Robust Dual-Column Nanospray Source for Improving Nano-LCMS Duty Cycle

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Introduction

On-column injections using packed-tip columns realize optimal chromatographic separation and sensitivity for nanoLC-MS experiments. The unique packed-tip column format adds no post-column volume to the LC plumbing thus providing the sharpest peak shape achievable given the packed column's specifications (internal diameter and stationary support). Qualitative and quantitative separations of complex biomolecular samples usually depend on long gradients to maximize the separation capacity of the column and improve the recovery of low concentration analytes. While nanobore columns enable nanoliter flow rates, the ability to sample load onto the column is limited by the backpressure of the column. Trap column injection is one approach to enable the ability to sample load at higher flow rates but the resultant peak broadening decreases sensitivity. Here, we present a hardware solution utilizing an automated XYZ translation stage digitally controlled by a graphical user interface. This system enables automated cycling between multiple columns, as controlled by an external device, most commonly the MS or the LC system. Duty cycle improvement is demonstrated by loading, washing and equilibrating one column off-line while the second column gradient elutes. Challenges to the dual column approach include optimizing the flow path to ensure column-to-column reproducibility and creating an optimized autosampler method to control for analyte carryover. Solutions to these challenges are presented for a Thermo LTQ MS connected to a direct flow nano-pump using commercially available peptide digests. With each column at voltage during MS acquisition, the presence of cross talk between the two columns was evaluated using syringe infusion.

Methods

Mass Spectrometer

- LTQ linear ion trap (Thermo Scientific)
  - Chromatographic experiments:
    - Full-scan MS: 300-1500 Da
    - Cross talk experiments: Targeted MS/MS scan for angiotensin I 433 Da MH^{3+} & 649 Da MH^{2+}
  - System carryover experiments:
    - Full MS scan
    - Top 3 MS/MS
    - Parent mass list generated from BSA digest chromatogram
- DPV-550 Digital PicoView® nanospray source (New Objective, Inc.)
  - Custom dual-column hardware with microtee high-voltage liquid junction
  - PV Aquire™ software with automated tip positioning control
  - Digital Divert™ box with contact closure controlled by LTQ mass spectrometer

Chromatography: Duty Cycle Experiments

- Eksigent nanoLC·Ultra 2D plus
  Channel 1
  - Flow rate: 1,000 nL/min.
  - Mobile phase A: 0.1% formic acid in water
  - Mobile phase B: 0.1% formic acid in acetonitrile
  - 35-Minute method:
    Valve washing at 98% A, 2%B – 5 min.
    Column washing at 10% A, 90% B – 4 min.
    Column equilibration at 98% A, 2% B – 21 min.
    Sample loading at 98% A, 2% B – 5 min.

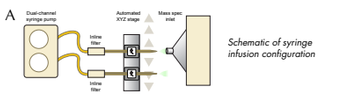
  Channel 2
  - Flow rate: 500 nL/min.
  - Mobile Phase A: 0.1% formic acid in water
  - Mobile Phase B: 0.1% formic acid in acetonitrile
  - Gradient: 30 minutes 2% – 50% B
- Column: PicoFrit column (360 µm OD x 75 µm ID x 15 µm tip) slurry packed to 10 cm with Proteopep II (C18, 5 µm, 300 Å)
- HTC Pal autosampler (Leap Technologies)
  - 6-Port injection valve (VICI)
  - 1.0 µL loop
- 10-Port column switching valve (VICI)

Cross Talk Experiments

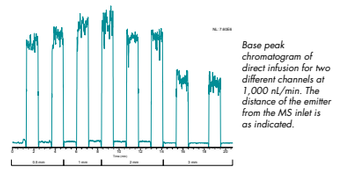
- Harvard Apparatus syringe pump for 2 syringe channels with 250 µL syringes
- Flow rates: 200 nL/min., 500 nL/min. and 1,000 nL/min.
- Emitters: Self-Pack PicoFrit® columns, 360 µm outer diameter, 75 µm inner diameter, 15 µm tip size, uncoated
- Files were collected at 0.5 mm, 1.0 mm, 2.0 mm and 3.0 mm distances between the tip of the emitter and the inlet to the mass spectrometer
- Samples
  - Blank: 50% water + 0.1% formic acid, 50 % acetonitrile + 0.1% formic acid
  - Analyte: 1 pmol/µL angiotensin I in 50% water + 0.1% formic acid, 50 % acetonitrile + 0.1% formic acid

DPV-550 dual-column hardware, showing the positioning of columns relative to LTQ inlet

Cross Talk | Interference from an inactive channel from which data is not being acquired



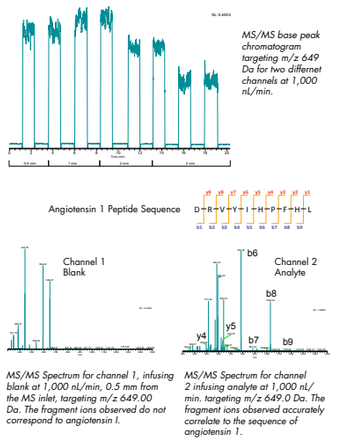
Full Scan Evaluation



MS Spectrum of blank acquisition on channel 1 at 0.5 mm from the MS inlet

MS Spectrum of analyte acquisition on channel 2 at 0.5 mm from the MS inlet

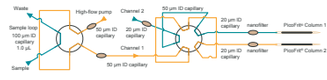
Targeted MS/MS Evaluation



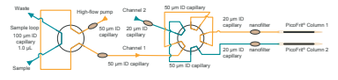
MS/MS Spectrum for channel 1, infusing blank at 1,000 nL/min, 0.5 mm from the MS inlet, targeting m/z 649.00 Da. The fragment ions observed do not correspond to angiotensin I.

MS/MS Spectrum for channel 2 infusing analyte at 1,000 nL/min. targeting m/z 649.0 Da. The fragment ions observed accurately correlate to the sequence of angiotensin 1.

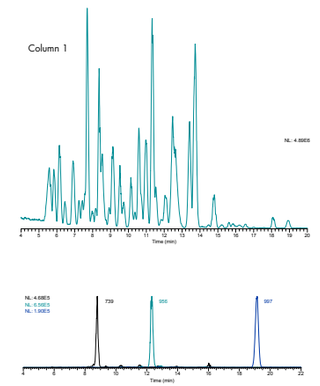
Duty Cycle | The ratio of useful chromatographic separation time to the total run time

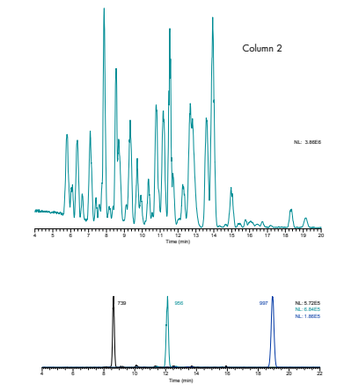


Flow path diagram for gradient elution on column 1. Column 2 is washed and equilibrated. At the end of the gradient, sample is loaded on column 2.



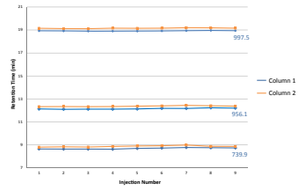
Flow path diagram for gradient elution on column 2. Column 1 is washed and equilibrated. At the end of the gradient, sample is loaded on column 1.





m/z (Da)		Column 1 Apex RT (min)	Column 1 Area	Column 1 Peak width (min)	Column 1 Capacity	Column 1 Asymmetry	Column 2 Apex RT (min)	Column 2 Area	Column 2 Peak width (min)	Column 2 Capacity	Column 2 Asymmetry
739.9	Average	8.68	4.48E+06	0.23	129.5	0.9	8.85	4.17E+06	0.27	111.3	1.0
	STDEV	0.06	5.17E+05	0.01	6.9	0.2	0.04	2.18E+05	0.01	4.8	0.2
	RSD %	0.7	11.5	5.2	5.3	27.5	0.5	5.2	4.4	4.3	17.9
956.1	Average	12.14	7.24E+06	0.28	109.0	1.1	12.35	8.09E+06	0.31	98.6	1.1
	STDEV	0.05	1.64E+06	0.01	4.1	0.3	0.03	7.92E+05	0.02	4.8	0.2
	RSD %	0.4	22.6	3.7	3.8	30.9	0.3	9.8	5.0	4.9	17.8
997.5	Average	18.92	2.38E+06	0.35	84.7	1.0	19.15	2.74E+06	0.41	73.9	1.0
	STDEV	0.02	3.76E+05	0.01	1.7	0.1	0.03	3.65E+05	0.01	1.9	0.1
	RSD %	0.1	15.8	2.0	2.0	9.0	0.1	13.3	2.6	2.6	13.0

Peptide specific peak capacity, retention time, and asymmetry data calculated for three different BSA peptides. The peaks were chosen from the beginning, middle and end of the chromatogram. The statistics were calculated for nine replicate injections on each column.



Plot of peptide specific retention times for three BSA peptides. The plot shows reproducible inter- and intra-column retention times for each peptide.

Conclusions

Demonstrated an absence of cross talk between the two channels at the flow rates, analyte concentration, and emitter positions evaluated

The cycle time per column decreased from 60 min. to 35 min. resulting in a 40% improvement in duty cycle

Minimized system carryover by incorporating a high organic washing step for the offline channel

Future Work

Investigate the advantages of column heating on the duty cycle

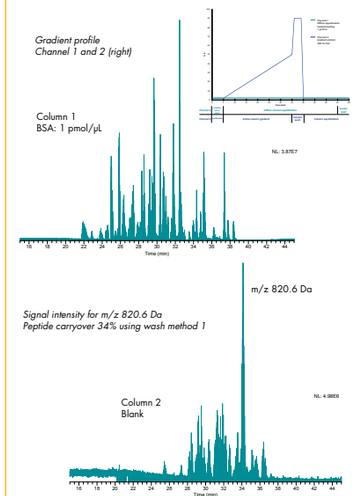
Incorporate a 4-port valve to optimize system plumbing

Further decrease system carryover using optimized washing conditions

System Carryover | Plumbing-associated residue from previous injection in active column

Carry over was evaluated by injecting1 pmol/µL BSA onto column1 followed by a blank injection onto column 2.

Method 1: Water Wash



Method 2: Organic Wash