**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)

**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

**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-dependent 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.
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

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