

Investigation of Elastomer Core Trap Column and Maximizing Sample Loading Capacity

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Introduction

Numerous injection strategies exist (direct, trapped, multi-dimensional) for nanobore LC/MS based analysis of complex peptide mixtures. Compared to direct on-column injection, sample trapped-column injection provides multiple advantages. By effectively desalting and concentrating samples on-line, sample traps improve analytical column longevity and throughput. Unfortunately trap-column injection suffers from comparatively degraded sensitivity and a loss of chromatographic performance. A novel elastomer-core trapping column format is evaluated in terms of chromatographic and mass spectrometric figures of merit. Using a BSA tryptic digest and a three peptide mixture, the performance of this sample trap configuration was assessed by evaluating loading capacity, operating back pressure, peak shape and sample recovery relative to a conventional format, fused-silica. In the process of evaluating trapping column format, the importance of matching the sorbent chemistry of the trapping column to the analytical column was observed and is demonstrated.

Methods

Chromatography

- o Leap Technologies HTC Pal Autosampler
 - VICI 6-port micro valve
 - 1.0 μ L Sample loop
- o Eksigent nanoLC-2D pump
 - Channel 1 - Isocratic loading
 - Mobile Phase A = 0.1% formic acid in 2% acetonitrile/98% water
 - Mobile Phase B = 0.1% formic acid in 2% acetonitrile/98% water
 - Sample trap loading - 5 μ L/min. for 5 minutes
 - On-Column loading - 2 μ L/min. for 10 minutes
 - Channel 2 – Gradient elution
 - Mobile Phase A = 0.1% Formic Acid in Water
 - Mobile Phase B = 0.1% Formic Acid in Acetonitrile
 - 300 nL/min. Gradient elution
 - 25 Minute gradient to 50% B
- o VICI® 10-port micro valve for column switching

Mass Spectrometry

- o Thermo LCQ Deca™ Ion Trap
- o 3 microscans/spectra
- o 375.00 – 1600.00 Da mass range for MS spectra
- o Top-3 data dependent mode with dynamic exclusion for MSMS spectra
- o New Objective Digital PicoView® Nanospray Source (DPV-150)
- o XCalibur™ v1.3 and Bioworks™ for data analysis

Columns

- o Analytical Column (AC)
 - 75 μ m ID PicoFrit® Column (New Objective) with a 15 μ m integral frit slurry packed to 10 cm with 5 μ m ZORBAX® 300SB-C18 (Agilent)
- o Trapping Columns
 - IntegraFrit™ Sample Trap Column (IFST)
 - 150 μ m ID packed to 20 mm (New Objective) with 5 μ m ZORBAX® 300SB-C18 (Agilent)
 - Column housing - PEEK™ sample trap column assembly containing a NanoFilter capsule with a 1 μ m titanium frit at the inlet and a PEEK capsule union at the outlet
 - PicoClear™ Sample Trap Column (PCST)
 - 300 μ m ID packed to 4.0mm with 5 μ m ZORBAX® 300SB-C18 (Agilent)
 - Column housing – PicoClear™ Union body (New Objective) with a 50 μ m ID IntegraFrit™ (New Objective) plumbed in each side

Samples

- o BSA digest (Waters MassPREP™)
 - 300 fmol/ μ L in 0.1% formic Acid
- o Equimolar mix of three peptides
 - Asn1, Val5-angiotensin II, angiotensin II and bradykinin (Sigma Aldrich)
 - 0.05 ng/ μ L, 0.10 ng/ μ L, 0.25 ng/ μ L, 0.50 ng/ μ L, 1.0 ng/ μ L and 10 ng/ μ L in 100% water



Figure 1 Upchurch Sample Trap Cartridge, assembled, with 150 mm ID x 20 mm IntegraFrit Sample Trap Column inserted



Figure 2 PicoClear Union with 300 μm ID x 4.0 mm packed bed inside PicoClear elastomer core

Figure 3 HTC Pal autosampler equipped with automatic VICI 6-port micro valve plumbed with 1.0 mL loop, Eksigent nanoLC-2D pump, VICI 10-port micro valve—for switching between trap loading and gradient elution—and LCQ Deca ion trap mass spectrometer.

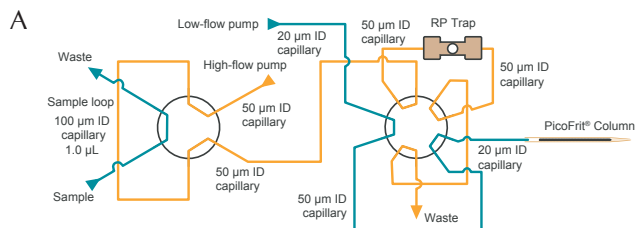
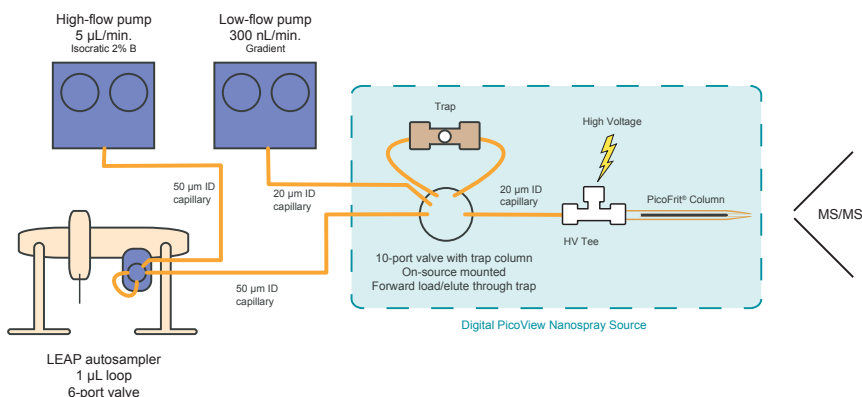


Figure 4 (A) Channel 1 on nanoLC-2D pump loading sample trap in forward direction, and (B) Channel 2 on nanoLC-2D pump gradient eluting off of the sample trap in the forward direction, onto the PicoFrit Analytical Column

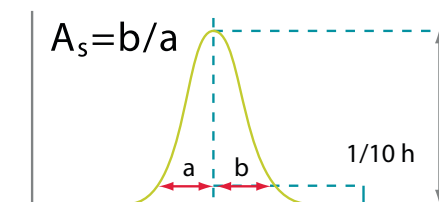
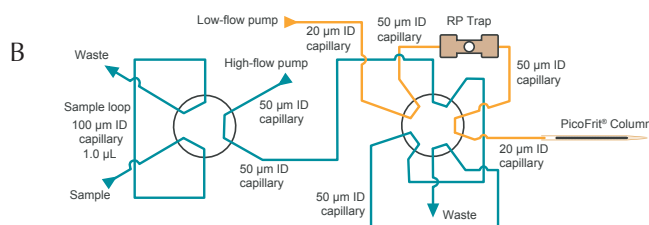
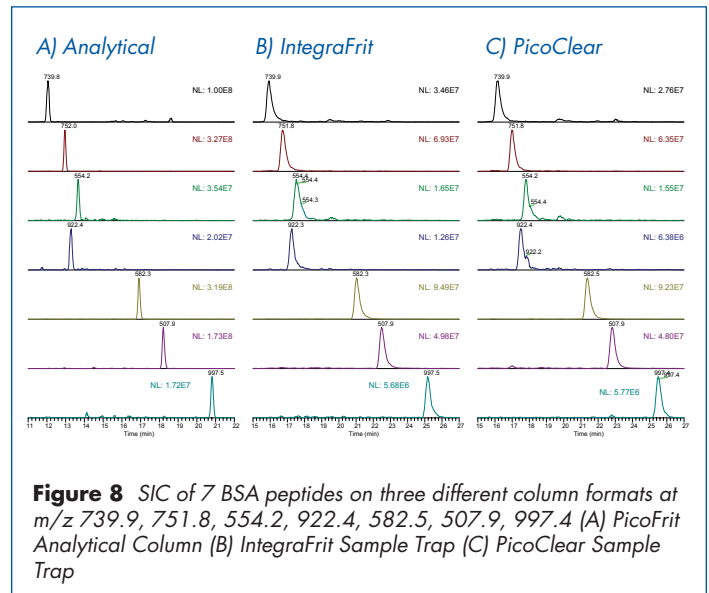
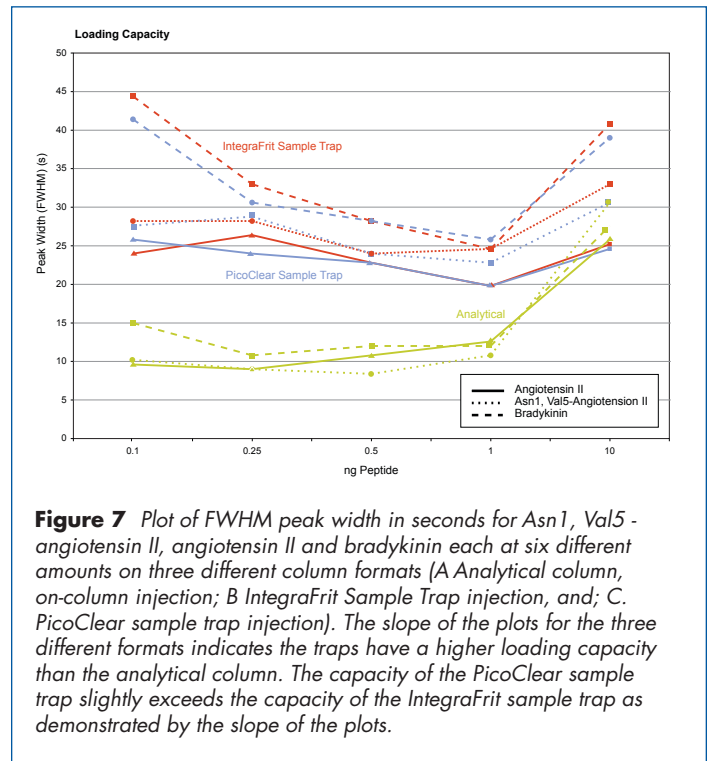
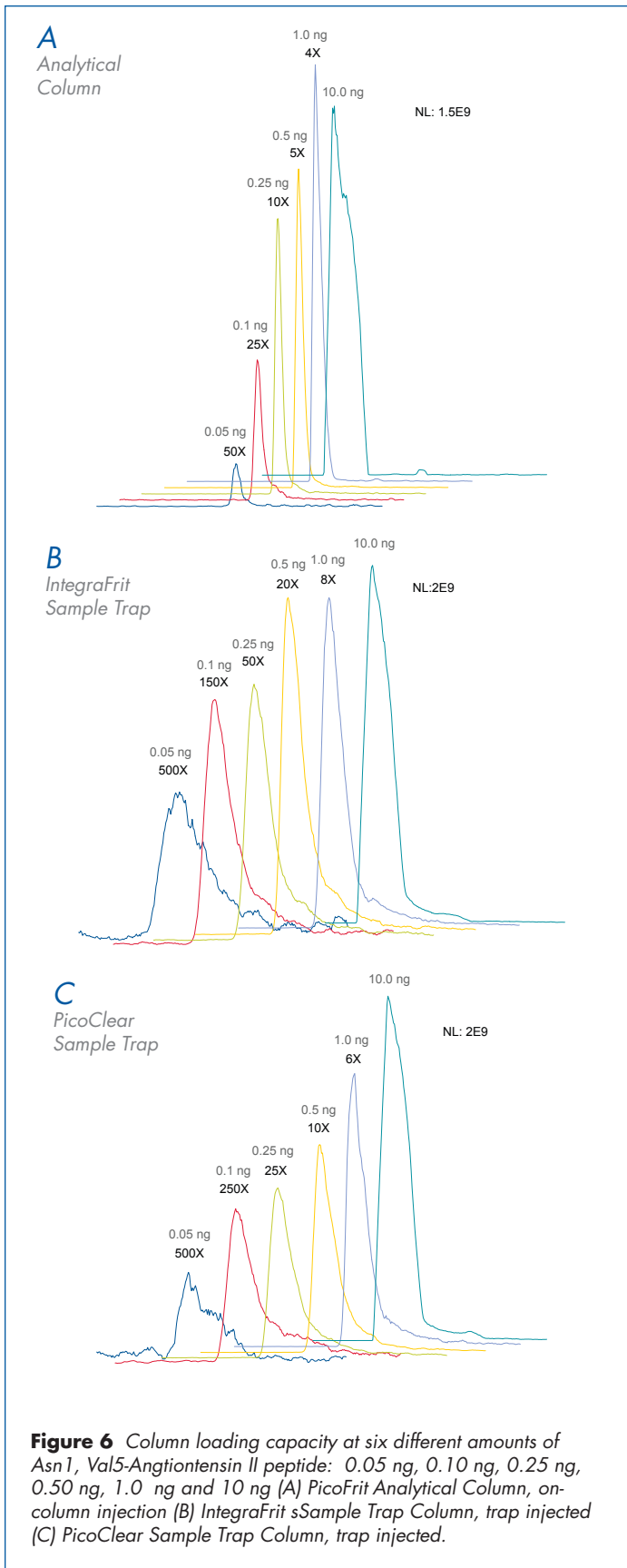


Figure 5 The ratio of w_b/w_a at 10% peak height indicates the asymmetry of a chromatographic peak. A perfectly symmetrical peak will have a value of 1, a tailing peak will have a value greater than 1 and a value less than 1 indicates fronting.



	BSA Peptide m/z (Da)	Apex RT (m)	Start RT (m)	End RT (m)	Mean Peak Width (m)	Mean Peak Width (s)	Mean Peak Asymmetry	Mean Peak Area	Mean Peak Height	Mean Peak S/N
Analytical Column	739.9	11.93	11.87	12.00	0.15	9.0	1.2	8.18E+08	1.22E+08	493
	752.0	12.79	12.73	12.86	0.13	8.0	0.9	1.93E+09	3.14E+08	1309
	554.4	13.52	13.45	13.60	0.15	9.0	1.3	4.53E+08	6.90E+07	129
	922.3	13.13	13.06	13.21	0.17	10.2	1.1	3.24E+08	4.51E+07	2627
	582.5	16.82	16.75	16.90	0.15	9.0	1.4	2.32E+09	3.44E+08	707
	508.0	18.10	18.01	18.16	0.16	9.8	1.2	1.30E+09	1.78E+08	400
997.5	20.68	20.59	20.76	0.16	9.8	1.1	1.28E+08	1.68E+07	198	
IntegraFrit Sample Trap Column	739.9	15.85	15.69	16.03	0.28	17.0	2.6	4.64E+08	3.68E+07	150
	752.0	16.67	16.54	16.84	0.30	18.2	2.6	1.05E+09	7.55E+07	392
	554.4	17.48	17.40	17.72	0.33	19.6	1.8	2.70E+08	1.84E+07	35
	922.3	17.16	17.08	17.33	0.25	15.0	2.8	1.50E+08	1.29E+07	69
	582.5	20.96	20.83	21.11	0.25	15.2	2.3	1.22E+09	1.05E+08	151
	508.0	22.36	22.27	22.56	0.30	18.0	2.5	7.40E+08	5.39E+07	102
997.5	25.12	25.00	25.30	0.29	17.4	2.4	7.96E+07	5.73E+06	54	
PicoClear Sample Trap Column	739.9	16.02	15.94	16.24	0.29	17.6	3.0	4.10E+08	3.20E+07	150
	752.0	16.90	16.80	17.08	0.28	16.6	2.9	8.78E+08	6.93E+07	479
	554.4	17.79	17.64	17.98	0.34	20.6	2.2	2.23E+08	1.42E+07	22
	922.3	17.47	17.35	17.62	0.28	16.8	2.3	1.04E+08	8.28E+06	51
	582.5	21.18	21.08	21.40	0.34	20.6	2.7	1.36E+09	8.57E+07	125
	508.0	22.65	22.54	22.87	0.35	21.2	2.3	7.38E+08	4.66E+07	81
997.5	25.37	25.24	25.51	0.30	17.8	1.9	9.80E+07	7.05E+06	112	

Figure 9 Separation of 300 fmol BSA digest on three different column formats in triplicate. Mean (n=3) peak width (FWHM) and mean peak asymmetry at 10% peak height is shown. Both trap column formats show an increase in peak width and peak tailing, as indicated by peak asymmetry values greater than 1, compared to on-column injection. The PicoClear sample trap column performs as well as the IntegraFrit sample trap in terms of peak shape, width and signal to noise.

PicoClear Sample Trap Column
300 fmol BSA

77.92% Sequence Coverage

MKWTFTISLL LFFSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLLIA FSQYLQCCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
 VASLRETYGD MADCCEKQEP ERNECFLSHK DSDPDLPKLK PDPNTLCDEF KADEKKFWGK YLYEIARRHP YFYAPELLEY ANKYNVGFQE CCQAEDKGAC
 LFPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKPKAE FVEVTKLVTD LTKVHKCCCH GDLLECAADDR ADLAKYICDN QDTISSKLEK
 CCDKPLLEKS HCAIEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAQDAFL GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
 KHLVDEPQNL IKQNCQDFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC CTRPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
 STQTALA

IntegraFrit Sample Trap Column
300 fmol BSA

72.16% Sequence Coverage

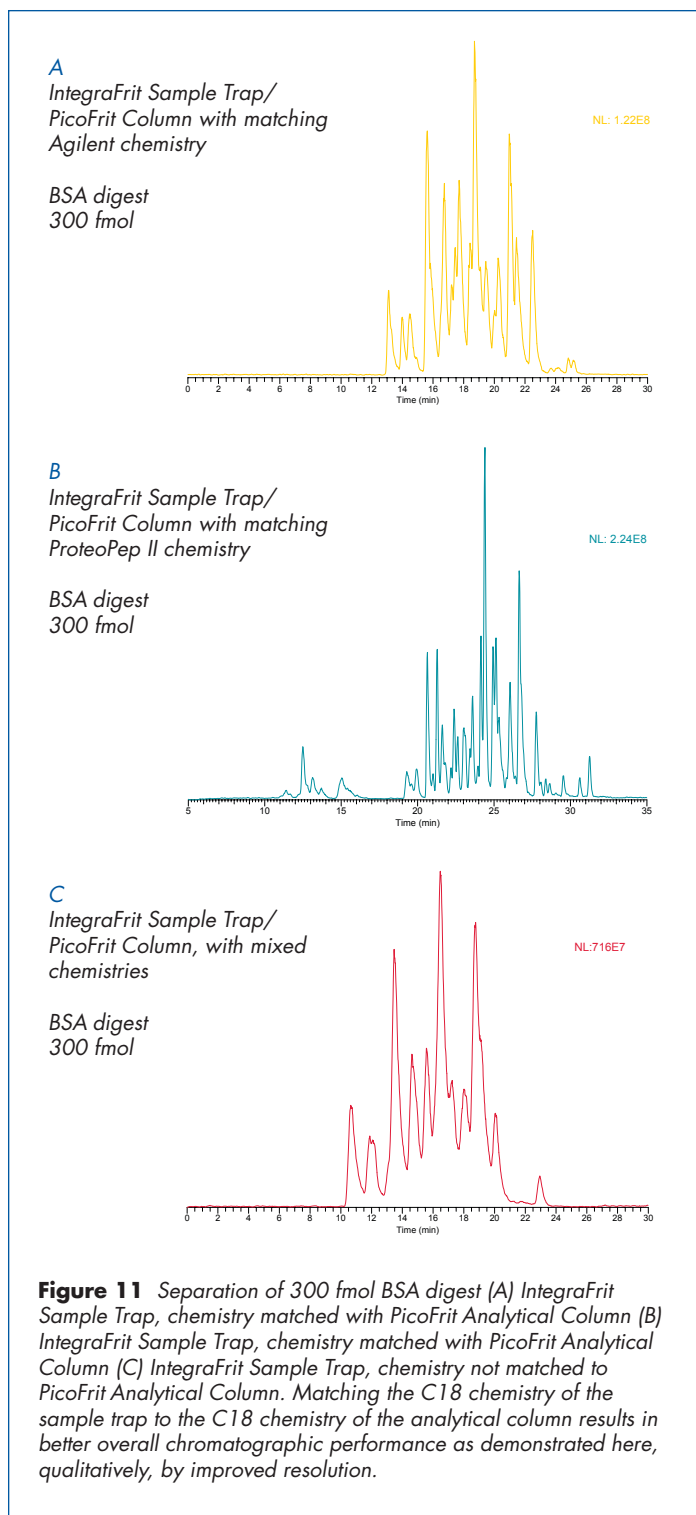
MKWTFTISLL LFFSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLLIA FSQYLQCCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
 VASLRETYGD MADCCEKQEP ERNECFLSHK DSDPDLPKLK PDPNTLCDEF KADEKKFWGK YLYEIARRHP YFYAPELLEY ANKYNVGFQE CCQAEDKGAC
 LFPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKPKAE FVEVTKLVTD LTKVHKCCCH GDLLECAADDR ADLAKYICDN QDTISSKLEK
 CCDKPLLEKS HCAIEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAQDAFL GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
 KHLVDEPQNL IKQNCQDFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC CTRPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
 STQTALA

Analytical Column
300 fmol BSA

70.84% Sequence Coverage

MKWTFTISLL LFFSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLLIA FSQYLQCCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
 VASLRETYGD MADCCEKQEP ERNECFLSHK DSDPDLPKLK PDPNTLCDEF KADEKKFWGK YLYEIARRHP YFYAPELLEY ANKYNVGFQE CCQAEDKGAC
 LFPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKPKAE FVEVTKLVTD LTKVHKCCCH GDLLECAADDR ADLAKYICDN QDTISSKLEK
 CCDKPLLEKS HCAIEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAQDAFL GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
 KHLVDEPQNL IKQNCQDFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC CTRPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
 STQTALA

Figure 10 Peptide sequence coverage of BSA digest on three column formats. Peptides not detected are indicated in black italics. Sequence coverage for each format is indicated as a percentage of amino acids identified from the total. The PicoClear sample trap shows improved sequence coverage at almost 78%, as compared to 72% for the IntegraFrit sample trap. This improvement in peptide recovery can be attributed to the different geometry (300 mm ID vs. 150 mm ID).



Results

- Matching the sorbent chemistry of the sample trap to the sorbent chemistry of the analytical column results in improved peak resolution.
- Employing sample traps in-line with an analytical column improves the overall loading capacity
- Sample traps improve peptide recovery as compared with on-column injection
- The PicoClear™ Sample Trap has a higher loading capacity than the IntegraFrit sample trap
- The PicoClear Sample Trap demonstrated improved peptide recovery compared to the IntegraFrit™ Sample Trap
- The PicoClear Sample Trap Column is a new column format offering improved overall performance as compared to a traditional fused-silica sample trap (IntegraFrit)

Future Work

- Investigate benefits of matching sample trap chemistry to analytical column chemistry further using other commercially available chemistries
- Evaluate PicoClear sample trap with different bed volumes to determine its effect on performance and recovery
- Optimize sample trap elution to minimize band broadening