DESIGN FEATURES AND PERFORMANCE OF A DIRECT-FLOW NANOSCALE HPLC SYSTEM COMBINED WITH COLUMNS PACKED WITH SUB 2 MICRON PARTICLES

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OVERVIEW

Purpose:

To design and demonstrate performance of a rugged, splitless nano– and capillary scale HPLC that yields excellent retention time reproducibility, can be used in trapping or direct-inject mode, and can withstand high operating pressures to maximize chromatographic resolution.

Methods:

75 µm ID nanocolumns packed with 1.7 µm C18 particles
Trapping and direct-inject mode
Mass spectrometry on Q-ToF instruments

Results:

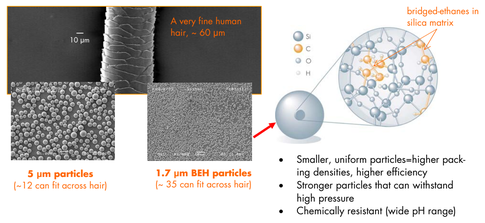
Minimal loss in resolution with trapping mode
Peptide retention time standard deviations typically < 0.1 min
Analysis of complex biological digest samples with minimal carryover
UPLC for nanoscale chromatography (3,000 to 10,000 psi)

INTRODUCTION

Due to higher MS sensitivity, nanoscale chromatography is often the method of choice for analysis of complex proteomic samples. Here we present results from the Waters nanoACQUITY UPLC™ system, a new nanoscale HPLC platform with the following features:

- Direct flow without splitting
- Based on ACQUITY UPLC™ platform
- Accurate gradient delivery for excellent retention time reproducibility
- Binary high-pressure mixing pump with wide dynamic flow range
- Operation at elevated pressures for nanocolumns packed with particles < 2 microns
- Heating Trapping Module (HTM) with simplified "forward flush" sample trapping scheme and integrated column heater
- Consumables that deliver consistent performance
- Nanocolumns packed with ACQUITY 1.7 µm bridged-ethyl hybrid (BEH) particles that provide higher separation efficiency
- Variable flow chromatography ("peak parking") capability
- Dedicated auxiliary pump for providing stable flow of "lockmass" solution to reference sprayer of Q-Tof NanoLockSpray source

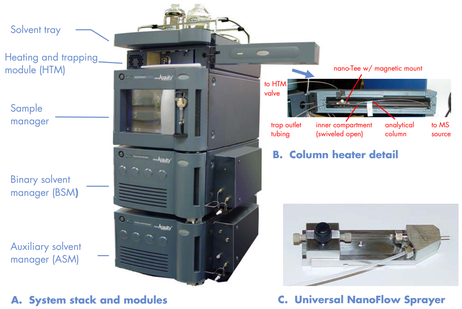
Figure 1. Columns packed with bridged-ethyl hybrid particles



- Smaller, uniform particles=higher packing densities, higher efficiency
- Stronger particles that can withstand high pressure
- Chemically resistant (wide pH range)

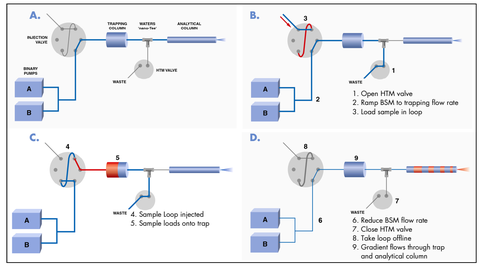
METHODS

Figure 2. nanoACQUITY UPLC system



Components of the nanoACQUITY UPLC system (Figure 2A) are designed for operating at pressures up to 10,000 psi. The column heater (Figure 2B) of the HTM is mounted on a pivot which positions the column outlet close to the MS source to minimize delay volume. All fused-silica tubing in the sample path is preassembled with PEEK protective cladding, fittings, and pre-cut polished ends to minimize dead volume. The Sample manager is completely enclosed and can maintain samples at 4 °C to 40 °C. The needle and sample loop can be flushed with both a strong and weak solvent wash, eliminating sample carryover. The NanoFlow Sprayer (Figure 2C) minimizes postcolumn dead volume in an easy to use design.

Figure 3. Simplified sample loading/trapping scheme



The wide dynamic flow range of the BSM makes it possible to configure the system for sample loading/trapping using the scheme shown in Figure 3. This greatly simplifies system fluidics, allows for robust operation in a "forward flush" mode, and eliminates the need for an extra loading pump.

RESULTS AND DISCUSSION

Figure 4. Direct inject vs. trapping performance

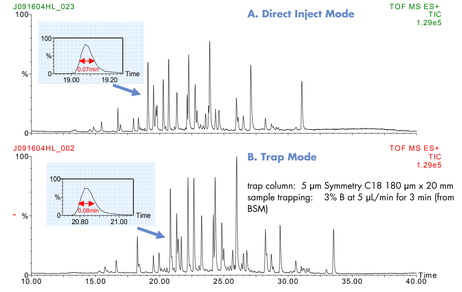


Figure 4 compares LC/MS traces for enolase digest acquired using the same analytical 1.7 µm BEH column for direct inject vs. trap modes. The data demonstrates that there is no significant loss in chromatographic resolution of the peptides with the addition of a trap column. The data also shows that sample losses are minimized.

Figure 5. Performance with different gradient lengths

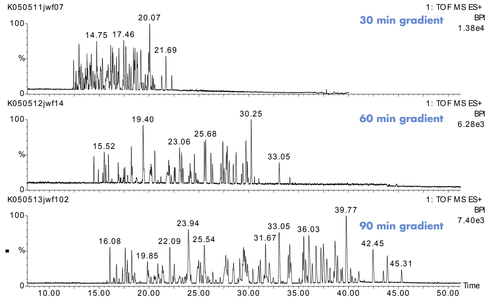


Figure 5 shows the effect of different gradient lengths on performance. The column pressure for these runs was approximately 5,200 PSI.

Figure 6. LC/MS reproducibility at 300 nL/min, n = 6

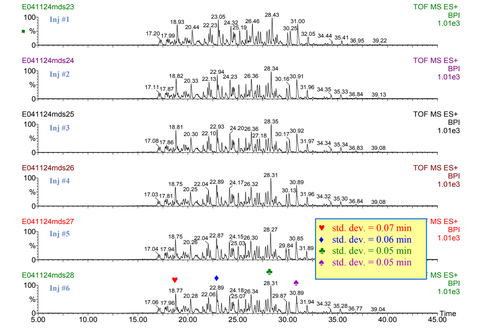


Figure 6 shows traces from six consecutive injections of a 5 protein digest, along with retention time std. dev. of four peptides. The data set demonstrates that the nanoACQUITY/Q-Tof yields good retention time reproducibility (std. dev. < 0.1 min) and reproducible MS response over an extended period of time. The 1.7 µm BEH column provides greater separation efficiency for peptides compared to columns packed with conventional particles (3 to 5 µm).

Figure 7. Biological samples: in-gel and in-solution digests

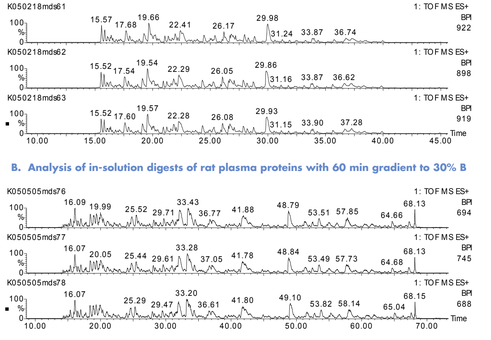
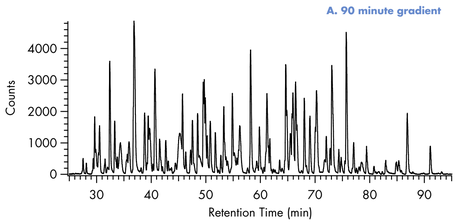


Figure 7 shows triplicate injections of in-gel and in-solution digests of biological tissue or fluid samples collected from rats. These chromatograms were obtained after 48 (Fig. 7A) and 57 (Fig. 7B) prior injections of similar samples on the column and trap. Highly reproducible retention times and ion intensities enable calculation of accurate peptide ratios with Protein Expression informatics.

Figure 8. UPLC on a nanoLC scale (9,000 psi)

MassPREP digest mixture (enolase, phosphorylase b, ADH, BSA), analytical column: 1.7 µm BEH C18 75 µm x 300 mm, 300 nL/min, gradient: 7-30% B for 90 min, trap column: 5 µm Symmetry C18 180 µm x 20 mm, sample trapping: 2% B at 4 µL/min for 3 min, column backpressure ~9,000 psi



MassPREP E. coli digest standard, analytical column: 1.7 µm BEH C18 75 µm x 300 mm, 300 nL/min, gradient: 7-30% B for 5 hours, trap column: 5 µm Symmetry C18 180 µm x 20 mm, sample trapping: 2% B at 4 µL/min for 3 min, column backpressure ~9,000 psi

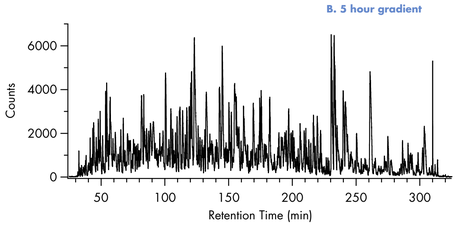


Figure 8 shows chromatograms from injections on a 30 cm BEH nanocolumn with system pressures exceeding 9,000 psi. Median peak widths (4σ) measured from SIC's of peptides throughout the gradient were 23 sec (Fig. 8A) and 54 sec (Fig. 8B), respectively.

CONCLUSIONS

Features and advantages of the nanoACQUITY UPLC include:

- Direct nanoflow delivery without splitting
- Highly reproducible retention times over extended analysis periods
- Binary high pressure pump with wide dynamic flow range
  - -supports analytical column flow rates from 100 to 5000 nL/min
  - -simplified sample loading/trapping configuration
- High pressure capabilities enabling the use of nanocolumns packed with particles < 2 µm and lengths up to 300 mm
- Columns packed with 1.7 µm bridged-ethyl hybrid particles
  - -higher efficiency, higher resolution, greater selectivity
- Reproducible ion signals for quantitative proteomics

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