

**SI&C**

# Promix™

Enter a New Era  
in Biomolecule  
Analysis with  
Promix™  
Columns



**“Unsurpassed Selectivity and Peak  
Capacity for Peptides and Proteins”**

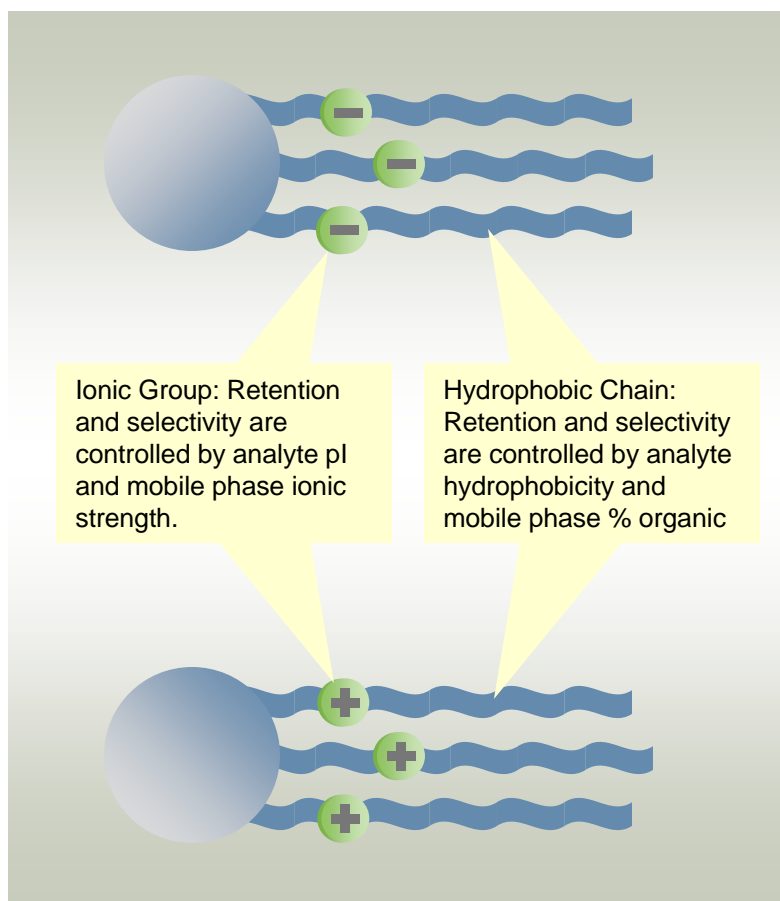
## Applications:

- Proteomics
- Peptide/Protein Analysis
- Peptide/Protein Purification

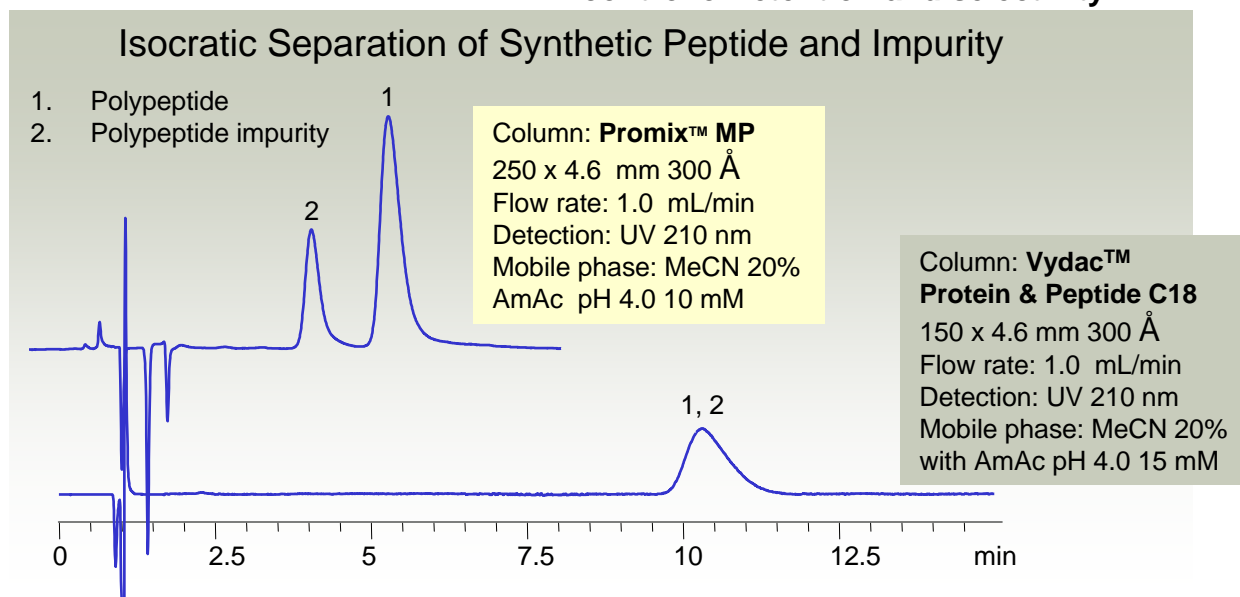
## Features & Benefits:

- **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

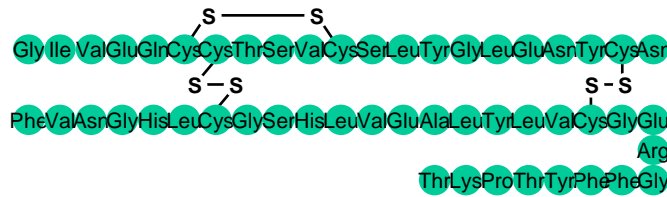
## Promix™ Technology



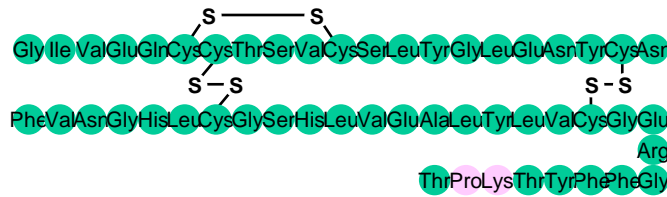
**Independent control of acid/buffer concentration and organic modifier offers almost limitless control of retention and selectivity!**



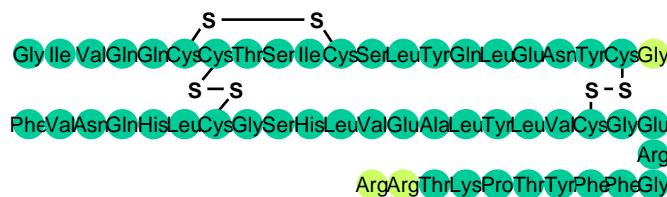
**Separation of Insulin Analogs:** Closely related peptides can be difficult to separate by reversed-phase. **By combining ionic and hydrophobic interactions, Promix MP is able to separate these analogs, differing in sequence by a single amino acid pair, using the slight differences in pI as illustrated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



**Normal human insulin**



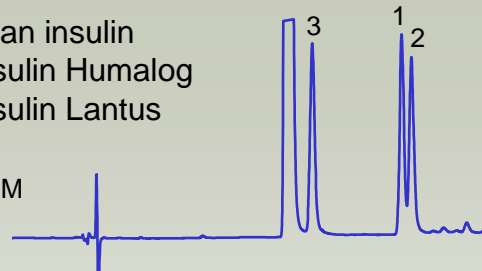
**Synthetic insulin Humalog**



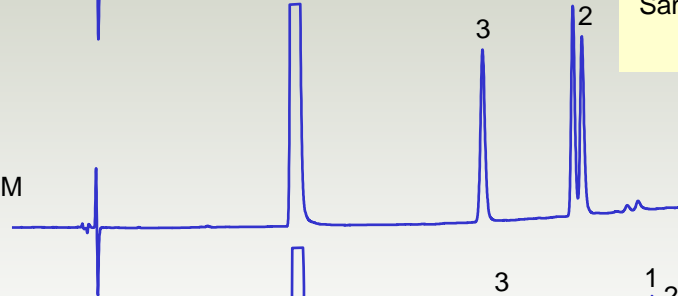
**Synthetic insulin Lantus**

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

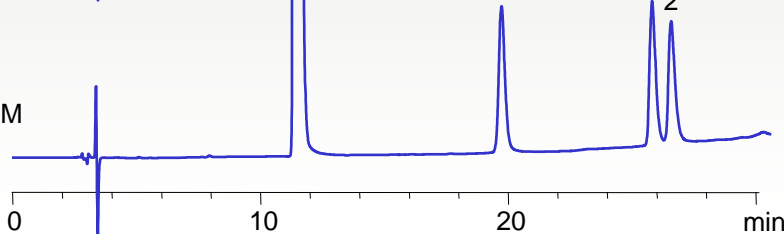
Buffer: AmFm 25 mM  
pH 3.0  
Resolution: 1.2



Buffer: AmAc 25 mM  
pH 3.5  
Resolution: 1.3



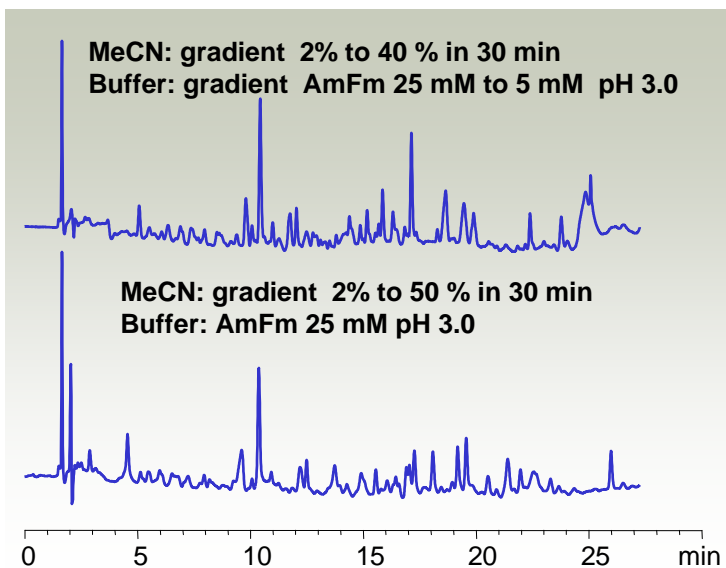
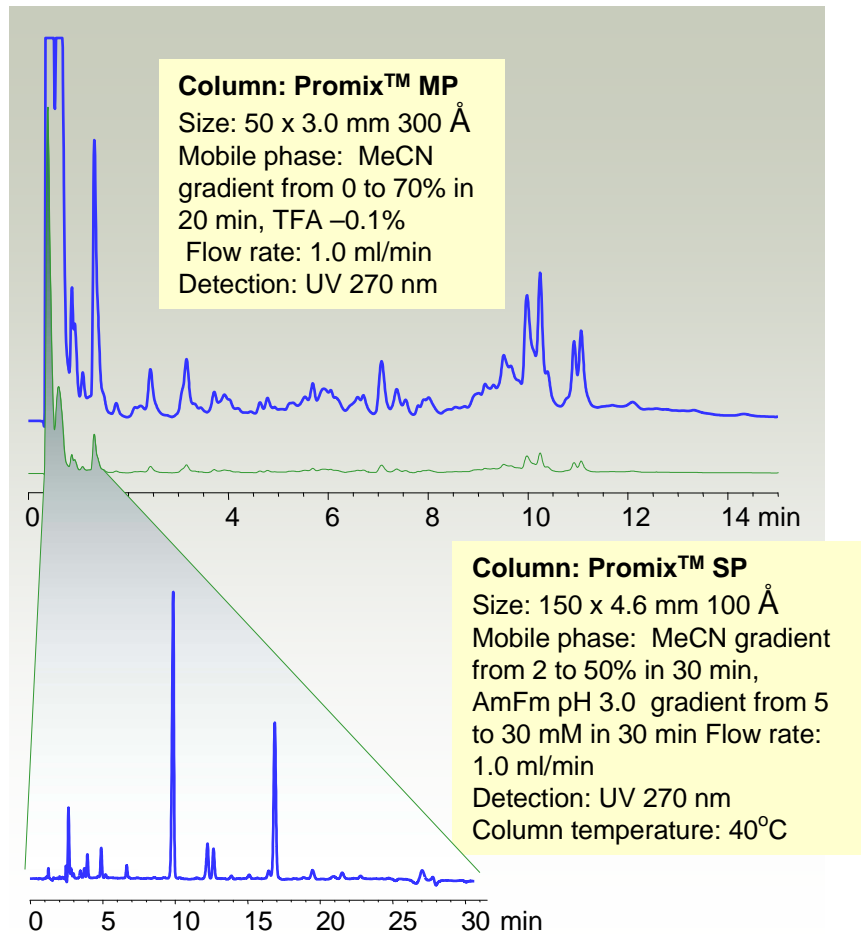
Buffer: AmAc 25 mM  
pH 4.0  
Resolution: 1.7



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:

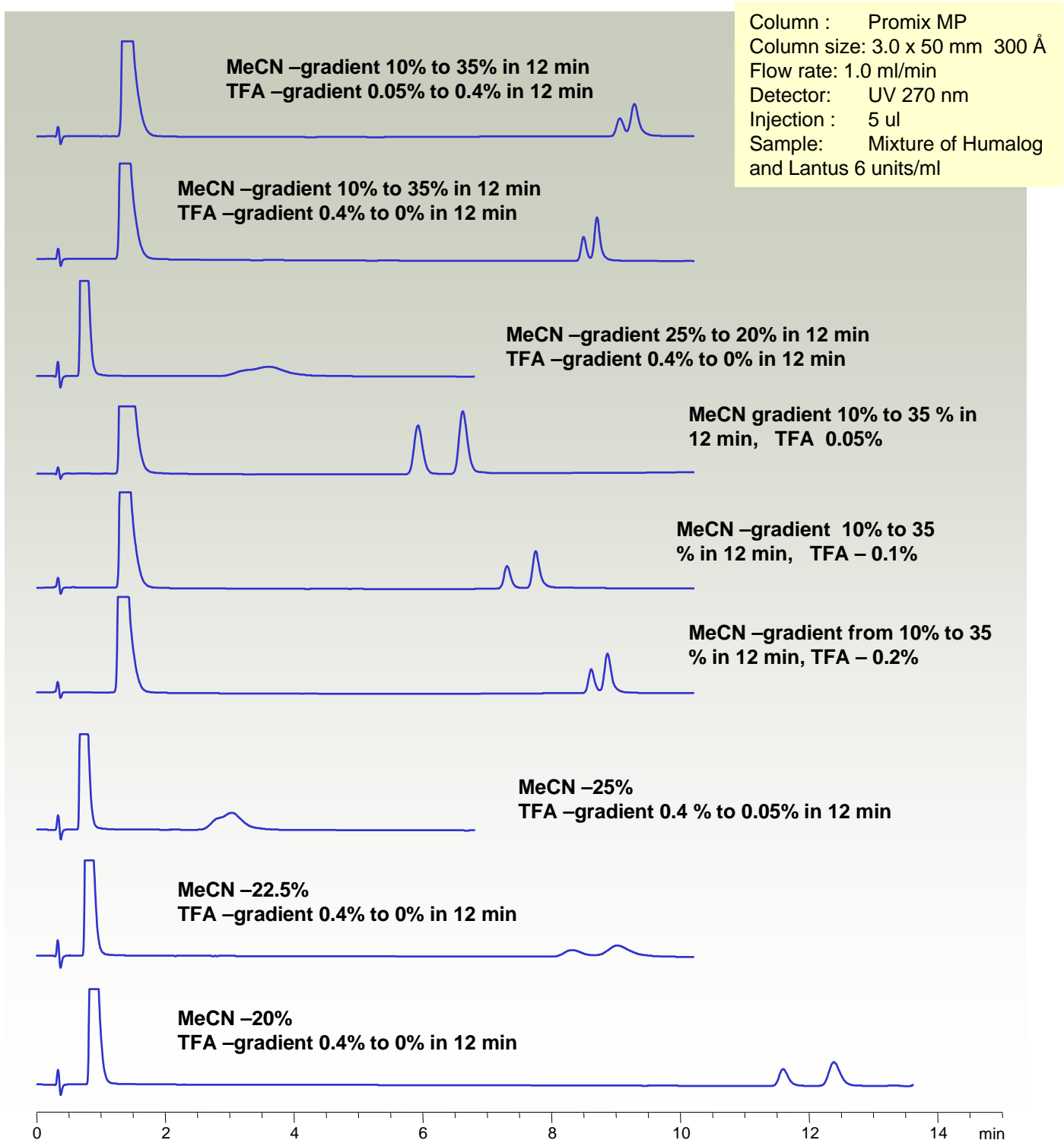
- organic gradient
- buffer gradient
- or both

**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.



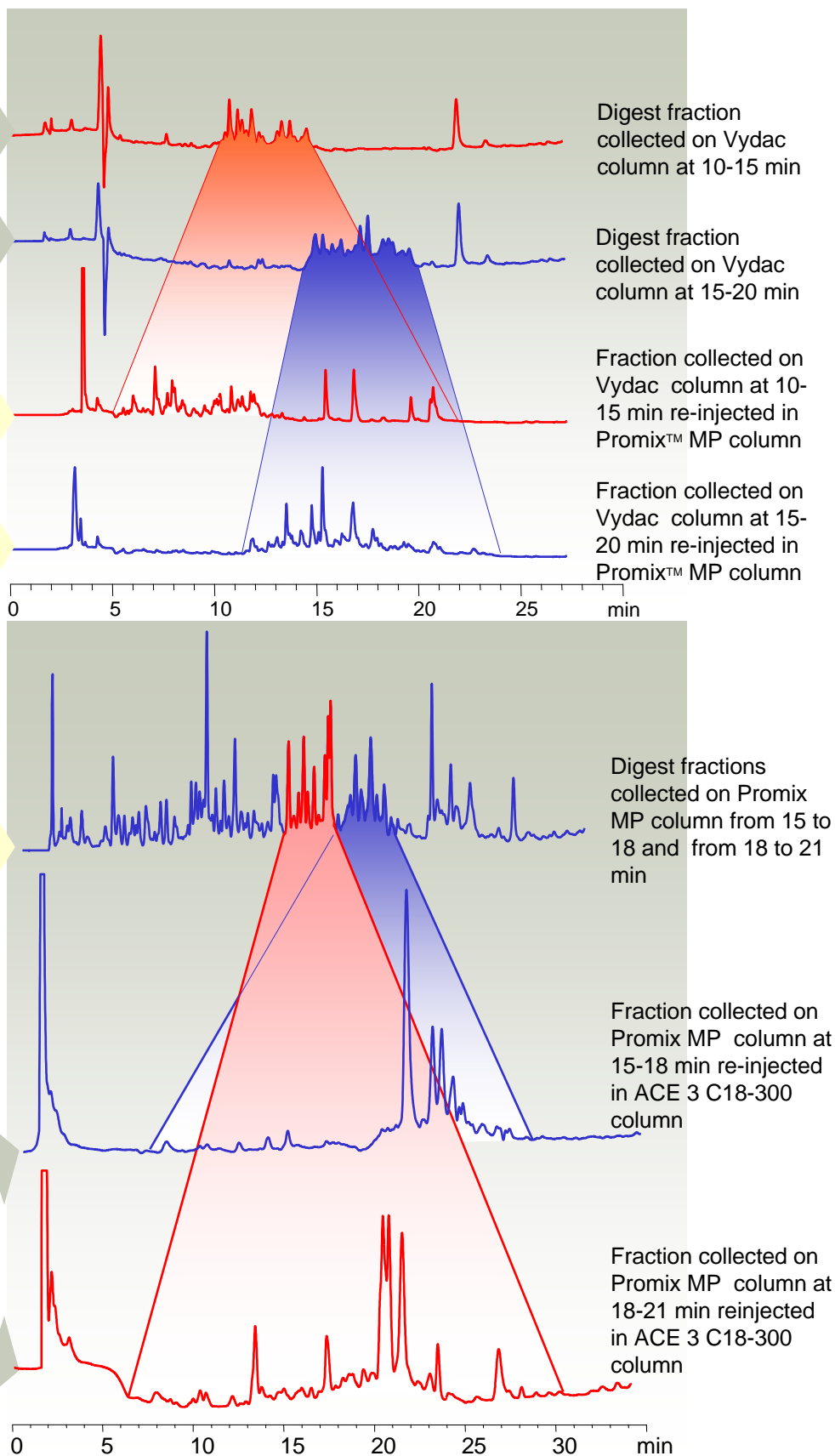
**Alternative Selectivity of Promix™ vs. RP 300Å Columns:** An indication of the alternative selectivity of Promix™ columns can be observed when the digest fraction collected from a Reversed-Phase column is reinjected onto a Promix™ column or visa versa. The elution time usually expands providing additional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

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

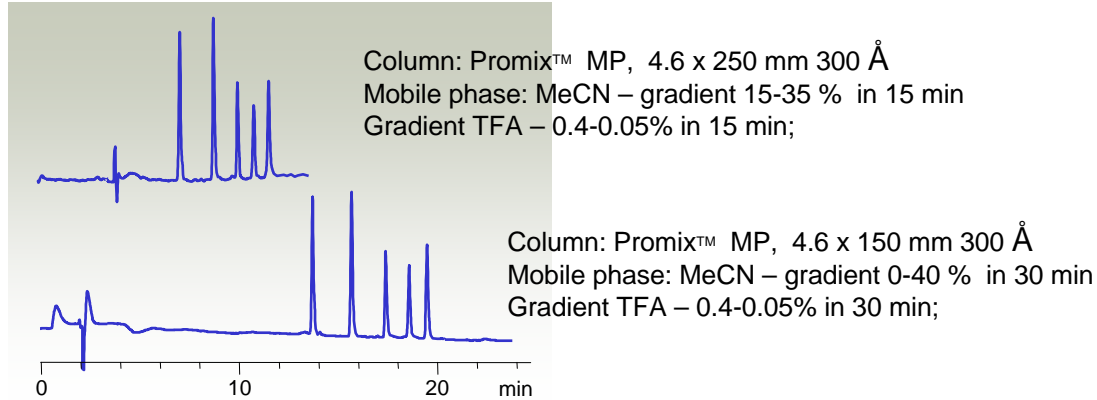
Column: ACE 3 C18-300  
 Size: 100 x 2.1 mm 300 Å  
 Flow rate: 0.3 ml/min  
 MeCN: gradient 0% to 50 % in  
 30 min  
 Buffer: TFA 0.1%  
 Detector: UV 270 nm  
 Injection: 75 uL



# Small Peptides Test Mixture (5 Angiotensins)

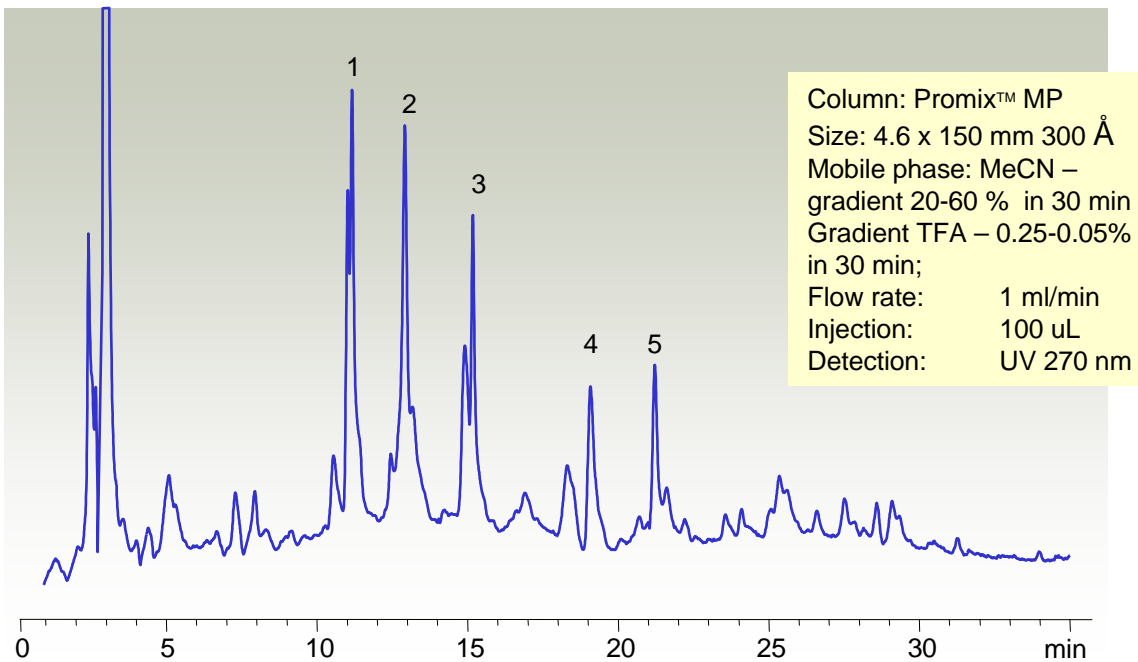
1. ARG-VAL-TYR-VAL-HIS-PRO-ILE
2. ARG-VAL-TYR-VAL-HIS-PRO-PHE
3. ALA-PRO-GLY-ASP-ARG-ILE-TYR-VAL-HIS-PRO-PHE
4. ASP-ARG-VAL-TYR-VAL-HIS-PRO-PHE-HIS LEU
5. ASP-ARG-VAL-TYR-ILE-HIS-PRO-PHE-HIS-LEU

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



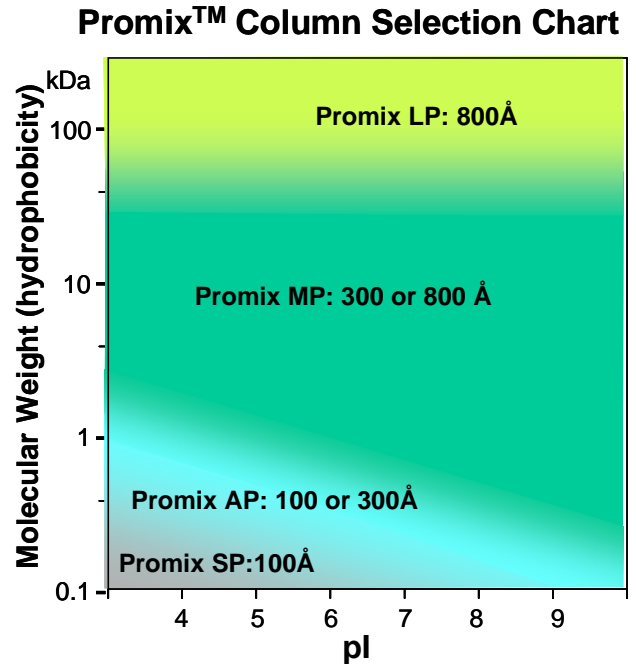
# Large Peptides Mixture

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 Selection and Use

- Use the following chart to select a column based on the analyte's properties
- 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
- Select the column i.d. based on sample loading and sensitivity needs
- Mobile Phase should be pH 2-4 with:
  - TFA (0.05-0.3%)
  - Formic Acid (0.1-0.9%)
  - Ammonium Acetate or Ammonium Formate
- Organic Modifier should be Acetonitrile
- Ionic strength, pH, and organic modifier gradients can be used to optimize the separation



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.

