4. Reversed-phase and Mixed-mode Hydrophilic Interaction/Cation-exchange HPLC of Peptides: Rival or Complementary Techniques?

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The ongoing development of HPLC methodology continues to meet a concomitant requirement for rapid and efficient separations of peptides from a wide variety of sources, including protein digests, synthetic peptide crude mixtures and cell extracts in such areas as biochemistry, immunology and biotechnology. To date, reversed-phase HPLC (RP-HPLC) has been the most widely-used mode of HPLC for peptide separations, taking advantage as it does of, frequently, just subtle variations in hydrophilicity/hydrophobicity between peptides to achieve resolution. Mixed-mode hydrophilic interaction/cation-exchange chromatography (HILIC/CEX) was developed as a novel separation technique which could complement and/or rival RP-HPLC for peptide separations. This approach (essentially CEX in the presence of high concentrations of organic modifier) combines the most advantageous aspects of two widely different separation mechanisms: a separation based on hydrophilicity/hydrophobicity differences between peptides overlaid on a separation based on net charge.

In this course, we will consider practical approaches to maximizing the peptide resolving effectiveness of both these HPLC modes, both as individual modes and as part of a multidimensional protocol. In addition, the appropriateness of utilizing peptide standards to monitor the effectiveness of RP-HPLC and HILIC/CEX for peptide applications is stressed.

Overview of the course

(1) RP-HPLC

(a) Conditions

- effect of packing variations (e.g., C_8, C_{18}, phenyl, polar endcapped, polar embedded)
- effect of organic modifier (MeOH, CH_3CN)
- effect of peptide conformation
- temperature effects
- effect of ion-pairing reagent (H_3PO_4, TFA, PFPA, HFBA, perchlorate)

(b) Samples

- synthetic peptide standards
- protein digests
- α-helical and β-sheet antimicrobial peptides
- crude synthetic peptides

(2) HILIC/CEX

(a) Conditions

- CEX column properties
- effect of acetonitrile concentration
- choice of eluting salt

(b) Samples

- synthetic peptide standards
- peptide mixtures

- proteins

(3) Miscellaneous roles of RP-HPLC and HILIC/CEX

- physicochemical probes of peptide structure
- development of *de novo* designed peptide antimicrobials