

Agilent MassHunter Optimizer for Ultivo LC/TQ

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?

Automated MS Method Development Software

MassHunter Optimizer lets you automatically optimize the data acquisition parameters for MRM mode (multiple-reaction monitoring) 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:

- Optimization of fragmentor voltage to maximize precursor ion intensity
- 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.
- Results Review shows you a graphical representation of the optimization results, allowing you to select the optimum fragmentor voltage and collision energy for selected product ions.

Additional Features

- Stores optimum precursor ion, product ions, and parameters in a compound database.
- Imports optimization results into the Agilent MassHunter Acquisition for Ultivo LC/TQ program.
- Lets you import compounds for optimization and update compound retention times from Data Acquisition methods
- Supports sample introduction via manual infusion (syringe pump), direct injection (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 source parameters using the Agilent Source Optimizer program. For more information, see the *online Help* and the *Familiarization Guide* for the MassHunter Acquisition program.

Startup

MassHunter Optimizer is automatically installed with the MassHunter Acquisition program.

To open Optimizer

You must first start the MassHunter Acquisition program before opening the MassHunter Optimizer program.

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



- Double-click the **Optimizer** icon on the desktop, *or*
- Click the Windows icon and then click **Agilent > Optimizer**.

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 5
- **“Status Bar”** on page 7

Tabs

- **“Optimizer Setup Tab”** on page 7
- **“Precursor Ion Selection Tab”** on page 7
- **“Product Ion Selection Tab”** on page 8
- **“Compound Setup Tab”** on page 8

Other Windows and Dialog Boxes

- **“Compound Browser Window”** on page 8

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 11.



Open Project Lets you open a project. See **“To open a project”** on page 11.



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 Compound Browser”** on page 28.



Delete Project(s) 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.

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 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 Compound Browser, which lets you select compounds for optimization from the database. See **“Compound Browser Window”** on page 8.



Import from Excel Lets you import compounds from an Excel file. See **“To import compounds from an Excel file”** on page 20.



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 21.

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 30



Stop Optimization Lets you terminate optimization before completion.

Generate Report Displays optimization results in PDF format.

Tools menu



Update RTs Lets you update compound retention times from Data Acquisition methods. See **“To update retention times from a MassHunter Acquisition method”** on page 22.

Review Results Opens the Review Results window.

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 in 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 13.

Precursor Ion Selection Tab

Lets you select ion 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 14.

Product Ion Selection Tab

Lets you set criteria to use in the search for product ions. See **“To select product ions”** on page 15.

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.

Compound Browser Window

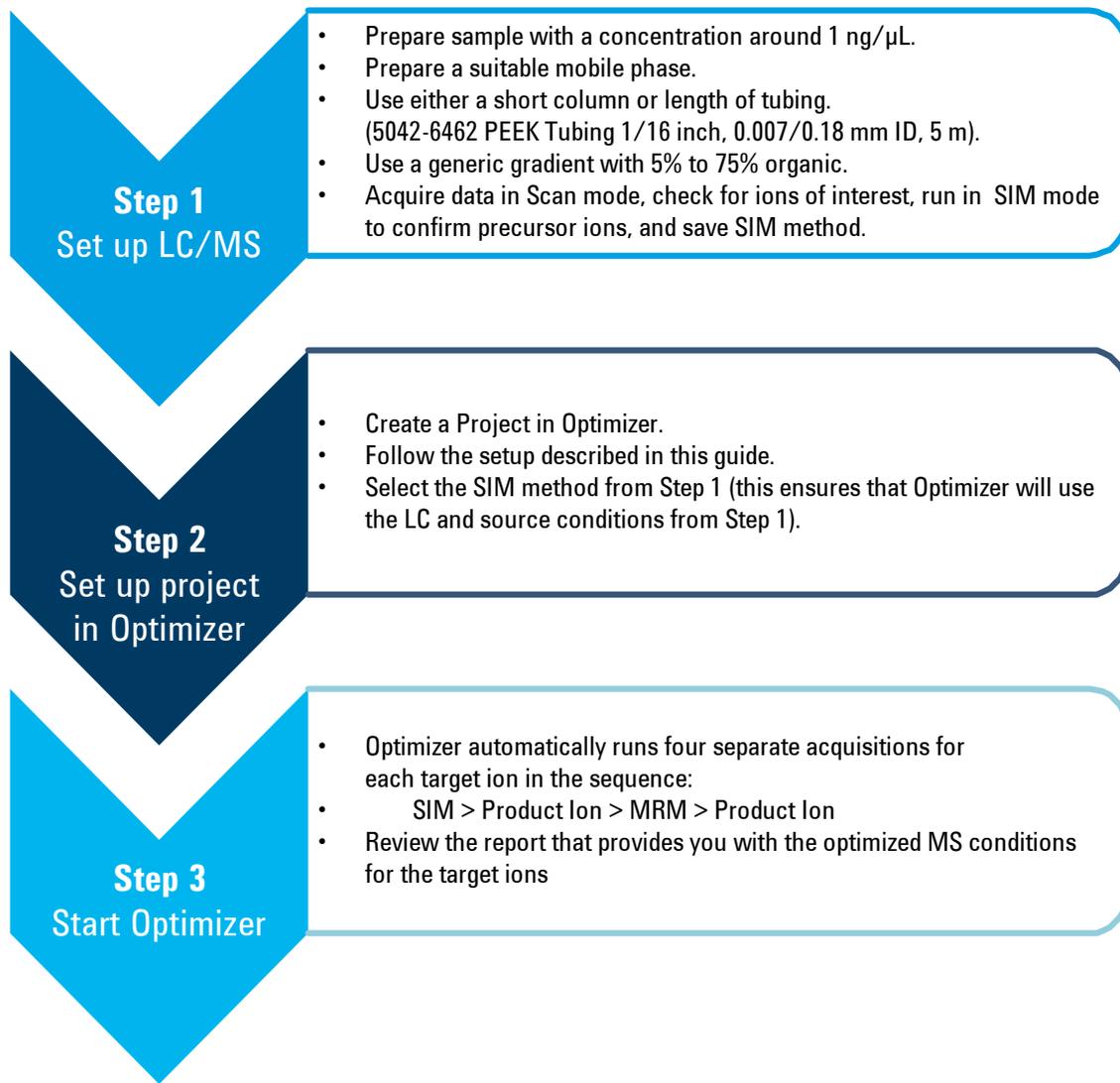


This window appears when you click the **Import from Database** toolbar button. It lets you select compounds for optimization from the database. The selected compounds are added to the Compound Setup tab. See **“To import compounds from a database”** on page 24.

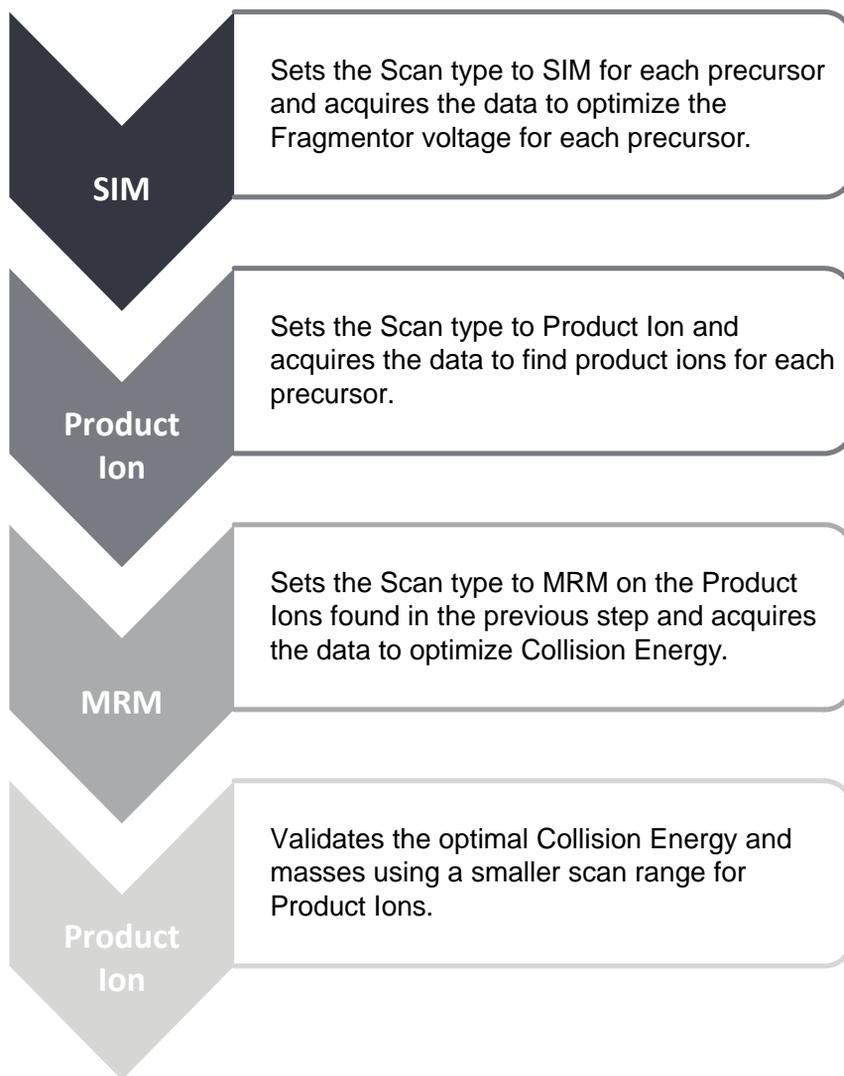
Compound Browser also lets you open a different database, save the current database with a new name, and make the current database the default database.

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.



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

Using Projects

- “To create a project” on page 11
- “To open a project” on page 11
- “To edit a project” on page 12
- “To save parameters to the current project” on page 12
- “To save parameters to a new project” on page 12

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 Ion Selection tab and Product Ion Selection tab
 - Compound Setup tab



- 3 Click the **Save Project** or **Save As Project** button 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 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 Ion Selection tab
 - Product Ion Selection tab
 - Compound Setup tab
- 3 Save the parameters. See “**To save parameters to the current project**” on page 12 or “**To save parameters to a new project**” on page 12.

To save parameters to the current project



- 1 Click the **Save Project** toolbar button.

To save parameters to a new project



- 1 Click the **Save As Project** toolbar button.
- 2 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.

“To set Optimizer acquisition parameters” on page 13

“To select data path and methods to use for optimization” on page 14

“To select precursor ions” on page 14

“To select product ions” on page 15

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 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.
- 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.

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 tab, select the acquisition methods with the appropriate LC and source parameters to use for optimization.
 - a Right-click the table and click **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 **Select Method** dialog box.
 - c Select the method of interest and click **Open**. 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 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 the  button.

- 3 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 value(s)** 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, for example water (H₂O). Enter a formula in the box, and click the  button to add it to the list.

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 17
- [“To use known ions for individual compounds”](#) on page 19

Using compound databases

- [“To open a database in Optimizer Window”](#) on page 20
- [“To save compounds to a database”](#) on page 20

Importing and exporting compound information from Excel

- [“To import compounds from an Excel file”](#) on page 20
- [“To export compound information to an Excel file”](#) on page 21

Using compound information from MassHunter Acquisition methods

- [“To import compounds from a MassHunter Acquisition method”](#) on page 21
- [“To update retention times from a MassHunter Acquisition method”](#) on page 22

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 20 or “**To import compounds from a database**” on page 24.
- 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 the table and click **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 24**.
 - 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 **asMethod**. 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 Setup tab and information from the Precursor Ion Selection and Product Ion Selection tabs to generate the ions.

Setting Up Compounds in Optimizer Window

To set up a compound list

- 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 the selected rows and click **Cut** or **Copy** from the shortcut menu.
 - c Right-click the new location for the cut or copied rows and click **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 Compound Browser**” on page 28 or “**To save parameters to the current project**” on page 12.
 - 7 (*optional*) Export the compound list as described in “**To export compound information to an Excel file**” on page 21.

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 17.
- 2 To add methods for the current compound:
 - a Right-click the **Compound** row of interest and click **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 13.
 - 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 the **Method** row for the compound of interest and click **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 the **Precursor Ion** row for the compound of interest and click **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.

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 Compound Browser, rename it with a unique name, and 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 the **Save Compounds** button 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 24.

To import compounds from an Excel file



- 1 Click the **Import from Excel** button 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.

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, and click **Save**.

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

All compounds in the data 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 Optimizer main toolbar.
- 4 Select the MassHunter Acquisition methods from **step 1** that contain the compounds of interest, and 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, 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 Compound Browser

Use these procedures to work with Compound Browser.

- “To open a database and make it the default database” on page 23
- “To import compounds from a database” on page 24
- “To select transitions and triggers for Dynamic and Triggered MRM” on page 26
- “To save a database in Compound Browser” on page 28
- “To filter compounds in Compound Browser” on page 28
- “To search compounds in Compound Browser” on page 29

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



2 Click the **Open Database** button on the Compound Browser toolbar. You instead can click **File > Open Database**.

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 the **Set Default Database** button on the Compound Browser toolbar. You can instead click **File > Set Default 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 Compound Browser](#)” on page 28.



- 1 Click the **Import from Database** toolbar button in the Optimizer window to open Compound Browser.
- 2 If not already open, open the database of interest as described on [page 23](#).
- 3 Select compounds for optimization from the database.
 - If desired, filter compounds as described on [page 28](#).
 - If desired, search compounds as described on [page 29](#).
 - 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 Compound 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 the **Select Transitions** button. 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 the **Select Transitions** button.
 - To select the secondary transitions, click the **Secondary Transitions** option, and then click the **Select Transitions** button.

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 26.

- 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 Compound 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:

- 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 click **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 select transitions and triggers for Dynamic and Triggered MRM

- 1 If not already open, open the database of interest as described on [page 23](#).
- 2 In Compound Browser, display compounds for optimization from the database.
 - If desired, filter compounds as described on [page 28](#).
 - If desired, search compounds as described on [page 29](#).
 - 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 (tMRM)

- 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 the **Select Transitions** button 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 (dMRM)

- 5 Select transitions for a *dynamic MRM method* as follows:
 - a Click **Primary transitions**.
 - b Click the **Select Transitions** button to display the transitions in the Compound table.
 - At this point, all transitions are displayed as primaries.
 - Typically, the transitions are ranked by abundance.

To save a database in Compound Browser

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

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

NOTE

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

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

To filter compounds in Compound Browser

- 1 If not already open, open the database of interest as described on [page 23](#).
- 2 Enter the Filter criteria in the upper left area of the Compound 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 the **Search/Filter** button.

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

To search compounds in Compound Browser

- 1 If not already open, open the database of interest as described on [page 23](#).
- 2 Clear the **Show All Records** check box.
- 3 Enter the **Search Text** in the upper right area of the Compound Browser Search/Filter tab, such as **Compound Name** and **Formula**. This can be either a partial or complete entry as described in [step 5](#).

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.

- 4 Mark the columns to search or click the top entry in the list (Select Columns) to search *all* columns.
- 5 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 3](#), *mark* the **Match entire word for each string** check box.
- 6 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.

- 7 Click the **Search/Filter** 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.

Optimization and Results

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

“To start optimization” on page 30

“To review optimization results” on page 31

“To manually set collision energy voltages” on page 31

“To import optimization results to Acquisition for dMRM/tMRM” on page 33

“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 13**.
- 2 Set up the compound list as described in “**To set up a compound list**” on page 17.
- 3 Start the optimization process:



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



- 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 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.

Collision Energy—The optimum voltage to apply to the ions in the collision cell for the compound, determined by MassHunter Optimizer.
- 5 Click **Optimization > Generate Report**.
- 6 Review the printed optimization report.
- 7 See **“To import optimization results to Acquisition for MRM time segments”** on page 36, to use optimization values for data acquisition.

To manually set collision energy voltages

- 1 Set optimization parameters and start optimization as described above.
- 2 Click **Tools > Review Results**. The Review Results dialog box opens. See **Figure 1** on page 32.
- 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 **Reset Collision Energy** button or the **Reset Fragmentor** button. Doing this overwrites the manual selections you made above.
- 5 To save the results, click . The changes that you made are saved. You can see these changes in the Compound Setup tab in the fourth level of the table.
- 6 Click the **X** in the upper right corner to close the Review Results command.

Optimization and Results

To manually set collision energy voltages



Figure 1 Review Results dialog box

To import optimization results to Acquisition for dMRM/tMRM

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



- 6 Set **Scan type** to **dMRM** or **tMRM** as shown above.
- 7 For triggered MRM, mark the **Triggered** check box in the lower right area of the Acquisition tab.



- 8 Notice that additional columns are now available in the Scan segments table, including **Primary**, **Trigger**, **Trigger Window**, **Trigger Threshold**, **Trigger Entrance**, and **Trigger Delay**.
- 9 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 dMRM and tMRM. 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.
- 10 Click **Compound Browser** in the left pane.

Optimization and Results

To import optimization results to Acquisition for dMRM/tMRM

- 11 When Compound Browser appears:
 - a If desired, filter the compounds as described on [page 28](#).
 - b If desired, search compounds as described on [page 29](#).
 - c Mark the transition ions of interest from the Compound table in the lower half of Compound 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 the **Select** button. 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.
- 12 If the transition ions selected in the table are the only ones you want to import at this time, click **Import**. This closes the Compound 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**.
- 13 To select additional compounds:
 - a To select compounds from a different database, open the database of interest as described on [page 23](#).

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 11](#) to select the transition ions of interest.
 - c Click **Add to Import List**.
- 14 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 click **Remove** from the shortcut menu.
- 15 Click the **Import** button. The selected transitions are imported into the current time segment.

- 16** Review the timings to make sure that not too many transitions are being monitored concurrently. The graph in the Acquisition section shows how many transitions are concurrent.

NOTE

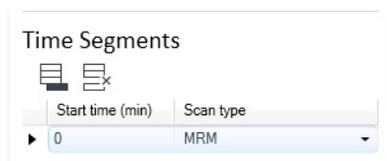
When too many transitions are active at the same time, the dwell time per transition may become too short (1-2 msec per transition or less). Under these conditions, the signal/noise ratio is low, and the quantitation accuracy and precision may suffer.

If necessary, change the retention time windows or delete transitions to increase the average dwell time.

- 17** (*optional*) Save the method.

To import optimization results to Acquisition for MRM time segments

- 1 Display the Method Editor window in the MassHunter Acquisition program.
- 2 Create or open the method of interest.
- 3 Click the **QQQ** tab in the Method Editor window.
- 4 Click the **Acquisition** section in the left pane.
- 5 Select the time segment of interest or create a new one.



- 6 Set **Scan Type** to **MRM**.
- 7 Click **Compound Browser** in the left pane.
- 8 When Compound Browser appears:
 - a If desired, filter the compounds as described on [page 28](#).
 - b If desired, search compounds as described on [page 29](#).
 - c Mark the transition ions of interest from the Compound table in the lower half of Compound 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 the **Select** button. 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 Compound 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 23](#).

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 the **Import** button. 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 C.01.00 revision or higher of the Agilent MassHunter Optimizer software, until superseded.

