

Agilent MassHunter Optimizer

Automated MS Method Development Software

Quick Start Guide

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Where to find more information

Use the online help for in-depth information not included in this *Quick Start Guide*.

Display online help by pressing the **F1** key to get more information about a window, tab, or dialog box.

What is MassHunter Optimizer?

Where to find more information

What is MassHunter Optimizer?

Automated MS Method Development Software

MassHunter Optimizer lets you automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) on a triple quad mass spectrometer instrument for each individual compound analyzed. Specifically, it automates the selection of the best precursor ions, the optimization of the fragmentor voltage for each precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify.

Automated Optimization

Automation of the following optimization tasks results in a significant time saving over manual optimization procedures:

- Selection of optimum precursor ion via rules, such as M+H, M+Na, M+K, and M+NH₄.
- Optimization of fragmentor voltage to maximize precursor ion intensity for up to 4 precursor ions (adducts and/or charge states) for each polarity in MS SIM
- Selection of multiple best product ions (MS/MS full scan)
- Optimization of collision energy to maximize product ion intensity (MS/MS full scan)
- Optionally perform fine fragmentor optimization for maximum sensitivity.
- Ion breakdown profile shows you a graphical representation of the optimization results and lets you select the optimum collision energy that work for your selective product ions.

What is MassHunter Optimizer?

Where to find more information

Additional Features

- Stores optimum precursor and product ions and parameters in a compound database.
- Imports optimization results into the Agilent MassHunter Data Acquisition for Triple Quad program.
- Lets you import compounds for optimization and update compound retention times from Data Acquisition methods
- Supports sample introduction via manual infusion (syringe pump), automatic infusion (via loop injection at lower flows), and injection analysis with column (to maximize optimization yield).
- Data that is acquired during the optimization run can be displayed with Agilent MassHunter Qualitative Analysis program.

NOTE

You can optimize other source and ion funnel parameters using the Agilent Ion Funnel and Source Optimization program. For more information, see *online Help* and the *Familiarization Guide* for the MassHunter Data Acquisition program.

Startup

To open Optimizer

Startup

MassHunter Optimizer is automatically installed with the MassHunter Data Acquisition program. Two versions of MassHunter Optimizer are installed on your system: one for small molecules and one for peptides.

To open Optimizer

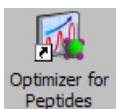
Open MassHunter Optimizer in *either* of the following ways:



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

To open Optimizer for Peptides

A version of MassHunter Optimizer is available for optimizing peptides. Open Optimizer for Peptides in *either* of the following ways:



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

NOTE

Projects are specific to the version of Optimizer that you use to create them. In other words, projects created with Optimizer for Peptides *cannot* be opened in MassHunter Optimizer and vice versa.

User Interface

To open Optimizer for Peptides

User Interface

The MassHunter Optimizer has the following user interface elements that let you enter parameters, set options, view status, and perform functions:

- **“Menus and Toolbar”** on page 6
- **“Status Bar”** on page 8

Tabs

- **“Optimizer Setup Tab”** on page 8
- **“Precursor Ion Selection Tab”** on page 9
- **“Product Ion Selection Tab”** on page 9
- **“Compound Setup Tab”** on page 9

Other Windows and Dialog Boxes

- **“Peptide Fragment Ion Predictor Dialog Box”** on page 10
- **“Database Browser Window”** on page 10
- **“Ion Breakdown Profile Dialog Box”** on page 10

Menus and Toolbar

The following menu items and toolbar buttons are available in MassHunter Optimizer main window.

File menu



New Project Clears the current settings and lets you set up a new project for use in MassHunter Optimizer. See **“To create a project”** on page 13.



Open Project Lets you open a project. See **“To open a project”** on page 13.



Save Project Lets you save changes to the current project.



Save As Project Lets you save the current project under a new name.



Save Compounds Lets you save compound information to a database, for use with other projects. See **“To save a database in Database Browser”** on page 30.



Delete Projects Lets you select projects and remove them.

Open Database Lets you open a different database. The newly opened database becomes the default database.

Exit Closes the MassHunter Optimizer window.

Edit menu

Copy Copies the selected rows, which can then be pasted into another position in the table.

Cut Cuts the selected rows from the table, which can then be pasted into another position in the table.

Paste Pastes the most recently copied or cut rows into the selected position in the table.

Delete Removes the selected rows from the table

Select All Selects all rows in the table.

User Interface

Menus and Toolbar

Fill Copies the value in the top cell down to the rest of the cells of a highlighted block or to the entire column.



Clear Results Lets you clear all fragmentor values, all collision energy values, or all results, including methods and all precursor and product ions for all compounds in the selected row and below in the table.

View menu

Show/Hide Columns Lets you select which columns of information to display in the table. At least one column in every row must be displayed.

Expand/Collapse All Rows Lets you display all rows in the table or collapse the table to show only Compound rows. This affects the currently selected row and all rows below it in the table.

Import/Export menu



Import from Database Opens Database Browser, which lets you select compounds for optimization from the database. See **“Database Browser Window”** on page 10.



Import from Excel Lets you import compounds from an Excel file. See **“To import compounds from an Excel file”** on page 23.



Import from Acquisition Methods Lets you import all compounds from Acquisition methods into the current project.



Export to Excel Lets you export the current compound list for use in Excel. See **“To export compound information to an Excel file”** on page 24.

Optimization menu



Start Optimization Starts the optimization process with the current parameters. The results are saved to the database and displayed in the Compound Setup tab. A report is automatically printed on completion. See **“To start optimization”** on page 40



Ion Breakdown Profile Runs optimization with the current parameters and displays the results in the Ion Breakdown Profile dialog box when optimization is complete. Collision energy values can be selected from the graphs, instead of using those determined by optimization. See **“To start optimization and display ion breakdown profiles”** on page 41.



Stop Optimization Lets you terminate optimization before completion.

User Interface

Status Bar

Tools menu



Update RT Lets you update compound retention times from Data Acquisition methods. See **“To update retention times from a MassHunter Data Acquisition method”** on page 25.

The following items only appear in Optimizer for Peptides.



Extract Peptide Opens the Peptide Fragment Ion Predictor dialog box, which lets you extract peptide sequences for optimization from a selected MassHunter Qualitative Analysis method. See **“Peptide Fragment Ion Predictor Dialog Box”** on page 10.



Convert .csv to .psq Lets you convert multiple peptide sequences with or without modification into multiple **.psq** files that can be imported into the MassHunter Qualitative Analysis program. See **“To convert multiple peptide sequences for import into MassHunter Qualitative Analysis”** on page 38.

Help menu

Help Topics Opens the online help window with the **Contents** tab displayed.

About Optimizer Displays version information for Optimizer.

Status Bar

The following items appear on the status bar at the bottom of Optimizer main window: Database, Project Name, Optimizer Status, Current Record progress bar, and All Records progress bar.

Optimizer Setup Tab

Lets you set the acquisition parameters used for optimization, including sample introduction method, Acquisition methods, and ranges for optimization of fragmentor and collision energy voltages. See **“To set Optimizer acquisition parameters”** on page 15.

Precursor Ion Selection Tab

Lets you select adduct species to include in the search for precursor ions. The default is **+H** for positive and **-H** for negative. See **“To select precursor ions”** on page 16.

Product Ion Selection Tab

Lets you set criteria to use in the search for product ions. See **“To select product ions”** on page 17.

Compound Setup Tab

Displays the list of compounds to optimize, and lets you enter precursor and product ion values, if known. You can also set different injection volumes for each compound to use for optimization, if needed. It also displays the results of optimization, when you mark the **Show results summary** check box. Sequences rather than compounds are used for optimization in Optimizer for Peptides.

Peptide Fragment Ion Predictor Dialog Box

Available in Optimizer for Peptides only.



This lets you select peptide sequences for optimization from a MassHunter Qualitative Analysis method. The selected sequences are added to the Compound Setup tab. After you click **Extract Peptide** shown at the left, you are prompted to select the method of interest. See **“To import peptide sequences from a MassHunter Qualitative Analysis method”** on page 34.

Database Browser Window



This window appears when you click **Import from Database**. It lets you select compounds for optimization from the database. The selected compounds are added to the Compound Setup tab. See **“To import compounds from a database”** on page 27.

Database Browser also lets you open a different database, save the current database with a new name, and make the current database the default database.

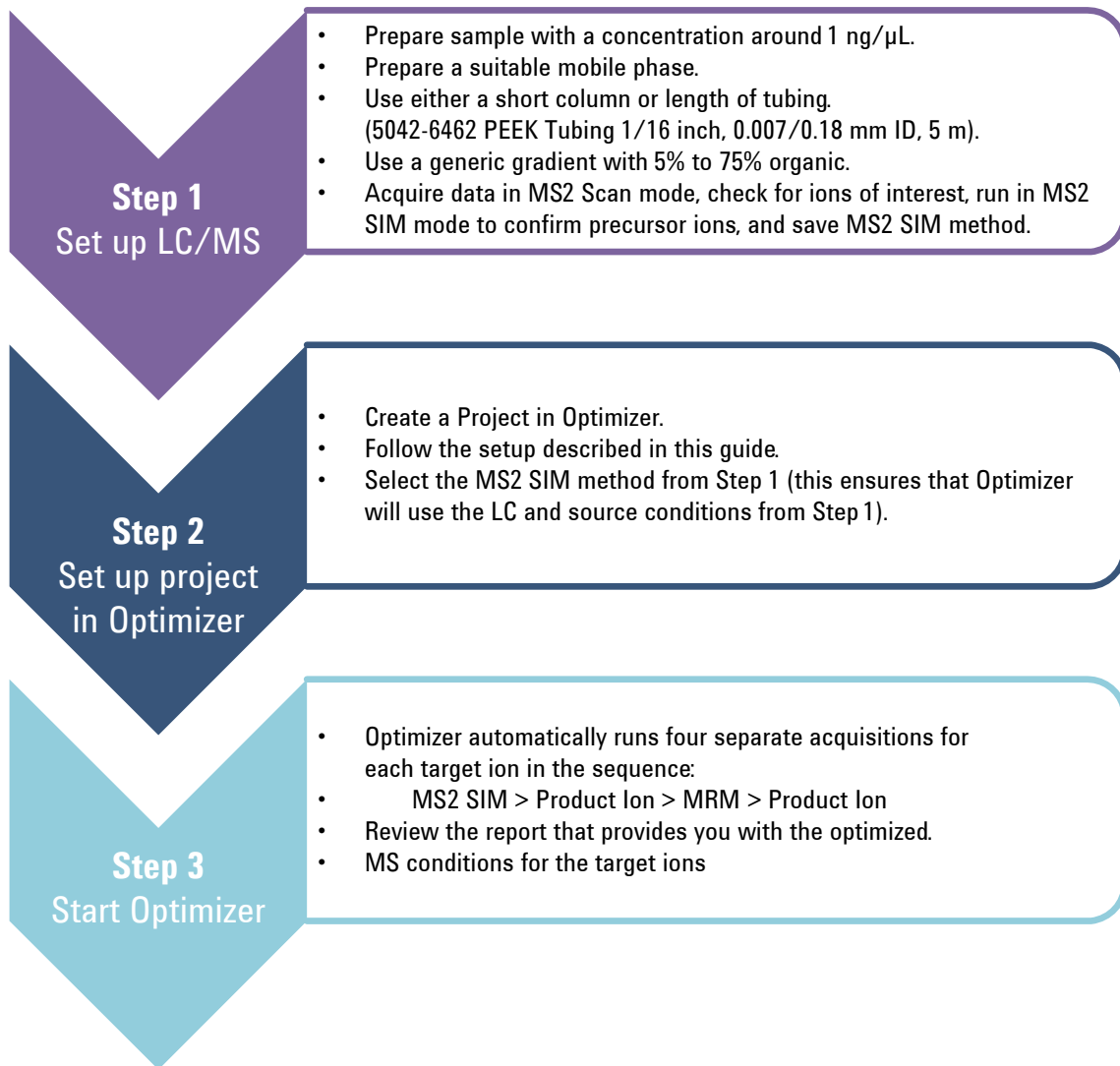
Ion Breakdown Profile Dialog Box



This dialog box automatically appears after optimization is complete when you click **Ion Breakdown Profile**. The product ion profiles determined by optimization are displayed and you can manually select collision energy values for each ion to replace the results of optimization. See **“To start optimization and display ion breakdown profiles”** and **“To manually set collision energy voltages”** on page 41.

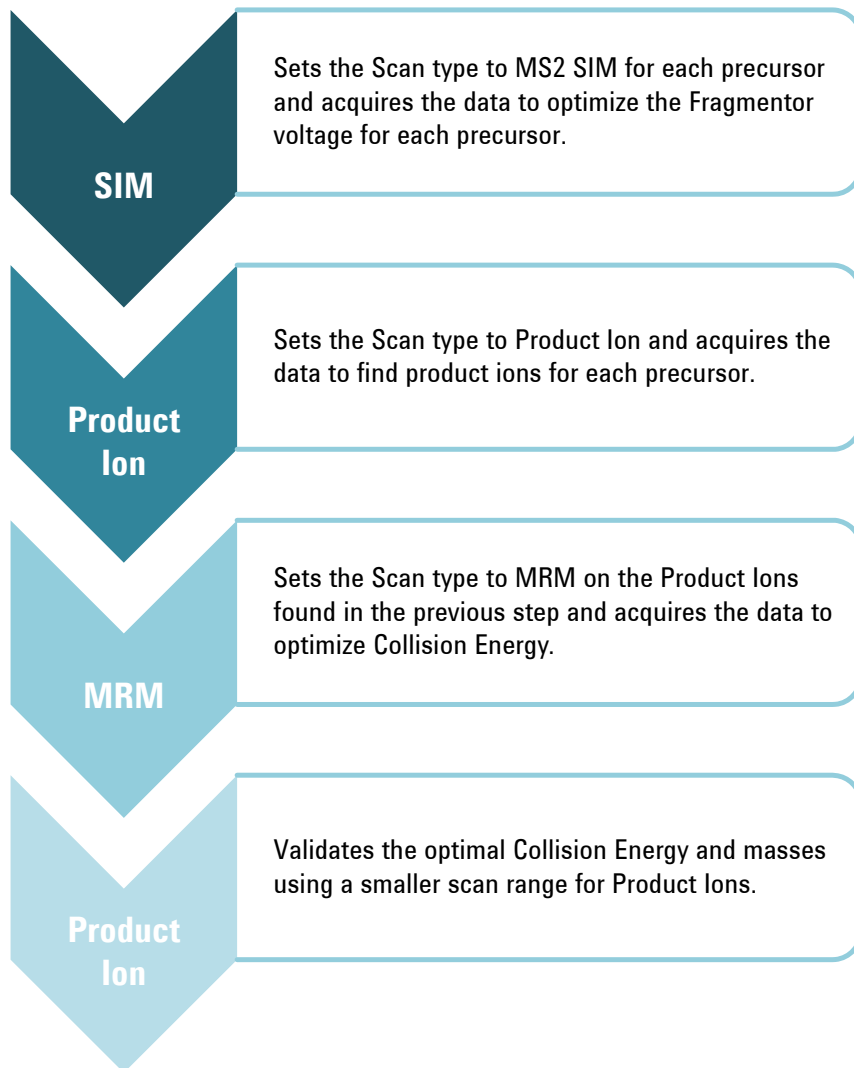
Getting Started

This figure shows the general workflow to use Optimizer.



When Optimizer starts, it automatically proceeds through the following scan types, in the order shown, for each precursor listed in the Compound Setup table.

Getting Started



Refer to the steps in the rest of this guide for detailed instructions on how to use Optimizer.

Using Projects

To create a project

Using Projects

- “To create a project” on page 13
- “To open a project” on page 13
- “To edit a project” on page 14
- “To save parameters to the current project” on page 14
- “To save parameters to a new project” on page 14

To create a project

Use this procedure to store all the values and settings used for optimization in a project.



1 Click **New Project** on the toolbar.

If current parameters have not already been saved, you are prompted to save the project. The current parameters are cleared.

2 Make the desired changes to parameters on the following tabs:

- Optimizer Setup tab
- Precursor and Product Ion Selection tabs
- Compound Setup tab



3 Click **Save Project** or **Save As Project** and enter a name for the project.

To open a project

Use this procedure to use optimization values and settings from an existing project.



1 Click **Open Project** on the toolbar.

2 Click to select the project on the Select Project Name dialog box.

3 Click **Open**.

Using Projects

To edit a project

To edit a project

- 1 Open the project to edit as described above.
- 2 Make the desired changes to parameters on the following tabs:
 - Optimizer Setup tab
 - Precursor and Product Ion Selection tabs
 - Compound Setup tab
- 3 Save the parameters. See **“To save parameters to the current project”** on page 14 or **“To save parameters to a new project”** on page 14.

To save parameters to the current project



- 1 Click **Save Project**.

To save parameters to a new project



- 1 Click **Save As Project**.
- 2 Enter a name for the project and click **Save**.

Setting Optimization Parameters

To set Optimizer acquisition parameters

Setting Optimization Parameters

Use the following procedures to set up the optimization parameters for MassHunter Optimizer.

“To set Optimizer acquisition parameters” on page 15

“To select data path and methods to use for optimization” on page 16

“To select precursor ions” on page 16

“To select product ions” on page 17

Workflow for Peptides

“To set Optimizer acquisition parameters for peptides” on page 18

“To select data path and methods to use for optimization” *below*

To set Optimizer acquisition parameters

- 1 Click the **Optimizer Setup** tab.
- 2 Set the desired **Sample introduction** method (**Injection**, **Automatic infusion**, or **Manual infusion**).
- 3 For all models *except* 6490 and 6495, set the Fragmentor ramp parameters as follows:
 - a Set the range for ramping the **Fragmentor** values.
 - b (*optional*) Mark the **Fragmentor Fine** check box and enter a **Step** size for Fine ramping. Fine optimization refines the coarse ramping values and provides better optimization but takes longer to run.
*For the 6490 and 6495, skip this step and continue at **step 4**. The fragmentor parameters are fixed, so they cannot be optimized. They are not displayed.*
- 4 Set the range for ramping the **Collision Energy** values.
- 5 Continue to select data path and methods to use for optimization as described below.

Setting Optimization Parameters

To select data path and methods to use for optimization

To select data path and methods to use for optimization


- 1 If not already displayed, click the **Optimizer Setup** tab.
- 2 Select a **Path for data files** to store the optimization run data. The data can be displayed later with the MassHunter Qualitative Analysis program.
- 3 In the table on the right side of the screen, select the acquisition methods with the appropriate LC and source parameters to use for optimization.
 - a Right-click in the table and select **Add Method** from the shortcut menu. This adds a row to the end of the table for a new method.
 - b Click the button on the right side of the **Acq Method** cell to open the Open Method dialog box.
 - c Select the method of interest and click **OK**. The **Polarity** and **Ion Source** will be filled in from the values set in the selected method.
 - d Check to make sure that the **Ion Source** from the method matches the physical configuration of your instrument.
 - e (*optional*) Repeat **step a** through **step d** to select additional methods for optimization.

Tip Additional options are available by right-clicking in the method table, such as **Delete Methods**, **Copy**, and **Fill**.

To select precursor ions

Use this procedure to specify precursor ions to use to optimize the fragmentor voltage. The information entered here will be used for all compounds, except for those that have precursor ions specified on the Compound Setup tab.

- 1 Click the **Precursor Ion Selection** tab.
- 2 Select the **Positive** and **Negative** adduct ions to include.

If necessary, add Positive or Negative adduct ions to the list by typing the value in the box and clicking .
- 3 Set the search priority of the precursor ions in *one* of the following ways:
 - *Mark* the **Use most abundant precursor ion** option to use the most abundant precursor ion.


Setting Optimization Parameters

To select product ions

- *Clear* the **Use most abundant precursor ion** option and use the **Up** and **Down** arrow buttons to set the search order (ions at the top of the list are given more priority).
- 4 To exclude certain masses from consideration, click **Exclude masses** at the bottom of the screen. Enter the **m/z values** to exclude separated by commas and/or enter a **Minimum abundance** value in counts.

To select product ions

Use this procedure to specify product ions to use to optimize the collision energy voltage. The information entered here will be used for all compounds, except for those that have product ions specified on the Compound Setup tab.

- 1 Click the **Product Ion Selection** tab.
- 2 Enter the **Maximum number of product ions** to find during the product ion scan phase.
 - For Triggered MRM applications, the recommended value is 10.
 - For MRM and Dynamic MRM applications, the recommended value is 4.
- 3 Enter a **Low mass cut-off** value. Select whether to specify the value as **Mass (m/z)** or as a **% Precursor mass (m/z)** and enter the cut-off value.
- 4 To exclude certain masses from consideration, click the **Exclude masses** option at the bottom of the screen. Enter the **m/z values** to exclude separated by commas and/or enter a **Minimum abundance** value in counts.
- 5 If desired, you can also enter **Neutral Losses** to exclude, e.g. water (H₂O). Enter a formula in the box and click  to add it to the list.

Setting Optimization Parameters

To set Optimizer acquisition parameters for peptides

To set Optimizer acquisition parameters for peptides

- 1 Click the **Optimizer Setup** tab in Optimizer for Peptides.
- 2 Set the desired **Sample introduction** method (**Injection**, **Automatic infusion**, or **Manual infusion**).
- 3 For all models *except* 6490 and 6495, set the Fragmentor Ramp Settings parameters as follows:
 - a Mark the **Fixed Fragmentor** check box and enter the fragmentor voltage to use for optimization. This value will be used for all compounds, unless you enter specific values for individual compounds on the Compound Setup tab.

Tip

Using a fixed fragmentor voltage saves time by skipping the SIM run to determine the optimal fragmentor voltage. However, if multiple adducts are specified, then a SIM run is made to determine the precursor ion to use.

- b Mark the **Overwrite existing** check box to use this value for all compounds, *even if* values are set for individual compounds on the Compound Setup tab.

*For the 6490 and 6495, skip this step and continue at **step 4**.*

- 4 In the Collision Energy area, mark the **Start from predicted value** check box, which lets Optimizer set a collision energy value, using the formula shown. In the formula, **m/z** is the mass of the precursor ion.
- 5 (optional) Mark the **Put instrument into standby mode at end** check box to put the instrument into standby mode at the end of the optimization run.

See “To select data path and methods to use for optimization” on page 16.

Setting Up Compounds in Optimizer Window

To set Optimizer acquisition parameters for peptides

Setting Up Compounds in Optimizer Window

Use the following procedures to set up compounds for use in MassHunter Optimizer.

Setting up compounds

- [“To set up a compound list”](#) on page 20
- [“To use known ions for individual compounds”](#) on page 22

Using compound databases

- [“To open a database in Optimizer Window”](#) on page 23
- [“To save compounds to a database”](#) on page 23

Importing and exporting compound information from Excel

- [“To import compounds from an Excel file”](#) on page 23
- [“To export compound information to an Excel file”](#) on page 24

Using compound information from MassHunter Data Acquisition methods

- [“To import compounds from a MassHunter Data Acquisition method”](#) on page 24
- [“To update retention times from a MassHunter Data Acquisition method”](#) on page 25

To set up a compound list

Use this procedure to set up the list of compounds to optimize and enter precursor and product ion values, if known.

- 1 Click the **Compound Setup** tab.
- 2 (*optional*) Import compounds as described in “**To import compounds from an Excel file**” on page 23 or “**To import compounds from a database**” on page 27.
- 3 Clear the **Show results summary** check box above the table while you set up the compound list.
- 4 To add a compound:
 - a Right-click in the table and select **Add Compound** from the shortcut menu to add a row to the end of the table.
 - b Enter the **Compound Name** for the new compound.
 - c (*optional*) If you want to be able to find groups of compounds in the database such as sulfa drugs or pesticides, assign a group name in the **Groups** column. You can then specify the desired group names when you filter compounds to import from the database as described on **page 27**.
 - d Enter the **Formula** of the compound. The **Mass** will be automatically calculated from the formula. If you don't know the formula, then enter the monoisotopic mass in the **Mass** column.
 - e Enter the **Sample Position** (physical location in the autosampler) for the new compound.
 - f To use the injection volume set in the Optimization method for optimization, for **Set Inj Vol**, select **As Method**. To set a specific injection volume for this compound, for **Set Inj Vol**, select **Specified Value**. Enter the desired injection volume in the **Inj Vol** column and the Product Ion Scan Injection Factor value in the **PI Scan Inj Factor** column. Note that the injection volume for the Phase 2 – Product Ion Scan optimization step is determined by multiplying the **Inj Vol** by the **PI Scan Inj Factor**.
 - g (*optional*) Adjust the **Optimization dwell time** value to set a shorter or longer cycle time if desired; otherwise a default value is used.
 - h (*optional*) To speed up optimization, specify precursor and product ions for the compound as described in “**To use known ions for individual compounds**” below. If no methods or ions are specified here, then optimization for the compound uses the methods from the Optimizer

Setting Up Compounds in Optimizer Window

To set up a compound list

Setup tab and information from the Precursor Ion Selection and Product Ion Selection tabs to generate the ions.

- i Repeat the steps above to add more compounds to the table.

NOTE

Compounds are global to all projects. Compound information such as name, group, formula, and mass in one project will be reflected in the entire database.

Tip

To cut or copy and paste compounds (rows) in the table:

- a Select the rows of interest in the table.
- b Right-click on the selected rows and select **Cut** or **Copy** from the shortcut menu.
- c Right-click on the new location for the cut or copied rows and select **Paste** from the shortcut menu.
- 5 Mark the **Select** columns for the compounds (rows) to use for optimization.
- 6 Save the compound list as described in **“To save a database in Database Browser”** on page 30 or **“To save parameters to the current project”** on page 14.
- 7 (*optional*) Export the compound list as described in **“To export compound information to an Excel file”** on page 24.

To use known ions for individual compounds

Use this procedure to optionally specify methods, precursor ions, and product ions for individual compounds on the Compound Setup tab. Using known ions speeds up the optimization process.

- 1 Add compounds to the Compound List as described in **“To set up a compound list”** on page 20.
- 2 To add methods for the current compound:
 - a Right-click in the **Compound** row of interest and select **Add Method** from the shortcut menu. This adds a Method row below the selected compound.
 - b Click the down arrow button on the right end of the **Acq Method** cell and select an acquisition method to use for the selected compound. If you don't see the method of interest, add it to the Method Table on the Optimizer Setup tab as described in **“To set Optimizer acquisition parameters”** on page 15.
 - c Repeat the above steps to add more methods for the current compound.
- 3 To specify a known precursor ion for the current compound:
 - a Right-click in the **Method** row for the compound of interest and select **Add Precursor Ion** from the shortcut menu. This adds a Precursor Ion row for the current compound.
 - b Enter a value for the **Precursor Ion**.
- 4 To specify product ions for the current compound:
 - a Right-click in the **Precursor Ion** row for the compound of interest and select **Add Product Ion** from the shortcut menu. This adds a Product Ion row for the current compound.
 - b Enter a value for the **Product Ion**.
 - c Repeat the above steps to add the product ions for the current compound and precursor ion.
- 5 Repeat **step 2** through **step 4** to specify methods and ions for other compounds in the Compound Table.

Setting Up Compounds in Optimizer Window

To open a database in Optimizer Window

To open a database in Optimizer Window

Use this procedure when you want to use a database other than the default Optimizer database.

- 1 In the Optimizer Window, click **File > Open Database**.
- 2 Select the database of interest and click **OK**.

When you open an Optimizer database, it becomes the default database. Any work done in Optimizer is saved to that database.

If you are building a unique database, open the database in Database Browser, rename it with a unique name, then save it as the default. As you build projects in Optimizer in this new database, you save those compounds to that uniquely named database.

To save compounds to a database



- To save compounds to the current database, click **Save Compounds** on the main toolbar.

Changes made to transitions and triggers are saved with the database.

You can import the compounds into other projects at a later time as described in **“To import compounds from a database”** on page 27.

To import compounds from an Excel file



- 1 Click **Import from Excel** on the main toolbar.
- 2 Select the **.xls**, **.xlsx**, or **.csv** file to import, and then click **Open**. All compounds from the file will be imported and listed on the Compound Setup tab.

The Select Method for Linking Compounds dialog box may be displayed to assign methods to the compounds.

Setting Up Compounds in Optimizer Window

To export compound information to an Excel file

To export compound information to an Excel file



- 1 Click **Export to Excel** on the main toolbar.
- 2 Enter a name to export the compound list in **.xls** format for use in Excel, click **Save**.

To import compounds from a MassHunter Data Acquisition method



- 1 Create MassHunter Data Acquisition methods for the compounds you want to import.
- 2 Click **Import from Acquisition Methods** in Optimizer.
- 3 Select the MassHunter Data Acquisition methods from **step 1** that contain the compounds of interest, and then click **Open**.

All compounds in the data acquisition methods will be added to the current project and can be seen on the Compound Setup tab.

Setting Up Compounds in Optimizer Window

To update retention times from a MassHunter Data Acquisition method

To update retention times from a MassHunter Data Acquisition method

- 1 Create MassHunter Data Acquisition methods for the compounds of interest.
- 2 Be sure that compounds whose retention times you want to update are part of a project. Only retention times for compounds that are part of a project will be updated. Retention times for compounds that are not in a project will not be updated, even if those compounds are in the database.



- 3 Click **Update RTs** on the Optimizer main toolbar.
- 4 Select the MassHunter Data Acquisition methods from **step 1** that contain the compounds of interest, and then click **Open**.
- 5 When the Update Retention Time Options dialog box appears:
 - a Select whether to **Update in current project** or to **Search database for eligible projects**.
 - b To update retention time windows, mark the **Update RT Windows** check box. If marked, the retention time windows in the acquisition method will be used to update the retention time windows in the database.
 - c If transition ions are found that do not match existing transition ions, you can add them by marking the **Add new transitions found** check box. Otherwise these transitions will be ignored.
 - d Click **OK**.
- 6 Several scenarios may result:

Projects are found that contain the same methods and transition ions In this case, select the project to update, and click **OK**.

or

No project matches for update In this case, you are prompted to select whether to add the transition ions to the **Current Project** or a **New Project**. Click the desired option, and then click **OK**.

All transition ions of the same compound will be updated with the first retention time in the acquisition method. The updated compound information can be seen on the Compound Setup tab.

- 7 Be sure to save the project to save the updated retention times.

Using Database Browser

To open a database and make it the default database

Using Database Browser

Use these procedures to work with Database Browser.

- “**To open a database and make it the default database**” on page 26
- “**To import compounds from a database**” on page 27
- “**To select transitions and triggers for Dynamic and Triggered MRM**” on page 29
- “**To save a database in Database Browser**” on page 30
- “**To filter compounds in Database Browser**” on page 31
- “**To search compounds in Database Browser**” on page 32

To open a database and make it the default database

Use this procedure when you want to use a database other than the default optimizer database.



1 If Database Browser is not already open, click **Import from Database** on the Optimizer window toolbar to open it.



2 Click **Open Database** on the Database Browser toolbar.

3 Select the database of interest and click **OK**.

4 You are asked if you want to make this database the default database.

NOTE

If you are importing additional compounds into an existing project, click **No**. Otherwise, unsaved changes made to the current project will be lost. All saved compound information in the previous project will still exist in the previous default database, but it won't be available for use with the new default database.

Tip



If you decide later to make this database the default database, click **Set Default Database** on the Database Browser toolbar.

Using Database Browser

To import compounds from a database

To import compounds from a database

Use this procedure to import compounds that have previously been saved as described in “**To save a database in Database Browser**” on page 30.



- 1 Click **Import from Database** in Optimizer window to open Database Browser.
- 2 If not already open, open the database of interest as described on [page 26](#).
- 3 Select compounds for optimization from the database.
 - If desired, filter compounds as described on [page 31](#).
 - If desired, search compounds as described on [page 32](#).
 - To display all compounds in the database, mark the **Show All Records** check box.
- 4 When the compounds from [step 3](#) appear in the Compound table in the lower half of Database Browser, select the transition ions to import in *one* of the following ways:
 - Individually mark the check boxes in the first column of the table for the transition ions you want to import.
 - Click the **Select top ranked transitions** option, enter the number of top transitions to import for each compound, and then click **Select Transitions**. You can rank the top transitions by either **Abundance** or **Response Factor**.
 - To select the primary transitions, click the **Primary Transitions** option, and then click **Select Transitions**.
 - To select the secondary transitions, click **Secondary Transitions**, and then click **Select Transitions**.

NOTE

To set up the primary and trigger transition ions to import, see “**To select transitions and triggers for Dynamic and Triggered MRM**” on page 29.

- 5 Do one of the following:
 - If the compounds in the table are the only ones you want to import at this time, click **Import**. This closes Database Browser and adds the compounds to the Compound Setup tab.
 - If you want to do additional search and filter operations on the current database or a different database, click **Add to Import List**.
- 6 Do one of the following:

Using Database Browser

To import compounds from a database

- To find more compounds in the *same* database, repeat **step 3** through **step 5**.
 - To select compounds from a *different* database, repeat **step 2** through **step 5**.
- 7** Click the **Import List** tab to review the list of compounds.
- Right-click and select **Remove** from the shortcut menu to remove any compounds you don't want to import.
- 8** Click **Import**.
- The imported compounds will appear on the Compound Setup tab.

To select transitions and triggers for Dynamic and Triggered MRM

- 1 If not already open, open the database of interest as described on [page 26](#).
 - 2 In Database Browser, display compounds for optimization from the database.
 - If desired, filter compounds as described on [page 31](#).
 - If desired, search compounds as described on [page 32](#).
 - To display *all* compounds in the database, mark the **Show All Records** check box.
 - 3 For any unwanted compound, clear the check box next to the **Compound Name** in the first column of the Compound table.
- Triggered MRM method**
- 4 Select transitions for a *triggered MRM method* as follows:
 - a In the **Select top ranked transitions** field, enter the maximum number of transitions per compound that you want to show in the import list. You can have two primary transitions, plus additional secondary transitions (see [step c below](#)).
 - b Click **Select Transitions** to display the transitions in the Compound table.
 - c In the **Set primary and trigger flags** area, specify the number of **Primary** transitions to use. The rest are assigned as Secondaries. Typically, two primaries are used.
 - d Click **Set Primaries and Trigger**. The most abundant transition is automatically assigned as the Trigger.
 - e Review the compound list and edit as needed.
 - A very abundant low m/z ion may be unsuitable as a Primary transition.
 - You can manually select a different transition for a compound as the Primary transition, and define a low m/z ion as a Secondary.
 - You may want to select two Primaries as triggers for a compound.
- Dynamic MRM method**
- 5 Select transitions for a *dynamic MRM method* as follows:
 - a Click **Primary transitions**.
 - b Click **Select Transitions** to display the transitions in the Compound table.
 - At this point, all transitions are displayed as primaries.
 - Typically, the transitions are ranked by abundance.
 - c Review the compound list and edit as needed.
 - Clear any unwanted transitions.

Using Database Browser

To save a database in Database Browser

- 6 When the list of transitions is set, click **Add to Import List** below the Compound table.
- 7 Do one of the following:
 - To find more compounds in the *same* database, repeat **step 2** through **step 6**.
 - To select compounds from a *different* database, repeat **step 1** through **step 6**.
- 8 Click the **Import List** tab to review the list of compounds.
 - Right-click and select **Remove** from the shortcut menu to remove any compounds you don't want to import.
- 9 Click **Import**.

The imported compounds will appear on the Compound Setup tab.

To save a database in Database Browser

Do one of the following to save compounds to a database:

- To save compounds to the current database, click **Save Database** on the Database Browser toolbar.
- To save compounds to a new database, click **Save As Database** on the Database Browser toolbar. When prompted, enter a name for the new database, and click **Save**.



NOTE

Changes made to transitions and triggers in Database Browser are *not* saved with the database.

Once saved, you can import the compounds into other projects at a later time.

Using Database Browser

To filter compounds in Database Browser

To filter compounds in Database Browser

- 1 If not already open, open the database of interest as described on [page 26](#).
- 2 Enter the Filter criteria in the upper left area of the Database Browser **Search/Filter** tab. Note that filter criteria are unavailable if **Show All Records** is selected.
 - Mark the **Enable Filters** check box.
 - Mark the check boxes for the filter criteria to use, such as **Optimized Compounds, Date, Group Name, Project Name, Polarity, Model,** and **Method**. Enter values for each selected criteria.
- 3 Click **Search/Filter**.

The compounds that pass the filter are displayed in the Compound table in the lower part of the screen.

To search compounds in Database Browser

- 1 If not already open, open the database of interest as described on [page 26](#).
- 2 Enter the **Search Text** in the upper right area of the Database Browser **Search/Filter** tab, such as **Compound Name** and **Formula**. This can be either a partial or complete entry as described in [step 4](#).

The best parameter to use in a search is the CAS number or the formula as you have specified. You will not get a match if you misspell the name or if the name is spelled differently in the database.

NOTE

The values you enter here for **Product** or **Precursor** ions are treated as *numeric values* rather than text. Matches are returned whose values fall between 0.7 amu below the entered mass and 0.3 amu above the specified mass.

- 3 Mark the columns to search or click the top entry in the list (Select Columns) to search *all* columns.
- 4 Do one of the following:
 - To find compounds that match only a partial name or formula, *clear* the **Match entire word for each string** check box.
 - To find only compounds that exactly match the text you entered in [step 2](#), *mark* the **Match entire word for each string** check box.
- 5 To ignore all filters for this search, *clear* the **Enable filters** check box.

NOTE

If you want to display *all items* in the database, mark the **Show All Records** check box. This automatically disables all the search and filter options.

- 6 Click **Search/Filter**.
 - The compounds that meet the search criteria are displayed in the Compound Table in the lower part of the screen.
 - Search text that was *not found* in the search is shown in red.

Setting Up Sequences

To set up a sequence list for peptides

Setting Up Sequences

Use the following procedures to set up peptide sequences for use in Optimizer for Peptides.

- “**To set up a sequence list for peptides**” below
- “**To import peptide sequences from a MassHunter Qualitative Analysis method**” on page 34
- “**To convert multiple peptide sequences for import into MassHunter Qualitative Analysis**” on page 38

To set up a sequence list for peptides

Use this procedure to set up the list of peptides to optimize in Optimizer for Peptides.

- 1 Click the **Compound Setup** tab.
- 2 Clear the **Show results summary** check box above the table while you set up the sequence list.
- 3 Import sequences as described in “**To import peptide sequences from a MassHunter Qualitative Analysis method**” on page 34.
- 4 (*optional*) If you want to be able to find groups of sequences in the database, assign a group name in the **Groups** column. You can then specify the desired group name when you filter sequences to import from the database.
- 5 Leave the **Formula** column blank. The **Mass** is imported from the method and displayed in the table automatically.
- 6 Enter the **Sample Position** for the sequence.
- 7 Mark the **Select** columns for the sequences (rows) that you want to use for optimization.
- 8 Mark the **Perform multi-compound run** check box. This lets you perform collision energy optimization when all the fragment ions are known, a typical case in peptides using ion prediction.
- 9 (*optional*) Adjust the **Optimization dwell time** value to set a shorter or longer cycle time if desired.

Setting Up Sequences

To import peptide sequences from a MassHunter Qualitative Analysis method

- 10 Click **Fill Values** to calculate the Cycle Time parameters and the peak width necessary to obtain ten data points required for optimization when using Fixed Fragmentor or CE value formula on the Optimizer Setup tab. See **“To set Optimizer acquisition parameters for peptides”** on page 18.
- 11 Save the sequence list as described in **“To save a database in Database Browser”** on page 30 or **“To save parameters to the current project”** on page 14.
- 12 (*optional*) Export the sequence list as described in **“To export compound information to an Excel file”** on page 24.

To import peptide sequences from a MassHunter Qualitative Analysis method

Applies to Optimizer for Peptides only.

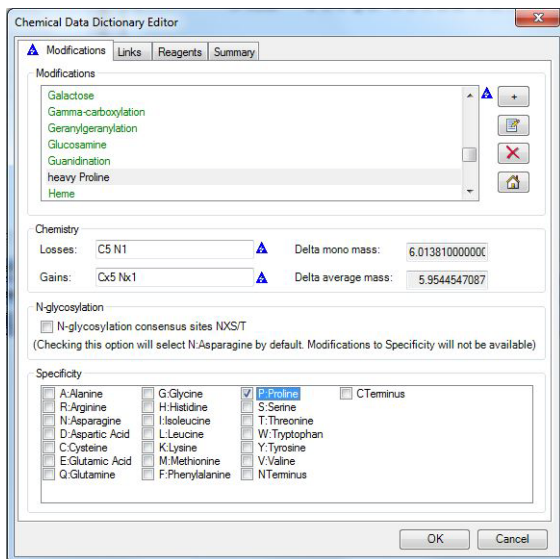
- 1 Create a MassHunter Qualitative Analysis method that contains the peptide sequences you want to import.
 - If desired, create the **.psq** files for MassHunter Qualitative Analysis as described in **“To convert multiple peptide sequences for import into MassHunter Qualitative Analysis”** on page 38.
- 2 If you need to add custom modifications:
 - a Select **Open Chemical Data Dictionary Editor** from the Tools menu.
 - b Click the **+** button on the **Modifications** tab to add a custom modification.
 - c Type in a name for a new modification, and then enter its **Chemistry** values.

Example

Assume you wish to add a custom modification “heavy Proline”, where the carbons and nitrogen in Proline are replaced with 13-C and 15-N. A Proline residue has five Carbons and one Nitrogen. Assuming complete labeling, specify **Losses** of C5 N1 and **Gains** of Cx5 Nx1 for a Delta mass of 6.01381. Mark the check box for Proline and clear all others to specify this custom modification for Proline only.

Setting Up Sequences

To import peptide sequences from a MassHunter Qualitative Analysis method



- d Click **OK** to close the Chemical Data Dictionary Editor.
- 3 Apply the custom modification to the sequences of interest as follows:
 - a Open the **Sequence Editor** window if it is not already open, and select the sequence of interest.
 - b Using the example above, select the Proline amino acid in the sequence, and then apply the custom modification created in **step 2**.
 - c Save the method, which contains the modified sequences.
- 4 Be sure to observe the following caveats if you modified the Chemical Data Dictionary as described in **step 2** above:
 - Close the MassHunter Qualitative Analysis program. Otherwise, the custom dictionary will not be modified.
 - If Optimizer was open while the custom modifications were added, restart Optimizer to make sure that it reads the new custom dictionary.
 - If you plan to use this method on a different computer, you need to add the same custom modifications to the Chemical Data Dictionary on that system. Otherwise Optimizer for Peptides won't recognize the custom modifications and an error will result.
- 5 Import the sequences into MassHunter Optimizer for Peptides as follows:
 - a Review the charge states in the **Precursor Ion Selection** tab. Make sure that the desired charge states are listed, and add them if they are not.

Setting Up Sequences

To import peptide sequences from a MassHunter Qualitative Analysis method

Tip For peptide analysis, charge state precursors 2+ and 3+ are usually preferred, and singly-charged precursors are ignored.



b Click **Extract Peptide** on the toolbar in Optimizer for Peptides.

Select the MassHunter Qualitative Analysis method from **step 1** that contains the peptide sequences of interest, and then click **Open**.

All sequences from the selected method are displayed in the **“Peptide Fragment Ion Predictor Dialog Box”** on page 10.

c Right-click in the table and select **Expand/Collapse All** from the shortcut menu to see the Precursor Ions and Charge states for each sequence.

d Mark the sequences that you want to use for optimization.

Tip To make it easier to edit long transitions lists, mark the **Show selected ions** option above the table to display only the selected ions.

- Within each peptide sequence, mark the precursor charge state to use for that peptide and *deselect* all charge states that you don't want to use. For example, you may want to examine only the 2+ charge state precursor because it produces abundant product ions, but the 3+ charge state precursor does not.
- If you decide to examine multiple charge states for a given peptide, then select all of the charge states of interest.
- Select the product ions of interest that result from each peptide precursor. Use the following check boxes in combination with selecting individual product ions in the table to make your selections.

- Show selected ions
- Show only y-ions
- Show only b-ions
- Show ions > than m/z value
- Show ions > than % of precursor

e Click **Add**. The selected peptide sequences from the method are imported and listed on the **“Compound Setup Tab”** on page 9.

6 Review the list of precursors and product ions.

a Right-click in the table and select **Expand/Collapse All Rows** from the shortcut menu to show all precursors and product ions.

b To edit the list, right-click on a product ion row, and select Delete Product Ions or Add Product Ion from the shortcut menu.

7 If you want to use only a single charge state for each peptide to optimize, then edit the charge states on the **Precursor Ion Selection** tab to make sure that only one charge state is selected for each peptide. That is, remove the charge

Setting Up Sequences

To import peptide sequences from a MassHunter Qualitative Analysis method

state selection from all but one charge state. For example, clear charge state selections except for Charge State 2.

NOTE

If more than one charge state is active, then during optimization, Optimizer tries to determine which charge state has the highest abundance.

8 (*optional*) Save the compounds.

Setting Up Sequences

To convert multiple peptide sequences for import into MassHunter Qualitative Analysis

To convert multiple peptide sequences for import into MassHunter Qualitative Analysis

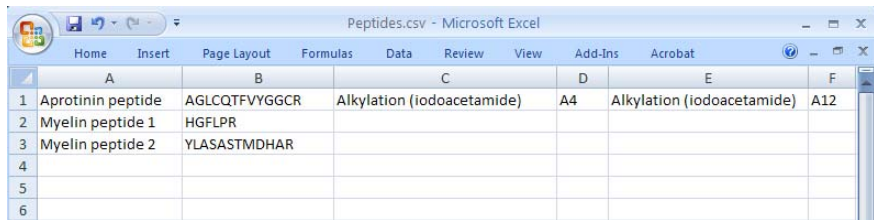
Applies to Optimizer for Peptides only.

Use this procedure to convert multiple peptide sequences with or without modification into multiple **.psq** files for import into MassHunter Qualitative Analysis program.

- 1 Create a comma separated (**.csv**) file that has one sequence per line, with the following format:

NameString, SequenceString, Modification1String, Location1String, Modification2String, Location2String

Example



	A	B	C	D	E	F
1	Aprotinin peptide	AGLCQTFVYGGCR	Alkylation (iodoacetamide)	A4	Alkylation (iodoacetamide)	A12
2	Myelin peptide 1	HGFLPR				
3	Myelin peptide 2	YLASASTMDHAR				
4						
5						
6						



- 2 Click **Convert .csv to .psq** in Optimizer for Peptides.
- 3 Select the **.csv** file created in **step 1**, and click **Open**.

The file is converted into multiple **.psq** files, one for each sequence, which are saved in a folder named **OutputPSQFiles** in the same folder that contains the original **.csv** file.

- 4 Import the **.psq** files into MassHunter Qualitative Analysis program and save them in a Data Analysis method.
- 5 Import the sequences for use in Optimizer for Peptides as described in **“To import peptide sequences from a MassHunter Qualitative Analysis method”** on page 34.

Optimization and Results

To convert multiple peptide sequences for import into MassHunter Qualitative Analysis

Optimization and Results

Use the following procedures to run the optimization procedure, evaluate the results, and import them into the MassHunter Data Acquisition program.

“To start optimization” on page 40

“To review optimization results” on page 40

“To start optimization and display ion breakdown profiles” on page 41

“To manually set collision energy voltages” on page 41

“To import optimization results to Acquisition for dMRM/tMRM” on page 43

“To import optimization results to Acquisition for MRM time segments” on page 46

Optimization and Results

To start optimization

To start optimization

- 1 Set the optimization parameters as described on [page 15](#).
- 2 Set up the compound list as described in [“To set up a compound list”](#) on page 20 or [“To set up a sequence list for peptides”](#) on page 33.
- 3 Start the optimization process in *either* of the following ways:



- Click **Start Optimization** on the toolbar.



- Click **Ion Breakdown Profile** on the toolbar if you want to display the product ion profiles determined by optimization before they are saved to the database. For more information, see [“To start optimization and display ion breakdown profiles”](#) on page 41.



- 4 If necessary, you can stop the optimization process by clicking **Stop Optimization** on the toolbar.
- 5 Review results as described below.
- 6 (*optional*) Use the MassHunter Qualitative Analysis program to look at the data acquired during the optimization run.

To review optimization results

- 1 Run the optimization procedure as described above.
- 2 Click the **Compound Setup** tab.
- 3 Mark the **Show results summary** check box above the table.
- 4 Review the following values for each transition in the Compound table:
Fragmentor—The optimum voltage to apply to the exit end of the capillary for the compound, determined by MassHunter Optimizer. (*Does not apply to instrument model 6490 LC/MS.*)
Collision Energy—The optimum voltage to apply to the ions in the collision cell for the compound, determined by MassHunter Optimizer.
- 5 Review the printed optimization report.
- 6 See [“To import optimization results to Acquisition for MRM time segments”](#) on page 46, to use optimization values for data acquisition.

Optimization and Results

To start optimization and display ion breakdown profiles

To start optimization and display ion breakdown profiles

Use this procedure to display the product ion profiles determined by optimization before they are saved to the database and to manually select collision energy values for each ion if desired.

1 Set the optimization parameters as described on [page 15](#).

2 Set up the compound list as described on [page 20](#).



3 Click **Ion Breakdown Profile** to start optimization.

4 When optimization is complete, the Ion Breakdown Profile dialog box is displayed. Review the graphs, which plot collision energy steps on the x-axis and abundance values on the y-axis for each product ion. The values shown in the table are the collision energy values determined by optimization. See an example of Ion Breakdown Profile results in [Figure 1](#) on the next page.

5 If desired, you can override the collision energy values as described below. Otherwise, click **Save & Exit** to close the Ion Breakdown Profile dialog box.

To manually set collision energy voltages

1 Set optimization parameters and start optimization as described above.

2 Click on the Ion Breakdown Profile graph to select the desired collision energy value. See an example Ion Breakdown Profile graph in [Figure 1](#) on the next page.

3 Apply the selected value to either **All ions** or only the **Selected ion** by clicking the appropriate option for **Apply manual selection to**.

4 If desired, you can reload the collision energy values set by optimization at any time by clicking **Load Optimizer Defaults**. Doing this overwrites the manual selections you made above.

5 Click **Save & Exit** to close the Ion Breakdown Profile dialog box.

Optimization and Results

To manually set collision energy voltages

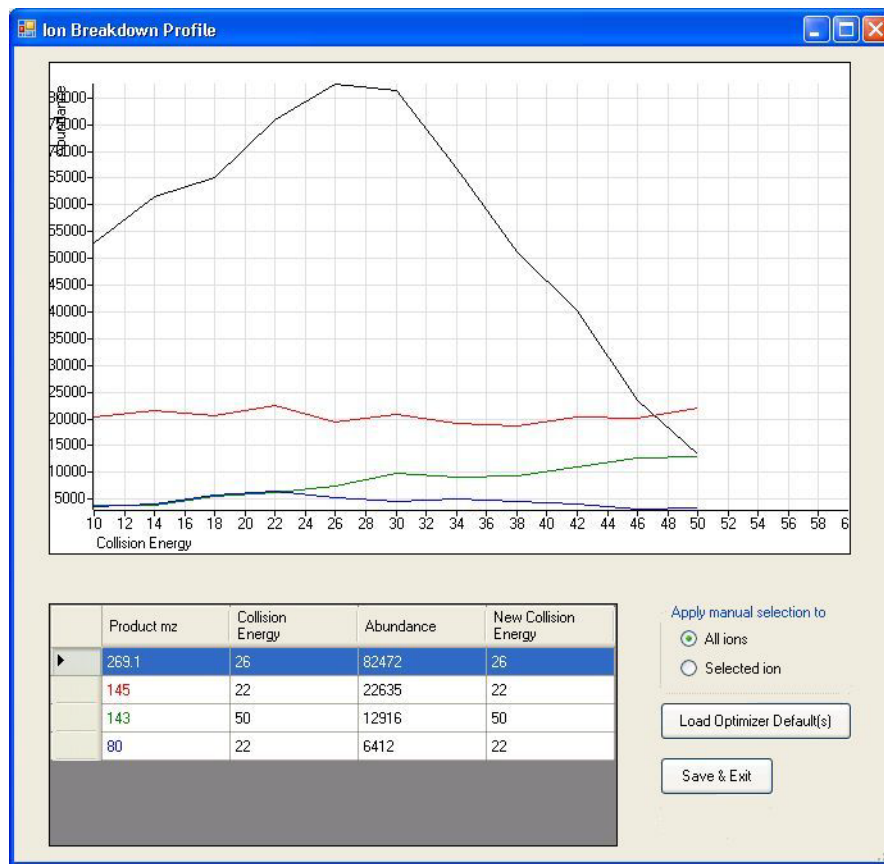


Figure 1. Ion Breakdown Profile Results

Optimization and Results

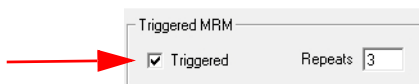
To import optimization results to Acquisition for dMRM/tMRM

To import optimization results to Acquisition for dMRM/tMRM

- 1 Display the **Method pane** in MassHunter Data Acquisition program.
- 2 Create or open the method of interest.
- 3 Click the **MS QQQ** tab in the Method Editor pane.
- 4 Click the **Acquisition** tab on the right side of the screen.
- 5 Select the time segment of interest or create a new one.

Time segments						
#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
▶ 1	0	Dynamic MRM	To MS	200	0	<input checked="" type="checkbox"/>

- 6 Set **Scan Type** to **Dynamic MRM** as shown above.
- 7 For triggered MRM:
 - a Mark the **Triggered** check box in the lower right area of the Acquisition tab.



- b Notice that additional columns are now available in the Scan segments table, including **Primary**, **Trigger**, **Trigger Window**, **Trigger MRM Threshold**, and **Trigger Entrance Delay**.
- 8 Use the expected retention time and RT window parameters to set the timetable for monitoring transitions. Usually only a single Time segment is used for Dynamic MRM and Triggered MRM. Note that the Cycle time remains constant.
 - As transitions are added to the list in real time, the Dwell time is reduced for each transition that is active to keep the Cycle time constant.
 - The reverse happens for transitions whose time has elapsed, and dwell times are *increased* for the remaining active transitions.
 - For a single compound on the list, one transition serves as the quantitation response. Other transitions for the same compound are used as qualifiers.
 - 9 Right-click in the **Acquisition** tab and select **Import from Data Browser** from the shortcut menu.

Optimization and Results

To import optimization results to Acquisition for dMRM/tMRM

- 10 When Database Browser appears:
 - a If desired, filter the compounds as described on [page 31](#).
 - b If desired, search compounds as described on [page 32](#).
 - c Mark the transition ions of interest from the Compound table in the lower half of Database Browser in *one* of the following ways:
 - Manually select the transition ions you want to import for each compound, *or*
 - Enter the top number of transitions to import for each compound, and then click **Select**. You can select the top transitions based on either Abundance or Response Factor. Clear the check box for any transitions that you don't want to use.
- 11 If the transition ions selected in the table are the only ones you want to import at this time, click **Import**. This closes the Data Browser window and adds the transition ions into the current time segment.
 - Otherwise, to do additional search and filter operations on the current database or a different database, click **Add to Import List**.
- 12 To select additional compounds:
 - a To select compounds from a different database, open the database of interest as described on [page 26](#).

NOTE

When asked if you want to make this database the default database, click **No**. Otherwise, unsaved changes made to the current project will be lost. All saved compound information in the previous project will still exist in the previous default database, but won't be available for use with the new default database.

- b Repeat **step 10** to select the transition ions of interest.
 - c Click **Add to Import List**.
- 13 Click the **Import List** tab to review the list of transition ions. To remove any transition ions you don't want to import, right-click and select **Remove** from the shortcut menu.
- 14 Click **Import**. The selected transitions are imported into the current time segment.
- 15 Review the timings to make sure that not too many transitions are being monitored concurrently:
 - a Right-click in the Scan segments table and select **Edit dMRM method** from the shortcut menu.

Optimization and Results

To import optimization results to Acquisition for dMRM/tMRM

- b When the Dynamic MRM Viewer appears, review how many transitions are active at any point in time during the run.

NOTE

When too many transitions are active at the same time, the dwell time per transition may become too short (1-2 msec per transition or less). Under these conditions, the signal/noise ratio is low, and the quantitation accuracy and precision may suffer.

- c If necessary, change the retention time windows or delete transitions to increase the average dwell time.
- d You can also use the **Split Method** feature in the **Options and Parameters** pane of the Dynamic MRM Viewer to divide the transitions from the current method across two or more methods.
 - Select the criteria for splitting the method from one of the following: **Minimum dwell time**, **Maximum concurrent MRM**, or **Number of new methods**.
 - Note that all transitions for the same compound are assigned to the same method.

16 (optional) Save the method.

Optimization and Results

To import optimization results to Acquisition for MRM time segments

To import optimization results to Acquisition for MRM time segments

- 1 Display the Method pane in the MassHunter Data Acquisition window.
- 2 Create or open the method of interest.
- 3 Click the **MS QQQ** tab in the Method Editor pane.
- 4 Click the **Acquisition** tab on the right side of the screen.
- 5 Select the time segment of interest or create a new one.

Time segments						
#	Start Time	Scan Type	Polarity	Div Valve	Delta EMV	Stored
1	0	MRM	Positive	To MS	200	<input checked="" type="checkbox"/>

- 6 Set **Scan Type** to **MRM**.
- 7 Right-click in the **Acquisition** tab and select **Import from Data Browser** from the shortcut menu.
- 8 When Database Browser appears:
 - a If desired, filter the compounds as described on [page 31](#).
 - b If desired, search compounds as described on [page 32](#).
 - c Mark the transition ions of interest from the Compound table in the lower half of Database Browser in *one* of the following ways:
 - Manually select the transition ions you want to import for each compound, *or*
 - Enter the top number of transitions to import for each compound and click **Select**. You can select the top transitions based on either Abundance or Response Factor. Deselect any transitions that you don't want to use by clearing their check box.
- 9 If the transition ions selected in the table are the only ones you want to import at this time, click **Import**. This closes the Data Browser window and adds the transition ions into the current time segment.
 - Otherwise, to do additional search and filter operations on the current database or a different database, click **Add to Import List**.
- 10 To select additional compounds:
 - a To select compounds from a different database, open the database of interest as described on [page 26](#).

Optimization and Results

To import optimization results to Acquisition for MRM time segments

NOTE

When asked if you want to make this database the default database, click **No**. Otherwise, unsaved changes made to the current project will be lost. All saved compound information in the previous project will still exist in the previous default database, but won't be available for use with the new default database.

- Repeat **step 8** to select the transition ions of interest.
 - Click **Add to Import List**.
 - b** Click the **Import List** tab to review the list of transition ions. To remove any transition ions you don't want to import, right-click and select **Remove** from the shortcut menu.
 - c** Click **Import**. The selected transitions are imported into the current time segment.
- 11** Repeat **step 5** through **step 10** for each time segment of interest.
- 12** (*optional*) Save the method.

In this guide

This Quick Start Guide describes how to use MassHunter Optimizer Automated MS Method Development software.

This guide is valid for the B.07.00 revision or higher of the Agilent MassHunter Optimizer Automated MS Method Development software, until superseded.



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