

PT23 A reproducible method for online RP/RP 2D nanoLC/MS

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2D, Chromatography, reproducible

Improved method of separating complex protein digests to provide more reproducible identification and absolute quantification.

An improved method for performing two-dimensional chromatography with MS for the analysis of proteomic samples has been developed. Peptides from a tryptic digest of *E. Coli* were analysed in both 1D and 2D, the samples were loaded for 1D, 2D-5 fraction, and 2D-10 fraction at 750 ng, 2.4µg, and 4.8µg respectively with three replicate injections. The 2D separation was using RP chromatography on a 150µm x 10cm XBridge™ C₁₈ (5µm) column with a discontinuous step gradient at high pH in the first dimension, followed by a low pH gradient separation on a 75µm x 15cm BEH C₁₈ (1.7µm) column in the second dimension. In order to maximize sample recovery an aqueous flow was delivered and mixed with the eluted organic-containing fractions prior to trapping on the second dimension trap column. Each separation was processed separately and then merged into one file prior to database searching with ProteinLynx Global Server with Identity^E informatics. Reproducible protein identification and absolute quantitation results were obtained from the *E. coli* samples and a high degree of intersection of protein identifications was observed.