

# High Performance Nanoflow Chromatography with the Tempo™ nano LC Systems

## Making reliable low flow separations a reality!

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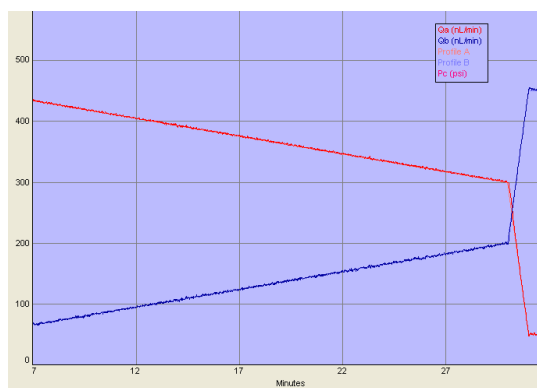
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From simple protein identification experiments to global protein expression workflows, proteomic applications require sensitive, reproducible, reliable chromatography. Tempo nano LC systems set a new performance standard for low flow separations.

### Technology Overview

The Tempo nano LC systems employ a direct pumping system with flow meters monitoring the flow rate in each mobile phase to a precision of <1 nL/min. Flow rate information is continuously fed to a microfluidic flow controller which rapidly adjusts a variable pressure source. This allows the system to maintain precise flow rates from 20-1000 nL/min. Each mobile phase is monitored independently before mixing, providing for precise gradient formation (Figure 1).

The simplicity in design of the Tempo nano LC systems represents a significant improvement in ease-of-use and reliability for the proteomics researcher. This direct pumping system eliminates the need for flow splitting, alleviating the flow inaccuracies of splitter based systems and reducing solvent consumption and waste.



**Figure 1. Real-time monitoring of flow rates for each mobile phase at 500 nL/min.** Microfluidic flow control provides a high level of flow accuracy for each channel, enabling very precise gradient formation.



Close coupling of the pump, gradient mixer and autosampler minimizes dispersion and delay time. Flow rate is unaffected by downstream pressure fluctuations, increasing the long term run to run stability of these low flow separations.

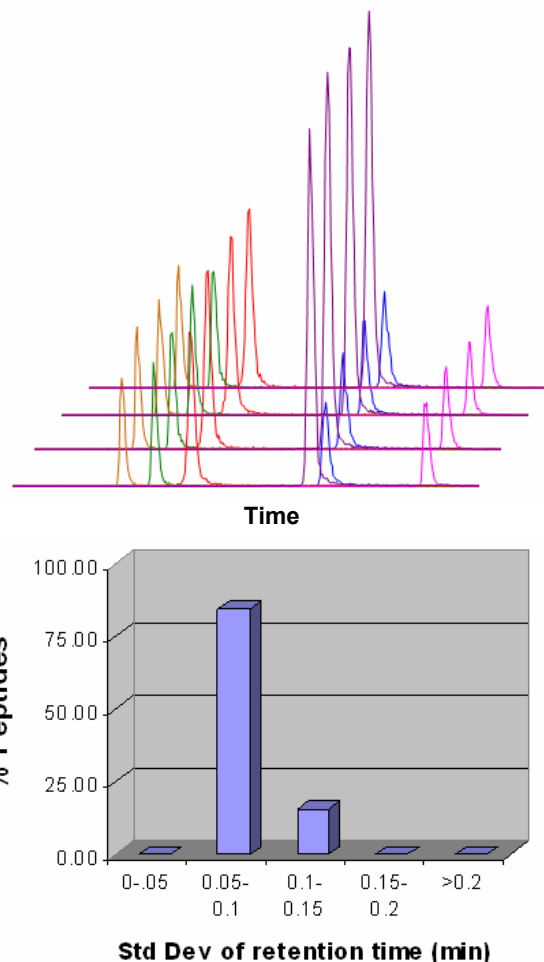
### Key Advantages of the Tempo™ nano LC Systems

- Direct pumping system and the measurement of flows with active feedback ensures precise gradient delivery, providing excellent run-to-run reproducibility
- Optimized for low flow rates between 20–1000 nL/minute, (and 1-20  $\mu$ L/min for the first dimension pump in MDLC system)
- Ultra low delay volume of 65 nL from mixer to injection valve enables rapid gradients and re-equilibration to shorten total cycle times
- Elimination of flow splitting reduces operating costs due to low solvent consumption and minimal waste production
- Full MDLC functionality available
- Easy-to-use software interface
- Simplified plumbing scheme and split-less design make for a reproducible, easy-to-use system

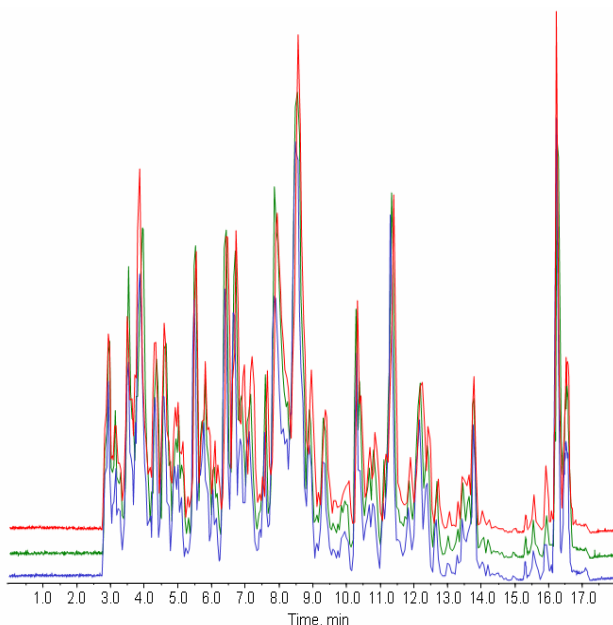
## Retention Time Reproducibility

Many LCMS applications, such as biomarker discovery by MS profiling, metabolomics, peptide quantitation, etc., require the comparison of chromatographic peaks across many replicate injections.

Quantitative peptide/protein analysis in complex mixtures is typically done using multiple reaction monitoring (MRM) on a 4000 Q TRAP® system and requires a high degree of reproducibility across many injections. For this type of quantitative work, the peak area of a peptide at a specific retention time from an unknown sample is compared to a standard concentration curve of a reference and the unknown concentration is calculated. Twenty replicate injections of BSA (5 fmol on column) were analyzed by MRM to assess the chromatographic reproducibility. Using a rapid 10 min gradient on a 75  $\mu$ m C18 column (PepMap C18, 300 nL/min), peak widths at half height of 6-10 seconds were observed with excellent peak shape (Figure 2). The retention time of each peak was measured and the variation was calculated for each peak. An average standard deviation of 0.08 minutes was determined (0.8%) (Figure 2).



**Figure 2. Reproducibility of nano LC Chromatography.** Selected MRM traces of peptides from BSA digest (5 fmol on column) show very consistent peak shape (top). Excellent retention time reproducibility was achieved across 20 replicate injections (bottom), the average standard deviation was 0.08 min (CV= 0.8%).



**Figure 3. Highly reproducible LCMS data can be obtained on complex mixtures.**

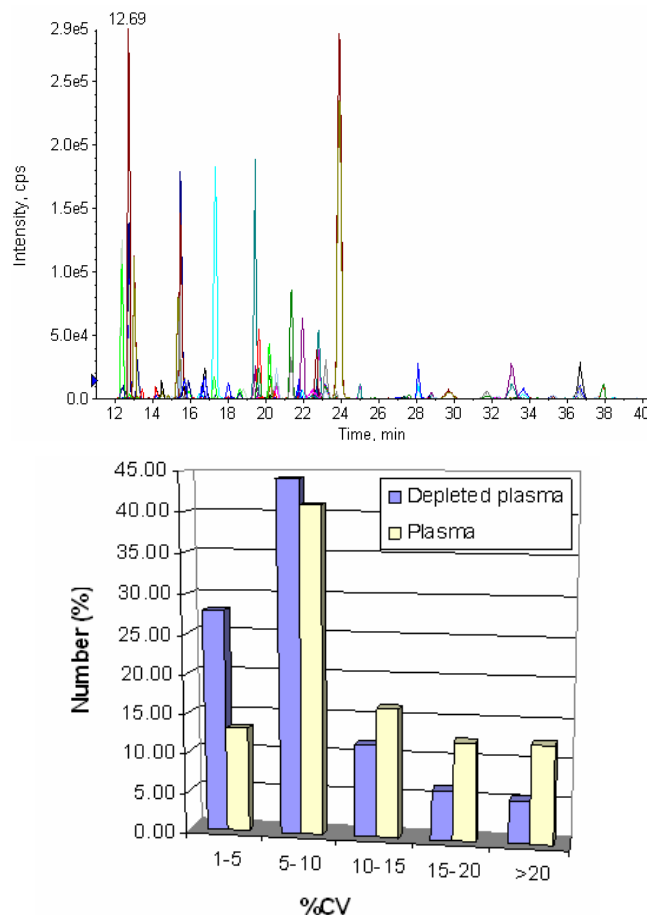
Figure 3 shows the LC/MS base peak intensity chromatograms for a protein digest consisting of ~30 digested proteins. This digest was analyzed repeatedly with a short 15 min gradient on a monolithic column (Merck, 100  $\mu$ m Chromolith, 1  $\mu$ L/min) on the QSTAR® XL system. Typical peak widths at half peak height of 6-12 seconds were observed across the gradient and good run-to-run reproducibility was achieved.

## Quantitative Robustness in Complex Samples

The analysis of many complex samples in a highly reproducible manner is a key component of protein biomarker discovery using MS profiling as well as for the validation of protein biomarkers. The reproducibility of an LC MRM method measuring 38 proteins in a single run was assessed by measuring 10 LC-MRM replicates of the same sample of either digested or depleted digested human plasma on a 4000 Q TRAP<sup>®</sup> system. The equivalent of 10 nL of plasma (digested with trypsin) was loaded directly onto a 75  $\mu$ m C18 column (PepMap, 300 nL/min) and eluted using a rapid 30 minute gradient. With no internal standard correction, the reproducibility of the peak areas was better than 10% for the majority of the MRM transitions (Figure 4) obtained for the 60 peptides monitored. The average %CV for all MRM peak areas was 8.6% for depleted plasma (loading equivalent of 10 nL of plasma) and 11.7% for non-depleted plasma (loading 1 nL equivalent) (Figure 4). Excellent peak shape was obtained on the majority of peptides monitored, 6-15 second peak widths at half height were obtained across the gradient.

## Conclusions

- Highly reproducible retention time and peak areas are obtained with the Tempo LC systems
- Rapid gradients on complex mixtures can be used for quantitative applications with high accuracy, high LC peak resolution and reduced cycle times
- Reproducible nanoflow experiments are now routine with the Tempo nano LC systems.
- Sets a new standard for rugged, reliable low flow chromatography



**Figure 4. Quantitative Reproducibility of a Highly Complex Mixture.** (Top) LC-MRM trace of a 1D nanoflow LC run of depleted digested human plasma, elution profile of 60 different peptides was monitored. Peak areas across 10 replicates (bottom) were highly reproducible, ~70% of the data obtained in depleted digested plasma had CVs better than 10%.

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