

Digitized Nanobore LC-MS: An Automated Control System with Emitter Divert for Column-Switching

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Introduction

Nanospray is an essential tool in high-sensitivity mass spectrometry; however, limitations with sample throughput present challenges with the routine use of nanospray for quantitative applications. Current trends toward MS-based biomarker quantitation impose strict requirements on nanobore LC-MS performance. A vision-based, self-tuning source was developed to interface with gradient chromatography and automatically control emitter-tip position¹. We extend this analytical format with an automated ESI column-switching methodology wherein a digital trigger control switches the emitter between two column positions. Vision-based control maintains the spray at an optimized inlet position from two different packed-tip columns as fine tuning of emitter position is optimized by this vision-based control.

Materials & Methods

Samples:

Continuous Infusion Experiments:

- Angiotensin, Bradykinin, Neurotesin peptides (Sigma) were prepared by serial dilution (0.1% formic acid) to a final concentration of 3 μM . (The angiotensin sample contained five different peptide variants at a concentration of 3 μM each.)

Switched LC Experiments:

- Bovine Serum Albumin (BSA) tryptic digest (Waters Mass Prep Standard) was prepared by serial dilution (0.1% formic acid) to 400 fmol/ μL

Continuous Infusion Pump & Monitor:

- Harvard syringe pump (PHD) with two 250 μL glass syringes (Hamilton)
- Scivex[®] in-line digital flow-rate monitor

Liquid Chromatography:

- Left Channel – Eksigent[®] Nanoflow LC
- Scivex Nanopeak 6-port valve, manual injection
- PicoFrit[®] Column (New Objective) 75 μm x 5 cm C18
- Right Channel – Agilent 1100 Capillary LC
- Rheodyne[®] Nanobore manual-injection 10-port valve
- Mobile Phase: Modifier A= 0.1% formic acid, Modifier B= 0.1% formic acid in Acetonitrile
- Gradient: 2% to 20% B (6 minutes), wash at 98% B (2 min), recondition at 2% B (\approx 5 min)

Mass Spectrometer:

- LCQ Deca[™] (Thermo Finnigan)
 - 3 Microscans/spectra
- Emitter-to-inlet distance: 2.5 mm
 - ESI voltage: 2.5 kV
- Digital PicoView[®] PV-150 nanospray source (New Objective) modified for switched spray

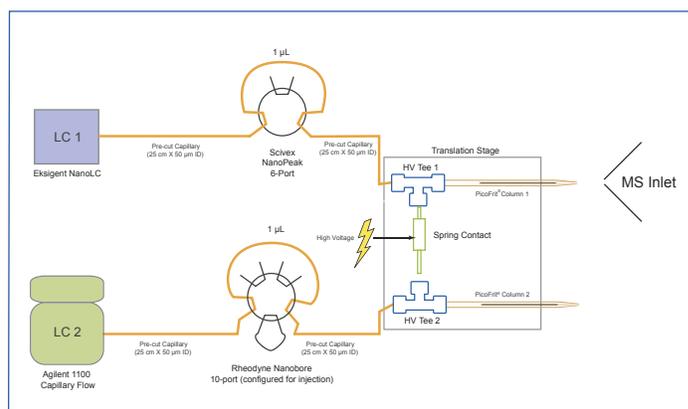


Figure 1A Schematic of the switched-emitter (PicoFrit[®] Column) source

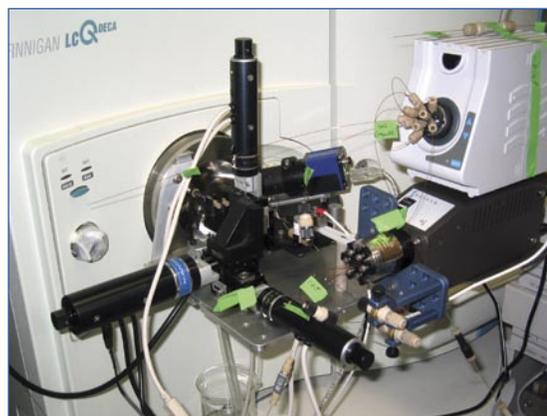


Figure 1B Digital PicoView (DPV-150) mounted on the mass spectrometer

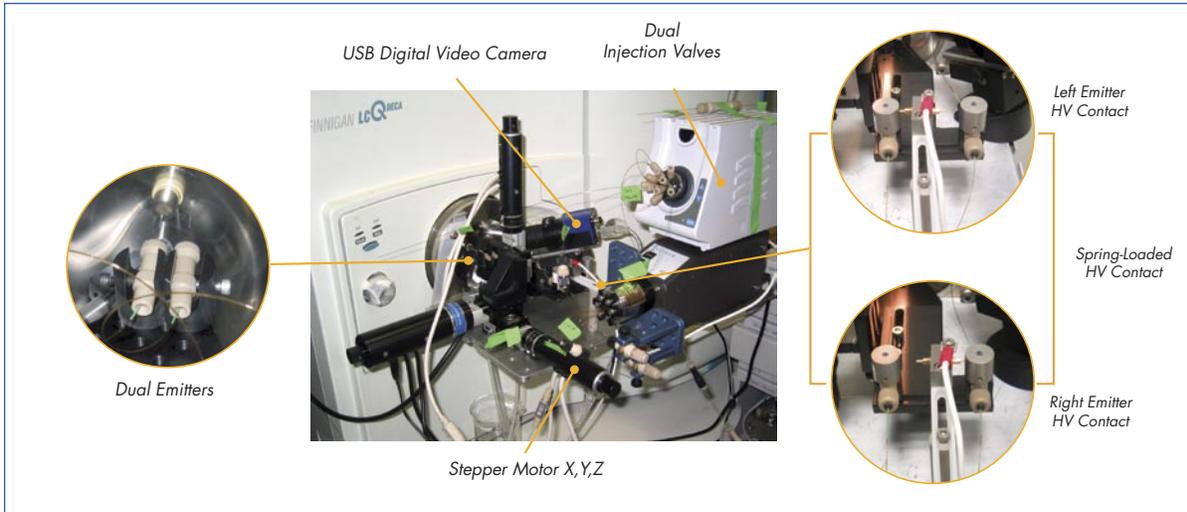


Figure 2 Photo of the modified source with a dual emitter head and high voltage switch contact mechanism. Switching high voltage eliminates direct carryover from each individual spray channel.

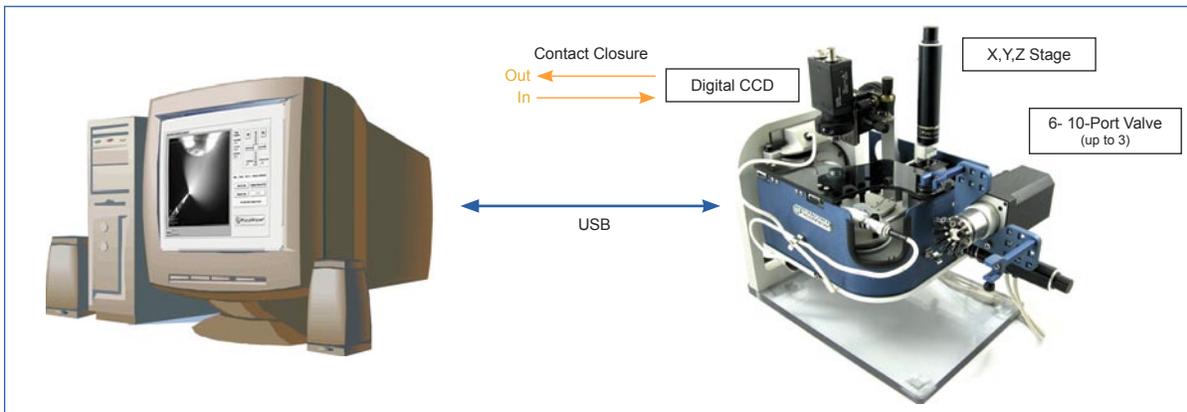


Figure 3 The digital nanospray source is controlled through a graphical user interface. Video, stage position, and external contact-closure control are all handled via USB interface.

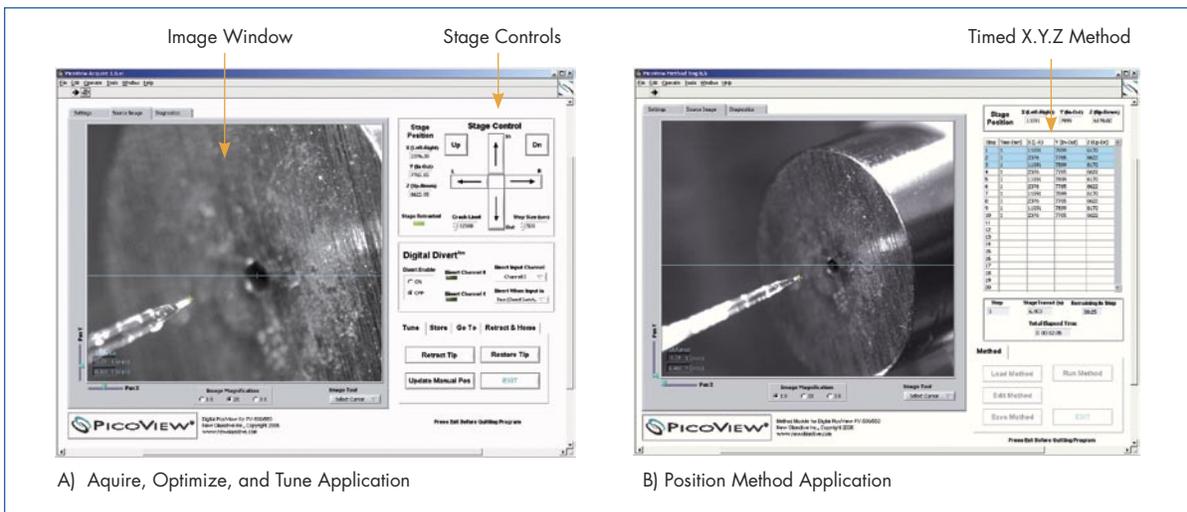


Figure 4 Screen shots of the A) Acquire and B) Method applications of the Digital PicoView® interface. Repeatable emitter positions in A) are enabled by quantitative measurement cursors. The stage may be positioned through the user interface using point and click tools. Timed position methods used in (B) are easily imported/exported via Excel files.

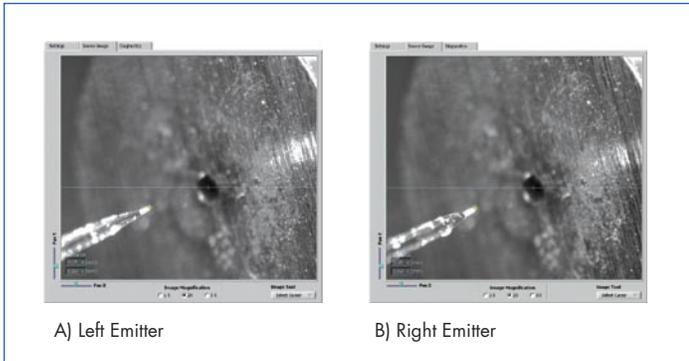


Figure 5 (A) Left and (B) Right emitters used in the continuous infusion study. Note the emitters are localized to a spray position within 10 μm via stage controls.

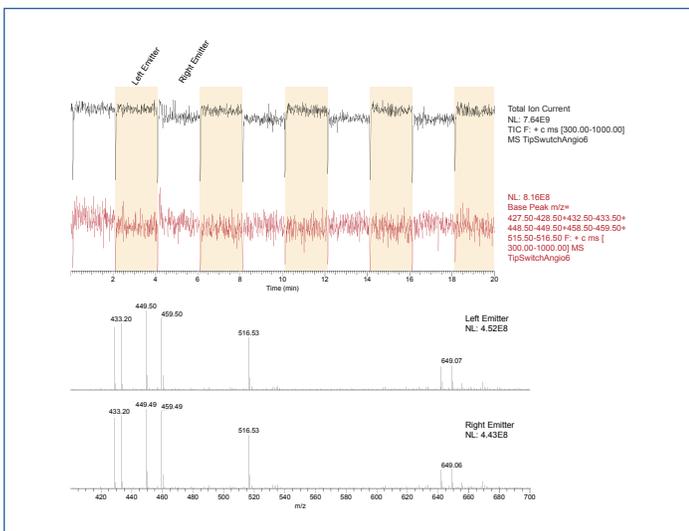


Figure 6 Total and selected ion current for switching between two channels. Each is spraying an identical mixture of five (5) angiotensin peptides (3 μM /peptide, 50% MeOH). The flow rate is 300 nL/min, voltage is 1.8 kV, dwell time for each emitter is 2 min.

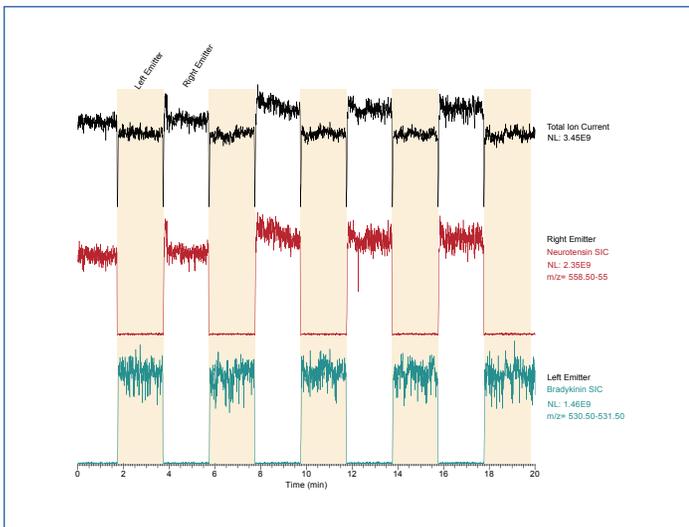


Figure 7 Total and selected ion current for switching between two channels of different sample. The left and right channels are spraying Neurotesin and Bradykinin, respectively, at a 5 μM /peptide (50% MeOH) concentration. The flow rate is 300 nL/min, voltage is 1.8 kV, dwell time for each emitter is 2 min.

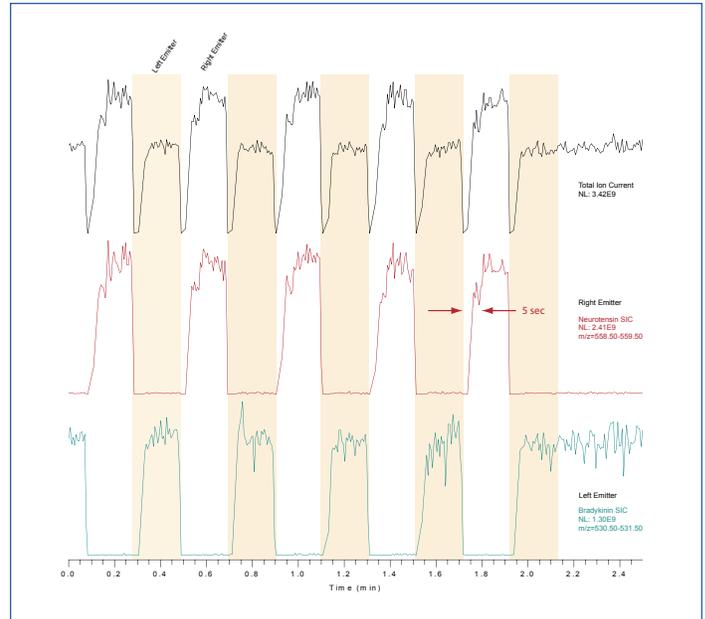


Figure 8 Total and selected ion current between two channels (same conditions as in Figure 7) with the dwell time reduced to 12 seconds. Note the approximate 5-sec. total dead-time to establish the new emitter position and stabilize spray.

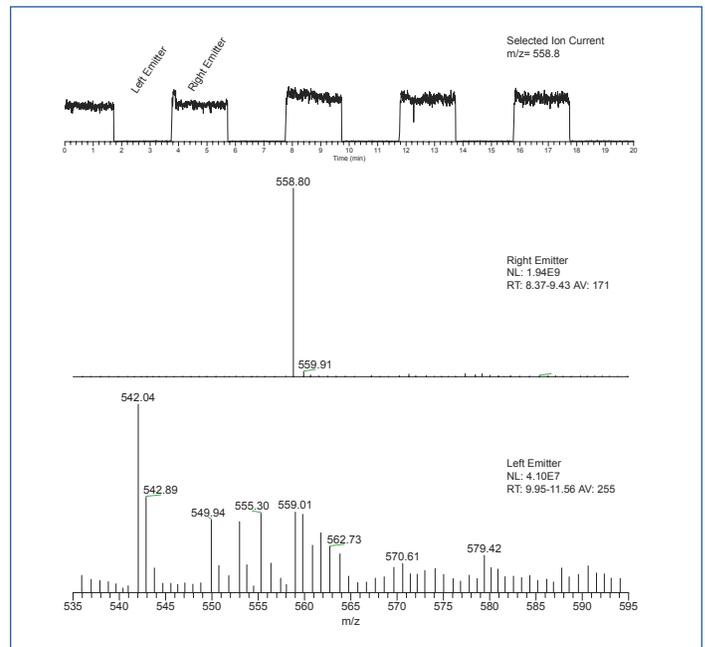


Figure 9 Selected ion current (A) and mass spectra (B, C) for the triply charged Neurotesin ion ($m/z = 538$) for the right emitter from Figure 7. Note the apparent carryover (C) is less than 1%, essentially representing the "memory effect" of the interface.

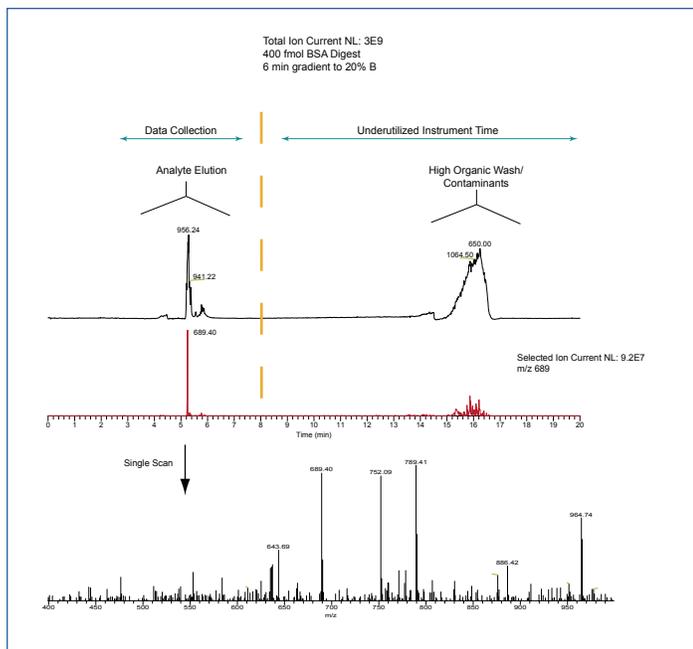


Figure 10 A typical analysis of a protein tryptic digest using fast gradient elution. Here a 6 minute gradient (20% B) is used with a 5cm long C18 PicoFrit column (400 nL/min) on the "left" LC channel. Nearly all peptides of interest elute within the first 8 minutes; the remainder is used to wash and re-condition.

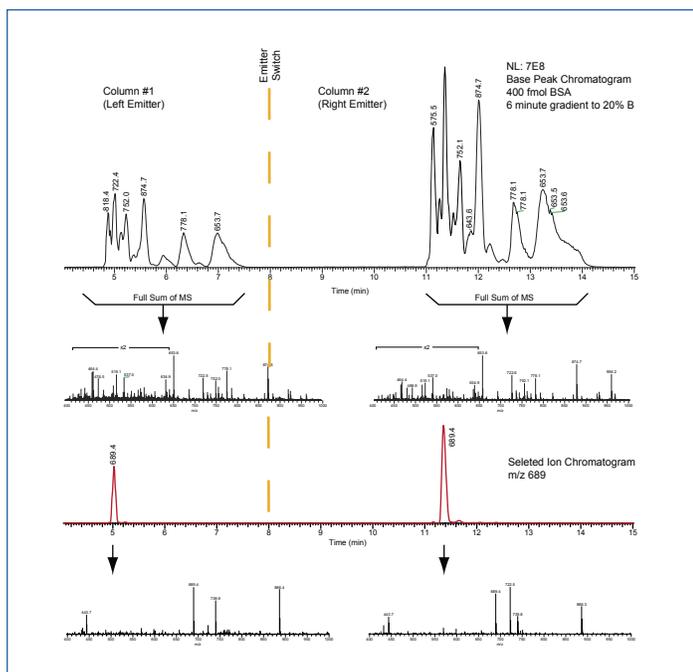


Figure 11 LC-MS acquisition using switched LC-nanospray channels. 400 fmol of BSA digest were injected into each channel at $t=0$. The emitter was switched from the left channel to the right channel at $t=8$ minutes. The right channel has an additional 8 minutes of delay time facilitating the switch. Flow rate = 400 nL/min., $V=2.5$ kV.

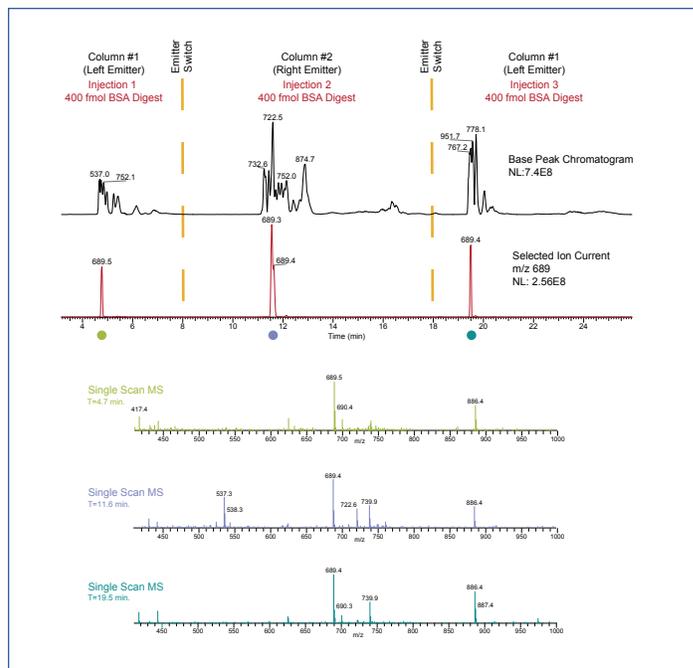


Figure 12 LC-MS acquisition using switched LC in a "left-right-left" mode. The emitter was switched from the left to right channel at $t=8$ minutes and then back to the left channel at $t=18$ minutes. The second injection into the left channel was performed at $t=15$ minutes. Flow rate = 400 nL/min in each channel, $V=2.5$ kV.

Observations & Conclusions

- Switching the high voltage simultaneously with emitter position is a viable method for switched-column nanobore LC-MS
- Carryover is reduced to that inherent to the memory effect of the heated-capillary interface as spray is only possible from a single channel at a time
- Nearly identical analyte-ion current (within 10%) was obtained by fine-tuning each channel's emitter position using continuous infusion
- Channel-switching yielded a dead time of approximately 6 seconds for the stage to locate and the emitter to re-establish spray
- Column-switching can greatly reduce the time lost to column-rinsing and conditioning
- Method development requires careful optimization of conditioning to ensure high reproducibility between column(s) & injections
- The two channels in gradient-LC mode yielded MS signal to better than 50% of each other (as determined by analyte SIC's). Given that each channel possessed different vendor LCs, each with different delay volumes, gradient-mixing schemes, and injection valves, the results are promising.

References

1. Valaskovic, G.A.; Murphy, J.P., III; Lee, M.S. (2004) *J. Am. Soc. Mass Spectrom.*, 15, 1201-1215.

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