Evolution, Development, and Application of a Dynamic, High-Throughput, Enterprise-Level Autosampler for High-Performance LC/MS/MS Bioanalysis

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Introduction

The biopharmaceutical industry faces increasing internal and external pressures to enhance productivity while decreasing operating costs. Reducing time to market, improving quality of critical predictions, and rapidly responding to changing portfolio needs are measures of R&D success. Bioanalytical Mass Spectrometry and associated methodologies are core technologies driving pharmaceutical drug discovery. These technologies consume an outsize portion of R&D budget and require highly skilled personnel to operate. We report here on new bioanalytical technology for HT-LC/MS analysis. The sample delivery hardware is simple and designed to be robust while enabling multiple separation modes. The software seamlessly integrates with drug discovery workflows and features an enterprise-level database that serves as repository for bioanalytical methods (knowledge) that is easily accessed and applied.

Materials and methods

- Dual Agilent 1290 Infinity and Eksigent ExpressHT micro-flow pumps
- SCIEX 5500 Triple Quad mass spectrometer with Analyst 1.6.3 software
- LeadSampler/LeadScape software equipped with high-pressure Rheodyne valves
- HALO C18 30x0.5mm 2.7µ micro-flow column
- HALO C18 20x2.1mm 2.7µ column
- Optimize Technologies C18 1.5x5mm 13µ trap cartridge
- Eksigent 65µ ESI electrode
- Column heater from Analytical Sales and Services set to 55C
- 75µ ID, 1/32” OD and 100µ ID, 1/16”OD tubing and fittings from Analytical Sales and Services
- PicoFuze probes from New Objective, Woburn MA
- A method development cocktail in 10% methanol containing quinidine, propranolol, diltiazem, verapamil, tolbutamide, terfenadine and diclofenac

System Design Features

A

Fig 1a. The system was designed to occupy the smallest possible footprint, enabling co-location of autosampler, pumps and mass spectrometer and substantial reduction in total system volume (~5µL)

B

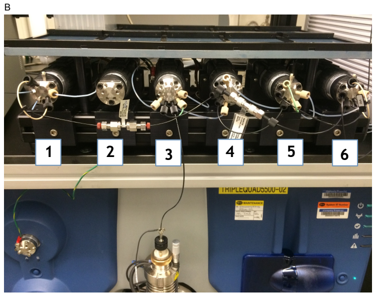

Fig 1b. Injector, diverter, and stream selector valves are located in front of the unit, allowing for short tubing runs and straightforward replacement when necessary. This design also places the stream selector in direct “line of sight” of the Turbo V and IonDrive source on SCIEX x500 series MS/MS instruments. Multiple injection ports and streams available on the platform enables the user to switch between UHPLC analysis (valve 6) with diverter (valve 4), optimization (valve 5), and micro-flow LC (valve 1) “on-the-fly” using a stream selector (valve 3).

Assessment of System Performance Across LC Methods

Table 1. Summary of LC methods performed on LeadSampler system

	Column	Flow rate	Cycle time	Injection volume
HT-Trap-and-elute	Optimize Technologies C18 13µ 1.5x5mm	600µL/min	15s	1µL
UHPLC gradient	HALO C18 2.7µ 20x2.1mm	800µL/min	30s	
Micro-Flow	HALO C18 2.7µ 30x0.5mm	75µL/min	60s	
	Reprosil-PUR C18 3µ 50x0.2mm (PicoFuze)	18µL/min		

A
B
C
D

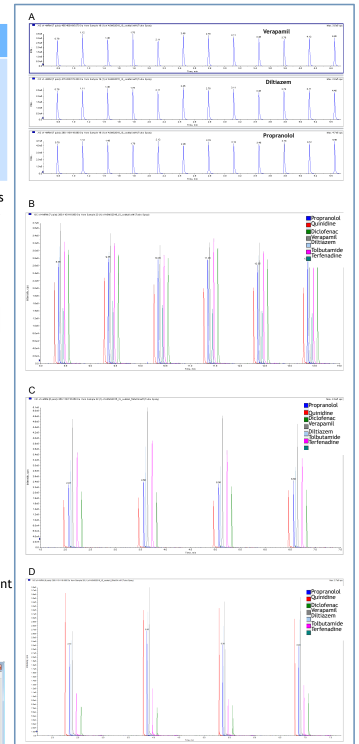

Fig 2a-d. Injections of method development cocktail using various LC methods on LeadSampler A) high-throughput (20s/injection) trap-and-elute, B) UHPLC gradient (60s/injection), C) Micro-flow at 75µL/min, and D) Micro-flow at 18µL/min using PicoFuze. Peak widths at base were at least 3s for all methods.

A

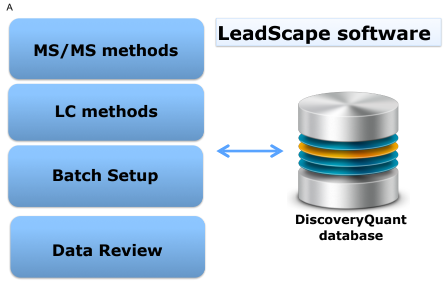

Fig 3a. Schematic of LeadScape modules and integration with DiscoveryQuant enterprise LC/MS/MS database

B

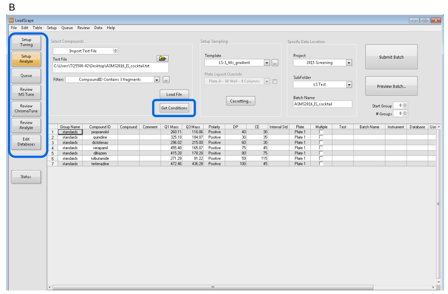

Fig 3b. Main panel of LeadScape software, which controls the LeadSampler and allows for communication with SCIEX Analyst software and mass spectrometers. Various modules (Compound Optimization, Sample Analysis, ChromaTune and Data Review) are integrated into LeadScape.

C

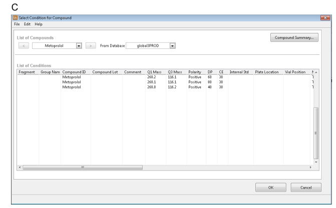

Fig 3c. Integration with DiscoveryQuant 3.0 database supports an enterprise LCMS/MS repository that can be queried through LeadScape software and shared within an organization.

Integrated Software Modules

A

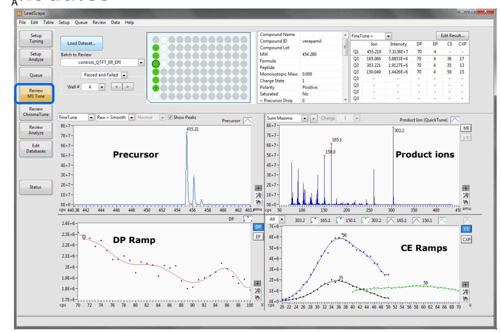

Fig 4a. Optimization Review module in LeadScape, FineTune MS/MS optimization of verapamil is shown. Optimization settings are fully customizable, and support various scan rates and types for SCIEX Triple Quad and Qtrap models.

B

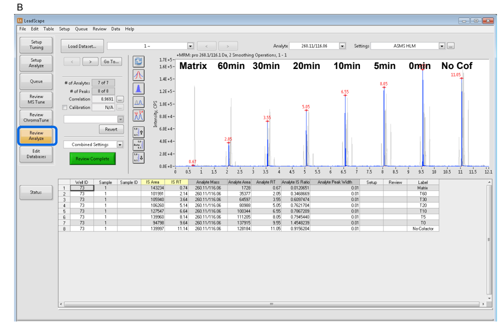

Fig 4b. Analysis Review module in LeadScape allows rapid processing of multi-injected files using customizable review templates and integration parameters. Shown is a metabolic stability study of propranolol in human liver microsomes with and without cofactor.

Conclusions

- A robust, low-profile LC/MS autosampler was designed primarily for enhanced throughput in both gradient and ‘trap-elute’ LC modes.
- Several distinct applications and LC chemistries were queued “on-the-fly” from a single platform, providing high-throughput and separation at low flow rates.
- MS/MS optimization data for several analytes was uploaded to an enterprise-level database.
- LeadScape software modules were used to review MS/MS optimization and sample data.
- LeadSampler/LeadScape provided support of various LC/MS/MS methods/tasks and integrated modules for data review and database query.