RESULTS AND DISCUSSION

Overview

Several alternative of 2D LC systems were evaluated:

- A geometric method for 2D LC orthogonality analysis was described
- RP-High/low pH is a promising tool for 2D proteome research

2D LC provides the highest practical peak capacity

Introduction

2D LC separation with tandem mass spectrometry (MS/MS) is a powerful tool for proteome research. 2D LC is expected to provide for a greater peak capacity (theoretical number of resolved components) than 1D LC, the peak capacity is typically calculated from equation 1.

However, a total 2D peak capacity can be calculated as multiplication of peak capacities in first and second dimensions only when the LC modes are ideally orthogonal. In this work we measured retention times of 196 tryptic peptides using 1D-MS in order to construct the normalized peptide retention maps (Figure 2) and compared the 2D LC orthogonality of 20 systems. A geometric approach for orthogonality description was developed, employing a surface area of retention plots utilized for 2D LC separation. Those retention maps were normalized according to equation 2 and plotted in a normalized 2D-LC separation space in Figure 3.

Figure 3: Geometrical approach to orthogonality description (A) 10 x 10 separation space, total 2D peak capacity=100. 100 data points were plotted in 2D space. (A) example of completely nonorthogonal 2D separation; 10% area is used.

Discussion of Figure 1: 2D retention plots

A) Total 2D peak capacity=100. 100 data points were plotted in 2D space. (type of RP stationary phase (Fig.1A) does not generate a high degree of orthogonality. The system with randomized retention data plot; in average 63% area is used.

B) Completely non-orthogonal 2D separation; 10% area is used.

C) Orthogonality of several RP modes tested is low. Varying ion-pairing buffer composition, or changing pH does not improve the orthogonality of SCX-RP system (Fig.1B). In average 70% area is used.

D) Completely orthogonal system with randomized retention data plot; in average 63% area is used.

E) Orthogonality of SCX-RP system. In part due to a secondary interaction of peptides with SEC sorbent. Partial loss of hydrophobic peptides was observed.

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