Optimization of Novel Fused-Core Silica Particle Nanobore Columns using a Pump Switching Motif for Mass Spectrometry

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Introduction

Nanobore LC-MS/MS has played a key role in protein identification and qualitative biomarker discovery. The recent emphasis on quantitative biomarker analysis and validation places different analytical demands on system performance such as selectivity, sensitivity, duty cycle, and ruggedness. The long gradients (> 30 min) used in traditional biomarker discovery are of limited application in repetitive quantitative analysis. Here we investigate the use of a novel superficially porous reverse phase media to enable faster chromatography separations. In combination with a novel pump-switching scheme to eliminate gradient delay, a sensitive nanobore LC-MS/MS assay is demonstrated with a cycle time of less than 10 minutes.

The compatibility of HALO Fused-Core silica particles with packed-emitter PicoFrit nanobore LC using a dual channel pump switching system is demonstrated. HALO particles consisting of a 0.5 um porous layer fused to a 1.7um solid core with a C18 bonded phase were packed into 75 µm ID PicoFrit columns having an integral 15 µm nanospray emitter. Decreased axial diffusion (porous layer limited) enables fast separations.

The pump-switching motif uses two pumps set to different isocratic conditions to achieve rapid gradient formation in-situ. The first pump, referred to as the loading channel pump, delivers a low strength mobile phase through the autosampler and valve mounted trapping column at a relatively high flow rate (1-5 uL/min). After the sample is loaded onto the trap column, a valve switches the flow to the trap column from a second pump, referred to as the elution channel pump. The elution channel pump delivers a relatively high strength mobile phase to the trap and analytical column at a lower flow rate (100 –500 nL/min). As the mobile phase transits from the switching valve to the trap column, a very steep gradient forms at the interface between the low and high strength mobile phases. The gradient is the result of both diffusion and any mixing that might occur elsewhere (e.g., valve ports). For the system used here, a gradient time of less than 30 seconds is suspected and chromatographic peak widths (half-height) on the order of 0.5 seconds are achieved.

Materials & Methods:

Instrumentation:

- Eksigent NanoLC –2D
  - Loading channel: (high flow)
    - o 2% ACN, 0.1% Formic Acid
    - o Isocratic 1-5 µL/min, 2.5 µL/min typical
  - Elution channel: (low flow)
    - o A=0.1% formic acid; B=0.1% formic acid in ACN
    - o Gradient elution: 25 min linear gradient, 2% to 50% B
    - o Pump-switched elution: isocratic 50% B
    - o 500 nL/min
- Leap HTC autosampler
  - 1 µL full loop injection with 6-port nanovalve (Valco),
- Thermo LCQ Deca 3-d Ion Trap
  - Full scan MS: 400-1500 m/z, 3 microscans/spectra
  - MS/MS: 433 m/z parent ion, fragment ions 500-800 m/z
  - Accelerated duty cycle MS (12/sec): 575-590 m/z, 1 microscan/spectra
- Digital PicoView nanospray source (New Objective) model DPV-150
  - Integral 10-port pump-switching micro valve (Valco Inc.)

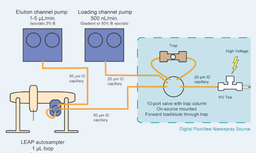
Columns:

- IntegraFrit trap column: 100 um ID x 25 mm (New Objective, Inc.)
  - 5 µm Aquasil C18 (ThermoFischer)
  - 2.7 µm HALO C18 (MAC-MOD Analytical)
- PicoFrit analytical column: 75 um x 100 mm; 15 um integral emitter (New Objective, Inc.)
  - 2.7 µm HALO C18 (MAC-MOD Analytical)

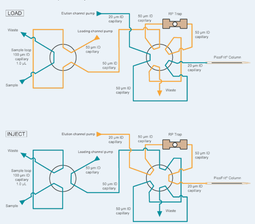
Samples:

- Waters Massprep BSA digest standard
  - Diluted to 300 fmol/µL, 30 fmol/µL (0.1% formic Acid)
- Human angiotensin I standard (Sigma-Aldrich)
  - Diluted (as stated in figures) in 0.1% formic acid
  - 1296 FW (433 m/z triply charged ion most abundant)
- Blank (0.1% Formic acid)

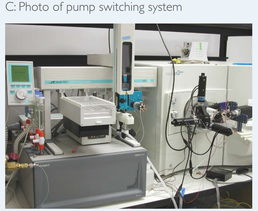
A: Overview of the LC-MS/MS system with novel pump switching scheme.



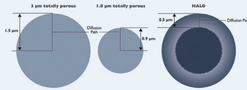
B: Details of the novel pump switching scheme showing valve positions during sample load and sample inject conditions.



C: Photo of pump switching system



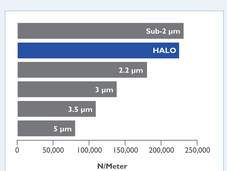
HALO Particles have short diffusion path



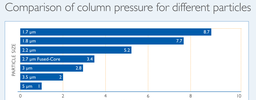
The shorter diffusion path of HALO particles reduces axial dispersion and minimizes peak broadening. This is particularly advantageous for chromatographic separations of molecules larger than ~400 DA and for high speed separations using higher mobile phase flow velocities.

Columns packed with HALO particles deliver high efficiency (separating power) at modest back pressure.

Comparison of column efficiency for different particles

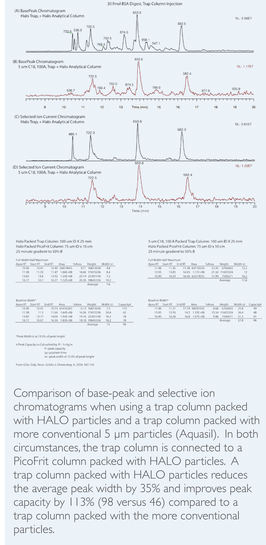


Comparison of column pressure for different particles



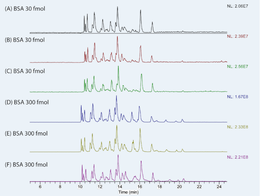
Columns packed with HALO particles deliver separation efficiency equal to columns packed with sub-2 µm particles, but at much more modest back pressure, permitting them to be easily used with conventional LC pumps.

BSA digest (30 fmol) injected on-column.



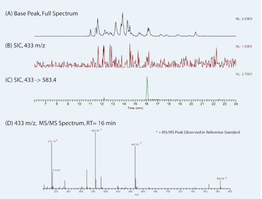
Comparison of base-peak and selective ion chromatograms when using a trap column packed with HALO particles and a trap column packed with more conventional 5 µm particles (Aquasil). In both circumstances, the trap column is connected to a PicoFrit column packed with HALO particles. A trap column packed with HALO particles reduces the average peak width by 35% and improves peak capacity by 11% (98 versus 46) compared to a trap column packed with the more conventional particles.

Injection Repeatability



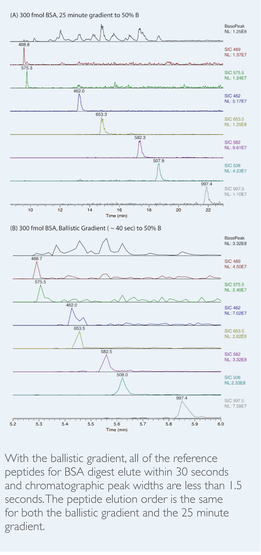
Chromatograms for the HALO packed trap/analytical column combination for 30 fmol and 300 fmol injections of BSA digest to show injection repeatability. A 25 min linear gradient from 2%B to 50% B was used. Three blank injections (not shown) were performed between the 30 and 300 fmol runs.

BSA digest (300 fmol/uL) spiked with Human angiotensin I peptide (MW=1,296 Da) at a level of 5 fmol/µL.



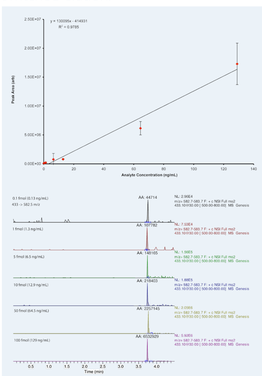
A full scan MS/MS (500-800 m/z) at retention time 16 min confirms the three most abundant angiotensin I product ions.

BSA digest: conventional versus ballistic gradient



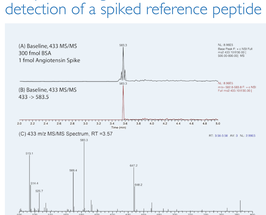
With the ballistic gradient, all of the reference peptides for BSA digest elute within 30 seconds and chromatographic peak widths are less than 1.5 seconds. The peptide elution order is the same for both the ballistic gradient and the 25 minute gradient.

Determination of MS/MS response to analyte concentration for pump switched elution.



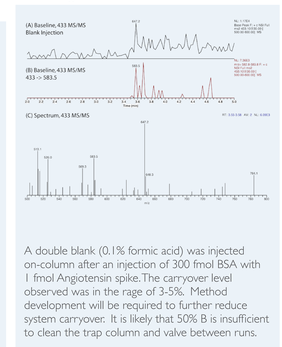
This plot of average peak area (n=3 for each concentration) vs. analyte concentration (ng/mL) for Human angiotensin I shows a reasonably good linear fit ($R^2 = 0.97$). The standards were prepared neat in 0.1% formic acid.

Pump switching elution for sensitive detection of a spiked reference peptide



A full scan MS/MS (500-800 m/z) at retention time 3.57 min confirms the three most abundant angiotensin I product ions.

Sample carry over observed with pump switching elution



A double blank (0.1% formic acid) was injected on-column after an injection of 300 fmol BSA with 1 fmol Angiotensin spike. The carryover level observed was in the rage of 3-5%. Method development will be required to further reduce system carryover. It is likely that 50% B is insufficient to clean the trap column and valve between runs.

Conclusions:

- 2.7 µm diameter HALO Fused-Core particles are suitable for use in a nanobore (100 µm ID x 25 mm) trap column format.
- A loading flow rate of 2.5 uL/min onto the trap required only modest system pressure (130 bar)
- Best chromatographic performance for the HALO packed PicoFrit column was obtained with a HALO packed trap column.
- Pump-switching system substantially decreased gradient delay.
- Pump-switched elution enabled fast run times. Nanobore LC run times of less than 10 minutes were readily obtained.
- Pump-switched elution results in chromatographic peaks of 0.5 sec FWHM. Fast duty cycle detection is required.
- Seven standard BSA digest peptides were observed to elute in less than 30 seconds using the pump switching system and followed the same elution order as a standard 25 minute linear gradient.
- Peaks for analytical standards (no matrix) followed a linear trend with analyte concentration over 3 orders of magnitude.
- A peptide standard spiked into a protein digest was readily detectable at a concentration of 1.2 ng/mL using MS/MS detection.