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Application Note

# QuanOptimize: A Software Tool that Enables Rapid, Consistent, and Accurate MRM Method Development for Large Numbers of Small Molecule Analytes

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This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

Discovery bioanalytical laboratories routinely develop methods for hundreds of compounds per week to support the various programs in their pipeline. Developing methods for each of these compounds individually can be time-consuming and tedious. Since the approach to small molecule method development is well understood, automation of this process is desirable. QuanOptimize automates the MRM method development for large sets of compounds. It can store the final method parameters to a database, automatically run sample lists using the optimized method, create data processing methods, and generate quantitative result with the click of a single button. QuanOptimize ensures consistent quality of methods across multiple users, reduces sample consumption and saves time by up to 5-fold.

Here, we discuss the use of QuanOptimize to develop methods for a set of 18 small molecule compounds using generic tune page and LC methods.

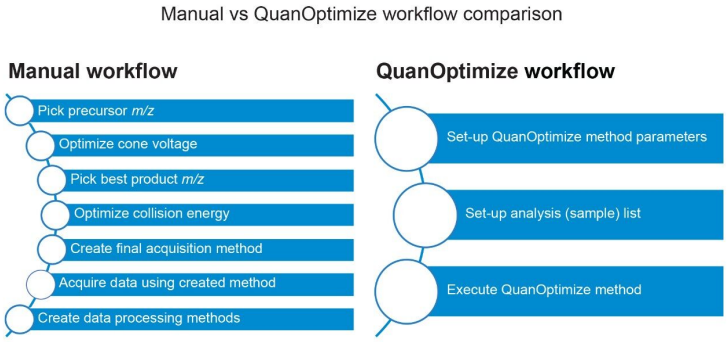

### Benefits

- Automated MRM method development for large number of analytes
- Automated generation of acquisition and data processing (TargetLynx) methods
- One-time, generic set-up for all compound types
- Consistent method quality across user experience levels
- Saves time and sample consumption

## Introduction

Developing accurate and robust multiple reaction monitoring (MRM) methods quickly and easily forms the cornerstone of any quantitative LC-MS/MS study. The typical drug discovery DMPK laboratory is charged with determining the pharmacokinetics and metabolic fate of 10s to 100s of compounds a week. This requires the rapid, accurate development of LC-MS methodology, including ionization mode, precursor/product ion selection, cone voltage, collision energy, etc. There are multiple approaches to develop MRM methods, and the choice of approach depends on the number and types of analytes for which methods need to be developed. For individual analyte assays, or in cases where the analyst is not sample limited, using an infusion-based method development tool like IntelliStart works well. LC injection-based approaches are preferred when developing methods for single analytes (when there is limited sample volume) or multiple analytes. When employing the manual injection-based method development workflow, the user would need to generate separate MS methods for each analyte, review the data, and pick the optimal cone voltage, product ions and collision energy for each product ion. This is challenging and time-consuming, especially for large sets of compounds. It also requires a degree of MS knowledge or training and familiarity with the MS instrumentation and software.

In cases where the scientist needs to develop methods for multiple compounds, using automated approach is desired, as it is faster and frees the scientist up to perform other tasks. QuanOptimize is the ideal solution for such laboratories as it allows the user to set up generic parameters which will be used by the software to automatically develop methods for a large compound set, without user intervention. The tool uses LC injection-based approach for method development, saves created methods to a database, runs sample lists using the created acquisition method, generates a data processing method and applies that data processing method to generate TargetLynx results. QuanOptimize uses significantly lower sample volumes compared to infusion-based methods and enables consistent quality of methods across different user experience levels whilst also saving time and effort for the scientist. Additionally, it allows the user to continue to develop methods remotely, which has become critical due to the current global pandemic.



## Results and Discussion

Development of MRM methods usually involves identifying the most abundant precursor ion, optimizing the cone voltage for that ion, identifying the best product ion for that precursor, and finally optimizing the collision energy for that MRM transition. QuanOptimize automates these steps to provide a final MRM method which can be used within MassLynx to acquire the required LC-MS data. As shown in Figure 1, the user can define the ionization mode (Box 1), cone voltage range (Box 2), collision energy range (Box 3), number of fragments per compound (Box 4) in the optimization tab of QuanOptimize method editor. The acquisition parameters (Box 5), tune files (Box 6), precursor adducts to be included (Box 7), product losses to be ignored (Box 8) and inlet methods to be used (Box 9) can all be defined within their respective tabs in the QuanOptimize method editor. In order to allow for enough data points across the peak, to perform cone voltage and collision energy optimization, it is recommended that a generic UPLC method on Waters ACQUITY Systems be set up with a low flow rate (e.g. 200 µL/min). This generally allows for a large enough peak width for the analyte of interest to accommodate data collection at different cone voltage and collision energy values. These method editor settings can be saved and reused across different compound sets without having to make any changes, thus simplifying the process and saving valuable time.

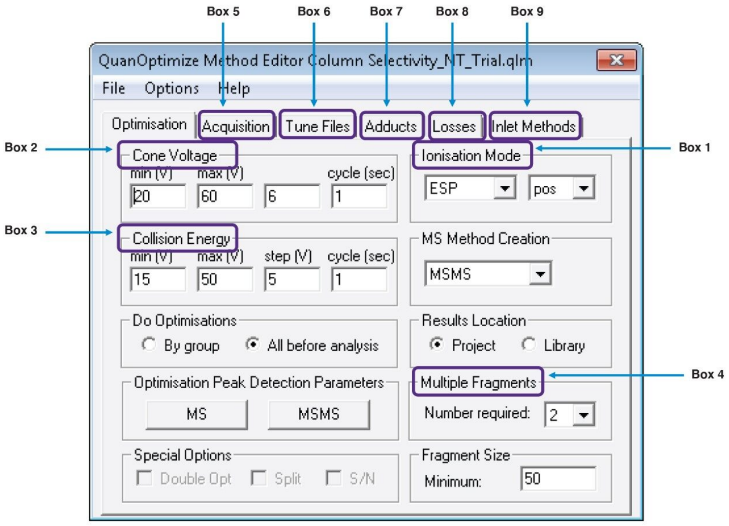



*Figure 1. QuanOptimize Method Editor – Allows the user to set up parameters to be used during automated method development using QuanOptimize.*

After saving the QuanOptimize method, the user can change the sample list view in MassLynx to AnalysisList by going to Samples→Format→Load→AnalysisList. This changes the columns displayed in the sample list in MassLynx to allow for a simpler interface to enter compound

information. A new sample list can be created for every batch of compounds and the user can input or import the compound name, molecular weight and sample location on the autosampler. Compounds can also be assigned to a Sample Group and Internal standards can be selected from the batch. Figure 2 shows the list of 18 small molecule compounds for which methods were developed using QuanOptimize for this study.

Spectrum Chromatogram Map Edit Samples

| | Compound | Mass | Bottle | Sample Group | Internal Std. |
|---|---|---|---|---|---|
| 1 | AMIODARONE HYDROCHLORIDE | 645.310 | 1:B,1 | A | |
| 2 | PHENYTOIN | 252.260 | 1:B,2 | A | |
| 3 | NIFEDIPINE | 346.300 | 1:B,3 | A | |
| 4 | HYDROCHLOROTHIAZIDE | 297.740 | 1:B,4 | A | |
| 5 | VALPROIC ACID SODIUM SALT | 166.190 | 1:B,5 | A | |
| 6 | ACETAMINOPHEN BIOXTRA | 151.160 | 1:B,6 | A | |
| 7 | BACLOFEN | 213.660 | 1:B,7 | A | |
| 8 | DABIGATRAN | 627.730 | 1:B,8 | A | |
| 9 | CLOPIDOGREL BISULFATE | 419.900 | 1:B,9 | A | |
| 10 | FAMOTIDINE | 337.440 | 1:B,10 | A | |
| 11 | SILDENAFIL CITRATE | 474.570 | 1:B,11 | A | |
| 12 | BUDESONIDE | 430.530 | 1:B,12 | A | |
| 13 | PROBENECID | 285.360 | 2:B,1 | A | |
| 14 | BUPROPION HYDROCHLORIDE | 276.200 | 2:B,2 | A | |
| 15 | QUETIAPINE FUMARATE | 383.500 | 2:B,3 | A | |
| 16 | LORATADINE | 382.800 | 2:B,4 | A | |
| 17 | MONTELUKAST SODIUM | 608.200 | 2:B,5 | A | |
| 18 | SALMETEROL | 603.800 | 2:B,6 | A | |

*Figure 2. AnalysisList – List of compounds with their molecular weights and sample locations.*

Once the sample list is set up and saved, the user can go to the QuanOptimize wizard by clicking Run QuanOptimize, select the QuanOptimize method, sample list and provide a unique ID and click Finish to start acquisition. Additionally, acquisition methods can be created, sample lists can be executed, and quantitation methods can be automatically created (Figure 3). QuanOptimize will go through the sample list and create MRM method for each compound on the sample list based on the parameter specified by the user in the QuanOptimize method, without any user intervention.

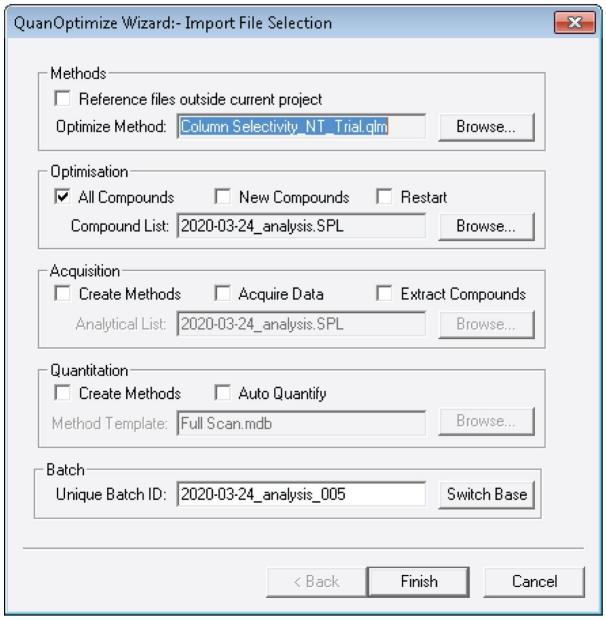



*Figure 3. QuanOptimize Wizard – User selects the QuanOptimize method and Compound list for which methods need to be developed.*

QuanOptimize method settings shown in Figure 1 were used for the list of 18 small molecules (shown in Figure 2) chosen for this study. A 2-minute LC method was chosen for this study. QuanOptimize injects every sample twice, requiring a total of 4 minutes/compound, leading to a total method development time of 72 minutes. In contrast, manual, infusion-based method development can take up to 15–20 minutes/compound, requiring a total of 360 minutes to develop methods for these compounds. Additionally, the time required to manually create data processing methods for each compound, and then generating the results file requires additional time. Using this automated approach, the method development time can be reduced by up to 5-fold (Figure 4), compared to infusion-based method development.

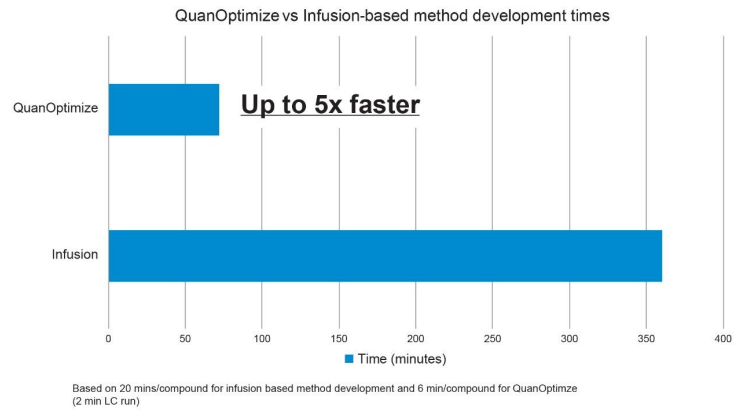



*Figure 4. Comparison of method development times for QuanOptimize vs Infusion-based approach.*

## Conclusion

QuanOptimize automates the MRM method development process for large numbers of small molecule compounds, ensures consistent quality of developed methods for scientists across all experience levels and saves valuable time and effort for scientists, allowing them to concentrate on the more critical aspects of their science. Additionally, QuanOptimize ensures continuity of laboratory work remotely as the scientist can load a large compound set for which methods need to be developed, set up the instrument and walk away. This can be extremely useful is situations where laboratory access may be restricted. For the set of 18 small molecules used for this study, QuanOptimize successfully completed MRM method development in under 1.25 hours without any manual intervention, reducing the method development time by 5-fold.

## Featured Products

ACQUITY UPLC I-Class PLUS System

Xevo TQ-XS Triple Quadrupole Mass Spectrometry

MassLynx MS Software

QuanOptimize

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