Chromatographic Optimization, Storage, and Re-use in a Combined High-throughput Compound Optimization Workflow using a Dual Arm Autosampler

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Introduction

Drug discovery and lead optimization involve diverse researchers performing assays on sets of thousands of compounds on the path to development. For each compound, a method for measurement must first be developed. Automated optimization of mass spectrometry parameters can be performed in a high-throughput manner on a dual arm auto-sampler. The system described here expands this approach to automate assessment of chromatographic parameters, (retention time, peak shape and intensity are evaluated). This approach stores these LC parameters alongside tuning parameters in a central database for later use.

Methods

A 96-well plate containing a set of five distinct compounds was prepared. Full optimization of the compounds' mass spectrometry parameters was performed, after which the compounds were automatically injected into pre-defined chromatography setups. Criteria were set for peak shape, intensity, and retention time. Low concentrations of the compounds of interest were chosen to increase the likelihood of a sub-optimal chromatographic result. A side-by-side visualization of the resulting data from the multiple chromatography setups was then displayed for each compound, along with a color to mark the pass/fail criteria. The parameters for the process were automatically stored within a database for later use. An Apricot Designs Dual Arm (ADDA) Autosampler, Shimadzu LC-20 pumping system, and an ABSCIEX 4000 QTRAP were used to perform the measurement. The ADDA™ software was used to analyze the results.

Experimental Conditions:

Sample Optimization (FIA Analysis)

Compounds in 50/50 MeOH/H_2O:
Propranolol (5µM), Quinidine (5µM), Verapamil (5µM), Gabapentin (5µM), Atenolol (5µM).
Injection Volume: 50uL

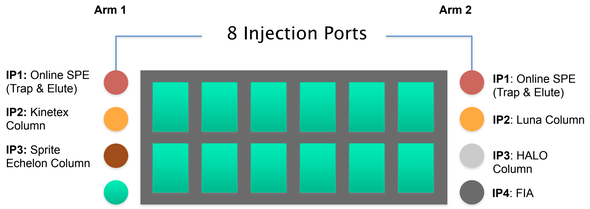

Figure 2. ADDA Injection Port configuration for experiment

LC Analysis

Pumps: Shimadzu LC-20 gradient pump
Injection Volume: 20µl

Gradient Conditions (all ports):
Aqueous: 0.1% formic acid in water
Organic: 0.1% formic acid in acetonitrile

Chromatune 1: Chromatographic Conditions
Column: Advanced Materials Technology HALO C18, 5µm, 20x2.1mm
Equilibration Time: 30 seconds
Flow rate: 1.0ml/min

Chromatune 2: Chromatographic Conditions
Column: Analytical Sales & Services Sprite Echelon C18, 4µm 20x2.1mm
Equilibration Time: 30 seconds
Flow rate: 1.0ml/min

Results

ChromaTune™ Analysis
MRM conditions for all compounds were automatically determined using the ADDA software, first by a quick tune and then a fine tune FIA-based method.

The instrument then automatically switched into ChromaTune™ mode, using a unique single-arm gradient method and a unique injection port to perform a test injection for the first column chemistry (HALO column) for all compound samples.

Subsequently, the instrument automatically performed the full gradient analysis on all compound samples for the Sprite column, giving gradient data for each compound on two separate column chemistries.

Both optimization and ChromaTune™ testing were performed for all compounds without human intervention.

ChromaTune™ Review panel
The samples were reviewed using the ChromaTune™ review panel. The results for each compound could be viewed by scrolling down the Compounds chart, and looking at the updated MRM chromatogram containing the results for each column placed in side-to-side comparison.

The software performed an automated tolerance check for five quality parameters, each of which had tolerance limits set in the software:
- Peak Height
- Peak Area
- Peak Width at Half Height
- Tailing
- Retention Time

Failed Tests were shown in red; successful results were shown in green (see Figure 4).

Database Storage & Retrieval
After optimization, the compound and ChromaTune™ optimization parameters were stored in the compound database for later retrieval.

Retrieved compounds were evaluated using the Compound Optimization Panel (see Figure 5). This panel allows a user to decide on the chromatographic conditions to choose for the compound based on a set of conditions from different ChromaTune™ runs.

The ChromaTune™ results for all compounds were observed.

Conclusions / Summary

A set of chromatographic optimization runs can be inserted into the optimization protocol for a set of compounds on a batch basis. These can be run automatically after the optimization of compound conditions.

Graphical software displays can be constructed which quickly and efficiently display the results of the chromatographic condition runs in order to compare relative column performance, and to ensure that the compound can be run successfully under gradient conditions.

The chromatographic information can be stored in a database for future review and usage by the laboratory for high-throughput analytical samples.

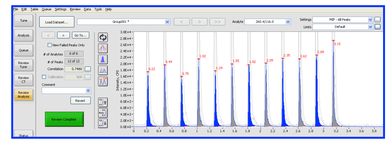

Figure 6: Example of analytical sample review within the ADDA Review software

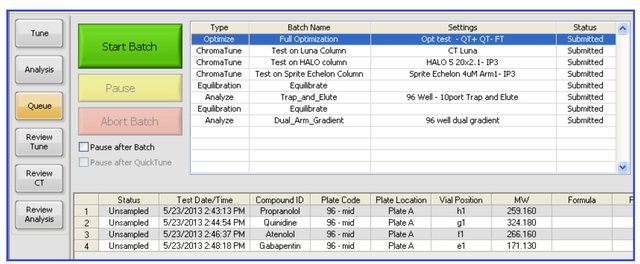

Figure 1: Example of a batch queue for samples to be optimized and analyzed. Each test is performed on the full set of samples specified within the batch plate map; thus plates of samples can be optimized and have their chromatographic data captured on a high-throughput basis.

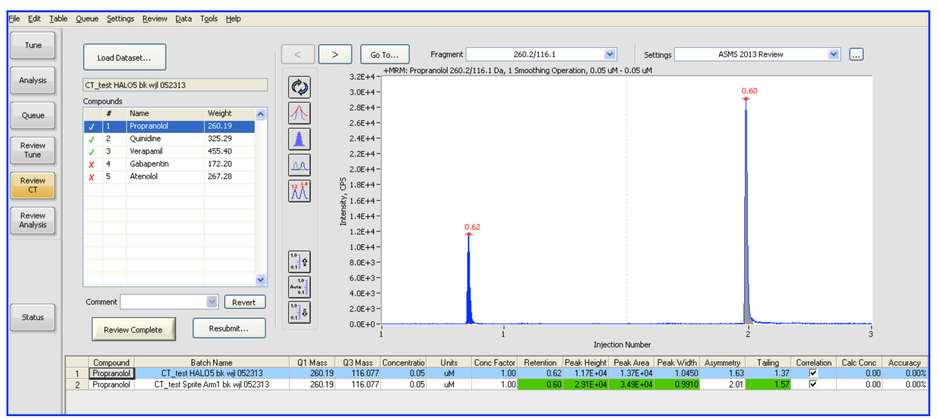

Figure 4: **ChromaTune™ quality visualization panel** of each compound set (with two ChromaTune™ setups selected). The peak intensity, along with peak shape and tailing are measured against set criteria. Results are shown in the chart under the peaks. As the user scrolls down the compound list, each set of ChromaTune™ results is updated in the graph, and on the chart. In this way, results from multiple runs can be compared using the same review criteria.

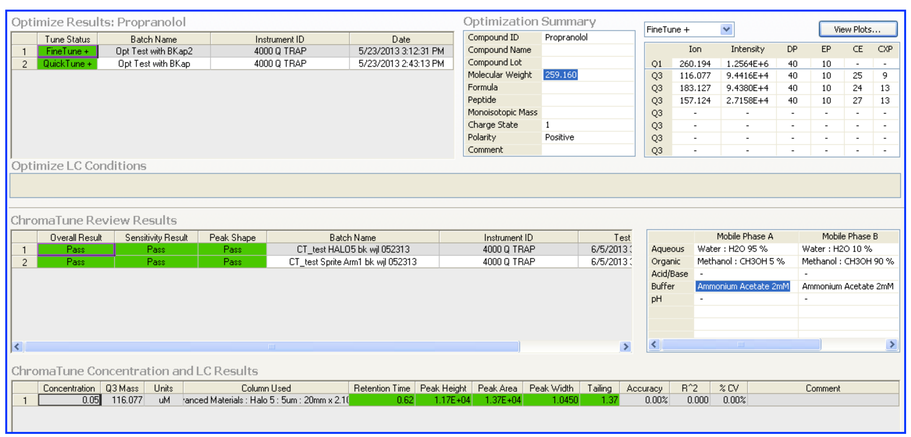

Figure 5: The **Compound Optimization panel** shows a summary of the full results of the compound optimization, along with a summary of the ChromaTune™ Review results run on that compound, each with peak characteristics shown in the lowest chart. LC conditions for each run are shown as well.