

Evaluation of Current High Throughput LC Capabilities

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Introduction

High-speed sample injections can significantly improve throughput in multi-sample processing, especially for analyses with short runtimes. Recently, a novel high-speed autosampler was introduced that features an injection time of just 7 seconds and an analysis cycle time of 14 seconds. The combination of this autosampler with core shell and small particle columns allows ultrahigh throughput to be achieved without any specialized instrument routines that add complexity to the analysis.

Analytical runs were evaluated with UV and a high-speed quadrupole that features a positive/negative ionization switching time of 15 msec and a high-speed scanning rate of 15,000 u/sec under ultra-high throughput conditions of up to four analytical runs per minute.

Background of Autosampler Development

- Reduction of analysis cycle time and increase of sample capacity
- Efficient sample analysis for pharmacokinetic and synthetic drug discovery
- Carryover reduction for high-sensitivity MS detection
- Open-access design for rapid synthesis-compound identification
- Reduction of column external volume for ultra-fast analysis
- Ultra-fast analysis by LC/MS and LC/MS/MS

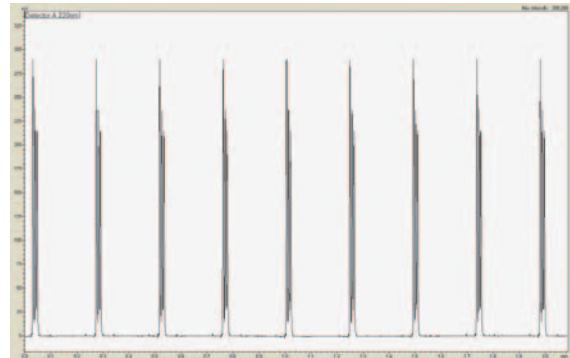
Nexera MP System

Enhanced LC/MS frontend HPLC performance with Nexera ultra-fast analysis technology

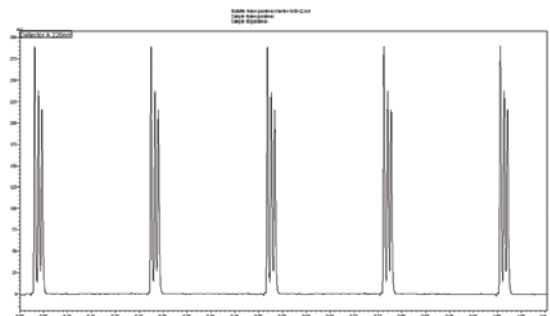
A newly-developed multiplate autosampler (SIL-30AC_{MP}) and a small column oven (CTO-30AS) comprise the new Nexera system.



High-throughput LC



Kinetex XB-C18, 60% ACN, 75°C, 4 mL/min, Paraben mix, runtime: 4.2 seconds, 7,940 psi, 2 minute timescale



1 minute timescale

High Throughput UHPLC/MS/MS

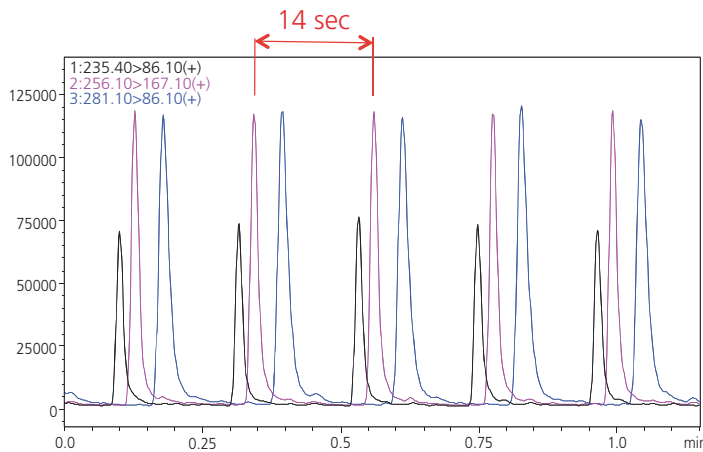
Requirements:

- High-speed scanning rates to obtain enough data points to reduce peak distortion (15,000 u/sec) and RSD values
- Fast polarity switching speeds (15 msec) to combine ionization modes without the need for additional runs
- Short pause time when switching measurements between compounds (minimum 1 msec) to allow multiple compounds to be analyzed
- Technology to keep ion momentum in collision cell to prevent crosstalk

High-throughput UHPLC/MS/MS Analysis

14 sec cycle time analysis

- Ultra-fast analysis by combination of SIL-30AC_{MP} and LC/MS/MS

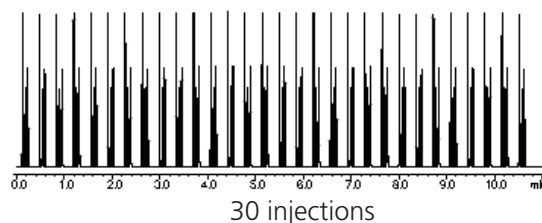
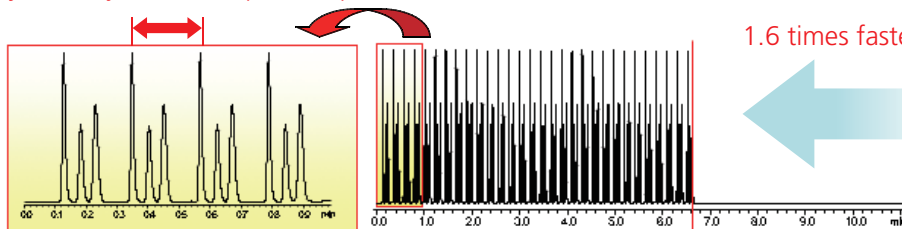


Event #	Compound	Q1 m/z	Q3 m/z
1	Lidocaine	235.4	86.1
2	Diphenhydramine	256.1	167.1
3	Imipramine	281.1	86.1

Column : Shim-pack XR-ODS II 1.5 mmID x 30 mm, 2.2 m
 MP : acetonitrile / water =25/75
 containing 0.1% formic acid
 Flow rate : 1.2 mL/min
 Ionization : ESI (+)

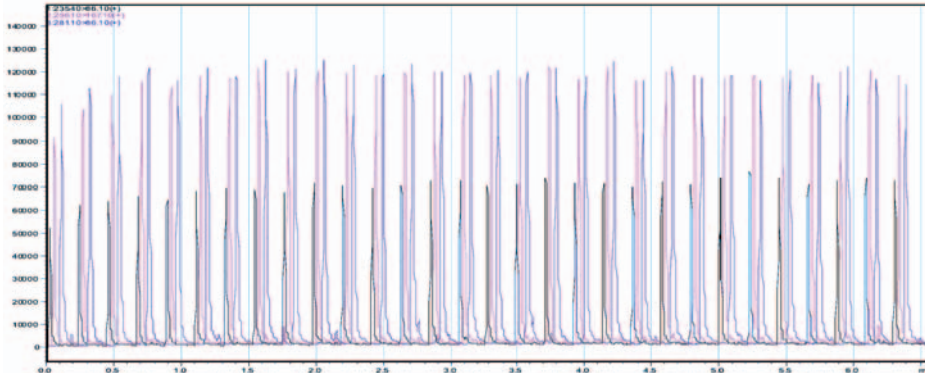
Increased Autosampler Throughput

Injection cycle: 14 sec peak to peak!



Conventional High-speed injector

Reproducibility Inj 21-30



	235.40-86.10	256.10-167.10	281.10-86.10
1	67219	117432	67219
2	69402	121586	69402
3	68270	116184	68270
4	71906	119314	71906
5	75066	121408	75066
6	68476	120635	68476
7	69790	123552	69790
8	68367	123627	68367
9	70299	123789	70299
10	69470	120516	69470
RSD	3.22	2.15	3.22

30 analyses in 6.5 minutes

Carryover Discussion

There are two ways to reduce carryover: 1) Remove it by rinsing or 2) Prevent it in the first place. Rinsing can be effective; however, with the need for increased throughput, taking the time needed for rinsing may not be the best option. Careful choice of materials used in the design and construction of an autosampler will go a long way to prevent carryover. A low carryover autosampler design is necessary for successful high-throughput conditions.

Carryover for ionic compounds can be reduced by:

- 1) Removing adsorbed sample from the system with rinsing solution – time penalty.

- 2) Controlling element adsorption by changing sample needle composition or by coating the needle with chemically inert materials.

Carryover for hydrophobic compounds can be reduced by:

- 1) Removing adsorbed sample from the rotor seal groove by rinsing or flushing the system with organic solvents – time penalty.
- 2) Controlling sample adsorption by changing the rotor seal material and geometry.

Carryover Evaluation

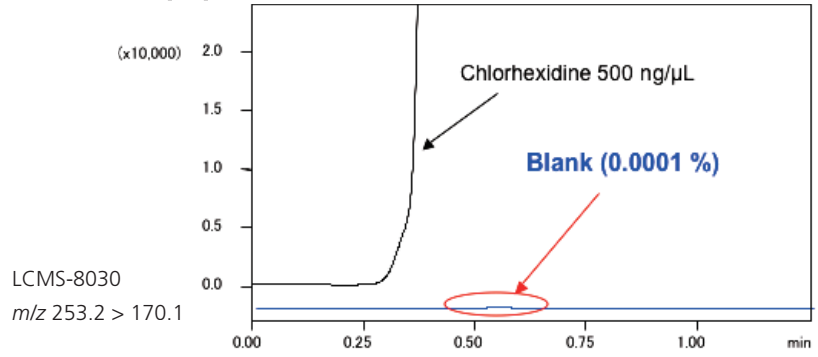
Ultralow carryover achieved without the need for rinsing

When rinsing is performed to keep carryover low, the total analysis time sometimes increases as the number of analyses increases. Nexera autosamplers excel in suppressing carryover even without rinsing.

Minimized Carryover to Support LC/MS/MS

Carryover level of chlorhexidine

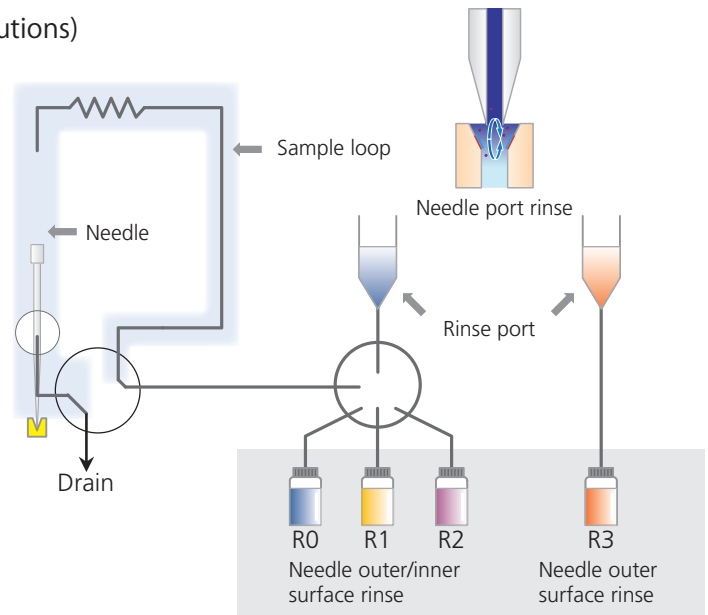
- ⇒ Very low carryover of a stubborn compound, chlorhexidine
- ⇒ Chlorhexidine 500 ug/mL carryover 0.0001%!



Multi Rinse Function

Multi-rinse function to minimize carryover (4 rinse solvents are available)

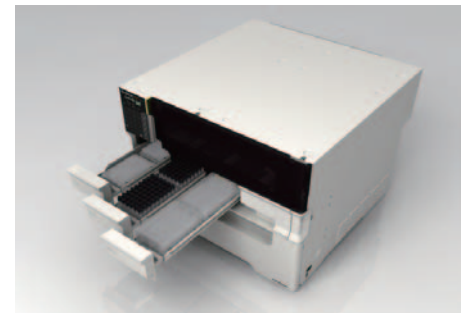
- ⇒ Rinse of needle surface (2 rinse solutions)
- ⇒ Rinse of needle inner surface and needle port (3 rinse solutions)
- ⇒ Support high-sensitivity LC/MS/MS analysis



More Capacity

Flexible use of plate types and easy access to samples

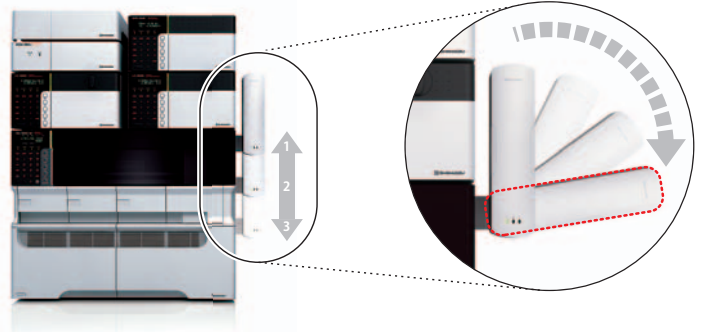
- ⇒ Accommodate 6 plates (384MTP 2304, 96MTP 576, 1.5 mL vial324)
- ⇒ Different plate types are available between 3 racks.
- ⇒ Mixed use of microplates and 1.5 mL vial racks is available.
- ⇒ Open-access design to set samples on analysis
- ⇒ Safety sample access in closed environment



More Flexibility (CTO-30AS Oven)

To maximize LC/MS performance

- ⇒ Adjustable oven position for nearest connection to LC/MS interface
- ⇒ 3 position-variable on levels
- ⇒ Flexible angle from horizontal to vertical
- ⇒ Minimized pipe length between LC/MS interface and column
- ⇒ 1 column (50 mm length), (room temp. + 10°C to 85°C)



Summary

Ultimate LC/MS frontend UHPLC

- More Speed
 - ⇒ *World's fastest cycle time (14 sec) to support* ultra-high throughput analysis
- More Reliable
 - ⇒ Expanded application range by precise low-volume injection (*1.0% RSD at 0.5 uL injection*)
 - ⇒ Minimized carryover to support high-sensitivity LC/MS/MS ($\leq 0.0015\%$)
- More Capacity
 - ⇒ Accommodates 2304 samples (*3 times higher capacity than SIL-30AC*)
- More Flexibility
 - ⇒ *Continuous analysis using 3 types of plates*
 - ⇒ Flexible sample setting by open access rack design
 - ⇒ Optional column oven for LC/MS