

# High Throughput LC/MS Optimization and Analysis using QuickQuan

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## Overview

QuickQuan software allows for the optimization of multiple compounds, acquisition of chromatographic data and generation of quantitative results in a single analytical run. Compound optimization parameters are stored to a database allowing for compounds to be analyzed in multiple assays. QuickQuan uses the database information to automatically generate acquisition and post-acquisition methods needed for multiple analytical runs.

## Introduction

A critical part of the drug discovery process is the need to assess the drug properties of new chemical entities (NCEs). Such evaluation includes understanding compound properties such as metabolic stability, solubility, and preliminary pharmacokinetics. LC-MS/MS has been widely adopted for the support of drug metabolism and bioanalytical studies and this technology is now an integral part of the high throughput compound assessment in early ADME. However the analysis of NCEs which have not previously been characterized by LC-MS/MS requires specialized software to enable the automated optimization of the LC-MS instrument in order to insure accurate, precise and sensitive quantitative data is acquired for each compound. QuickQuan software has been developed for our LC-MS/MS systems to meet this requirement and to permit automated LC-MS/MS analysis of plates of compounds with optimization of polarity, collision energy, tube lens voltage, and product ion transitions. QuickQuan also allows for optimization in H-SRM mode which increases detection limits and analytical specificity.

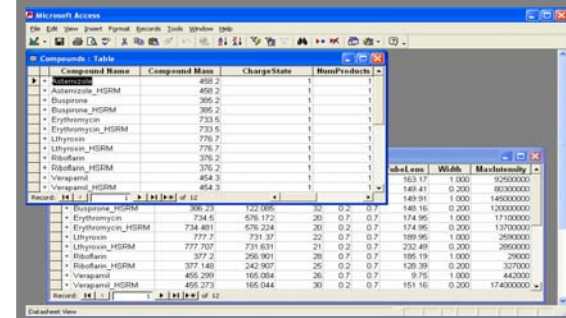
All compound optimization information is stored in a database. Automated HPLC conditions are generated in real-time for assays using optimized compounds. QuickQuan manages and downloads assays to the LC-MS/MS system, monitoring each sequence and generating quantitative results specific for the compounds in each sequence. This feature saves the user from setting up LC and post-processing conditions for each compound individually. Hence, the quantitative analysis data of compounds, for which initially only the molecular weight of the compound was known, are fully automated. QuickQuan supports real-time optimization for Thermo Electron TSQ Quantum platform mass spectrometers

## Results

QuickQuan uses a database to store the optimization information for each compound separately. The database is used to generate the SRM method conditions for each compound during data acquisition and generating the processing components used for post acquisition data analysis.

FIGURE 1. Database

Compound start parameters such as name and molecular weight allow for automated optimization of SRM information.



A tune plate represents the compounds to optimize. The compounds can be optimized individually or grouped together to allow for a higher number of compounds to be optimized in a single pass. The compounds to be optimized can be selected from the database or imported from an external source. If a suitable autosampler is used the autosampler can be used to infuse the compound into the LC-flow.

FIGURE 2. Compound Optimization Plate

Optimization of compounds can be grouped in any order within a tune plate. HSRM is an option with a single click of a button.

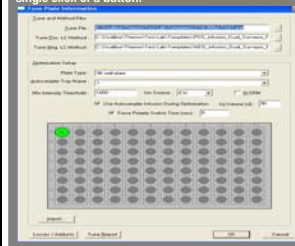


FIGURE 3. Compound Vial Information

Each vial within the Tune plate allows for single or multiple compounds to be optimized in a single pass, simply click of a button.



The CTC autosampler with an additional trio valve option is used to automatically switch between optimization by infusion and chromatography, and to increase analytical through-put by column switching. During the optimization the analyte is infused into the LC-flow for fast and optimal optimization. During the optimization step certain non-specific losses can be excluded from optimization. Adducts can be monitored and selected if they are observed. Before the analytical sequences are started injector valve 1 and the stream selector are switched. Injector 2 is used for the analytical sequences.

FIGURE 4. Multiple valve

The use of multiple valves to automatically switch between optimization by infusion and chromatography, also to increase analytical through-

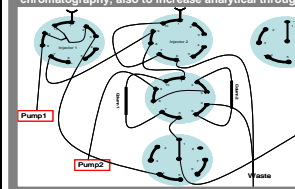
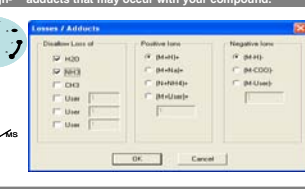


FIGURE 5. Losses/Adducts

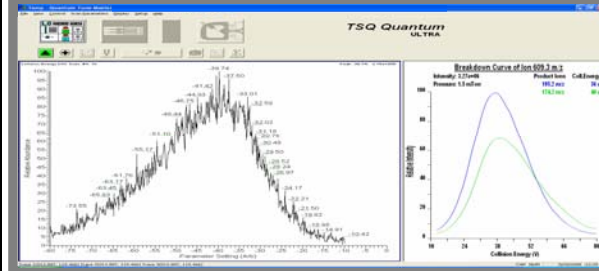
Some losses are not very compound specific and are not desired. The instrument is able to monitor adducts that may occur with your compound.



Optimization of compounds is achieved through QuickQuan communicating with the LC-MS Xcalibur data system. Optimization information such as polarity, tube lens, product ion selection and optimal collision energy is determined individually for each compound and stored to the QuickQuan database.

FIGURE 6. Automated Optimization

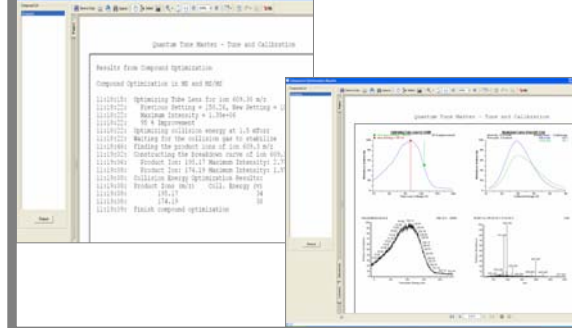
Optimization of compounds determines the polarity, tube lens, collision energy, source parameters and product ion transitions. Optimization can be performed using HSRM (Q1 = 0.2) or unit resolution (Q1 = 0.7).



After tuning each compound a tune report in PDF format is created for each compound. The tune reports are stored in the database. With the incorporated viewer the quality of the tuning can be easily checked without exiting the software

FIGURE 7. Optimization report

Optimization reports consists of the polarity, tube lens, collision energy, and product ion transitions tune parameters as well as a full scan product ion spectrum that was used to determine SRM transitions.



QuickQuan downloads assays imported from an external Excel spreadsheet to the LC-MS/MS system. The assay allows for the analysis of multiple plates, the definition of an internal standard compound and the selection of the LC-MS method for acquisition.

With the multiple valve setup on the CTC (see Fig. 4) the through-put is doubled by using two LC pumps and columns. While one column is equilibrating the analytical acquisition is done on the other column. The software is flexible enough to have the choice of spreading the analysis of the one drugset over two columns or injecting a different drugset on each column so the compounds always see the same column. These methods are easily setup with the ThermoPal driver for the CTC autosampler. QuickQuan provides the option of optimizing multiple compounds in a single run or downloading an assay for high-throughput acquisition. The database can be used for multiple assays allowing for the same compounds to run under the same conditions without manual user interaction.

FIGURE 8. Acquisition Setup Parameters

The assay to be downloaded to the LC-MS/MS system is imported from an external Excel spreadsheet.

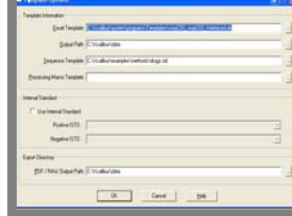
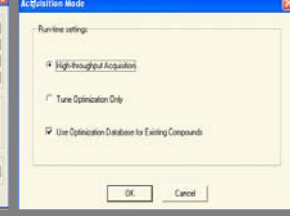


FIGURE 9. Run-time events

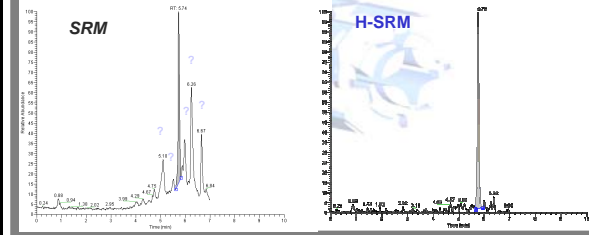
Determines the path that the current run will take. Optimization or Acquisition separately or together.



The software is designed for the Finnigan TSQ Quantum platform with the HyperQuads technology which enables you to use HSRM (Highly selective Selected Reaction Monitoring). The advantages of HSRM are a reduction of isobaric chemical noise, increased confidence in the analysis, improved LOQs, which are clearly demonstrated in Fig. 10.

Figure 10. SRM vs. H-SRM

QuickQuan has the option of setting up HSRM experiments because we use the Finnigan TSQ Quantum Platform with the Hyperbolic Quadrupoles.



At the completion of sample analysis, the post-processing of the data will start automatically. Peak detection and integration of areas and amounts will be generated which will give quantitative information about the amount of the target compound in each sample. After one batch of samples has been processed, information about the next batch of samples for analysis will be automatically downloaded to the LCMS and the cycle continues until the entire assay has completed. All post-processing methods are generated automatically by QuickQuan for each set of compounds allowing the user to manually reprocess their data through the LC-MS XCalibur data system if required. Reports can be obtained through Xreports, for customized reports, and Excel for easy LIMS system import.

## Conclusions

QuickQuan drives the acquisition, processing, and reporting of quantitative sequences using standard template methods. QuickQuan allows for the complete optimization of compounds resulting in the detection of polarity, collision energy, tube lens and SRM method conditions for individual compounds. QuickQuan manages compound information through a database, allowing for multiple compounds to be used in an automated environment in multiple assays without user management of specific compound information. QuickQuan controls the analysis of samples and associated data acquired, finding the highest concentrated peak and integrating the peak using the information about the compound from the central database. Automated instrument and post-processing methods are created and managed in real-time.

FIGURE 11. Post Acquisition Results

