

Fundamental Studies on the Application of Nanobore LC-MS for the Analysis of Small Drug Molecules

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Introduction

The success of nanoscale formats for LC-MS has been driven primarily by the need to analyze low-concentration samples and/or samples where quantities are limited, yet the need for higher sensitivity and higher throughput continues to grow. Miniaturization of the ESI LC-MS format has become the standard for proteomic analysis. This format provides a powerful approach to manipulate and deliver small quantities of sample with significant improvements in sensitivity. Here we demonstrate the novel application of nanobore LC-MS to the analysis of a spiked standard in plasma using a protein precipitation sample preparation protocol. Variable-flow LC-MS “peak parking” is applied to improve nanospray response without negatively impacting analysis time.

Methods

Protein Precipitation Sample Preparation Protocol:

1. Aliquot 100 μ l plasma spike (10 ng/ml) into Eppendorf[®] tube
2. Add 400 μ L of ACN
3. Vortex
4. Spin at 14,000 rpm for 10 min
5. Transfer 400 μ L into Eppendorf tube
6. Dry down
7. Reconstitute in 150 μ L of solvent (95:5, ACN:Water)
8. Spin at 14,000 rpm for 10 min
9. Injection volume: 1 μ L

MS: Thermo LCQ Deca[™] with New Objective PicoView[®] source
Full scan: 300 – 1500 m/z, μ Scans: 3

LC: Eksigent NanoLC

Mobile Phase A: Water, 5% ACN with 0.1% Formic Acid
Mobile Phase B: 95% ACN with 0.1% Formic Acid

Gradient: Hold at 10% B for 0.5 min; ramp to 90% B over 3.5 min; return to 10% B over 0.5 min and hold for 8.5 min

Flow rate: 250 nL/min

Peak Parking Flow rate: 150, 50 nL/min

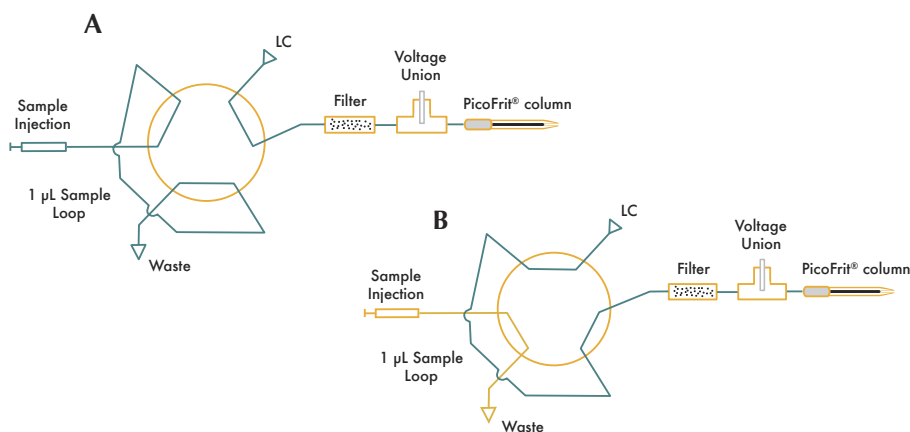
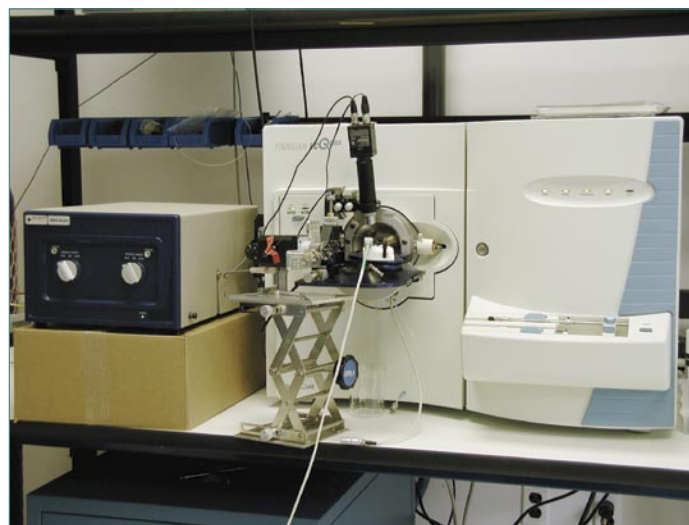
Peak Parking event: triggered manually at 7 min; released at 9 min

Column:

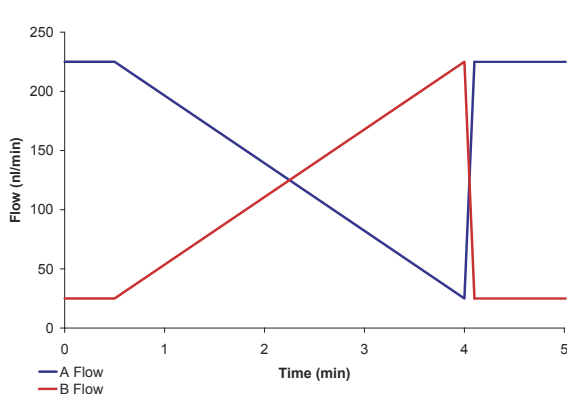
PicoFrit[®] column: 2.5 cm x 75 μ m with a 15 μ m tip
2.5 cm packed bed of Waters Symmetry[™] C18, 3.5 μ m

System Configuration

The experimental apparatus consisted of an Eksigent gradient NanoLC delivering mobile phase to a Valco injection valve and New Objective PicoView® nanospray source on a Thermo Finnigan™ LCQ Deca™ ion trap mass spectrometer.

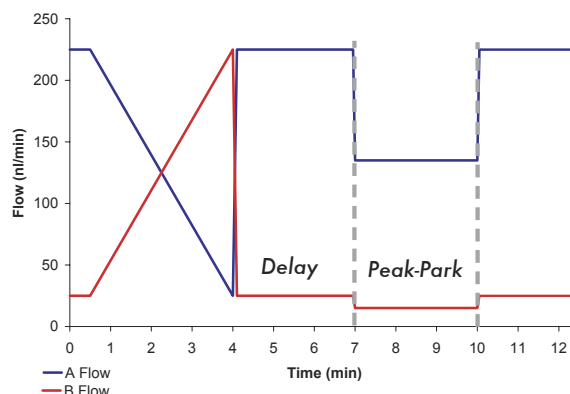


A standard 6-port injection scheme (1 µL volume) was used with in-line filtration (1 µm porosity) to preserve column integrity. ESI high voltage was applied pre-column directly to the mobile phase through a platinum wire micro-electrode. Pre-column volume was kept to a minimum ($\leq 1 \mu\text{L}$) to minimize the gradient delay.



Gradient

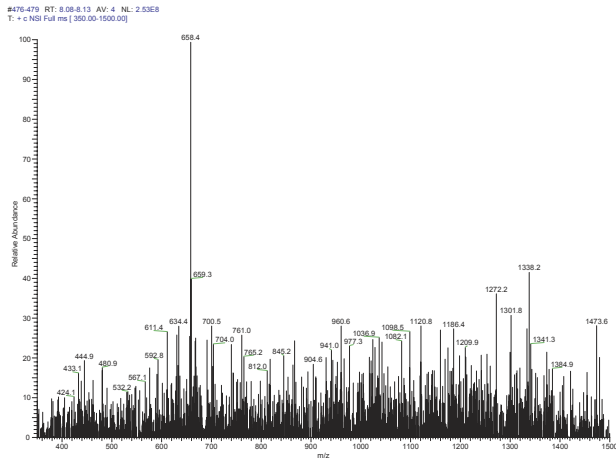
The conventional gradient at 250 nL/min went from 10% B to 90% B over a 3.5 minute period of time. The gradient delay between pump and column was approximately 3 minutes.



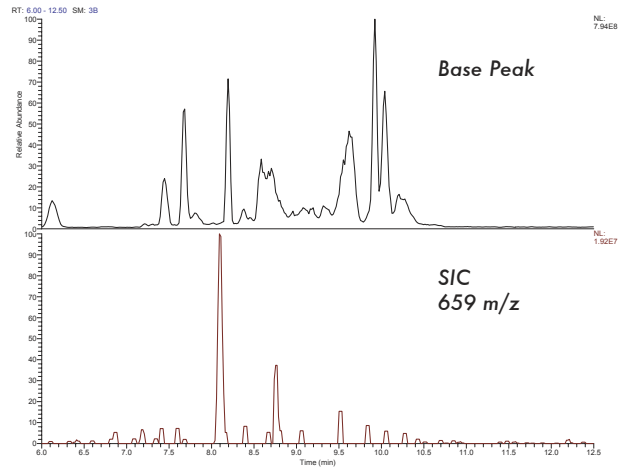
Peak Parking Gradient

The peak-parking gradient used an initial flow rate of 250 nL/min lowering to 50 or 150 nL/min between 7 and 10 minutes. The low-flow window was chosen to correlate with the elution of the target compound. The offset is generated by the gradient delay.

Gradient LC-MS

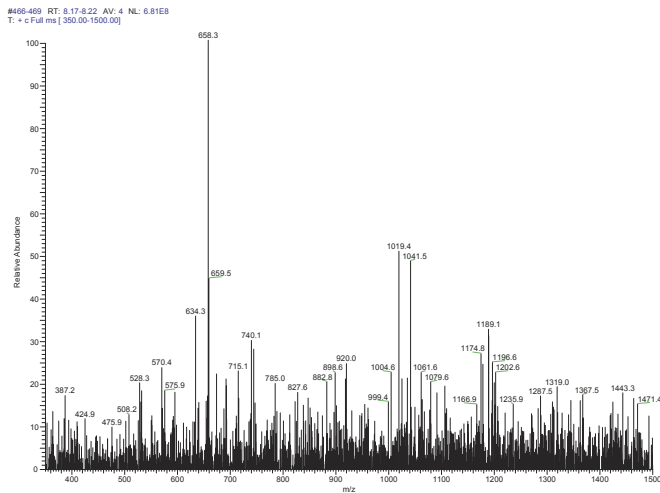


Full-scan MS
Average 9 scans
w/ background subtraction

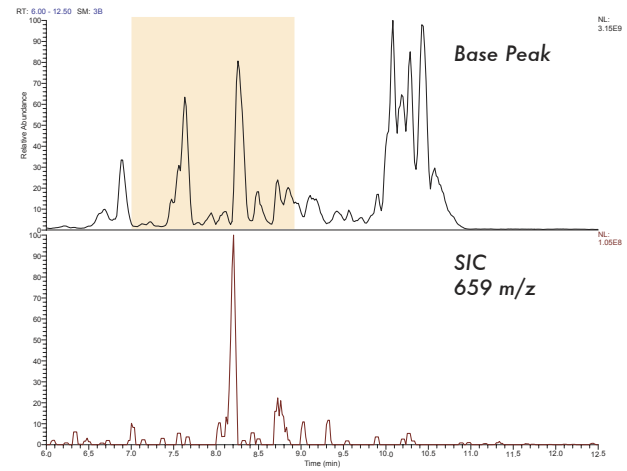


Normal gradient
250 nL/min
1 μ L injection
10 pg on-column

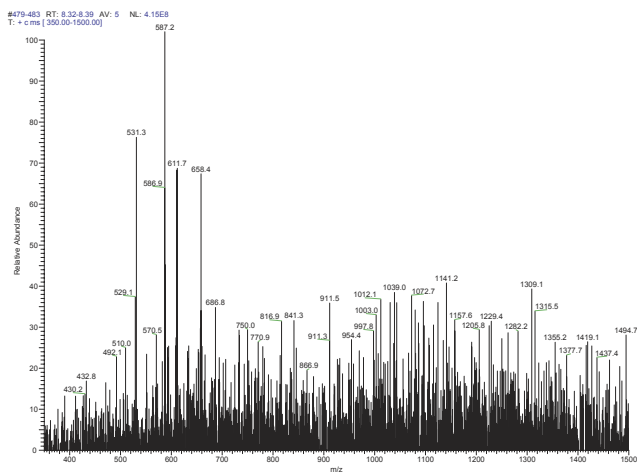
Nano-Flow Peak Parking



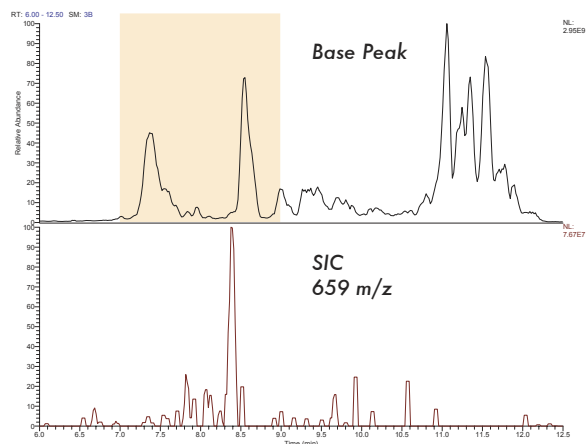
Full-scan MS
Average 10 scans
w/ background subtraction



Peak parking gradient
250 nL/min to 150 nL/min
1 μ L injection
10 pg on-column



Full-scan MS
Average 10 scans
w/ background subtraction



Peak parking gradient
250 nL/min to 50 nL/min
1 μ L injection
10 μ g on-column

Conclusions

- Using fast gradient elution with nanobore LC-MS enables operation on a chromatographic time scale compatible with traditional small molecule LC-MS
- A finite amount of delay in gradient delivery can be used advantageously to establish a peak-parking “window” after the gradient has been formed by the pump
- Qualitatively, one can go to very low flow rates using variable flow LC methods
- Peak parking with a window format can be used to lower the flow rate to “true” nanospray flow rates without dramatically increasing run time
- Nanobore LC-MS consumes approximately 40 fold less sample when compared to a traditional narrow bore (mm) column format
- Typically there is no loss, and in some cases an increase, in analyte ion intensity as the flow rate is reduced to 50 nL/min

Future Work

- Determine the impact of nanospray flow rates on matrix effects such as ion suppression
- Determine the “robustness” factor for different sample prep methodologies
- Establish performance criteria for quantitative analytical methods

Acknowledgements

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