MassHunter Optimizer Software for Automated MRM Method Development Using the Agilent 6400 Series Triple Quadrupole Mass Spectrometers

Technical Overview

Introduction

Triple-quadrupole mass spectrometers are considered the gold standard for quantitative mass spectrometry analyses for a wide variety of applications, especially if the analyte of interest has to be quantified in a complex biological matrix such as plasma, blood, or urine. The selectivity, sensitivity, dynamic range and linearity of LC/MS/MS enable the development of rigorous and robust analytical methods for target compound quantitation.

The majority of the triple-quadrupole instrument parameters can be set by using the Autotune functions within the MassHunter software. However, there are a number of compound specific parameters that can be automatically determined with MassHunter Optimizer software. These parameters can be stored in a database and subsequently used by the MassHunter Acquisition software for specific method development.

In order to rapidly achieve optimum results for each target compound, the software automatically optimizes fragmentor voltage and collision energy (CE) for up to four Multiple Reaction Monitoring (MRM) transitions after determining the m/z values for precursor and product ions.

MassHunter Optimizer software is unique in that it supports four different workflows (manual injection, loop injection, HPLC without and with column). While existing optimization software was mainly designed for the injection and HPLC without column approaches, MassHunter Optimizer was developed to achieve unprecedented optimization yields and robustness via the HPLC with column workflow.

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Description

MassHunter Optimizer is enabled using the tabbed screens in its user interface. On the first screen, the names and nominal masses or formulas for one or more compounds are provided. See **Figure 1**. Compound lists can also be imported from Microsoft® Excel.
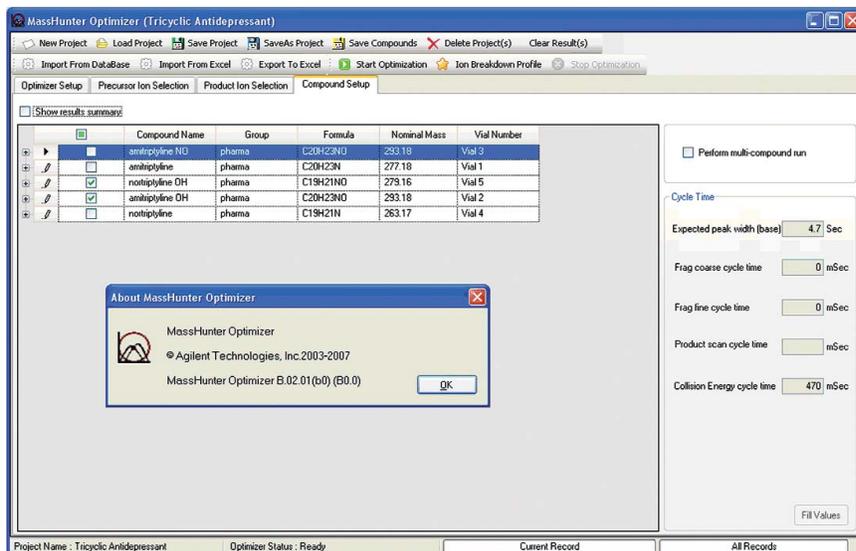
Figure 1: List of 5 tricyclic antidepressants and metabolites shown in the Compound Setup screen.

On the Precursor Ion Selection screen, conditions for selecting the appropriate precursor ion can be set. See **Figure 2**. By default, the software will select the most abundant ion for optimization unless the checkmark is removed. The charge carriers to be considered can be specified and specific ions can be excluded if useful. Charge state limitations may be applied as well.
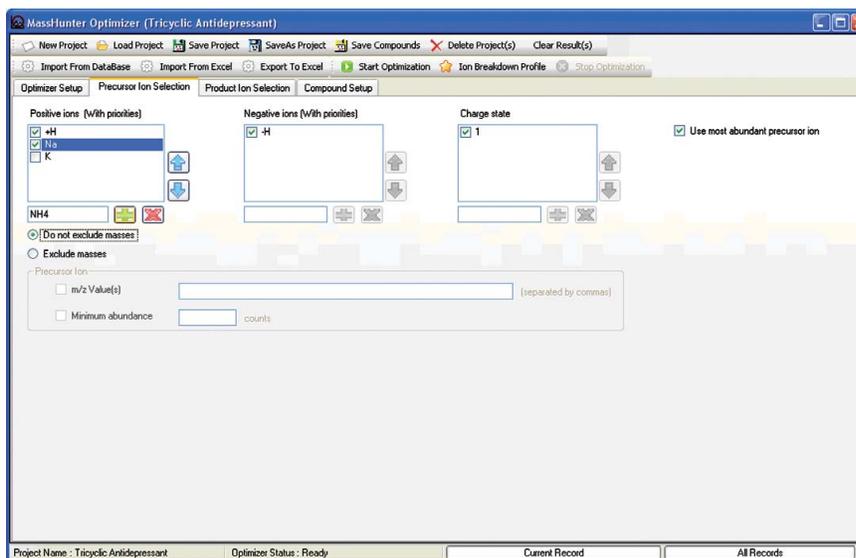
Figure 2: MassHunter Precursor Ion Selection screen.

To avoid non specific product ions being selected for optimization, either a low mass cut-off value can be specified in absolute *m/z*, or relative to precursor *m/z*, and/or certain *m/z* values can be excluded in the third tab, e.g. if they derive from a non specific fragmentation of the precursor ion, or if their abundance is too low. See **Figure 3**.
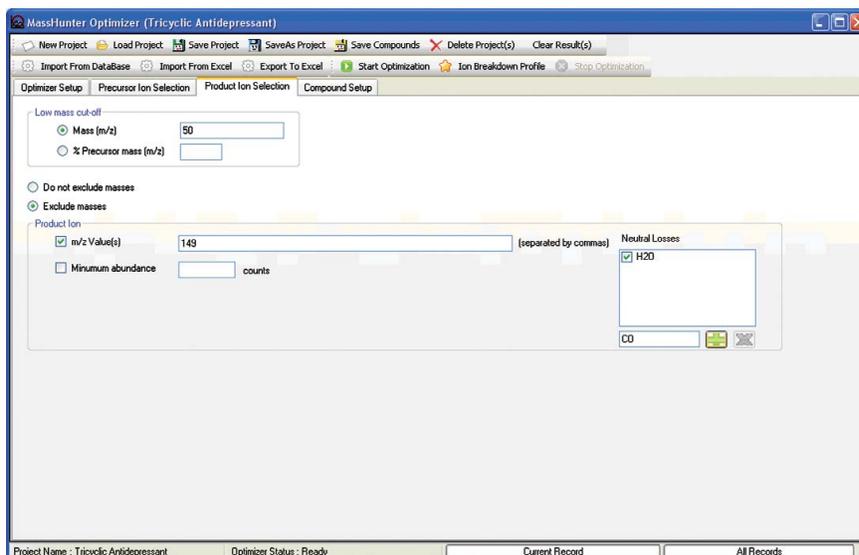
Figure 3: Product Ion Selection screen.

On the fourth tab, the user defines the mode of sample introduction to the system. See **Figure 4**. In order to provide maximum flexibility, four options are available: manual infusion via syringe, automatic infusion using loop injection, and sample injection with or without HPLC column.

Note that MassHunter Optimizer was designed specifically to support the HPLC with column workflow. While it requires more time per run, the yield and robustness of optimization is much higher. In order to be useful, the optimization software must routinely optimize nearly 100% of compounds submitted for analysis. MassHunter Optimizer is the only product on the market which fulfills this requirement.

On the same menu page, the fragmentor voltage and collision energy ranges are defined together with the step width for optional fragmentor fine tuning. Previously determined values in the database can be kept or overwritten during the optimization process.
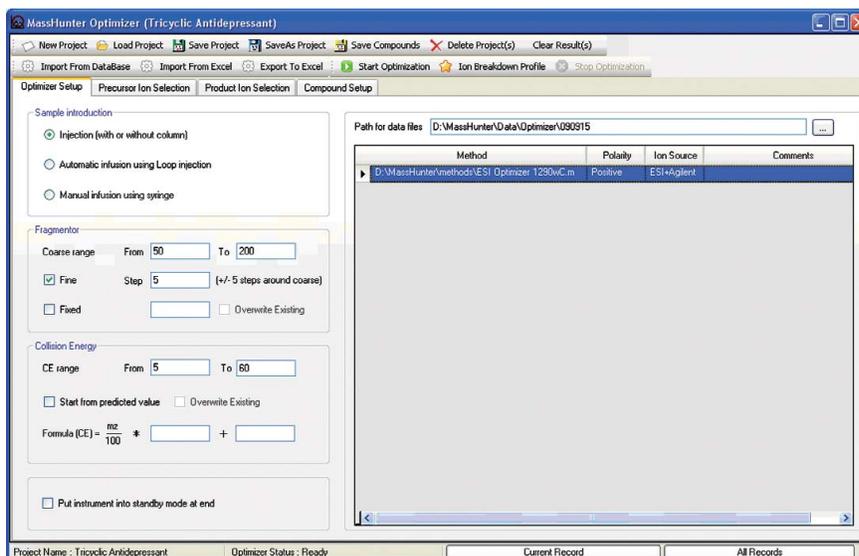
Figure 4: Optimizer Setup screen.

The system first detects the coarse fragmentor voltage, which results in a maximum ion intensity of the selected MRM (**Figure 5, bottom**), and then optionally refines the fragmentor voltage by stepping in smaller increments to get the optimum system response (**Figure 5, top**).

Figure 5: Fragmentor voltage, coarse (bottom) and fine tune (top).
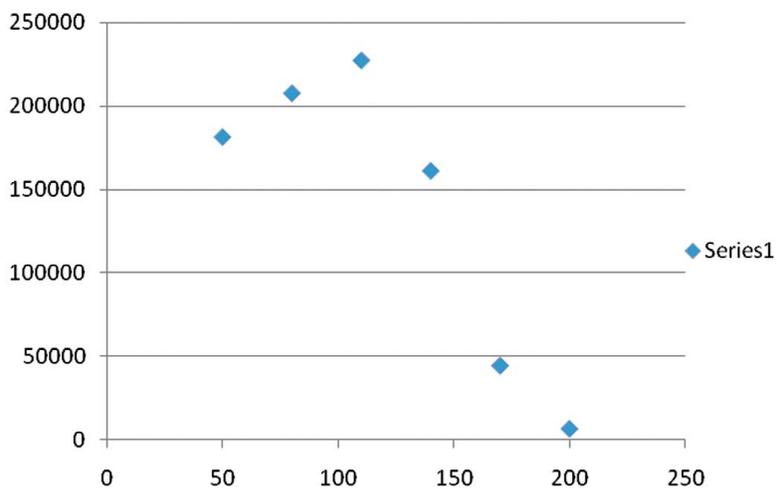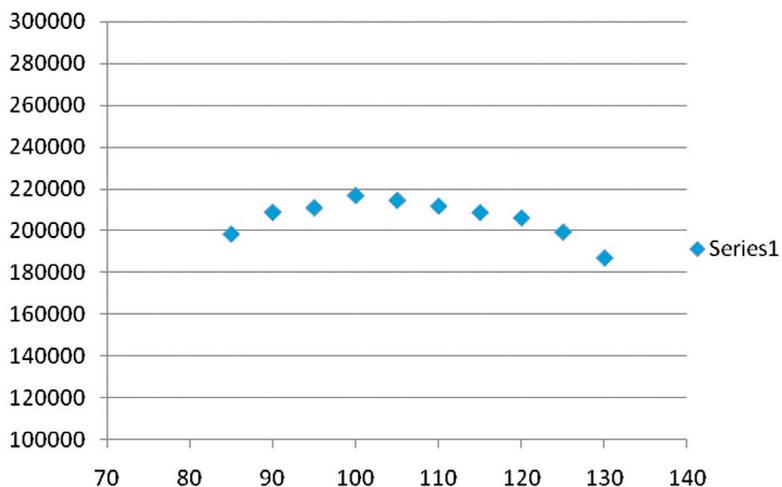
After optimizing the fragmentor voltage, the optimum collision energy setting is determined by applying modified CE settings and keeping the setting with the highest signal intensity. **Figure 6** displays an overlay of the signals generated by applying different collision energies to one MRM transition.
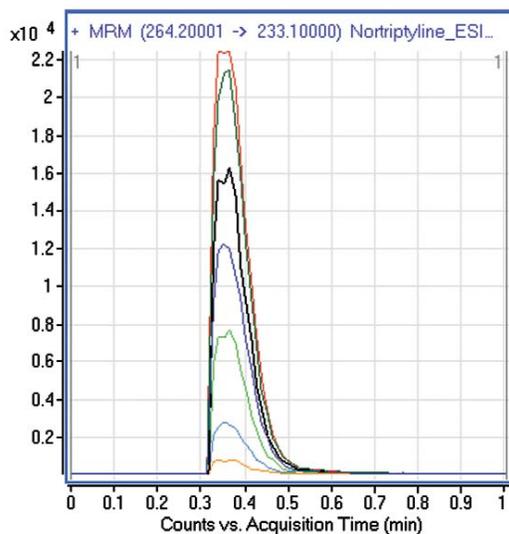
Figure 6: Overlay of different CE settings for one MRM transition for Nortriptyline.

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In order to facilitate the setting of an optimum CE, especially when multiple MRM transitions need to be monitored at high sensitivity, the user can decide on the best settings based on an ion breakdown profile as shown in **Figure 7**.
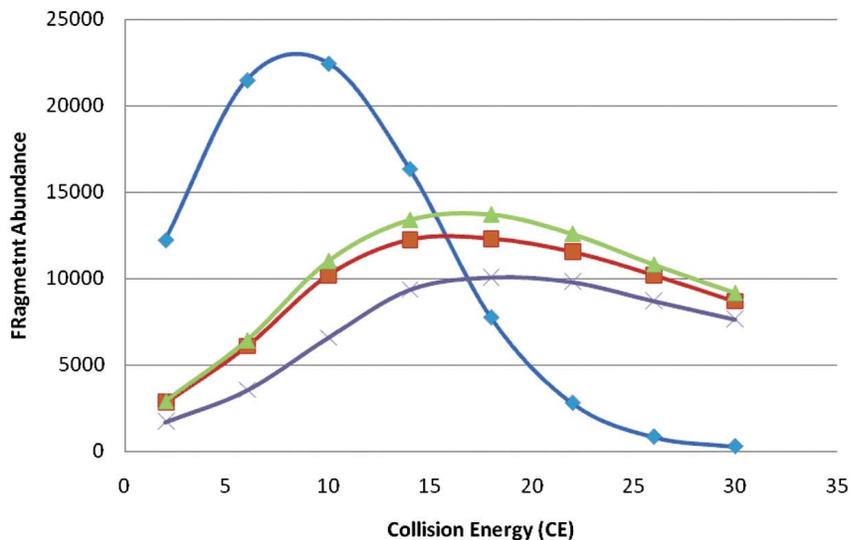
Figure 7: Ion breakdown profile for multiple MRMs.

Finally, Optimizer can display the product ions selected together with the optimized MRM conditions. See **Figure 8**. By pressing the "Export to Excel" button (third left in second row), the results can be exported to a Microsoft Excel file.

The final optimized MRM conditions can be stored in a database or loaded into the MassHunter Acquisition software for performing quantitative analyses. This procedure is described in a separate Technical Note.[1]
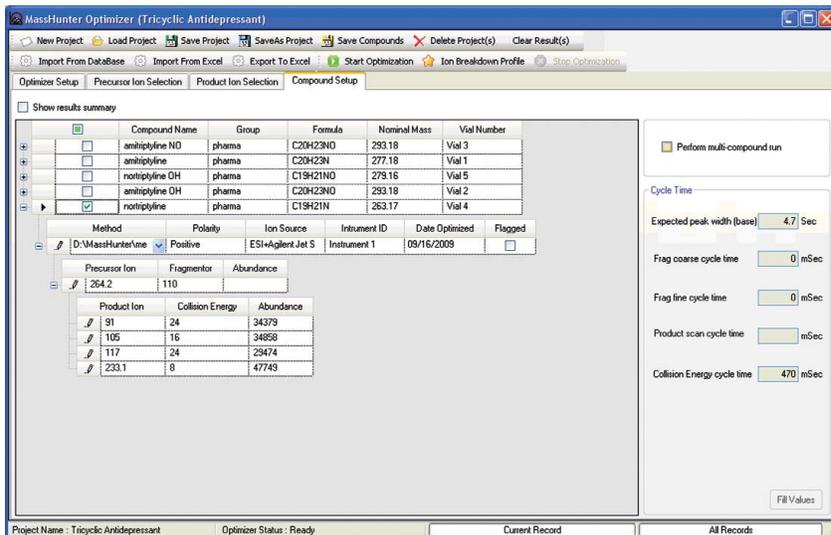
Figure 8: Display of selected MRM transitions from the Optimizer output.

Summary

For each specified compound, the MassHunter Optimizer software can perform the following steps:

1. Determine the presence of precursor ions based on the adduct(s) selected using SIM and optimize fragmentor voltage within a user predefined range.

2. Fine tune the fragmentor voltage (optional).

3. Use the optimal fragmentor voltage and inject each analyte using Product Ion Scan mode to determine the most abundant product ions while varying the CE to determine optimal product ions.

4. Further optimize and fine tune the CE for those product ions using MRM mode.

5. Perform a narrow mass scan for each product ion to more accurately determine the actual m/z value, for use in subsequent analytical methods.

The results obtained during the optimization process can be used to setup a MassHunter acquisition experiment with optimum MRM conditions and/or to export to a database to facilitate MRM method development for the target compounds.

Comparing the MRM settings determined by MassHunter Optimizer software with manual optimization results shows that in more than 80% of all cases, the automated results of Optimizer could not be significantly improved by manual optimization.

While the MassHunter Optimizer software supports all possible workflows, it is specifically designed to support the HPLC with column workflow, resulting in high optimization yields and robustness.

References:

1. "Pesticide Dynamic MRM Compound Database for Screening and Identification using the Agilent LC/MS Triple Quadrupole Systems," Agilent publication number 5990-4255EN, **2009**.

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