High Throughput Sample Analysis Using Multiple Column LC/MS/MS and Automated Method Optimization Techniques

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OVERVIEW

Automaton is an application that works with Analyst® to automatically optimize the mass spectrometer for specific compounds, perform a batch acquisition for these compounds, and then carry out any desired quantitative processing on the acquired data. An integrated compound database is provided which stores the results of automatic method optimization for future use.

Two-Stream Batch is a software plug-in that has been specifically designed to synchronize an autosampler, selector valves and and API mass spectrometer in such a way that the autosampler will inject samples alternately into two LC streams in parallel at a specified time schedule.

These two software applications work together to provide unattended operation for high throughput analyses.

INTRODUCTION

In the drug discovery and development markets a common application is the optimization of LC/MS/MS methods for specific compounds in a series of samples, and the subsequent quantitative analysis of the target compound. Coupled to this is the interest in high throughput applications that can double the throughput by injecting on multiple columns as fast as possible. Automaton is a computer program that works with Analyst® to automatically optimize the MS and MS/MS parameters for specific compounds, perform a batch acquisition using the generated method, and then carry out any desired quantitative processing on the acquired data. The optimum values of both the MS and MS/MS parameters are stored in a database so that other Analyst® components and applications can access them. Along side Automaton, is a software plug-in, Two-Stream Batch that has been specifically designed to synchronize an autosampler, selector valves and an API mass spectrometer in such a way that the autosampler will inject samples alternately into two LC streams in parallel at a specified time schedule. The faster method development, overlapping LC runs and targeted data acquisition will result in higher throughput for the overall system.

MATERIALS AND METHODS

A 96 well plate, containing 8 different compounds and a simulated PK sample set for each compound were analyzed. Automaton software was used to automatically create and optimize the MS/MS methods for each compound, and then execute the quantitative batch for each compound. Working together with Automaton, is Two-Stream Batch, a software program that allows two columns to be used for the analysis, ultimately leading to higher throughput for the overall analysis.

Chromatography/Mass Spectrometry:

- 8 different tranquilizers were used for the study. Simulated PK samples were prepared from the stock standards through serial dilutions in mobile phase.
- Mobile phase: Gradient elution (water + 0.1% formic acid, ACN + 0.1% formic acid) over 5 minutes.
- LC/MS/MS analysis was conducted on an API 4000™ LC/MS/MS system with Analyst® software.
- A Leap CTC PAL autosampler equipped with a new TrioVS valve system for column switching capabilities and an Agilent 1100 binary pump were used to deliver samples to the mass spectrometer through a Chromolith SpeedROD (RP-18 4.6 x 50 mm) at a flowrate of 1.0 mL/min.

RESULTS

Figure 1. Automaton User Interface

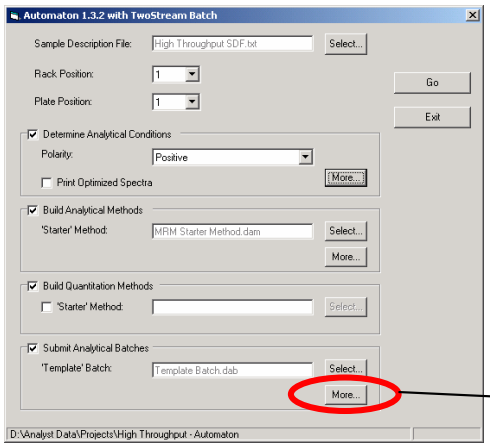
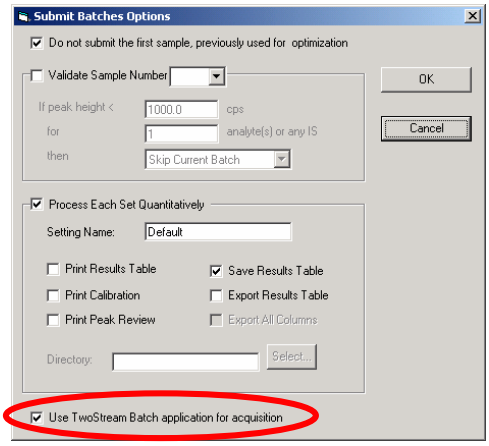


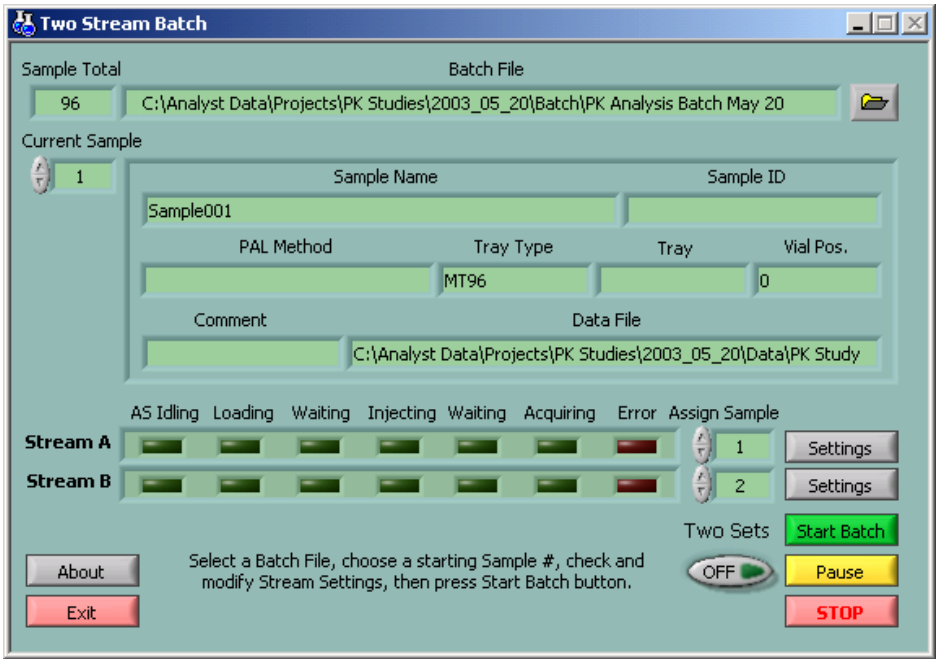
Figure 2. Batch Options



The main screen of Automaton allows the user to input all the appropriate information for the compounds to be optimized and submitted for analysis. After MS and MS/MS parameters are obtained, MRM methods are automatically created and the samples are submitted to Analyst®.

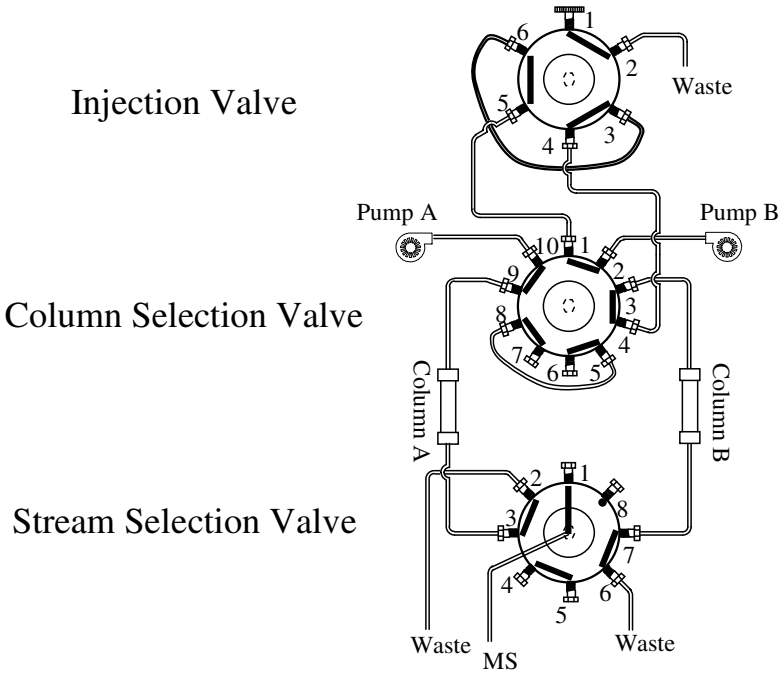
To increase the overall throughput of the analysis, the Two-Stream Batch application can be selected. Using this option, samples will be injected on two columns running in parallel, ultimately reducing the overall runtime of the 96 well plate analysis.

Figure 3. Two-Stream Batch User Interface



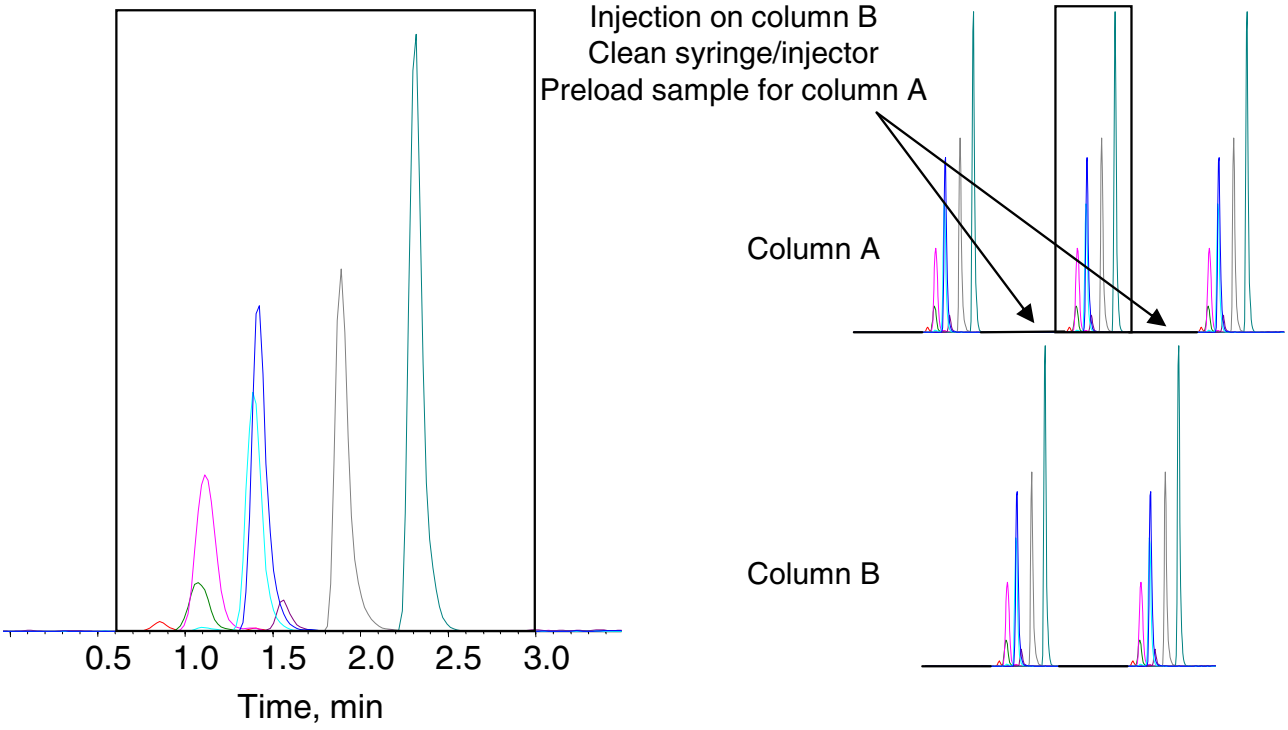
Two-Stream Batch helps to maximize the throughput of the overall analysis. Once the the MRM methods are created by Automaton, Two-Stream Batch takes over and submits the samples to one of two columns. The 'dead' time can be factored out of a chromatographic run, spending this time cleaning the syringe and injection port and finally preloading the next injection.

Figure 4. Schematic Diagram of the TrioVS Valve System



The two-stream system consists of a Leap TrioVS valve system on a CTC PAL autosampler. The Two-Stream Batch application controls injections and all the corresponding switching times.

Figure 5. Column Switching Scheme



The autosampler will inject the samples alternately into two LC streams at a specified time schedule and the mass spectrometer will acquire data for only a small section of the LC run for each sample. In this example all 8 compounds can be eluted over a 5 minute gradient analysis on one column. The time before and after compound elution can be saved through the multiple LC stream analysis method by selecting a 2.5 minute window.

Figure 6. Sample Definition

A representation of the plate arrangement used for this experiment. Each row of the plate contains a different target compound standard and the associated samples. The optimized methods are generated from injections made in the first column. The batch is executed using the appropriate method and each sample is injected on alternate columns as shown in Figure 5.

Standards for optimization

Analytical Samples

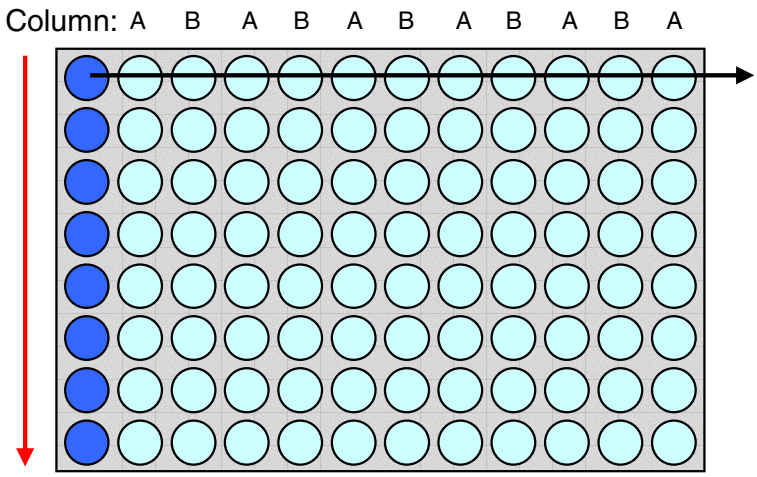


Table 1. High Throughput Automaton Method Efficiency

Method	# of Streams	# of Sample	Total Analysis Time
Automaton	1	88	440 minutes
HT-Automaton	2	88	220 minutes

The original method runtime was 5 minutes (rapid gradient). A retention time window of 2.5 minutes (as shown in Figure 5) was used in the Two-Stream Batch set-up (0.5 – 3.0 minutes) as the MS analysis time. The overlapping LC runs and targeted data acquisition results in higher throughput for the overall system.

CONCLUSIONS

- Automaton software provides the rapid determination of MS and MS/MS parameters for new compounds, performs a batch acquisition for these compounds, and carries out quantitative processing on the acquired data.

- The optimized parameters are stored in a compound database for future use.

- Two-Stream Batch provides a quick and easy solution for reducing the overall runtime of a set of samples. By cutting out the time around the peaks of interest, spending this time washing the syringe/valve and preloading the next sample, the mass spectrometer only acquires the data for the specified retention time window.

- These two software applications work together to provide unattended operation for high throughput analyses.

TRADEMARKS