



# Superior HPLC Packing Materials: High Resolution and Excellent Durability for Peptide and Protein Separation



# New: DAISOGEL BIO Series



For both, the research and the big scale of peptide and protein manufacturing spherical silica must be tailor made, fine tuned. The new line up of DAISOGEL shows **extended acidic** and **alkalic resistance**, **high loadability** combined with exceptional **durability**.

- The perfect choices for biopharmaceutical applications
- Novel chemical bonding assures stability under severe alkalic and acidic conditions



# DAISOGEL BIO Series

**SP-120-BIO** for smaller molecules, smaller peptides

**SP-200-BIO** for bigger peptides (MW ca. 5000)

**SP-300-BIO** for proteins (MW ca. 20000)

silica	pore size (nm)	particle size ( $\mu$ m)	bonded phase
SP (spherical) ultra pure	12 20 30	5 10 15 20	ODS C8 C4



# Estimation of the Size of Proteins



$$\text{Size (diameter)} = 0.62 \times (\text{mol. weight})^{0.59}$$

Molecular weight	Estimated diameter of the molecule (Å)	Recommended pore size (Å)
1,000	36.5	120
5,000	94.4	200
20,000	213.8	300

**Molecules must be smaller than the pore size to take advantage on all chromatographically accessible surface area!**

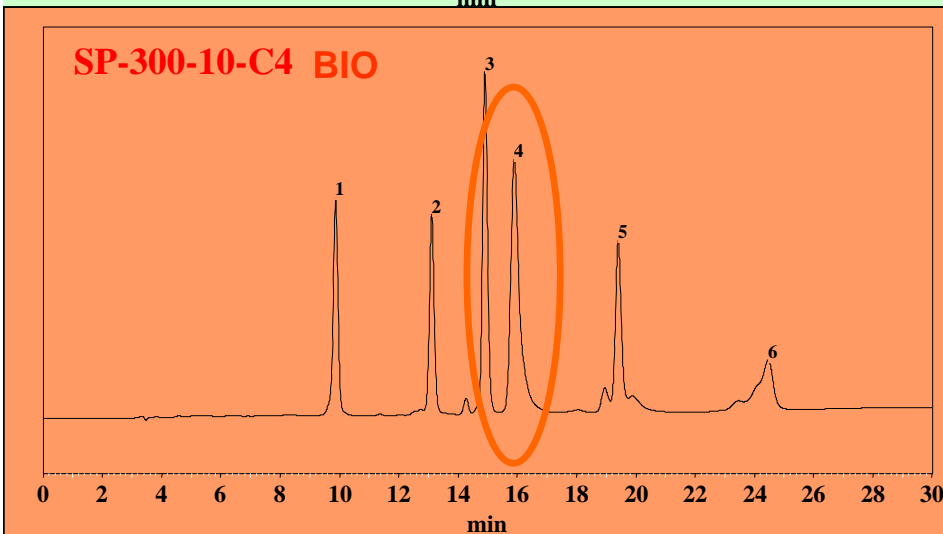
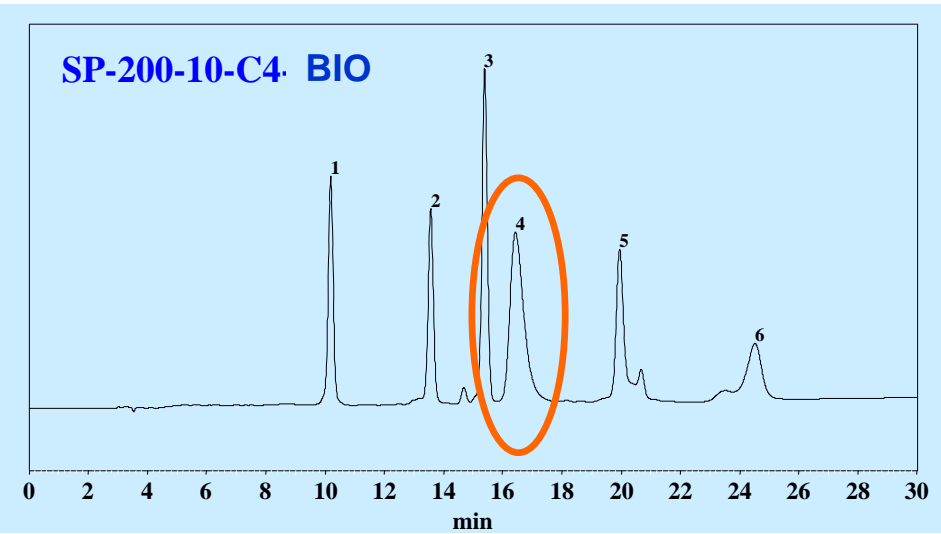
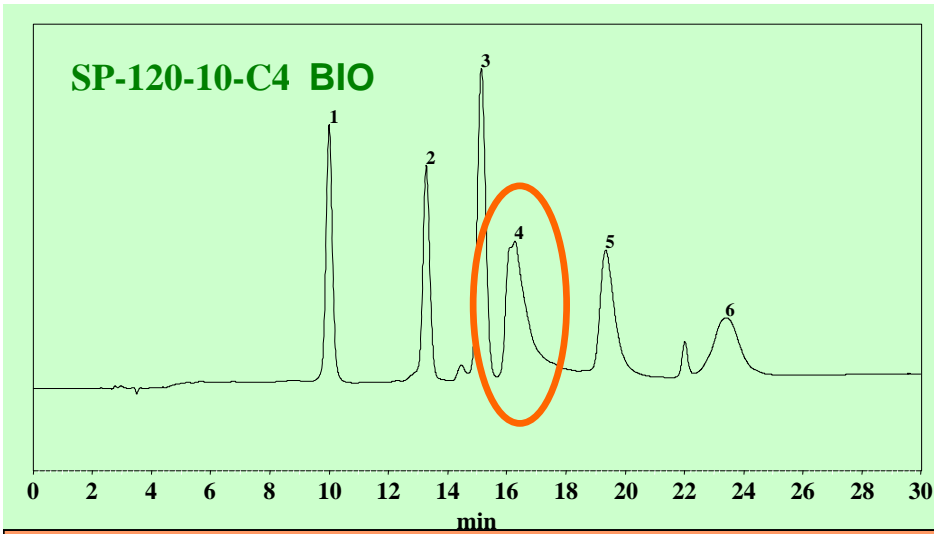


# BIO Series: SP-300-C4-BIO



## Protein separation on different particle sizes

Pay attention to the shape of the 4<sup>th</sup> peak (BSA)



Column: 6 mm I.D. x 250 mm length;  
Temperature: 35°C; Detector: UV 220 nm;  
Mobile phase: A) CH<sub>3</sub>CN/H<sub>2</sub>O/TFA = 20/80/0.1,  
B) CH<sub>3</sub>CN/H<sub>2</sub>O/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.

### Protein standards

1. Ribonuclease A (M.W. 13,700)
2. Cytochrome C (M.W. 12,400)
3. Lysozyme (M.W. 14,300)
4. BSA (M.W. 67,000)
5. Myoglobin (M.W. 18,800)
6. Ovalbumin (M.W. 45,300)



# SP-300-C4-BIO



Designed for protein purification

- **30 nm enlarged, narrow range pore diameter**  
Suitable for **bulky bio-molecules, e.g. proteins**
- **Available in 5, 10, 15, 20  $\mu\text{m}$  spherical ultra-high purity silica particles**  
High resolution and excellent peak shape  
Applicable for preparative purification
- **Specially designed C4 ligand configuration combined with new endcapping technology**  
Higher surface bonding density (**C4 > 6.0  $\mu\text{mol}/\text{m}^2$** )  
Improvement in phase stability against extreme acidic and alkalic environments

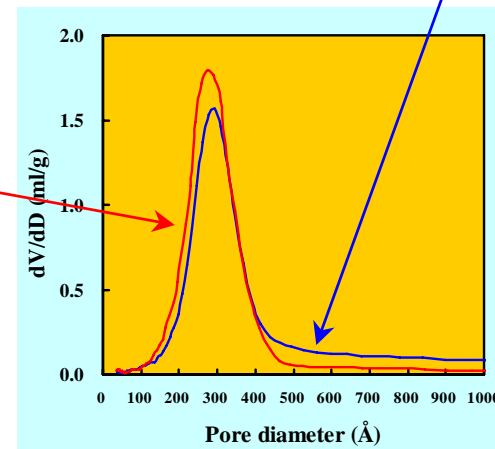
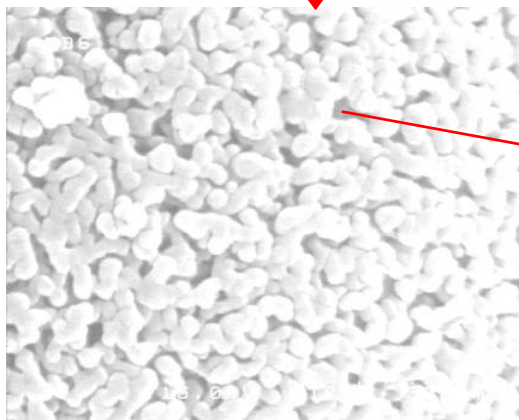
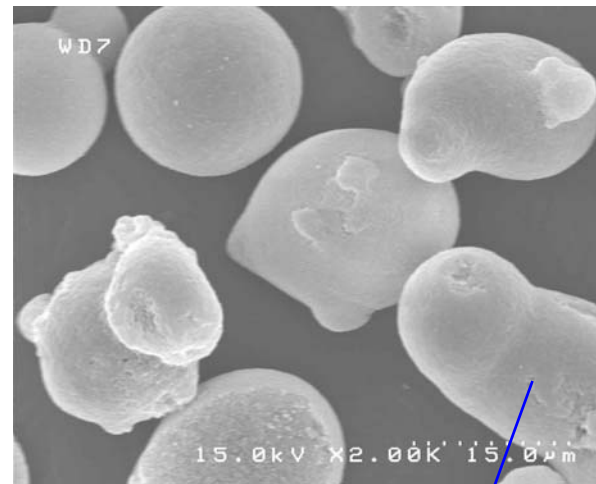
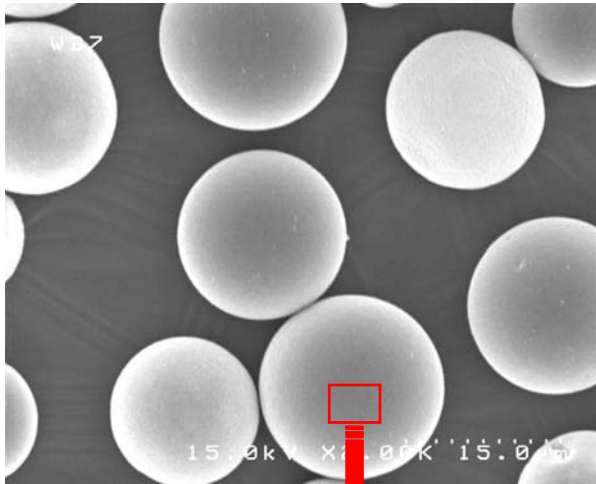


# SP-300-P

## Morphology and pore distribution

### DAISOGEL SP-300-15-P

### V 300-15

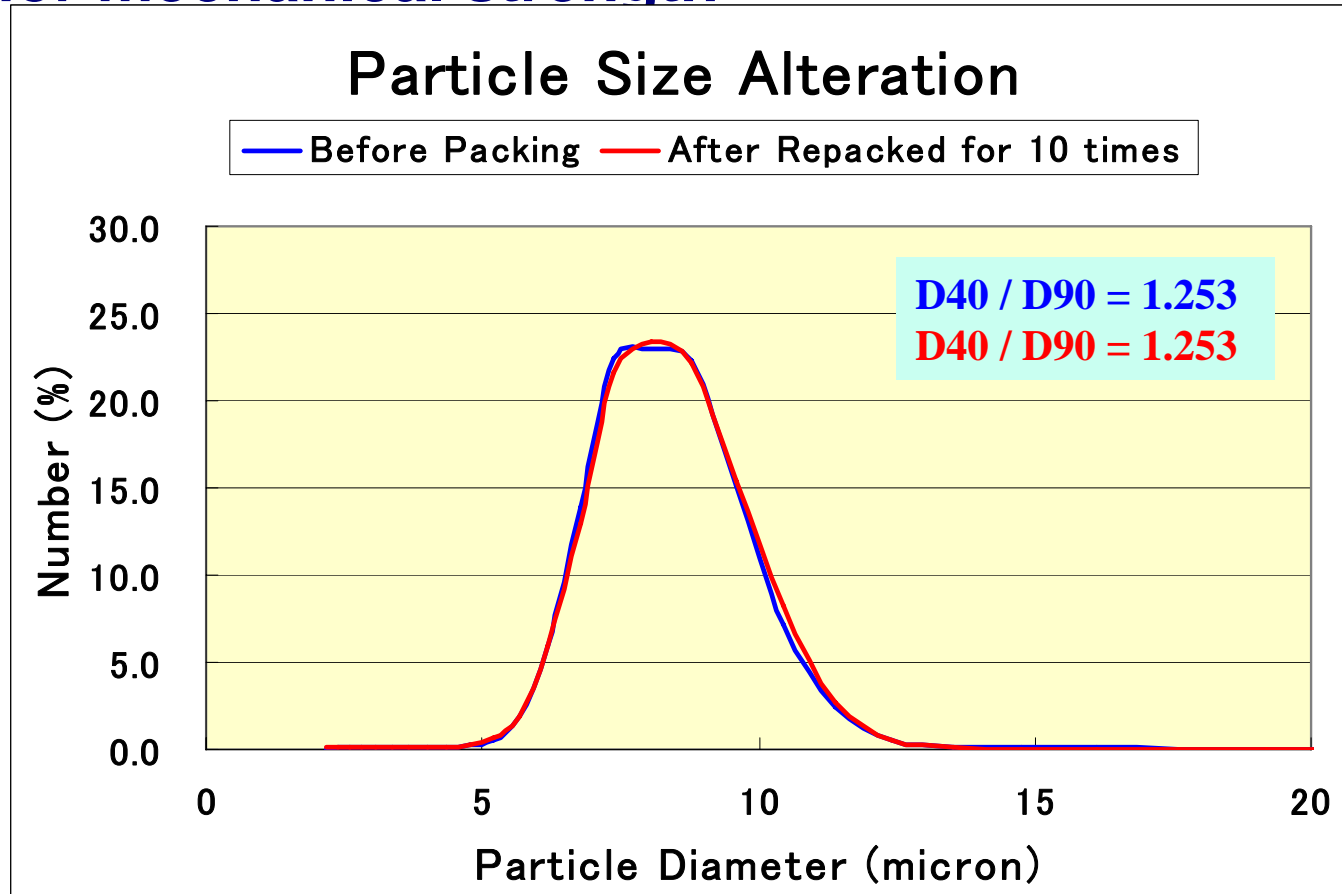




# SP-300-P



## Superior mechanical strength



**Material:SP-300-10P; Column: Dynamic Axial Compression column (50 mm I.D.)**

**Packing solvent: 2-Propanol; Piston pressure: 10 MPa**

**Particle size distribution measured by Coulter Counter.**

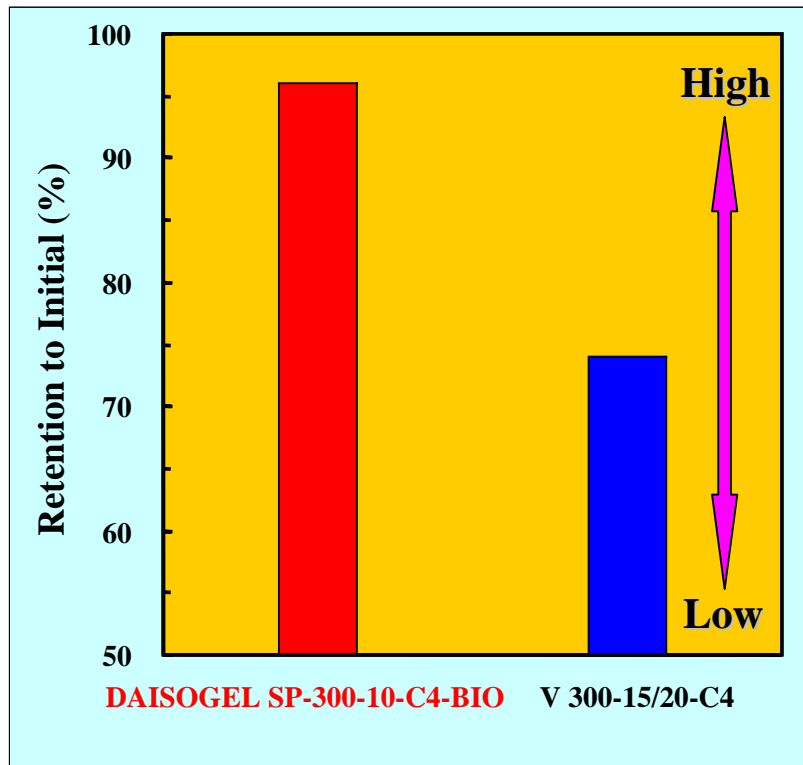


# SP-300-C4-BIO

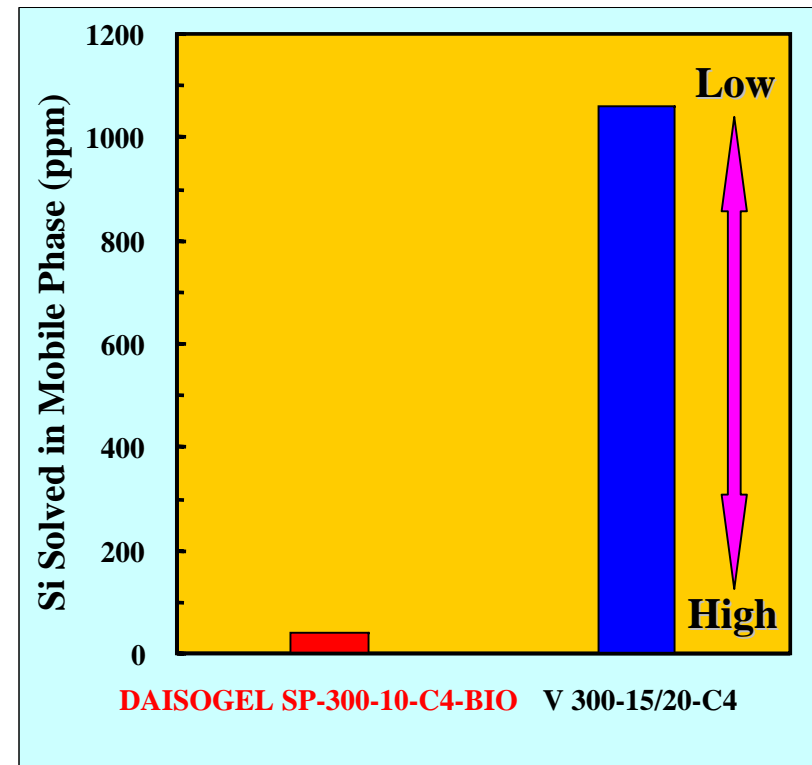


## Extraordinary durability

### Acidic resistance (pH=1)



### Alkalic resistance (pH=12)



Column size: 6 mm I.D. x 250 mm length;

Mobile phase: CH<sub>3</sub>CN/1% TFA (pH=1) = 10/90;

Temperature: 70°C; Flow: 0.9 ml/min; Time for purge: 15 h.

Column size: 6 mm I.D. x 250 mm length;

Mobile phase: CH<sub>3</sub>CN/20mM Na<sub>3</sub>PO<sub>4</sub>-NaOH (pH=12) = 10/90;

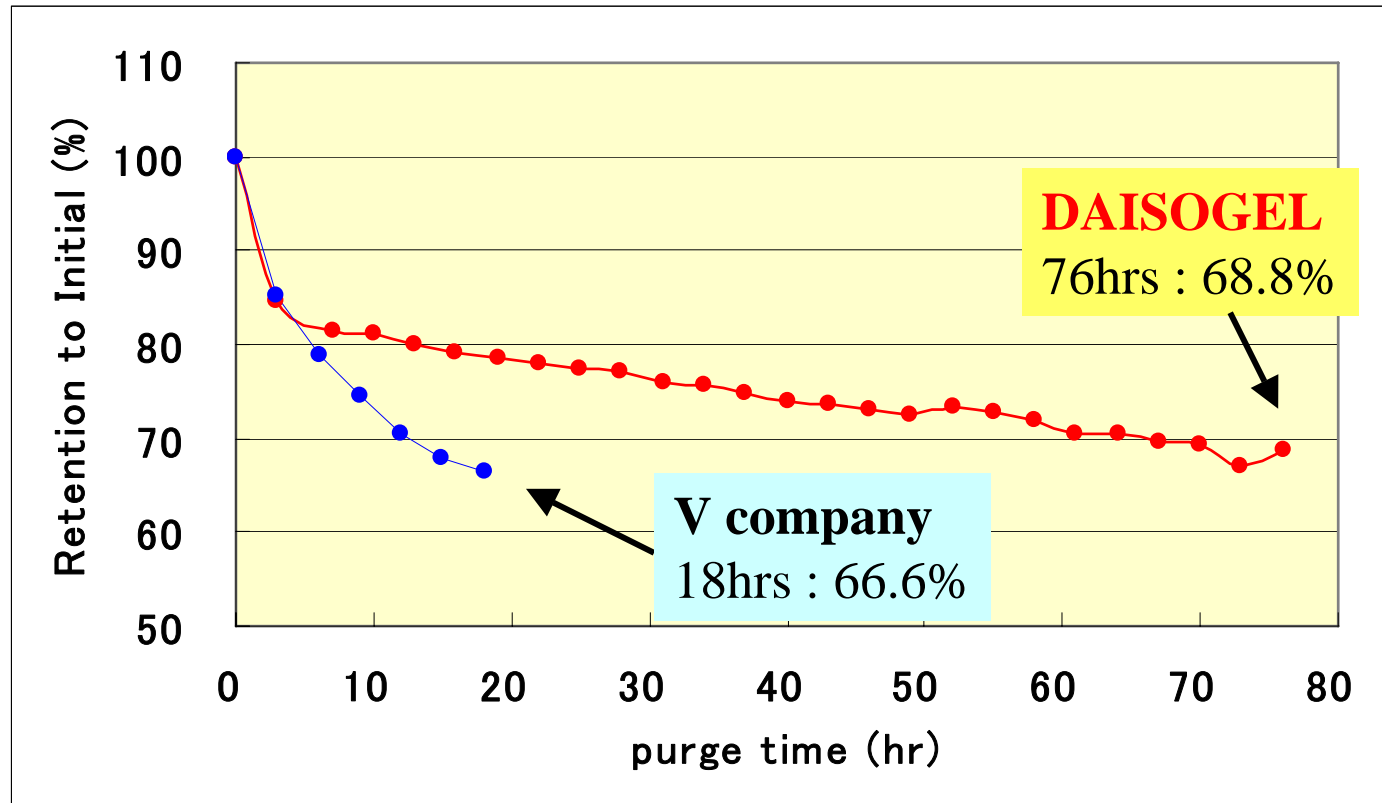
Temperature: 40°C; Flow: 1.7 ml/min; Time for purge: 3 h.



# SP-300-C4-BIO



**Durability comparison with V company 300-15/20-C4 in alkalic environment - *retention time* -**



Alkalic duration test conditions

Mobile phase: CH<sub>3</sub>CN/0.01N-NaOH aq. (pH=12) = 10/90; Flow rate: 1.0 ml/min; Temp.: 25°C ; Purge time: 3h



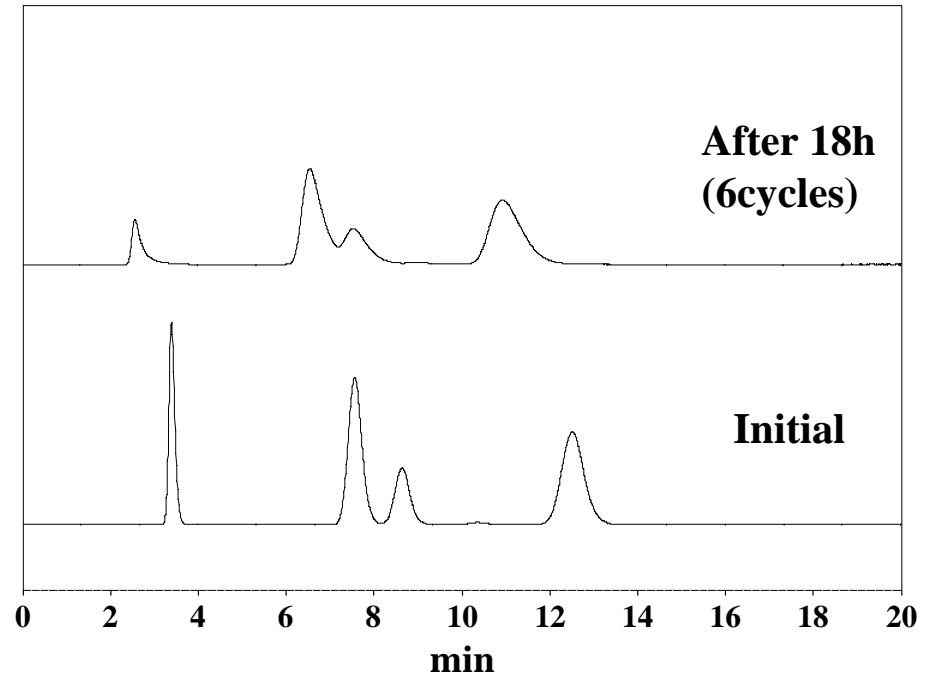
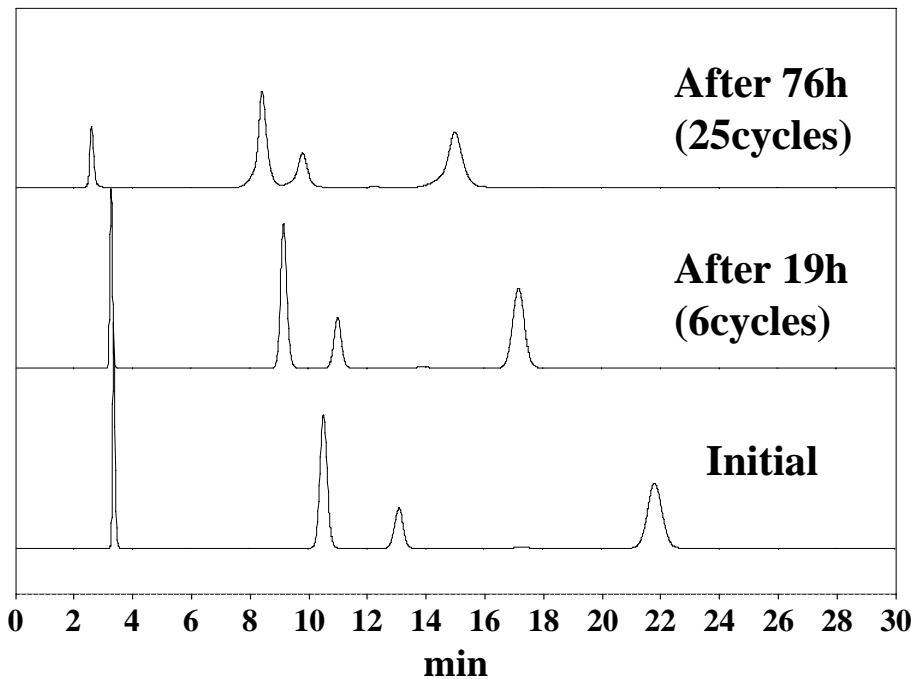
# SP-300-C4-BIO



**Durability comparison with V company 300-15/20-C4 in  
alkalic environment - chromatogram -**

**DAISOGEL SP-300-10-C4-BIO**

**V company 300-15/20-C4**



Alkalic duration test conditions

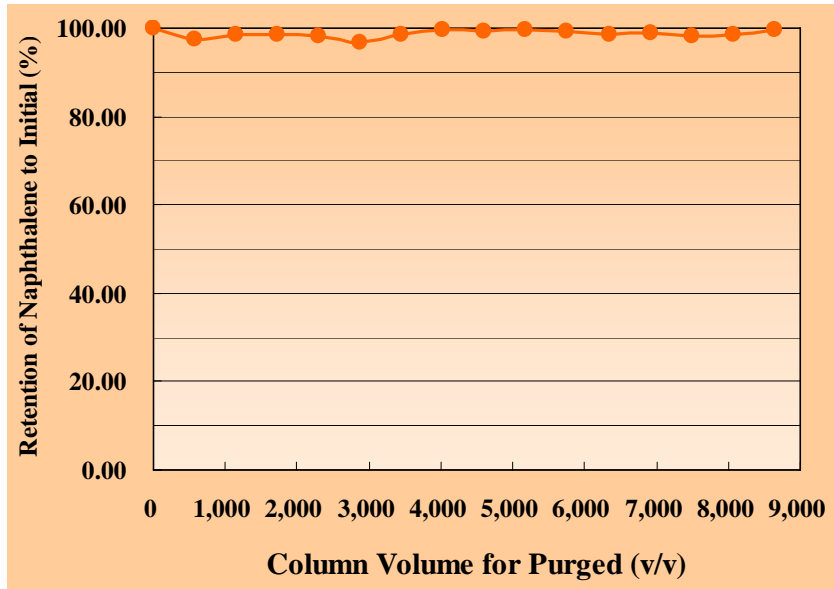
Mobile phase: CH<sub>3</sub>CN/0.01N-NaOH aq. (pH=12) = 10/90; Flow rate: 1.0 ml/min; Temp.: 25°C ; Purge time: 3h



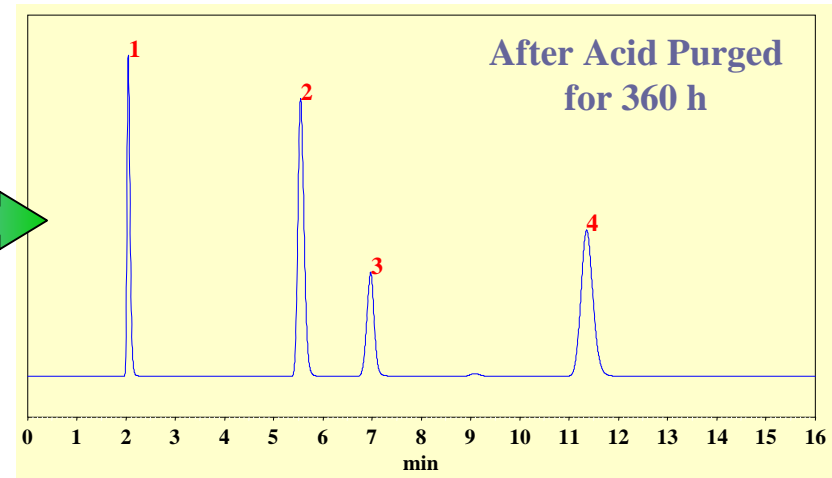
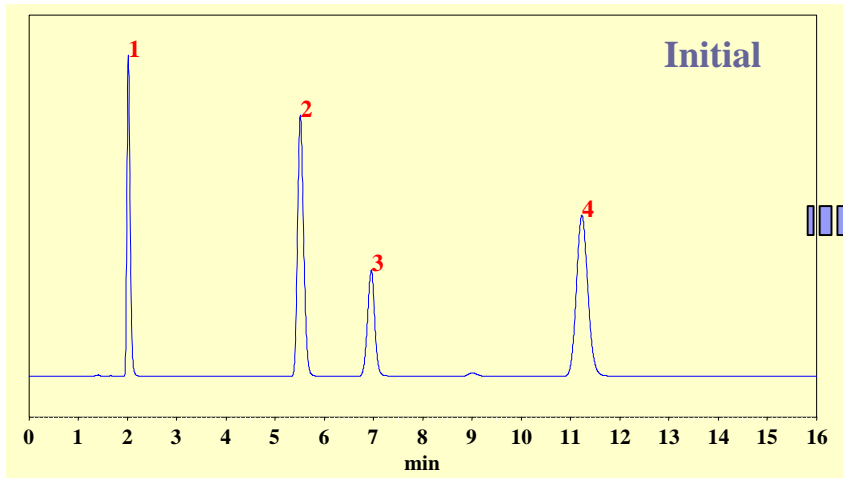
# SP-300-C4-BIO



## Extreme durability under severe acidic conditions



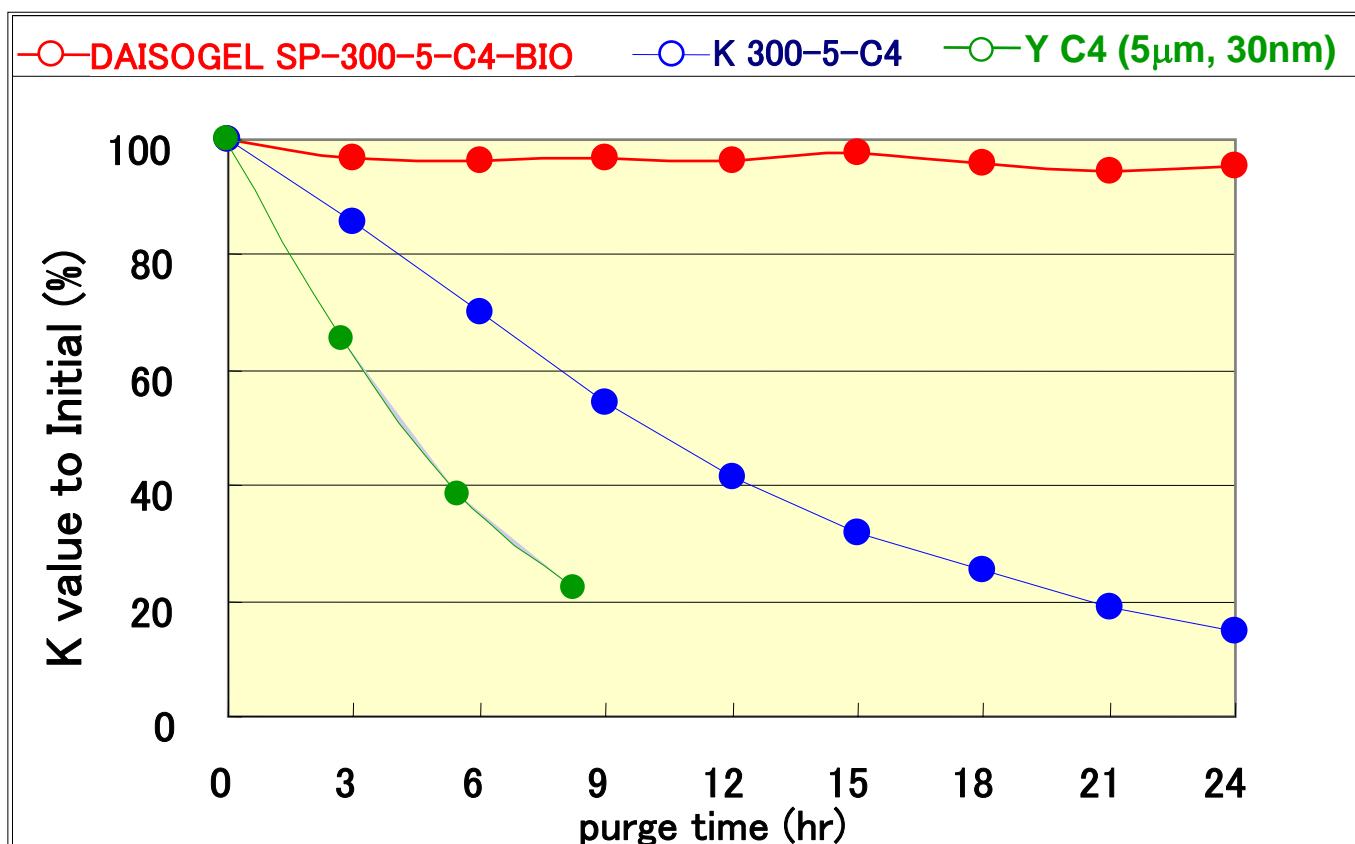
**Column:** SP-300-5-C4-BIO  
**Size:** 4.6 mm I.D. x 150 mm  
**Acidic Duration Test Condition**  
**Mobile Phase:** CH<sub>3</sub>CN/0.05% TFA aq. (pH=2.0) = 50/50;  
**Flow Rate:** 1.0 ml/min;  
**Temperature:** Ambient  
**Chromatographic Test Condition**  
**Mobile Phase:** CH<sub>3</sub>OH/H<sub>2</sub>O=35/65;  
**Flow Rate:** 1.0 ml/min; **Temperature:** 40°C;  
**Detector:** UV 254 nm  
**Analyst:** 1. Uracil, 2. Methyl Benzoate,  
3. Toluene, 4. Naphthalene





# SP-300-C4-BIO

Durability comparison with K 300-5-C4 and Y C4 (5 $\mu$ m, 30nm) in acidic environment - *K* value -



**DAISOGEL**  
24hrs : 95.5%

**K** 24hrs :  
14.7%

**Y**  
9hrs : 23.2%

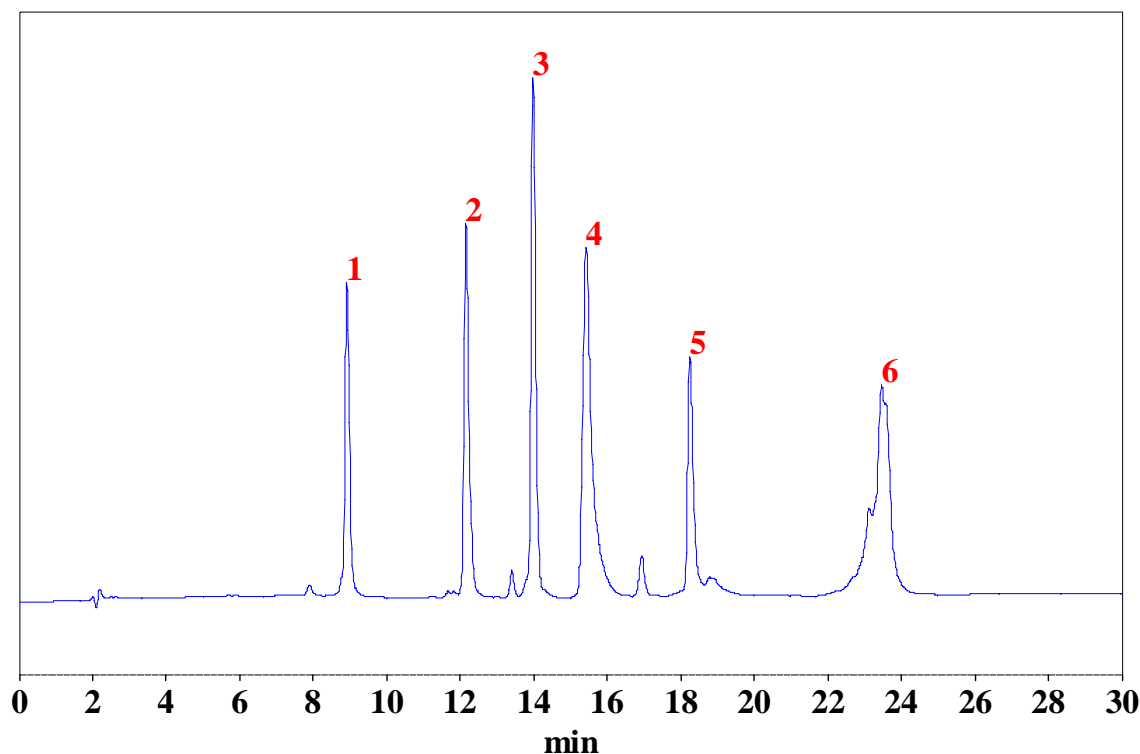
Accelerated acidic duration test condition

Mobile phase: CH<sub>3</sub>CN/1.0% TFA aq. (pH=1.0) = 10/90; Flow rate: 1.0 ml/min; Temp.: 70°C ; Purge time: 3h



# SP-300-C4-BIO

## Standard protein separation



1. Ribonuclease A (M.W.=13,700)

2. Cytochrome c (M.W.=12,400)

3. Lysozyme (M.W.=14,300)

4. BSA (M.W.=67,000)

5. Myoglobin (M.W.=18,800)

6. Ovalbumin (M.W.=45,300)

Column : DAISOGEL SP-300-5-C4-BIO (150 mm x 4.6 mm I.D.)

Mobile phase : A) CH<sub>3</sub>CN / TFA = 1000/1, B) H<sub>2</sub>O / TFA = 1000/1

A / B = 20 / 80 → A / B = 60 / 40 (0-25 min) B = 100% (25-30 min)

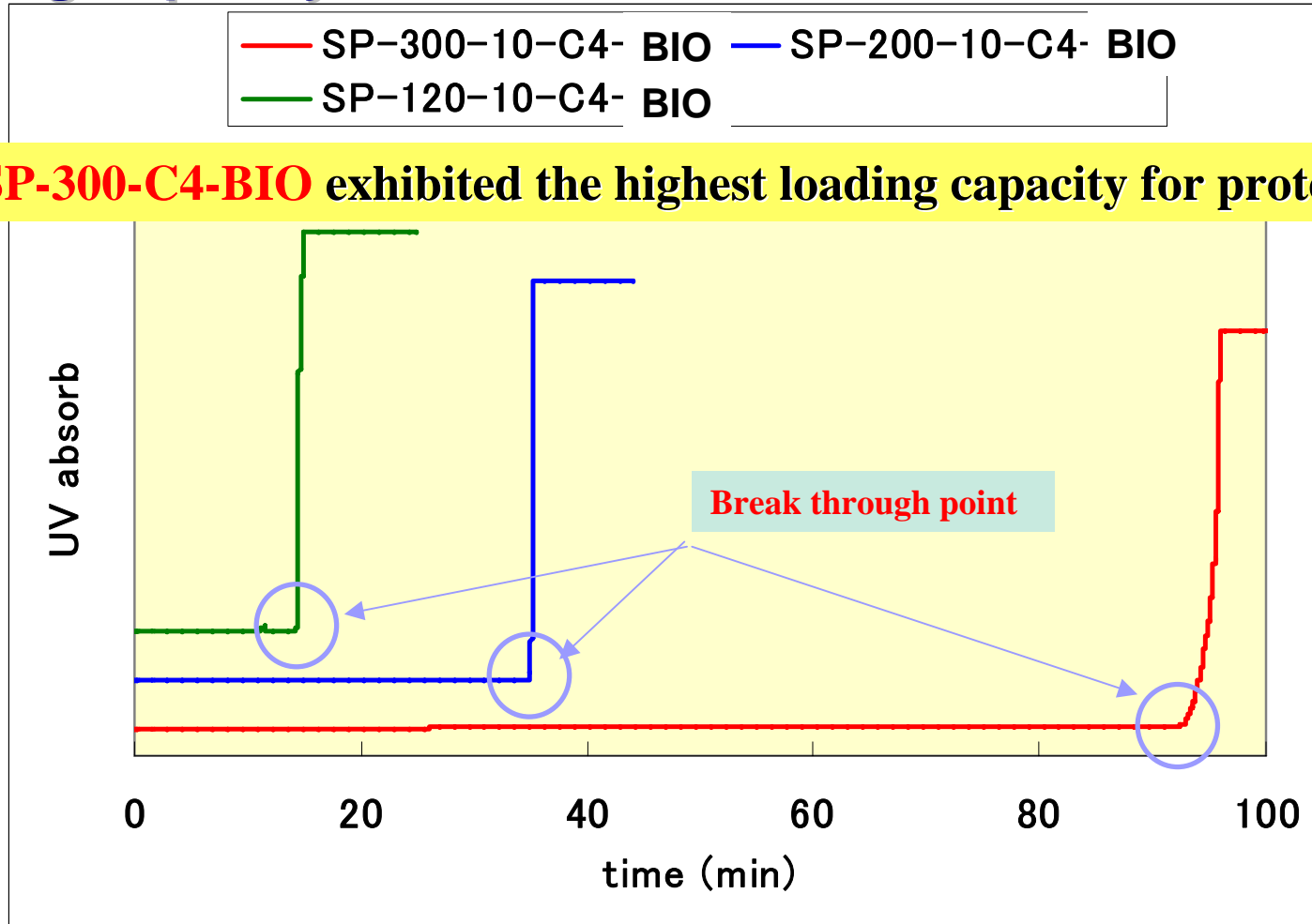
Flow rate : 1.0 mL / min; Temperature: 35 ° C; Detection: UV220 nm



# BIO Series: SP-300-C4-BIO



## Loading capacity for BSA



Column: 6 mm I.D. x 250 mm length; Temperature: 35°C; Detector: UV 220 nm; Flow rate: 1.0 ml/min.  
Feed : 10 mg/mL BSA in 0.1% TFAaq



# Summary

**SP-300-C4-BIO is the ideal silica for protein separation**

- **300 Å pore size provides complete access for large molecules (proteins) to the whole surface area**
- **C4 (butyl group) bonded silica is less hydrophobic than ODS, causing less aggregation and absorption**
- **Proprietary modification technique and enhanced endcapping makes this silica extremely durable under severe alkalic and acidic conditions**
- **Strong mechanical strength allows repeated packings to DAC columns**



**Best choice: SP-300-C4-BIO is the ideal silica for preparative/process scale protein separation!**