

# Comparing Manual Compound Optimizations Against Automated Optimizations Obtained by QuickQuan on the TSQ Quantum Access

Nicholas Duczak and Jim Koers, Thermo Fisher Scientific, San Jose CA

## Introduction

One of the rate limiting steps in LC-MS/MS sample analysis is the time required to create accurate and sensitive instrument methods. To ensure the best possible performance, operators may spend an extended period of time optimizing the mass spectrometer for compounds of interest. In laboratories where higher throughput is required, the allotted time for instrument optimization is minimal. Automated optimization packages are frequently utilized to increase throughput, but these solutions compromise assay sensitivity for the sake of speed.

## Goal

To determine the sensitivity difference in methods created by QuickQuan™ HTS software versus methods created from manual optimization procedures.

## Experimental Conditions / Methods

### Quantum Optimization

For the experiment, four compounds (Minoxidil, Imipramine, Paroxetine and Nefazodone) were optimized using the automated infusion optimization procedure available in QuickQuan (Figure 1). Optimal gas flow rates and temperatures were determined through the Quantum EZ-Tune interface (Figure 2). A 5 ng/mL neat standard was prepared in 50/50 methanol to water and samples were analyzed through QuickQuan.

After results were obtained from QuickQuan, the instrument parameters (gas flows, temperatures, and product ion energies) were optimized for these four compounds manually. The same sample was then injected using the manually optimized parameters under identical LC conditions and ion source positioning to compare intensity results between the two techniques.

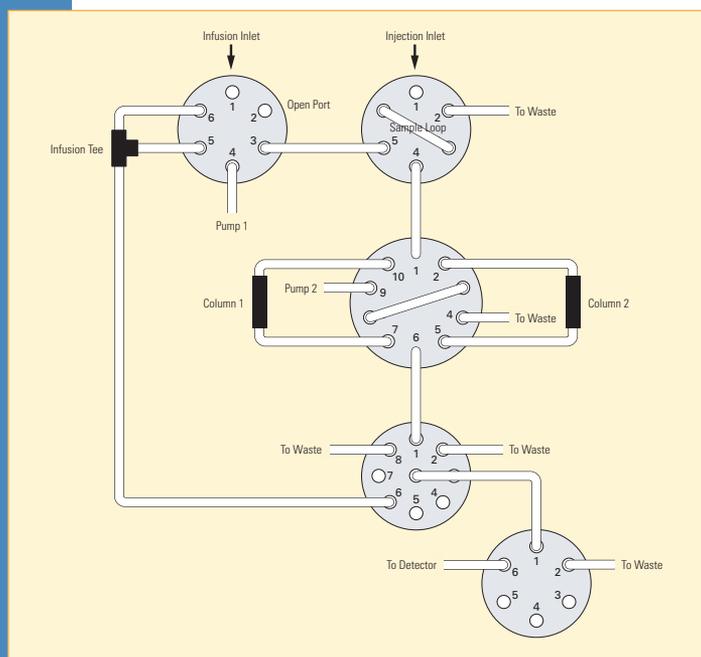


Figure 1: Diagram of the QuickQuan Total Solution package. QuickQuan utilized the autosampler and a dedicated injection valve to infuse compounds for MS optimization. During the manual and automated optimizations, Mobile phase composition and flow were kept identical; however the manual optimization required the infusion of compound via the syringe pump on the front of the TSQ Quantum Access.

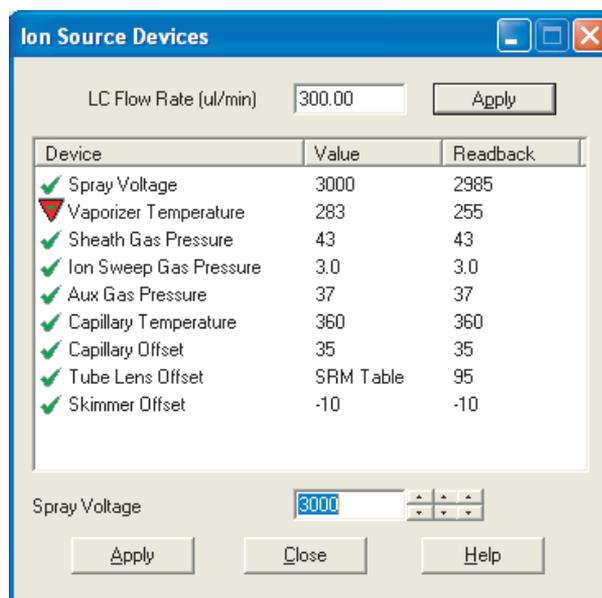


Figure 2: For QuickQuan auto-optimization, the appropriate gas and temperature setting were determined using the Quantum EZ Tune interface. EZ tune automatically calculated the source parameters when given the LC flow rate of the experiment

### Sample Analysis

Mobile Phase A was water with 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid. Solvent was pumped at 400  $\mu\text{L}/\text{min}$  and chromatographic separations were achieved using a Thermo Scientific

Hypersil GOLD™ (50 $\times$ 2; 5  $\mu\text{M}$ ) column. Compounds were eluted using a linear gradient starting at 10% B to 95% B in 1 minute, allowing a hold at 95% B for 1 minute. MS analysis was performed on a TSQ Quantum Access™ using heated electrospray (HESI).

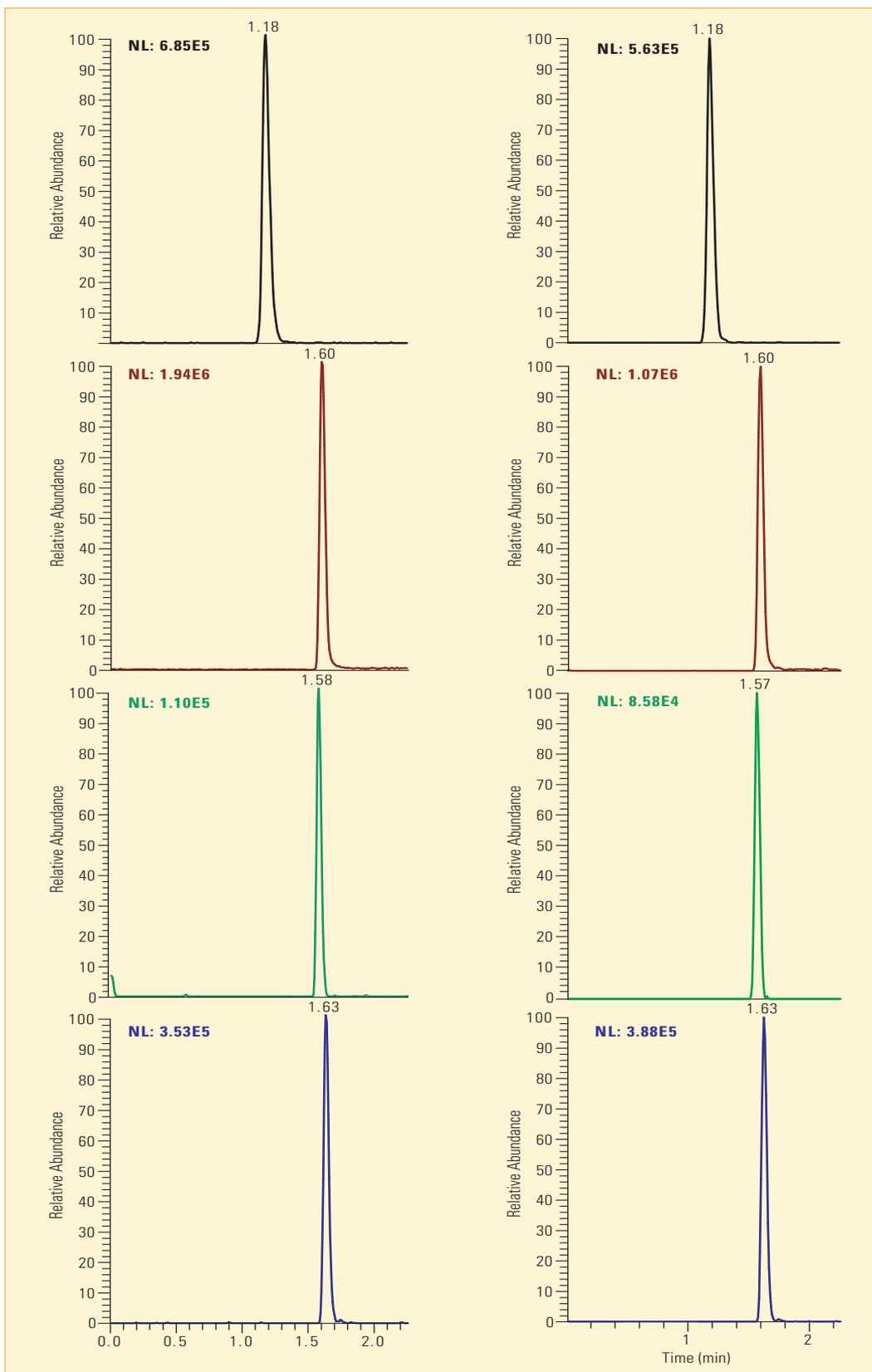


Figure 3: Comparative chromatograms from an on column injection of the compounds using the manually determined method (left) and the QuickQuan generated method (right)

## MS Conditions

For both QuickQuan and manual optimizations:  
Instrument: TSQ Quantum Access with Heated Electrospray (HESI)  
Ionization Mode: Positive  
Scan Type: Selective Reaction Monitoring (SRM)  
Scan Time: 0.020 seconds

## Source parameters for QuickQuan and manual optimizations:

Parameter	QuickQuan and EZ Tune	Manual Optimization
Spray Voltage	4500	4500
Vaporizer Temperature	283	320
Sheath Gas Pressure	43	50
Ion Sweep Gas Pressure	3	3
Auxiliary Gas Pressure	37	30
Transfer Tube Temperature	360 °C	375 °C

## Results and Discussion

Figure 3 shows the extracted ion chromatograms for both the QuickQuan (right) and manual (left) derived methods. In both cases, the most intense product ion determined for each compound was identical (Figure 4). The percentage of change in intensity is displayed in Figure 5. Manual

optimization of the four compounds required a longer time (approximately 30 minutes) when compared to the time required for QuickQuan to perform the procedure (approximately 5 minutes).

QuickQuan Results		Manual Optimization Results	
Most Intense SRM Transition	Collision Energy	Most Intense SRM Transition	Collision Energy
210→193	14	210→193	15
281→86	17	281→86	15
330→192	20	330→192	20
470→274	28	470→274	25

Figure 4: Table comparing optimization results. In both cases, the most intense product ion for each compound was identical.

SRM Transition	QuickQuan Optimizations	Manual Optimizations	Percent Difference
210→193	5.63E+05	6.85E+05	21.67%
281→86	1.07E+06	1.94E+06	81.31%
330→192	8.58E+04	1.10E+05	28.21%
470→274	3.88E+05	3.53E+05	-9.02%

Figure 5: Table comparing intensities obtained from the QuickQuan generated method against a manually created method. All LC and autosampler conditions were kept identical.

## Conclusion

In conclusion, QuickQuan provided an equivalent optimization result to the manual procedure, in one-sixth the time. Manual optimization only showed a slight improvement in on-column response for one of the four compounds tested. In order to achieve this increase in signal, an extra 25 minutes of manual tuning was required. Also in comparison, the user did not need to

tend to the Quantum during the QuickQuan optimization procedure. In high throughput environments where a large number of compounds are analyzed daily, the time required for manual optimization is extremely inefficient. QuickQuan proves to be an excellent solution for compound optimization when sample throughput is a high priority.

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