Alternative Approach to Protein Separation by HPLC

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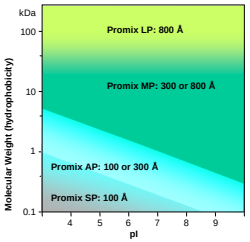
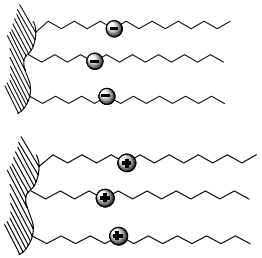
Three main technologies are widely accepted in protein and peptide separation: reversed-phase, ion-exchange, and size-exclusion. The diversity of proteins and peptides and the constant need for better separation of bio-molecules of similar structure require new and alternative means of separation. We found that peptides and proteins can be efficiently resolved with a new alternative chromatography technology. This technology is based on a combination of two interactions, hydrophobic and ionic, working at the same time. This approach is possible due to a new type of separation media constructed as a chemical combination of a hydrophobic functional group and an ionic one. The stationary phase was specially modified for separation of large molecules. Using this phase we achieved unparalleled selectivity and peak capacity. For example, molecules of insulin differing only in the position of two amino acids can be separated and identified. Peak capacity of protein digests is significantly higher in this mixed-mode separation in comparison to either separation mode alone. Similar to traditional ion separations the buffer concentration plays an important role in this new technology by altering the degree of ionic interaction of the biomolecules with stationary phase. The amount of organic modifier is equally important for the degree of hydrophobic interaction. Independent modification of the amount of buffer and organic modifier creates an infinite number of separation conditions suitable for many types of biomolecules.

Promix™ Column Selection Chart

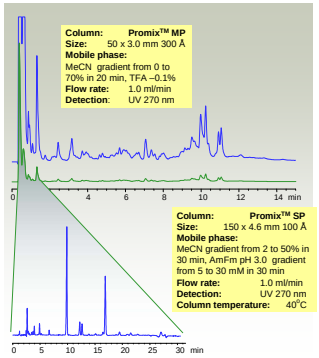


Schematic structure of Promix Mixed-mode Stationary Phases

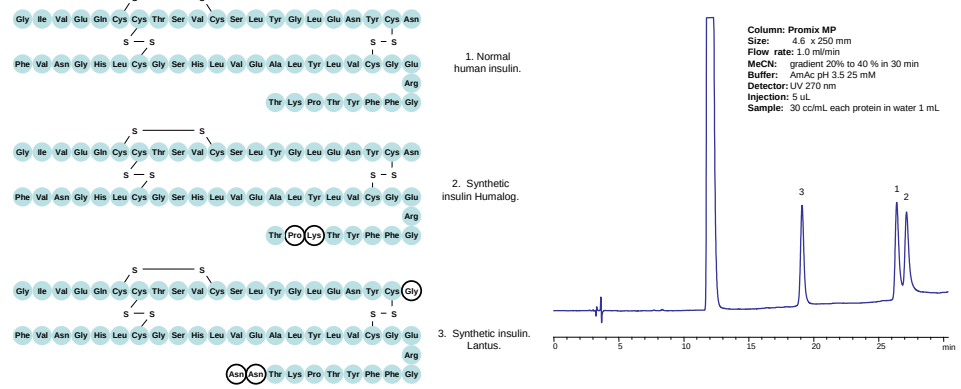


Promix SP
Promix AP

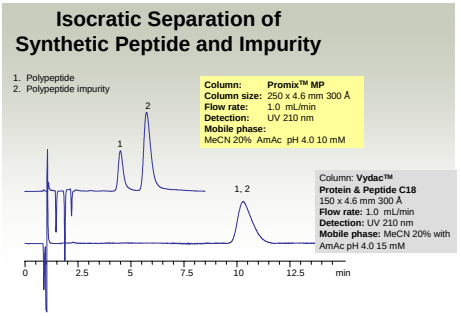
Promix MP
Promix LP



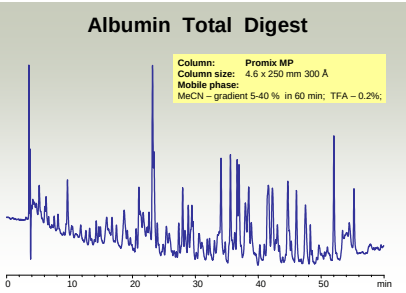
Separation of Synthetic and Natural Insulins



Isocratic Separation of Synthetic Peptide and Impurity



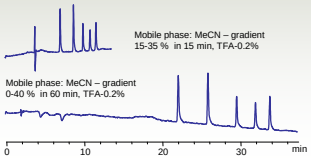
Albumin Total Digest



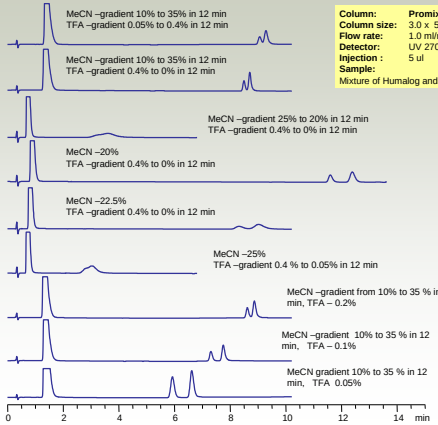
Small Peptides Test Mixture
(5 Angiotensins)

1. ARG-VAL-TYR-VAL-HIS-PRO-ILE
2. ARG-VAL-TYR-VAL-HIS-PRO-PHE
3. ALA-PRO-GLY-ASP-ARG-ILE-TYR-VAL-HIS-PRO-PHE
4. ASP-ARG-VAL-TYR-VAL-HIS-PRO-PHE-HIS LEU
5. ASP-ARG-VAL-TYR-ILE-HIS-PRO-PHE-HIS-LEU

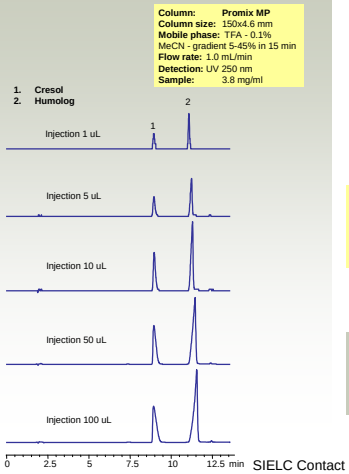
Column: Promix MP
Size: 4.6 x 150 mm
Flow rate: 1.0 mL/min
Injection: 40 uL
Detection: UV 270 nm



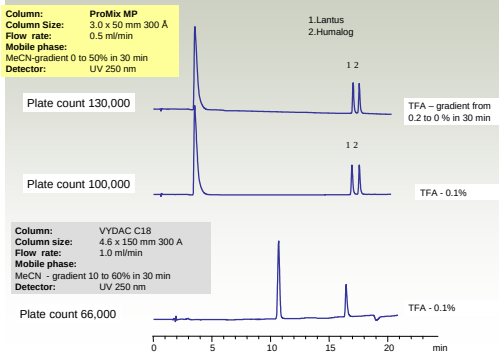
Lantus and Humalog Separation at Different HPLC Conditions.



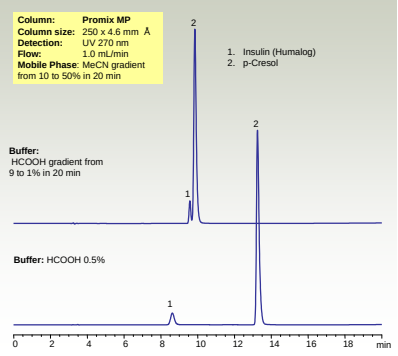
Column Loading Study



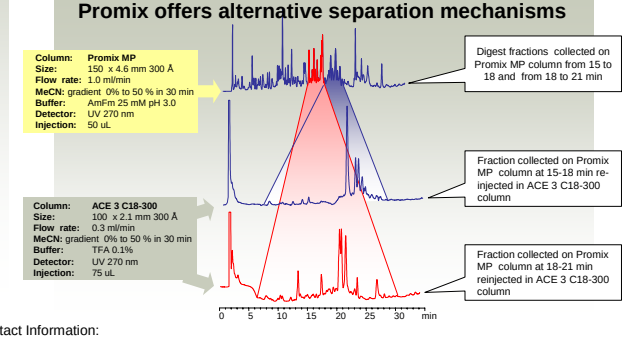
Separation of Synthetic Insulins in Gradient Conditions.



Independent retention control of small molecules and peptides.



Promix offers alternative separation mechanisms



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