NEW OBJECTIVE

Efficient Multi-Column Coupling for Enhanced nLC-ESI-MS Separation

Christopher J. Toher, Adam W. Perala, Carla J. Marshall-Waggett, Gary A. Valaskovic

New Objective, Inc., Woburn, MA

Introduction

Coupling columns of same or different resin materials is often employed in complex proteomic digest analysis. Despite enhanced separation, these multidimensional columns are costly, time-consuming to produce, and initiate post-column loss by dead-volume introduction. Confounding factors of column-coupling can be eliminated via transparent, true zero-dead-volume (ZDV) unions that achieve flush connections and rapid swap-out facility during system maintenance. In the current investigation, two conventional 10 cm-bedded columns were coupled and connected to the bed terminus of a third 10 cm nanobore column with integrally fritted tip. Analytical merit of this extended column was compared with a single 30 cm-bedded column with integrally fritted tip and the same resin material. These novel unions supported chromatographic data collection with zero dead-volume, negligible resolution loss, and comparable caliber as the single 30 cm-bedded column. The performance of a 35 cm column fabricated from 15- and 20-columns is also detailed.

Methods & Materials

Instrumentation & Components

- Ion-trap mass spectrometer (LCQ Deca™, Thermo Fisher Scientific)
- Customized nanospray source (Digital PicoView® 150, New Objective, Inc.)
- NanoLC Pump (Eksigent™)
- Six-port automatic nano-valve (Scivex) with 0.5uL sample loop
- PicoFrit® columns (360 µm OD, 75 µm ID, 15 µm tip ID, New Objective), each containing ProteoPep™ II (New Objective) 5.0 µm-diameter particles packed to 10 cm-, 20-, and 30 cm- bed lengths
- IntegraFrit™ Columns (360 µm OD, 75 µm ID, New Objective), containing ProteoPep™ II (New Objective) 5.0 µm-diameter particles packed to 10 cm-, 15- cm, and 20 cm-bed lengths

Sample Preparation

- A commercially available bovine serum albumin (BSA) standard was diluted to 200, 100, and 20 fmol/µL in an aqueous solvent of 2% ACN, 0.1% formic acid
- A commercially available mixture of 5 angiotensins was diluted to 0.1 ng/peptide concentration with 2% ACN, 0.1% formic acid aqueous solvent
- Samples were analyzed via online nanobore ESI-MS in positive-ion-mode

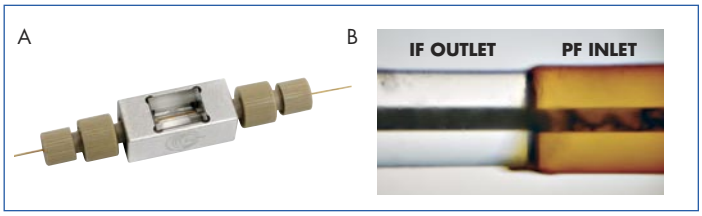

Figure 1 *A) PicoClear™ Union, and B) Expanded view of the zero-dead-volume connection achieved inside the clear union body. Note the excellent column-to-column alignment.*

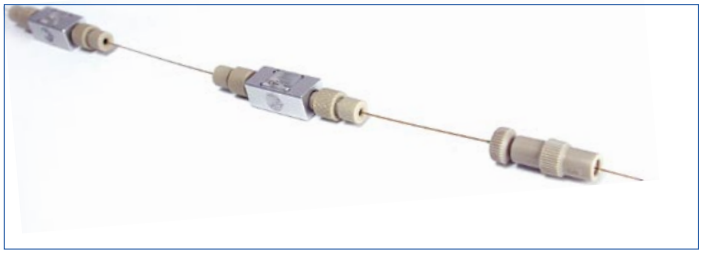

Figure 2 *Two 10 cm IntegraFrit™ columns configured with a 10 cm PicoFrit® column via two PicoClear™ Unions to form a single 30 cm column*

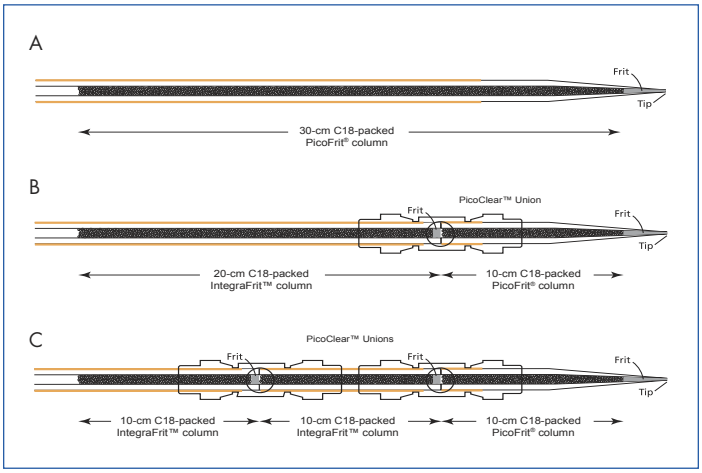

Figure 3 *Schematic diagrams of PicoClear™ Union-Column combinations. A) PicoFrit® column with 30 cm bed, B) 20 cm IntergaFrit™ coupled to a 10 cm PicoFrit column with a PicoClear Union, and C) Two 10 cm IntegraFrit columns coupled to a 10 cm PicoFrit column via two PicoClear Unions*

Results

All column combinations were employed in analyzing the angiotensin standard. Data collected using the 30 cm ProteoPep™ II (PP2)-packed PicoFrit® Column resulted in FWHMs between 8.4 – 10.2 seconds. The 20 cm IntegraFrit™ column + 10 cm PicoFrit column combination displayed FWHMs between 13.2 – 14.4 seconds. The two 10 cm IntegraFrit column + 10 cm PicoFrit displayed FWHMs between 12.6 – 14.4 seconds. Figure 4 illustrates three chromatograms from each column combination for analyzing the angiotensin standard; 0.25 ng total peptide were subjected to a 300 nL/min. flow rate over a 70 min. gradient from 2% - 50% organic modifier concentration.

Figure 5 illustrates the three chromatograms produced in the BSA digest analysis through each column combination; 100 fmol BSA was subjected to a gradient identical to that used for angiotensin. Data collected using the 30 cm ProteoPep II (PP2)-packed PicoFrit column allowed 71.8% sequence coverage. The 20 cm IntegraFrit column + 10 cm PicoFrit column supported 58.6% sequence coverage. The two 10 cm IntegraFrit column + 10 cm PicoFrit column yielded 65.1%. sequence coverage. Figure 6 shows the rich data possible when combining a long column (35 cm) with a long gradient (90 min.) Narrow peak widths (FWHM 10-14 sec.) yield an average peak capacity of 239 (Figure 10).

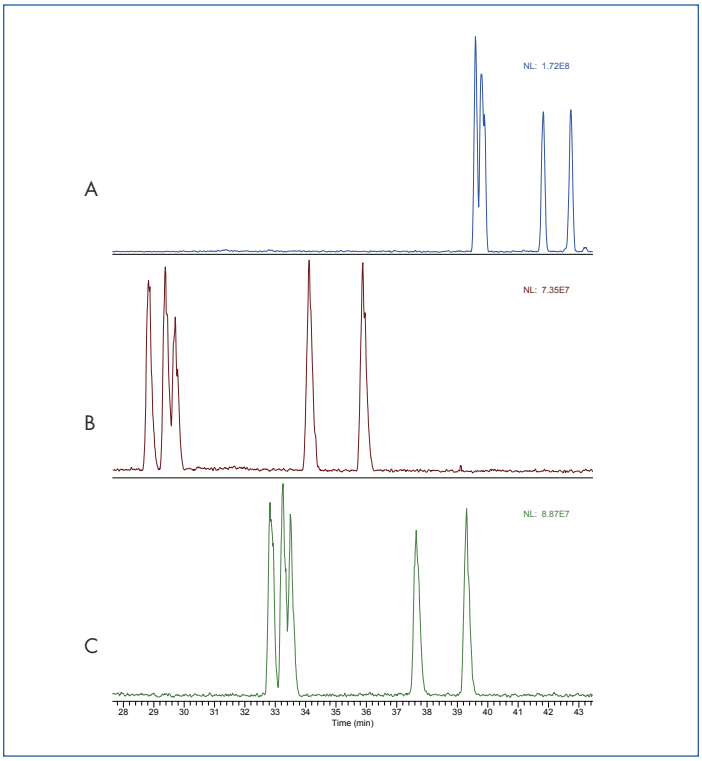

Figure 4 *Angiotensin chromatographic data collected with each column-union configuration A) Chromatogram collected with a 30 cm PicoFrit® Column, B) chromatogram collected with 20 cm IntegrFrit™ Column + 10 cm PicoFrit Column, and C) chromatogram collected using two 10 cm IntegraFrit Columns + 10 cm PicoFrit column*

Injection: 0.25 ng total peptide, Flow rate: 300 nL/min.., Gradient: 2% - 50% B over 70 min.

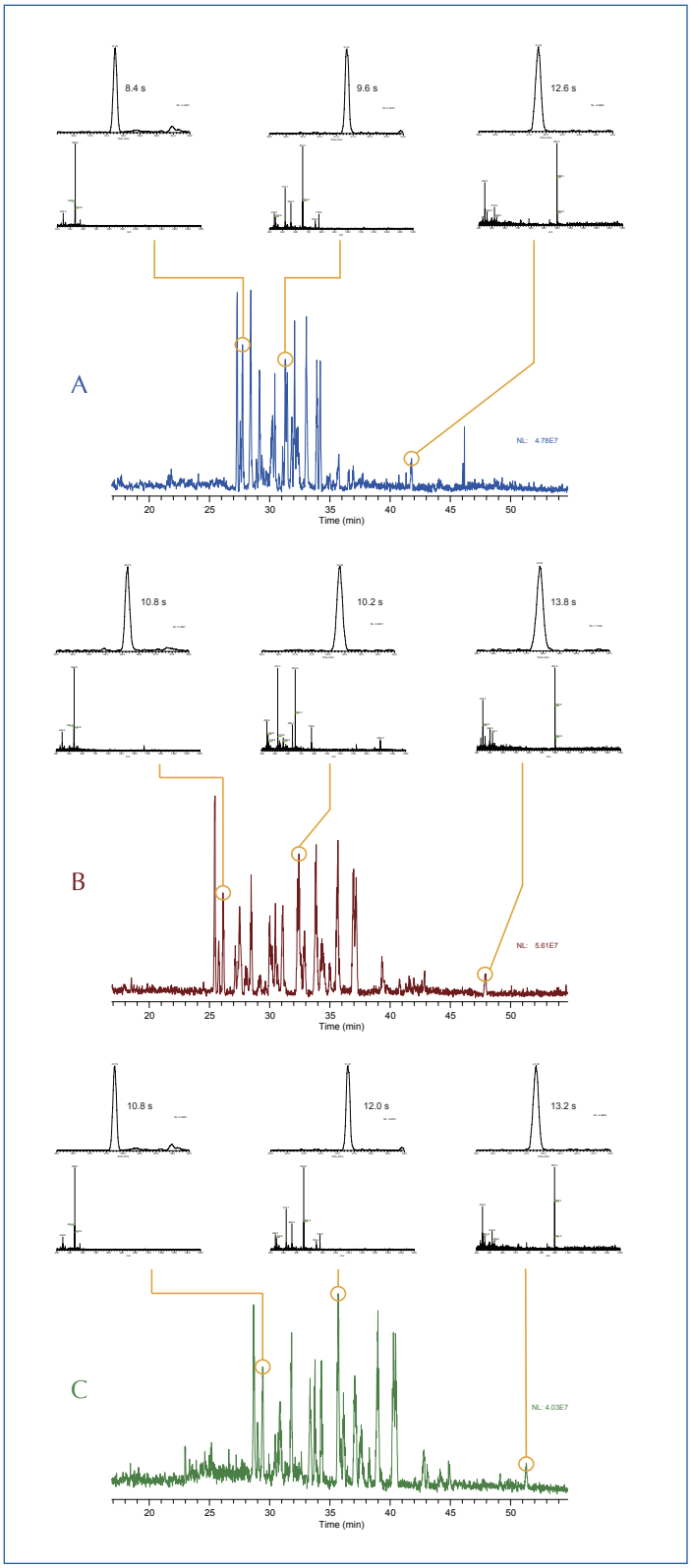

Figure 5 *Expanded regions of BSA tryptic digest chromatographic peaks. A) Chromatogrphic region, as collected with 30 cm PicoFrit® Column, B) Chromatographic region, as collected with 20 cm IntegraFrit™ + 10 cm PicoFrit, and C) Chromatographic region, as collected using two 10 cm IntegraFrit Columns coupled to a 10 cm PicoFrit column.*

Injection: 100 fmol BSA, Flow rate: 300 nL/min., Gradient: 2% - 50% B over 70 min.

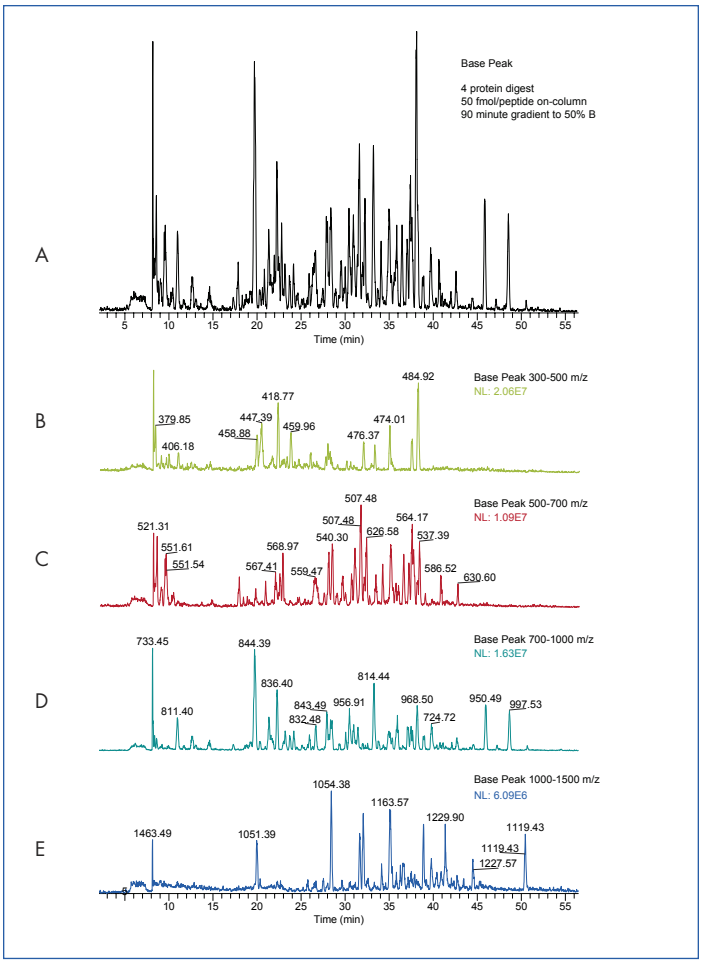

Figure 6 *Long gradient (90 min to 50% B) base-peak chromatogram (A) obtained using a 35 cm x 75 µm ID column fabricated by coupling a 20 cm long PicoFrit® column to a 15 cm long IntegraFrit™ Column through a PicoClear™ connector. Packing material was 5 µm ProteoPep II C18 (300 A). Mobile phase flow rate was 400 nL/min. The sample was a four protein tryptic digest (50 fmol/peptide on-column). Chromatograms (B-E) are base-peak reconstructions for the mass ranges indicated in the right hand column.*

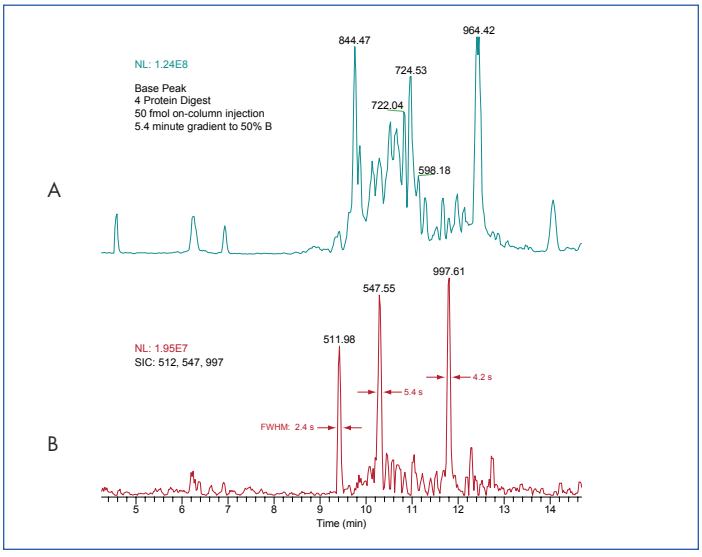

Figure 7 *Conditions as in Figure 6. Here the gradient is fast (5.4 min to 50% B). Base peak (A) and selected ion current of three marker analyte ions are shown in (B). Full width at half height is labeled in (B).*

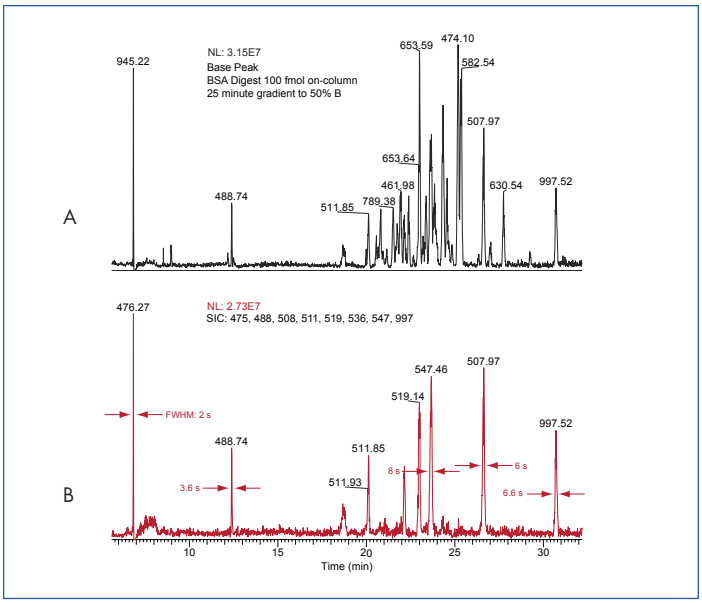

Figure 8 *Same column as in Figure 6 running a BSA digest (100 fmol on-column) with a 25 min gradient to 50% B, The selected ion current of 8 marker ions, along with respective peak widths, is shown in (B).*

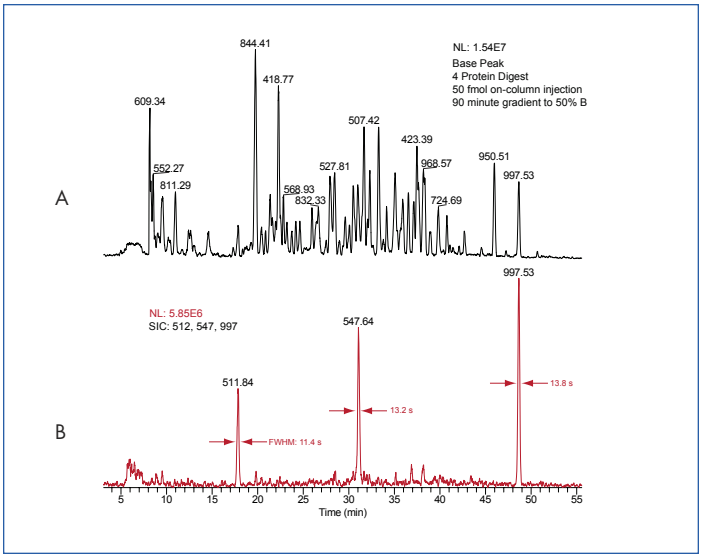

Figure 9 *Same run as in Figure 6 showing the selected ion current of the marker ions used in Figure 7*

A

Peak Capacity
75 µm x 35 cm long Integrafrit-PicoFrit Column

Fast Gradient: 5.4 min to 50% B
4 Protein Digest: 50 fmol/peptide

m/z	Apex RT	Start RT	End RT	Height	Baseline Width (min)	Baseline Width (sec)	Capacity*
511.9	9.42	9.38	9.49	2.01E+07	0.11	6.6	46
547.5	10.31	10.24	10.35	2.42E+07	0.11	6.6	46
997.5	11.78	11.74	11.87	2.68E+07	0.13	7.8	39
			AVERAGE	2.37E+07	0.12	7.0	44

Medium Gradient: 25 min to 50% B
BSA Digest: 100 fmol/peptide

m/z	Apex RT	Start RT	End RT	Height	Baseline Width (min)	Baseline Width (Sec)	Capacity*
476.2	6.84	6.81	6.85	2.66E+07	0.04	2.4	NI
488.8	12.39	12.36	12.44	1.03E+07	0.08	4.8	NI
511.9	20.13	20.03	20.21	9.34E+06	0.18	10.8	140
476.4	22.15	22.05	22.23	8.01E+06	0.18	10.8	140
519	23	22.9	23.08	1.56E+07	0.18	10.8	140
547.5	23.68	23.53	23.76	1.88E+07	0.23	13.8	110
507.9	26.63	26.51	26.73	2.00E+07	0.22	13.2	115
997	30.71	30.62	30.82	1.24E+07	0.2	12	126
			AVERAGE	1.51E+07	0.16	9.8	128

Long Gradient: 90 min to 50%B
4 Protein Digest: 50 fmol/peptide

m/z	Apex RT	Start RT	End RT	Height	Baseline Width (min)	Baseline Width (sec)	Capacity*
511.9	17.88	17.66	18.01	3.36E+06	0.35	21	258
547.5	31.09	30.88	31.29	4.43E+06	0.41	24.6	221
997.5	48.65	48.46	48.84	6.38E+06	0.38	22.8	238
			AVERAGE	4.72E+06	0.38	22.8	239

Capacity = 1 +(Tg/w)
Tg= gradient time (min)
W=peak width at 13.5% from baseline

* From Gilar, Daly, Neue, Gebler; J. *Chromatog. A.* **2004**, 183-192.

B

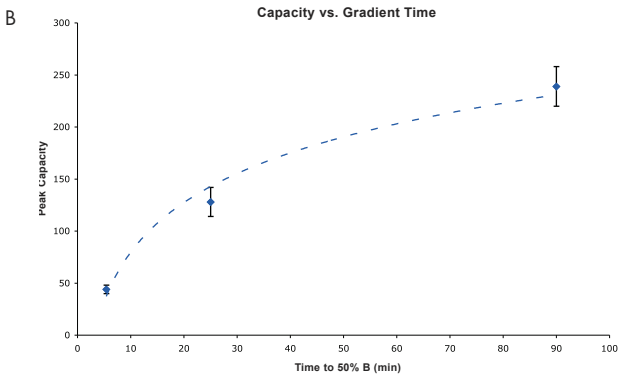

Figure 10 *Summary of peak width (baseline) and peak-capacity measurements from the data obtained in Figures 7, 8, 9. (B) Plot of peak capacity vs. gradient elution time (to 50% B) for the 35 cm long column.*

Angiotensin	MW	Sequence
[Ile^7]-Angiotensin III	897.1	RVYIHPI
[Val^4]-Angiotensin III	917.1	RVYVHPF
[Asn^1,Val^5]-Angiotensin II	1,031.0	NRVYVHPF
[Val^5]-Angiotensin I	1,282.5	DRVYVHPFHLA
Angiotensin I	1,296.0	DRVYIHPFHL

Table 2 *5-Angiotensin composition*

Conclusions

- Minimal resolution loss and post-column loss were observed for columns combined using transparent, true ZDV unions
- Long 75 µm ID nanobore columns (30+ cm) are easily fabricated
- Negligible sequence coverage differences were recorded between each column, although the integral 30 cm column provided the best overall score
- Coupling columns enables peak capacities >200 when running longer gradients
- Transparent, true zero-dead-volume (ZDV) unions ensure clean connections between columns without dead volume
- Connecting columns containing different resins will be explored in future work
- Nanobore columns having "semi-disposable" integral guard columns are a viable next stepObservations & Conclusions
- Switching the high voltage simultaneously with emitter position is a viable method for switched-column nanobore LC-MS
- Carryover is reduced to that inherent to the memory effect of the heated-capillary interface as spray is only possible from a single channel at a time
- Nearly identical analyte-ion current (within 10%) was obtained by fine-tuning each channel's emitter position using continuous infusion
- Channel-switching yielded a dead time of approximately 6 seconds for the stage to locate and the emitter to re-establish spray
- Column-switching can greatly reduce the time lost to column-rinsing and conditioning
- Method development requires careful optimization of conditioning to ensure high reproducibility between column(s) and injections
- The two channels in gradient-LC mode yielded MS signal to better than 50% of each other (as determined by analtye SIC's). Given that each channel possessed different vendor LCs, each with different delay volumes, gradient-mixing schemes, and injection valves, the results are promising.

Originally published June 2007, at the American Society of Mass Spectrometry Meeting in Indianapolis, Indiana, U.S.A.

New Objective, Inc.
2 Consitution Way
Woburn, MA 01801 USA
781 933 9560 tel
781 933 9564 fax
www.newobjective.com