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Technical Report

Nexera™ QX: Improving the Efficiency of Routine LC-MS/MS Assay with Multiplex UHPLC Platform

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Abstract:

For many modern analytical laboratories, high-throughput analysis is pivotal. LC-MS/MS shows enormous capabilities for increasing sample throughput by monitoring multiple analytes simultaneously. Over the duration of the method, the time frames used for injection, column wash, and column equilibration time frequently represent a waste of time which negatively affects the throughput of the analysis. A multi-channel LC configuration can provide the capability to maintain the same analytical conditions while increasing sample throughput by reducing the detector idle time. The Nexera QX, a two-channel system coupled with a triple quadrupole MS detector, provides reliable and robust results running an LC-MS/MS method on two parallel flow lines and contributes to effortless usability in increasing the overall analytical throughput.

Keywords: Nexera QX, high-throughput LC-MS/MS analysis, multi-channel LC system, multiplex LC-MS analysis

1. Overview of High-throughput Analysis

Performing a high-throughput analysis requires careful design. Usually, the analytical method is initially developed without regard to sample throughput and then later adapted for high-throughput. That usually means speeding up all steps in the analytical workflow, from sample preparation to results report. Generally, to perform high-throughput experiments, analytical methods must be adapted and, in most cases, analytical performance (e.g., detection limit, reproducibility) is not as good as conventional analysis[1]. The use of a multi-channel system allows increasing the throughput without modifying the original analytical method by overlapping two separate flow lines (Fig. 1).

In this report, we introduce the capability of Nexera QX (Fig. 2), a dual-channel LC-MS/MS system. This novel system increases the throughput of a traditional LC-MS/MS analytical method, minimizing downtime, and is executed via a simple software operation. We evaluated system robustness for consistent results regardless of the flow path used. Performance testing was comprised of retention time reproducibility, linearity and carryover for both flow paths.

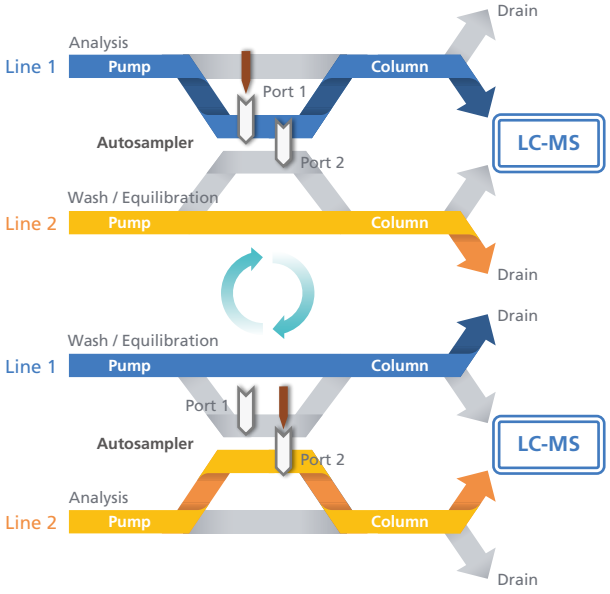

Fig. 1 Nexera QX Flow Diagram

2. Multi-channel LC System

As described in Fig. 2 and 3, the Nexera QX system consists of two flow lines, both operating in high-pressure gradient mode, with a dual-channel autosampler and a triple quadrupole MS detector. Shimadzu QX Solutions software made it possible to overlap two identical analytical methods running in lines 1 and 2, increasing the overall analysis throughput by reducing the detector idle time.

Fig. 2 Nexera QX and LCMS-8060NX

1 Analytical & Measuring Instruments Division

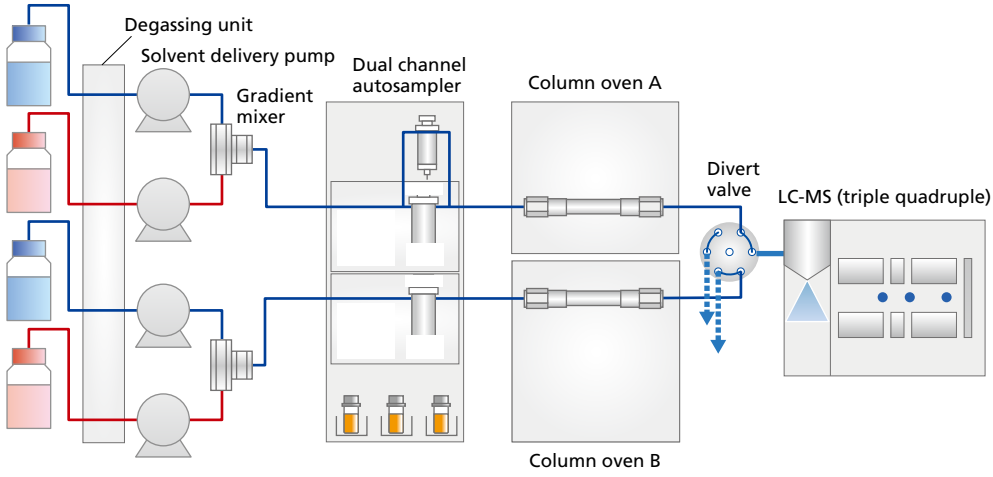

Fig. 3 Nexera QX Flow Diagram

3. Retention Time Reproducibility

Evaluation of retention time reproducibility was carried out by analysis of antiarrhythmic agents with reverse phase chromatographic separation (Shim-pack™ XR-ODS III 50 × 2.0 mm 1.6 µm) and MRM acquisition mode (positive ionization). The analytical method run time of eight minutes includes three minutes of acquisition time and five minutes of column wash and equilibration time. By overlapping lines 1 and 2, it was possible to increase the overall throughput by a factor of 1.4. The retention time reproducibility was excellent, and the t_R differences between lines 1 and 2 were negligible for all analytes (Table 2).

Table 1 Analytical Conditions

System	:	Nexera QX (dual-channel)
Column	:	Shim-pack XR-ODS III (50 × 2.0 mm 1.6 µm)
Flowrate	:	0.5 mL/min
Mobile phase A	:	Water:Acetonitrile 95:5 + 0.05% Formic Acid
Mobile phase B	:	Acetonitrile + Formic Acid 0.05%
Column temp.	:	40°C
Injection volume	:	0.5 µL
Needle rinse	:	Internal rinse A+B+C+A (A: Mobile Phase A; B: Water; C: Acetonitrile/Methanol/Isopropanol)
Elution mode	:	Gradient – 0%B (0–1 min) – 5%B (3 min) – 90%B (3.1–6 min) – 0%B (6.1–8 min)
Acquisition time (MS)	:	3 min
Analytical time (LC)	:	8 min
Detector	:	LCMS-8060
Acquisition mode	:	MRM (positive)
Verapamil	:	455.10>303.30, 455.10>150.25
Nor-Verapamil	:	440.95>150.00, 440.95>165.05
Propranolol	:	260.35>56.20, 260.35>116.10
Atenolol	:	267.25>190.10, 267.25>145.10
Metoprolol	:	268.15>116.10, 268.15>74.50
Sotalol	:	273.25>133.15, 273.25>106.20

Table 2 Retention Rime Differences Between Line 1 and Line 2 (t_R = avg. of 6 replicates)

Molecule	Line 1 (min)	RSD% (n=6)	Line 2 (min)	RSD% (n=6)	Δt_R (min)
Verapamil	2.464	0.35	2.441	0.23	0.023
Nor-Verapamil	2.442	0.35	2.418	0.25	0.024
Propranolol	2.24	0.36	2.171	0.27	0.069
Atenolol	0.366	2	0.346	1.3	0.02
Metoprolol	2.027	0.45	1.938	0.28	0.089
Sotalol	0.314	2.5	0.288	3.2	0.026

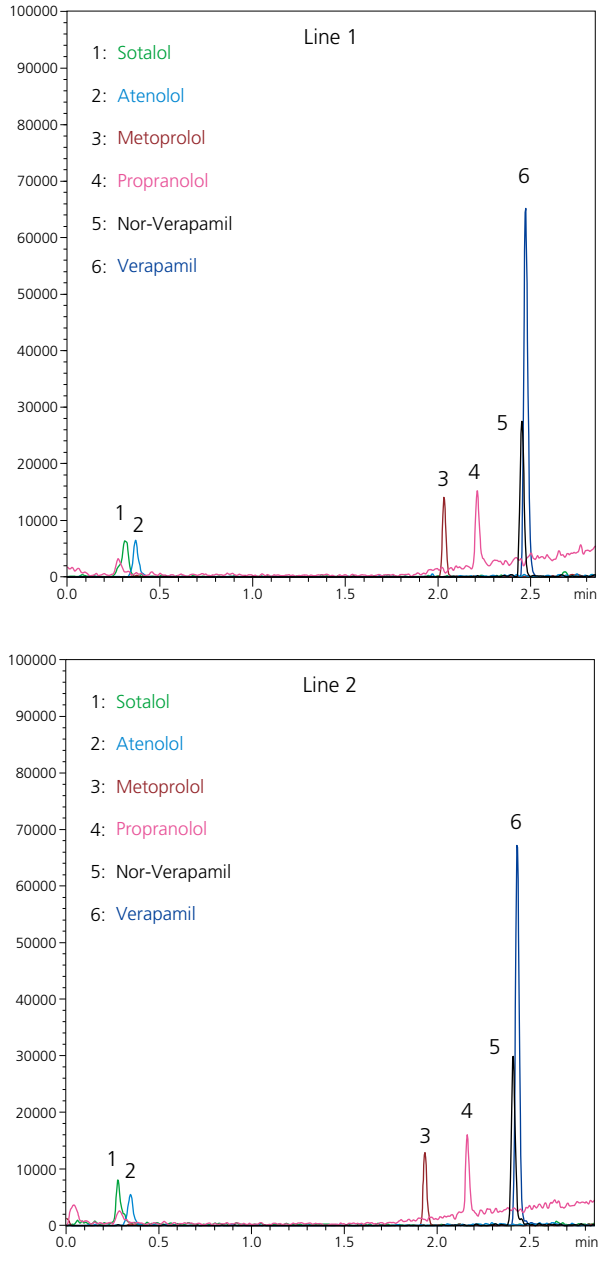

Fig. 4 Chromatogram of Antiarrhythmic Agents 10 µg/L Standard Solution

4. Linearity

Linearity was assessed for verapamil from 6 to 750 µg/L (external standard calibration), and the calibration curves were acquired both using lines 1 and 2. The resulting trend was excellent ($r^2 > 0.997$), and accuracy was within 85–115% range for each of the six levels (calibration curve is shown in Fig. 5). No differences were observed when running the calibration curve in line 1 or line 2.

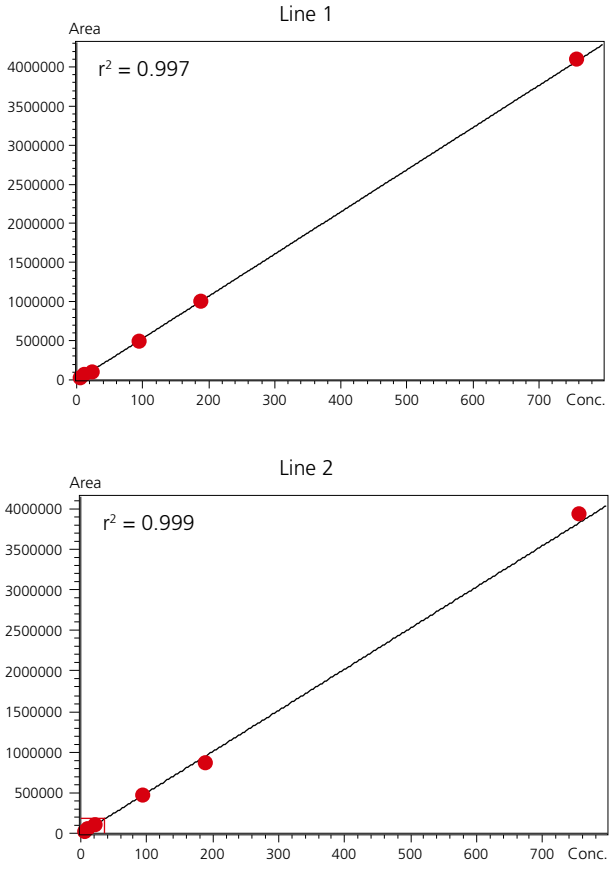

Fig. 5 Calibration Curve of Verapamil, 6 – 750 µg/L

5. Carryover

Carryover was evaluated for both lines using a chlorhexidine sample (5000 µg/L), with a reversed phase method (isocratic elution with methanol 40% + Formic acid 0.1%, Shim-pack XR-ODS III, 5.0 × 2.0 mm 1.6 µm) at 0.4 mL/min flow rate and 1 µL injection volume. After injecting the chlorhexidine standard solution and acquiring the signal in MRM mode (*m/z* 253.30 → *m/z* 110.65) a subsequent blank sample (methanol) was injected using the same analytical conditions. The needle was rinsed both internally and externally, internal rinse was performed using a sequence of 3 different solvents R1+R2+R3 (R1: Methanol/Acetonitrile/Water/Isopropanol + Formic Acid 1%) (R2: water) (R3: Methanol 40% + Formic acid 0.1%). The results showed negligible carryover (<0.00056% and <0.00064%) and no differences were observed between the two lines (Fig. 6 and Fig. 7).

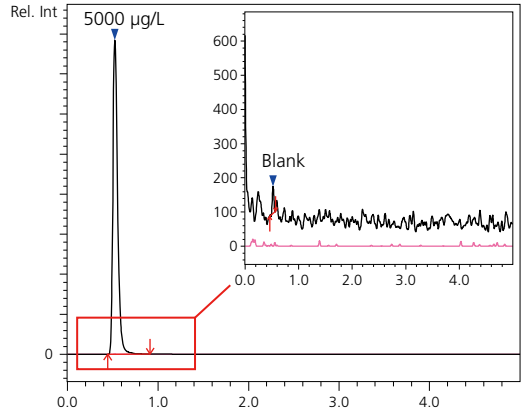

Fig. 6 Carryover Evaluation in Line 1

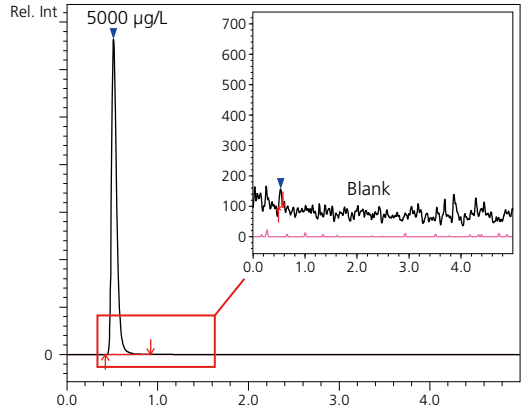

Fig. 7 Carryover Evaluation in Line 2

6. Conclusions

- The Nexera QX system, used in a dual-channel configuration, gave increased sample throughput without any modification to chromatographic conditions.
- Chromatographic performance differences in terms of retention time, linearity and carryover between the two lines were negligible, proving the robustness of the system for high-throughput analysis.

References

[1] *Medical Applications of Mass Spectrometry*, Chapter 4, 2008, Elsevier B.V.

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