Next Generation LCMS Multiplexing Platform for High Throughput Assays with Advanced Analytical Intelligence

Logan Miller[1], Yuka Fujito[1], Theresa Meli[2], Sarah Bartok[2], Kerry Hill[1], Timothy Lee[1], Arthur Harmon-Glaus[1], Andy Sasaki[1]
[1]Shimadzu Scientific Instruments, Inc., Columbia, MD; [2]Quest Diagnostics Nichols Institute, Chantilly, VA

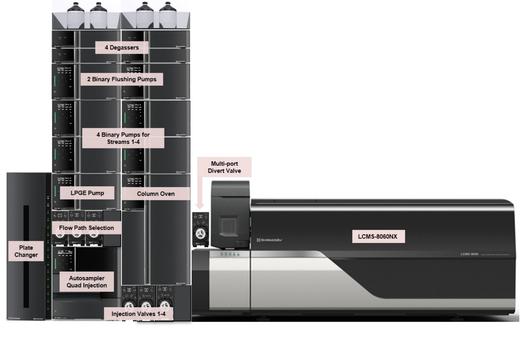
Introduction

The Nexera QX Multiplexing LCMS system is the next generation LCMS platform that can alleviate many of the commonly associated problems with existing high throughput LCMS assays. With advanced Analytical Intelligence (AI) features including dedicated analytical pumps to provide high concentration sample detection and both autosampler needle rinsing as well as full analytical flow path rinsing, downtime can be minimized to keep the instrument operational longer without any maintenance. Additionally, with QX Solution software a seamless operation of the instrumentation with simple user interfaces and customizable batch importing options is achieved. The incorporated AI features can activate when unknown samples have surpassed user defined thresholds and automatically employ dedicated rinsing methodologies to clean the effected streams to eliminate analyte carryover.

Methods

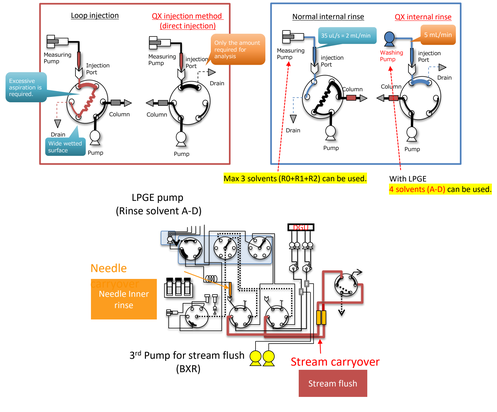
The Nexera QX four channel multiplexing system (autosampler, column oven, four binary pumps and four single pumps, LPGE pump for the needle inner rinse) coupled with triple quadruple LCMS (Shimadzu LCMS-8060NX) was used. Urine samples were extracted following an established solid-phase extraction procedure. Calibration range for the analysis was between 0.2/0.8 ng/mL and 500/2000 ng/mL depending upon the analyte within the opiate drug class panel. Dedicated methods were established for both needle and stream rinsing to eliminate high patient sample carryover. The Nexera QX allows up to 5ml/min of active rinsing with four solvent selection to customize aqueous/organic washes to eliminate carryover. In extreme cases a ternary gradient can be applied to rinse columns with a stronger organic solvent.

System Configuration

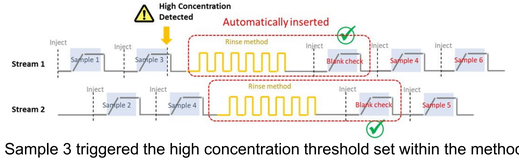


New Approach to Carryover Reduction

New approaches to carryover reduction include implementation of four solvent selection internal needle rinsing with a robust analytical pump, this allows higher flow rates and more solvent combinations compared to previous autosamplers. Additionally, QX offers true ternary gradient formation using dedicated flushing pumps which can introduce a more aggressive organic solvent rinse to equilibration portion of gradient to drastically reduce column carryover.



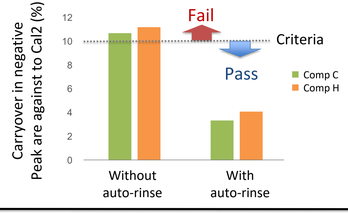
Advanced Analytical Intelligence

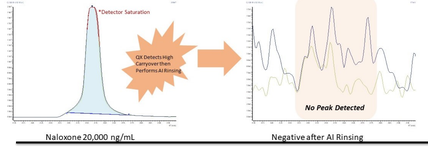


Sample 3 triggered the high concentration threshold set within the method, sample 4 was already injected on its own flow path. Both flow paths had rinsing methodology applied (known carryover and suspected carryover), after which both flow paths injected a blank sample to ensure no carryover present. Once blank check has passed the system will reinject the suspected sample (sample 4) and continue batch. This progress occurs fully without any user intervention.

Results

Autosampler carryover was eliminated by employing a short needle inner rinse time problem that flushed the needle with a mixture of mobile phase A, mobile phase B, and an organic rinse solvent. This AI rinse program was able to reduce carryover in a negative sample following a high concentration sample (70,000 ng/mL) of target from over 10% peak area of calibrator cut-off to less than 5%. Acceptance criteria for carryover defined within scope of application was less than 10% of cut-off level, the Nexera QX was easily able to achieve this criteria even after high concentration injections from 30,000 to 100,000 ng/ml.

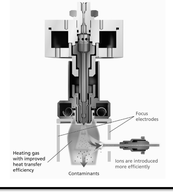


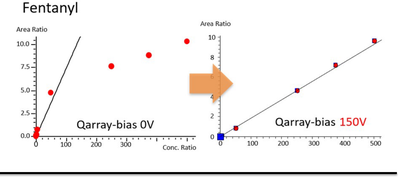


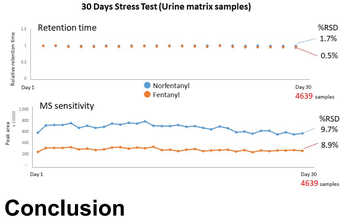
With the Nexera QX system high-throughput LCMS can be achieved while maintaining very low levels of carryover. Carryover is typically very challenging in multiplexing because if a high concentration sample is injected there is possibility that it could affect subsequent injections during the batch. For example a fortified sample of naloxone at 20,000 ng/mL (10x higher then method ULOQ) was injected, QX applied dedicated rinsing program which resulted in subsequent negative samples to show no carryover.

Compounds that are typically challenging to achieve linear calibration relationships such as fentanyl benefited from the new IonFocus source and Qarray bias to reduce ionization competition which typically creates quadratic calibration curves.

The newly designed NX source with IonFocus electrodes affords further probe positions from inlet thereby reducing matrix introduction while showing no loss in sensitivity. Along with the redesigned optics which further reduce matrix contamination of quadrupoles, higher ion sampling efficiencies are achieved.







The AI features along with hardware design maximizes robustness of LCMS system to provide continuous analysis of samples over the course of 30 days which resulted in overall %RSD for norfentanyl/fentanyl retention time of 1.7/0.5% respectively as well as a %RSD of raw peak area for calibration standard of 9.7/8.9%.

During continuous usage no maintenance of the system was required, and no loss of sensitivity was seen with the enhance robustness afforded by the mass spectrometer.

Conclusion

The innovation in the hardware design along with AI software features provide an extremely robust and simple user interface allowing maximum productivity. Customizable rinsing options provide a flexible approach to combat nearly any carryover scenario that may be encountered, further reducing loss of productivity by requiring re-injections post batch completion. While optimizing linearity of response increases confidence in observed results. QX Solution software provides a seamless experience to end user regardless if functioning within R&D environment or day-to-day routine production.