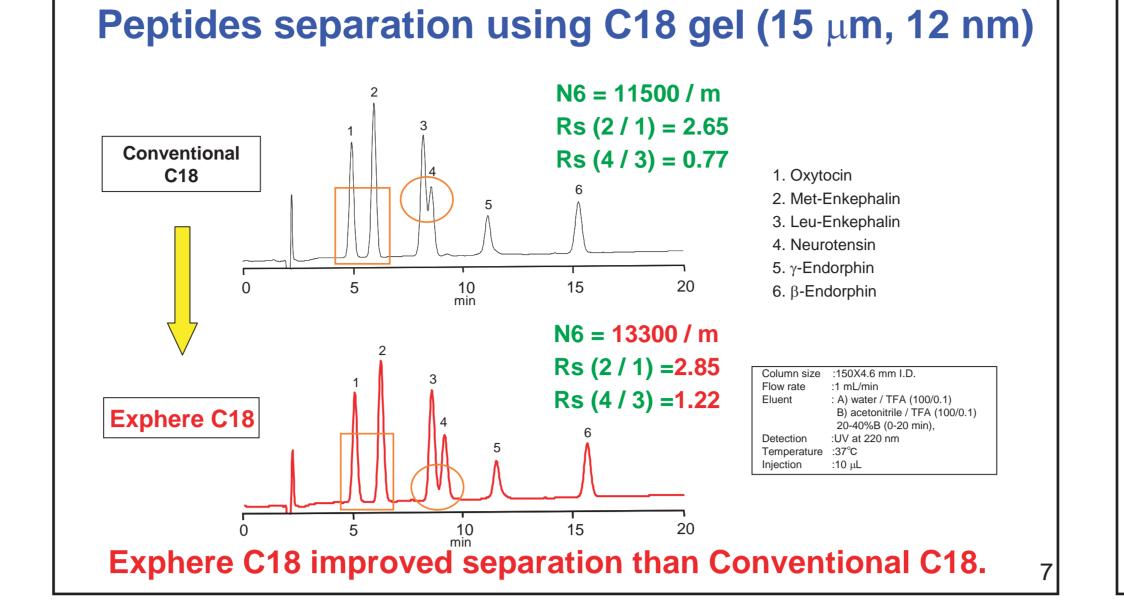


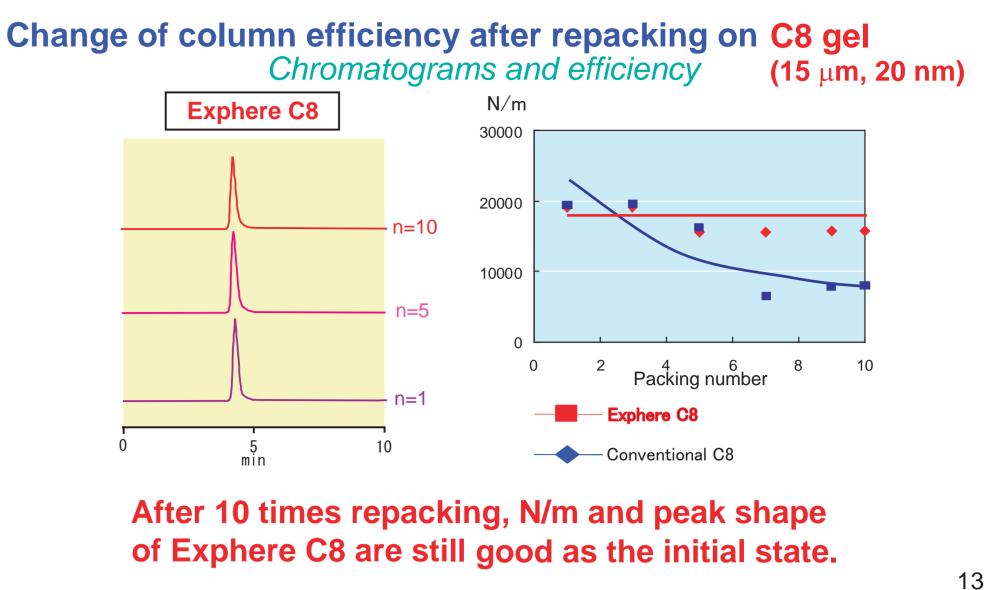
High mechanical stability reversed phase gel with chemical resistant for purification of peptides and proteins Naohiro Kuriyama, Noriko Shoji, Yoshihiko Yamada, Kiyoshi Morishita and Masakatsu Omote YMC Co., Ltd., Ishikawa, Japan

Introduction

A new high strength silica gel and a bonding technology based preparative bulk packing materials for HPLC have been developed to provide improved recovery, selectivity, and longer life time for the preparative peptide separations. The novel preparative silica particle was successfully prepared by the new generation process, which allows the higher gel density than typical silica gel and the particle size distribution would be practically mono-dispersed character. For the effective reversed phase peptide separations, pore size and pore volume of these new particle were optimized depending on the molecular weight of peptides. To enhance chemical stability and selectivity under the typical peptide purification conditions, the combination of chemical bonding method and functional group density was optimized for maximum performance.

By repeated packing and unpacking of this synthesized gel with large dynamic axial compression column, it was demonstrated that no fine has appeared and no back pressure increasing has occurred comparing to commercially available packing materials. Also cost effective peptide purification with high loadability, productivity, and recovery was achieved with significant small and large peptides.



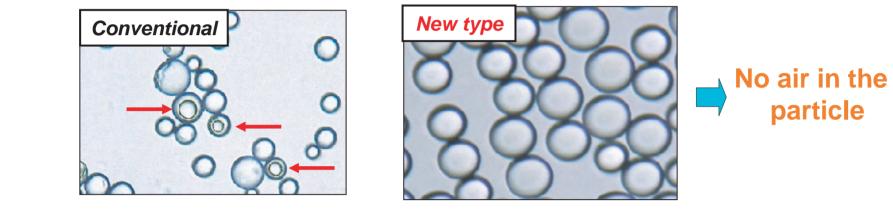


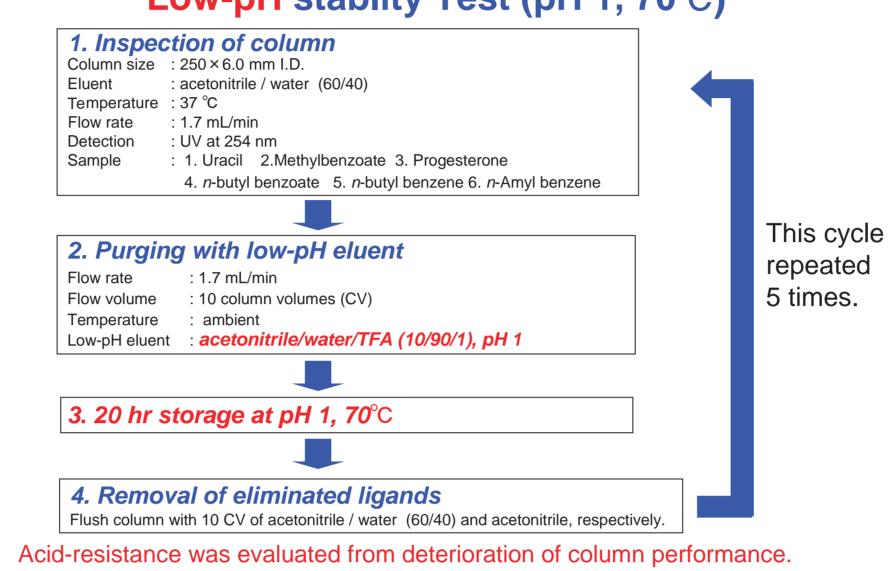
New generation silica gel images **SEM** image Conventiona

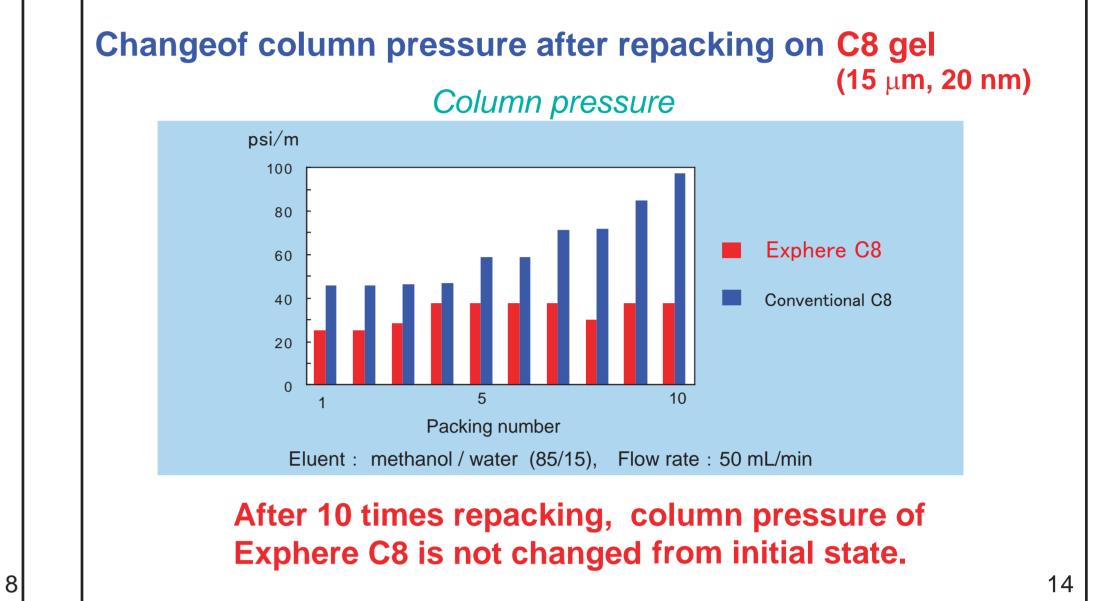
Low-pH stablity Test (pH 1, 70°C)

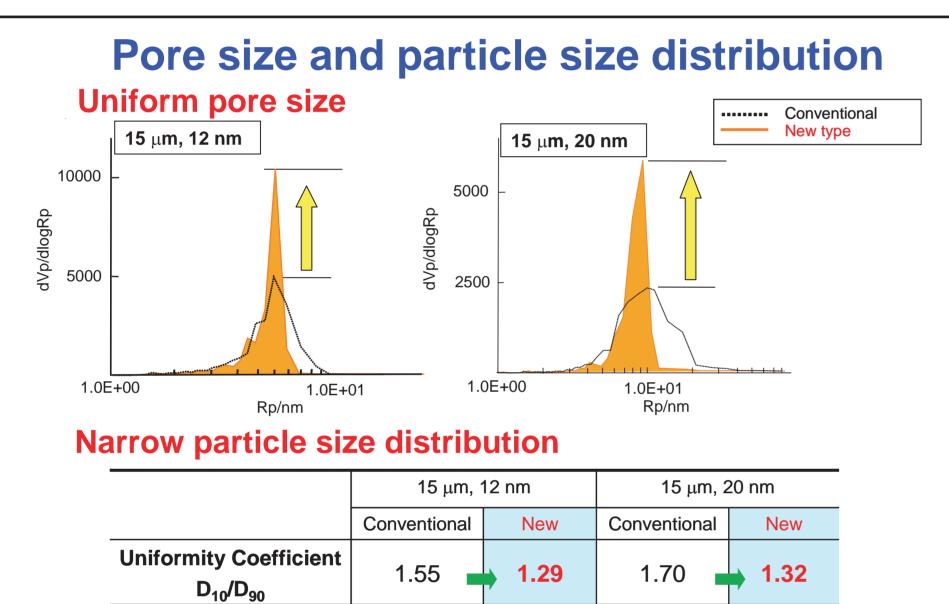


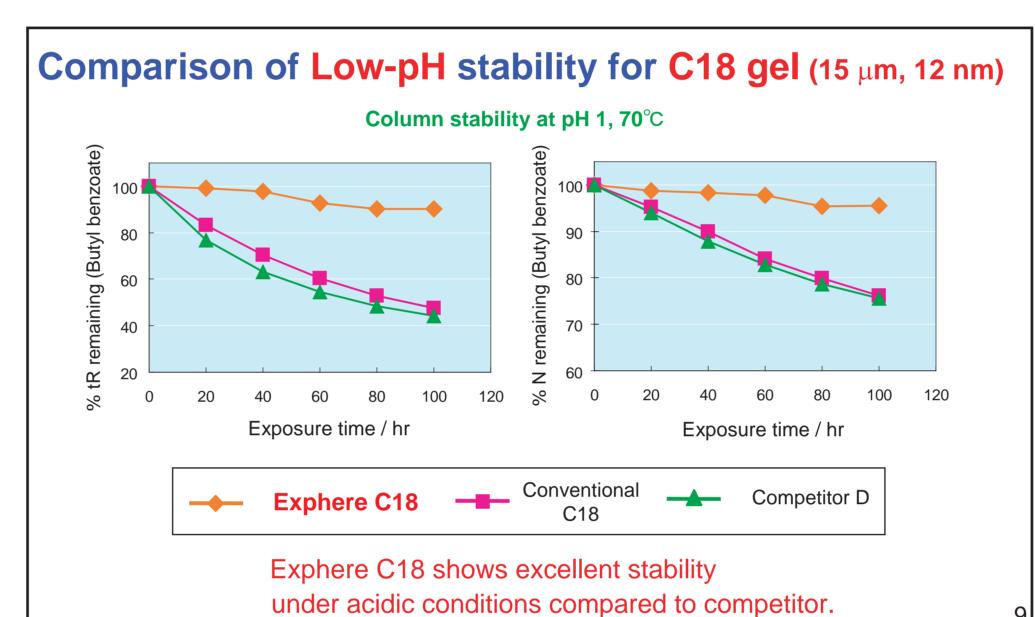
Perfect spherical shape and smooth surface

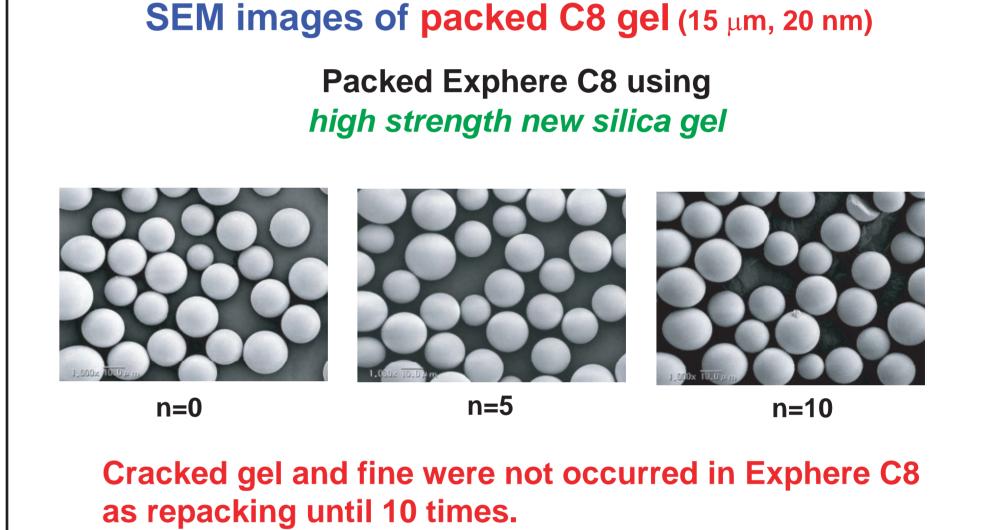








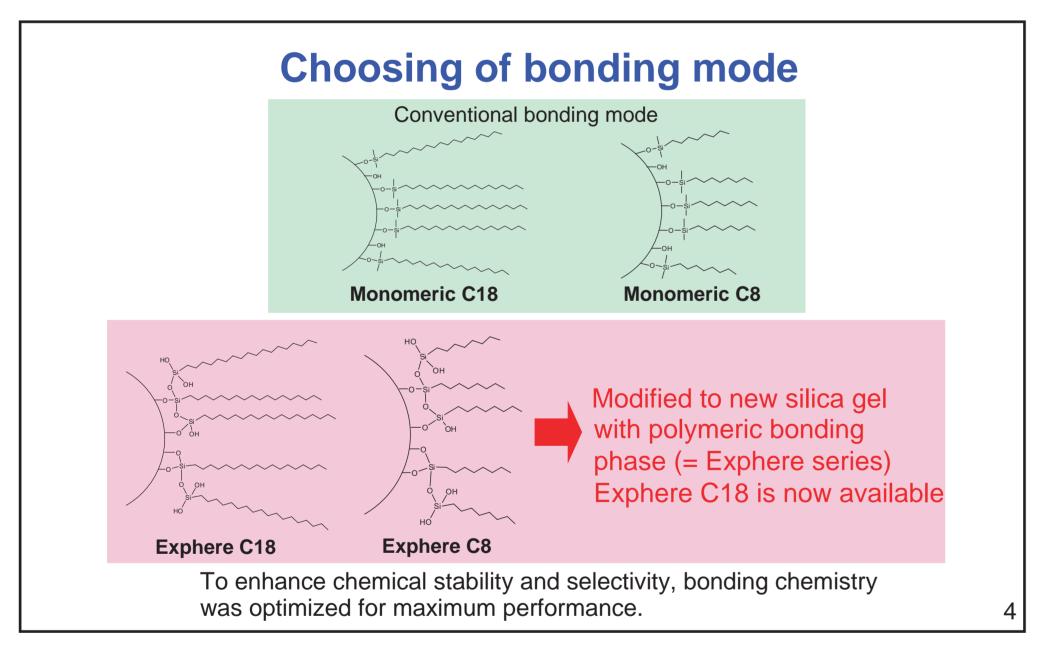


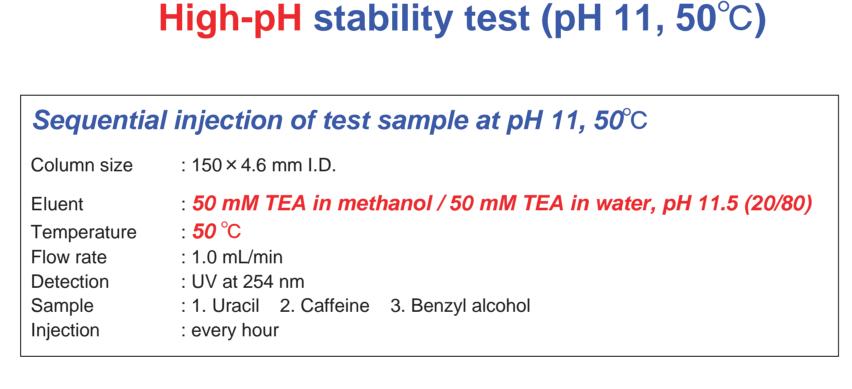




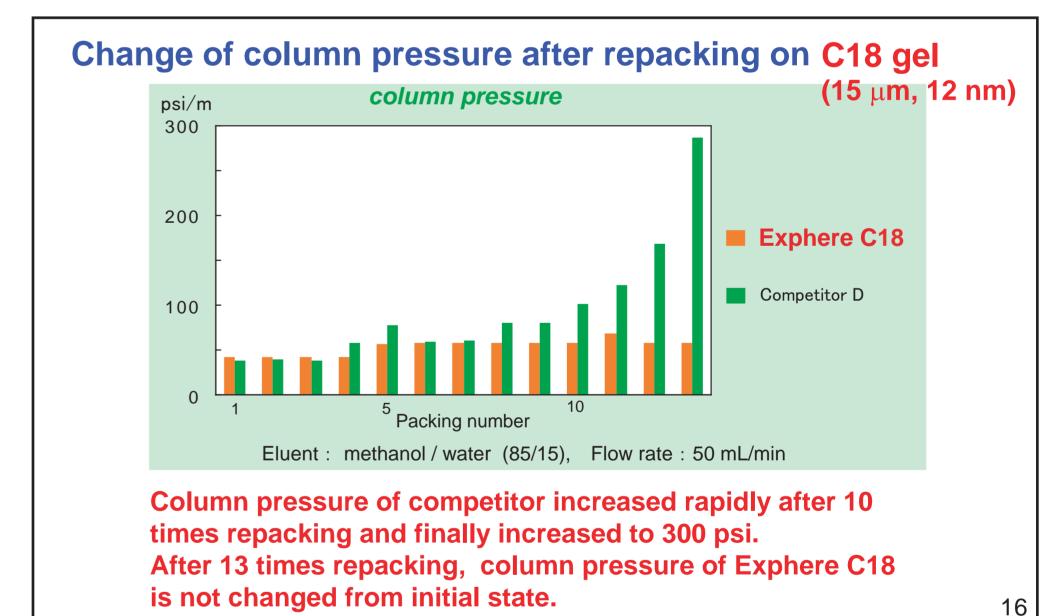
17

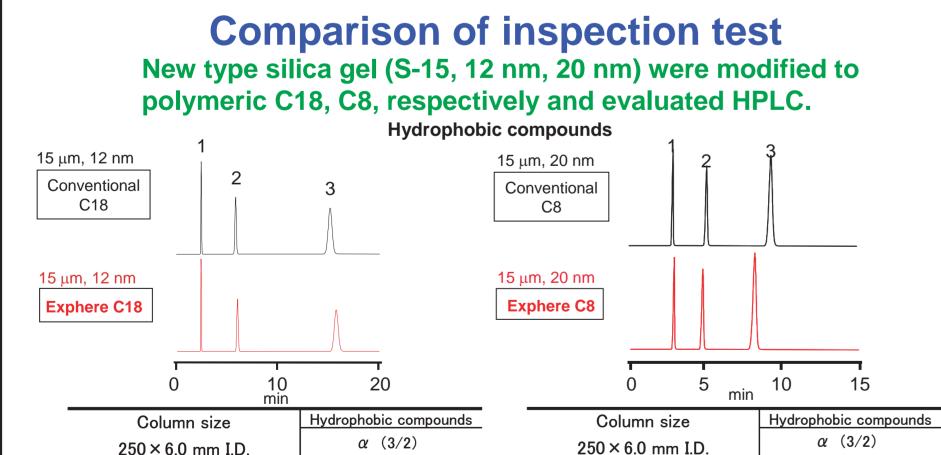
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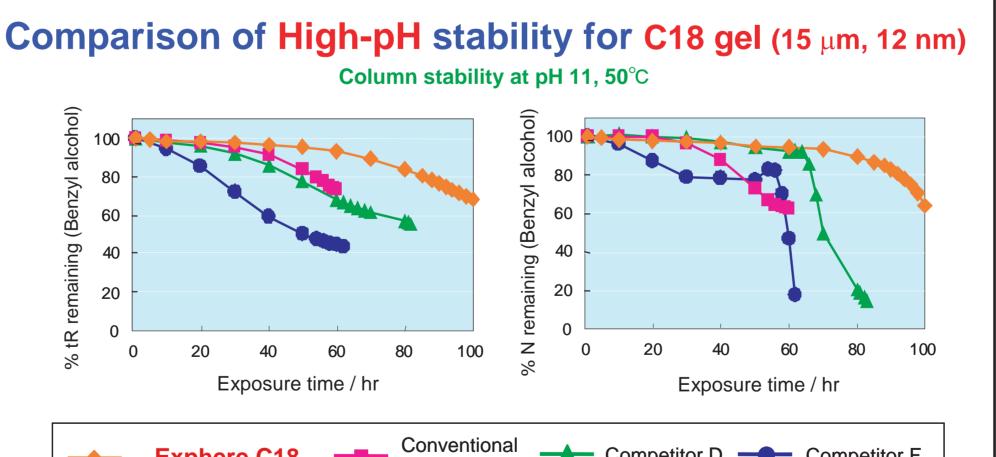




Alkaline-resistance was evaluated from deterioration of column performance













Modification to polymeric gels show similar separation pattern as conventional gel.									
Exphere C18	3.71	Exphere C8	2.82						
Conventional C18	3.75	Conventional C8	2.89						

		Exprere C18	_	C18			Competitor F
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Exphere C18 has improved stability of both retention and column efficiency under high-pH conditions.

Analysis of insulin Column size :150 × 4.6 mm I.D. **Insulin from Bovine (MW 5700)** Flow rate : 1.0 mL/mir : A) water / TFA (100/0.1) 15 μm, 20 nm 15 μm, 12 nm B) acetonitrile/ TFA (100/0.1 10-90%B(0-20 mir Conventional Conventional Detection : UV at 220 nm **C**8 C18 Temperature: 37°C Injection : 10 i 10 min 15 μm, 12 nm 15 μm, 20 nm Exphere C18 **Exphere C8** Retention time of insulin was same as conventional gel.

Mechanical stability test of 15 µm gel Packing conditions

: 100 × 50 mml.D. Column size Packing pressure : 930 psi (6.5 MPa)

HPLC conditions for inspection of packing procedure

: toluene (80 mL/mL) Sample : 500 μL Injection : 50 mL/min Flow rate : ambient Temperature : UV at 254 nm Detection : methanol / water (85/15) Eluent

Repeatedly repacked and evaluated the change of efficiency and column pressure.



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10

Summary

Performance of HPLC

Exphere series are new bulk products using new silica gel with polymeric bonding phase. The column of Exphere C8 shows lower pressure than conventional columns. Exphere C18 showed improved peak shape of peptides. In addition, the column using new silica gel shows same retention time of insulin as conventional gel.

Chemical stability test

Newly developed gel shows excellent stability under acidic and alkaline conditions due to modification to polymeric C18.

Mechanical stability test

Exphere series show high mechanical stability, therefore peak shapes and column performance are as good as the initial state after 10 times repacking procedure. High mechanical stability ensures longer life time and reduce your cost.

