



# **Agilent G3793AA MassHunter Optimizer**

## ***Automated MS Method Development Software***

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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.



**Agilent Technologies**

## What is MassHunter Optimizer?

### *Automated MS Method Development Software*

MassHunter Optimizer software provides a way to 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 fragment 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+NH<sub>4</sub>.
- 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 fragment ions (MS/MS full scan)
- Optimization of collision energy to maximize fragment ion intensity (MS/MS full scan)
- Optionally performed fine fragmentor optimization for maximum sensitivity.

### **Additional Features**

- Stores optimum precursor and fragment ions and parameters in a compound database.
- Imports optimization results into Agilent MassHunter Workstation triple quad Data Acquisition software.
- Lets you import compounds for optimization and update compound retention times from Agilent MassHunter Workstation triple quad 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).

- Displays data acquired during the optimization run with Agilent MassHunter Qualitative Analysis software.

## Installation

If the MassHunter Optimizer software is not already installed on your system, install it as follows. Note that compatible Agilent MassHunter Acquisition software must be installed on the same system.

- 1 Put the disk labeled Agilent G3793AA into the disk drive.
- 2 Click the Windows **Start** button and select **Run**.
- 3 Type **x:\setup**, where **x** is the letter of the disk drive, and click **OK**.
- 4 Follow the instructions on the screen to install the software.

Note that two versions of MassHunter Optimizer software are installed on your system: one for small molecules and one for peptides. See [Startup](#) instructions below.

### To remove the software

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

## Startup

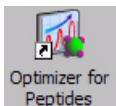
Open MassHunter Optimizer software in *either* of the following ways:



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

### To optimize peptides

A version of MassHunter Optimizer software is available for optimizing peptides. Open Optimizer for Peptides software 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 software that you use to create them. In other words, projects created with Optimizer for Peptides software *cannot* be opened in MassHunter Optimizer software and vice versa.

## User Interface

The MassHunter Optimizer software has the following UI elements that let you enter parameters, set options, and perform functions:

- [“Menus and Toolbar”](#) on page 6

### Tabs

- [“Optimizer Setup Tab”](#) on page 8
- [“Precursor Ion Selection Tab”](#) on page 8
- [“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 9
- [“Database Browser Window”](#) on page 9
- [“Ion Breakdown Profile Dialog Box”](#) on page 9

## Menus and Toolbar

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

### File menu



**New Project** Clears the current settings and lets you set up a new project for use in MassHunter Optimizer software. See [“To create a project” on page 12.](#)



**Open Project** Lets you open a project. See [“To open a project” on page 12.](#)



**Save Project** Lets you save changes to the current project.



**SaveAs 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 compounds to a database” on page 24.](#)



**Delete Projects** Lets you select projects and remove them.

**Exit** Closes the MassHunter Optimizer software 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.

**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** Lets you clear all fragmentors, all collision energies, 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 the Database Browser window, which lets you select compounds for optimization from the database. See [“Database Browser Window” on page 9](#).



**Import from Excel** Lets you import compounds from an Excel file (.xls format). See [“To import compounds from an Excel file” on page 27](#).



**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 in .xls format for use in Excel. See [“To export compound information to an Excel file” on page 27](#).

### 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 33](#).



**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 34](#).



**Stop Optimization** Lets you terminate optimization before completion.

## Tools menu



**Update RT** Lets you update compound retention times from Acquisition methods. See [“To update retention times from a MassHunter Acquisition method”](#) on page 26.

*The following items on the Tools menu only appear in Optimizer for Peptides software.*



**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 9.



**Convert .csv to .psq** Lets you convert multiple peptide sequences with or without modification into multiple **.PSQ** files that can be imported into MassHunter Qualitative Analysis software. See [“To convert multiple peptide sequences for import into MassHunter Qualitative Analysis”](#) on page 31.

## Help menu

**Help Topics** Opens the online help window for MassHunter Optimizer software with the Contents tab displayed.

**About Optimizer** Displays version information for MassHunter Optimizer software.

## Optimizer Setup Tab

Lets you set the acquisition parameters used for optimization, including sample introduction method, Acquisition methods, and range for optimization of fragmentor and collision energy voltages. See [“To set Optimizer acquisition parameters”](#) on page 14.

## 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 15.



## Product Ion Selection Tab

Lets you set criteria to use in the search for product ions. See [“To select product ions” on page 16.](#)

## Compound Setup Tab

Displays the list of compounds to optimize, and lets you enter precursor and product ion values, if known. 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 software.

## Peptide Fragment Ion Predictor Dialog Box

*Available in Optimizer for Peptides software only.*



This dialog box appears when you click the **Extract Peptide** toolbar button. It lets you select peptide sequences for optimization from a MassHunter Qualitative Analysis method. The selected sequences are added to the Compound Setup tab. See [“To import peptide sequences from a MassHunter Qualitative Analysis method” on page 30.](#)

## Database Browser Window



This window appears when you click the **Import from Database** toolbar button. 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 21.](#) The Database Browser window 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 the **Ion Breakdown Profile** toolbar button. 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 34.](#)

## Getting Started

The following topics will help you get started using MassHunter Optimizer software.

### Using Projects

- [“To create a project” on page 12](#)
- [“To open a project” on page 12](#)
- [“To edit a project” on page 12](#)
- [“To save parameters” on page 12](#)

### Setting Optimization Parameters

- [“To set Optimizer acquisition parameters” on page 14](#)
- [“To select data path and methods to use for optimization” on page 14](#)
- [“To select precursor ions” on page 15](#)
- [“To select product ions” on page 16](#)
- [“To set Optimizer acquisition parameters for peptides” on page 16](#)

### Setting Up Compounds and Working with Databases

- [“To set up a compound list” on page 18](#)
- [“To use known ions for individual compounds” on page 20](#)
- [“To import compounds from a database” on page 21](#)
- [“To filter compounds in Database Browser” on page 23](#)
- [“To search compounds in Database Browser” on page 23](#)
- [“To open a database and make it the default database” on page 24](#)
- [“To save compounds to a database” on page 24](#)
- [“To import compounds from a MassHunter Acquisition method” on page 24](#)
- [“To update retention times from a MassHunter Acquisition method” on page 26](#)
- [“To import compounds from an Excel file” on page 27](#)
- [“To export compound information to an Excel file” on page 27](#)

### Setting Up Sequences

- [“To set up a sequence list for peptides” on page 28](#)
- [“To import peptide sequences from a MassHunter Qualitative Analysis method” on page 30](#)
- [“To convert multiple peptide sequences for import into MassHunter Qualitative Analysis” on page 31](#)

## **Optimization and Results**

- [“To start optimization”](#) on page 33
- [“To review optimization results”](#) on page 33
- [“To start optimization and display ion breakdown profiles”](#) on page 34
- [“To manually set collision energy voltages”](#) on page 34
- [“To import optimization results to Acquisition for MRM time segments”](#) on page 36

## Using Projects

### To create a project

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



- 1 Click the **New Project** button 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 the **Save As Project** button and enter a name for the new project.

### To open a project

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



- 1 Click the **Open Project** button on the toolbar.
- 2 Click to select the project on the Select Project Name dialog box.
- 3 Click the **Open** button.

### 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 as described below.

### To save parameters



- 1 To save the changes to the current project, click the **Save Project** toolbar button.
- 2 To save the project under a *new* name for later use:



- a** Click the **Save As Project** toolbar button.
- b** Enter a name for the project and click the **Save** button.

## Setting Optimization Parameters

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

[“To set Optimizer acquisition parameters” below](#)

[“To select data path and methods to use for optimization” below](#)

[“To select precursor ions” on page 15](#)

[“To select product ions” on page 16](#)

### Workflow for Peptides

[“To set Optimizer acquisition parameters for peptides” on page 16](#)

[“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 Set the 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 course ramping values and provides better optimization but takes longer to run.
  - c Set the range for ramping the **Collision Energy** values.
- 4 Continue to select data path and methods to use for optimization as described below.

### 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 Agilent MassHunter Qualitative Analysis software.
- 3 In the table on the right side of the screen, select the acquisition methods to use for optimization as follows. *These methods will be used*

*to optimize all compounds, except for those that have specific methods selected on the Compound Setup tab.*


- 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 **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 Steps 3a - d to select additional methods for optimization.

**Tip** Additional options are available by right-clicking in the method table, such as **Delete Methods**, **Copy**, **Cut**, **Paste**, 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 ions** and **Negative ions** to include.

If necessary, add Positive or Negative ions to the list by typing the value in the box and clicking the  button.

- 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.
  - *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 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 in the box.
- 3 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.
- 4 If desired, you can also enter **Neutral Losses** to exclude, e.g. water (H<sub>2</sub>O). Enter a formula in the box and click the  button to add it to the list.

## To set Optimizer acquisition parameters for peptides

- 1 Click the **Optimizer Setup** tab in Optimizer for Peptides software.
- 2 Set the desired **Sample introduction** method (**Injection**, **Automatic infusion**, or **Manual infusion**).
- 3 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.

### NOTE

Using a fixed fragmentor voltage saves time by skipping the SIM run that determines the optimal fragmentor. However, if more than one adduct is specified, the SIM run has to be performed to determine the precursor ion to use.

- a 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.
- 4 Mark the **Start from predicted value** check box, which lets you let the optimizer software set a collision energy value, using the formula shown. In the formula, **m/z** represents the mass of the precursor ion.



- 5 Continue “To select data path and methods to use for optimization” on page 14.

## Setting Up Compounds and Working with Databases

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

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

### Using compound databases

- [“To import compounds from a database”](#) on page 21
- [“To filter compounds in Database Browser”](#) on page 23
- [“To search compounds in Database Browser”](#) on page 23
- [“To open a database and make it the default database”](#) on page 24
- [“To save compounds to a database”](#) on page 24

### Using compound information from MassHunter Acquisition methods

- [“To import compounds from a MassHunter Acquisition method”](#) on page 24
- [“To update retention times from a MassHunter Acquisition method”](#) on page 26

### Importing and exporting compound information from Excel

- [“To import compounds from an Excel file”](#) on page 27
- [“To export compound information to an Excel file”](#) on page 27

### 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 27 or [“To import compounds from a database”](#) on page 21.
- 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 21](#).
  - 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 **Nominal Mass** column.
  - e Enter the **Sample Position** for the new compound.
  - f (*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 Setup tab and information from the Precursor Ion Selection and Product Ion Selection tabs to generate the ions.
  - g 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.

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- 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) that you want to use for optimization.
  - 6 Save the compound list as described in ["To save compounds to a database"](#) on page 24 or ["To save parameters"](#) on page 12.
  - 7 (*optional*) Export the compound list as described in ["To export compound information to an Excel file"](#) on page 27.

## 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 18.
- 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 **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 14.
  - 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**. Leave the Fragmentor and Abundance cells blank, as anything you enter will be overwritten by values determined by optimization. After optimization, you can change the Fragmentor value if necessary.
- 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**. Leave the Collision Energy and Abundance cells blank, as anything you enter will be overwritten by values determined by optimization. After optimization, you can change the Collision Energy value if necessary.
  - c Repeat the above steps to add the product ions for the current compound and precursor ion.
- 5 Repeat Steps 2 - 4 to specify methods and ions for other compounds in the Compound Table.

## To import compounds from a database

Use this procedure to import compounds that have previously been saved as described in [“To save compounds to a database”](#) on page 24.



- 1 Click the **Import from Database** toolbar button to open the Database Browser.
- 2 If not already open, open the database of interest as described on [page 24](#).

### NOTE

You are asked if you want to make this database the default database. 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 won't be available for use with the new default database.

- 3 Select compounds for optimization from the database as described in the following sections.
  - [“To filter compounds in Database Browser”](#) on page 23
  - [“To search compounds in Database Browser”](#) on page 23
- 4 When the compounds from Step 3 appear in the Compound table, select the transition ions to import in *either* 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 the **Select** button. You can select the top transitions based on either **Abundance** or **Response Factor**.
- 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 the Data Browser window 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:
  - To find more compounds in the *same* database, repeat Steps 3 - 5.
  - To select compounds from a *different* database, repeat Steps 2 - 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 the **Import** button.

The Imported compounds will appear on the Compound Setup tab.

## To filter compounds in Database Browser

- 1 If not already open, open the database of interest as described on [page 24](#).
- 2 Enter the Filter criteria in the upper left area of the Database Browser Search Filter tab, such as **Optimized Compounds**, **Date range**, **Group Name**, **Project Name**, **Method**, and **Polarity**.
  - If the filter criteria are currently disabled, mark the **Enable filters** check box.
  - If you want the search to return *all items* in the database, clear all filters and mark the **Show All Records** check box.
- 3 Click the **Search** button.  
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 24](#).
- 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.
- 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** checkbooks.
- 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 the **Search** button.  
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.

## 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 the Database Browser window is not already open, click the **Import from Database** button on the toolbar to open it.



- 2 Click the **Open Database** button on the Database Browser toolbar.
- 3 Select the database of interest and click **OK**.

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



If you don't say yes now, you can still make it the default database later, by clicking the **Set Default Database** toolbar button on the Database Browser toolbar.

## To save compounds to a database

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



- To save compounds to the current database:
  - Click the **Save Compounds** button on the main toolbar, or
  - Click the **Save Database** button on the Database Browser toolbar.
- To save compounds to a *new* database, click **SaveAs Database** button on the Database Browser toolbar, enter a new name, and click the **Save** button.



You can import the compounds into other projects at a later time as described in [“To import compounds from a database”](#) on page 21.

## To import compounds from a MassHunter Acquisition method

- 1 Create MassHunter Acquisition methods for the compounds you want to import.



- 2 Click the **Import from Acquisition Methods** toolbar button in MassHunter optimizer software.



- 3** Select the MassHunter Acquisition methods from Step 1 that contain the compounds of interest, then click the **Open** button.

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

## To update retention times from a MassHunter Acquisition method

- 1 Create MassHunter 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 the **Update RTs** button on the MassHunter optimizer software main toolbar.
- 4 Select the MassHunter Acquisition methods from Step 1 that contain the compounds of interest, then click the **Open** button.
- 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, 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.

## To import compounds from an Excel file



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

## To export compound information to an Excel file



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

## Setting Up Sequences

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

- [“To set up a sequence list for peptides”](#) below
- [“To import peptide sequences from a MassHunter Qualitative Analysis method”](#) on page 30
- [“To convert multiple peptide sequences for import into MassHunter Qualitative Analysis”](#) on page 31

### To set up a sequence list for peptides

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

- 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 30.
- 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 Click the **Fill Values** button 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 16.
- 10 Save the sequence list as described in [“To save compounds to a database”](#) on page 24 or [“To save parameters”](#) on page 12.

- 11 (*optional*) Export the sequence list as described in “[To export compound information to an Excel file](#)” on page 27.

## To import peptide sequences from a MassHunter Qualitative Analysis method

*Applies to Optimizer for Peptides software 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 the next page.



- 2 Click the **Extract Peptide** toolbar button in Optimizer for Peptides software.
- 3 Select the MassHunter Qualitative Analysis method from Step 1 that contains the peptide sequences of interest, then click the **Open** button.

All sequences from the selected method are displayed in the Peptide Fragment Ion Predictor dialog box.

- 4 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.
- 5 Mark the sequences that you want to use for optimization.
- 6 (optional) Mark the **Show selected ions** option above the table, if you want to display only the ions you selected in Step 5.
- 7 Click the **Add** button. The selected peptide sequences from the method will be imported and listed on the Compound Setup tab.

## To convert multiple peptide sequences for import into MassHunter Qualitative Analysis

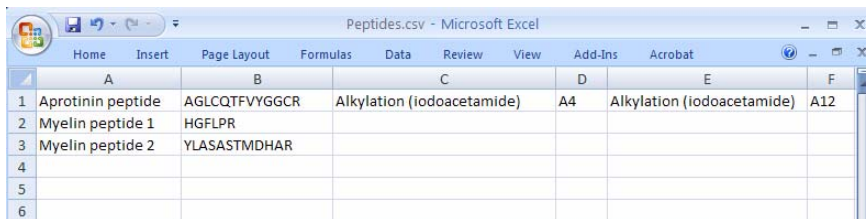
*Applies to Optimizer for Peptides software only.*

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

- 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 the **Convert .csv to .psq** toolbar button in Optimizer for Peptides software.

- 3 Select the **.CSV** file created in Step 1, and click the **Open** button.

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 software and save them in a Data Analysis method.
- 5 Import the sequences for use in Optimizer for Peptides software as described in [“To import peptide sequences from a MassHunter Qualitative Analysis method”](#) on page 30.

## Optimization and Results

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

[“To start optimization”](#) on page 33

[“To review optimization results”](#) on page 33

[“To start optimization and display ion breakdown profiles”](#) on page 34

[“To manually set collision energy voltages”](#) on page 34

[“To import optimization results to Acquisition for MRM time segments”](#) on page 36



## To start optimization

- 1 Set the optimization parameters as described on [page 14](#).
- 2 Set up the compound list as described in [“To set up a compound list”](#) on page 18 or [“To set up a sequence list for peptides”](#) on page 28.
- 3 Start the optimization process in *either* of the following ways:



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



- Click the **Ion Breakdown Profile** button 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 34.



- 4 If necessary, you can stop the optimization process by clicking the **Stop Optimization** button on the toolbar.
- 5 Review results as described below.
- 6 (*optional*) Use Agilent MassHunter Qualitative Analysis software 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 compound in the Compound table:

**Fragmentor**—The optimum voltage to apply to the exit end of the capillary for the compound, determined by MassHunter Optimizer software.

**Collision Energy**—The optimum voltage to apply to the ions in the collision cell for the compound, determined by MassHunter Optimizer software.

- 5 Review the printed optimization report.
- 6 See [“To import optimization results to Acquisition for MRM time segments”](#) on page 36, to use optimization values for data acquisition.

## 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.

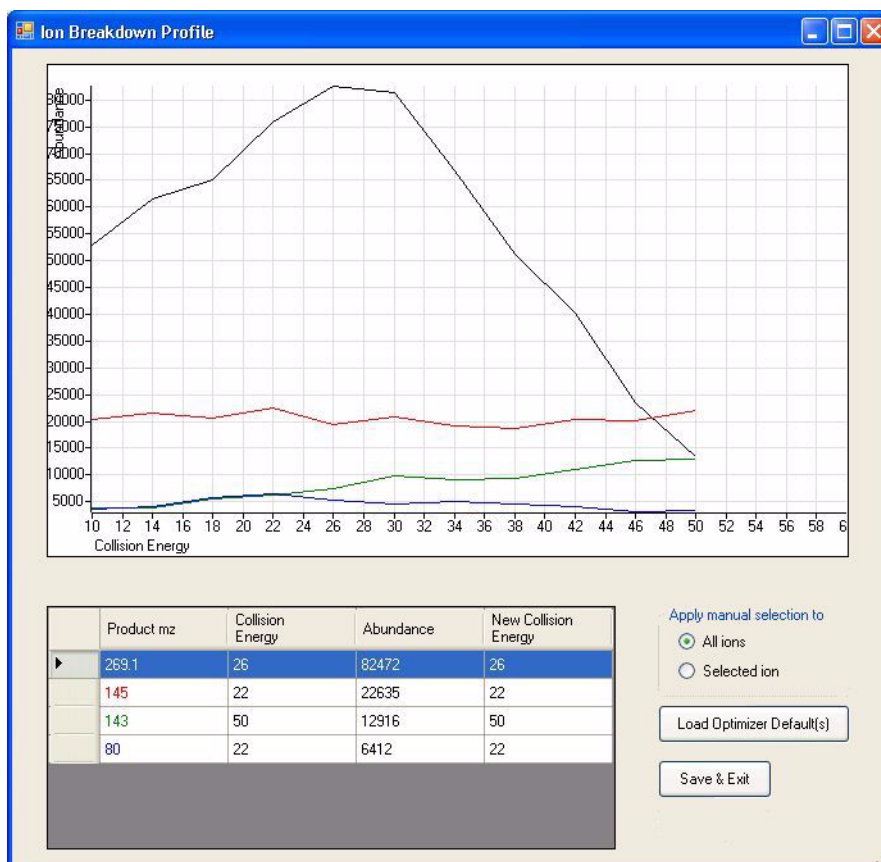
- 1 Set the optimization parameters as described on [page 14](#).
- 2 Set up the compound list as described on [page 18](#).



- 3 Click the **Ion Breakdown Profile** toolbar button 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 the **Load Optimizer Defaults** button. Doing this overwrites the manual selections you made above.
- 5 Click the **Save & Exit** button to close the Ion Breakdown Profile dialog box.



**Figure 1** Ion Breakdown Profile Results

## To import optimization results to Acquisition for MRM time segments

- 1 Display the Method pane in MassHunter Workstation Data Acquisition software.
- 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 Optimizer** from the shortcut menu.
- 8 When the Database Browser window appears:
  - a If desired, filter the compounds as described on [page 23](#).
  - b If desired, search compounds as described on [page 23](#).
  - c Mark the transition ions of interest from the Compound table in the lower half of the Database Browser window 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 the **Select** button. You can select the top transitions based on either Abundance or Response Factor. Any unwanted transitions can be deselected by clearing their check box.
  - d Do *one* of the following:
    - 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.
    - If you want to do additional search and filter operations on the current database or a different database, click **Add to Import List**.
  - e To select additional compounds:
    - To select compounds from a different database, open the database of interest.
    - Repeat Steps 8a - c to select the transition ions of interest.
    - Click **Add to Import List**.

**f** 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.

**g** Click the **Import** button.

The selected transitions are imported into the current time segment.

**9** Repeat step 5 through step 8 for each time segment of interest.

**10** (*optional*) Save the method.



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## **In this guide**

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

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