An end-to-end software algorithm for LC/MS/MS method development, optimization, and QA/QC deployment

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Introduction

The act of developing targeted triple quadrupole LC/MS (LC/TQ) methods from start to finish is a complex and time-consuming, multi-step workflow. Method development becomes even more challenging if former mass spectrometry parameters for each analyte were not established – especially in the case of novel compounds. In such cases, "by-hand" optimization and characterization is needed to obtain the most effective MRM parameters.

Using the new **6475 triple quadrupole LC/MS system with MassHunter 12, intelligent workflows were added to ensure that users, particularly in routine analysis QA/QC lab environments, can maximize their operational efficiency.** One aspect of LC/MS/MS analysis that may benefit from efficiency improvements is with the assisting and automation of method optimization. MassHunter 12 features an embedded 21CFR compliant, method-oriented, intelligent optimizer; allowing users to optimize MRMs and ion source parameters in a fully-automated or semi-automated fashion.

Using a modular end-to-end workflow approach, users may input chemical formula information which will result in (1) optimized MRM transitions for each compound and (2) optimal ion source parameters for the overall method. A unique feature of this algorithm is the ability to take advantage of the LC/TQ's speed to allow the simultaneous optimization of multiple compounds on a per-method basis, such that pre-production method development time is dramatically reduced compared to compound-by-compound approaches.

Experimental

Chemical formulas of the neutral analytes found in the LCMS 7-Analyte Sys Suitability Standard (amitriptyline hydrochloride, diethyl phthalate, diamyl phthalate, dihexyl phthalate, dioctyl phthalate, 8-bromoguanosine hydrate, and 4-chlorocinnamic acid) were entered into the method development interface to automatically calculate the potential [M+H]+ or [M-H]- precursor ions.

The optimization workflow was done unattended in two major phases. First, MRM optimization: precursor fragmentor voltage, RT determination (optional) Product Ion selection, and MRM collision energy voltage. Then, ion source optimization: drying gas heater, sheath gas heater, capillary voltage, nebulizer pressure, drying gas flow, sheath gas flow and nozzle voltage.

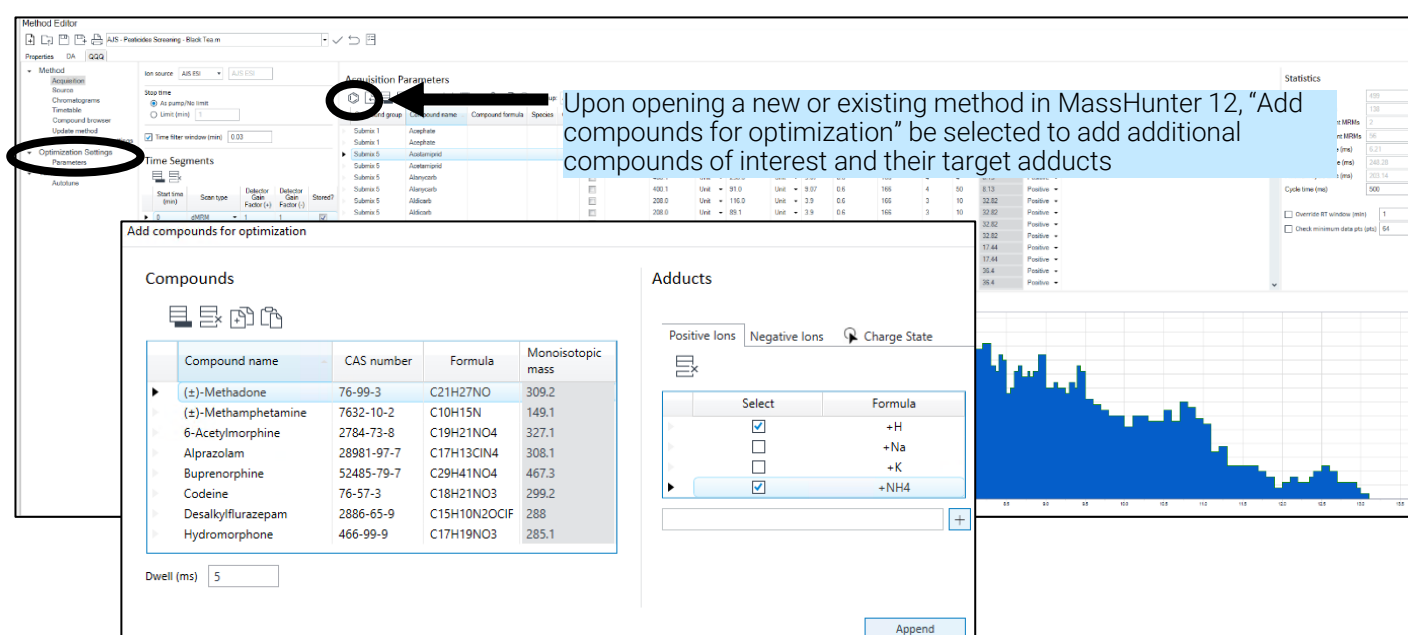**Fully optimized 7-analyte standard mixture chromatogram created from scratch**
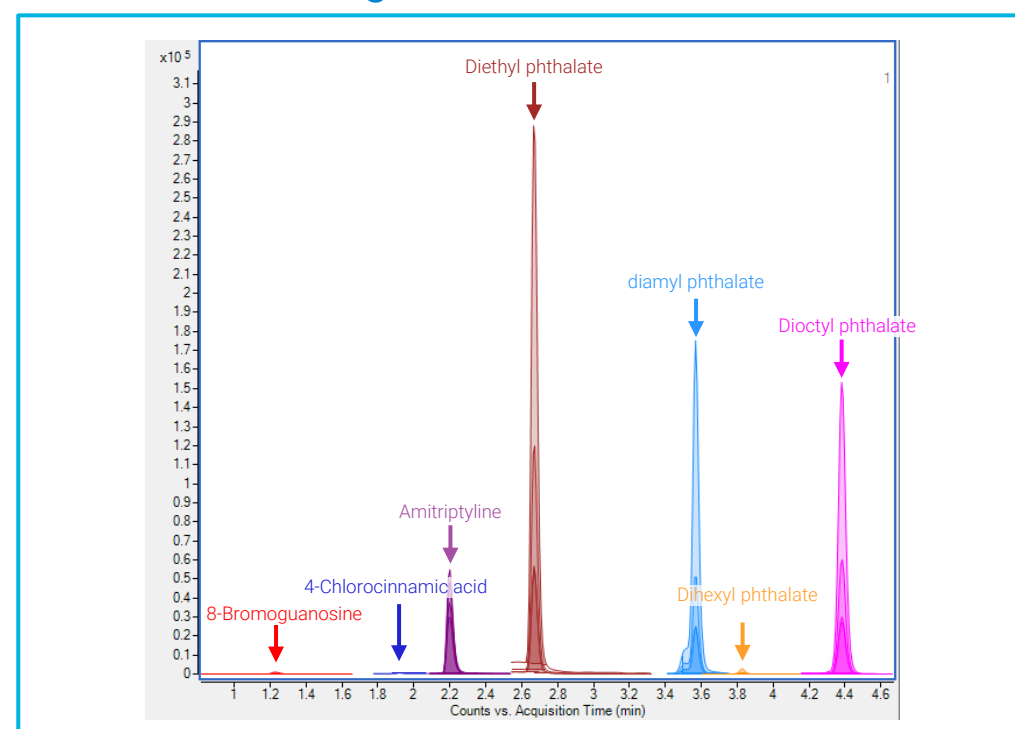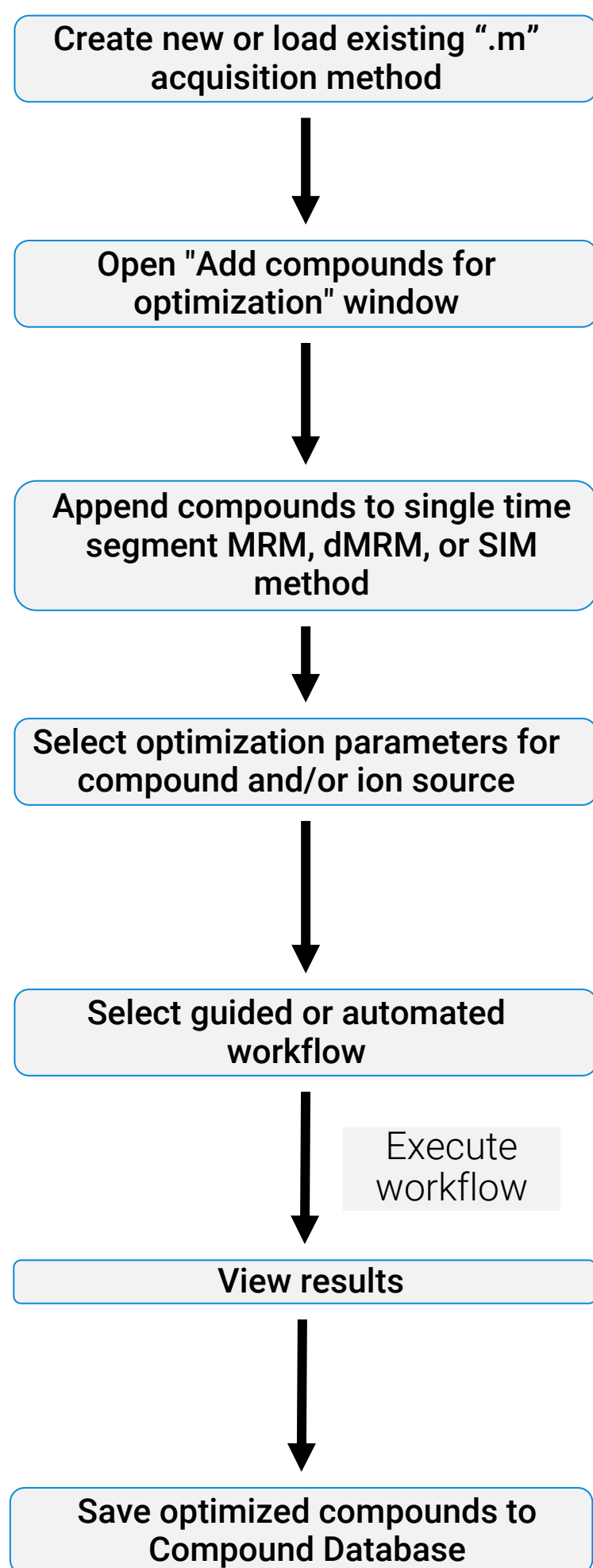

The new Agilent 6475 triple quadrupole LC/MS system and MassHunter 12 Data Acquisition software
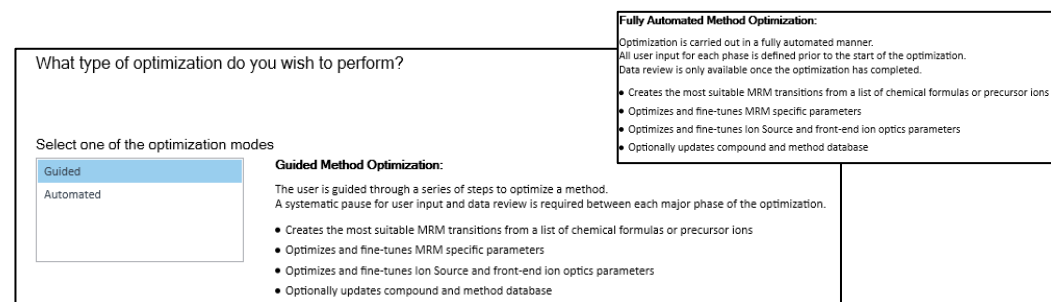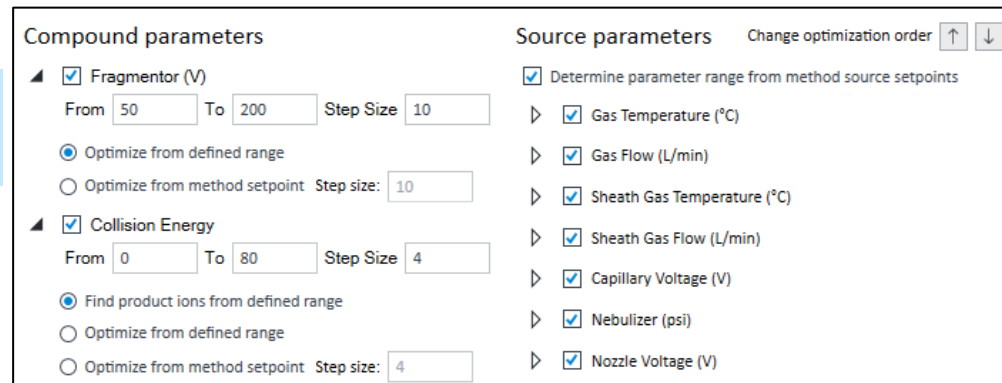
The End-to-End Optimization Workflow

The new intelligent optimization software algorithm, provides a complete, user-friendly, workflow approach to the tedious task of method development. Through this approach, a user may create a new method by inputting neutral analyte formulas, importing an existing MRM list from CSV, adding uncharacterized analytes to an existing method, or fine-tuning existing MRM or source parameters.

Compound and source parameters to be optimized are selected via the "Optimization parameters" page in the Method Editor with customizable user-input ranges and step sizes. An example of the optimization workflow is described in the diagram below.


Create new or load existing ".m" acquisition method

Upon opening a new or existing method in MassHunter 12, "Add compounds for optimization" be selected to add additional compounds of interest and their target adducts

Open "Add compounds for optimization" window

Append compounds to single time segment MRM, dMRM, or SIM method

Specific compound parameters can be selected for optimization with a defined range and step size

Select optimization parameters for compound and/or ion source

Select guided or automated workflow

What type of optimization do you wish to perform?

The user is presented with two choices to execute optimization with detailed descriptions

Execute workflow

Results are presented in an exploratory UI and PDF report to show effects of parameter adjustments. Ion source parameters can have "weights" applied to emphasize more critical analytes

View results

Save optimized compounds to Compound Database
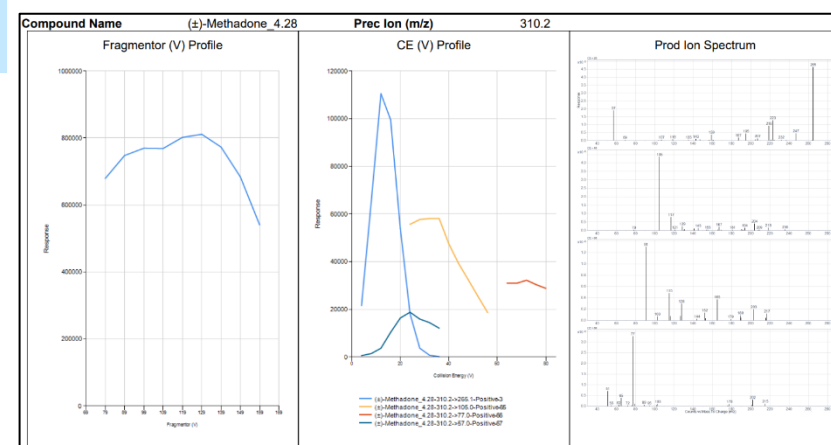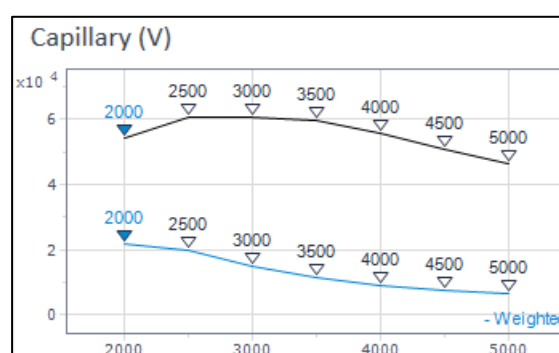
Guided Optimization Workflow

Through this workflow, optimization is carried out in two major steps.

1. MRM specific parameters are optimized to find the ideal product ions, fragmentor voltage, and collision energy voltage and retention time assignment (applied to injection with column) per analyte. The workflow will then pause to allow reviewing of data quality as it pertains to each compound.
2. Once ready, ion source parameters are optimized on a "global" basis to maximize the total ion current (unweighted - TIC based optimization) or with applied weights to priority analytes (weighted - EIC based optimization)

The method will be updated throughout the optimization process in stepwise fashion to create the final method. The user can save their transitions to the compound database to later apply to other methods.
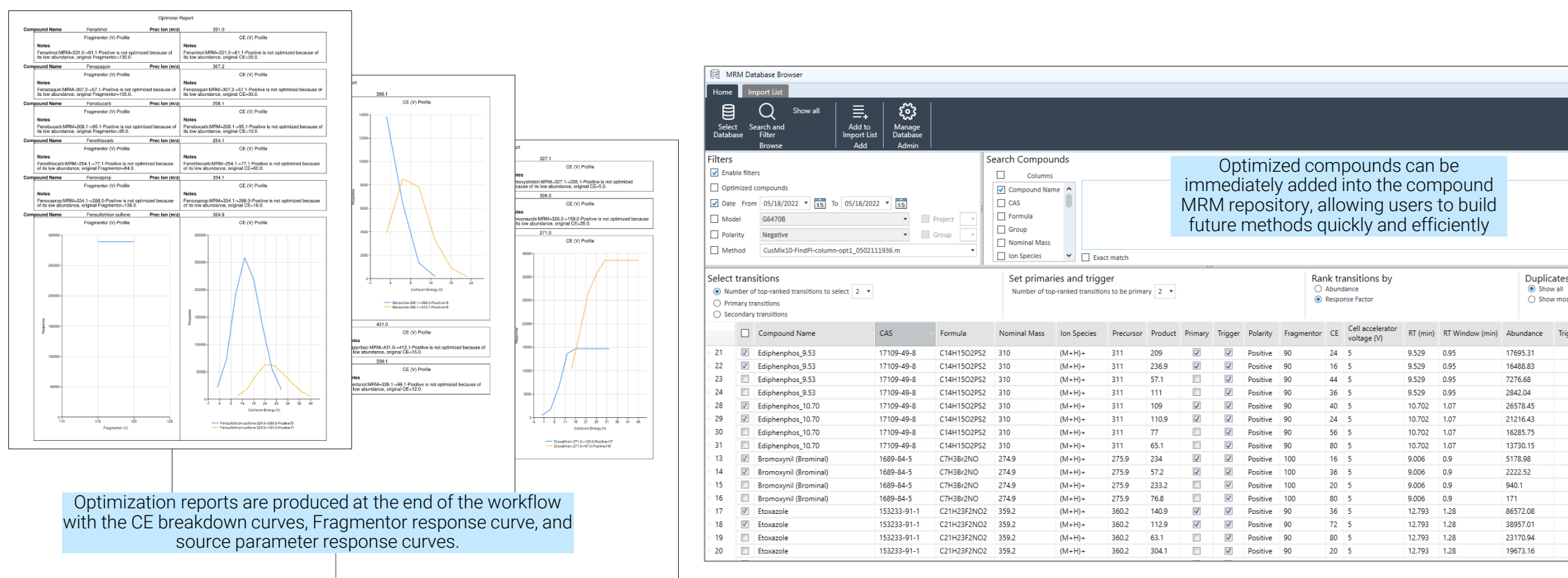
Automated Optimization Workflow

Through this workflow, optimization is carried out without pause, allowing the user optimize multiple methods in sequence.

Like Guided optimization, this workflow will also provide the user with a method that will include the best MRM transitions, collision energies, and voltages based on the parameters selected for optimization. It will also provide the user with fine-tuned source settings created specifically for that method. It can, however, run multiple-methods instead of a single one.

During this process, everything is completely automated and will transition from MRM to ion source optimization immediately. The user will not have the ability to view and update results or change the instrument settings.

Finalized results are printed in an optimization report, stored as method revisions, and saved in a compound repository

Optimization reports are produced at the end of the workflow with the CE breakdown curves, Fragmentor response curve, and source parameter response curves.

Optimized compounds can be immediately added into the compound MRM repository, allowing users to build future methods quickly and efficiently

Conclusions

- The new 6475 triple quadrupole LC/MS system with MassHunter 12 includes an intelligent MRM and source optimizer that is built into the method editor. The final result is a dMRM method with fully optimized parameters, MRM transitions, and retention time assignments.

- **The intelligent optimizer can be used to (1) Create a new method from scratch, (2) Add new compounds to an existing method, (3) Fine tune or verify parameters of an existing method**

- Optimization results can be reviewed by the user after workflow completed. Any changes to the method are saved in an auditable fashion in accordance with to 21 CFR Part 11 compliance

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