Agilent MassHunter Optimizer for GC Triple Quad

Quick Start

Notices

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CAUTION

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Welcome to the Agilent MassHunter Optimizer

The Agilent MassHunter Optimizer for GC Triple Quad will assist you in developing optimized MRM transitions for creating MRM and dMRM methods.

Starting with a GC/MS method that provides good chromatographic compound separation, the MassHunter Optimizer will first identify precursor ions and product ions, and then optimize collision energies for each promising precursor-product combination to identify the best MRM parameters.

In This Book

This document contains an overview of the MassHunter Optimizer for GC Triple Quad software. In the following pages you will find information on:

- Running the Optimizer in an automatic mode
- Icons found in the Optimizer software
- Default settings in the Optimizer

Refer to your Online Help for more details.

Running the Optimizer in an Automated Mode

The following pages include an example of how to run the Optimizer in an automated mode. In this example, we begin with Scan data and use default settings, to optimize MRMs for new compounds. Later, as you become more familiar with the process, you may modify it to meet your specific needs. See the Online Help for more details.

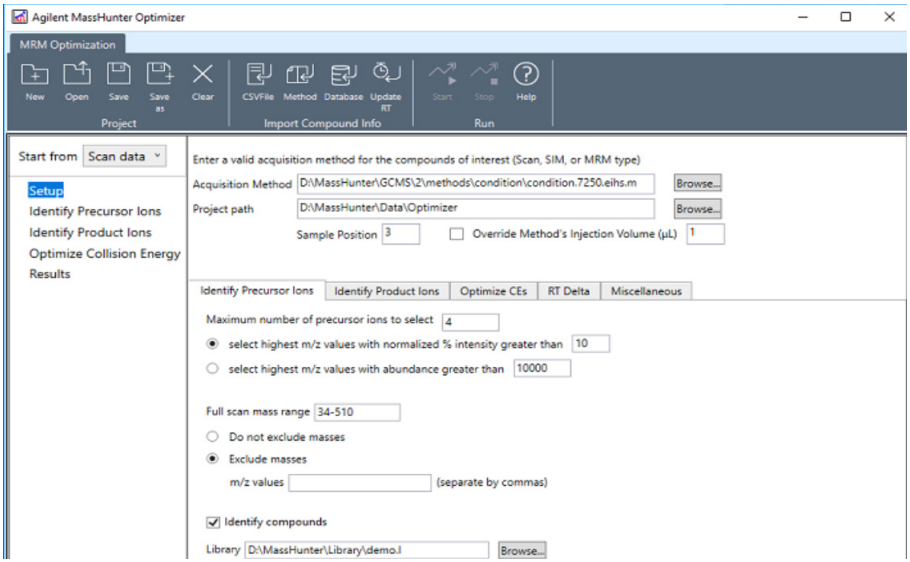
To run in an automated mode you will:

1. **Set up a Project**. To do this you will define the high level properties of the project, such as the project name, path, and acquisition method.
2. Identify the **compounds of interest**.
3. Click **Start Optimization**.
4. **Save** your results.

Each of these steps is described in more detail on the following pages.

MassHunter Optimizer Opening Screen

With MassHunter running, double-click the Optimizer startup icon  to display the opening screen.



Ribbon Toolbar Icons


Create a new project


Open a previously saved project


Save a project


Save as a different project


Clear the project


Import compound information from a CSV file


Import compound information from an acquisition method (SIM, MRM, or dMRM)


Import compound information from a Database


Import retention times from a Quant report or an MRM/dMRM data file


Run optimization automatically. This button will be enabled when the prerequisite for auto run is met.


Stop optimization. This button will be enabled after optimization is started.

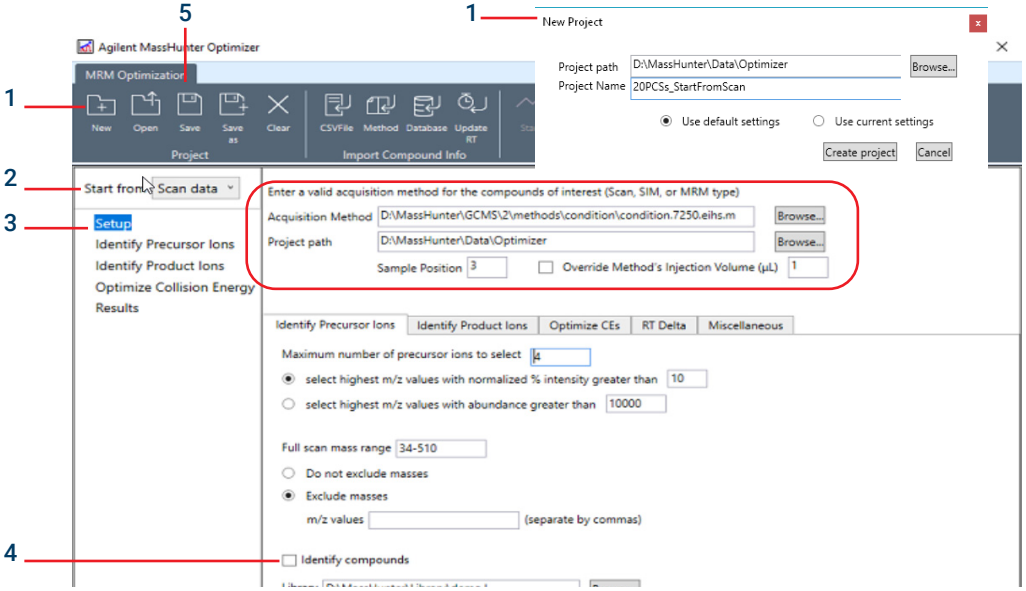

Online Help

Continue reading the next page to learn how to setup a new project.

1 Setup a Project

To Setup a project to run in an automated mode using defaults:

1. On the opening screen, click **New** to display the **New Project** dialog. Keep the default **Project path** (MassHunter\Data\Optimizer), or browse and enter an alternate, then enter a **Project name** (E.G. Demo1), select **Use default settings**, and click **Create project**. The Optimizer creates a folder that will contain all the data for this project. (E.G. MassHunter\Data\Optimizer\Demo1_Project)
2. Select **Scan data**.
3. Select **Setup**, then enter the GC **Acquisition Method** name, **Project path**, and **Sample position**. This acquisition method should sufficiently separate the compounds of interest, include enough cooling and equilibrium time to ensure the retention time is stable between injections, and provide a sample concentration high enough to give strong peaks. The sample vial containing the compounds of interest should be placed in the position specified here.
4. On the **Identify Precursor Ions** tab, by default, **Identify compounds** is selected. You could click **Browse** and navigate to the library you would like to use for compound identification, or uncheck the option to continue without identifying a library. For this example, we will manually enter the compound names, so uncheck the option to continue.
5. For this example, we are using the defaults provided under each of the five tabs shown on the main screen (**Identify Precursor Ions**, **Identify Product Ions**, **Optimize CEs**, **RT Delta**, and **Miscellaneous**). So this completes the initial setup. Click **Save** . You may review the default settings for each of these tabs beginning with **"Precursor Ion Default Settings"** on page 11.
6. For now, continue with **"2 Identify the Compounds of Interest"** on page 7.



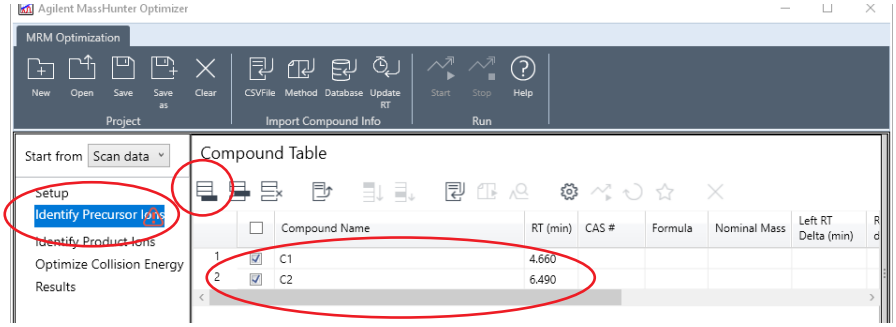
2 Identify the Compounds of Interest

1 After setting up the project, select **Identify Precursor Ions**, then, in the Compound Table, click **Add a compound**, and enter a **Compound Name** and the **Retention Time** for each compound of interest. (Alternatively, you could use the or buttons to import or acquire a full scan data. See the Online Help for details.)

   The Optimizer can now identify the precursor ions for these compounds.

   The compound name, although required, will not be used as an identification attribute, so you may enter C1, C2, C3, etc. However, specifying the correct retention time of a compound is critical for correct identification of the chromatogram component of the compound thus the precursor ions.

2 When you have entered all of the compounds of interest, move on to **"3 Begin the Optimization Process"** on page 8.

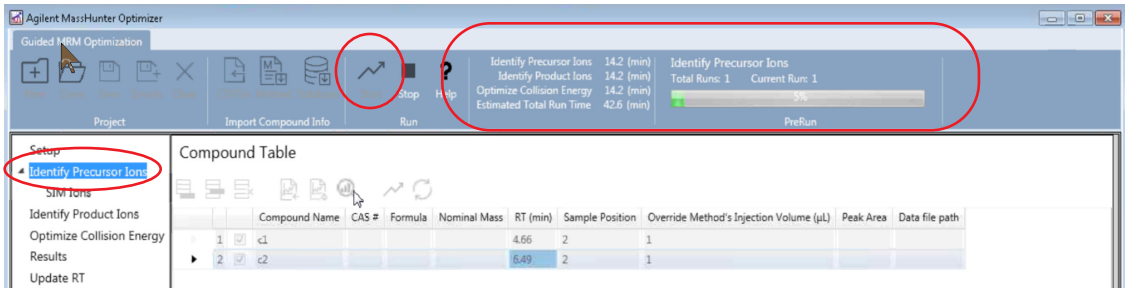


3 Begin the Optimization Process

1 As soon as one compound name and retention time is entered in the table, the **Start Optimization** icon in the Ribbon toolbar will be enabled. When all compounds are added, click .

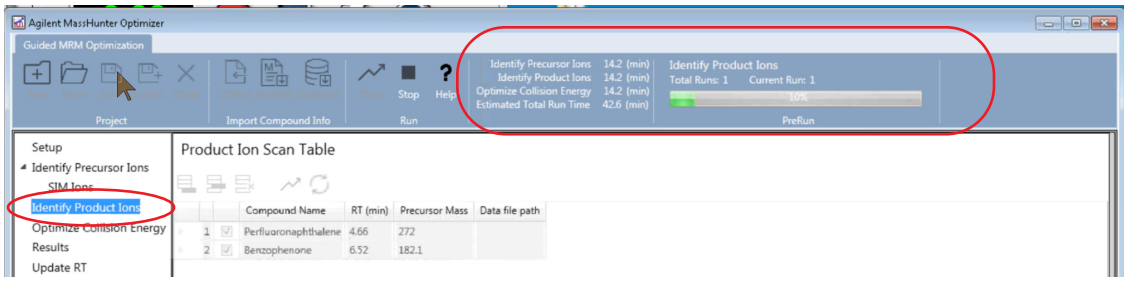
2 A confirmation dialog is displayed, click **Start** to continue. MassHunter Optimizer will begin to acquire and analyze full scan data and identify precursor ions according to precursor Ion settings.

As the precursor ions are being identified, the status of the run is displayed in the toolbar.



The Optimizer will substitute the time segments and scan segments to create new methods as the optimization progresses.

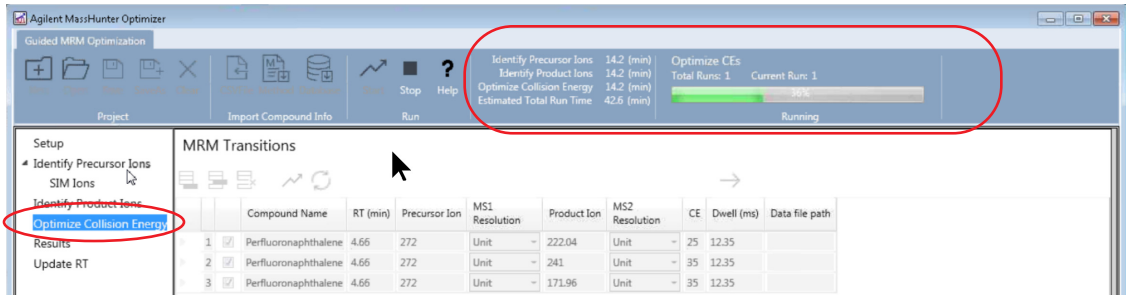
3 When the Optimizer is running in an automated mode, after identifying the precursor ions, it automatically begins to identify the Product ions. You may select **Identify Product Ions** to view the **Product Ion Scan Table** as it is populated.



- MassHunter Optimizer automatically creates and executes a sequence of methods to determine the product ions associated with each of the precursor ions determined in the previous step. Each product ion method consists of a series of product ions scans for a single precursor ion at multiple collision energies.
- MassHunter Optimizer automatically analyzes the resulting data and presents you with recommended sets of precursor and product ions for the next phase, which you may modify.

4 When running in an automatic mode, after identifying the Precursor Ions and Product Ions, the Optimizer will move on to the next step and **Optimize the Collision Energy**. During this phase:

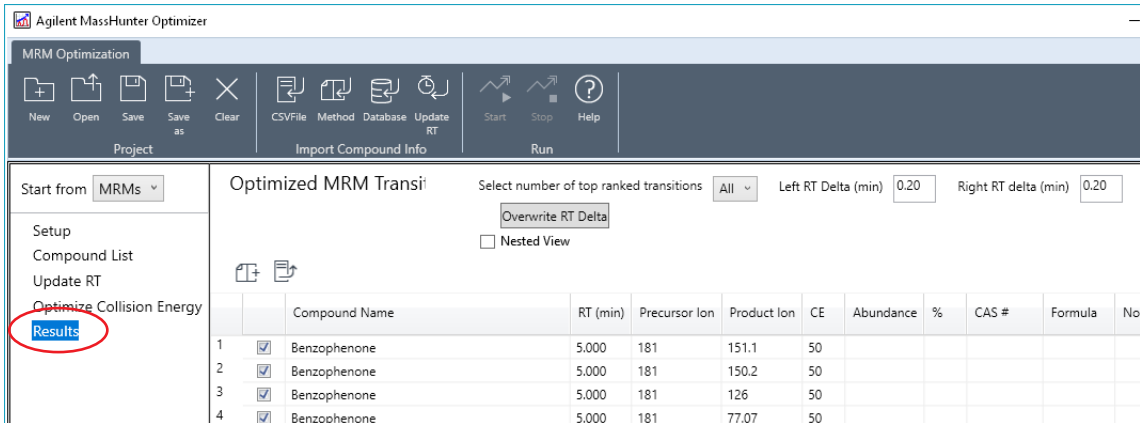
- MassHunter Optimizer automatically creates and executes a series of methods (in MRM mode) to determine the optimum collision energy for each precursor/product combination.
- MassHunter Optimizer automatically analyzes the resulting MRM data and presents the results in tabular format.



4 Save Your Results

When the optimization is complete, select **Results** and review the findings. From here you may:

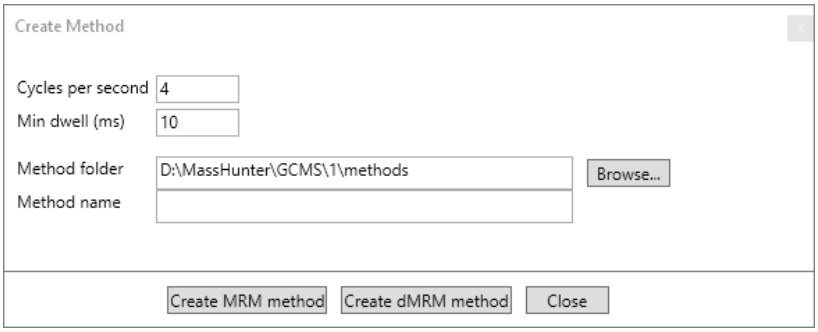
- Create an optimized MRM or dMRM acquisition method or
- Save the transitions to a CSV file



1 For this example, we selected to save the results to an MRM or dMRM method. The **Create Method** dialog displays.

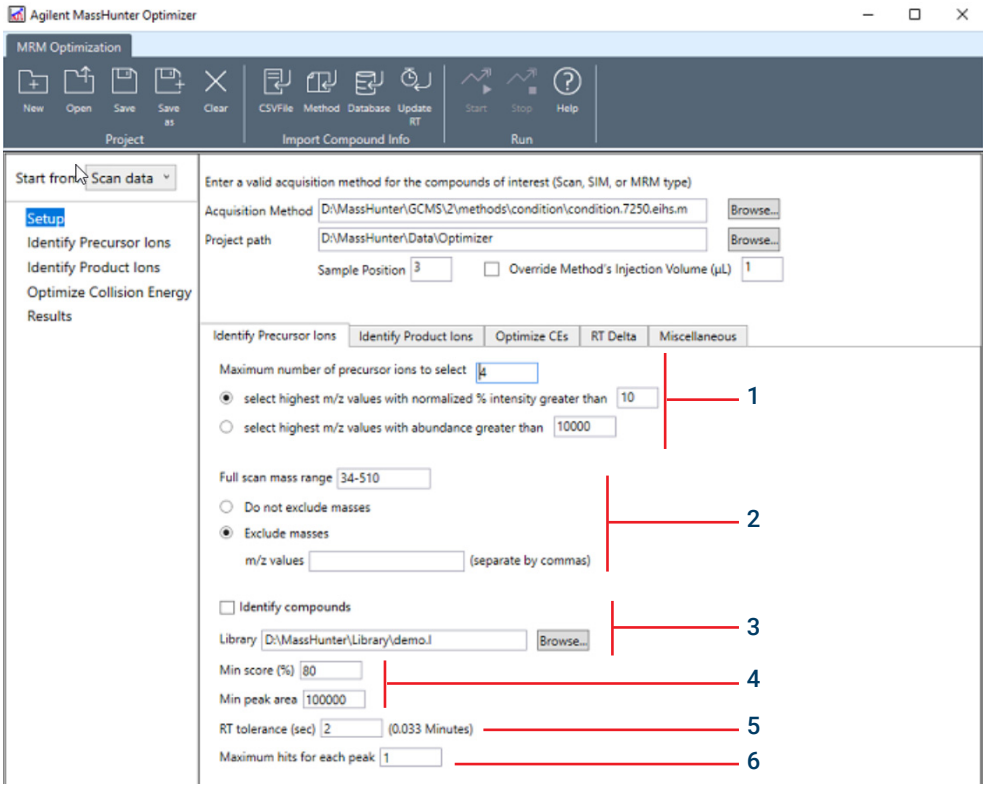
2 The default cycles per second are calculated according to the narrowest peak, assuming the number of data points for the narrowest baseline peak is 20. Typical baseline peak widths of 3-6 seconds, result in about 3-6 cycles per second. Enter a **Method name**.

3 Click **Create MRM method** or **Create dMRM method** to complete the process.



Precursor Ion Default Settings

Precursor Ion settings (defaults shown here) provide the criteria for identifying compounds and selecting the best precursor ions. You may adjust these settings as applicable for your Project.



1 Maximum number of precursor ions to select automatically. You may select precursor ions by relative or absolute abundance, weighted by mass (higher mass weights more).

2 Mass range to be scanned. You may exclude masses.

3 You may search the NIST library to identify the compounds for chromatogram peaks specified by RTs. If unchecked, compounds will be named cmpd_1,2,3... and the library score will not be available.

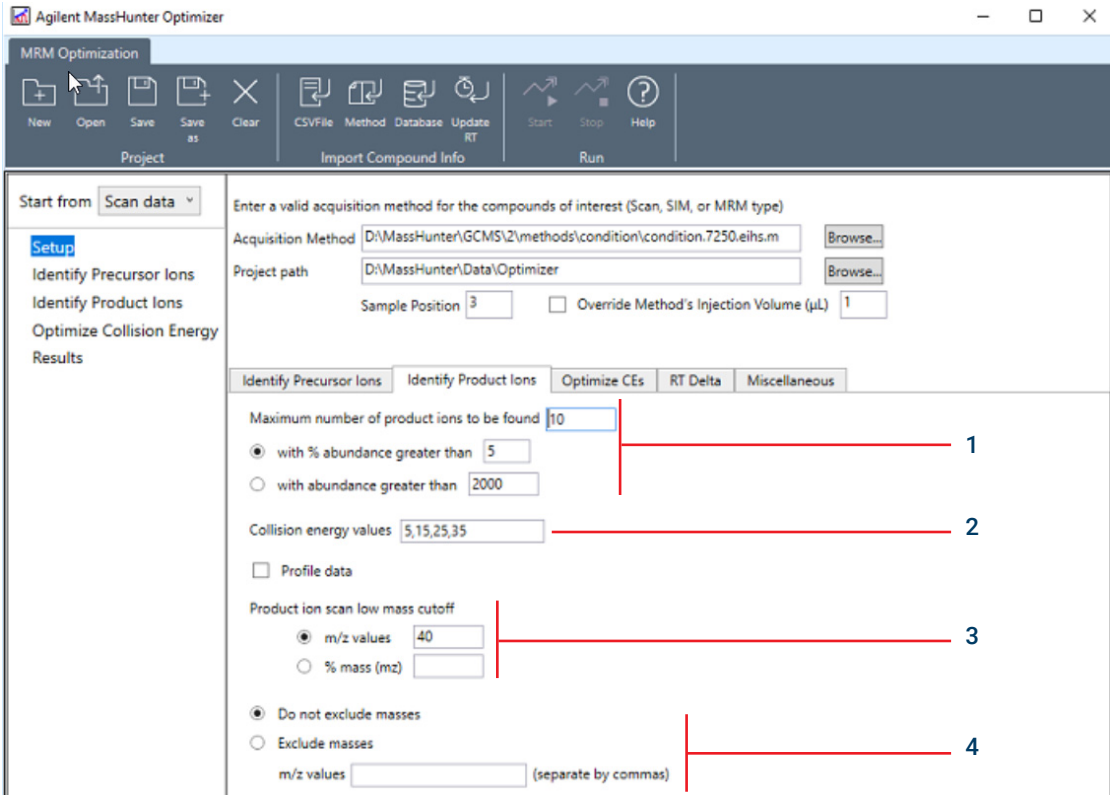
4 You may specify a minimum library match score and a minimum peak area.

5 A compound is identified by RT and RT tolerance (the most abundant deconvoluted component with highest library score within the RT window will be selected). The more accurate the RT, the smaller the RT tolerance can be.

6 More than one hit can be selected for each RT.

Product Ion Default Settings

For each precursor ion, product ions will be scanned using a maximum of 4 different collision energies. The most abundant ions and optimal CE value will be selected. Defaults are shown here.



1 The maximum number of product ions to be found.You may select the product ions by relative or absolute abundance.

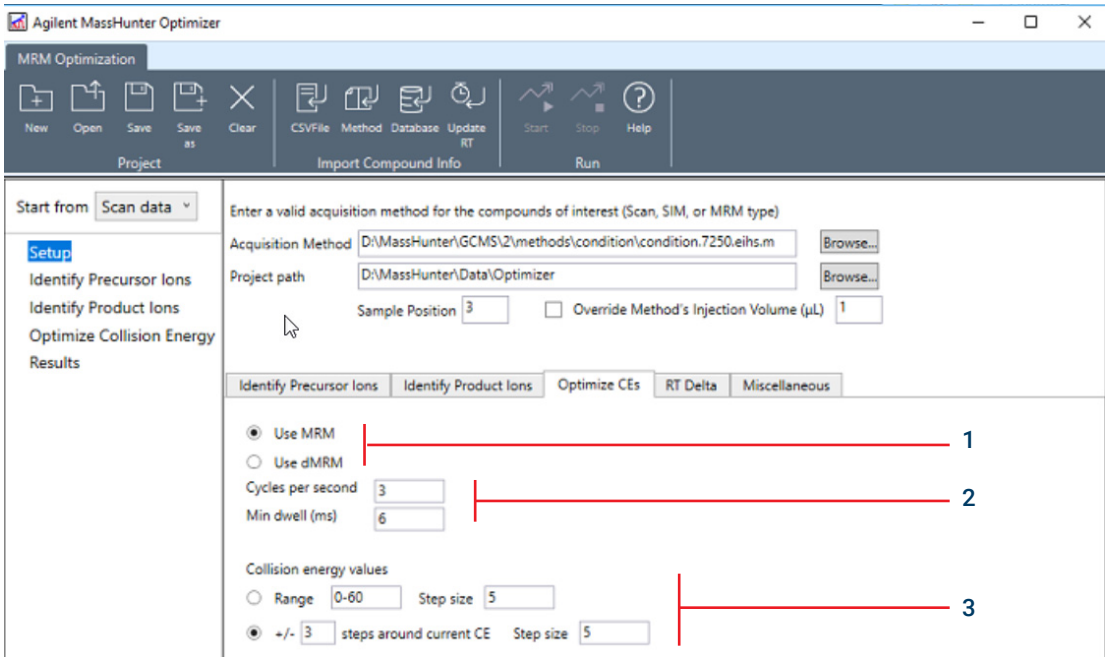
2 Enter at least 1 but no more 4 comma separated collision energy values to be used to perform the product ion scan for each precursor ion.

3 A low mass cutoff of product ion scan will be applied. The low mass cutoff can be specified as an absolute m/z value, or a specified percentage of the precursor mass.

4 You may exclude or include masses.

Optimized Collision Energy (CE) Defaults

The collision energy of MRM transitions is optimized by acquiring MRM or dMRM data for multiple CEs and selecting the optimal CE for each transition. Defaults are shown here.



1 Select the type of method you want: MRM or dMRM.

2 The cycles per second and minimum dwell time. The cycle time and min dwell will be used to create either an MRM or dMRM method.

The maximum number of scan segments in a time segment will depend on the minimum dwell of scan segments. The bigger the minimum dwell time, the fewer the scan segments will be allowed.

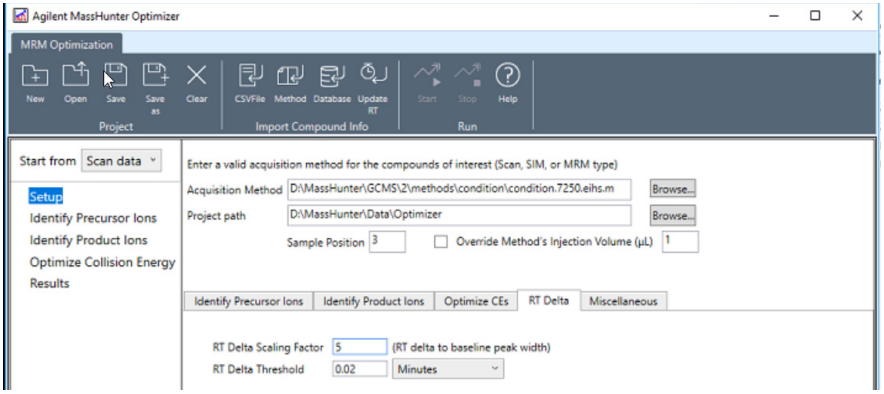
3 The CE range and the step size. You may use the current CE (coarsely optimized) and take +/- N steps from the current CE.

If the number of transitions of a compound multiplied by the number of CE steps is greater than the maximum allowed scan segments (affected minimum dwell time), the MRM or dMRM method will be split into two or more runs.

Retention Time Delta Defaults

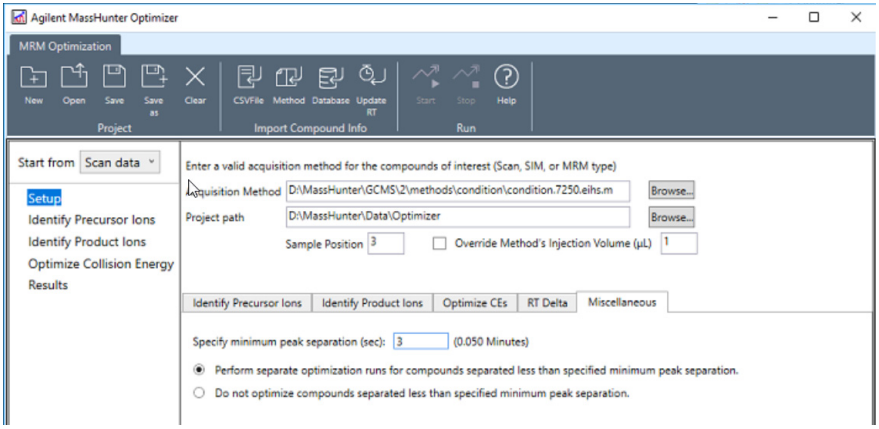
When peaks are identified in the Identify Precursor Ions step, or in the SIM ions step, or in the Update RT step, the RT and RT delta of the compounds will be updated. This panel allows you to specify how RT delta will be calculated.

The RT deltas will be determined by multiplying a scaling factor to the baseline peak width of the chromatogram peak. Defaults are shown here.



Miscellaneous Defaults

Here you may specify the minimum peak separation. If two peaks are separated less than the limit, they will be considered as coeluting. You may, optionally, optimize coeluting compounds in separate runs, or ignore the coeluting compounds. Defaults are shown here.



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