

High Throughput LC-MS/MS Analysis of Metabolic Stability Incubations

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Key Words

- Aria TLX-4
- TurboFlow Technology
- QuickQuan Software
- TSQ Quantum Ultra
- Drug Discovery

Introduction

High throughput screening (HTS) assays are commonly used in the discovery stage of the drug development process where a large number of compounds are screened. Compound optimization and sample analysis are factors that limit throughput. This study addresses mass spectrometry optimization and sample analysis problems by using the Aria TLX-4 multiplexed HPLC system, powered by TurboFlow™ technology, and Thermo Scientific QuickQuan software for the compound optimization process.

Goal

To show how throughput in drug discovery can be dramatically increased by using QuickQuan™ fully-automated optimization software with the Thermo Scientific Aria TLX-4 system.

Experimental Conditions / Methods

Eight compounds were used to compare the new compound optimization algorithm against manual optimization. For the quantitation study, standard curves were made for each compound from 1 ng/mL to 1000 ng/mL. One replicate of samples was injected into a single laminar HPLC system. The second set of samples was injected into one of four channels of an Aria™ TLX-4 system. The chromatographic peaks were integrated. A linear regression was performed on the area ratios of analyte divided by the internal standard (Figure 1).

Results and Discussion

The accuracy and precision of the analyses were essentially identical. Both techniques gave accuracies within 10% deviation from theoretical and precisions below 10% relative standard deviation. The throughput was significantly increased using Thermo Scientific QuickQuan software with the Aria TLX-4 system. Compound optimization by the manual method required approximately two hours of dedicated operator time for all ten compounds. The method using the new automated algorithm required only thirteen minutes, resulting in nearly a ten-fold time savings (Figure 2). The samples run using an HPLC method on a single analytical column required 48-hours. The samples run using a TurboFlow HPLC method utilizing four parallel LC channels required only 12-hours, representing a four-fold time savings (Figure 3). Throughput increased four-fold for the entire process, with minimal user intervention.

Conclusion

The combination of an Aria TLX-4 HPLC system, QuickQuan software and a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer significantly increases sample throughput without compromising quality.

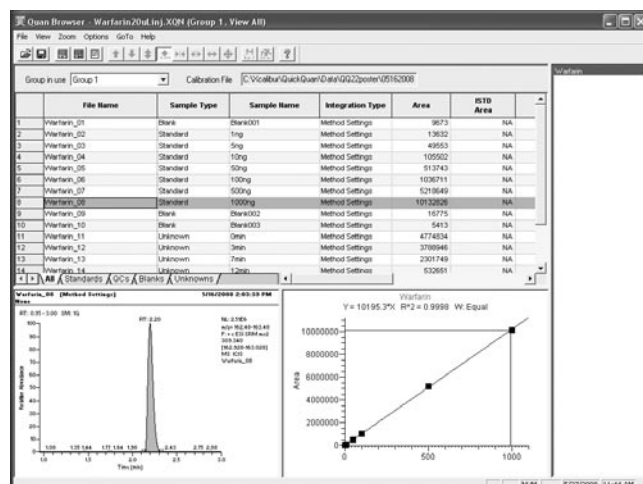


Figure 1: Sequences are shown in the Results Summary tab. Data can then be viewed in either Qual or Quan browser.

Manual Optimization 10 Compounds = 2 hours	QuickQuan Optimization 10 Compounds = 16 minutes
User sets up parameters in software manually	Compounds added to QuickQuan database
↓	↓
User re-plumbs the system and starts co-infusion from LC system	Aria LC and autosampler methods specified in QuickQuan
↓	↓
User infuses compounds manually using a syringe pump	Optimization plate information imported into QuickQuan software
↓	↓
User manually chooses best optimization parameters and creates instrument method	QuickQuan opens Aria, and submits optimization batch
	Aria system infuses solution, QuickQuan reports optimization results in .pdf format
	Optimization Speed
	QuickQuan: 2 minutes per compound
	Manual Optimization: 15 minutes per compound
	Time Savings: 13 minutes per compound

Figure 2: Comparison of QuickQuan automated compound optimization with manual optimization. QuickQuan reduces optimization time from 2 hours to 16 minutes.

Typical Single Channel LC System 48 Hours	QuickQuan Software with Multiplexing 12 Hours
Sample list prepared manually	Sample sequence is imported into QuickQuan software
↓	↓
Samples run on a single LC system	LC method, autosampler method, and multiplexing options are specified in QuickQuan software
↓	↓
Processing method created manually	QuickQuan submits batches to Aria
↓	↓
Data is quantitated manually	Aria runs batches, Quickquan provides Qual and Quan browser data, as well as Microsoft® Excel® reports
↓	Acquisition Speed
Reports generated manually	Traditional LC/MS system: 48 hours
	QuickQuan with Aria: 12 hours
	Overall Time Savings: 36 hours

Figure 3: Acquisition workflows: with QuickQuan software, sequences are built and automatically exported to Aria system software for acquisition. Data processing is handled automatically by QuickQuan. Acquisition speed is increased four-fold over a traditional LC system.

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