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

MS: Thermo LCQ Deca™with New Objective PicoView® source

Full scan: 300 – 1500 m/z, µScans: 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

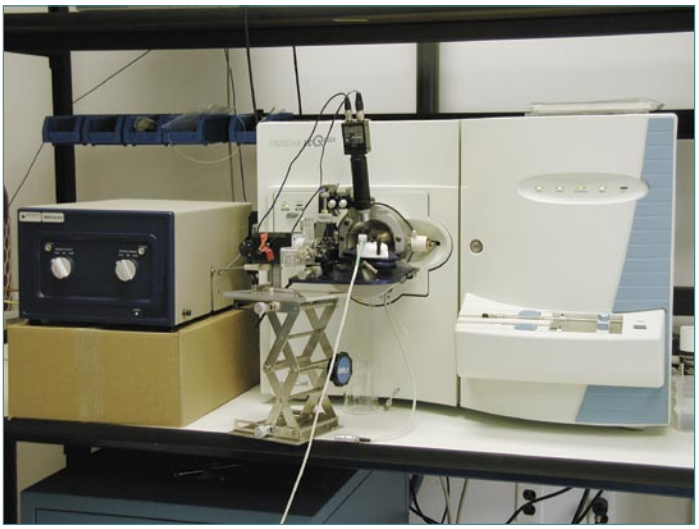
Flow rate: 250 nL/min
Peak Parking Flow rate: 150, 50 nL/min
Peak Parking event: triggered manually at 7 min; released at 9 min

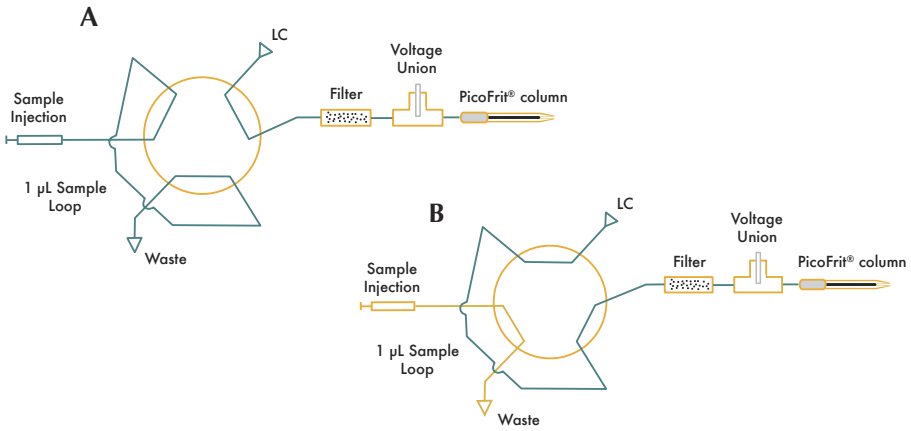
Column:

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

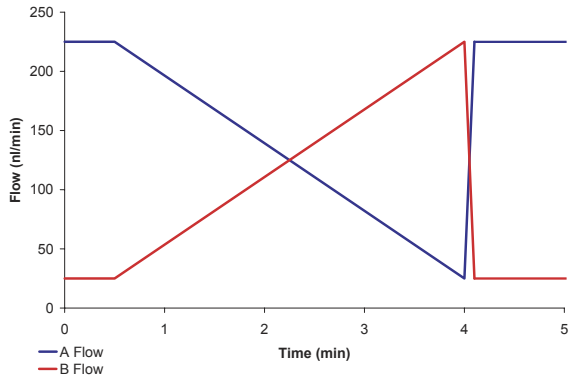
System Configuration

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.



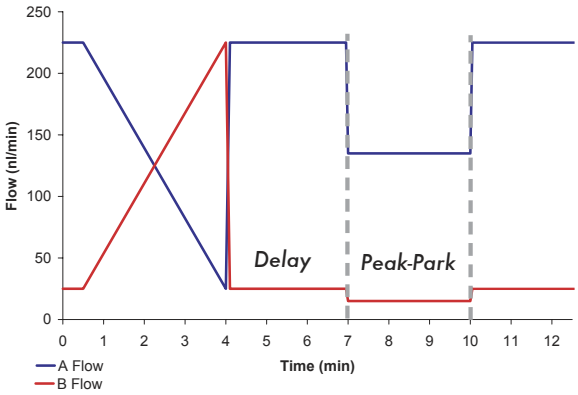


A standard 6-port injection scheme (1 µL volume) was used with in-line filtration (1 µm porosity) to preserve column integrity. ESI high voltage was applied pre-column directly to the mobile phase through a platinum wire micro-electrode. Pre-column volume was kept to a minimum (≤ 1 µL) to minimize the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



Gradient

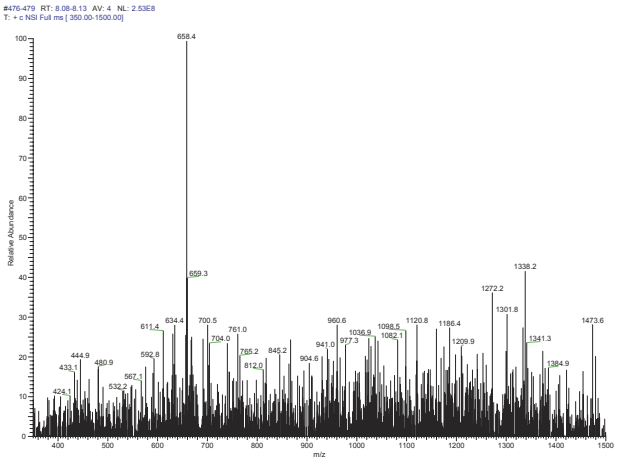
The conventional gradient at 250 nL/min went from 10% B to 90% B over a 3.5 minute period of time. The gradient delay between pump and column was approximately 3 minutes.



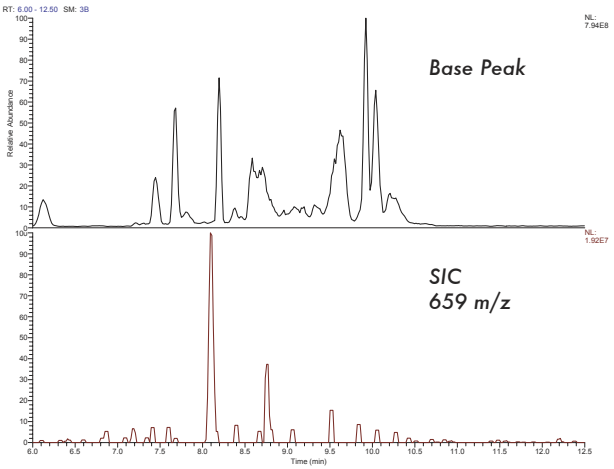
Peak Parking Gradient

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 chose to correlate with the elution of the target compound. The offset is generated by the gradient delay.

Gradient LC-MS

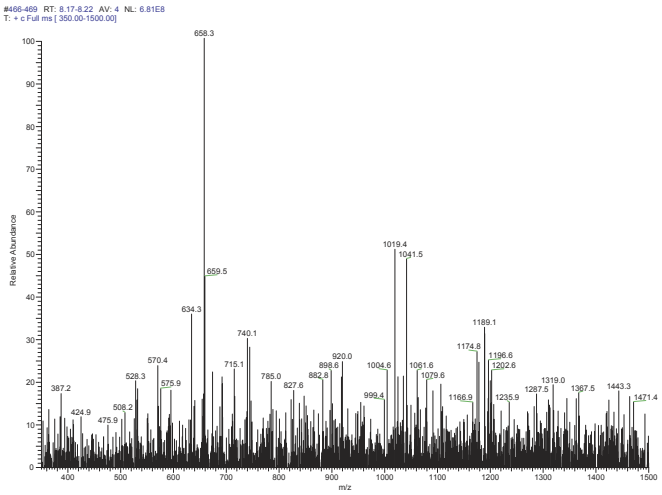


Full-scan MS
Average 9 scans
w/ background subtraction



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

Nano-Flow Peak Parking



Full-scan MS
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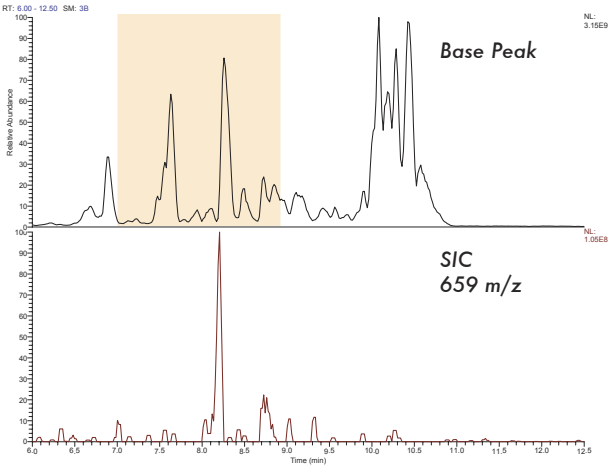
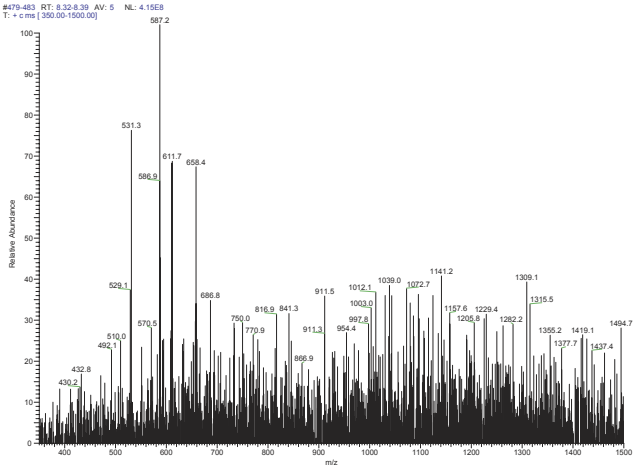
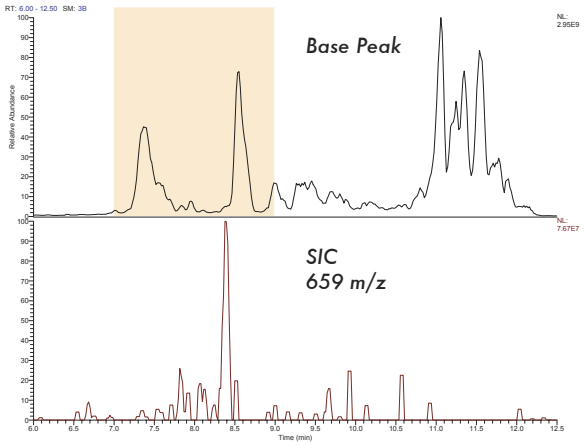
w/ background subtraction



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

Conclusions

- Using fast gradient elution with nanobore LC-MS enables operation on a chromatographic time scale compatable 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

Acknowlegements
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