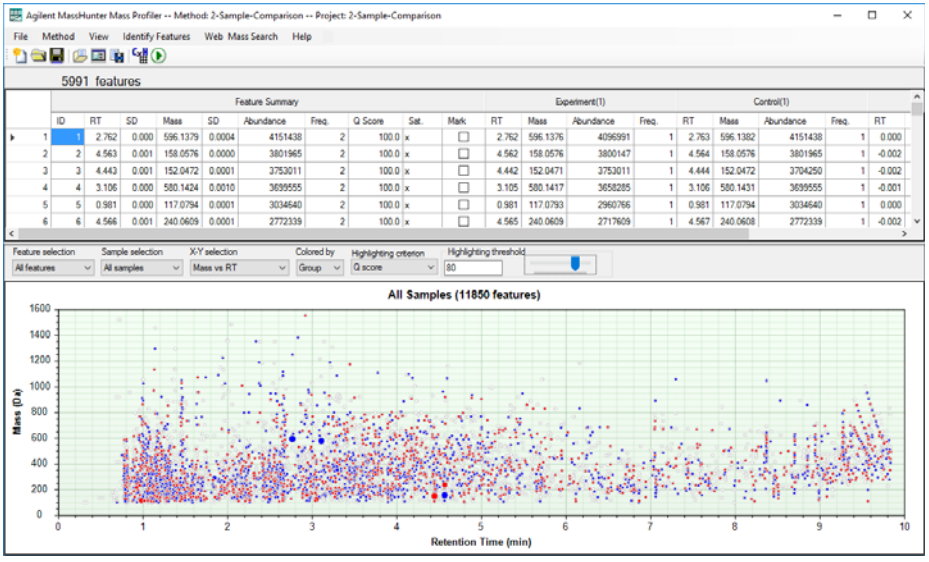


Figure 21 Adjusted view of the 2-Sample-Comparison project - 5,991 features are identified in the Feature Table. Since the features are not necessarily present in both groups, the Feature Plot shows a total of 11,850 features.

9 Adjust the parameters of the Feature Plot to obtain the view shown in Figure 21 and to become familiar with the Feature Plot parameters.

- **a** Select **All samples** for **Sample Selection**.
- **b** Select **Mass vs RT** for **X-Y selection**.
- **c** Select **Group** for **Colored by**.
- **d** Select **Q score** for **Highlighting criterion**.
- **e** Type `80` for **Highlighting threshold**.

Exercise 2. Edit post-alignment filtering parameters

In this exercise you adjust the post-alignment filtering parameters.

1 Click the **Edit Method** button or click **Method > Edit Method**.

2 Select the *Statistics & Filters* tab.

3 Mark **Q-Score >=**.

4 Type `100` for **Q-Score >=** in order to require the highest quality features.

5 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 5,991 to 1,561.

6 Click the **Edit Method** button or click **Method > Edit Method**.

7 Select the *Statistics & Filters* tab.

8 Mark **Apply** under the *Expression* section.

9 Click **Both** under the *Expression* section.

10 Type `4.0` for **Fold change >=** in the *Group difference* section.

11 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 1,561 to 4.

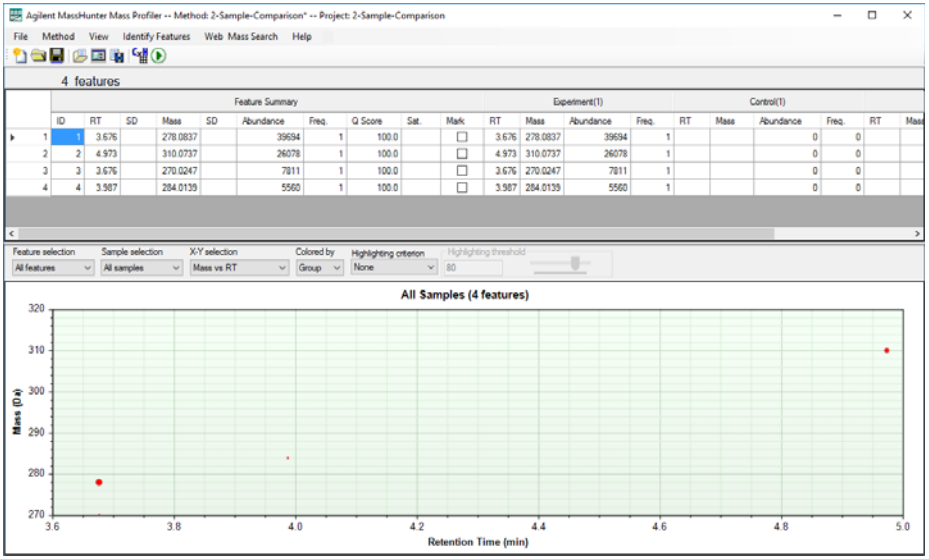


Figure 22 Reprocessed view of the 2-Sample-Comparison project - 4 features

Exercise 3. Identify features

In this exercise you identify the four features from Exercise 2 using ID Browser.

1 Click the **Identify Features > For All Features in Table**.

2 Clear **Molecular formula generator (MFG)** in the **Compound Identification Wizard** dialog box.

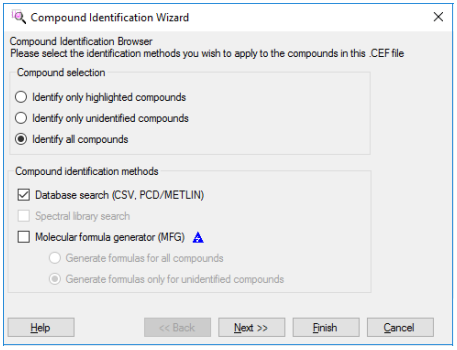


Figure 23 **Compound Identification Wizard** dialog box

3 Click **Next**.

4 Verify that the database path on the *Database* tab is set to **\MassHunter\PCDL\default.csv**.

5 Click **Finish**.

6 Verify that 4 out of the 4 compounds are identified as Sulfamethazine, Sulfadimethoxine, Sulfamethizole, and Sulfochloropyridazine with match scores from 77-95 (see Figure 24 on page 44)

7 Click **Save and Return** to return the identified feature information to Mass Profiler. Two new columns, **Name** and **Formula**, are created in the Feature Table and contain the compound names and formulas (see Figure 25 on page 44).

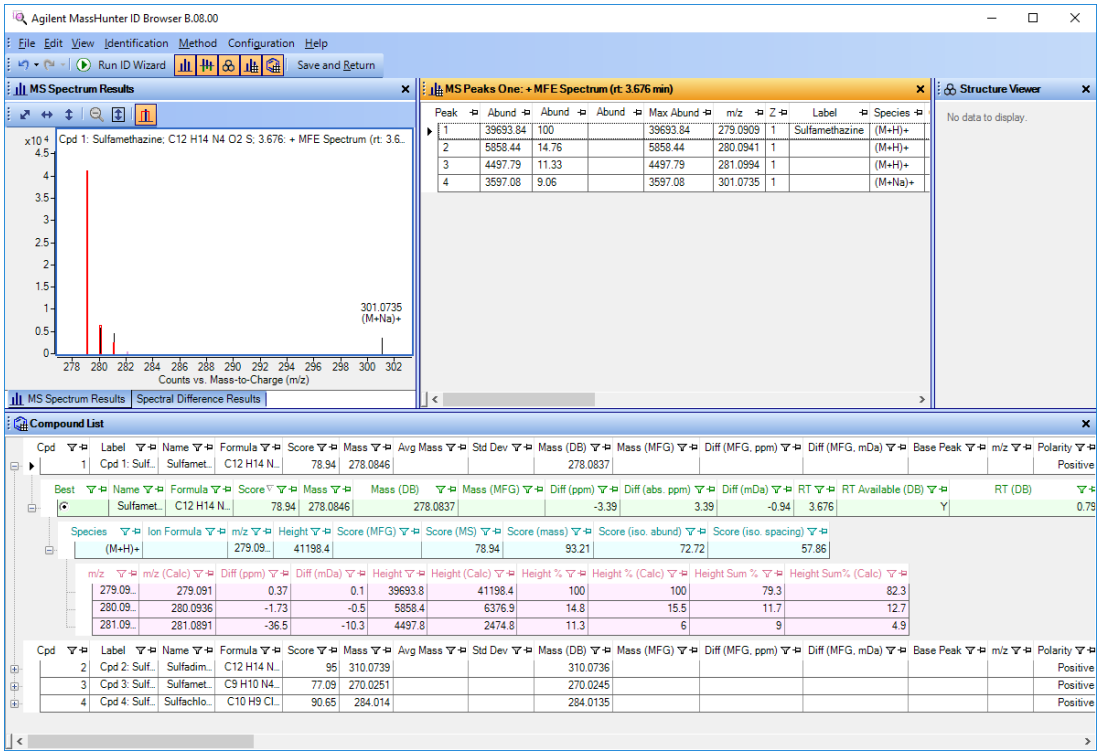


Figure 24 ID Browser results for the four differentially expressed features

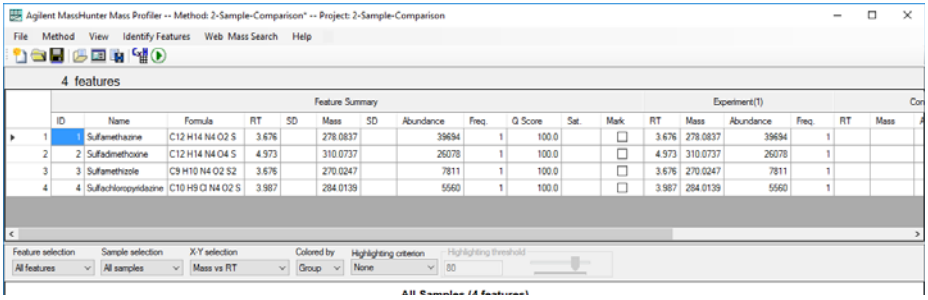


Figure 25 Identified features of the 2-Sample-Comparison project

Exercise 4. View feature details

In this exercise you review the feature details in the main window.

1 Click **View > Switch to Feature-Details Mode** to replace the Feature Plot with Feature Details.

2 Double-click on the row number of the first sulfa drug in the Feature Table. The Feature Details associated with this feature populate the lower half of the main window (see Figure 26).

The upper of the two new tables is titled *Feature 1 - All Samples*, and contains entries for the *Fruit_Tea_1_10000* data in red and *Fruit_Tea* data in blue. The lower table is titled *Fruit_Tea_1_10000* and shows three ions and the neutral mass of the feature calculated at 278.0837.

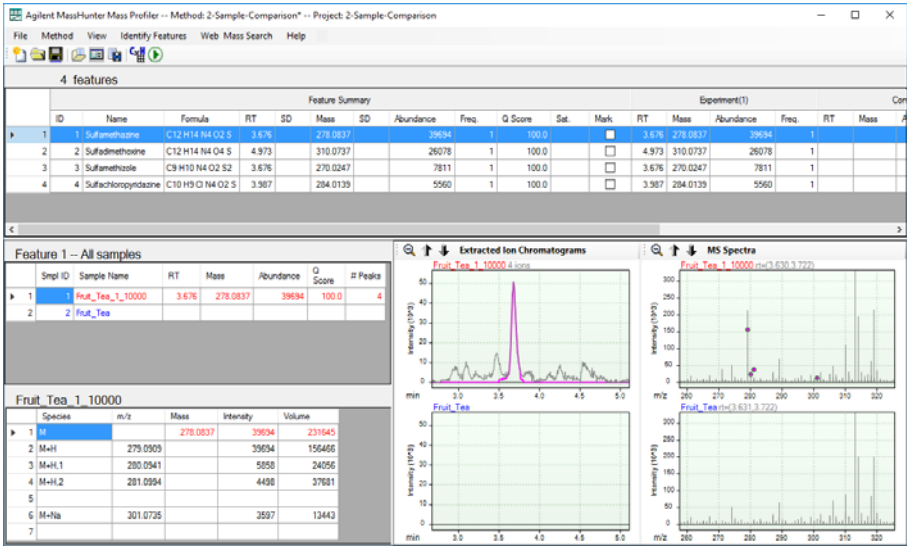


Figure 26 Feature Details view of the 2-Sample-Comparison project

3 Verify that on the right side of the Feature Details view two sets of chromatograms and mass spectra are shown. The chromatogram for *Fruit_Tea_1_10000* is on top and shows a chromatographic peak, which is not visible in the chromatogram of the *Fruit_Tea* sample. The average mass spectrum on the top has the four ions associated with the feature marked with red dots, while the lower spectrum does not.

4 Double-click on the row number of the first ion in the ion table. Observe how the chromatograms change when extracting the ion species individually (in this example “M+H,” “M+H,1,” “M+H,2,” and “M+Na”).

5 Repeat steps 2, 3, and 4 for the remaining three sulfa drugs. Explore the graphics using zoom and unzoom to compare the chromatogram and mass spectrum for each feature in detail.

6 Double-click the row number 2 in the **Feature Details by Sample** table to update the **Feature Details by Ion** table with the ions from the Fruit_Tea.d (control) sample.

7 Double-click on the row numbers in the ion table and observe how the chromatograms change when extracting the ion species individually.

8 Click **File > Save Project**. A copy of the most recent method parameters used to create the results is embedded with the project.

9 Review the project, Feature Table, Feature Plot, and Feature Details as described earlier in this guide in the sections: “View results in the Feature Table” on page 20, “View results in the Feature Plot” on page 21, and “View results in the Feature Details” on page 22.

Exercise B: 2-Sample-Group-Comparison

The goal of this exercise is to use untargeted feature finding for the comparison of two groups of samples and see if the experiment can differentiate the samples by grouping. Three exercises are in this example:

- “Exercise 1. Create 2-Sample-Group-Comparison project and run the method” on page 47
- “Exercise 2. Edit post-alignment filtering parameters” on page 49
- “Exercise 3. Analyze your groups using PCA” on page 51

The folder **2-Sample-Group-Comparison** contains the following:

- Folder **Control** that contains ten control data files and folder **Experiment** that contains ten experiment data files
- Method file **2-Sample-Group-Comparison.mpm**

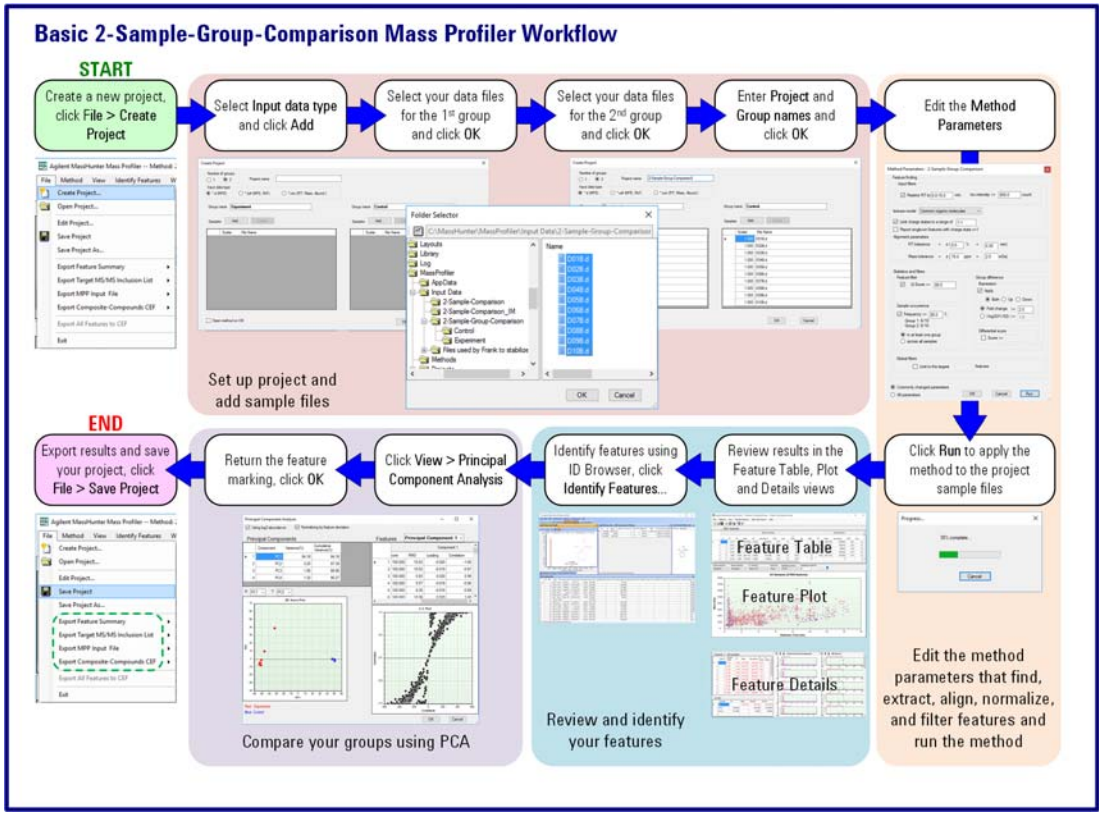


Figure 27 The basic Mass Profiler workflow for the 2-Sample-Group-Comparison project

Exercise 1. Create 2-Sample-Group-Comparison project and run the method

In this exercise you set up and process the example **2-Sample-Group-Comparison** project.

1 Copy the example files and folders to the appropriate MassHunter folders on your computer. By default, the **\MassHunter** folder is on the **D:** drive on acquisition systems, and on the **C:** drive on offline systems.

   a Copy the method file **2-Sample-Group-Comparison.mpm** to **\MassHunter\MassProfiler\Methods**.

   b Create a folder named **2-Sample-Group-Comparison** within the folder **\MassHunter\MassProfiler\Input Data**.

c Copy the folders **Control** and **Experiment** and their contents to **\MassHunter\MassProfiler\Input Data\2-Sample-Group-Comparison**.

2 Double-click the **Mass Profiler** icon located on your desktop.

3 Create the example project.

a Click **File > Create Project** or the **Create Project** button. The **Create Project** dialog box is opened.

b Verify that the **Number of groups** is set to 2.

c Type `2-Sample-Group-Comparison` for the **Project Name**.

d Select ***.d (MFE)** for the **Input data type**

e Click **Add** in the *Experiment* group section on the left.

f Navigate to the folder **\MassHunter\MassProfiler\Input Data\2-Sample-Group-Comparison\Experiment** in the **Folder Selector** dialog box.

g Select all ten data files in the **Folder Selector** dialog box.

h Click **OK** to load the data files into the project.

i Click **Add** in the *Control* group section on the right.

j Navigate to the folder **\MassHunter\MassProfiler\Input Data\2-Sample-Group-Comparison\Control** in the **Folder Selector** dialog box.

k Select all ten data files in the **Folder Selector** dialog box.

l Click **OK** to load the data files into the project.

m Clear **Open method on OK**. For this exercise a previously created example method is used to process the data files.

n Click **OK**.

4 Load and edit the example method.

a Click the **Load and Open Method** button or click **Method > Load and Open Method**.

b Select **2-Sample-Group-Comparison.mpm**.

c Click **Open**.

d Verify **Commonly changed parameters** is selected at the bottom of the dialog box.

e Verify that *Feature filter* **Q-Score >=** is cleared.

f Verify that *Sample occurrence* **Frequency >=** is cleared.

5 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features found is 14,057 (see Figure 28). This is a large number of features because no post-alignment filtering is set.

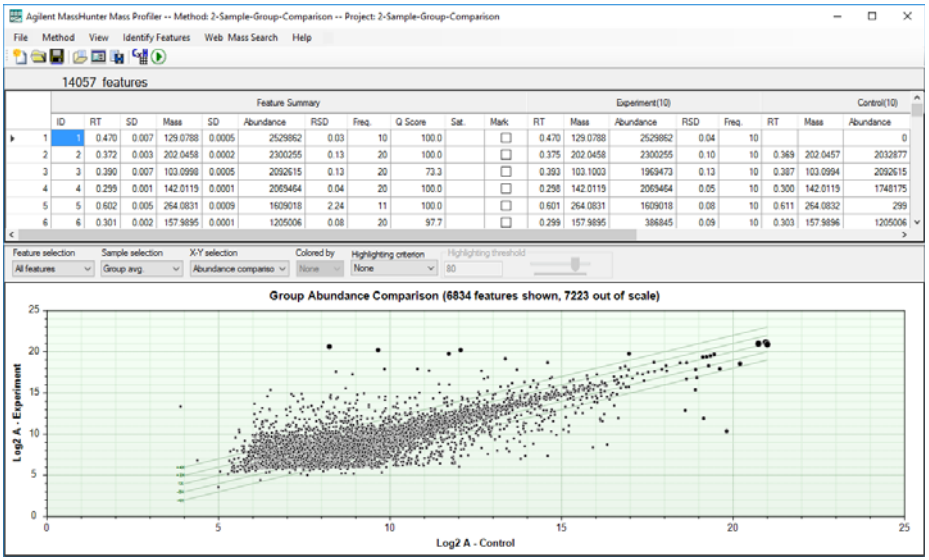


Figure 28 Initial view of the 2-Sample-Group-Comparison project - 14,057 features

Exercise 2. Edit post-alignment filtering parameters

In this exercise you adjust the post-alignment filtering parameters.

1 Click the **Edit Method** button or click **Method > Edit Method**.

2 Mark *Feature filter* **Q-Score >=**.

3 Type 80 for **Q-Score >=**.

4 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 14,057 to 7,005.

5 Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.

6 Mark *Sample occurrence* **Frequency >=**.

7 Type `80` for **Frequency >=**.

8 Click **in at least one group**, this requires that a feature is present in at least 80% of the samples in at least one group.

9 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 7,005 to 4,658.

10 Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.

11 Mark **Apply** under the *Expression* section.

12 Click **Both** under the *Expression* section.

13 Type `2.0` for **Fold change >=** in the *Abundance difference, Group abundance* section.

14 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 4,658 to 2,941.

15 Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.

16 Click **Up** under the *Expression* section.

17 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 2,941 to 2,227.

18 Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.

19 Click **Down** under the *Expression* section.

20 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 2,227 to 714.

21 Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.

22 Click **Both** under the *Expression* section.

23 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features increases from 714 back to 2,941 as shown in Figure 29.

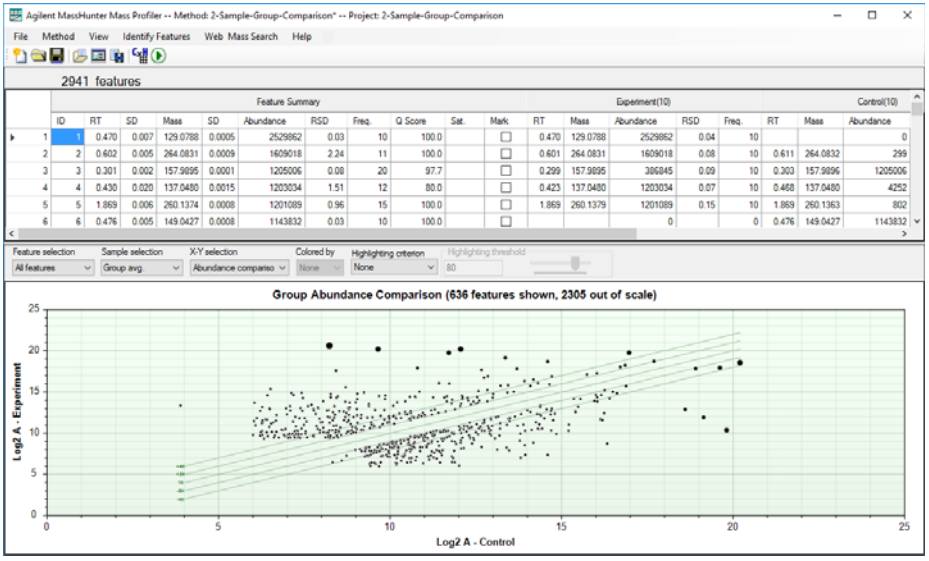


Figure 29 2-Sample-Group-Comparison project with 2,941 features

Exercise 3. Analyze your groups using PCA

In this exercise you perform a principal component analysis on the 2,941 features from "Exercise 2. Edit post-alignment filtering parameters". The goal of the PCA analysis in this exercise is to see whether the features allow the sample groups to be differentiated and then identify a subset of abundant features that account for the differentiation.

1 Click **View > Principal Component Analysis > For All Features in Table**. Since the method applied to the project has been optimized to reduce the occurrence of features that are less significant, as illustrated by the Feature

Plot shown in Figure 29, all of the features in the Feature Table are analyzed using PCA.

2 Review the PCA results (Figure 30) to become familiar with the panes and actions available in the **Principal Component Analysis** dialog box. See "Perform a PCA analysis" on page 13 for more information.

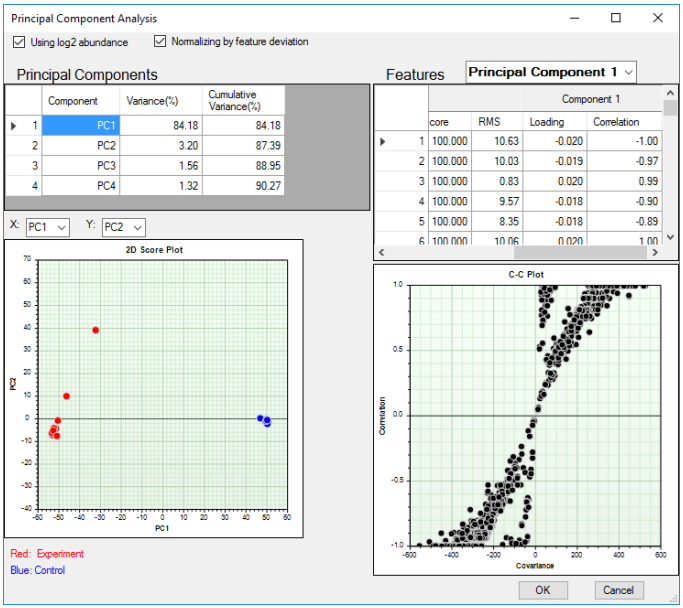


Figure 30 Initial PCA for the project after filtering the features in Exercise 2

3 Clear **Normalizing by feature deviation** in the **Principal Component Analysis** dialog box.

4 Clear **Using log2 abundance** in the **Principal Component Analysis** dialog box.

The choice of whether to use **Normalizing by feature deviation** or **Using log2 abundance** depends on where you publish your results.

In the initial PCA analysis (Figure 30) the cumulative variance accounted for by the first four principal components is 84.18%, 87.39%, 88.95%, and 90.27%.

By removing the abundance normalization options (Figure 31 on page 53) the variance accounted for by the first principal component increases and the cumulative variance improves to 91.27%, 94.43%, 96.95%, and 97.86%.

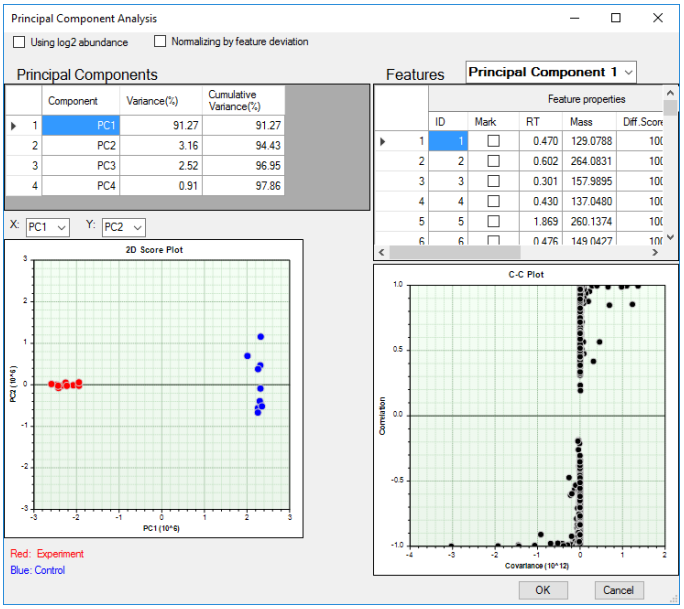


Figure 31 PCA for the project after clearing **Using log2 abundance** and **Normalizing by feature deviation**

5 Click the **Diff. Score** column heading two times to sort the **Feature: Principal Component 1** feature table by decreasing score. Note that 2,922 of the 2,941 features have a Diff. Score greater than or equal to 90.

6 Mark the features that have the greatest correlation and covariance in the C-C Plot:

- **a** Right-click and select **Unzoom** in the C-C Plot.
- **b** Right-click and select **Mark Features in Zoomed Area** (Figure 33 on page 54, left). Notice that all of the features are marked in the feature table above the C-C- Plot.
- **c** Click and drag the pointer from approximately (-0.5, -1) to (0.5, 1) in the C-C Plot as shown in Figure 32 on page 54. Since drawing the zoom window to exact values is not very accurate your numbers in the next step can be different.

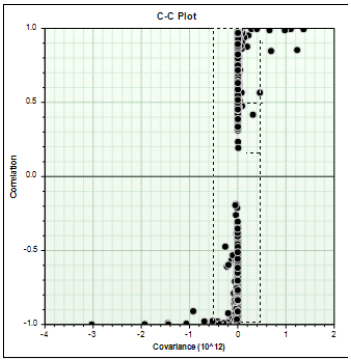


Figure 32 Zoom in on the C-C Plot

d Right-click and select **Clear Marks for Features in Zoomed Area** (Figure 33, right).

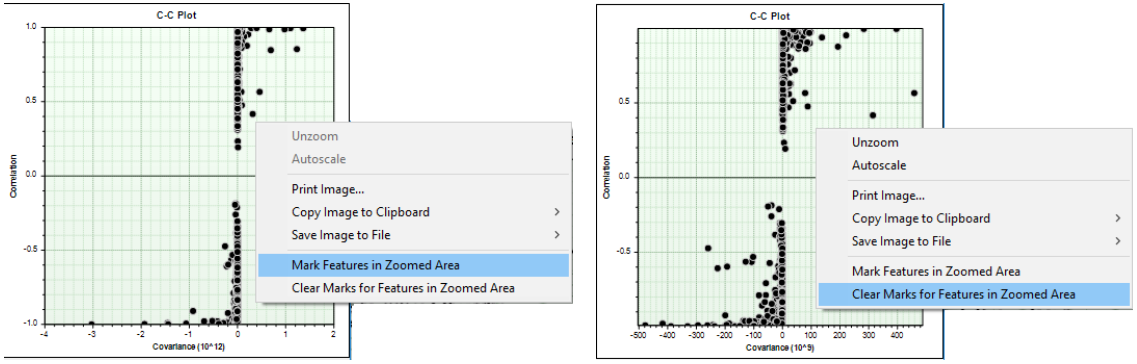


Figure 33 Using the C-C Plot to mark all features (left) and then clear the features that have less correlation and covariance (right).

7 Click the **Mark** column heading to sort the **Feature: Principal Component 1** feature table by mark. Note that about 20 of the 2,941 features are marked and account for a covariance greater than ~0.5 and a correlation greater than ~1.0, and note that these feature are not necessarily just the features with the greatest Diff. Score.

8 Click **OK** to return the *PCA Mark* information to the Feature Table in the main window.

9 Click **View > Principal Component Analysis > For PCA Marked Features in Table**. The principal component analysis is rerun on the features selected from the initial PCA analysis with results that show improved group separation.

By removing the abundance normalization (Figure 34) and the use of the log2 abundance, the variance accounted for by the first principal component increases and the cumulative variances for the four Principal Components increase to 96.00%, 98.27%, 99.29%, and 99.67% compared to the variances in step 4 on page 52.

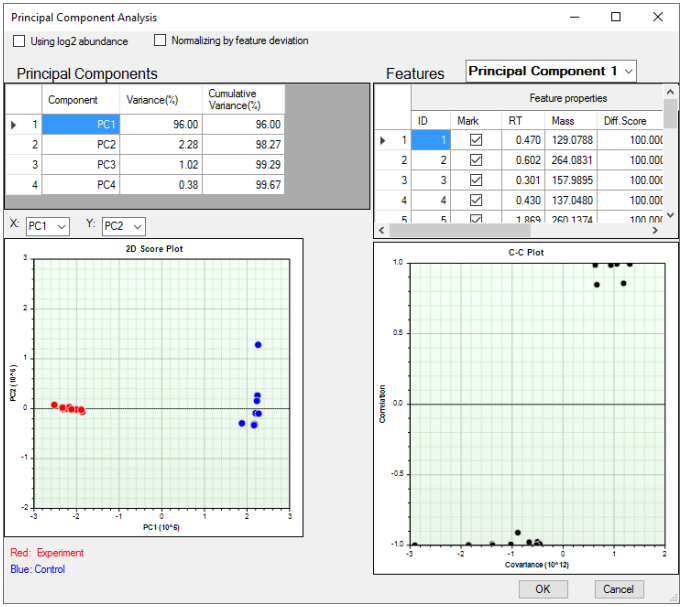


Figure 34 PCA for the PCA Marked features after clearing **Using log2 abundance** and **Normalizing by feature deviation**

10 Click **OK** to return to the main window.

11 Review the project, Feature Table, Feature Plot, and Feature Details as described earlier in this guide in the sections: "View results in the Feature Table" on page 20, "View results in the Feature Plot" on page 21, and "View results in the Feature Details" on page 22.

12 Click **File > Export Composite-Compounds CEF > From Table - PCA Marked Features** to save the features you marked in the PCA as a CEF file. See "Export Mass Profiler Data" on page 33 for additional information.

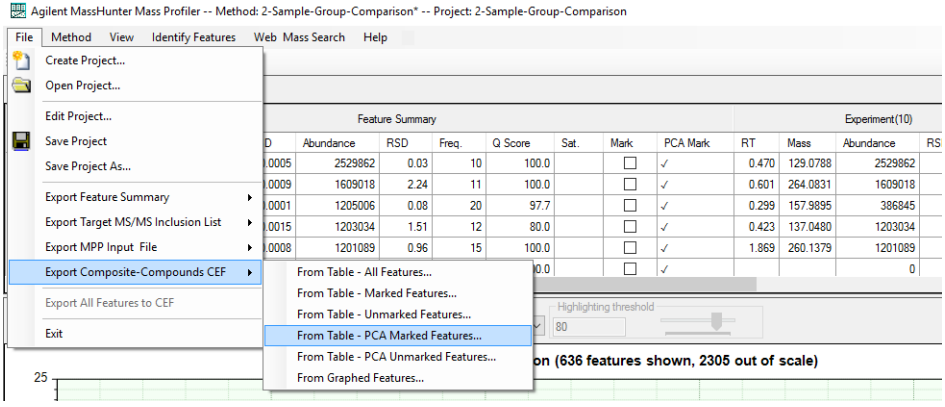


Figure 35 Export PCA marked features from the Feature Table

Example C: 2-Sample-Comparison_IM of IM-MS data

The goal of this exercise is to use untargeted feature finding for the comparison of two samples acquired using an LC/IM-MS and find and identify the compound differences between the samples. The sample data files are similar to that used in Example A. Four exercises are in this example:

- "Exercise 1. Create a project and run method" on page 57
- "Exercise 2. Edit post-alignment filtering parameters" on page 60
- "Exercise 3. Identify features" on page 61
- "Exercise 4. View feature details" on page 63

The folder **2-Sample-Comparison_IM** contains the following files for this example:

- Method file **2-Sample_Comparison_IM.mpm**
- **Fruit_Tea_IM.d** (a raw data file from a fruit tea extract)
- **Fruit_Tea_1_1000_IM.d** (a raw data file from the fruit tea extract spiked with a low concentration of four sulfa drugs)

Exercise 1. Create a project and run method

In this exercise you create a new project to view the example **2-Sample-Comparison_IM** data files that were acquired using an LC/IM-MS.

1. Copy the example files and folders to the appropriate MassHunter folders on your computer. By default, the **\MassHunter** folder is on the **D:** drive on acquisition systems, and on the **C:** drive on offline systems.
   a. Copy the method file **2-Sample_Comparison_IM.mpm** in the **2-Sample-Comparison** folder to **\MassHunter\MassProfiler\Methods**.
   b. Create a folder named **2-Sample-Comparison_IM** within the folder **\MassHunter\MassProfiler\Input Data**.
   c. Copy the **Fruit_Tea_IM.d** and **Fruit_Tea_1_1000_IM.d** data folders in the **2-Sample-Comparison** folder to **\MassHunter\MassProfiler\Input Data\2-Sample-Comparison_IM**.
2. Double-click the **Mass Profiler** icon  located on your desktop.
3. Create the example project.
   a. Click **File > Create Project** or the **Create Project**  button. The **Create Project** dialog box is opened.
   b. Verify that the **Number of groups** is set to 2.
   c. Type `2-Sample-Comparison_IM` for the **Project Name**.
   d. Select ***.d (MFE)** for the **Input data type**
   e. Click **Add** in the *Experiment* group section on the left.
   f. Navigate to the folder **\MassHunter\MassProfiler\Input Data\2-Sample-Comparison_IM** in the **Folder Selector** dialog box.
   g. Select **Fruit_Tea_1_1000_IM.d** in the **Folder Selector** dialog box.

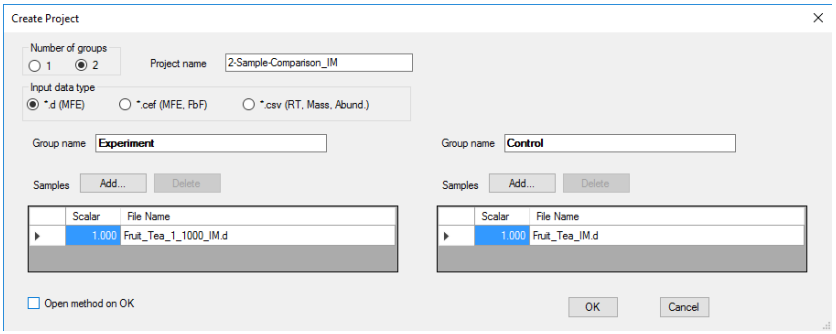


Figure 36 **Create Project** dialog box for the 2-Sample-Comparison_IM project

h Click **OK** to load the data file into the project.

i Click **Add** in the *Control* group section on the right.

j Select **Fruit_Tea_IM.d** in the **Folder Selector** dialog box.

k Click **OK** to load the data file into the project.

l Clear **Open method on OK**. For this exercise a previously created example method is used to process the data files.

m Click **OK**.

4 Load and edit the example method.

a Click the **Load and Open Method** button or click **Method > Load and Open Method**.

b Select **2-Sample_Comparison_IM.mpm**.

c Click **Open**.

d Verify **Commonly changed parameters** is selected at the bottom of the dialog box.

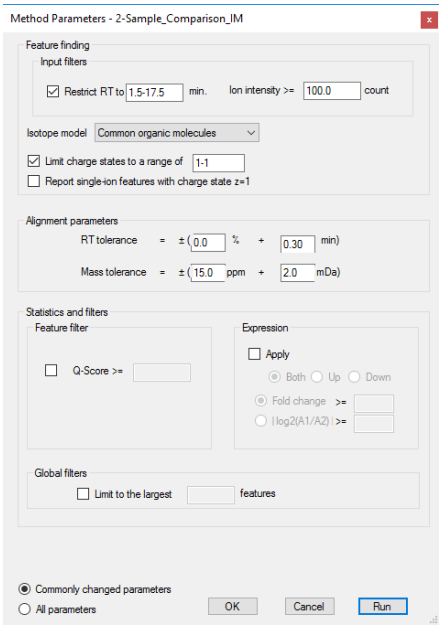


Figure 37 **Method Parameters** dialog box for 2-Sample_Comparison_IM.mpm

e Verify that *Input filters* **Restrict RT to** is marked and set to 1.5 to 17.5 to exclude the regions before the injection peak and when the column is flushed with high organic mobile phase.

f Verify that *Input filters* **Ion Intensity >=** is set to 100. For LC/IM-MS data the minimum ion intensity is set to a lower value (100 is the default).

g Click **All parameters** at the bottom of the dialog box.

h Verify that **Max ion volume** is selected for the *Measure of abundance* on the *Feature Finding/Loading* tab. Maximum ion volume is the preferred measure of abundance for LC/IM-MS data and the default setting. Summing the ion intensities across *m/z*, DT, and RT improves the dynamic range of feature finding.

i Click **Commonly changed parameters** at the bottom of the dialog box.

5 Click **Run**.

Mass Profiler processes the project with the method parameters and updates the Feature Table and Feature Plot in the main window. The number of features found is 41,919 (see Figure 38 on page 60). This is a large number of features because no post-alignment filtering is set.

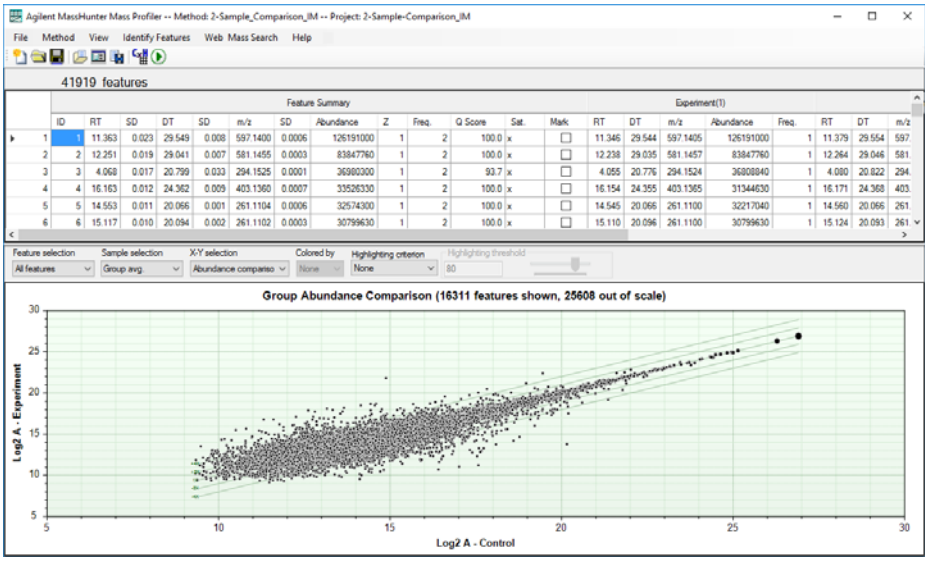


Figure 38 Initial view of the 2-Sample-Comparison_IM project - 41,919 features are identified in the Feature Table.

Exercise 2. Edit post-alignment filtering parameters

In this exercise you adjust the post-alignment filtering parameters.

1. Click the **Edit Method** button or click **Method > Edit Method**.
2. Mark *Feature filter* **Q-Score >=**.
3. Type `100` for **Q-Score >=**.
4. Click **Run**.

   Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 41,919 to 1,733.
5. Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.
6. Mark **Apply** under the *Expression* section.
7. Click **Both** under the *Expression* section.
8. Type `4` for **Fold change >=**.
9. Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 1,733 to 22.

10 Click the **Edit Method** button or click **Method > Edit Method** to re-open the Method Editor window.

11 Click **Up** in the *Expression* section.

12 Click **Run**.

Mass Profiler reprocesses the project with the changed parameters and updates the Feature Table and Feature Plot in the main window. The number of features decreases from 22 to 12 (see Figure 39).

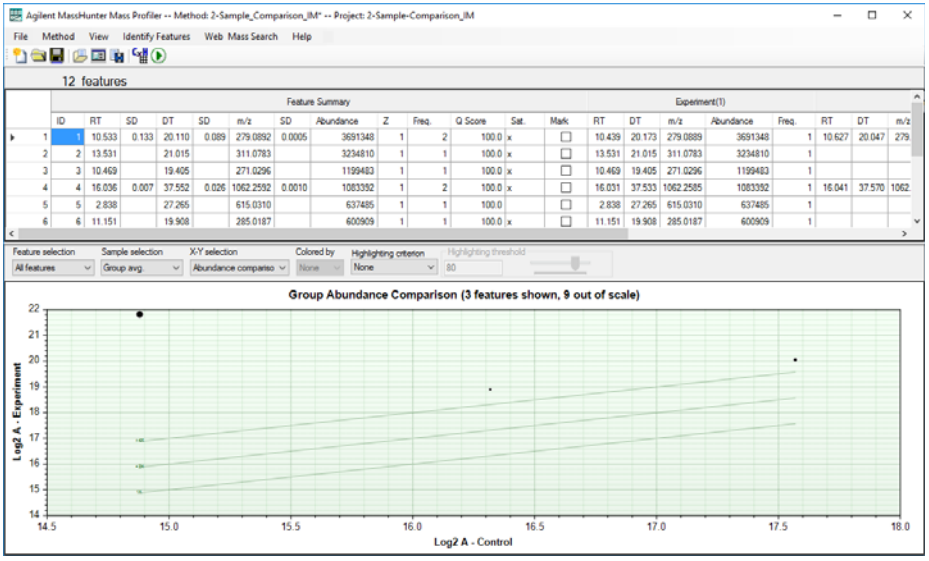


Figure 39 Reprocessed view of the 2-Sample-Comparison_IM project - 12 features

Exercise 3. Identify features

In this exercise you identify the twelve features from Exercise 2 using ID Browser. IMFE features from LC/IM-MS data files can be identified via Accurate Mass Database Search and/or Molecular Formula Generator in ID

Browser. In this example, identification is done via accurate mass and optional retention time, but without drift time or collisional cross section (CCS) consideration.

1 Click the **Identify Features > For All Features in Table**.

2 Clear the **Molecular formula generator (MFG)** mark in the **Compound Identification Wizard** dialog box.

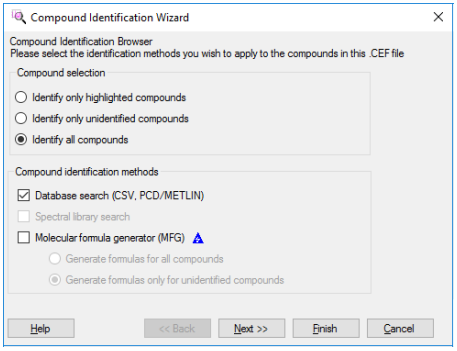


Figure 40 **Compound Identification Wizard** dialog box

3 Click **Next**.

4 Type `15` for the mass *Match tolerance* in **ppm** on the *Search Criteria* tab.

5 Verify that the database path on the *Database* tab is set to **\MassHunter\PCDL\default.csv**.

6 Click **Finish**.

7 Verify that 4 out of the 12 compounds are identified as Sulfamethazine, Sulfadimethoxine, Sulfamethizole, and Sulfochloropyridazine (see Figure 41 on page 63)

8 Click **Save and Return** to return the identified feature information to Mass Profiler. Two new columns, **Name** and **Formula**, are created in the Feature Table and contain the compound names and formulas (see Figure 42 on page 63).

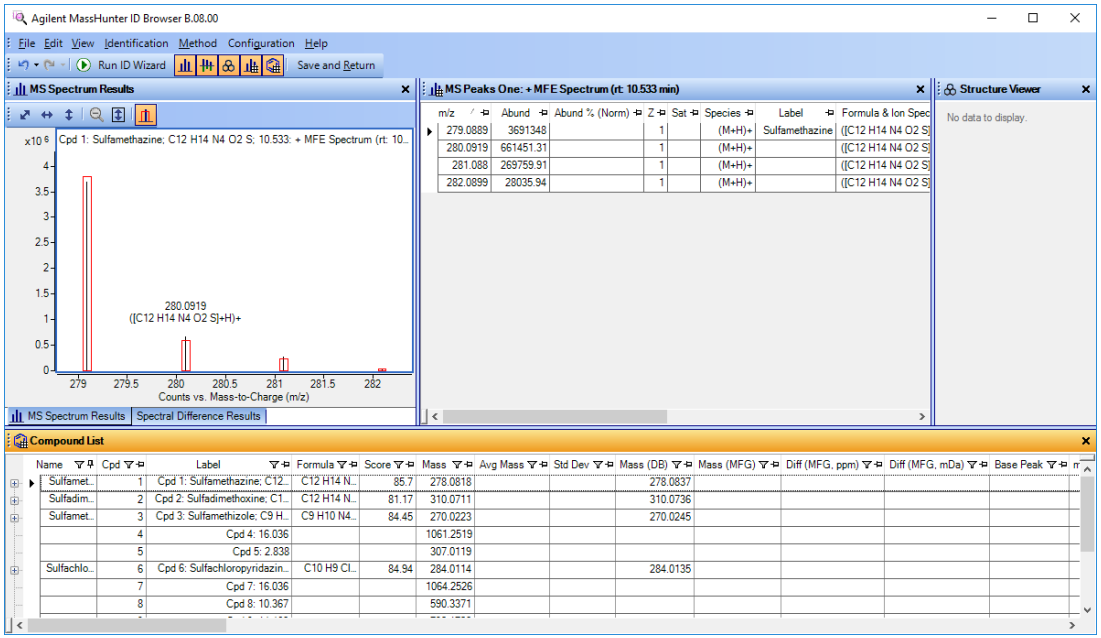


Figure 41 ID Browser results for the 2-Sample-Comparison_IM project

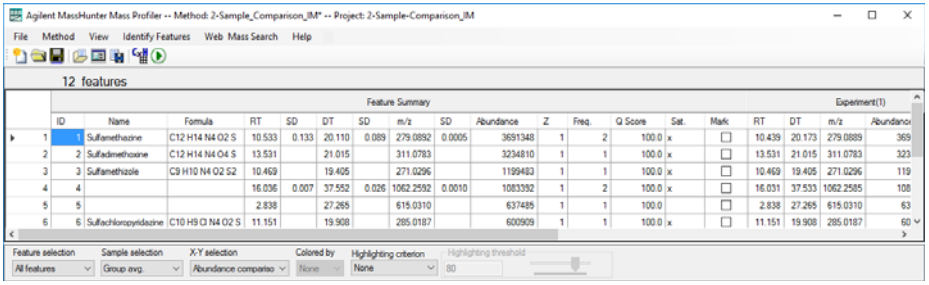


Figure 42 Identified features of the 2-Sample-Comparison_IM project

Exercise 4. View feature details

In this exercise you review the feature details in the main window.

1 Click **View > Switch to Feature-Details Mode** to replace the Feature Plot with Feature Details.

2 Double-click on the row number of the sulfochloropyridazine feature in the Feature Table. The Feature Details associated with this feature populate the lower half of the main window (see Figure 43).

The upper of the two new tables is titled *Feature 1 - All Samples*, and contains entries for the *Fruit_Tea_1_1000_IM* data in red and *Fruit_Tea_IM* data in blue. The lower table is titled *Fruit_Tea_1_1000_IM* and shows five ions. For LC/IM-MS data a neutral mass is not calculated, since differently adducted or charged ion clusters are not combined. The ions are presented with both the drift time and retention time.

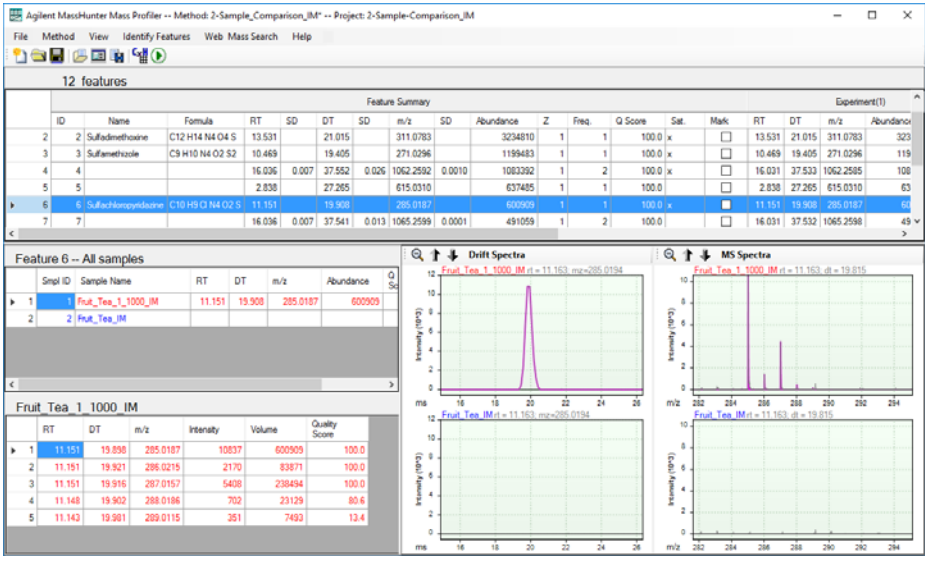


Figure 43 Feature Details view of the 2-Sample-Comparison_IM project

3 Verify that on the right side of the Feature Details view two sets of drift spectra and mass spectra are shown. The drift spectra for *Fruit_Tea_1_1000_IM* is on top.

4 Click **File > Save Project**. A copy of the most recent method parameters used to create the results is embedded with the project.

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In this guide

The *Agilent G3297AA MassHunter Mass Profiler Software - Familiarizaton Guide* presents instructions to learn to use the MassHunter Mass Profiler Software.

This guide is valid for the B.08.00 revision or higher of MassHunter Mass Profiler Software, until superseded.

Revision A, December 2016

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Agilent Technologies