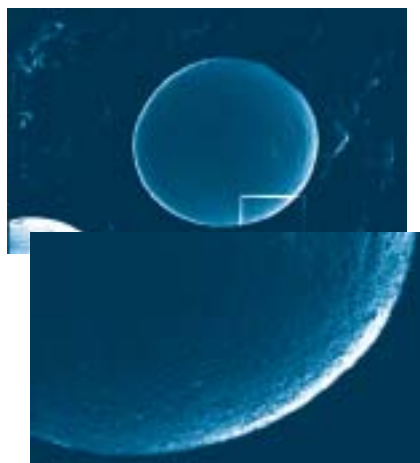


# Separation techniques (Proteins)

## Reversed Phase Chromatography-Uptisphere



### Uptisphere Wide-Pore HPLC

Our laboratory staff is always concerned to meet the different requirements of our customers. They are searching and developing new technologies on a daily basis. Uptisphere ODB and HDO, our first C18 chemistries, created by our chemists in the mid-nineties, are meeting an ever-growing success.

A large number of analysis methods of pharmaceuticals compounds requiring the most sensitive techniques such as LC/MS, MS/MS... have been validated with one of these 2 phases.

Mono-functional bonding processes, combined with our proprietary end-capping techniques, guarantee perfect reproducibility for these C18 phases.

Proteins, peptides and other large biomolecules are generally not suited to separation using supports based upon 120 Å silica. 300 and 500 Å silica have been successfully used to support many purification requirements for biomolecules.

Uptisphere 300 and 500 Å silica are manufactured under the same demanding protocols and the same philosophy as the smaller particles and provide an excellent support for the purification of biomolecules.

The philosophy is not to create new bonding for the fun but in the final stage to cover all the needs for any kind of applications.

Advantages :

- ◆ High efficiency
- ◆ Ultrapure silica
- ◆ pH stability : between 2 to 7.5
- ◆ Perfect reproducibility

Results of the efficiency tests :

- 5 µm < Efficiency > 80 000 plates/m
- 10 µm < Efficiency > 40 000 plates/m
- 15 µm < Efficiency > 25 000 plates/m
- 20 µm < Efficiency > 15 000 plates/m

Silica purity > 99.995 % guarantees a minimum of non specific interactions.

Biochromatography Uptisphere® silicas are totally compatible with ordinary mobile phases for the separation of biomolecules.

Their pH is very stable, especially in an acidic environment. WOD, WRP, WC8, WC4 and our new WTF Uptisphere® phases are available in 15 and 20 µm for purification.

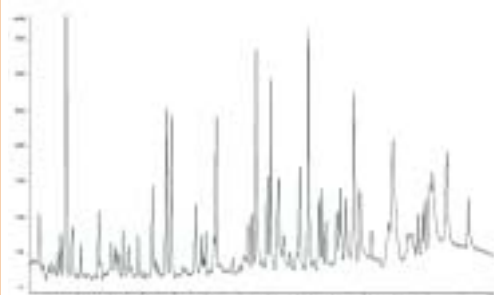
| Bonding            | WOD                   | WRP | WC8 | WC4 | WTF            | WT4 | XTF                  |
|--------------------|-----------------------|-----|-----|-----|----------------|-----|----------------------|
| Pore size          | 300Å                  |     |     |     |                |     | 500 Å                |
| Surface area       | 100 m <sup>2</sup> /g |     |     |     |                |     | 60 m <sup>2</sup> /g |
| Bonding Technology | monofunctional        |     |     |     | Polyfunctional |     | Trifunctional        |
| Carbon Content     | 10 %                  | 9 % | 8 % | 4 % | 8 %            | 3 % | na                   |
| End-Capping        | oui                   | na  | oui | oui | oui            | oui | oui                  |
| 3 µm               | x                     |     |     | x   |                |     |                      |
| 5 µm               | x                     | x   | x   | x   | x              | x   | x                    |
| 10 µm              | x                     | x   | x   | x   |                |     |                      |
| 15 µm              | x                     | x   | x   | x   |                |     |                      |
| 20 µm              | x                     |     |     | x   |                |     |                      |

# Separation techniques (Proteins)

## Reversed Phase Chromatography-Uptisphere

### Trypsin digest of Monoclonal IgG

Uptisphere 300 Å 5µ C18-ODB, 250 x 3.9 mm



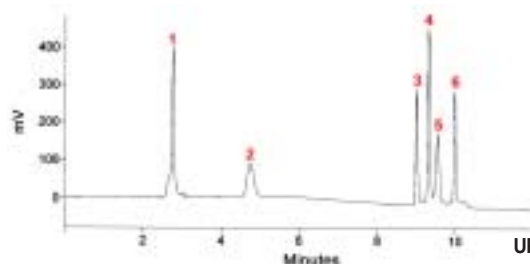
A : TFA 0.115%  
B : MeCN + TFA 0,1%  
Flow rate : 0.5 ml/min  
0-5% : 0.1 cv ; 45% : 20 cv ; 80% : 3 cv

Courtesy of LFB

UP5WOD\$25QS

### Efficiency of our UP5WTF column for the separation of a peptide mixture

Uptisphere 300 Å 5µ C18 UP5WTF, 250 x 4.6 mm



Flow rate : 1 mL/min  
Mobile phase : 20 % CH<sub>3</sub>CN / 80 % H<sub>2</sub>O, 0,04 % TFA  
Gradient : 20 à 90 % en min  
Pressure : 2250 psi  
U.V. : 210 nm  
K-22-R, A-20-V and H-27-L are proprietary peptides

UP5WTF-25QS

| µm               | mm           | WC4          | WC8         | WOD          | WRP | WTF |
|------------------|--------------|--------------|-------------|--------------|-----|-----|
| <b>2.0 mm ID</b> |              |              |             |              |     |     |
| 3                | 20 x 2.0 mm  | UP3WC4#2HS   |             | UP3WOD#2HS   |     |     |
|                  | 30 x 2.0 mm  | UP3WC4#3HS   |             | UP3WOD#3HS   |     |     |
|                  | 50 x 2.0 mm  | UP3WC4#5HS   |             | UP3WOD#5HS   |     |     |
|                  | 100 x 2.0 mm | UP3WC4#10QS  |             | UP3WOD#10QS  |     |     |
|                  | 125 x 2.0 mm | UP3WC4#12QS  |             | UP3WOD#12QS  |     |     |
|                  | 150 x 2.0 mm | UP3WC4#15QS  | UP3WC4#15QS | UP3WOD#15QS  |     |     |
| Guard Kit        | 100 x 2.0 mm | UP3WC4#10QK  |             | UP3WOD#10QK  |     |     |
|                  | 125 x 2.0 mm | UP3WC4#12QK  |             | UP3WOD#12QK  |     |     |
|                  | 150 x 2.0 mm | UP3WC4#15QK  |             | UP3WOD#15QK  |     |     |
| <b>3.0 mm ID</b> |              |              |             |              |     |     |
| 3                | 50 x 3.0 mm  | UP3WC4\$5HS  |             | UP3WOD\$5HS  |     |     |
|                  | 100 x 3.0 mm | UP3WC4\$10QS |             | UP3WOD\$10QS |     |     |
|                  | 125 x 3.0 mm | UP3WC4\$12QS |             | UP3WOD\$12QS |     |     |
|                  | 150 x 3.0 mm | UP3WC4\$15QS |             | UP3WOD\$15QS |     |     |
| Guard Kit        | 100 x 3.0 mm | UP3WC4\$10QK |             | UP3WOD\$10QK |     |     |
|                  | 125 x 3.0 mm | UP3WC4\$12QK |             | UP3WOD\$12QK |     |     |
|                  | 150 x 3.0 mm | UP3WC4\$15QK |             | UP3WOD\$15QK |     |     |
| <b>4.0 mm ID</b> |              |              |             |              |     |     |
| 3                | 30 x 4.0 mm  | UP3WC4*3HS   |             | UP3WOD*3HS   |     |     |
|                  | 100 x 4.0 mm | UP3WC4*10QS  |             | UP3WOD*10QS  |     |     |
|                  | 125 x 4.0 mm | UP3WC4*12QS  |             | UP3WOD*12QS  |     |     |
|                  | 150 x 4.0 mm | UP3WC4*15QS  |             | UP3WOD*15QS  |     |     |
| Guard Kit        | 100 x 4.0 mm | UP3WC4*10QK  |             | UP3WOD*10QK  |     |     |
|                  | 125 x 4.0 mm | UP3WC4*12QK  |             | UP3WOD*12QK  |     |     |
|                  | 150 x 4.0 mm | UP3WC4*15QK  |             | UP3WOD*15QK  |     |     |
| <b>4.6 mm ID</b> |              |              |             |              |     |     |
|                  | 20 x 4.6 mm  | UP3WC4-2HS   |             | UP3WOD-2HS   |     |     |
|                  | 30 x 4.6 mm  | UP3WC4-3HS   |             | UP3WOD-3HS   |     |     |
|                  | 50 x 4.6 mm  | UP3WC4-5HS   |             | UP3WOD-5HS   |     |     |
|                  | 75 x 4.6 mm  | UP3WC4-7HS   |             | UP3WOD-7HS   |     |     |
|                  | 100 x 4.6 mm | UP3WC4-10QS  |             | UP3WOD-10QS  |     |     |
|                  | 125 x 4.6 mm | UP3WC4-12QS  |             | UP3WOD-12QS  |     |     |
|                  | 150 x 4.6 mm | UP3WC4-15QS  |             | UP3WOD-15QS  |     |     |
| Guard Kit        | 100 x 4.6 mm | UP3WC4-10QK  |             | UP3WOD-10QK  |     |     |
|                  | 125 x 4.6 mm | UP3WC4-12QK  |             | UP3WOD-12QK  |     |     |
|                  | 150 x 4.6 mm | UP3WC4-15QK  |             | UP3WOD-15QK  |     |     |

Last 2 digits

QS : Quide seal Modulo-cart ; QT : Expert QT Modulo-cart ; QK : Expert QK Modulo-cart. See page B144

# Separation techniques (Proteins)

## Reversed Phase Chromatography-Uptisphere

### 2.0 mm ID

|   |              |             |             |             |             |             |
|---|--------------|-------------|-------------|-------------|-------------|-------------|
| 5 | 20 x 2.0 mm  | UP5WC4#2HS  |             | UP5WOD#2HS  |             | UP5WTF#2HS  |
|   | 30 x 2.0 mm  | UP5WC4#3HS  |             | UP5WOD#3HS  |             | UP5WTF#3HS  |
|   | 50 x 2.0 mm  | UP5WC4#5HS  |             | UP5WOD#5HS  |             | UP5WTF#5HS  |
|   | 100 x 2.0 mm | UP5WC4#10QS | UP5WC8#10QS | UP5WOD#10QS | UP5WRP#10QS | UP5WTF#10QS |
|   | 125 x 2.0 mm | UP5WC4#12QS | UP5WC8#12QS | UP5WOD#12QS | UP5WRP#12QS | UP5WTF#12QS |
|   | 150 x 2.0 mm | UP5WC4#15QS | UP5WC8#15QS | UP5WOD#15QS | UP5WRP#15QS | UP5WTF#15QS |
|   | 250 x 2.0 mm | UP5WC4#25QS | UP5WC8#25QS | UP5WOD#25QS | UP5WRP#25QS | UP5WTF#25QS |

|           |              |             |             |             |             |             |
|-----------|--------------|-------------|-------------|-------------|-------------|-------------|
| Guard Kit | 100 x 2.0 mm | UP5WC4#10QK | UP5WC8#10QK | UP5WOD#10QK | UP5WRP#10QK | UP5WTF#10QK |
|           | 125 x 2.0 mm | UP5WC4#12QK | UP5WC8#12QK | UP5WOD#12QK | UP5WRP#12QK | UP5WTF#12QK |
|           | 150 x 2.0 mm | UP5WC4#15QK | UP5WC8#15QK | UP5WOD#15QK | UP5WRP#15QK | UP5WTF#15QK |
|           | 250 x 2.0 mm | UP5WC4#25QK | UP5WC8#25QK | UP5WOD#25QK | UP5WRP#25QK | UP5WTF#25QK |

### 3.0 mm ID

|   |              |              |              |              |              |              |
|---|--------------|--------------|--------------|--------------|--------------|--------------|
| 5 | 50 x 3.0 mm  | UP5WC4\$5HS  |              | UP5WOD\$5HS  |              | UP5WTF\$5HS  |
|   | 100 x 3.0 mm | UP5WC4\$10QS | UP5WC8\$10QS | UP5WOD\$10QS | UP5WRP\$10QS | UP5WTF\$10QS |
|   | 125 x 3.0 mm | UP5WC4\$12QS | UP5WC8\$12QS | UP5WOD\$12QS | UP5WRP\$12QS | UP5WTF\$12QS |
|   | 150 x 3.0 mm | UP5WC4\$15QS | UP5WC8\$15QS | UP5WOD\$15QS | UP5WRP\$15QS | UP5WTF\$15QS |
|   | 250 x 3.0 mm | UP5WC4\$25QS | UP5WC8\$25QS | UP5WOD\$25QS | UP5WRP\$25QS | UP5WTF\$25QS |

|           |              |              |              |              |              |              |
|-----------|--------------|--------------|--------------|--------------|--------------|--------------|
| Guard Kit | 100 x 3.0 mm | UP5WC4\$10QK | UP5WC8\$10QK | UP5WOD\$10QK | UP5WRP\$10QK | UP5WTF\$10QK |
|           | 125 x 3.0 mm | UP5WC4\$12QK | UP5WC8\$12QK | UP5WOD\$12QK | UP5WRP\$12QK | UP5WTF\$12QK |
|           | 150 x 3.0 mm | UP5WC4\$15QK | UP5WC8\$15QK | UP5WOD\$15QK | UP5WRP\$15QK | UP5WTF\$15QK |
|           | 250 x 3.0 mm | UP5WC4\$25QK | UP5WC8\$25QK | UP5WOD\$25QK | UP5WRP\$25QK | UP5WTF\$25QK |

### 4.0 mm ID

|   |              |             |             |             |             |             |
|---|--------------|-------------|-------------|-------------|-------------|-------------|
| 5 | 30 x 4.0 mm  | UP5WC4*3HS  |             | UP5WOD*3HS  |             | UP5WTF*3HS  |
|   | 100 x 4.0 mm | UP5WC4*10QS | UP5WC8*10QS | UP5WOD*10QS | UP5WRP*10QS | UP5WTF*10QS |
|   | 125 x 4.0 mm | UP5WC4*12QS | UP5WC8*12QS | UP5WOD*12QS | UP5WRP*12QS | UP5WTF*12QS |
|   | 150 x 4.0 mm | UP5WC4*15QS | UP5WC8*15QS | UP5WOD*15QS | UP5WRP*15QS | UP5WTF*15QS |
|   | 250 x 4.0 mm | UP5WC4*25QS | UP5WC8*25QS | UP5WOD*25QS | UP5WRP*25QS | UP5WTF*25QS |

|           |              |             |             |             |             |             |
|-----------|--------------|-------------|-------------|-------------|-------------|-------------|
| Guard Kit | 100 x 4.0 mm | UP5WC4*10QK | UP5WC8*10QK | UP5WOD*10QK | UP5WRP*10QK | UP5WTF*10QK |
|           | 125 x 4.0 mm | UP5WC4*12QK | UP5WC8*12QK | UP5WOD*12QK | UP5WRP*12QK | UP5WTF*12QK |
|           | 150 x 4.0 mm | UP5WC4*15QK | UP5WC8*15QK | UP5WOD*15QK | UP5WRP*15QK | UP5WTF*15QK |
|           | 250 x 4.0 mm | UP5WC4*25QK | UP5WC8*25QK | UP5WOD*25QK | UP5WRP*25QK | UP5WTF*25QK |

### 4.6 mm ID

|   |              |             |             |             |             |             |
|---|--------------|-------------|-------------|-------------|-------------|-------------|
| 5 | 20 x 4.6 mm  | UP5WC4-2HS  |             | UP5WOD-2HS  |             | UP5WTF-2HS  |
|   | 30 x 4.6 mm  | UP5WC4-3HS  |             | UP5WOD-3HS  |             | UP5WTF-3HS  |
|   | 50 x 4.6 mm  | UP5WC4-5HS  |             | UP5WOD-5HS  |             | UP5WTF-5HS  |
|   | 75 x 4.6 mm  | UP5WC4-7HS  |             | UP5WOD-7HS  |             | UP5WTF-7HS  |
|   | 100 x 4.6 mm | UP5WC4-10QS | UP5WC8-10QS | UP5WOD-10QS | UP5WRP-10QS | UP5WTF-10QS |
|   | 125 x 4.6 mm | UP5WC4-12QS | UP5WC8-12QS | UP5WOD-12QS | UP5WRP-12QS | UP5WTF-12QS |
|   | 150 x 4.6 mm | UP5WC4-15QS | UP5WC8-15QS | UP5WOD-15QS | UP5WRP-15QS | UP5WTF-15QS |
|   | 250 x 4.6 mm | UP5WC4-25QS | UP5WC8-25QS | UP5WOD-25QS | UP5WRP-25QS | UP5WTF-25QS |

|           |              |             |             |             |             |             |
|-----------|--------------|-------------|-------------|-------------|-------------|-------------|
| Guard Kit | 100 x 4.6 mm | UP5WC4-10QK | UP5WC8-10QK | UP5WOD-10QK | UP5WRP-10QK | UP5WTF-10QK |
|           | 125 x 4.6 mm | UP5WC4-12QK | UP5WC8-12QK | UP5WOD-12QK | UP5WRP-12QK | UP5WTF-12QK |
|           | 150 x 4.6 mm | UP5WC4-15QK | UP5WC8-15QK | UP5WOD-15QK | UP5WRP-15QK | UP5WTF-15QK |
|           | 250 x 4.6 mm | UP5WC4-25QK | UP5WC8-25QK | UP5WOD-25QK | UP5WRP-25QK | UP5WTF-25QK |

### B.142

#### 4.0 mm ID

|           |              |              |              |              |              |  |
|-----------|--------------|--------------|--------------|--------------|--------------|--|
| 10        | 250 x 4.0 mm | UP10WC4*25QS | UP10WC8*25QS | UP10WOD*25QS | UP10WRP*25QS |  |
| Guard Kit | 250 x 4.0 mm | UP10WC4*25QK | UP10WC8*25QK | UP10WOD*25QK | UP10WRP*25QK |  |

#### 4.6 mm ID

|           |              |              |              |              |              |  |
|-----------|--------------|--------------|--------------|--------------|--------------|--|
| 10        | 250 x 4.6 mm | UP10WC4-25QS | UP10WC8-25QS | UP10WOD-25QS | UP10WRP-25QS |  |
| Guard Kit | 250 x 4.6 mm | UP10WC4-25QK | UP10WC8-25QK | UP10WOD-25QK | UP10WRP-25QK |  |

### Microcolumns

#### 1.0 mm ID

|   |              |              |  |              |  |  |
|---|--------------|--------------|--|--------------|--|--|
| 3 | 20 x 1.0 mm  | UP3WC4D2MC   |  | UP3WODD2MC   |  |  |
|   | 20 x 1.0 mm  | UP3WC4D2MCE  |  | UP3WODD2MCE  |  |  |
|   | 50 x 1.0 mm  | UP3WC4D5MC   |  | UP3WODD5MC   |  |  |
|   | 50 x 1.0 mm  | UP3WC4D5MCE  |  | UP3WODD5MCE  |  |  |
|   | 50 x 1.0 mm  | UP3WC4D5MCK  |  | UP3WODD5MCK  |  |  |
|   | 150 x 1.0 mm | UP3WC4D15MC  |  | UP3WODD15MC  |  |  |
|   | 150 x 1.0 mm | UP3WC4D15MCE |  | UP3WODD15MCE |  |  |
|   | 150 x 1.0 mm | UP3WC4D15MCK |  | UP3WODD15MCK |  |  |

Last 2 digits

QS : Quide seal Modulo-cart ; QT : Expert QT Modulo-cart ; QK : Expert QK Modulo-cart. See page B144

# Separation techniques (Proteins)

## Reversed Phase Chromatography-Uptisphere

|   |              |              |              |              |
|---|--------------|--------------|--------------|--------------|
| 5 | 20 x 1.0 mm  | UP5WC4D2MC   | UP5WODD2MC   | UP5WTFD2MC   |
|   | 20 x 1.0 mm  | UP5WC4D2MCE  | UP5WODD2MCE  | UP5WTFD2MCE  |
|   | 50 x 1.0 mm  | UP5WC4D5MC   | UP5WODD5MC   | UP5WTFD5MC   |
|   | 50 x 1.0 mm  | UP5WC4D5MCE  | UP5WODD5MCE  | UP5WTFD5MCE  |
|   | 50 x 1.0 mm  | UP5WC4D5MCK  | UP5WODD5MCK  | UP5WTFD5MCK  |
|   | 150 x 1.0 mm | UP5WC4D15MC  | UP5WODD15MC  | UP5WTFD15MC  |
|   | 150 x 1.0 mm | UP5WC4D15MCE | UP5WODD15MCE | UP5WTFD15MCE |
|   | 150 x 1.0 mm | UP5WC4D15MCK | UP5WODD15MCK | UP5WTFD15MCK |

### Nanocolumns

#### 0.3 mm ID

|   |              |           |           |  |
|---|--------------|-----------|-----------|--|
| 3 | 50 x 0.3 mm  | UP3WC4A5  | UP3WODA5  |  |
|   | 100 x 0.3 mm | UP3WC4A10 | UP3WODA10 |  |
|   | 150 x 0.3 mm | UP3WC4A15 | UP3WODA15 |  |

#### 0.5 mm ID

|   |              |           |           |  |
|---|--------------|-----------|-----------|--|
| 3 | 50 x 0.5 mm  | UP3WC4B5  | UP3WODB5  |  |
|   | 100 x 0.5 mm | UP3WC4B10 | UP3WODB10 |  |
|   | 150 x 0.5 mm | UP3WC4B15 | UP3WODB15 |  |

#### 0.3 mm ID

|   |              |           |           |            |
|---|--------------|-----------|-----------|------------|
| 5 | 50 x 0.3 mm  | UP5WC4A5  | UP5WODA5  | UP5WTF A5  |
|   | 100 x 0.3 mm | UP5WC4A10 | UP5WODA10 | UP5WTF A10 |
|   | 150 x 0.3 mm | UP5WC4A15 | UP5WODA15 | UP5WTF A15 |

#### 0.5 mm ID

|   |              |           |           |            |
|---|--------------|-----------|-----------|------------|
| 5 | 50 x 0.5 mm  | UP5WC4B5  | UP5WODB5  | UP5WTF B5  |
|   | 100 x 0.5 mm | UP5WC4B10 | UP5WODB10 | UP5WTF B10 |
|   | 150 x 0.5 mm | UP5WC4B15 | UP5WODB15 | UP5WTF B15 |

### Nano / Micro columns

Thanks to its high mechanical resistance and narrow particle size distribution, the keys to get excellent nano and micro columns, Uptisphere is the guarantee of an irreproachable quality of packings.

- ◆ Very high sensibility
- ◆ Ideal for trace analysis
- ◆ Very low solvent consumption
- ◆ Zero dead volume
- ◆ Easy frits replacement
- ◆ Direct guard column

Uptisphere nano and micro columns are available under 2 configurations :

- ◆ Glass lined PEEK tubing
- ◆ 316L stainless steel tubing



# Separation techniques (Proteins)

## Reversed Phase Chromatography-Uptisphere



our new HPLC column hardware

### Quick seal Modulo-cart concept

We are every day concerned about offering the most recent technological innovations.

It is neither a column  
or a cartridge.

Quintessence of the modern HPLC connectic, the Modulo-cart Quick seal gives the way to what we, chromatographists, should all focuss our attention on :  
the analysis.

### Its advantages ?

- ◆ Real handtight connections
- ◆ The couple PEEK spiked carbon on inox guarantees a long lifetime for the system.
- ◆ The caps frits are easily replaceable
- ◆ 15 % increased efficiency compared to the current systems
- ◆ Direct connections between the guard cartridge and the analytical column
- ◆ No insert, no additional ring
- ◆ No cartridge drying during storage

Quick seal Modulo-cart are available in a wide range of length (33 mm to 300 mm) and internal diameters (1.0 mm to 4.6 mm).



Quick seal Modulo-cart  
**QS** : (the last 2 digits in the Cat.#)



**Description**  
Quick seal connection kit  
(1 inlet connector + 1 outlet connector)

**Cat.#**      **Qty**

**CH953820**      1 u



**Description**  
Quick seal guard cartridge holder - **10 mm**

**Cat.#**      **Qty**

**CH979540**      1 u

### Description

**Cat.#**      **Qty**

Quick seal guard cartridges 10 x 1.0 mm

**Q95961**      3 u

**Q95960**      10 u

Quick seal guard cartridges 10 x 2.0 mm

**CH980671**      3 u

**CH980670**      10 u

Quick seal guard cartridges 10 x 4.0 mm

**CH979521**      3 u

**CH979520**      10 u



**10 mm Expert QK Modulo-cart**  
**QK** : (the 2 last digits in the Cat.#)



**20 mm Expert QT Modulo-cart**  
**QT** : (the last 2 digits in the Cat.#)

The Expert system is the guarantee of an excellent lifetime of the stationnary phase. This concept, with an integrated guard cartridge, protects the analytical Modulo-cart efficiently against chemical pollution through the sample. A periodical replacement of the pre-column is necessary for an optimal protection.

# Separation techniques (Proteins)

## Reversed Phase Chromatography-Uptisphere

### Different ways of using the Quick seal

#### Ready to use

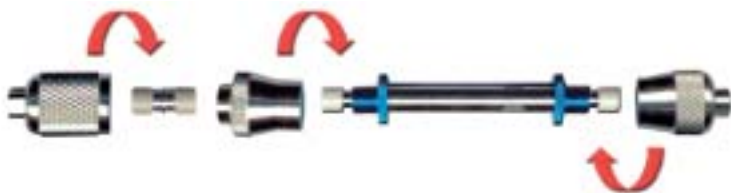
#### Standard mode assembly



The connection kit P/N CH953820 fits the Quick seal Modulo-cart with a 1.0



#### Guard cartridge assembly



⚠ The guard cartridge holder must be adapted to the length of the guard cartridge chosen.



#### Coupling assembly



It enables the coupling of different lengths and/or internal diameters and/or chemistries. Typical application : coupling of a C18 and SCX chemistry for the analysis of drugs in biological fluids.



#### Direct mass connection



The internal diameter of the mass connection tube of the mass connector is 50µm i.d.



# Separation techniques (Proteins)

## Reversed Phase Chromatography Zorbax

### Zorbax Wide-Pore HPLC Columns

#### 300Å StableBond

Ideal for the separations of proteins and peptides

The Zorbax StableBond columns use patented, unique, monofunctional silanes with bulky diisobutyl or diisopropyl side chain groups that sterically protect the key siloxane bond from hydrolytic attack at low pH.

Zorbax 300 Å StableBond columns are ideal for the separations of proteins and peptides

- ◆ Porosity : 300 Å
- ◆ Excellent access to the bonded phase
- ◆ Monofunctional, non End-Capped
- ◆ Unmatched in their durability at low pH
- ◆ Four different bonded phases
- ◆ Available in capillary and nano dimensions

These columns are available in four different bonded phases (C18, C8, C3 and CN) for selectivity and recovery optimization of proteins and polypeptides. Moreover, their exceptional stability at high temperature allow their use up to 80-90°C for complex protein and protein digest separations.

| Description            | Size (mm)  | Particle size (µm) | 300SB-C18<br>UPS L1 | 300SB-C8<br>UPS L7 | 300SB-CN<br>USP L10 | 300SB-C3   |
|------------------------|------------|--------------------|---------------------|--------------------|---------------------|------------|
| SemiPreparative        | 9.4 x 250  | 5                  | 880995-202          | 880995-206         | 880995-205          | 880995-209 |
| Analytical             | 4.6 x 250  | 5                  | 880995-902          | 880995-906         | 880995-905          | 880995-909 |
| Analytical             | 4.6 x 150  | 5                  | 883995-902          | 883995-906         | 883995-905          | 883995-909 |
| Analytical             | 4.6 x 50   | 5                  | 860950-902          | 860950-906         | 860950-905          | 860950-909 |
| Rapid Resolution       | 4.6 x 150  | 35                 | 863973-902          | 863973-906         | 863973-905          | 863973-909 |
| Rapid Resolution       | 4.6 x 100  | 3.5                | 861973-902          | 861973-906         |                     |            |
| Rapid Resolution       | 4.6 x 50   | 35                 | 865973-902          | 865973-906         | 865973-905          | 865973-909 |
| Solvent Saver Plus     | 30 x 150   | 35                 | 863974-302          | 863974-306         |                     | 863974-309 |
| Solvent Saver Plus     | 3.0 x 100  | 3.5                |                     | 861973-306         |                     |            |
| Narrow Bore            | 2.1 x 250  | 5                  | 881750-902          |                    |                     |            |
| Narrow Bore            | 2.1 x 150  | 5                  | 883750-902          | 883750-906         | 883750-905          | 883750-909 |
| Narrow Bore RR'        | 2.1 x 150  | 35                 |                     | 863750-906         |                     |            |
| Narrow Bore RR         | 2.1 x 100  | 3.5                | 861775-902          | 861775-906         |                     |            |
| Narrow Bore RR         | 2.1 x 50   | 3.5                | 865750-902          | 865750-906         |                     |            |
| MicroBore              | 1.0 x 250  | 5                  | 861630-902          |                    |                     |            |
| MicroBore RR           | 1.0 x 150  | 3.5                | 863630-902          | 863630-906         |                     |            |
| MicroBore RR           | 1.0 x 50   | 3.5                | 865630-902          | 865630-906         |                     |            |
| MicroBore Guard, 3/pk  | 1.0 x 17   | 5                  | 5185-5920           | 5185-5920          |                     |            |
| Guard Cartridges, 2/pk | 9.4 x 15   | 7                  | 820675-124          | 820675-124         | 820675-124          | 820675-124 |
| Guard Cartridges, 4/pk | 4.6 x 12.5 | 5                  | 820950-921          | 820950-918         | 820950-923          | 820950-924 |
| Guard Cartridges, 4/pk | 2.1 x 125  | 5                  | 821125-918          | 821125-918         | 821125-924          | 821125-924 |
| Guard Hardware Kit     | 9.4 x 15   |                    | 840140-901          | 840140-901         | 840140-901          | 840140-901 |
| Guard Hardware Kit     | 4.6 x 12.5 |                    | 820888-901          | 820888-901         | 820888-901          | 820888-901 |
| Guard Hardware Kit     | 2.1 x 12.5 |                    | 820888-901          | 820888-901         | 820888-901          | 820888-901 |

# Separation techniques (Proteins)

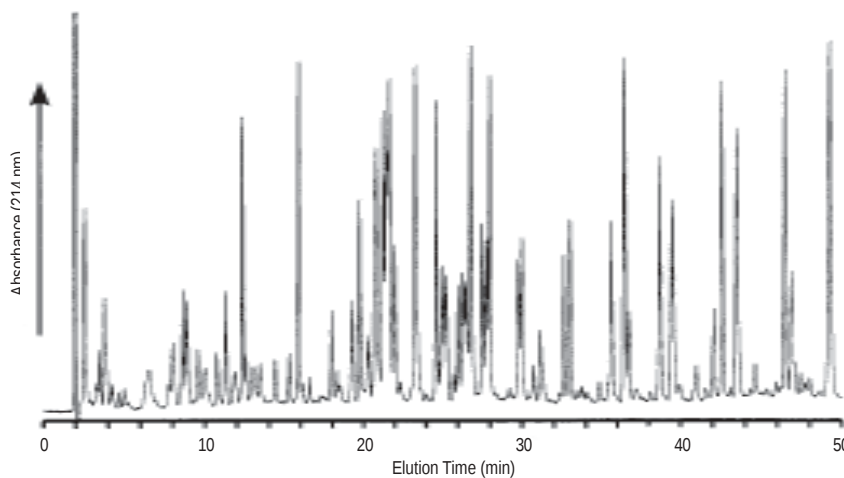
## Reversed Phase Chromatography Zorbax

### Efficiency

High resolution of couple digest of proteins and peptides.

Sample :  
BSA trypsin Hydrolysate

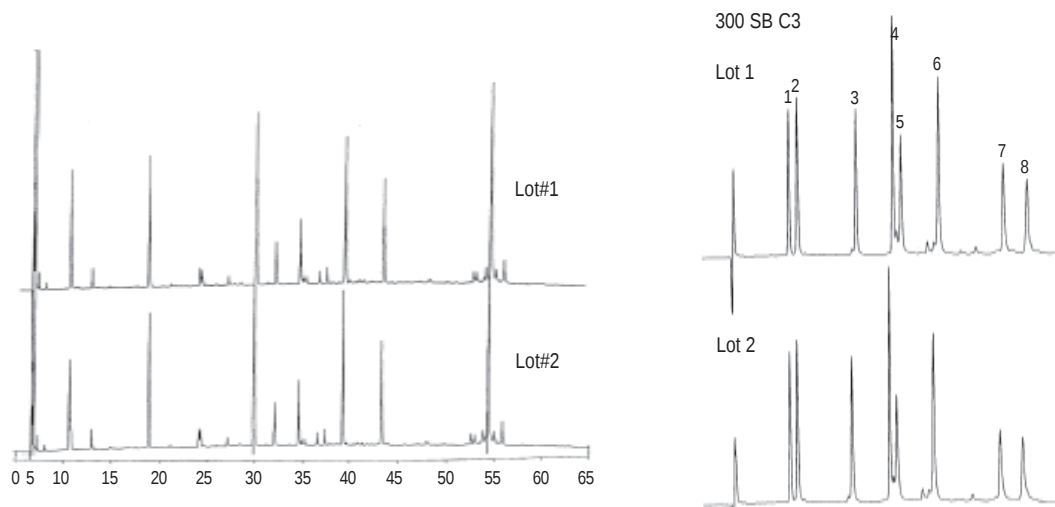
Column : Zorbax® 300SB-C8, 150 x 2.1 mm  
Gradient : 2 % à 62 % B : 70 min  
A : 0.1 % TFA B : 0.1 % TFA : 80 % ACN/20 % eau  
Temperature : 50°C  
Flow rate : 0,2 ml/min  
Injection : 50 pmol BSA 4 M urea



Spherical Zorbax StableBond beads distribution is very tight (granulometry and porosity), which implies high efficiency.

### Reproducibility

Sample :  
1. Leucine Enkephalin  
2. Angiotensin  
3. RNase  
4. Insulin  
5. Cytochrome C  
6. Lysozyme  
7. Myoglobin  
8. Carbonic Anhydrase



Zorbax 300 StableBond Columns are based on the Ultrapure (>99.995%) silica Zorbex Rx. The monofunctional bounding guarantee a perfect reproducibility.

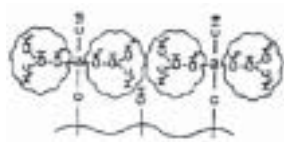
Agilent Technologies is the only company to produce both silica and bounding, and to insure the bounding chemistry. Each step is strictly controlled which guarantees a perfect lot to lot reproducibility.

# Separation techniques (Proteins)

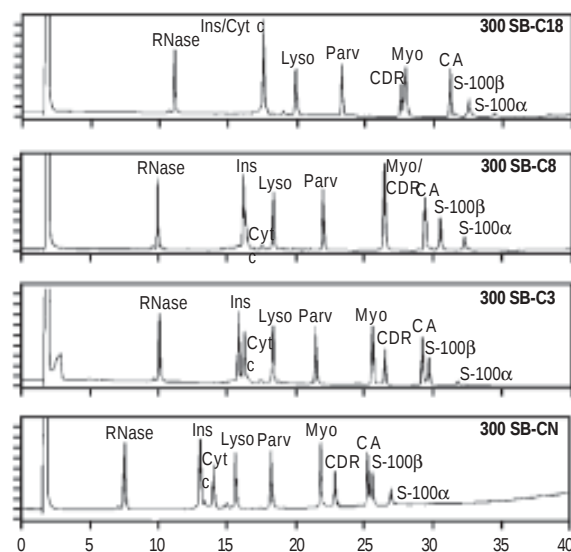
## Reversed Phase Chromatography Zorbax

### Selectivity

Effect of bonded phase on selectivity



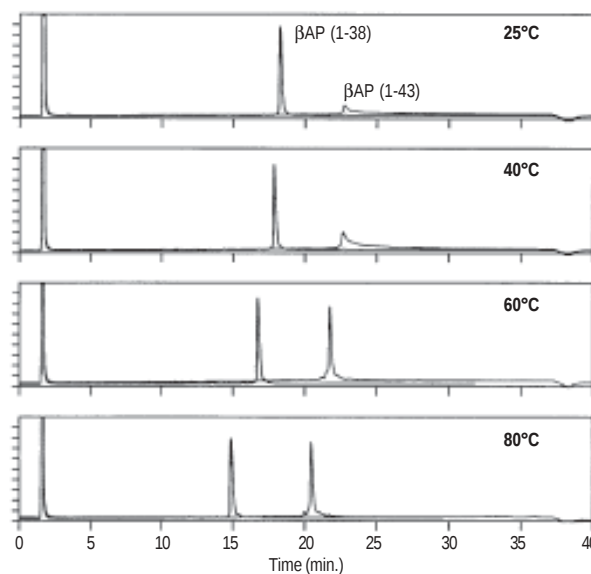
Colonne : Zorbax® 300SB-C18, 300SB-C8, 300SB-C3, 300SB-CN  
(150 x 4.6 mm)  
Mobile phase : 25-70 % B : 40 min,  
A : 0.1 % TFA : H<sub>2</sub>O, B : 0.09 % TFA : 80 % ACN/20 % H<sub>2</sub>O  
Injection 3 µg of each polypeptide, 1ml/min, 60°C  
Detection : UV (210 nm)



Based on Ultra-pure Zorbax® Rx silica  
the stableBond bonding chemistry allows a stability acidic conditions.

### Temperature stability

Collect of hydrophobic peptide depending on temperature



Column : Zorbax® 300SB-C18, (150 x 4.6 mm) (Cat.# 883995.902)  
Mobile phase : 25-45 % B in 35 min,  
A : 0.1 % TFA : H<sub>2</sub>O, B : 0.09 % TFA in ACN  
Injection 10 µL (5 µg in 6M urea/5% HOAc), 1mL/min  
Detection : UV (210 nm)

Zorbax 300 StableBond columns allow to work at high temperature  
with no loss of quality or shelflife.

# Separation techniques (Proteins)

## Reversed Phase Chromatography Zorbax

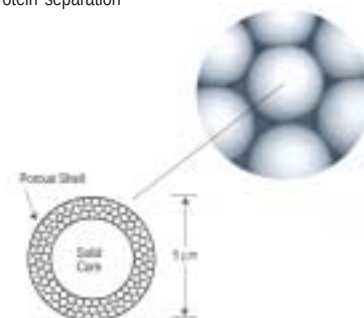
### Poroshell 300SB

Ideal for fast separations of proteins and peptides

Typically, peptides and proteins, slow diffusing analytes, are typically separated slowly to reduce the potential peak broadening. Poroshell using a unique particle made with a thin layer of porous silica on a solid core of silica allows a fast high-resolution separations of peptides and proteins up to 500-1000 kDa.

- ◆ Porosity : 300 Å
- ◆ Fast high-resolution separations of biomolecules
- ◆ Long column life at low pH with StableBond bonded phases
- ◆ Excellent MS compatibility with 2.1 mm, 1 mm and 0.5 mm ID column
- ◆ Three different bonded phases 300SB-C18, 300SB-C8 and 300SB-C3

Innovative Poroshell Particles are ideal for Ultra-fast protein separation



The unique Poroshell particle design consists of a solid core of high purity silica surrounded by a thin layer of porous, high-purity silica.

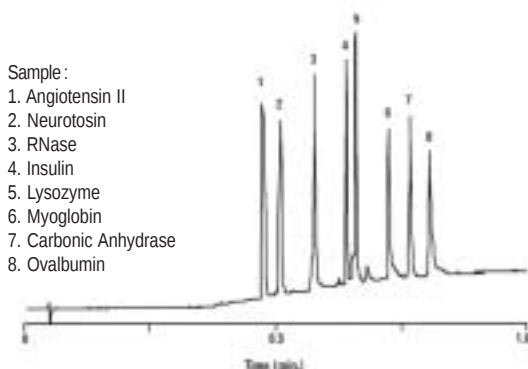
Pores in the thin, outer layer measure 300Å in diameter and are bonded with sterically protected ligands (SB-C18, SB-C8 or SB-C3) for maximum column life with low pH (i.e., IFA and formic acid) mobile phases. During separation, proteins diffuse rapidly in and out of the porous shell - allowing for the use of high flow velocities - thereby eluting as sharp peaks in just seconds.

| Column Description               | Size (mm)  | Particle Size (µm) | 300SB-C18  | 300SB-C8   | 300SB-C3   |
|----------------------------------|------------|--------------------|------------|------------|------------|
| <b>Standard Columns</b>          |            |                    |            |            |            |
| Narrow Bore                      | 2.1 x 75   | 5                  | 660750-902 | 660750-906 | 660750-909 |
| MicroBore                        | 1.0 x 75   | 5                  | 661750-902 | 661750-906 | 661750-909 |
| Capillary                        | 0.5 x 75   | 5                  |            | 5065-4468  |            |
| Guard Cartridges, 4/pk           | 2.1 x 12.5 | 5                  | 821075-920 | 821075-918 | 821075-924 |
| Guard Hardware Kit               | 2.1 x 12.5 |                    | 820888-901 | 820888-901 | 820888-901 |
| MicroBore Guard Cartridges, 3/pk | 1.0 x 17   | 5                  | 5185-5968  | 5185-5968  | 5185-5968  |

### Poroshell columns can separate proteins and peptides in seconds

Column : Poroshell 300 SB-C18  
2.1 x 75 mm, 5 µm  
Cat.# 660750-902  
Mobile phase :  
A : 0.1 % TFA in H<sub>2</sub>O  
B : 0.07 % TFA in ACN  
Gradient : 5-100 % B in 1.0 min  
Flow rate : 3.0 ml/min  
Temperature : 70°C  
Pressure : 260 bar  
Detection : UV 215 nm

Sample :  
1. Angiotensin II  
2. Neurotensin  
3. RNase  
4. Insulin  
5. Lysozyme  
6. Myoglobin  
7. Carbonic Anhydrase  
8. Ovalbumin

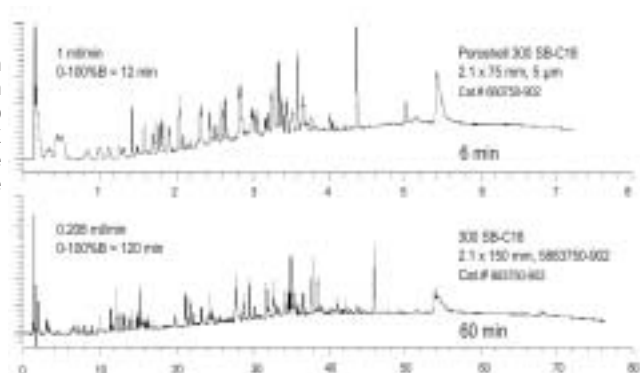


### Reduce Peptide Map Analysis Time by 90 % with ZORBAX Poroshell 300SB

Instrument : Agilent 1100 Binary Systems  
Mobile Phase :  
A: 95 % H<sub>2</sub>O, 5 % ACN, 0.1 % TFA  
B : 5 % H<sub>2</sub>O, 95 % ACN, 0.07 % TFA  
Flow rate : As shown  
Piston Stroke : 20 µl

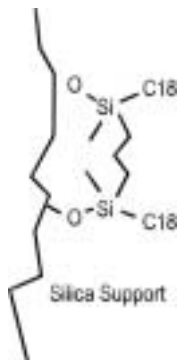
Detection : UV:215 nm  
Temperature : 70°C  
Agilent 1100 well-plate auto sampler with delay volume reduction  
Injection Volume : 20 µl (0.22 µg/1 µl)  
Sample : BSA Tryptic Digest (15 hours, 70 pmol)

A single chromatographic run of a protein tryptic digest can require an hour or more to complete. With ZORBAX Poroshell columns, the same complex separation can be completed in 1/10th the time.



# Separation techniques (Proteins)

## Reversed Phase Chromatography Zorbax



The combination of the unique bidentate structure and double endcapping permits long life at high pH.

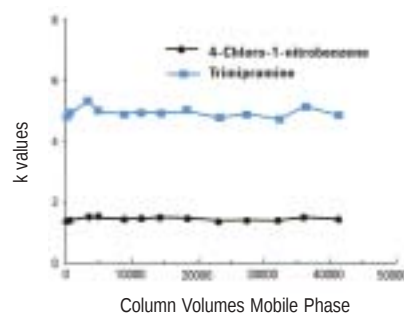
### 300Å Extend-C18

Separations of proteins and peptides from pH 2 to pH 11.5

Zorbax 300Å Extend-C18 is a wide-pore HPLC column for high efficiency separations of peptides from pH 2-11.5. The unique, bidentate bonded phase provides excellent lifetime and reproducibility at high and low pH. At high pH, retention and selectivity of peptides and polypeptides can change dramatically as a result of changes in charge on molecules. Excellent recoveries of hydrophobic polypeptides have been achieved at room temperature and high pH.

- ◆ Porosity : 300 Å
- ◆ Separation of peptide from pH 2-11.5
- ◆ Different selectivity possible at high and low pH
- ◆ High efficiency and good recovery of hydrophobic peptides at high pH
- ◆ Ideal for LC/MS with ammonium hydroxide-modified mobile phase

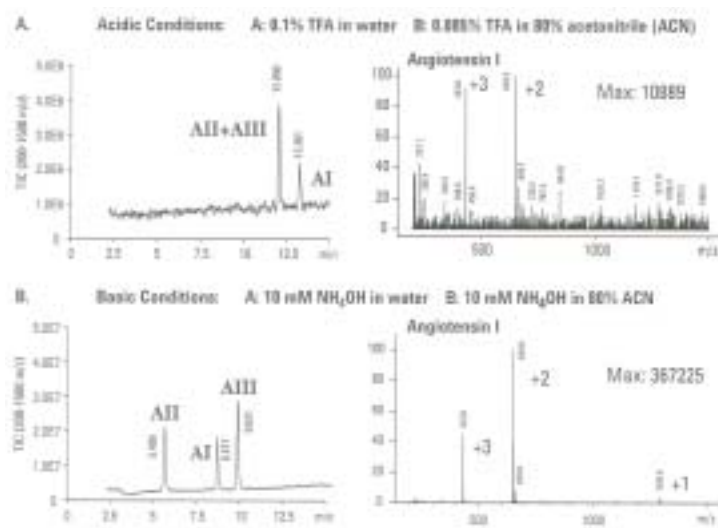
### Long life at high pH with Extend-C18



Column : Zorbax Extend-C18  
4.6 x 150 mm, 5 µm  
Cat.# 773450-902  
Mobile Phase :  
20 % 20 mM NH<sub>4</sub>OH, pH 10.5  
80 % methanol  
Flow rate : 1.5 ml/min  
Aging at 24°C, tests at 40°C

Each 10 000 column volume is approximately one working month.

### LC/MS analysis of Angiotensin on Extend-C18



Column : Zorbax Extend-C18  
2.1 x 150 mm, 5 µm  
Cat.# 773700-902  
Flow rate : 0.2 ml/min  
Temperature : 35°C  
Mobile Phase : as indicated  
Gradient : 15-50 % B in 15 min  
LC/MS : Pos. Ion ESI- V<sub>f</sub> 70 V, V<sub>cap</sub> 4.5 kV  
- 36 psi, 12 L/min., 325°C

Sample :  
Angiotensin I, II, III  
2.5 µl sample (50 pmol each)  
Angiotensin I : Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu  
Angiotensin II : Asp-Arg-Val-Tyr-Ile-His-Pro-Phe  
Angiotensin III : Arg-Val-Tyr-Ile-His-Pro-Phe

Both small and large peptides demonstrate selectivity changes at high and low pH. At high pH, due to a change in charge, all three Angiotensins can be resolved. In addition, the spectral clarity of Angiotensin I is dramatically improved at high pH with the ammonium hydroxide mobile phase. The Extend-C18 column can be used for the analysis of small peptides at high pH as well.

# Separation techniques (Proteins)

## Reversed Phase Chromatography Zorbax

| Column                  | Size (µm)  | Particle | Cat.#      |
|-------------------------|------------|----------|------------|
| <b>Standard Columns</b> |            |          |            |
| Analytical              | 4.6 x 250  | 5        | 770995-902 |
| Analytical              | 4.6 x 150  | 5        | 773995-902 |
| Rapid Resolution        | 4.6 x 150  | 3.5      | 763973-902 |
| Rapid Resolution        | 4.6 x 100  | 3.5      | 761973-902 |
| Rapid Resolution        | 4.6 x 50   | 3.5      | 765973-902 |
| Narrow Bore RR*         | 2.1 x 150  | 3.5      | 763750-902 |
| Narrow Bore RR          | 2.1 x 100  | 3.5      | 761775-902 |
| Narrow Bore RR          | 2.1 x 50   | 3.5      | 765750-902 |
| Guard Cartridges, 4/pk  | 4.6 x 12.5 | 5        | 820950-932 |
| Guard Cartridges, 4/pk  | 2.1 x 12.5 | 5        | 821125-932 |
| Guard Hardware Kit      |            |          | 820888-901 |

### Capillary Glass-lined Columns

|              |           |     |           |
|--------------|-----------|-----|-----------|
| Capillary RR | 0.3 x 150 | 3.5 | 5065-4464 |
| Capillary RR | 0.3 x 100 | 3.5 | 5065-4465 |
| Capillary RR | 0.3 x 75  | 3.5 | 5065-4466 |
| Capillary RR | 0.3 x 50  | 3.5 | 5065-5567 |

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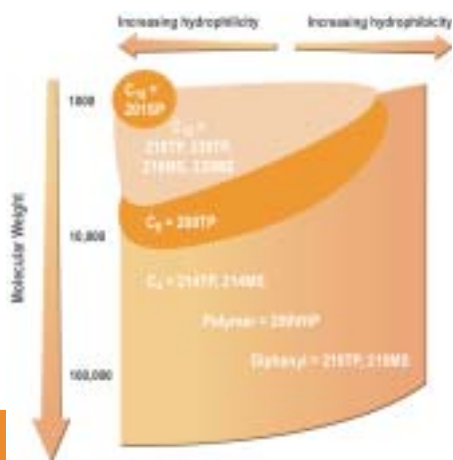
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# Separation techniques (Proteins)

## Reversed Phase Chromatography Vydac

### Vydac® 300Å

Vydac® 300Å silica process results in very high purity metal free spheroidal silica with well controlled characteristics and a large pore size. Derivatized by the covalent surface addition of hydrocarbon chains of various lengths, Vydac® silica proves to be an ideal medium for high performance reversed phase chromatography of proteins, peptides and other large molecules.



See items catalog number in table.

Polymeric reversed phase media (214TP, 208TP, 218TP, 219TP) are produced using polyfunctional alkyl silanes to attach the hydrophobic phase to the silica. This result in high carbon coverage and complex crosslinking polymerization of the hydrocarbon phase. The practical benefits of polymeric phases are :

- ♦ Very long column life time
- ♦ No measurable column leaching

Monomeric reversed phase media (238TP) are produced using monofunctional alkylsilanes. Monomeric phases offer :

- ♦ A simpler monocarbon phase
- ♦ Distinctly different selectivity for many samples

The reversed phase column for a polypeptide separation should be selected bases on the hydrophobicity of the polypeptide being chromatographed and molecular weight as a secondary consideration.

All the Vydac® columns are available from the nanocapillary to the preparative scale.

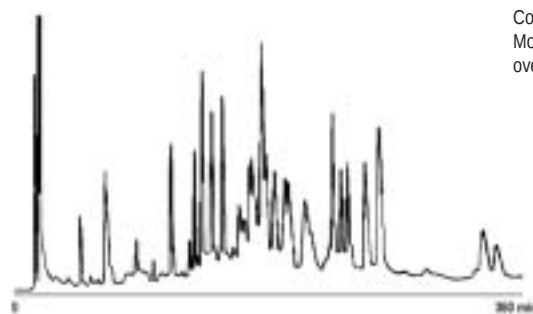
### Vydac® 214TP column

300Å C<sub>4</sub>, polymeric end-capped for the separation of :

- ♦ Polypeptides larger than 4 - 5 kDa

It is optimized for the very hydrophobic polypeptides of any size.

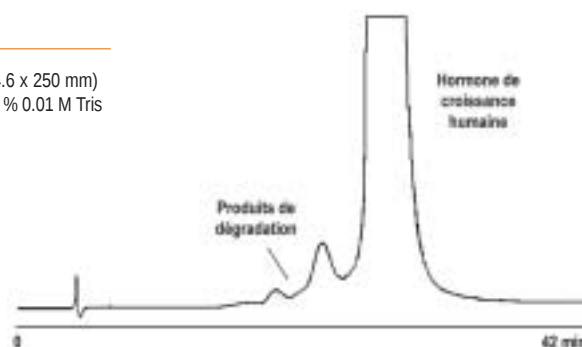
#### Ribosomal Proteins



Column : Vydac 214TP54 (5  $\mu$ m - 4.6 x 250 mm)  
Mobile Phase : 10-38 % isopropanol with 0.1 % over 355 min.

#### HGH

Column : Vydac 214TP54 (5  $\mu$ m - 4.6 x 250 mm)  
Mobile Phase : 29 % isopropanol/71 % 0.01 M Tris buffer, pH 7.5



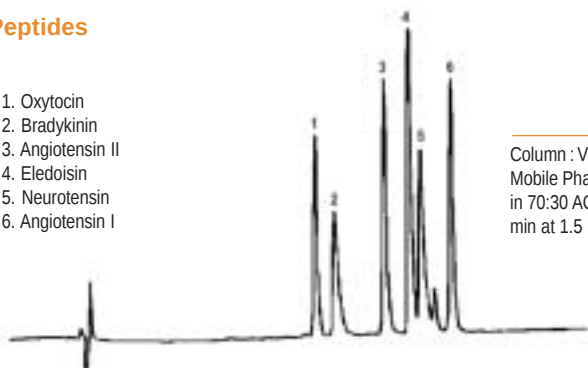
### Vydac® 208TP column

300Å C8, polymeric end-capped for the separation of :

- ◆ Polypeptides up to 10 - 20 kDa
- ◆ Enzymatic digest fragments
- ◆ Natural and synthetic peptides

#### Peptides

1. Oxytocin
2. Bradykinin
3. Angiotensin II
4. Eledoisin
5. Neurotensin
6. Angiotensin I



Column : Vydac 208TP54 (5µm C8 - 4.6 x 250 mm)  
Mobile Phase : A.0.1% aqueous TFA ; B..1 % TFA in 70:30 ACN:H2O ; Gradient from 0 -100 % in 30 min at 1.5 ml/min.

### Vydac® 218TP column

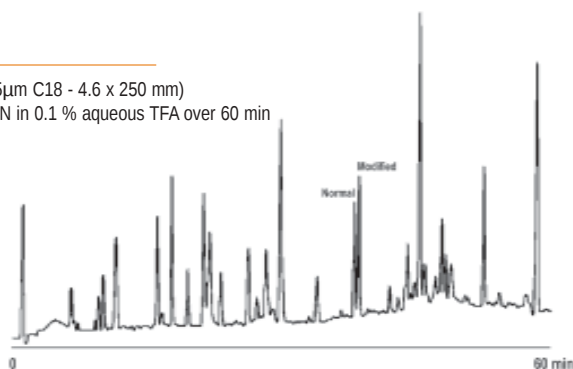
The most popular Vydac column

300Å C18, polymeric end-capped for the separation of :

- ◆ Small peptides less than 4 - 5 kDa
- ◆ Enzymatic digest fragments
- ◆ Natural and synthetic peptides

#### Trypsic Hydrolysate

Column : Vydac 218TP54 (5µm C18 - 4.6 x 250 mm)  
Mobile Phase : 5 - 70 % ACN in 0.1 % aqueous TFA over 60 min



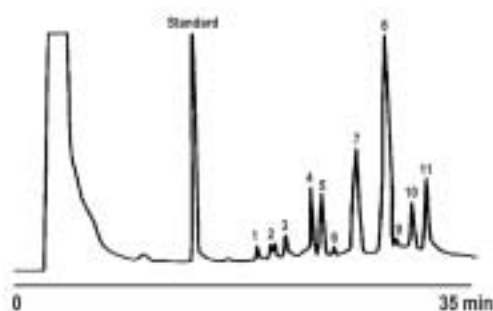
#### Human Apolipoproteins

Column : Vydac 218TP54 (5µm C18 - 4.6 x 250 mm)  
Mobile Phase : 1.2 ml/min 50°C  
A : 25 % ACN/0.1 % TFA in water  
B: 58 % ACN/0.1 % TFA in water  
Gradient : 0 to 100 % B in 33 min

Sample :

- |              |              |
|--------------|--------------|
| 1. apo-IIIa  | 7. apoA-Ia   |
| 2. apoC-IIIb | 8. apoA-Ib   |
| 3. apoC-IIa  | 9. apoA-IIc  |
| 4. apoC-IIc  | 10. apoA-IIa |
| 5. apoC-I    | 11. apoA-IIb |
| 6. apoC-IIb  |              |

Standard : human Insulin



See items catalog number in table p. B156

# Separation techniques (Proteins)

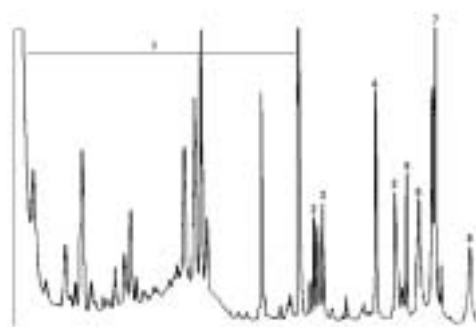
## Reversed Phase Chromatography Vydac

### Vydac® 219TP column

Combines moderate retentivity with unique selectivity  
300Å diphenyl, polymeric end-capped for the separation of :

- ◆ Polypeptides with aromatic side chains
- ◆ Large, hydrophobic proteins
- ◆ Membrane spanning peptides
- ◆ Lipid peptides
- ◆ Fusion proteins from inclusion bodies

#### Peptides/proteins mixture



- Sample :
1. Horse cytochrome C tryptic digest fragments
  2. Whrale myoglobin CNBr digest fragments
  3. Ribonuclease
  4. Cytochrome C
  5. Lysosyme
  6. BSA
  7. Myoglobin
  8. Ovalbumin

Mobile Phase :  
Gradient from 0-70 % Acn with 0.1 % TFA 67.5 min

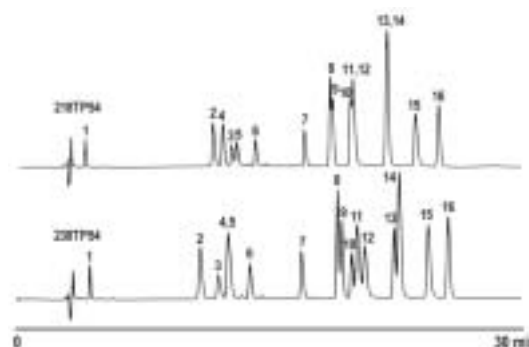
### Vydac® 238TP

300A C18, monomeric. It is an alternative to the polymeric 218TP with subtle differences in selectivity. The combination of these adsorbents can reveal analytes that may be hidden on a single C18 column.

#### Synthetic Peptides

- Sample
1. GY
  2. VYV
  3. neurotensin fragment 1-8 (pELYENKPR)
  4. acRGGGGLGLGK-NH2
  5. RGAGGLGLGK-NH2
  6. acRGAGGLGLGK-NH2
  7. acRGVGLGLGK-NH2
  8. oxytocin (CYIQNCPLG-NH2)
  9. met enkephalin (YGGFM)
  10. bradykinin (RPPGFSPFR)
  11. acRGVGLGLGK-NH2
  12. neurotensin fragment 8-13 (RRPYIL)
  13. angiotensin II (DRVYIHPF)
  14. leu enkephalin (YGGFL)
  15. neurotensin (pELYENKPRRPYIL)
  16. angiotensin I (DRVYIHPFHL)

Mobile Phase :  
10% to 40% ACN avec 0.1% TFA (w/v) over 30 minutes



See items catalog number in table p. B156

### LC/MS grade columns

- ◆ TFA independent

TFA containing mobile phases have a suppressive effect on ion generation, reducing the sensitivity and analytical reliability of LC/MS techniques. To overcome this effect, Vydac developed new adsorbents that produce peptide and protein separations with excellent peak sharpness and symmetry using only a fraction of the TFA concentration normally required : the Vydac® MS.

These Vydac MS adsorbents are based on Vydac's TP 300Å pore size silica.

### EVEREST™

High resolution Reversed-Phase 300 Å silica for columns & process media, which performs 15% better than the existing Vydac products.

Everest™ columns contain a new generation silica based 300A monomeric C18 adsorbent that provides exceptionally high resolution for protein and peptide separation. Their unique selectivity and sensitivity are a result of new bonding technology developed by Vydac that improves C18 surface coverage and deactivates residual silanols.

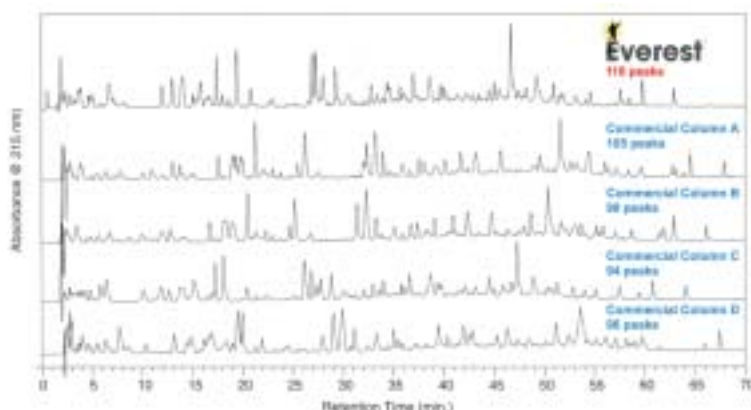
- ◆ High resolution
- ◆ Unique selectivity for both hydrophilic and hydrophobic peptides
- ◆ High sensitivity
- ◆ Excellent reproducibility
- ◆ Long lifetime
- ◆ High peak counts

Everest™ 300 Å C18 columns are recommended as the first choice for all new peptide separation applications, especially those involving highly complex samples such as peptide fingerprinting or proteomic analyses. Everest™ C18 is also available in pre-packed nano/capillary columns for LC/MS applications.

Applications :

- ◆ Peptide Mapping
- ◆ Synthetic Peptides
- ◆ High Loading Applications

#### Tryptic digest of Bovine serum Albumin on a EVEREST™ C18 column



Column : 300 Å pore size, 5 µm, 4.6 mm id. x 150 mm  
 Mobile Phase :  
 A : 0.1 % v/v TFA in water  
 B : 0.085 % v/v TFA in acetonitrile  
 Gradient : 4 % B for 5 min, 4-40 % B in 75 min,  
 40-90 % B in 10 min, 90 % B for 10 min, equilibrate at  
 4 % B for 20 min.

Flow rate : 1.0 ml/min  
 Detector : UV at 215 nm  
 Temperature : 22°C  
 Peptide load : 30 µl of a 5.8 µg/µl digest (174 µg)

Everest™ outperforms commercial columns at higher peptide loads by providing higher-resolution separations

See items catalog number in table p.B157

# Separation techniques (Proteins)

## Reversed Phase Chromatography Vydac

| Dimensions         | Cat.#              | Size µm |
|--------------------|--------------------|---------|
| <b>Vydac 214TP</b> |                    |         |
| 50 x 4.6 mm        | <b>214TP3405</b>   | 3       |
| 100 x 4.6 mm       | <b>214TP3410</b>   | 3       |
| 50 x 1.0 mm        | <b>214TP5105</b>   | 5       |
| 150 x 1.0 mm       | <b>214TP5115</b>   | 5       |
| 250 x 1.0 mm       | <b>214TP51</b>     | 5       |
| 50 x 2.1 mm        | <b>214TP5205</b>   | 5       |
| 150 x 2.1 mm       | <b>214TP5215</b>   | 5       |
| 250 x 2.1 mm       | <b>214TP52</b>     | 5       |
| 150 x 3.2 mm       | <b>214TP5315</b>   | 5       |
| 250 x 3.2 mm       | <b>214TP53</b>     | 5       |
| 50 x 4.6 mm        | <b>214TP5405</b>   | 5       |
| 150 x 4.6 mm       | <b>214TP5415</b>   | 5       |
| 250 x 4.6 mm       | <b>214TP54</b>     | 5       |
| 250 x 10 mm        | <b>214TP510</b>    | 5       |
| 250 x 4.6 mm       | <b>214TP104</b>    | 10      |
| 250 x 10 mm        | <b>214TP1010</b>   | 10      |
| 250 x 22 mm        | <b>214TP1022</b>   | 10      |
| 250 x 22 mm        | <b>214TP101522</b> | 10-15   |

|                    |                    |       |
|--------------------|--------------------|-------|
| <b>Vydac 208TP</b> |                    |       |
| 50 x 4.6 mm        | <b>208TP3405</b>   | 3     |
| 100 x 4.6 mm       | <b>208TP3410</b>   | 3     |
| 50 x 1.0 mm        | <b>208TP5105</b>   | 5     |
| 150 x 1.0 mm       | <b>208TP5115</b>   | 5     |
| 250 x 1.0 mm       | <b>208TP51</b>     | 5     |
| 50 x 2.1 mm        | <b>208TP5205</b>   | 5     |
| 150 x 2.1 mm       | <b>208TP5215</b>   | 5     |
| 250 x 2.1 mm       | <b>208TP52</b>     | 5     |
| 150 x 3.2 mm       | <b>208TP5315</b>   | 5     |
| 250 x 3.2 mm       | <b>208TP53</b>     | 5     |
| 50 x 4.6 mm        | <b>208TP5405</b>   | 5     |
| 150 x 4.6 mm       | <b>208TP5415</b>   | 5     |
| 250 x 4.6 mm       | <b>208TP54</b>     | 5     |
| 250 x 10 mm        | <b>208TP510</b>    | 5     |
| 250 x 4.6 mm       | <b>208TP104</b>    | 10    |
| 250 x 10 mm        | <b>208TP1010</b>   | 10    |
| 250 x 22 mm        | <b>208TP1022</b>   | 10    |
| 250 x 22 mm        | <b>208TP101522</b> | 10-15 |

|                    |                    |       |
|--------------------|--------------------|-------|
| <b>Vydac 218TP</b> |                    |       |
| 50 x 4.6 mm        | <b>218TP3405</b>   | 3     |
| 100 x 4.6 mm       | <b>218TP3410</b>   | 3     |
| 50 x 1.0 mm        | <b>218TP5105</b>   | 5     |
| 150 x 1.0 mm       | <b>218TP5115</b>   | 5     |
| 250 x 1.0 mm       | <b>218TP51</b>     | 5     |
| 50 x 2.1 mm        | <b>218TP5205</b>   | 5     |
| 150 x 2.1 mm       | <b>218TP5215</b>   | 5     |
| 250 x 2.1 mm       | <b>218TP52</b>     | 5     |
| 150 x 3.2 mm       | <b>218TP5315</b>   | 5     |
| 250 x 3.2 mm       | <b>218TP53</b>     | 5     |
| 50 x 4.6 mm        | <b>218TP5405</b>   | 5     |
| 150 x 4.6 mm       | <b>218TP5415</b>   | 5     |
| 250 x 4.6 mm       | <b>218TP54</b>     | 5     |
| 250 x 10 mm        | <b>218TP510</b>    | 5     |
| 250 x 4.6 mm       | <b>218TP104</b>    | 10    |
| 250 x 10 mm        | <b>218TP1010</b>   | 10    |
| 250 x 22 mm        | <b>218TP1022</b>   | 10    |
| 250 x 22 mm        | <b>218TP101522</b> | 10-15 |

| Dimensions         | Cat.#              | Size µm |
|--------------------|--------------------|---------|
| <b>Vydac 238TP</b> |                    |         |
| 50 x 4.6 mm        | <b>238TP3405</b>   | 3       |
| 100 x 4.6 mm       | <b>238TP3410</b>   | 3       |
| 50 x 1.0 mm        | <b>238TP5105</b>   | 5       |
| 150 x 1.0 mm       | <b>238TP5115</b>   | 5       |
| 250 x 1.0 mm       | <b>238TP51</b>     | 5       |
| 50 x 2.1 mm        | <b>238TP5205</b>   | 5       |
| 150 x 2.1 mm       | <b>238TP5215</b>   | 5       |
| 250 x 2.1 mm       | <b>238TP52</b>     | 5       |
| 150 x 3.2 mm       | <b>238TP5315</b>   | 5       |
| 250 x 3.2 mm       | <b>238TP53</b>     | 5       |
| 50 x 4.6 mm        | <b>238TP5405</b>   | 5       |
| 150 x 4.6 mm       | <b>238TP5415</b>   | 5       |
| 250 x 4.6 mm       | <b>238TP54</b>     | 5       |
| 250 x 10 mm        | <b>238TP510</b>    | 5       |
| 250 x 4.6 mm       | <b>238TP104</b>    | 10      |
| 250 x 10 mm        | <b>238TP1010</b>   | 10      |
| 250 x 22 mm        | <b>238TP1022</b>   | 10      |
| 250 x 22 mm        | <b>238TP101522</b> | 10-15   |

|                    |                    |       |
|--------------------|--------------------|-------|
| <b>Vydac 219TP</b> |                    |       |
| 50 x 4.6 mm        | <b>219TP3405</b>   | 3     |
| 100 x 4.6 mm       | <b>219TP3410</b>   | 3     |
| 50 x 1.0 mm        | <b>219TP5105</b>   | 5     |
| 150 x 1.0 mm       | <b>219TP5115</b>   | 5     |
| 250 x 1.0 mm       | <b>219TP51</b>     | 5     |
| 50 x 2.1 mm        | <b>219TP5205</b>   | 5     |
| 150 x 2.1 mm       | <b>219TP5215</b>   | 5     |
| 250 x 2.1 mm       | <b>219TP52</b>     | 5     |
| 150 x 3.2 mm       | <b>219TP5315</b>   | 5     |
| 250 x 3.2 mm       | <b>219TP53</b>     | 5     |
| 50 x 4.6 mm        | <b>219TP5405</b>   | 5     |
| 150 x 4.6 mm       | <b>219TP5415</b>   | 5     |
| 250 x 4.6 mm       | <b>219TP54</b>     | 5     |
| 250 x 10 mm        | <b>219TP5105</b>   |       |
| 250 x 4.6 mm       | <b>219TP104</b>    | 10    |
| 250 x 10 mm        | <b>219TP1010</b>   | 10    |
| 250 x 22 mm        | <b>219TP1022</b>   | 10    |
| 250 x 22 mm        | <b>219TP101522</b> | 10-15 |

|                                 |                     |   |
|---------------------------------|---------------------|---|
| <b>micro column Vydac 208MS</b> |                     |   |
| 50 x 75 mm                      | <b>208MS5.07505</b> | 5 |
| 100 x 75 mm                     | <b>208MS5.07510</b> | 5 |
| 150 x 75 mm                     | <b>208MS5.07515</b> | 5 |
| 250 x 75 mm                     | <b>208MS5.07525</b> | 5 |
| 50 x 150 mm                     | <b>208MS5.1505</b>  | 5 |
| 100 x 150 mm                    | <b>208MS5.1510</b>  | 5 |
| 150 x 150 mm                    | <b>208MS5.1515</b>  | 5 |
| 250 x 150 mm                    | <b>208MS5.1525</b>  | 5 |
| 50 x 300 mm                     | <b>208MS5.305</b>   | 5 |
| 100 x 300 mm                    | <b>208MS5.310</b>   | 5 |
| 150 x 300 mm                    | <b>208MS5.315</b>   | 5 |
| 250 x 300 mm                    | <b>208MS5.325</b>   | 5 |
| 50 x 500 mm                     | <b>208MS5.505</b>   | 5 |
| 100 x 500 mm                    | <b>208MS5.510</b>   | 5 |
| 150 x 500 mm                    | <b>208MS5.515</b>   | 5 |
| 250 x 500 mm                    | <b>208MS5.525</b>   | 5 |

# Separation techniques (Proteins)

## Reversed Phase Chromatography Vydac

| Dimensions                      | Cat.#        | Size µm |
|---------------------------------|--------------|---------|
| <b>micro column Vydac 214MS</b> |              |         |
| 50 x 75 mm                      | 214MS5.07505 | 5       |
| 100 x 75 mm                     | 214MS5.07510 | 5       |
| 150 x 75 mm                     | 214MS5.07515 | 5       |
| 250 x 75 mm                     | 214MS5.07525 | 5       |
| 50 x 150 mm                     | 214MS5.1505  | 5       |
| 100 x 150 mm                    | 214MS5.1510  | 5       |
| 150 x 150 mm                    | 214MS5.1515  | 5       |
| 250 x 150 mm                    | 214MS5.1525  | 5       |
| 50 x 300 mm                     | 214MS5.305   | 5       |
| 100 x 300 mm                    | 214MS5.310   | 5       |
| 150 x 300 mm                    | 214MS5.315   | 5       |
| 250 x 300 mm                    | 214MS5.325   | 5       |
| 50 x 500 mm                     | 214MS5.505   | 5       |
| 100 x 500 mm                    | 214MS5.510   | 5       |
| 150 x 500 mm                    | 214MS5.515   | 5       |
| 250 x 500 mm                    | 214MS5.525   | 5       |

### micro column Vydac 218MS

|              |              |   |
|--------------|--------------|---|
| 50 x 75 mm   | 218MS5.07505 | 5 |
| 150 x 75 mm  | 218MS5.07515 | 5 |
| 250 x 75 mm  | 218MS5.07525 | 5 |
| 50 x 150 mm  | 218MS5.1505  | 5 |
| 150 x 150 mm | 218MS5.1515  | 5 |
| 250 x 150 mm | 218MS5.1525  | 5 |
| 50 x 300 mm  | 218MS5.305   | 5 |
| 150 x 300 mm | 218MS5.315   | 5 |
| 250 x 300 mm | 218MS5.325   | 5 |
| 50 x 500 mm  | 218MS5.505   | 5 |
| 100 x 500 mm | 218MS5.510   | 5 |
| 150 x 500 mm | 218MS5.515   | 5 |
| 250 x 500 mm | 218MS5.525   | 5 |

### micro column Vydac 219MS

|              |              |   |
|--------------|--------------|---|
| 50 x 75 mm   | 219MS5.07505 | 5 |
| 150 x 75 mm  | 219MS5.07515 | 5 |
| 250 x 75 mm  | 219MS5.07525 | 5 |
| 50 x 150 mm  | 219MS5.1505  | 5 |
| 100 x 150 mm | 219MS5.1510  | 5 |
| 150 x 150 mm | 219MS5.1515  | 5 |
| 250 x 150 mm | 219MS5.1525  | 5 |
| 100 x 300 mm | 219MS5.310   | 5 |
| 150 x 300 mm | 219MS5.315   | 5 |
| 250 x 300 mm | 219MS5.325   | 5 |
| 50 x 500 mm  | 219MS5.505   | 5 |
| 100 x 500 mm | 219MS5.510   | 5 |
| 150 x 500 mm | 219MS5.515   | 5 |
| 250 x 500 mm | 219MS5.525   | 5 |

### micro column Vydac 238MS

|              |              |   |
|--------------|--------------|---|
| 100 x 75 mm  | 238MS5.07510 | 5 |
| 150 x 75 mm  | 238MS5.07515 | 5 |
| 250 x 75 mm  | 238MS5.07525 | 5 |
| 50 x 150 mm  | 238MS5.1505  | 5 |
| 100 x 150 mm | 238MS5.1510  | 5 |
| 150 x 150 mm | 238MS5.1515  | 5 |
| 250 x 150 mm | 238MS5.1525  | 5 |
| 50 x 300 mm  | 238MS5.305   | 5 |
| 100 x 300 mm | 238MS5.310   | 5 |
| 150 x 300 mm | 238MS5.315   | 5 |
| 250 x 300 mm | 238MS5.325   | 5 |
| 50 x 500 mm  | 238MS5.505   | 5 |
| 100 x 500 mm | 238MS5.510   | 5 |
| 150 x 500 mm | 238MS5.515   | 5 |
| 250 x 500 mm | 238MS5.525   | 5 |

| Dimensions                      | Cat.#        | Size µm |
|---------------------------------|--------------|---------|
| <b>micro column Vydac 238MS</b> |              |         |
| 50 x 75 mm                      | 238EV5.07505 | 5       |
| 100 x 75 mm                     | 238EV5.07510 | 5       |
| 150 x 75 mm                     | 238EV5.07515 | 5       |
| 250 x 75 mm                     | 238EV5.07525 | 5       |
| 50 x 150 mm                     | 238EV5.1505  | 5       |
| 100 x 150 mm                    | 238EV5.1510  | 5       |
| 150 x 150 mm                    | 238EV5.1515  | 5       |
| 250 x 150 mm                    | 238EV5.1525  | 5       |
| 50 x 300 mm                     | 238EV5.305   | 5       |
| 100 x 300 mm                    | 238EV5.310   | 5       |
| 150 x 300 mm                    | 238EV5.315   | 5       |
| 250 x 300 mm                    | 238EV5.325   | 5       |
| 50 x 500 mm                     | 238EV5.505   | 5       |
| 100 x 500 mm                    | 238EV5.510   | 5       |
| 150 x 500 mm                    | 238EV5.515   | 5       |
| 250 x 500 mm                    | 238EV5.525   | 5       |

### Vydac Everest™ column

|                 |            |   |
|-----------------|------------|---|
| 1.0 mm x 50 mm  | 238EV5105  | 5 |
| 1.0mm x 100 mm  | 238EV5110  | 5 |
| 1.0 mm x 150 mm | 238EV5115  | 5 |
| 1.0 mm x 250 mm | 238EV51    | 5 |
| 2.1 mm x 150 mm | 238EV5215  | 5 |
| 2.1 mm x 150 mm | T238EV5215 | 5 |
| 2.1 mm x 250 mm | 238EV52    | 5 |
| 2.1 mm x 250 mm | T238EV52   | 5 |
| 4.6 mm x 150 mm | T238EV5415 | 5 |
| 4.6 mm x 250 mm | 238EV54    | 5 |
| 4.6 mm x 250 mm | T238EV54   | 5 |

### Guard column for Vydac column

| Phase | µm | int mm | Kit<br>(cartridge + holder) | Cartridge   |
|-------|----|--------|-----------------------------|-------------|
| 201HS | 5  | 2.1    | 201GK52H                    | 201GD52H    |
|       | 5  | 4.6    | 201GK54H                    | 201GD54H    |
|       | 10 | 4.6    | 201GK104H                   | 201GD104H   |
|       | 12 | 10     | 201FSK1210H                 | 201GCC1210H |
| 208HS | 5  | 2.1    | 208GK52H                    | 208GD52H    |
|       | 5  | 4.6    | 208GK54H                    | 208GD54H    |
|       | 10 | 4.6    | 208GK104H                   | 208GD104H   |
|       | 12 | 10     | 208FSK1210H                 | 208GCC1210H |
| 201TP | 5  | 2.1    | 201GK52T                    | 201GD52T    |
|       | 5  | 4.6    | 201GK54T                    | 201GD54T    |
|       | 10 | 4.6    | 201GK104T                   | 201GD54T    |
| 202TP | 5  | 2.1    | 202GK52T                    | 202GD52T    |
|       |    |        | 202GK54T                    | 202GD54T    |
| 208TP | 5  | 2.1    | 208GK52T                    | 208GD52T    |
|       | 5  | 4.6    | 208GK54T                    | 208GD54T    |
|       | 10 | 4.6    | 208GK104T                   | 208GD104T   |
|       | 12 | 10     | 208FSK1210T                 | 208GCC1210T |
| 214TP | 5  | 2.1    | 214GK52T                    | 214GD52T    |
|       | 5  | 4.6    | 214GK54T                    | 214GD54T    |
|       | 10 | 4.6    | 214GK104T                   | 214GD104T   |
|       | 12 | 10     | 214FSK1210T                 | 214GCC1210T |
| 218TP | 5  | 2.1    | 218GK52T                    | 218GD52T    |
|       | 5  | 4.6    | 218GK54T                    | 218GD54T    |
|       | 10 | 4.6    | 218GK104T                   | 218GD104T   |
|       | 12 | 10     | 218FSK1210T                 | 218GCC1210T |
| 219TP | 5  | 2.1    | 219GK52T                    | 219GD52T    |
|       | 5  | 4.6    | 219GK54T                    | 219GD54T    |
|       | 10 | 4.6    | 219GK104T                   | 219GD104T   |
|       | 12 | 10     | 219FSK1210T                 | 219GCC1210T |
| 228TP | 10 | 4.6    | 228GK104T                   | 228GD104T   |
| 238TP | 5  | 2.1    | 238GK52T                    | 238GD52T    |
|       | 5  | 4.6    | 238GK54T                    | 238GD54T    |
|       | 10 | 4.6    | 238GK104T                   | 238GD104T   |
|       | 12 | 10     | 238FSK1210T                 | 238GCC1210T |

# Separation techniques (Proteins)

## Reversed Phase Chromatography Agilent

### Macroporous Reversed-Phase C18 Column



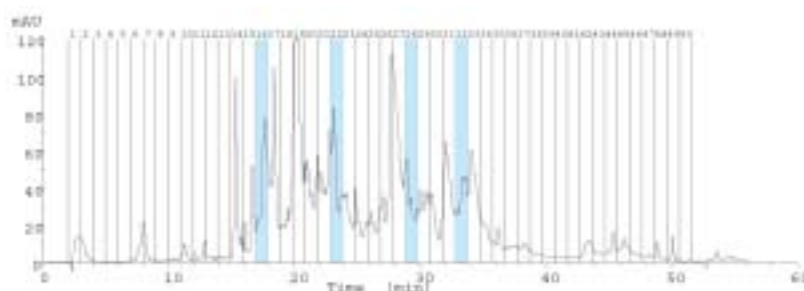
#### Separation of complex protein mixtures

The unique feature of this Macroporous Reversed-Phase C18 column includes high protein recoveries\*- Up to 98%-, consistent reproducibility, increased column loads and enhanced resolution compared to conventional silica based RP HPLC columns.

- ◆ Increased loadability, reproducibility and resolution/selectivity compared to conventional RP-C18 columns and other LC packings.
- ◆ Compatible with Agilent LC 1100, standard HPLC equipment, and mobile phases.
- ◆ Ability to resolve complex mixtures (like immunodepleted serum) and cut them into many distinct fractions.
- ◆ Ideal for fractionating or desalting immunodepleted serum for biomarker discovery.
- ◆ Opportunity to replace gel electrophoresis, or even enhance gel separations (reduce their complexity).

The Agilent mRP-C18 column is designed specifically for the separation of complex protein mixtures for protein/polypeptide fractionation prior to downstream analysis. The mRP-C18 column performs optimally under gradient separations at low pH, with mobile phases such as acetonitrile/aqueous trifluoroacetic acid combinations widely used for protein and peptide reversed-phase separations. This column, used with the recommended gradient and elevated temperatures between 70°C and 80°C, will provide excellent reproducibility, high protein mass recovery and high resolution.

The separation characteristics of the Agilent mRP-C18 column make it a robust proteomic tool to fractionate complex protein samples. High protein recovery, enhanced resolution, high column loading and run-to-run reproducibility make the mRP-C18 column ideal for proteomic sample fractionation and/or desalting/concentration. Complex proteomic samples, such as serum, should be immunodepleted to remove high abundant proteins.



B.158

| Sample | Number of proteins Identified |
|--------|-------------------------------|
|--------|-------------------------------|

|                                     |     |
|-------------------------------------|-----|
| Human serum                         | 40  |
| Immunodepleted Human Serum          | 181 |
| Fraction 16                         | 71  |
| Fraction 22                         | 97  |
| Fraction 28                         | 99  |
| Fraction 32                         | 181 |
| Combined Fractions (16, 22, 28, 32) | 394 |

| Description | Cat.# |
|-------------|-------|
|-------------|-------|

|             |           |
|-------------|-----------|
| 4.6 x 50 mm | 5188-5231 |
|-------------|-----------|

### Atoll Polymeric phase

Atoll is a family of spherical macro-porous co-polymers which are extremely pure and very highly cross-linked. Atoll is extremely pure and has been designed to support applications such as solid phase sample preparation and HPLC analysis of biomolecules.

- ◆ Ultra pure
- ◆ High mechanical resistance
- ◆ Pharmaceutical grade

Rigorous manufacturing protocols, combined with a judicious choice of monomer ratio, guarantees Atoll's excellent mechanical stability. The Atoll polymeric support does not "swell" and can be used with all ordinary solvents.

- ◆ pH range : 0 - 14

Atoll polymeric supports do not suffer from irreversible absorption (a problem inherent with traditional silica based supports). Sample bio-activity is therefore maintained and sample output optimised an essential feature of this purification tool.

**Atoll NPR** : non-porous resin with a 2 µm particle size dedicated to ultra-fast analyses of biomolecules in LC/MS, MS/MS...

**Atoll MP** : porous resin recommended for the purification of pharmaceutical molecules, small basic molecules and peptides

**Atoll MP3** : macro-porous resin adapted to the separation of hydrophilic peptides, proteins, recombinant proteins and bio-purification.

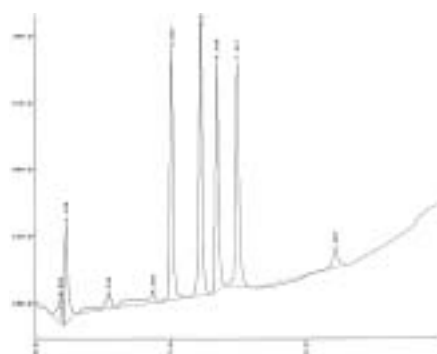
Atoll polymers are available in several sizes and hardwares (depending on the application).

Columns, cartridges and 96 well plates for sample preparation and purification

- ◆ Nano / Micro columns
- ◆ Modulo-cart Quick seal
- ◆ Modulo-cart prep
- ◆ Easy-pack
- ◆ Low pressure glass column
- ◆ Columns and cartridges for flash chromatography



|                    | Atoll NPR             | Atoll MP              | Atoll MP3             |
|--------------------|-----------------------|-----------------------|-----------------------|
| Pore size          | 0                     | n.a.                  | 300 Å                 |
| Surface area       | 2.5 m <sup>2</sup> /g | 500 m <sup>2</sup> /g | 500 m <sup>2</sup> /g |
| Bonding Technology | non - functional      |                       |                       |
| 2 µm               | x                     |                       |                       |
| 3 µm               |                       | on request            |                       |
| 5 µm               |                       | x                     | x                     |
| 10 µm              |                       | on request            |                       |
| 15 µm              |                       | x                     | x                     |
| 30 µm              |                       | on request            |                       |



#### Proteins mixture

Column : Atoll 5 µm, 50 x 4.6 mm  
 Mobile phase :  
 H<sub>2</sub>O + 0.1% TFA to ACN  
 + 0.1% TFA (15min)  
 Flow rate : 1 ml/min  
 Detection : UV 254 nm

1. Ribonuclease A
2. Cytochrome C
3. Holo-Transferrin
4. Apomyoglobin

# Separation techniques (Proteins)

## Reversed Phase Chromatography Uptisphere

### Modulo-cart Quick seal Atoll [QS]



| $\mu\text{m}$ | mm        | Atoll NPR   | Atoll MP    | Atoll MP3    |
|---------------|-----------|-------------|-------------|--------------|
| 2,0 mm I.D.   |           |             |             |              |
| 2             | 33 x 2.0  | ATL2NPR#3QS |             |              |
|               | 50 x 2.0  | ATL2NPR#5QS |             |              |
| 5             | 33 x 2.0  |             | ATL5MP#3QS  | ATL5MP3#3QS  |
|               | 50 x 2.0  |             | ATL5MP#5QS  | ATL5MP3#5QS  |
|               | 75 x 2.0  |             | ATL5MP#7QS  | ATL5MP3#7QS  |
|               | 100 x 2.0 |             | ATL5MP#10QS | ATL5MP3#10QS |
|               | 150 x 2.0 |             | ATL5MP#15QS | ATL5MP3#15QS |
|               | 250 x 2.0 |             | ATL5MP#25QS | ATL5MP3#25QS |
| 4,6 mm I.D.   |           |             |             |              |
| 2             | 33 x 4.6  | ATL2NPR-3QS |             |              |
|               | 50 x 4.6  | ATL2NPR-5QS |             |              |
| 5             | 33 x 4.6  |             | ATL5MP-3QS  | ATL5MP3-3QS  |
|               | 50 x 4.6  |             | ATL5MP-5QS  | ATL5MP3-5QS  |
|               | 75 x 4.6  |             | ATL5MP-7QS  | ATL5MP3-7QS  |
|               | 100 x 4.6 |             | ATL5MP-10QS | ATL5MP3-10QS |
|               | 150 x 4.6 |             | ATL5MP-15QS | ATL5MP3-15QS |
|               | 250 x 4.6 |             | ATL5MP-25QS | ATL5MP3-25QS |



| $\mu\text{m}$ | mm        | Atoll NPR   | Atoll MP    | Atoll MP3    |
|---------------|-----------|-------------|-------------|--------------|
| 2,0 mm I.D.   |           |             |             |              |
| 2             | 33 x 2.0  | ATL2NPR#3QK |             |              |
|               | 50 x 2.0  | ATL2NPR#5QK |             |              |
| 5             | 33 x 2.0  |             | ATL5MP#3QK  | ATL5MP3#3QK  |
|               | 50 x 2.0  |             | ATL5MP#5QK  | ATL5MP3#5QK  |
|               | 75 x 2.0  |             | ATL5MP#7QK  | ATL5MP3#7QK  |
|               | 100 x 2.0 |             | ATL5MP#10QK | ATL5MP3#10QK |
|               | 150 x 2.0 |             | ATL5MP#15QK | ATL5MP3#15QK |
|               | 250 x 2.0 |             | ATL5MP#25QK | ATL5MP3#25QK |
| 4,6 mm I.D.   |           |             |             |              |
| 2             | 33 x 4.6  | ATL2NPR-3QK |             |              |
|               | 50 x 4.6  | ATL2NPR-5QK |             |              |
| 5             | 33 x 4.6  |             | ATL5MP-3QK  | ATL5MP3-3QK  |
|               | 50 x 4.6  |             | ATL5MP-5QK  | ATL5MP3-5QK  |
|               | 75 x 4.6  |             | ATL5MP-7QK  | ATL5MP3-7QK  |
|               | 100 x 4.6 |             | ATL5MP-10QK | ATL5MP3-10QK |
|               | 150 x 4.6 |             | ATL5MP-15QK | ATL5MP3-15QK |
|               | 250 x 4.6 |             | ATL5MP-25QK | ATL5MP3-25QK |

### Biomolecules Purifications

| $\mu\text{m}$ | mm         | Atoll NPR   | Atoll MP     | Atoll MP3 |
|---------------|------------|-------------|--------------|-----------|
| 10,0 mm I.D.  |            |             |              |           |
| 15            | 250 x 10.0 | ATL15MP.25M | ATL15MP3.25M | PSD       |
| 21,2 mm I.D.  |            |             |              |           |
| 15            | 250 x 21.2 | ATL15MP/25M | ATL15MP3/25M | PSD       |
| 28,0 mm I.D.  |            |             |              |           |
| 15            | 250 x 28.0 | ATL15MP&25M | ATL15MP3&25M | PSD       |

### HILIC "Hilic Interaction Chromatography"

Rapid separation and analysis of hydrophilic compounds can be accomplished by normal phase liquid chromatography (NPLC) or hydrophilic interaction chromatography (HILIC). In NPLC, the elution of molecules occurs by introduction a more polar or aqueous mobile phase. HILIC is a variant of NPLC in which the stationary phase is polar and the mobile phase is mostly organic. Water present in the mobile phase forms a stagnant aqueous layer on the surface of the stationary phase, enabling partition between the mobile phase and this aqueous layer.

#### TSKgel Amide-80

The TSKgel Amide-80 from Tosoh Bioscience is an excellent choice for separating hydrophilic compounds such as peptides, carbohydrates and polar natural products.

Figure 1 shows the comparison of selectivity between a mix of free amino acids and peptides separated by RPC and HILIC with detection by ESI-MS.

On the RP-packing no retention of the free amino acids or of the glutamyl peptides could be observed, only the more hydrophobic leucine and the tripeptide WGY were retained. However, the separation of free amino acids and hydrophilic glutamyl di- and tripeptides was achieved in 85 min by HILIC on the TSKgel Amide-80 column without derivatization.

#### Characteristics :

The TSKgel Amide-80 column is packed with 5  $\mu\text{m}$  or 10  $\mu\text{m}$  spherical silica particles covalently bonded with carbamoyl groups.

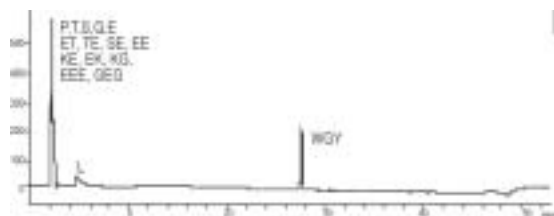
The polar functional groups of the sample, such as hydroxy groups, form hydrogen bonds with the polar groups (amino groups) of the stationary phase. The number of hydroxy groups, conformation and solubility of the sample in the mobile phase will determine the order of elution. The retention mechanism differs from other HILIC or Amide columns, which mainly include RP phases with embedded polar groups. On the other hand the TSKgel Amide-80 packing is superior to aminoalkyl silica gel in chemical stability and will not react with reducing sugars

in carbohydrate analysis. Besides detection by ESI-MS, the stability of the TSKgel Amide-80 stationary phase also enables detection by Evaporative Light Scattering (ELS).

#### TSKgel Amide-80 column : typical properties

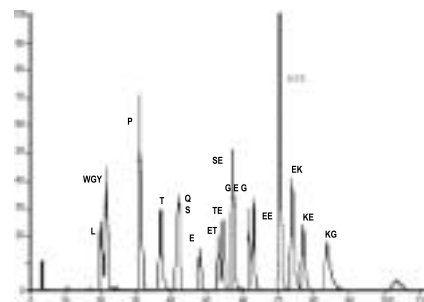
| Product      | Cat.#        | ID   | Length | Porosity | Particle size     | Min. Number        |             |      |                            |  |
|--------------|--------------|------|--------|----------|-------------------|--------------------|-------------|------|----------------------------|--|
| Flow (ml/mn) | Max.Pressure | (mm) | (mm)   | (Å)      | ( $\mu\text{m}$ ) | Theoretical Plates | Rang.       | Max  | Drop (kg/cm <sup>2</sup> ) |  |
| Amide-80     | <b>20009</b> | 1.0  | 5      | 5        | 80                | n/a                | 0.15 - 0.18 | 0.22 | 250                        |  |
| Amide-80     | <b>19697</b> | 2.0  | 25     | 5        | 80                | n/a                | 0.15 - 0.18 | 0.22 | 250                        |  |
| Amide-80     | <b>13071</b> | 4.6  | 25     | 5        | 80                | 8,000              | 0.5 - 1.0   | 1.2  | 150                        |  |
| Amide-80     | <b>14459</b> | 7.8  | 30     | 10       | 80                | 5,000              | 1.0 - 2.0   | 3.0  | 70                         |  |
| Amide-80     | <b>14460</b> | 21.5 | 30     | 10       | 80                | 6,000              | 2.0 - 4.0   | 8.0  | 30                         |  |

#### RP-HPLC of a mixture of free amino acids, di and tripeptides



A mixture of 6 free amino acids (1.9 - 3  $\mu\text{mol/ml}$ ), 7 dipeptides (0.4 - 1.2  $\mu\text{mol/ml}$ ) and 3 tripeptides (0.4 - 0.9  $\mu\text{mol/ml}$ ) was analyzed by HPLC on a RP-C18 column packing with hydrophilic endcapping. No retention of the hydrophilic free amino acids nor of the glutamyl peptides could be observed, only the more hydrophobic leucine as well as the tripeptide WGY were retained by the RP column packing (Fig. 1)

#### HILIC-ESI-MS base peak chromatogram of free amino acids, di and tripeptides



However, the separation of free amino acids and of hydrophilic glutamyl di- and tripeptides over 85 min was achieved by HILIC (carbamoyl column packing) without derivatization ( Fig. 2)

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