SIELC

Promix™

Enter a New Era in Biomolecule Analysis with Promix™ Columns

“Unsurpassed Selectivity and Peak Capacity for Peptides and Proteins”

Applications:

•Proteomics

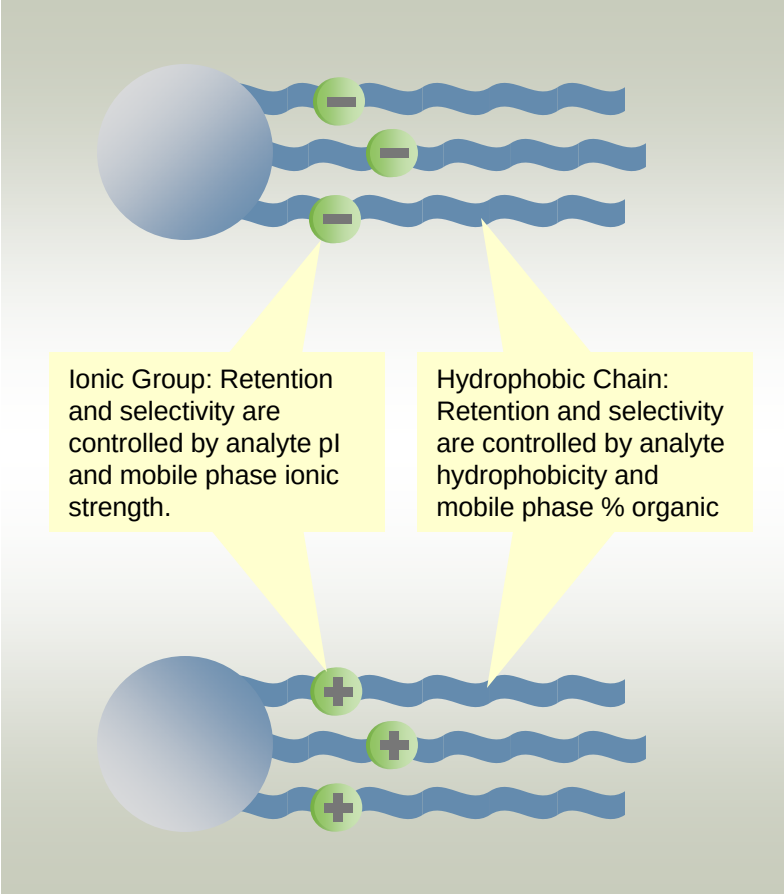
•Peptide/Protein Analysis

•Peptide/Protein Purification

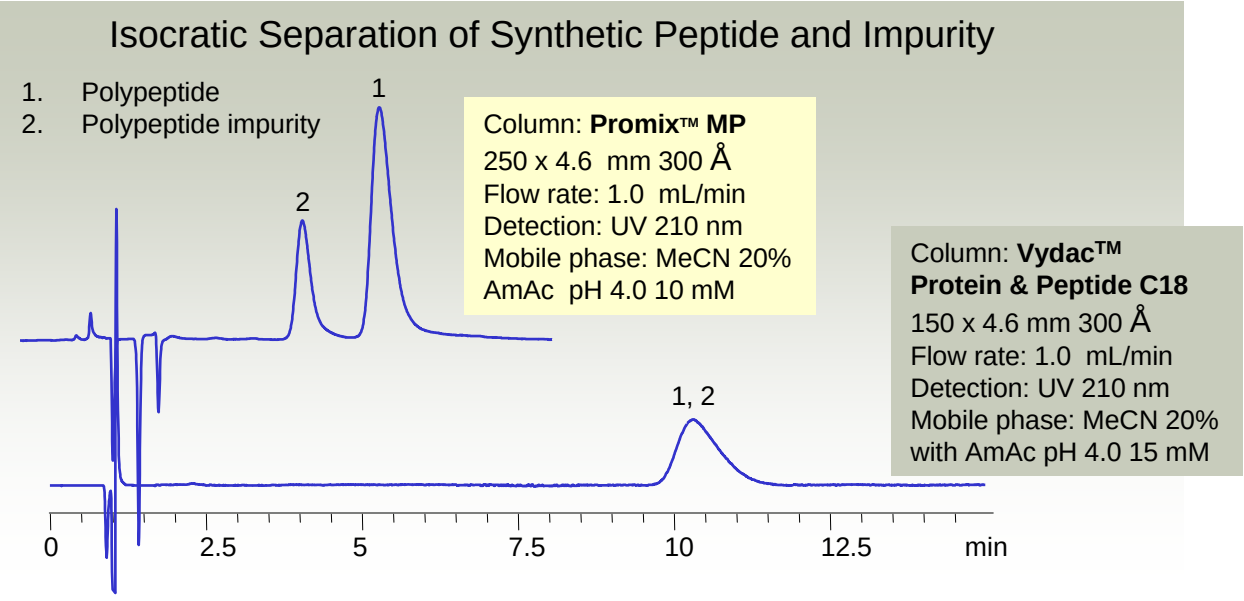
Features & Benefits:

- **2-D HPLC with a Single Column**
  - •Ionic Interactions
  - •Reversed-Phase
- Separate peptides and proteins that differ only by a **single amino acid**.
- **Enhanced selectivity** for closely related peptides and proteins.
- **Increased peak capacity** compared to reversed-phase and ion exchange.
- **Increased LC-MS sensitivity** using formic acid and ammonium acetate.
- **Completely Scalable** from capillary to prep

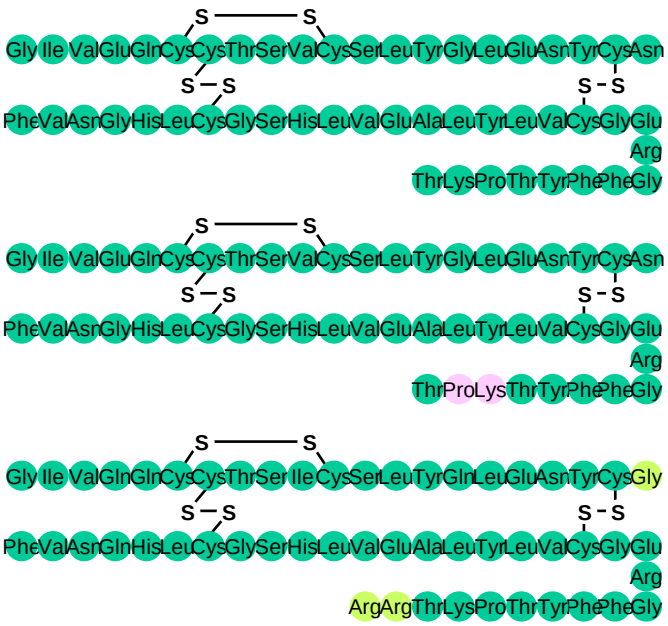
Promix™ Technology



Independent control of acid/buffer concentration and organic modifier offers almost limitless control of retention and selectivity!



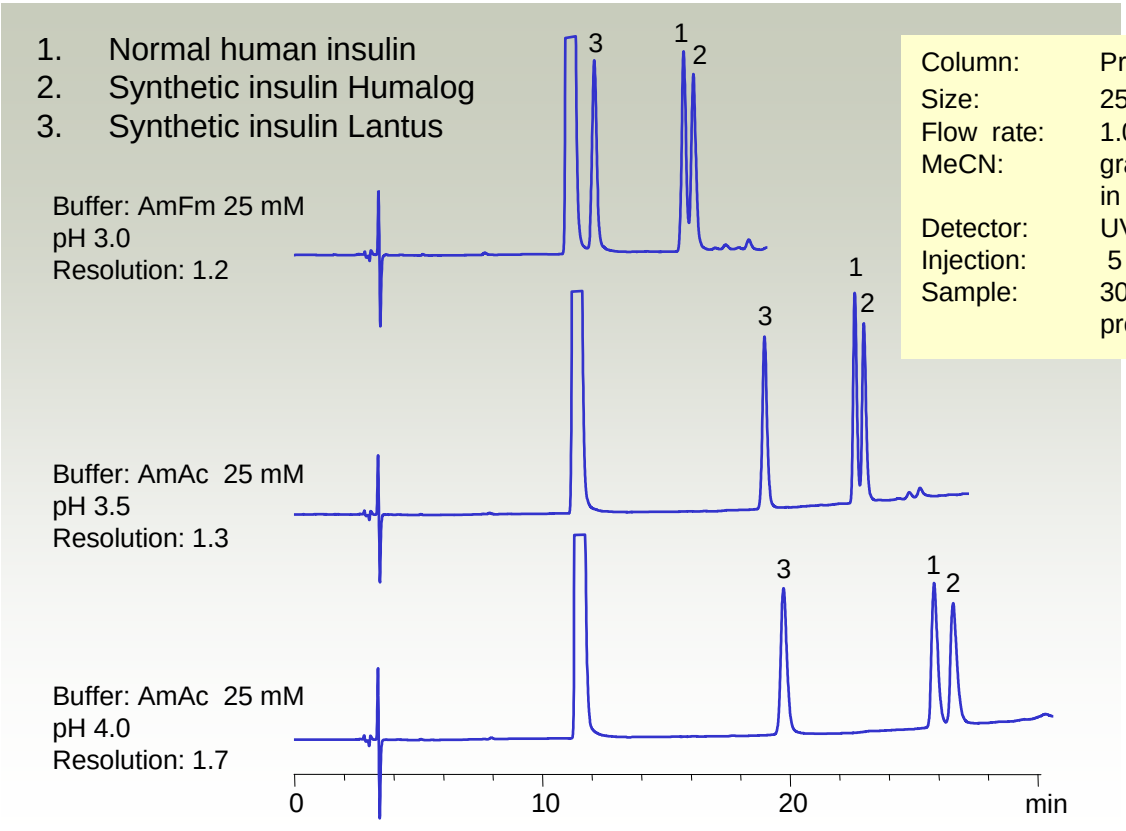
Separation of Insulin Analogs: Closely related peptides can be difficult to separate by reversed-phase. **By combining ionic and hydrophobic interactions, Promix MP is able to separate these analogs, differing in sequence by a single amino acid pair**, using the slight differences in pI as illustrated below.



Normal human insulin

Synthetic insulin Humalog

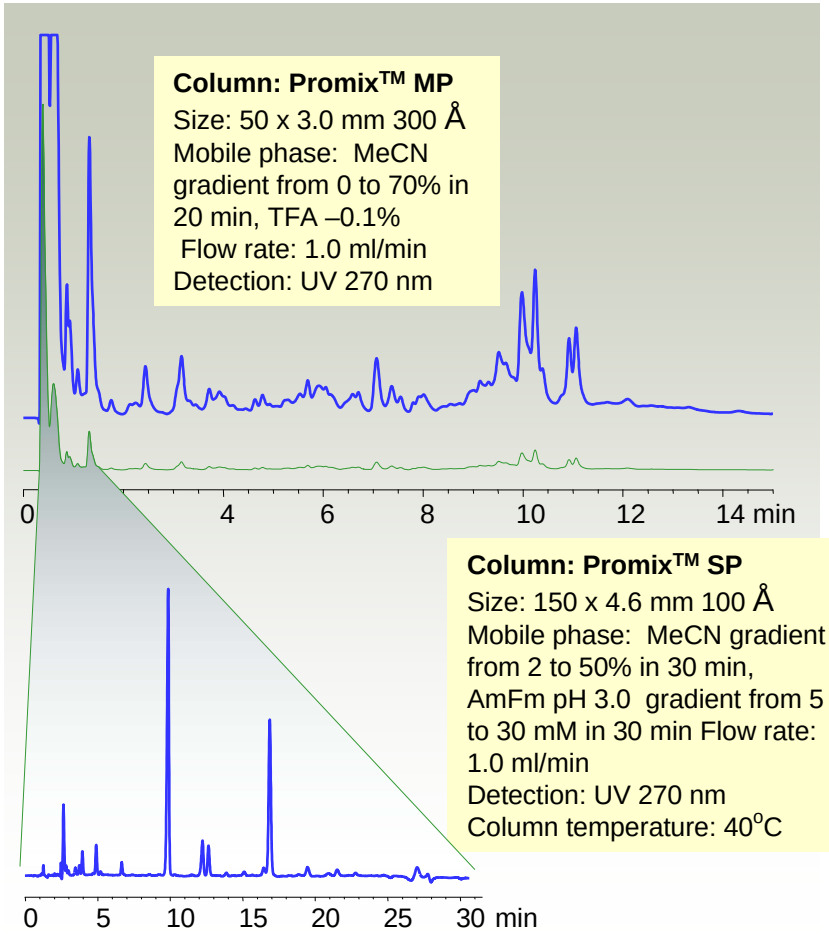
Synthetic insulin Lantus

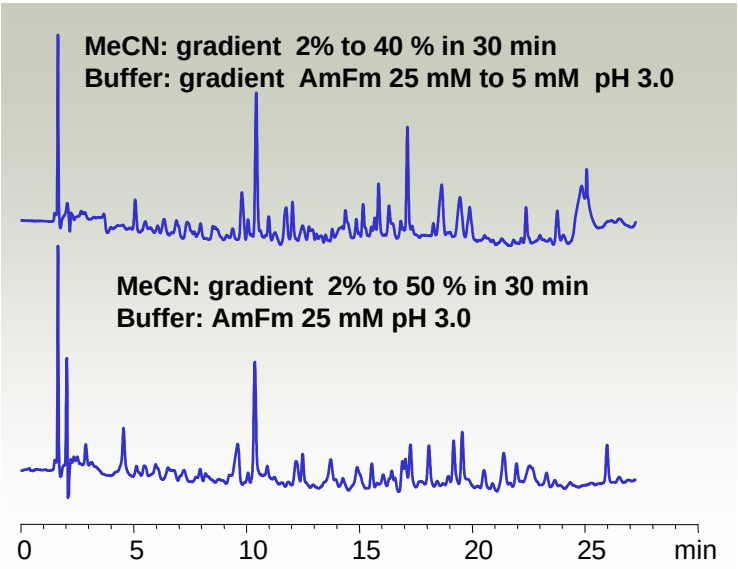


Column:	Promix™ MP
Size:	250 x 4.6 mm 300 Å
Flow rate:	1.0 ml/min
MeCN:	gradient 20% to 40 % in 30 min
Detector:	UV 270 nm
Injection:	5 uL
Sample:	30 units/mL each protein in water 1 mL

Separation of a Complex Mixture

containing very small peptides and very large peptides can be achieved using two different Promix columns. Very small peptides eluted in first 1.5 min on Promix™ MP column were collected and resolved using Promix SP column.





Albumin Digest Selectivity Affected by Buffer Concentration:

Complex mixtures such as protein digests have different profiles at different ionic strengths. Multiple gradients can be applied to complex samples:

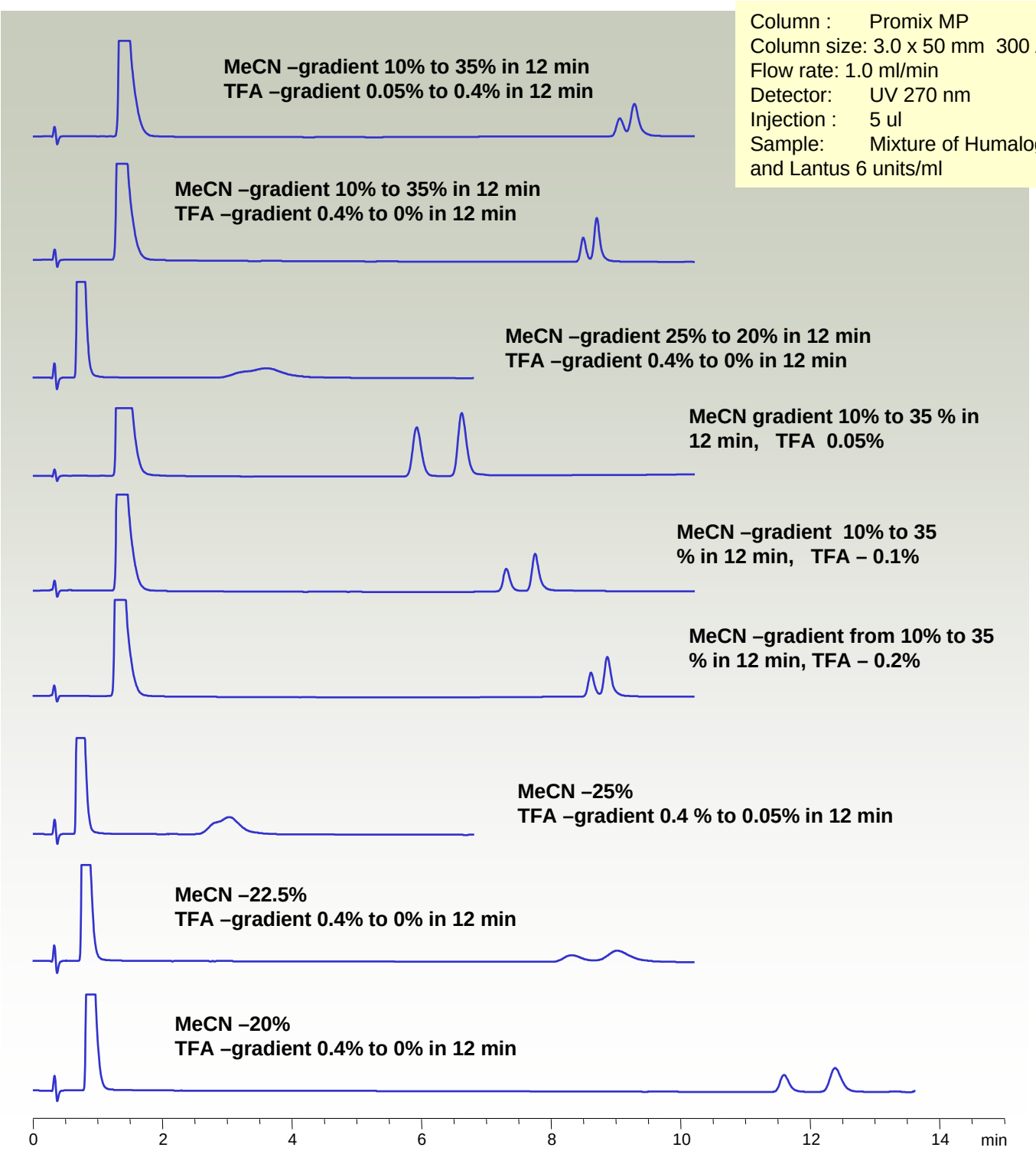
- •organic gradient
- •buffer gradient
- •or both

Each mode provides a different selectivity and elution profile.

Column: Promix™ MP
150 x 4.6 mm 300 Å
Flow rate: 1.0 ml/min
Detector: UV 270 nm
Injection: 50 uL
Sample: 0.3 mg/ml

Separation Study of Lantus and Humalog

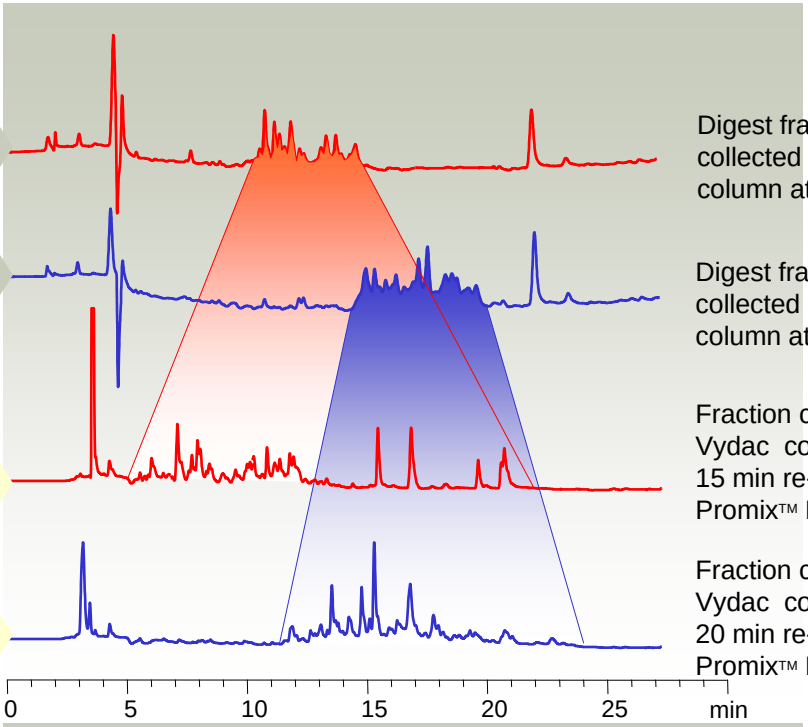
Similarly to a traditional ion separation, the buffer concentration plays an important role in the mixed-mode technology altering the degree of ionic interaction of the biomolecules with the stationary phase. The amount of the organic modifier is also important in changing the degree of hydrophobic interaction. Independent adjustment of the amount of buffer and organic modifier creates infinite number of separation conditions that are suitable for many types of biomolecules. Generally three types of gradient separation mode available on Promix columns. Gradient of MeCN with isocratic buffer concentration, gradient of buffer concentration with isocratic MeCN concentration, and dual gradient of both MeCN and buffer.



Alternative Selectivity of Promix™ vs. RP 300Å Columns: An indication of the alternative selectivity of Promix™ columns can be observed when the digest fraction collected from a Reversed-Phase column is reinjected onto a Promix™ column or visa versa. The elution time usually expands providing additional resolution and peak capacity.

Column: Vydac
Size: 150 x 4.6 mm 300 Å
Mobile phase: MeCN gradient from 0 to 70% in 30 min, TFA –0.1%
Flow rate: 1.0 ml/min
Detection: UV 270 nm
Column temperature: ambient

Column: Promix™ MP
Size: 250 x 4.6 mm 300 Å
Mobile phase: MeCN gradient from 0 to 70% in 30 min, TFA – 0.1%
Flow rate: 1.0 ml/min
Detection: UV 270 nm
Column temperature: 40C



Digest fraction collected on Vydac column at 10-15 min

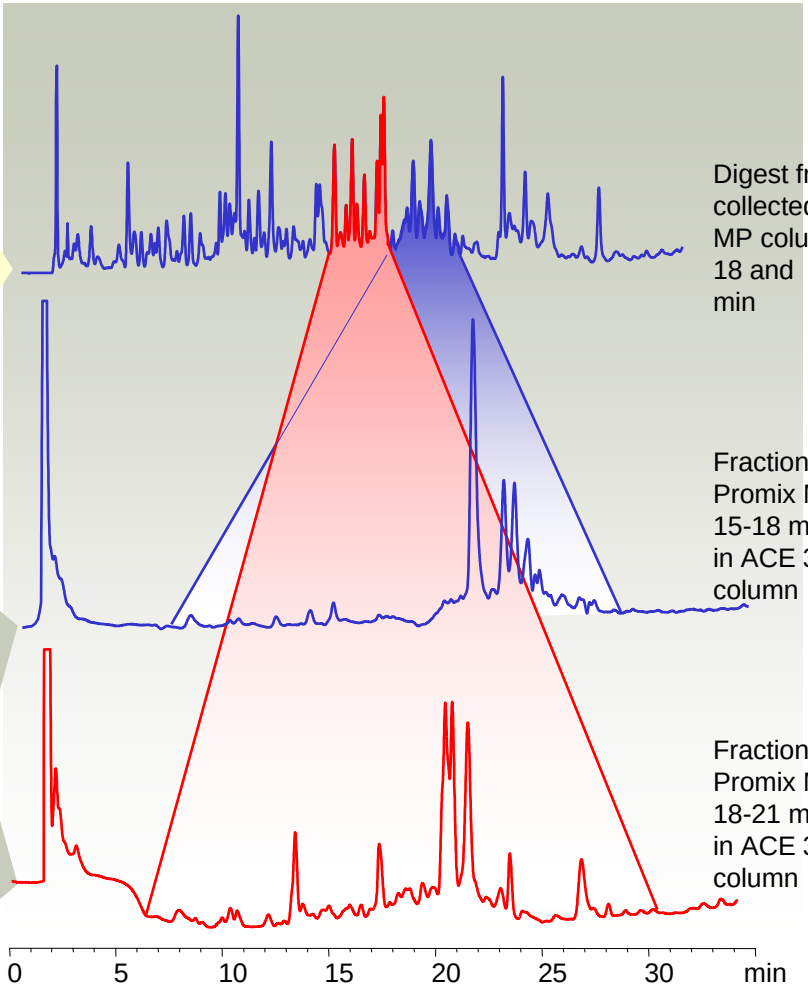
Digest fraction collected on Vydac column at 15-20 min

Fraction collected on Vydac column at 10-15 min re-injected in Promix™ MP column

Fraction collected on Vydac column at 15-20 min re-injected in Promix™ MP column

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



Digest fractions collected on Promix MP column from 15 to 18 and from 18 to 21 min

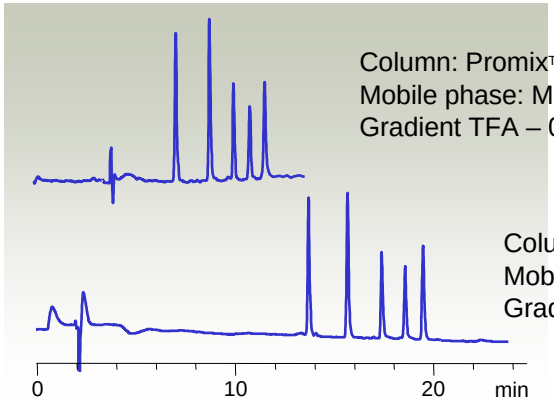
Fraction collected on Promix MP column at 15-18 min re-injected in ACE 3 C18-300 column

Fraction collected on Promix MP column at 18-21 min reinjected in ACE 3 C18-300 column

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

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

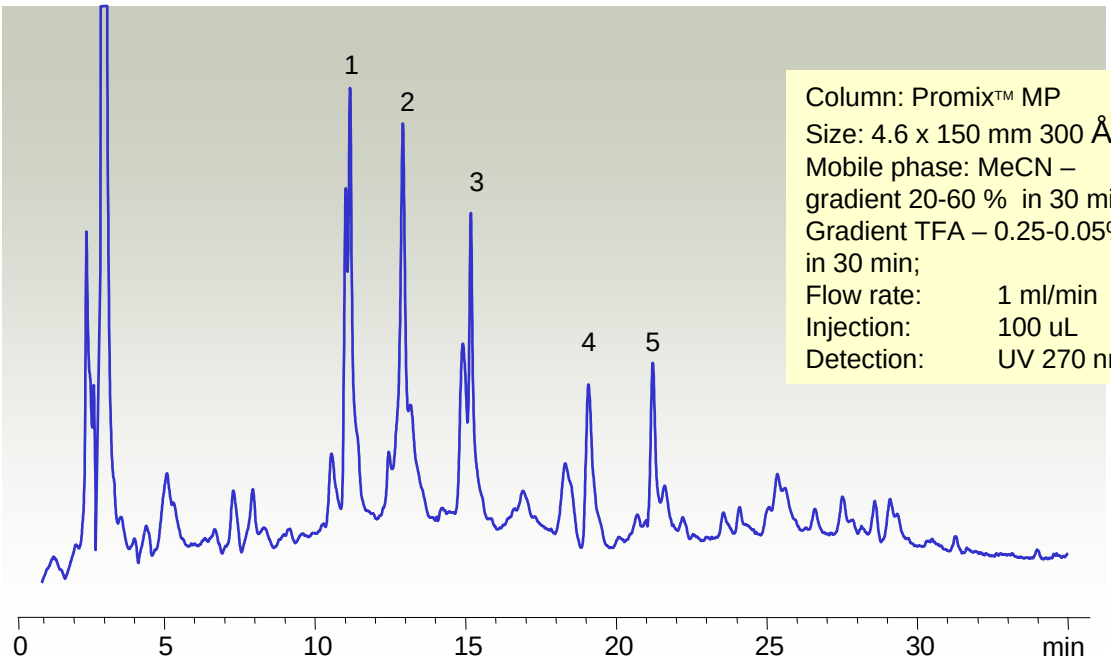


Column: Promix™ MP, 4.6 x 250 mm 300 Å
Mobile phase: MeCN – gradient 15-35 % in 15 min
Gradient TFA – 0.4-0.05% in 15 min;

Column: Promix™ MP, 4.6 x 150 mm 300 Å
Mobile phase: MeCN – gradient 0-40 % in 30 min
Gradient TFA – 0.4-0.05% in 30 min;

Large Peptides Mixture

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)

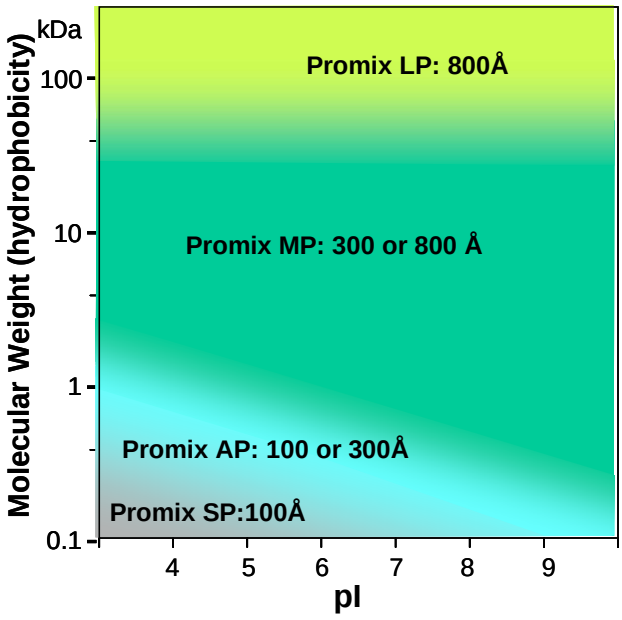


Column: Promix™ MP
Size: 4.6 x 150 mm 300 Å
Mobile phase: MeCN – gradient 20-60 % in 30 min
Gradient TFA – 0.25-0.05% in 30 min;
Flow rate: 1 ml/min
Injection: 100 uL
Detection: UV 270 nm

Column Selection and Use

1. Use the following chart to select a column based on the analyte's properties
2. Select the column length based on sample complexity
   - 150-250 mm for proteomics, protein digests, and complex samples
   - 50-150 mm for synthetic analysis and purification
3. Select the column i.d. based on sample loading and sensitivity needs
4. Mobile Phase should be pH 2-4 with:
   - TFA (0.05-0.3%)
   - Formic Acid (0.1-0.9%)
   - Ammonium Acetate or Ammonium Formate
5. Organic Modifier should be Acetonitrile
6. Ionic strength, pH, and organic modifier gradients can be used to optimize the separation

Promix™ Column Selection Chart



To order:

By phone 847-229-2629

By fax 847-655-6079

By mail mail@sielc.com

Create your Part Number:

We recommend to select stationary phase with pore size 100 Å for Promix LP; pore size 100 or 300 Å for Promix AP; pore size 300 or 800 Å for Promix MP and pore size 800 Å or none porous for Promix LP.

MP -46 . 150 . 05 10

Type of packing

Promix LP	LP
Promix MP	MP
Promix AP	AP
Promix SP	SP

Column ID

22 mm	220
4.6 mm	46
3.0 mm	30
2.0 mm	20
1.0 mm	10
0.5 mm	05
0.25 mm	03
0.1 mm	01

Column length

250 mm	250
150 mm	150
100 mm	100
50 mm	050
25 mm	025
10 mm	010

Packing size

3 um	03
5 um	05
10 um	10
20 um	20

Pores size

100 Å	10
300 Å	30
800 Å	80
None-porous	00