Alternative Approach to Protein Separation by HPLC

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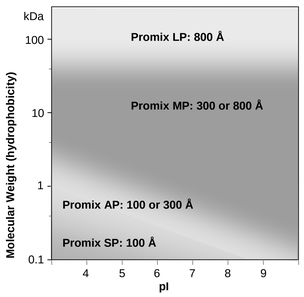
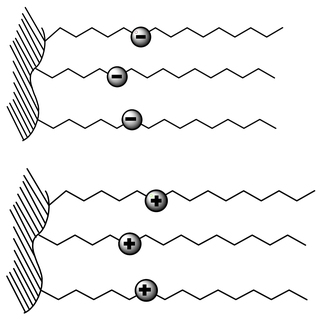
Three main technologies are widely acceptable in protein and peptide separation: reverse phase, ion-exchange, and size exclusion. Diversity of proteins and peptides and constant needs for better separation of bio-molecules of similar structure requires new and alternative ways to separate. We found that peptides and proteins can be efficiently resolved with new alternative chromatography technology. This technology based on a combination of two interactions working at the same time - hydrophobic interaction and ionic interaction. This approach became possible due to a new type of separation media which construct as a chemical combination of hydrophobic functional group and ionic one. The stationary phase was specially modified for separation of big molecules. Using this phase we achieved unparallel selectivity and peak capacity. For example molecules of insulin different only in positioning of two amino acids can be separated and identified. Peak capacity of protein digest is significantly higher in this mixed-mode separation if compare with either mode of separation when applied alone. Similarly to traditional ion separation the buffer concentration play an important role in this new technology altering the degree of ionic interaction of the biomolecules with stationary phase. Amount of organic modifier is equally important for degree of hydrophobic interaction. Modification independently of amount of buffer and organic modifier create infinite number of separation conditions that suitable for many type of biomolecules.

Promix™ Column Selection Chart

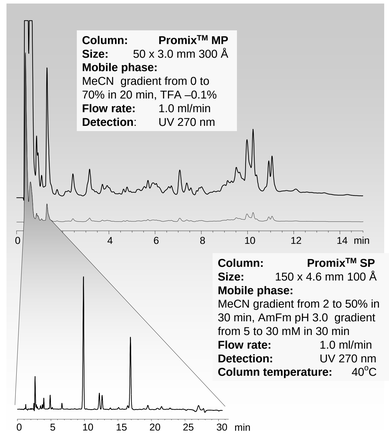


Schematic structure Promix Mixed-mode Stationary Phases

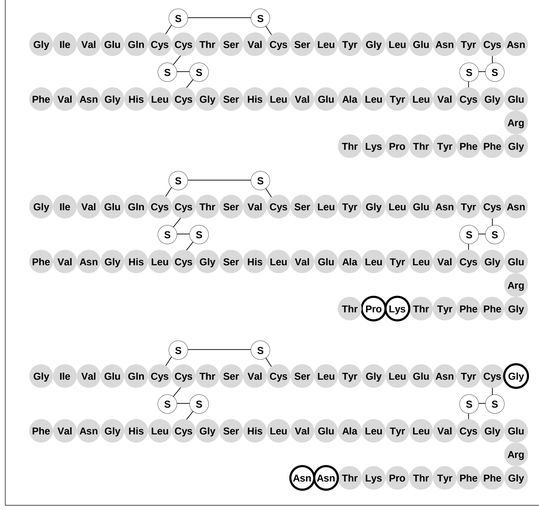


Promix SP
Promix AP

Promix MP
Promix LP



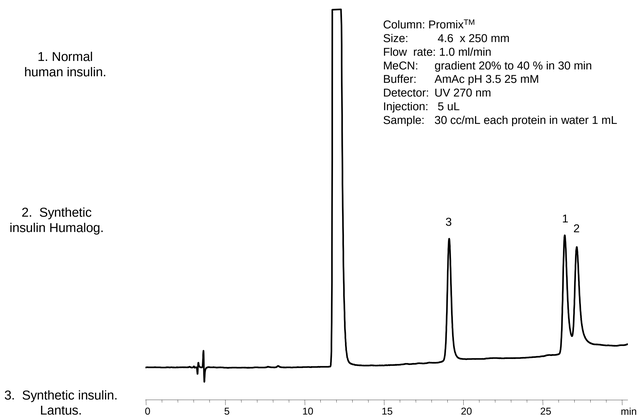
Separation of Synthetic and Natural Insulins



1. Normal human insulin.

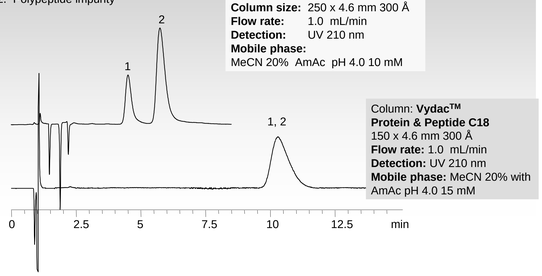
2. Synthetic insulin Humalog.

3. Synthetic insulin. Lantus.



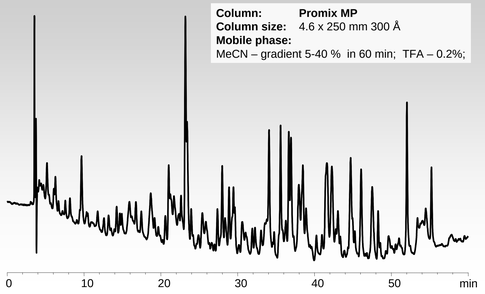
Isocratic Separation of Synthetic Peptide and Impurity

1. Polypeptide
2. Polypeptide impurity



Column: **Vydac™**
Protein & Peptide C18
150 x 4.6 mm 300 Å
Flow rate: 1.0 mL/min
Detection: UV 210 nm
Mobile phase: MeCN 20% with AmAc pH 4.0 15 mM

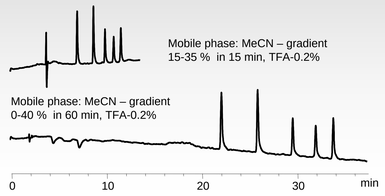
Albumin Total Digest



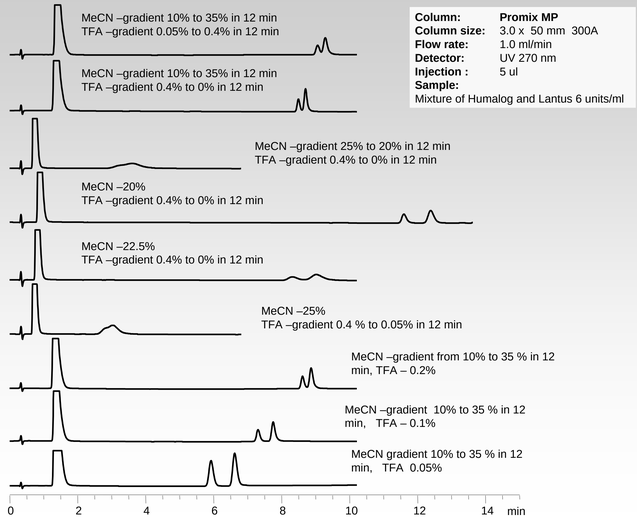
Small Peptides Test Mixture
(5 Angiotensins)

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



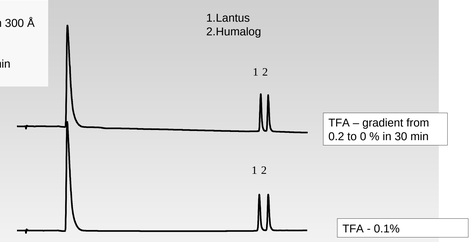
Lantus and Humalog Separation at Different HPLC Conditions.



Column: Promix MP
Column size: 3.0 x 50 mm 300A
Flow rate: 1.0 ml/min
Detector: UV 270 nm
Injection : 5 ul
Sample:
Mixture of Humalog and Lantus 6 units/ml

Separation of Synthetic Insulins in Gradient Conditions.

Column: ProMix MP
Column Size: 3.0 x 50 mm 300 Å
Flow rate: 0.5 ml/min
Mobile phase:
MeCN-gradient 0 to 50% in 30 min
Detector: UV 250 nm



Column: VYDAC C18
Column size: 4.6 x 150 mm 300 A
Flow rate: 1.0 ml/min
Mobile phase:
MeCN - gradient 10 to 60% in 30 min
Detector: UV 250 nm

Independent retention control of small molecules and peptides.

Column: Promix MP
Column size: 250 x 4.6 mm Å
Detection: UV 270 nm
Flow: 1.0 mL/min
Mobile Phase: MeCN gradient from 10 to 50% in 20 min

Column Loading Study

Column: Promix MP
Column size: 150x4.6 mm
Mobile phase: TFA - 0.1%
MeCN - gradient 5-45% in 15 min
Flow rate: 1.0 mL/min
Detection: UV 250 nm
Sample: 3.8 mg/ml

1. **Cresol**
2. **Humolog**

Promix offers alternative separation mechanism

Column: Promix MP
Size: 150 x 4.6 mm 300 Å
Flow rate: 1.0 ml/min
MeCN: gradient 0% to 50 % in 30 min
Buffer: AmFm 25 mM pH 3.0
Detector: UV 270 nm
Injection: 50 uL

Column: ACE 3 C18-300
Size: 100 x 2.1 mm 300 Å
Flow rate: 0.3 ml/min
MeCN: gradient 0% to 50 % in 30 min
Buffer: TFA 0.1%
Detector: UV 270 nm
Injection: 75 uL

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