Minimizing Post-Column Band Broadening in Nanobore LC-MS/MS for Bottom-up Proteomic Applications

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Introduction

Peptide based proteomic analysis critically depends on the separation performance of nanobore chromatography for high sensitivity, typically using column diameters of 75 µm ID or less. For single dimension separations of simple mixtures, figures of merit such as chromatographic peak width, height, and tailing factors directly impact fundamental analytical results of sequence coverage, the number of protein "hits", and quality of protein identification. This is even more the case for multi-dimensional separations, where a poor separation in the first dimension can obscure detection of low abundance peptides in the second dimension. Here we investigate different combinations of nanobore column, MS coupling methodology, and nanospray emitter designs to optimize separation factors for separations in peptide based proteomics.

Methods

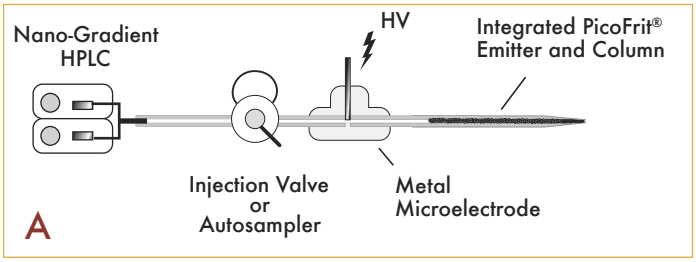
Preparation of Peptide Digests:
A mixture of Sigma peptides Angiotensin I (A-9650, MW 1296.5), Angiotensin II (A-9525, MW 1046.2), Neurotensin (N-6383, MW 1672.9), Bradykinin (B-3259, MW 1060.2), and Oxytocin (O-9679, MW 1007) was prepared by dissolving each peptide in 0.1% formic acid. Appropriate amounts of each solution were combined for a final concentration of 50 fmol/µL of each peptide.

NanoLC-ESI Ion Trap MS:
A FinniganTM Surveyor® HPLC (Thermo Electron, San Jose, CA) was employed with nanoflow delivery system in front of either a 75 µm ID x 10 cm C18 PicoFrit® with a 15 µm ID pulled tip (New Objective, Woburn, MA) on the Finnigan nanospray source or a 75 µm ID x 10 cm BioBasic® C18 capillary column (Thermo Electron, Bellefonte, PA) on a newly designed nanospray source incorporating grounding and post-column HV junction and employing a 5 cm long pre-cut spray tip with 30 µm ID pulled tip (New Objective, Woburn, MA). 2 µL of a peptide mixure was manually injected with a 2 µL loop in the injection valve. The peptides were eluted with a with a linear gradient of 0-75% B over 30 minutes at a flow rate of 250 nL/min (A = 0.1% formic acid in water, B = 0.1% formic acid in acetonitrile). Eluting peptides were analyzed by a Finnigan LCQ Deca XP Plus™ ion trap mass spectrometer (Thermo Electron). The mass spectrometer was operated in full scan mode.

Different ID post-column tip connections were studied on a second LC-MS system comprised of a NanoLC (Eksigent Technologies, Livermore, CA) delivering mobile phase to a PicoView® nanospray source (New Objective). Nanobore IntegraFrit™ columns (BioBasic C18, 75 µm ID x 10 cm, New Objective) were directly connected to 20, 75, and 100 µm ID fused-silica emitters with tip ID's ranging from 10 to 15 µm. High voltage was applied via a platinum microelectrode on the high-pressure side of the column. Full scan data acquisition was performed on a Finnigan LCQ Deca™ ion trap mass spectrometer (Thermo Electron).

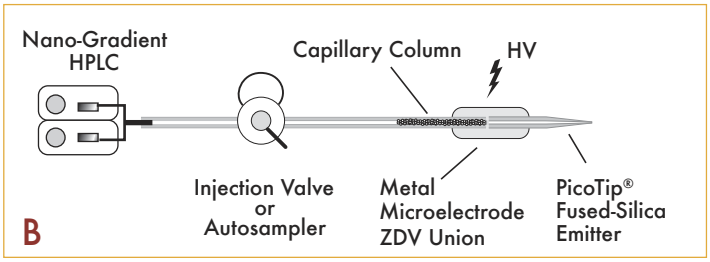
Data Analysis:
For studying the peptide peak characteristics, peak smoothing was done using a 7 point Gaussian algorithm with the ICIS™ algorithm for peak detection. The peak width (FWHM) at half maximum and peak assymetry were determined manually.

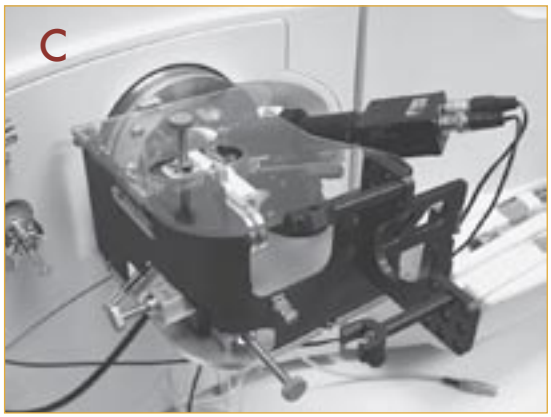
Apparatus



(A) Experimental set-up for the use of integrated emitter-column format (PicoFrit®); HV is applied through a noble metal electrode directly to the mobile phase, pre-column

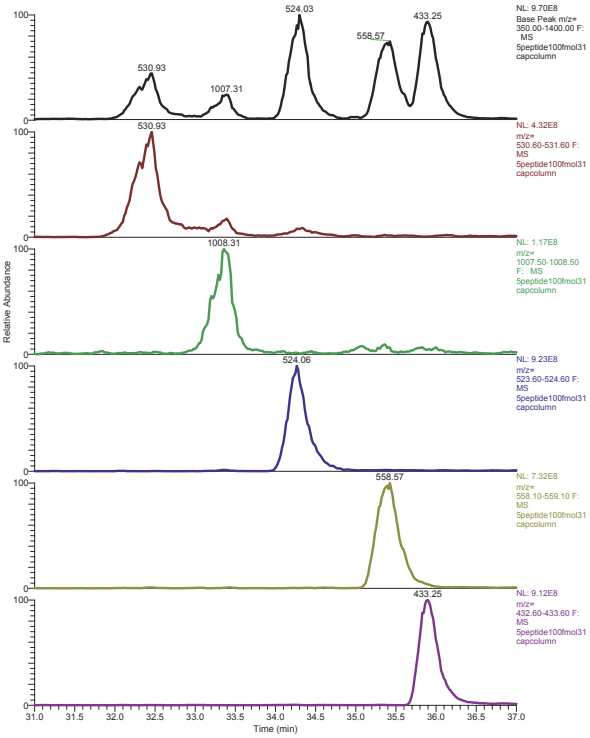
(B) Experimental set-up for capillary/nanobore column with butt-connected fused-silica emitter; HV connection is post-column



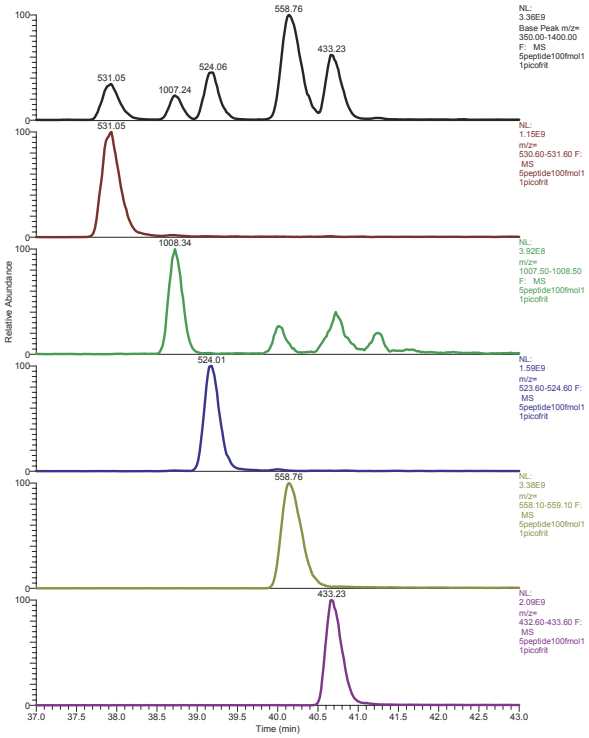


(C) Photo of PicoView® 150 nanospray source on LCQ Deca™ (D) Photo of Thermo Finnigan™ nanospray source with integrated grounding and HV junction used for capillary column experiments on the LCQ Deca XP Plus™

Results: Capillary & PF Column



Capillary Column: 75 µm x 10 cm with a 30 µm tip made from 75 µm tubing, with BioBasic® C18, 5 µm, 300 Å pore



PicoFrit® Column: 75 µm x 10 cm x 15 µm tip with BioBasic® C18, 5 µm, 300 Å pore

PicoFrit® Column: 75 µm x 10 cm

Run #	1	2	3	4	5	6	7	8		
	50% Peak Width [min]	50% Peak Width [min]	50% Peak Width [min]	50% Peak Width [min]	50% Peak Width [min]	50% Peak Width [min]	50% Peak Width [min]	50% Peak Width [min]	Peak Height (AVE)	C.V.
Peak 1	0.217	0.246	0.220	0.218	0.284	0.219	0.233	0.235	1.3877E+09	11.81%
Peak 2	0.152	0.184	0.154	0.188	0.186	0.169	0.185	0.234	6.5404E+08	19.51%
Peak 3	0.181	0.198	0.202	0.269	0.235	0.262	0.234	0.250	1.5867E+09	11.48%
Peak 4	0.263	0.264	0.268	0.305	0.384	0.331	0.326	0.298	3.5034E+09	18.22%
Peak 5	0.215	0.197	0.200	0.270	0.251	0.265	0.261	0.268	2.4276E+09	11.25%
AVG [sec]	12.328	13.064	12.520	15.012	16.086	14.954	14.866	15.418	1.9119E+09	14.45%
	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio		
Peak 1	1.78	2.23	1.76	1.36	2.10	3.60	1.99	2.71		
Peak 2	1.36	1.22	1.01	1.87	1.20	1.47	1.00	1.09		
Peak 3	1.30	1.55	1.45	1.70	2.25	1.42	1.60	1.65		
Peak 4	1.65	1.79	2.00	1.92	3.73	2.18	1.85	2.08		
Peak 5	1.57	1.44	1.87	1.64	2.00	1.74	2.43	1.54		
AVG	1.53	1.65	1.62	1.70	2.25	2.08	1.77	1.81		

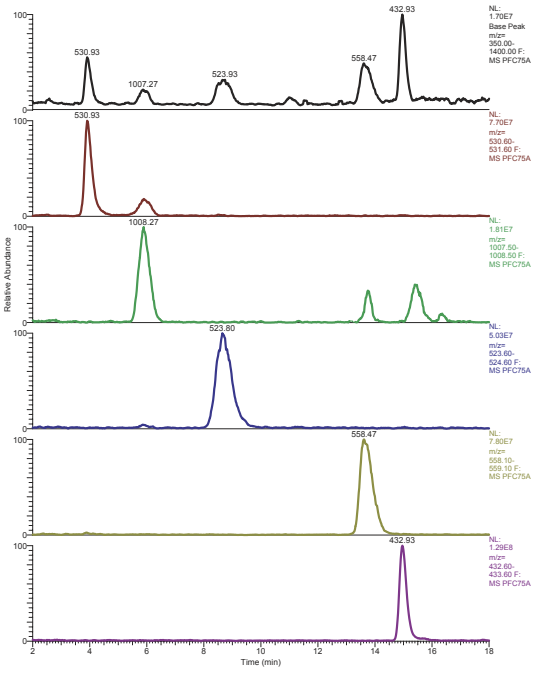
Capillary Column: 75 µm x 10 cm

Run #	1	2	3	4	5		
	50% Peak Width	50% Peak Width	50% Peak Width	50% Peak Width	50% Peak Width	Peak Height (AVE)	C.V.
Peak 1	0.260	0.389	0.366	0.291	0.290	3.05E+08	46.7%
Peak 2	0.226	0.280	0.262	0.242	0.241	2.14E+08	43.3%
Peak 3	0.173	0.258	0.202	0.255	0.250	6.75E+08	40.1%
Peak 4	0.176	0.204	0.390	0.291	0.271	4.49E+08	36.8%
Peak 5	0.209	0.289	0.238	0.204	0.235	6.26E+08	41.1%
AVG [sec]	12.536	17.038	17.502	15.402	15.444	4.54E+08	41.6%
	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio	Symmetry Ratio		
Peak 1	1.37	1.75	1.44	2.55	0.76		
Peak 2	1.40	1.35	2.00	2.74	0.68		
Peak 3	1.55	1.97	1.86	1.34	0.84		
Peak 4	1.39	1.35	2.07	1.25	1.21		
Peak 5	1.27	1.29	2.38	1.40	1.83		
AVG	1.40	1.54	1.95	1.86	1.07		

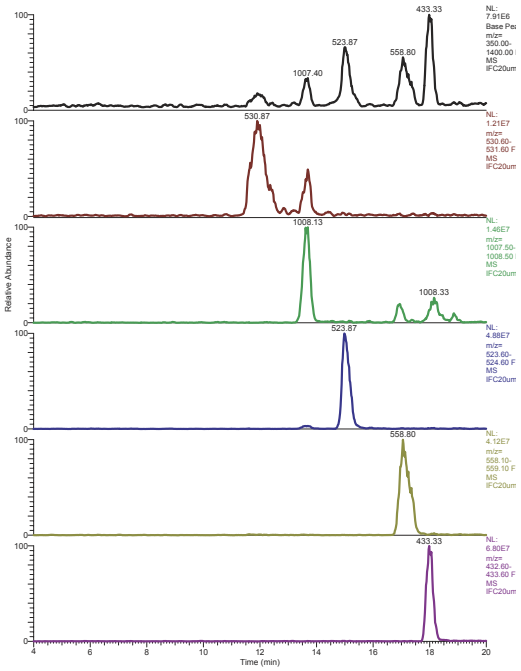
	Peak Height	C.V.	Peak Width	Symmetry Ratio
PicoFrit® BioBasic® C18 75 µm x 10 cm	1.919E+09	14.45%	14.3	1.802
Capillary BioBasic® C18 75 µm x 10 cm	4.54E+08	41.60%	15.6	1.561

Peak height, coefficient of variation, width at half maximum and symmetry data (at 10%) for repetitive injections performed with each column configuration. The summary at the bottom is an average of data accross all five peaks. The improved C.V. for the integral emitter-column PicoFrit® format is believed to be generated by spray stability provided by the smaller tip diameter. Instrument: LCQ Deca XP Plus™, 100 fmol/peptide injection, 250 nL/min, gradient eultion

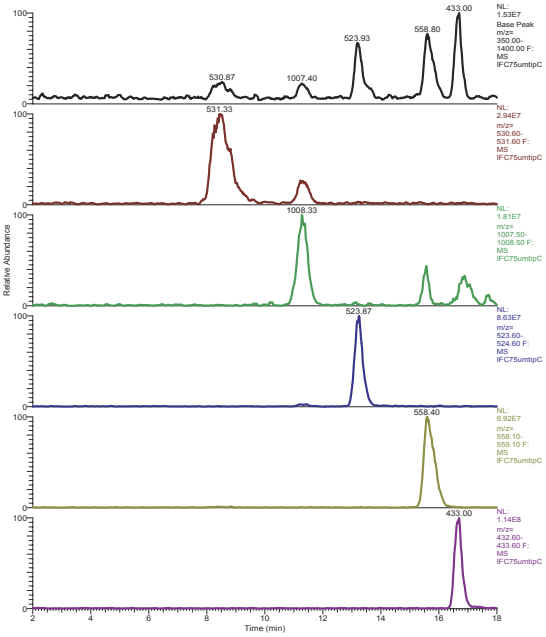
Results: IF Column & Tip Size



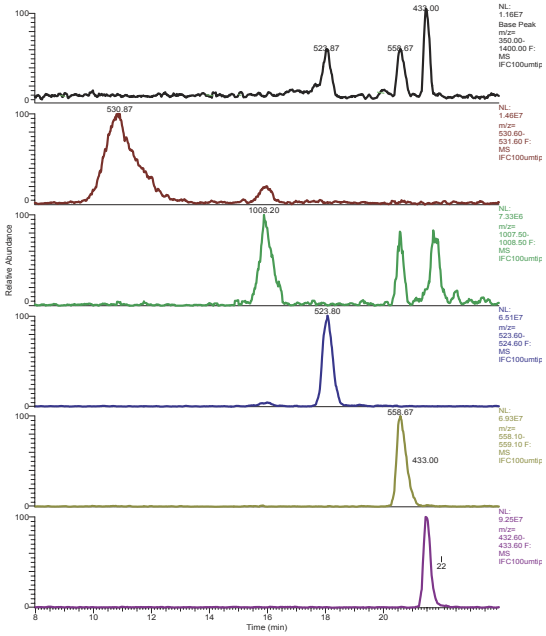
PicoFrit® Column: 75 µm x 10 cm
15 µm tip



IntegraFrit™ Column: 75 µm x 10 cm
10 µm tip x 20 µm ID tubing



IntegraFri™ Column: 75 µm x 10 cm
15 µm tip x 75 µm ID tubing

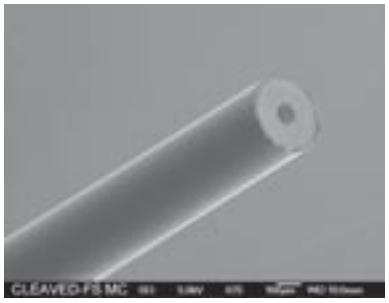


IntegraFrit™ Column: 75 µm x 10 cm
15 µm tip x 100 µm ID tubing

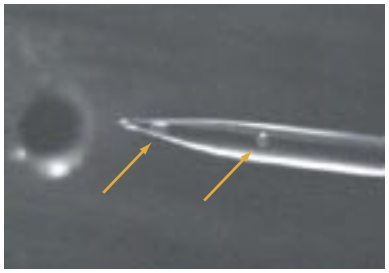
	Tip Size x Tubing ID (µm)	Avg. Peak Height	Avg. Peak Width (sec.)
PicoFrit® Column	15	7.70E+07	23.6
IntegraFrit™ Column	10 x 20	3.70E+07	22.6
IntegraFrit™ Column	15 x 75	5.80E+07	26.8
IntegraFrit™ Column	15 x 100	5.00+07	32.52

Peak height and width at half maximum for repetitive injections (n=3) performed with each column configuration. The summary at the bottom is an average of data accross all five peaks. Instrument: LCQ Deca™, 100 fmol/peptide injection, 250 nL/min, gradient eultion

Keys to Success



- A high quality cleave or machine cut fused-silica tubing is critical. Poor tubing end-quality creates connections that deteriorate chromatographic perfor- mance, robbing sensitivity.



- Using an emitter with the appropriate tip diameter made from suitably small ID tubing (or an integrated emitter-column) prevents the formation of air bubbles that generate instability in the spray.

Conclusions

- High performance nanobore LC-MS is possible with either connected-emitter or integrated-emitter configurations.

- The highest possible performance is obtained using the integrated emitter- column format (PicoFrit®):
  - Improved peak height
    - 2 to 4 fold greater MS signal
    - Improved sensitivity, S/N
  - Improved C.V. for repetitive injections
    - Improved spray stability
    - Better suited for Quantitative & Semi Quantitative Analysis
  - Decreased peak width

- High performance with the connected-emitter format is obtainable provided:
  - Post-column connections made with square cut fused-silica
    - Peak width similar to integrated emitter is possible
    - Improved peak symmetry
  - Emitter tubing ID is less than or equal to the column ID
    - Smaller ID's create back-pressure to suppress bubble formation
    - Reduced turbulence at junction to maintain peak shape
  - Smaller diameter tip IDs are preferable
    - Improved spray stability
    - Reduced bubble formation

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