Promix™ Columns

Two-Dimensional Separation of Proteins & Peptides

SIELC Technologies, Inc., Wheeling, IL 60090, USA

The ever-increasing utility of proteomics in biotechnology has spurred demand for effective HPLC separation of mixtures involving proteins and peptides. However, analysis is complicated by the complexity and homology of these biomolecules.

Promix™ Technology enables two-dimensional separation of peptides and proteins for purification and proteomics applications. Columns combine both ionic and reverse-phase interactions to **maximize resolution**. Promix can separate peptides that differ in a single amino acid.

Promix offers **enhanced selectivity** for closely related peptides and proteins, and **increased peak capacity** compared to ion-exchange and reverse-phase columns alone. Promix is **completely scalable** from capillary to preparatory applications. Columns are compatible with formic acid and ammonium acetate, which makes them **perfectly suited for LC-MS**.

Separation of Insulin Analogs

Synthetic insulin analogs differ from normal insulin by as little as a single amino acid pair (right).

Combining ionic and hydrophobic interactions enables Promix MP to separate these analogs by exploiting slight differences in isoelectric charge (pI) (bottom).

Independent adjustment of ionic and organic concentrations provides a diverse array of separation conditions.
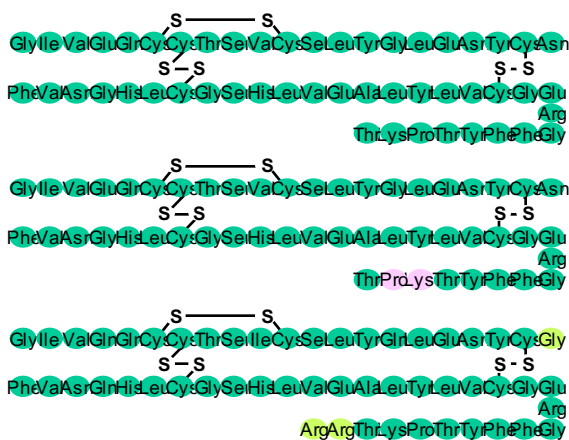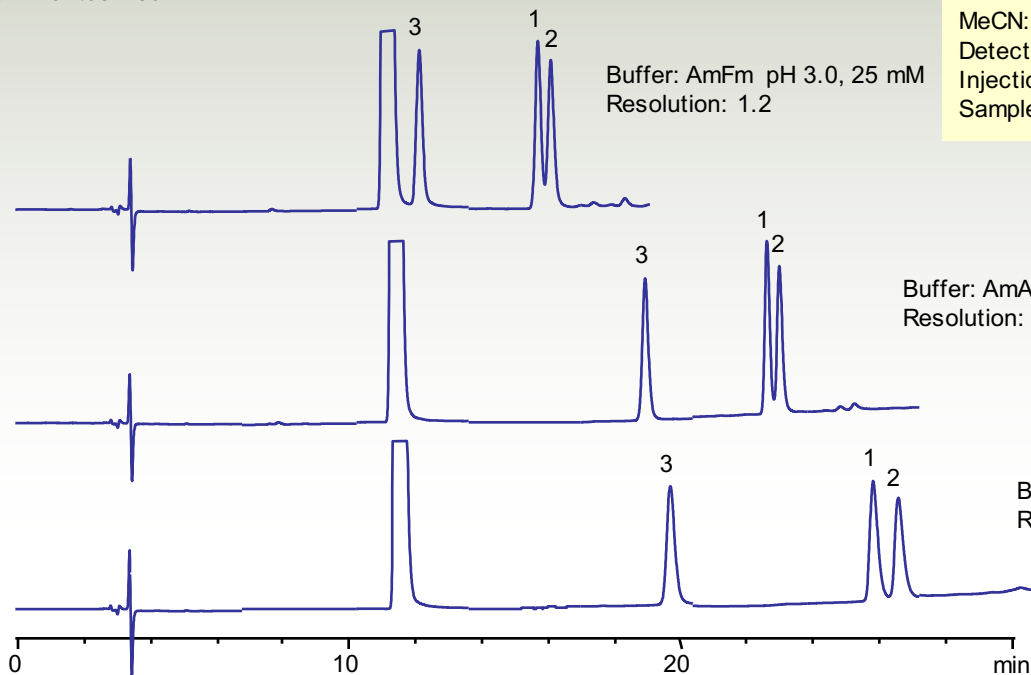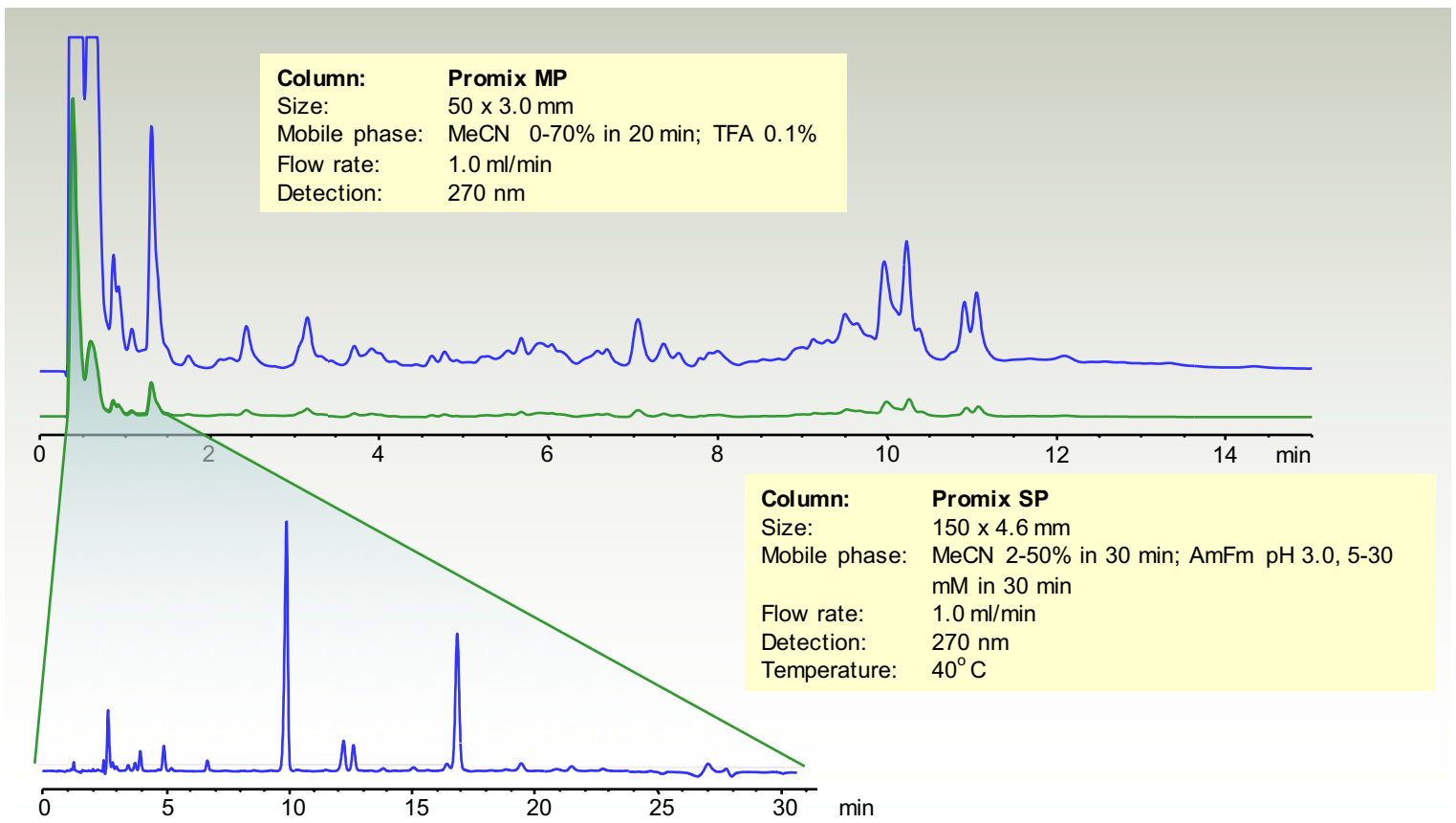

Normal human insulin
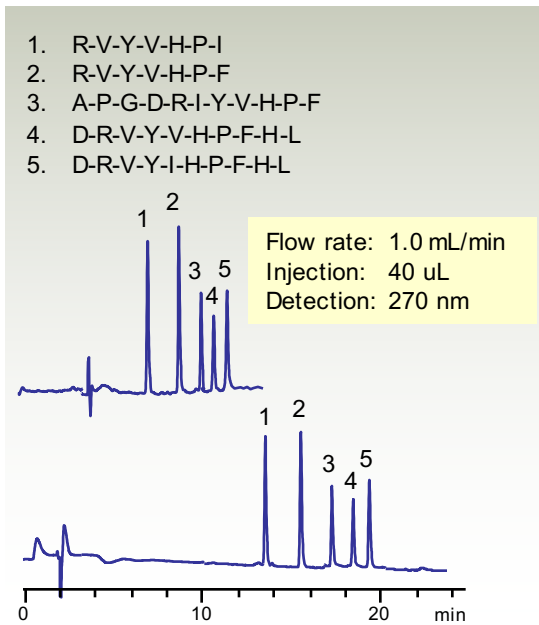
Synthetic insulin Humalog

Synthetic insulin Lantus

1. Normal human insulin
2. Humalog insulin
3. Lantus insulin

Column:	Promix™ MP
Size:	250 x 4.6 mm
Flow rate:	1.0 ml/min
MeCN:	20-40% in 30 min
Detector:	270 nm
Injection:	5 uL
Sample:	30 units/mL in water

Buffer: AmFm pH 3.0, 25 mM
Resolution: 1.2

Buffer: AmAc pH 3.5, 25 mM
Resolution: 1.3

Buffer: AmAc pH 4.0, 25 mM
Resolution: 1.7

Column: Promix MP
Size: 50 x 3.0 mm
Mobile phase: MeCN 0-70% in 20 min; TFA 0.1%
Flow rate: 1.0 ml/min
Detection: 270 nm

Column: Promix SP
Size: 150 x 4.6 mm
Mobile phase: MeCN 2-50% in 30 min; AmFm pH 3.0, 5-30 mM in 30 min
Flow rate: 1.0 ml/min
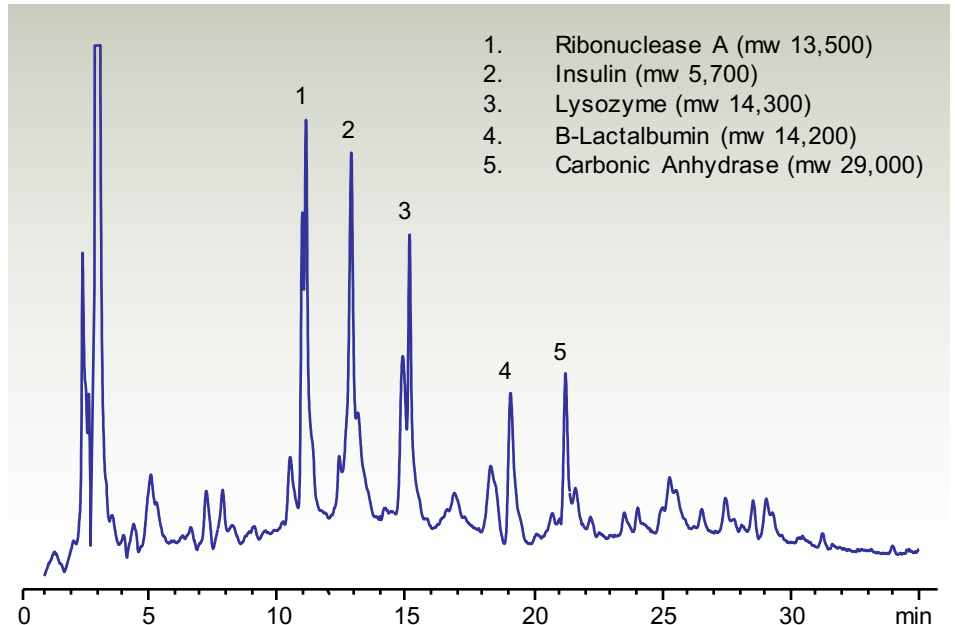Detection: 270 nm
Temperature: 40° C

Separation of a Complex Mixture containing both large and small peptides can be achieved using two different Promix columns. Small peptides eluted in the first 1.5 min using Promix MP were collected and resolved using Promix SP.

1. R-V-Y-V-H-P-I
2. R-V-Y-V-H-P-F
3. A-P-G-D-R-I-Y-V-H-P-F
4. D-R-V-Y-V-H-P-F-H-L
5. D-R-V-Y-I-H-P-F-H-L

Flow rate: 1.0 mL/min
Injection: 40 uL
Detection: 270 nm

Separation of small oligopeptides.

Top:
Column: Promix MP, 4.6 x 250 mm
Mobile phase: MeCN 15-35% in 15 min;
TFA 0.4-0.05% in 15 min

Bottom:
Column: Promix MP, 4.6 x 150 mm
Mobile phase: MeCN 0-40% in 30 min;
TFA 0.4-0.05% in 30 min

1. Ribonuclease A (mw 13,500)
2. Insulin (mw 5,700)
3. Lysozyme (mw 14,300)
4. B-Lactalbumin (mw 14,200)
5. Carbonic Anhydrase (mw 29,000)

Separation of large (>5 kDa) peptides.

Column: Promix MP
Size: 4.6 x 150 mm
Mobile phase: MeCN 20-60 % in 30 min; TFA 0.25-0.05% in 30 min
Flow rate: 1 ml/min
Injection: 100 uL
Detection: 270 nm

Column size: 250 x 4.6 mm
Flow rate: 1.0 mL/min
Detection: 210 nm

Column: **Promix AP**
Mobile phase: Gradient of MeCN from 15% to 40% in 20 min
with H_2SO_4 0.2%

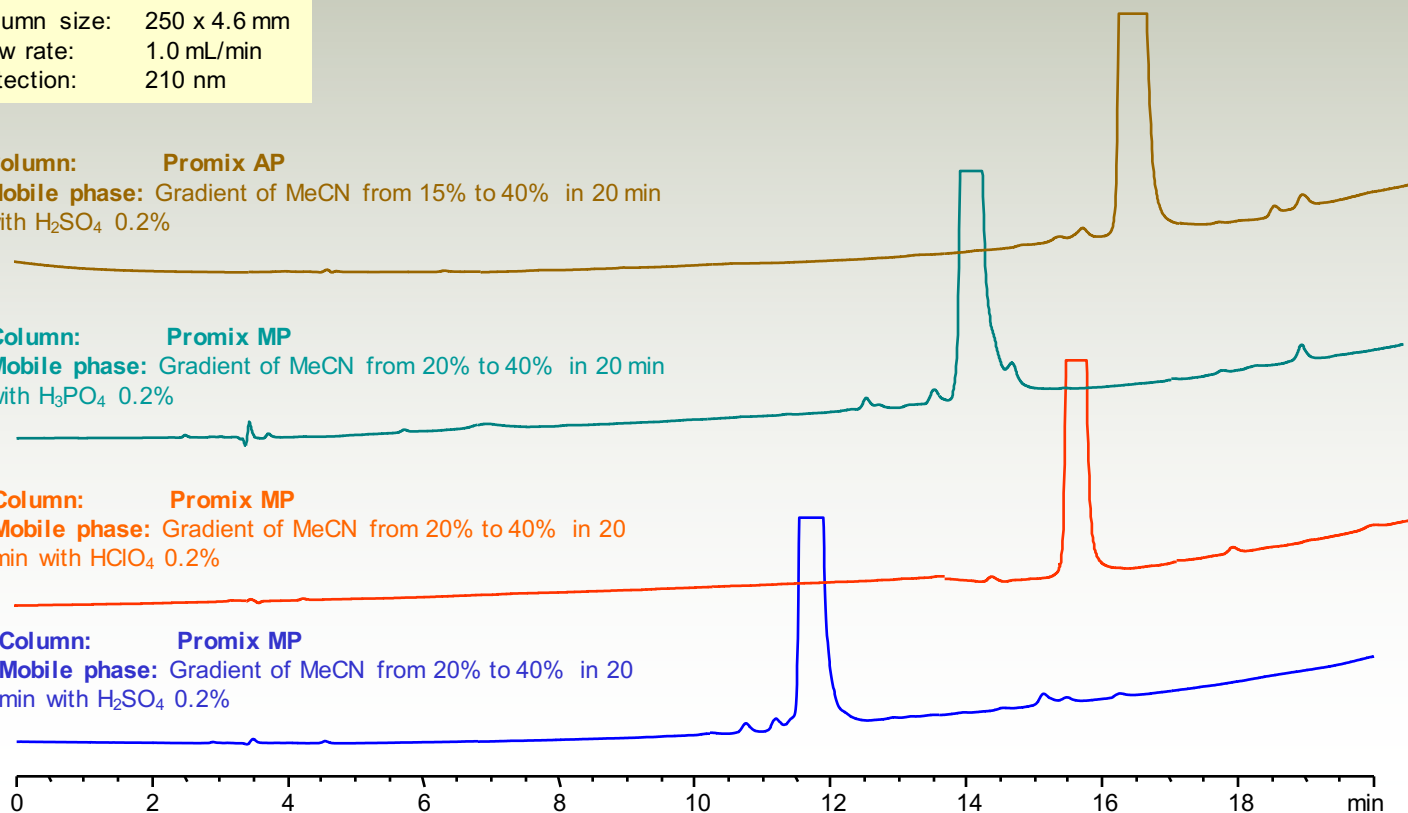Column: **Promix MP**
Mobile phase: Gradient of MeCN from 20% to 40% in 20 min
with H_3PO_4 0.2%

Column: **Promix MP**
Mobile phase: Gradient of MeCN from 20% to 40% in 20
min with $HClO_4$ 0.2%
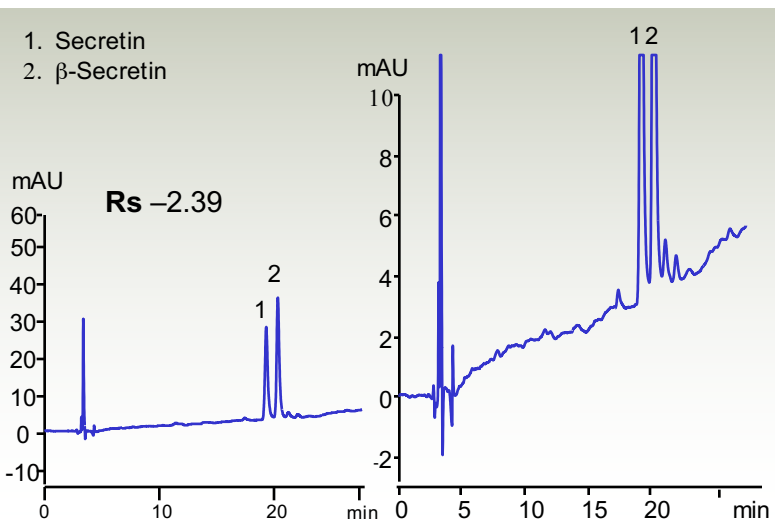
Column: **Promix MP**
Mobile phase: Gradient of MeCN from 20% to 40% in 20
min with H_2SO_4 0.2%

Selectivity adjustment by buffer type. The choice of organic and ionic buffers, and their concentrations, significantly influences elution profile.

Effect of Buffer Concentration on Albumin Selectivity.
Complex mixtures such as protein digests exhibit different profiles at different ionic strengths. Multiple gradients can be applied to complex samples: organic gradients, ionic gradients, or both. Each provides a different selectivity and elution profile.
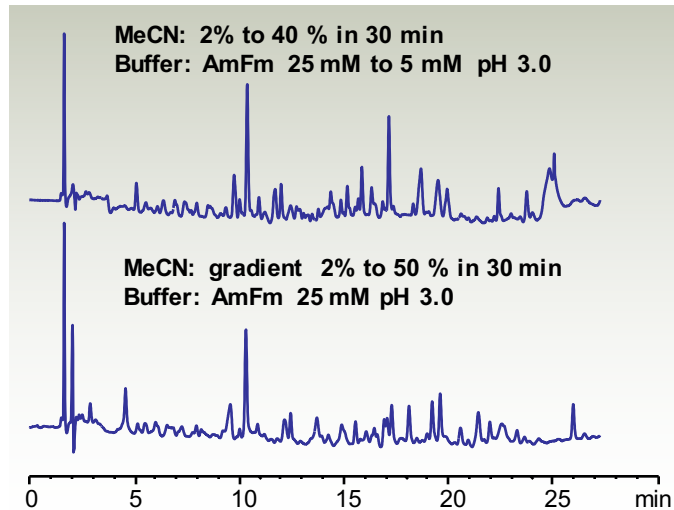
Separation of Secretin and b-Secretin.
Secretin is a protein comprised of the following 11 amino acids:
H-S-D-G-T-S-E-L-S-R-L-R-D-S-A-R-L-Q-R-L-L-Q-G-L-V.

1. Secretin
2. β-Secretin

Rs –2.39

MeCN: 2% to 40 % in 30 min
Buffer: AmFm 25 mM to 5 mM pH 3.0

MeCN: gradient 2% to 50 % in 30 min
Buffer: AmFm 25 mM pH 3.0

Column: Promix MP
Column size: 250 x 4.6 mm
Mobile phase: MeCN 10-25% in 30 min, H3PO4 pH 2.0, 10 mM
Detection: 210 nm
Flow: 1.0 mL/min
Temperature: 45ºC

Column: **Promix MP**
Size: 150 x 4.6 mm
Flow rate: 1.0 ml/min
Detector: 270 nm
Injection: 50 uL
Sample: 0.3 mg/ml