

#### Introduction

Teknokroma introduces in the market the new line of Europa HPLC columns.

After the versatility of our popular **mediterranea**™ **Sea 18** column that enables you to deal successfully with the inmense variety of separations in the fields of pharmaceuticals, life sciences, environment, foods, etc. Teknokroma has focused all its efforts and all its know-how, accumulated through more than 30 years of chromatographic research and development, in offering the best reverse phase HPLC packing for identification and purification of peptides and protein compounds.

Manufactured using novel proprietary technologies, analytical and preparative Europa columns are simply the best reverse phase columns available today.

As a result of these, we launch into the market the Line of Europa HPLC columns, one of the best columns in the field of analysis of biomolecules.

The Europa HPLC columns for peptides and proteins, provide the best performance and unsurpassed efficiency, reliability and reproducibility.

There is still a consensus that the best material to use as chromatographic packing continues to be silica. The particles of silica material are physically resistant, enable multiple functions. present maximum levels of efficiency and are also compatible with practically all solvents.

Teknokroma has dedicated years of research and development in obtaining the best silica particle on the market. The silica particle on which the Europa columns is based is the result of an optimisation process, starting with extremely pure materials with unusually low metal content, and obtaining a perfectly spherical, rigid and inert particle.

Furthermore, the propietary "porification process" (Surface Enhanced Accessibility, SEA) for Europa silica has achieved high surface area without sacrificing important properties like physical resistence and high loading capacity- making it ideal for preparative-scale processing.

In addition, the Surface Enhanced Accessibility manufacturing process creates a porous structure that ensures maximum transfer speeds for solutes between the stationary and mobile phases-resulting in higher separation efficiency.

Our "Ultra-Fast" Europa columns are made in 3-5 cm length in order to get quick analytical results, whereas the "High Efficiency" columns are normally in 15-25 cm lengths to obtain best resolution.

The Teknokroma Europa Columns are uniquely designed with optimized pore size distribution; 120Å for Peptide and 300Å for the Protein Columns.

Europa columns are available for:

Peptides: Europa C18 with 0.21, 0.30, 0.40, 0.46, 0.78, 1.0 and 2.12 cm.

**Proteins:** Europa C18, C8 and C4 with 0.21, 0.30, 0.40, 0.46, 0.78, and 2.12 cm.

#### Purity of silica

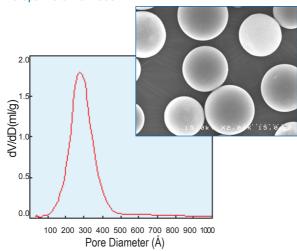
The responsibility for chromatographic separation of peptides and proteins is found inside the particle-within the pores. To obtain a very homogeneous pore distribution the least possible number of nanopores is essential.

For most reverse-phase silica packings, these nanopores are not properly chemically bonded, endcapped or deactivated. So when nanopores are accessible to the peptides and proteins, surfacepeptide and protein interactions frequently dominate. These interactions often result in a decrease of column efficiency.

# K Europa HPLC Column for Peptides and Proteins

#### Europa Protein C4 Pore Distribution

#### Europa Protein C 4 300



#### **Deactivation Process**

Thanks to our propietary new Multifunctional Endcapping Deactivation (MED) technology used with our popular HPLC columns Mediterranea™ Sea 18, we obtain with the Europa packing a specially designed C4, C8 and C18 ligand configuration, that blocks practically all the active centres that may have remained on the surface of the silica.

As a result of this, Europa columns have an unusual low level of silanol activity, helping you to obtain symetrical peaks for the most basic and acidic compounds. The improved high density bonding and full endcapping make them suitable to separate or purify low molecular weight compounds (especially small peptides when using Europa Peptide column 120 Å) and separate or purify high molecular weight compounds, especially proteins when using Europa Protein column 300 Å.

Europa C 18 bonding chemistries will help you to achieve an extraordinary resistance and column lifetime when running at extreme pH levels.

#### Wide pH Range

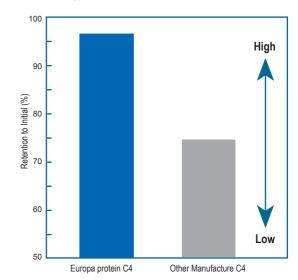
Using Europa C 18 packing materials it is possible to work with eluents from pH 1 to pH 12. Such unusual pH resistance values have been achieved as a result of phase bonding efficiency and a propietary endcapping process which provides a protective shield against acidic and basic eluents.

Europa columns ensure greater separation efficiency, resistance to extreme pH conditions and can be used for an extended period of time.

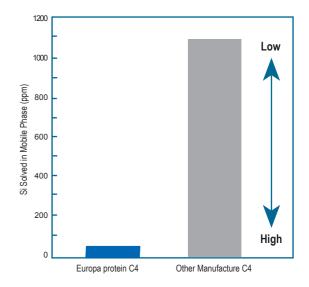
#### Europa Protein C4 Phase Stability

Phase stability of Europa Protein C4 columns has been checked purging one 25 x 0.78 cm column either with CH3CN/1%TFA 10:90 (pH=1)during 15 hours at 0.9 ml/min or with CH3CN/20 mM Na3PO4 10:90 (pH=12) during 3 hours at 1.7 ml/min.

#### Acid Resistance pH=1



#### Alkalil Resistance pH=12

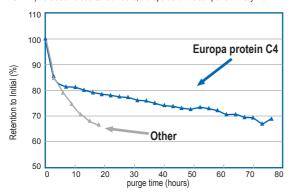


## Europa HPLC Column for Peptides and Proteins K

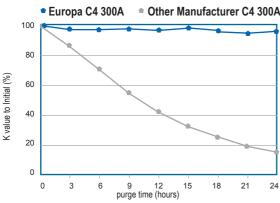
#### Durability comparison in Alkaline Medium/RT

The graphic bellow shows the durability of the column after more than 80 hours of purge time passing through one Europa Protein C4 column a flow rate of 1.0 ml of alkaline solution at pH 12, CH3CN/0.01NaOH 10/90.

There is represented in the graphic the retention time of naftalene after every three hours of purge, using CH3CN / H2O 35:65 at 1.7 ml/min and 40°C (UV detection at 254 nm). It is seen that after 80 hours, Europa columns still perform very well.

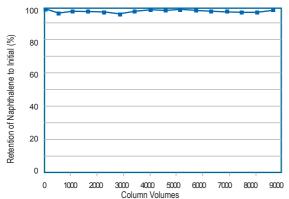


#### Durability comparison in Acidic Medium / K value



Durability of Europa C4 has also been compare against other manufacturers using a  $15 \times 0.46$  cm column and CH3CN / 1.0% TFA in water 10:90 (pH=1) at  $70^{\circ}$ C, and checking K values for naftalene every 3 hours.

### **Durability under Acidic Contition**



Retention time for naphtalene using the same chromatographic conditions has also been controlled after up to 9000 column volumes of CH3CN / 0.05% TFA in water (pH=2) at a flow rate of 1.0 ml/min at room temperature. Column size was 15 x 0.46 cm

#### Europa C18 Peptide HPLC columns

We invite you to try our Europa C18 peptide column when you experience unsatisfactory results with your favorite column.

Europa C18 Peptide columns are suitable to separate or purify low molecular weight compounds, especially small peptides.

Europa HPLC columns for peptides provide a high performance that is unsurpassed in efficiency, reliability and reproducibility. Manufactured using novel proprietary technologies, analytical and preparative Europa columns are simply the best reverse phase columns available today. Europa columns ensure greater separation efficiency, resistance to extreme pH conditions and longer column life.

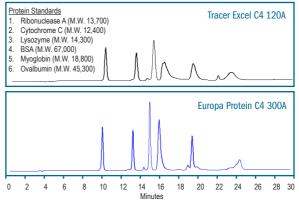
Our "Ultra-Fast" columns are made in 3-5 cm length in order to get quick analytical results, whereas the "High Efficiency" columns are normally in 15-25cm lengths to obtain the best resolution.

#### Specifications:

- Ultra high purity, totally spherical silica gel
- High density bonding for extreme performance proprietary fully end-capped silica
- Porous Size: 120 Å, narrow particle size distribution
- Surface Area 300 m<sup>2</sup>/g
- % of Carbon 19 %
- · High loading capacity of crude peptides
- · Stable under basic and extreme acidic conditions
- Packed with 5µm sized silica particles

Microbore Columns are available in: 0.21, 0.30 cm ID Analytical Columns are available in: 0.40 and 0.46 cm ID Semi-Prep Columns are available in: 0.78 and 1.0 cm Prep Columns are available in: 2.1 cm ID Larger diameter available by request

#### Influence of Pore size in Peak Shape



Column: 7.8 mm I.D. x 250 mm Length; Temperature: 35°C; Detector: UV 220 nm; Mobile Phase: A) CH3CN/H2O/TFA = 20/80/0.1, B) CH3CN/H2O/TFA = 60/40/0.1, Linear Gradient from A to B in 25 min and hold for 10 min; Flow Rate: 1.7 ml/min.