TIC-Mediated Flow Rate Variation for Flow-Injection Nanospray Analysis

Carla J. Waggett[1], David Neyer[2], Adam W. Perala[1], Christopher J. Toher[1], Gary A. Valaskovic[1],
[1] New Objective, Inc., Woburn, MA [2] Eksigent Technologies, Inc., Livermore, CA

Introduction

Nanospray typically involves offline operation in static mode (flow rate ≈ 20 nL/min.) or online using nanobore LC. A third mode, microscale flow injection, has received scant attention due to throughput and carryover limitations. The recent development of reliable nL/min. flow generation coupled with mass-directed variable (parked) flow alleviates these experimental deficiencies.

Upon injection, the system operates at a microspray flow rate and automatically decreases to nanospray flow rate when the requisite total ion count (TIC) is detected; upon run completion, high-flow conditions are restored. Post-injection delay time and carryover are minimized by increasing inter-injection wash volume. Signal acquisition at true nanospray flow rates enables extensive characterization by MS/MS for sub-microliter nanoscale injection.

Methods & Materials

Instrumentation & Components

- Ion trap mass spectrometer (LCQ Deca™, Thermo Electron)
- Xcaliber™ software for MS and pump control (Thermo Electron)
- Nanospray source (PicoView® 150, New Objective)
- NanoLC™ pump (Eksigent)
- IntegraFrit™ Sample Trap for back pressure (New Objective)
- Inline NanoFilter Assembly with 1 µm filter capsule (UpChurch)
- SilicaTip™ FS360-20-10-D (New Objective)
- 10-Port nano-valve (Valco)
- Pre-cut fused-silica tubing (New Objective)

Figure 1 *(Left) PicoView® 150 on LCQ Deca™, and (right) IntegraFrit™ Sample Trap and cartridge*

Sample Preparation

- A 10 ng/µL solution was prepared by diluting a commercially available 5.0 µg angiotensin digest with 500 µL of a 50:50 mixture of ACN/HPLC-grade water containing 0.1% formic acid.
- Isocratic chromatography employed a 50:50 mixture of ACN organic modifier and HPLC-grade water. Both solutions contained 0.1% formic acid.
- A 1 µM solution of ubiquitin was prepared by diluting a 10 µM solution with 50% acetonitrile / 0.1% formic acid

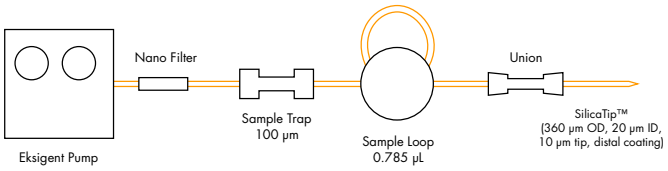

Figure 2 Diagram of the flow injection system

Results

An Eksigent NanoLC™ pump delivered an isocratic 50:50 mixture of ACN/HPLC-grade water containing 0.1% formic acid. Instrumental setup is displayed in Figure 2. Data were acquired using a data-dependant contact closure directing a parked-pump flow rate (ca. 50 nL/min) upon reaching a specific TIC threshold. The pump maintained the low flow rate until the TIC dropped below threshold, restoring high-flow conditions.

The angiotensin mixture was injected into a flow path maintained at 500 nL/min. Sample elution took <2 minutes with a total analysis time of <4 minutes (Figure 3A). When injected into a 50 nL/min. eluent flow, the sample eluted in 12 minutes; a 28-minute run duration resulted from the system swept volume (Figure 3B). In a configuration using TIC-mediated flow rate variation, the sample was injected into an initial flow rate of 500 nL/min. Upon detecting the threshold TIC, the flow rate decreased to 50 nL/min; the sample was then allowed to elute at the lower flow rate. The sample eluted over 14 minutes with a total analysis time of 17 minutes (Figure 3C). The subsequent drop in TIC restored the 500 nL/min. flow rate following sample elution. Example spectra from each run are displayed in Figure 4.

Originally published on June 7 , 2005, at the 54th Annual American Society for Mass Spectrometry Conference in San Antonio, TX, U.S.A.

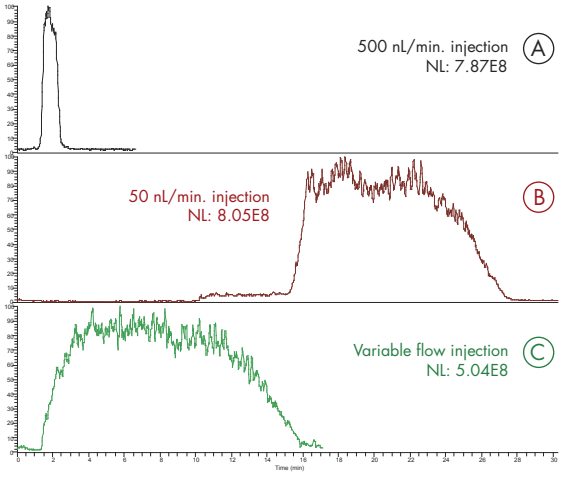

Figure 3 *Flow rate comparison for angiotensin sample: A) Sample retention for 500 nL/min. constant flow rate; B) Sample retention for 50 nL/min. constant flow rate; C) Sample retention for MS signal-mediated variation in flow rate*

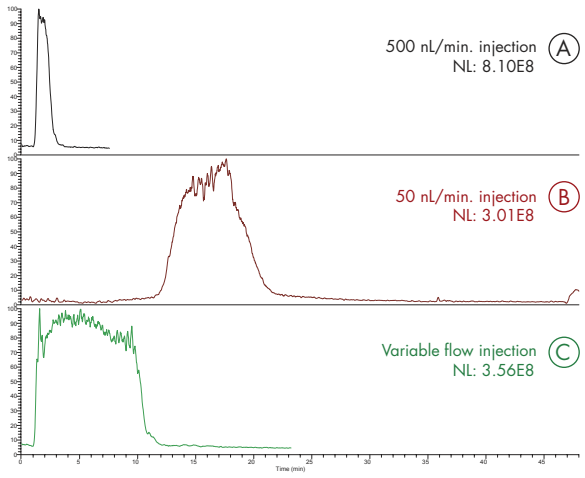

Figure 5 *Carryover comparison for a ubiquitin sample: A) Sample retention for 500 nL/min. constant flow rate; B) Sample retention for 50 nL/min, constant flow rate; C) Sample retention for MS signal-mediated variation in flow rate*

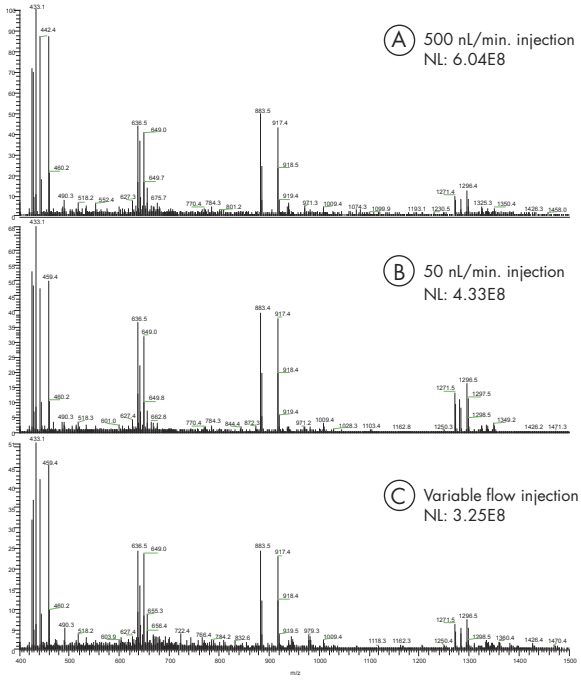

Figure 4 *Flow rate comparison for angiotensin sample: A) Spectra for 500 nL/min. constant flow rate; B) Spectra for 50nL/min. constant flow rate; C) Sample retention for MS signal-mediated variation in flow rate*

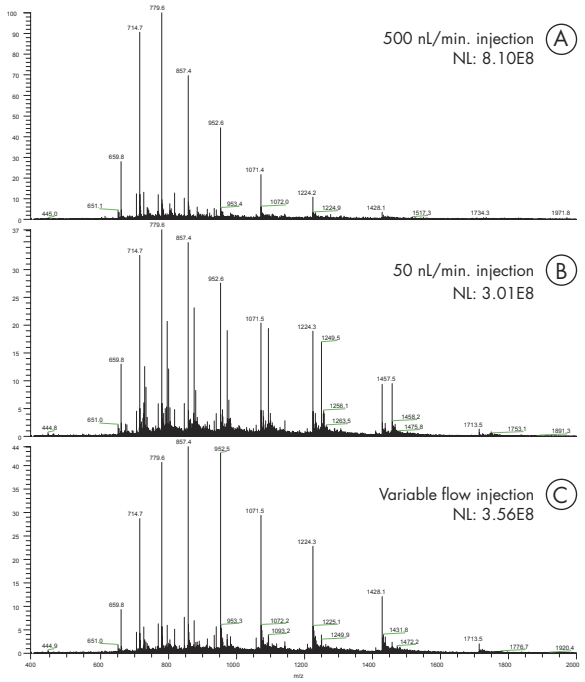

Figure 6 *Carryover comparison for a ubiquitin sample: A) Spectra for 500 nL/min. constant flow rate; B) Spectra for 50 nL/min, constant flow rate; C) Spectra for MS signal-mediated variation in flow rate*

Results (cont'd)

Ubiquitin was employed to assess carryover and turnaround time at the three flow rates. When the 1 µM solution was injected into a controlled 500 nL/min. flow path, the sample eluted in <2 minutes; after elution, an additional 3 minutes elapsed before the system was ready for another injection (Figure 5A.) Injection into a 50 nL/min. eluent flow increased run time by 26 minutes before the next injection was possible; a total run time of 50 minutes resulted (Figure 5B). Using TIC-mediated flow, an additional 3 minutes was needed after higher flow rate was restored, yielding a total run time of 16 minutes (Figure 5C). Example spectra from each run are displayed in Figure 6.

Conclusions

- A 4-minute analysis duration resulted from a 500 nL/min. controlled flow rate using angiotensin
- A 28-minute analysis duration resulted from a 50 nL/min. controlled flow rate using angiotensin
- A 17-minute analysis duration was observed for a TIC-mediated variable flow rate using angiotensin
- Pre-injection time was reduced from 15 minutes to <2 minutes
- Turnaround time for carryover was reduced from 26 minutes to 3 minutes using ubiquitin
- A TIC-mediated variable flow configuration facilitated sample analysis and subsequent MS/MS evaluation

Acknowledgements
The authors graciously acknowledge Eksigent Technologies for use of the NanoLC pumping system.