

Increased stability and sensitivity using direct pumping for nanospray LC/MS of peptides

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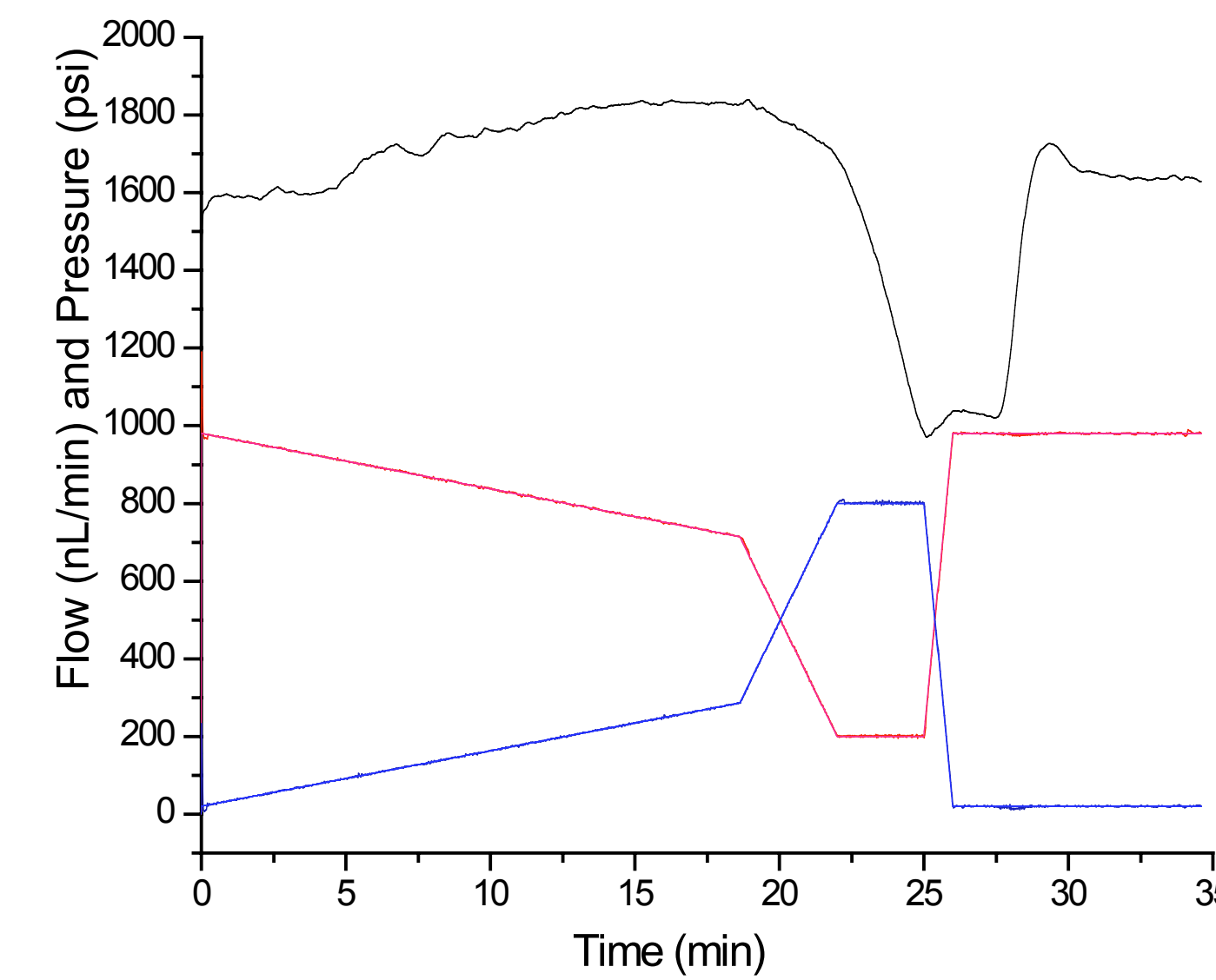
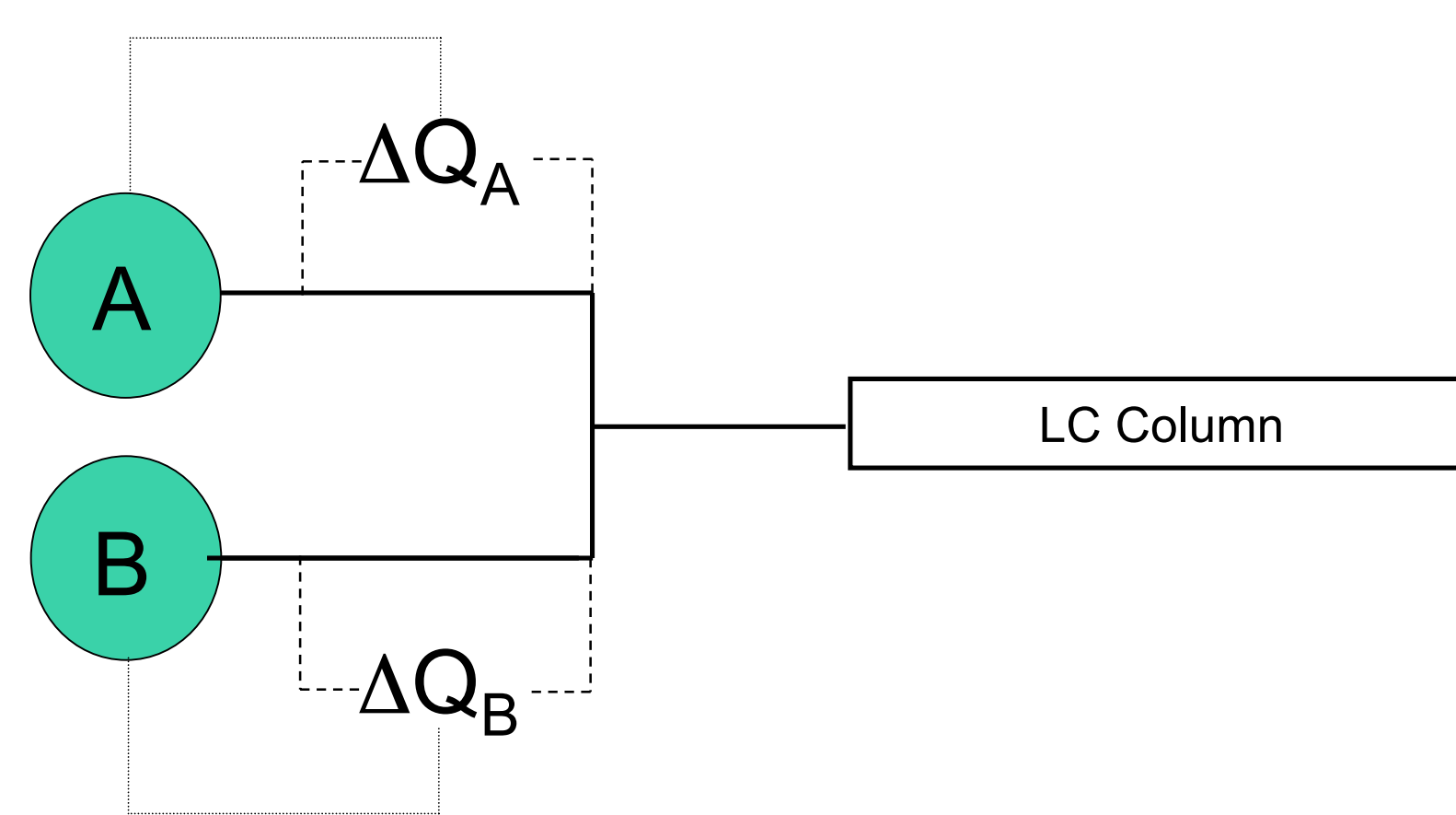
Abstract

Sensitive detection and identification of proteomic samples typically employs nanoflow LC using capillary columns (50-100 micron diameter) with a nanospray interface to a tandem mass spectrometer. Hybrid MS systems allow one to sensitively determine mass and sequence information for identification of the eluting peptides. A challenge in such analyses has been delivering stable and reproducible gradient flows at the 50-500 nL/min range used for such chromatography.

We describe the coupling of a direct pumping nanoflow chromatography instrument with a hybrid quadrupole/time-of-flight mass spectrometer for peptide analysis. We have observed increased gradient reproducibility along with improved baseline flow and spray stability over more conventional splitter-based LC systems. Experiments to examine stability, reproducibility, and sensitivity have been conducted.

Experimental

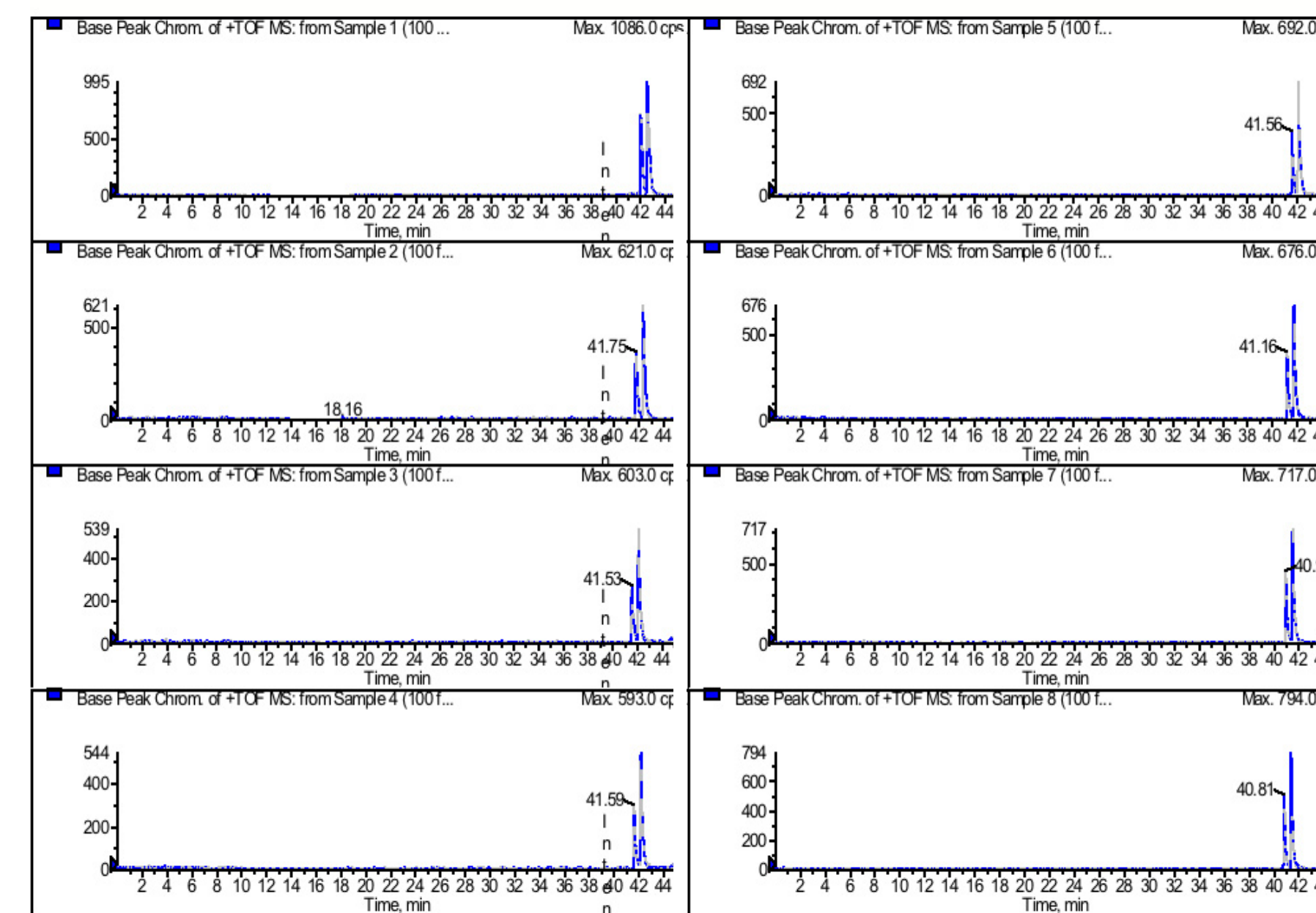
Instrumentation: A novel, binary gradient, nanoflow LC system (NanoLC-1D™, Eksigent Technologies) was coupled to a hybrid quadrupole/time-of-flight mass spectrometer (Q-Star Pulsar i, Applied Biosystems/MDS Sciex). Mixtures of water and acetonitrile (with 0.1% formic acid) at flow rates of 50-400 nL/min were used for both chromatography and direct infusion experiments. Peptide samples and protein digests were injected using a micro-volume autosampler (FAMOS, Dionex) and separated on 75 micron diameter C18 columns (Vydac, Dionex). The hybrid MS system was equipped with a nanospray interface using 10 micron pulled capillary emitters (New Objective). The TOF detector was used to determine accurate masses for both the parent peptides and the MS/MS fragments.



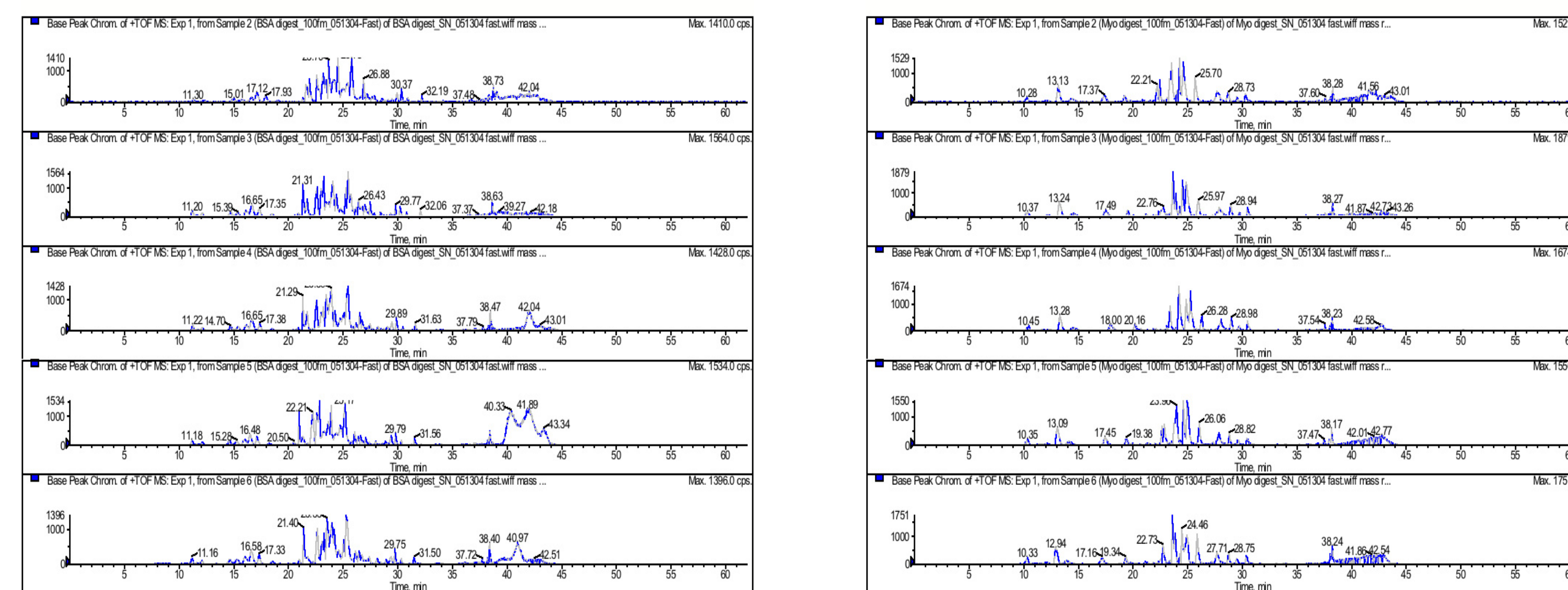
The direct pumping NanoLC-1D system uses flow measurement and feedback for each mobile phase to maintain reproducible controlled flows that are independent of column conditions and back pressure. In B, the flow profiles and measurements for a typical gradient run are shown along with the column pressure. The changes in column pressure represent changes in viscosity as the composition changes and can be used to determine column stability and swept volumes.

Reproducible retention times of peptide and protein digest samples

Repeated injections of both peptide standards and tryptic digests of model proteins were used to evaluate retention time reproducibility. Separations were conducted at flow rates of 200-300 nL/min. 1 μ L full loop injections of samples were coordinated with the LC gradient and MS acquisition using external contact closures.



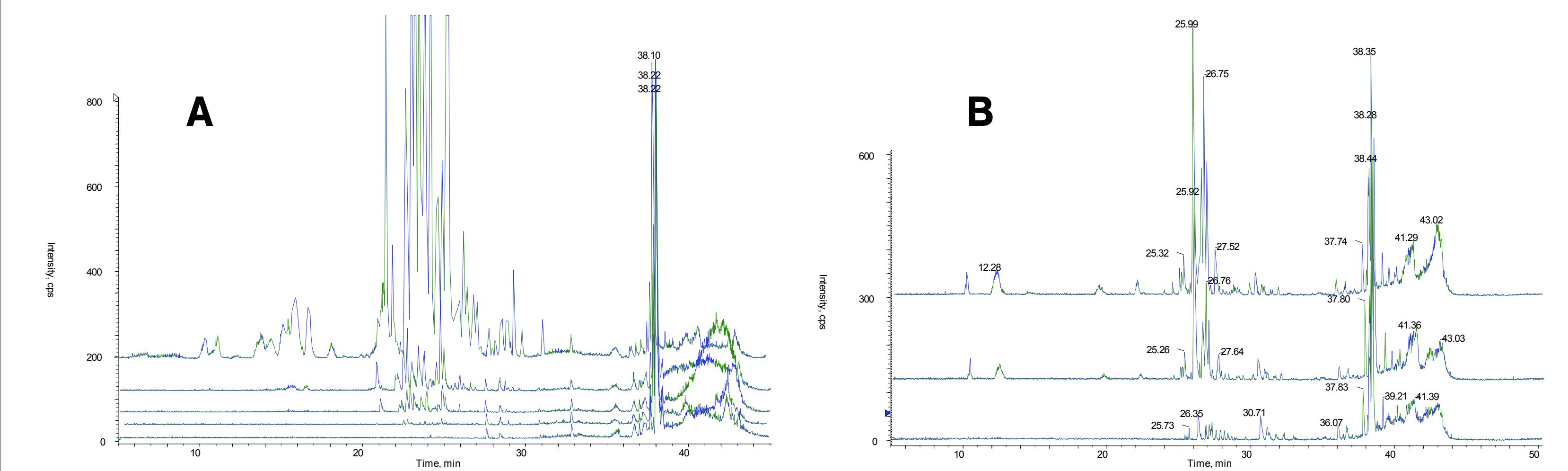
Eight repeated injections and separations of Angiotensin I and Angiotensin II. Standard deviations in retention time were 0.41 min. providing RSD's less than 1%. Mobile phases: A–98/2, B–2/98, water/acetonitrile with 0.1% formic acid. Column: Vydac 218MS 3 μ m, 0.075 x 150 mm.



Repeated injections of both BSA and Myoglobin tryptic digests. Mobile phases as above. Column: LCPackings PepMap 3 μ m, 0.075 x 150 mm. Gradient: 0 min–5% B, 25min–40% B, 27 min–85% B, 32 min–85% B, 34 min–5% B.

Stable nanospray provides improved sensitivity for protein digests

The direct pumping of the NanoLC coupled with the Qstar Pulsar I provides very stable nanospray signals. The stability of the signal allows one to pick out very low level peptide signals. In comparison to other capillary LC systems, we observed an approximately 5 fold increase in sensitivity.



Base peak chromatograms showing low level detection of protein digests. A: BSA with on-column injections of 100, 10, 5, 1 fmols and a blank injection. B: Myoglobin separations for 10, 5, and 1 fmol on-column.

Conclusions

- A direct pumping nanoLC coupled with a hybrid quadrupole-TOF MS instrument provides a robust setup for sensitive proteomics experiments.
- High reproducibility was observed in chromatograms of both peptide standards and protein digests.
- Very stable nanospray and TIC levels allowed increased sensitivity over conventional nano LC systems for low-level proteomics samples.