Waters

NANOLC/MS IN QUANTITATIVE AND QUALITATIVE PROTEOMICS

I. Kass[2], S. A. Cohen[1], J. W. Finch[1], H. Liu[1], G. Gerhardt[1], K. Fadgen[1]
[1]Waters Corporation, Milford, MA 01757, USA
[2]Waters Corporation, 100 Cummings Center, Suite 407N, Beverly, MA 01915, USA

INTRODUCTION

Advances in instrumentation have become one of the most significant driving forces in advancing proteomics analyses. Among the many improvements is the widespread adaptation of nanoscale liquid chromatography (nanoLC) where the increase in MS sensitivity obtained in microscale separations is often critical for the analysis of complex proteomic samples.

Here we describe a design for nanoLC/MS that overcomes serious reproducibility problems associated with traditional split flow solvent delivery systems. Specified flow rates in the range of 200–5000 nl/min are compatible with 75–320 µm i.d. columns, and by avoiding stream splitting, provides reliable, reproducible results. Separations with either simple peptide mixtures or digests of complex cellular extracts or biofluids can be accomplished with component retention times varying less than 10 seconds (typical standard deviations <6 seconds) over extended sample sets. Examples with gradient times ranging from 30–120 minutes will be shown. This performance is critical for comparing samples quantitatively, and ensuring that differences amongst samples and controls are not an artifact of the separation step.

System components have also been designed to operate with new small particle columns that require careful control of system bandspread to limit extra-column effects that can cause excessive peak broadening. Comparisons of complex mixture analysis performed on 3.0 and 1.7 µm particle columns shows that separations with the smaller particle columns yield peak widths half of the large particle ones. The enhanced peak capacity attained is highly beneficial in the analysis of complex sample mixtures, simplifying the interpretation of MS and MS/MS data, and the reduced peak volumes also result in significantly greater peak response, routinely achieving low femtomole to attomole sensitivity on 75 µm i.d. columns. Both quantitative and qualitative comparisons of complex extract digests show clear advantages stemming from the increases in resolution and reproducibility achieved, and are eminently suited for biomarker identification studies.

Capillary- and Nano-Scale

Column Internal Diameter	Flow Rate
300 µm	4 µL/min
150 µm	1 µL/min
75 µm	250 µL/min

Flow Rates and Column Internal Diameters for Capillary and Nanoflow. Capillary-scale separations are done in a flow rate range of 1 µl/min to 10 µl/min on columns with internal diameters (i.d.) ranging from 300 µm to 100 µm. Nanoflow is <1 µl/min on columns with i.d. ≤75 µm.

ADVANTAGES OF DIRECT FLOW VS. FLOW SPLITTING

Ease of Use

- Direct-flow delivery of nanoACQUITY UPLC™ system produces consistent liquid flow
- Does not require constant manual check of flow rate, change of pre-split flow tubing, or manual change of pre-split flow to achieve proper flow rate
- Columns with different particle size, chemistries, lengths, etc. can be used without having to recheck or alter system parameters

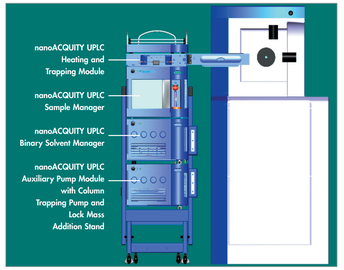
Better Reproducibility

- With flow splitting, flow rate can change with changes in pressure resistance of the two flow streams, causing shifts in retention time from run-to-run
- Build up of particulate matter on inlet of column, etc.
- Partial blockage or restriction in fluidic path (tubing, fittings, valves, frit filters, etc.)
- Partial blockage or restriction of spray tip

High Pressure Capabilities

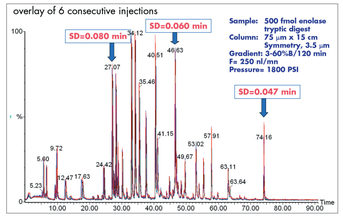
- Smaller particle size (higher efficiency) nanocolumns
- Longer nanocolumns
- Higher flow rates

Waters® nanoACQUITY UPLC™ Q-Tof Premier™ System Schematic



The nanoACQUITY UPLC System is designed for nanoflow with the Q-Tof Premier with integrated features for column trapping and NanoLockSpray™ addition. The nanoACQUITY UPLC binary solvent manager provides direct, non-spilt flow rates for columns with internal diameters ranging from 75 µm to 300 µm, typically 200 nl/min to 5 µl/min.

nanoACQUITY UPLC Reproducibility on Conventional HPLC Particles

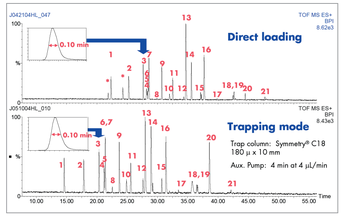


The retention time reproducibility at nanoflow on a 75 µm i.d. column achieves values typically seen with analytical-scale separations on 3.9 mm i.d. columns.

Comparison of Performance of Direct-Loading Mode vs. Trapping-Column

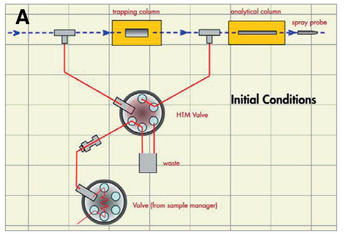
100 fmol Enolase Digest Separated with A 75 µm x 100 mm ACQUITY UPLC™ nanocolumn

5% B to 55% B in 30 minutes

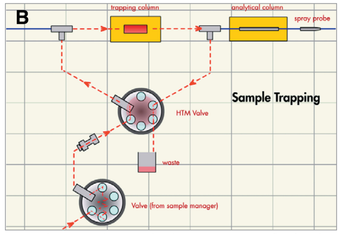


*No Effect on Resolution with Column Trapping. Sample was loaded directly from the Sample Manager in the top panel labeled "Direct Loading" and then separated with a linear water/acetonitrile/formic acid gradient. The lower panel shows the separation in "Trapping Mode." Chromatographic motif and resolution are retained. The * indicate loss of 2 hydrophilic peptides in column trapping mode. These peptides may not have been retained by the trapping column.*

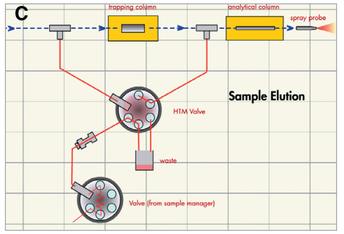
nanoACQUITY UPLC Heating and Trapping Module



Column Trapping is used to load, concentrate and desalt large sample volumes before separation at nanoflow. In preparation for sample loading, the nanoUPLC eluent flows from the Binary Solvent Module to the trapping column and analytical column as indicated by the blue dashed lines. The Sample Manager Injector and Heating and Trapping Module (HTM) valves are not in the flow path, as indicated by the solid red lines.



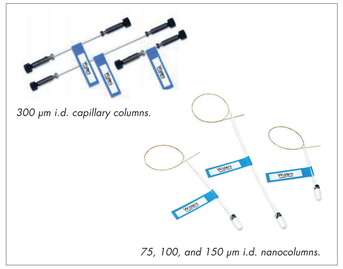
For sample trapping, the HTM valve changes state to permit flow from the trapping pump on the Auxiliary Pump Module through the Sample Manager to load sample on to the trapping column (red dashed lines). Flow from the Binary Solvent Module is stopped during sample loading. Sample is loaded on to the trapping column; salts and other non-retained solutes elute from the trapping column to waste.



After trapping the sample on the trapping column, the HTM valve changes state and gradient flow from the Binary Solvent Manager commences. Sample moves from the trapping column to the analytical column for analysis by MS.

NanoEase™ Analytical Capillary and nanoColumns

- Novel frit technology and design
- Consistent backpressure
- Robust and easy to handle
- Superior chromatographic performance
- Long column lifetime
- Easily interfaces with LC/MS/MS system
- Available in variety of WatersTM stationary phases: Symmetry® C_{18}, Atlantis™ dC_{18}, Xterra® MS C_{18}
- New: ACQUITY UPLC™ 1.7 µm bridged-hybrid particle C_{18}



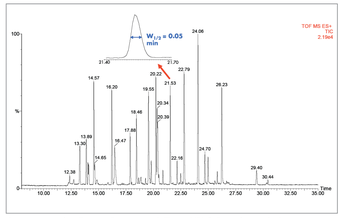
300 µm i.d. capillary columns.

75, 100, and 150 µm i.d. nanocolumns.

nanoUPLC Performance with1.7 µm ACQUITY UPLC Particles with Direct Nanoflow

5% B to 40% B in 30 minutes, 350 nL/min flow rate, 50 fmol enolase digest, 0.5 sec/spectra

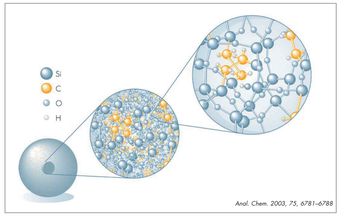
75 µm id x 150 mm packed-pulled tip nanocolumn at 8,000 psi



Separation of a Tryptic Digest on 1.7 µm UPLC Particles with the nanoACQUITY UPLC System at operating at 8,000 psi. Average peak width at half-height is 0.05 min, indicating the ability to achieve higher peak capacity separations.

ACQUITY UPLC™ New 2nd Generation Hybrid Bridged EthylSiloxane/Silica Hybrid Particles

- Bridged Ethanes in Hybrid Matrix
- Improved Strength
- 1.7 mm particle size
  Highly uniform
  Very narrow size distribution
- Improved Efficiencies
- Improved Peak Shape
- Wider pH Range
- Used in ACQUITY UPLC™ Columns



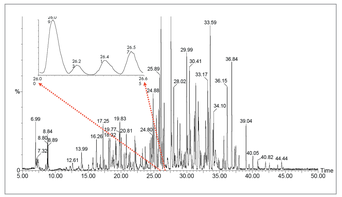
The UPLC particle combines the attributes of a silica-based particle with those of a polymeric support. The UPLC particle has excellent performance at higher operating pressures while retaining good loading capacity and resolution.

Increased Sensitivity with 1.7 µm ACQUITY UPLC Particles with Direct Nanoflow

5% B to 40% B in 60 minutes, 350 nL/min flow rate, 0.5 sec/spectra

5 protein MassPREP™ digest standard mixture, 20 fmol each of enolase, phosphorylase b, hemoglobin, ADH, BSA

75 µm id x 135 mm packed-pulled tip nanocolumn at 6,200 psi



Increased Information with nanoACQUITY UPLC. Better resolution and increased peak capacity allow isolation of low-abundance peptides in this separation of a tryptic digest of five standard proteins. The inset shows a section of the separation encompassing 0.65 minutes. This section shows 4 peptides that are baseline resolved. If permitted to coelute into the MS, it is possible that information could have been lost.

Waters® nanoACQUITY UPLC™ and Q-Tof Premier™ A complete and integrated proteomics LC/MS/MS system

nano Acquity Ultra Performance LC™

CONCLUSIONS

- Innovative Technology for Nanoflow

  Based on award winning ACQUITY UPLC Familiarity of Separation System and Principles
- Confident Results

  Reliable, Reproducible Direct Nanoflow

  Advanced Diagnostics Support

  nanoLockSpray Support for Optimized Exact Mass Measurement
- Increased Productivity

  Improved Resolution

  Better Reproducibility

  More Information per Separation