Nanobore RPHPLC: Determining The Role of Selectivity in Method Development

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Introduction
Nanobore chromatography coupled with ESI mass spectrometry has proven to be an excellent approach for both peptide identification and protein quantitation in the fields of proteomics and biomarker discovery. The growth of quantitation and quantitation approaches employing nanobore RPHPLC intensifies the need for robust separations with excellent RSDs on the nanobore scale. In recent years, the introduction of sub-2 µm phase-porous phases and UHPLC has enabled scientists to perform highly efficient separations, necessary for the analysis of highly complex biological matrices. Regardless of particle size, predicting the selectivity of a specific stationary phase for a specific analyte is virtually impossible. Selecting the correct stationary phase for analytical separation is a key part of method development and method optimization. One way we survey a sample of the many different stationary phases available on nanobore column formats is to evaluate their selectivity. All experimental conditions were held constant while the stationary phase—or the separation it generated—under these conditions—was the variable. HPLC of a commercially available IBA digest was repeated onto 1.5 µm, 5 µm (10 columns) and separated by an acetonitrile gradient of 12% to 30% in 30 minutes. All columns were comprised of the same packer material and packed with the same type of particle size. Separations were evaluated based on their retention factor and separation factors, as well as peak shape and the RSD of these values over a series of 10 injections.

Materials and Methods

Chromatographic
• Reverse phase HPLC-PDA system
• Waters 2690 Series HPLC system
• 4 deca-pentadecafluorooctyl phenyl-2-hexylphenyl polychlorinated biphenyl sol-gel; 240 nm absorbance
• Waters 600 controller/7900 mass spectrometer
• Waters 2487 variable wavelength detector
• Waters 996 photodiode array detector

Analytical Columns
• 25 µm ID packed columns with a 10 µm integral void, slurry packed to 12 cm with selected dimensions

Samples
• IBA peptide digest (Waters Masslynx)
• 20 µL (12% acetonitrile), 10 µL (7% acetonitrile)
• Step gradient: 0.1% formic acid/0.1% acetic acid from 0% to 100% over 40 min, eluting at 1 mL/min

Separation of two peptide isoforms on eight different reversed phases: separation in peak shape, retention factor and intensity time of the four peptides is apparent amongst the different columns. Selectivity of different stationary phases demonstrated in the nanobore column format.

Conclusions
• Selectivity of different stationary phases demonstrated in the nanobore column format
• Separations using Proton Graphite-Carbon (hexapods) require further investigation
• Additional stationary phases for reversed-phase separation in the nanobore-column format will be evaluated

Acknowledgments
Special thanks to members of Waters Proteomics, Barry Connolly and Bruce Whitman of New Objective for their contributions in this study.

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