Multi-Method LCMS Assay Multiplexing with Advanced Analytical Intelligence Capabilities to Increase Laboratory Throughput

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1. Overview

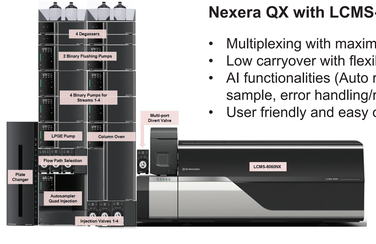
This study shows that multiplexing multiple assays was achieved with good quantitative accuracy and reproducibility. This also can reduce the overall analysis time by eliminating the time and effort required to change mobile phase and columns, rather than performing a single-method analysis in multiple assays.

2. Introduction

Increased throughput is one of the greatest interest in multi-sample processing in clinical laboratories. Multiplex analysis offers significant advantages with respect to time, reagent cost, sample requirements, and the amount of data that can be generated. In high-volume assays, the same analytical method is usually assigned to all streams of a multiplex system to accelerate sample processing. However, there are also middle to low volume assays, and there is a need to combine these assays to run multiple methods on a single LC in order to maximize LCMS utilization.
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. Using QX Solution software, a seamless control of the system can be achieved even while multiplexing multiple assays within a single batch, allowing a single instrument ultimate method flexibility.

3. Methods

The Nexera QX four channel multiplexing system coupled with triple quadruple LCMS (Shimadzu LCMS-8060NX) was used. Two different analytical methods (Method 1: Benzodiazepines, Method 2: Bath salt) using different mobile phases and columns are equipped, multiplex analysis was performed in multi-method mode.
Urine samples were extracted following an established solid-phase extraction procedure for each assay developed on the system.
The Nexera QX allows active rinsing with four solvent selection to customize aqueous/organic washes to eliminate carryover. The system can automatically activate a ternary gradient for complete flushing of the analytical flow path, with methods dedicated to each assay being run overlapped. Dedicated methods were established for both needle and stream rinsing to eliminate high patient sample carryover.



Nexera QX with LCMS-8060NX

- Multiplexing with maximum four streams
- Low carryover with flexible needle/stream rinse
- AI functionalities (Auto rinse after high concentration sample, error handling/recovery)
- User friendly and easy operation software

New Approach to Carryover Reduction in Nexera QX

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.
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.

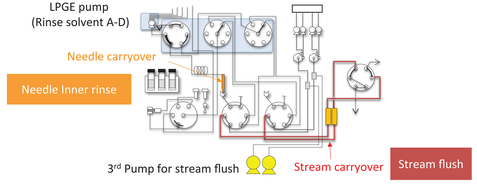
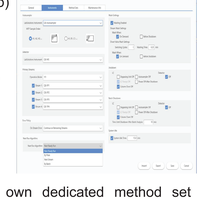


Figure 1. Diagram of Nexera QX

Multi-Method Setting and Batch Analysis in QX Solution

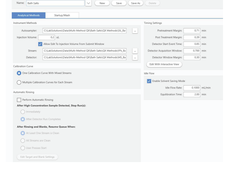
Timing calculator for each method set provides a simple view prior to running samples what throughput increase can be achieved based upon input variables for the method. Some variables are automatically applied based upon the base methods used for the assay.

(b)



QX Solution provides four run algorithms (optimizes run order based on setting selected).
In this study, stream 1/2 and stream 3/4 were assigned to Benzodiazepines and Bath salt method respectively.

(c)



Each assay has its own dedicated method set parameters that can be customized. Settings including automatic idle flow rates, automatic carryover rinsing and washing. Each stream can then be specified to run a set assay. Dedicated rinse methods with different rinse solvents for each method were assigned.

Figure 2. QX Solution method setting UI

QX Solution provides a two-tier batch runs status for multi-method run; the upper showing the overall status of each submitted batch and the lower showing the actively running and completed samples. A status column shows the real-time status for each line of the submitted batches.

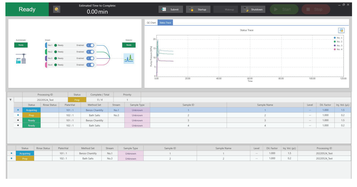


Figure 3. QX Solution main UI

4. Results

4-1. Reducing Analysis Time by Overlapping Multiple Methods

Analytical conditions of each method were summarized in Table 1. These two methods use different mobile phase and column. Therefore, after the first assay finished the system required columns to be changed as well as mobile phases in single method multiplex analysis.
In multimethod approach, the system used 2-channels for benzodiazepines and 2-channels for bath salts, effectively performing overlapping 2-plex analysis of both assays simultaneously with 4-plex configuration.

Table 1. Method conditions

Methods	LC run time	MS acquisition window	Mobile phase conditions	Columns
Benzodiazepines (Stream 1, 2)	5.0 min.	1.9-3.9 min.	MP-A: 0.1% formic acid-water MP-B: 0.1% formic acid-methanol	Kinetex Biphenyl (2.6um 50x3.0mm)
Bath salt (Stream 3, 4)	2.5 min.	1.2-2.0 min.	MP-A: 0.1% formic acid-water containing 10 mM ammonium formate MP-B: 0.1% formic acid-methanol	Kinetex C18 (2.6um 50x3.0mm)

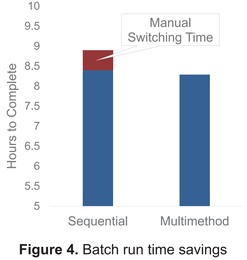


Figure 4. Batch run time savings with multi-method

Using the multimethod approach when factoring in the manual process that would be required to switch system when running sequentially, multimethod saved approximately 30 minutes of run time per 2 plates (1 plate for each assay) in this method combination (Figure 4).
The Nexera QX system provided the capability to overlap multiple methods on one system without the user having to pause within batches of samples to change mobile phases and columns.
The unattended operation of the system while multiplexing different methods together achieved an overall faster batch run time.
Depending on the combination of methods, further time reduction would be possible by multiplexing based on the balance of LC run time and MS acquisition window.

4-2. Quantitative Data

Linearity of the calibration curves and accuracy of the calibrators and QC samples were summarized in Table 2. In multi-method operation, quantitative analysis with good accuracy was possible in both methods.

Table 2. Linearity of calibration curves and accuracy of calibrators and QC samples

Methods	Compound	Calibration curve		QC Accuracy (%)		
		R^2	Accuracy (%)	QC 1	QC 2	QC 3
Benzodiazepine	Temazepam	0.996	89-105	113	120	93
	7-aminoclonazepam	0.999	96-105	129	102	93
	Lorazepam	0.998	95-109	100	95	98
	Nordiazepam	0.996	95-110	112	106	93
	OH-flurazepam	0.999	97-103	114	115	96
	OH-triazolam	0.996	92-108	114	114	93
	OH-alprazolam	0.997	93-107	105	107	108
	Oxazepam	0.999	95-104	90	105	96
	OH-midazolam	0.998	95-104	108	106	100
Bath salt	Alpha-PVP	0.998	95-103	98	103	-
	Butylone	0.999	89-117	90	98	-
	MDPV	0.999	92-116	96	97	-
	Mephedrone	0.998	88-119	100	111	-
	Methylone	0.993	89-107	101	109	-
	Pentylone	0.998	85-124	96	95	-

4-3. Reproducibility

Figure 5 shows the QC chart capabilities of QX with each color indicating a different analyte being monitored by the system. After each sample has been collected, the resulting peak area (or height) is displayed in the chart (standards are identified by squares while all other samples are circles).
In this study, excellent reproducibility of internal standard areas were achieved over the course of 1000 injections (the periodic dips in intensity correspond to blank samples, no internal standard in sample). Overall, the stability and robustness of the multimethod system was excellent routinely achieving less then 5% RSD for retention time for each analyte across all streams as well as less then 10% RSD for internal standard area.

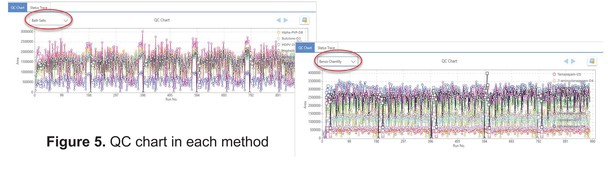


Figure 5. QC chart in each method

5. Conclusion

Multiplexing multiple assays (2 methods 2-plex) using Nexera QX with QX Solution was successfully done with excellent linearity, accuracy and reproducibility. The time savings of 30 minutes per 2-plate was confirmed compared to the conventional single-method multiplex analysis.