

Nanobore RPHPLC: Determining The Role of Selectivity in Method Development

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Nanobore chromatography coupled with ESI mass spectrometry has proven to be an excellent approach for both protein identification and protein quantitation in the fields of proteomics and biomarker discovery. The growth of quantitation and quantitative approaches employing nanobore LC-MS stimulates the need for robust separations with excellent RSDs on the nanobore scale. In recent years, the introduction of sub-2 μ m stationary phases and UHPLC has enabled scientists to perform highly efficient separations, which are 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 your analytical separation is a key part of method development and method optimization. Here we survey a sample of the many different stationary phases available in nanobore column format to evaluate their effect on selectivity. To do so, we held all experimental variables constant and used the stationary phase—or the separation it generated under these conditions—as the variable. 100 fmol of a commercially available BSA digest was injected onto a 10cm, 75 μ m ID column and separated by an acetonitrile gradient of 2 – 50% B at 300 nl/min over 30 minutes. 5 μ m particle-size resins were employed to maintain robustness and flexibility. Multiple chemistries, including C18, C18AQ, C8, PFP and CN, on the same silica support were evaluated. Data was collected on a three dimensional ion-trap in full scan mode. Separations were evaluated based on their retention factor and separation factor, as well as peak shape and the RSD of these values over a series of 10 injections.