

Fully Automatable Two-dimensional HILIC–RP Liquid Chromatography with Online Tandem Mass Spectrometry for Shotgun Proteomics

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Multidimensional liquid chromatography (MDLC) which multiplies the resolution power of individual dimension with high orthogonality is a very efficient front-end separation method for analyzing the digests of complex biological samples. Among the existing two dimensional liquid chromatography (2DLC) systems, the combination of hydrophilic interaction liquid chromatography (HILIC) followed by low-pH reversed-phase (RP)LC (HILIC-RP) has very high orthogonality and is a very promising 2DLC method. Herein, a fully automatable two-dimensional (2D) liquid chromatography system was developed for shotgun proteomics analyses, which coupling the hydrophilic interaction liquid chromatography (HILIC) TSKgel Amide 80 (a non-ionic type) with the low-pH reversed-phase (RP) chromatography. The performance of the 2D HILIC-RP LC platform was investigated at both pH 6.8 (neutral pH) and pH 2.7 (acidic pH) of the first dimension HILIC column by duplicate analyses of a *Rat pheochromocytoma* lysates. Online coupling of the neutral-pH HILIC and RP systems outperformed the acidic HILIC–RP combination, resulting in 18.4% (1914 versus 1617 non-redundant proteins) and 41.6% (12,989 versus 9172 unique peptides) increases in the number of identified proteins and peptides. To further test the established 2D HILIC-RP platform, we identified 2648 non-redundant proteins from triplicate analyses of a *Saccharomyces cerevisiae* lysate, with the detected protein abundances spanning from approximately 41 to 106 copies per cell, which contained up to 2164 different validated protein species with a dynamic range of concentrations up to approximately 104. Herein, this study established a fully automated 2D liquid chromatography platform to enable online coupling of different HILIC and RP chromatography systems, thereby expanding the choice and application of multidimensional liquid chromatography for shotgun proteomics.

Keywords:

Two-Dimensional Liquid Chromatography / HILIC–RP / Orthogonality / Proteomics

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