

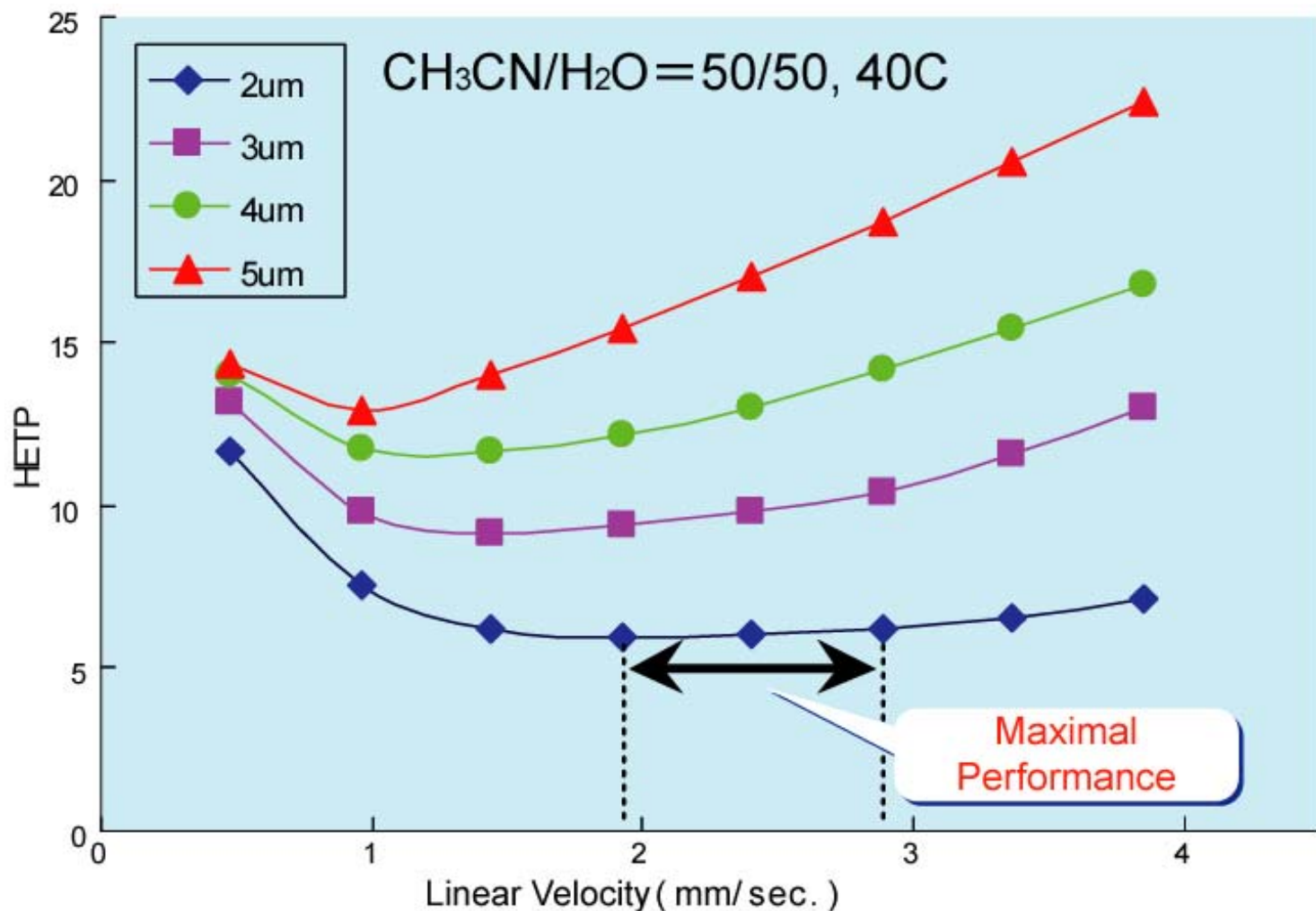
High-Speed Separation

Inertsil ODS-3 2 μ m

Base Silica Physical Properties and Chemical Modification

Base Silica:	High Purity Silica Gel 99.999%
Surface Area:	450 m ² /g
Pore Size:	100 Å
Pore Volume:	1.05 ml/g
Bonded Phase:	Octadecyl Groups
Carbon Loading:	15%
Endcapped:	Yes
Column Sizes:	50 × 2.1 mm I.D. 50 × 3.0 mm I.D.
Maximum Operating Pressure:	50 MPa
Guaranteed Theoretical Plates:	160,000/m (GLS Standard Column Performance Test)

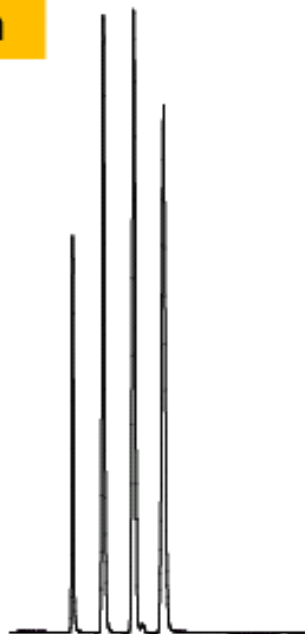
Van Deemter Plot of HETP vs Flow Rate



Column: 50 X 2.1 mm I.D.

Comparison of 2 μm versus 5 μm

2 μm

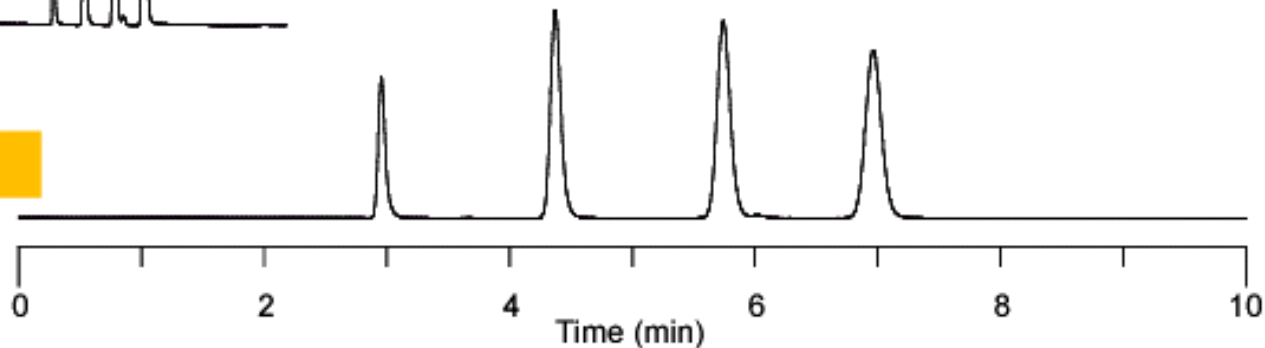


Particle Size	2 μm	5 μm
Column Size	50 \times 3.0mm I.D.	150 \times 3.0mm I.D.
Flow Rate	0.8 mL/min	0.4 mL/min
Retention Time	1.19min	6.96min
Theoretical Plates	8,846	13,206
Peak Height	433mAU	179mAU
Resolution	5.0	5.4
Pressure	19.5MPa	3.5MPa

Analysis time was reduced 1/6!
Peak height was increased 2.5 fold!

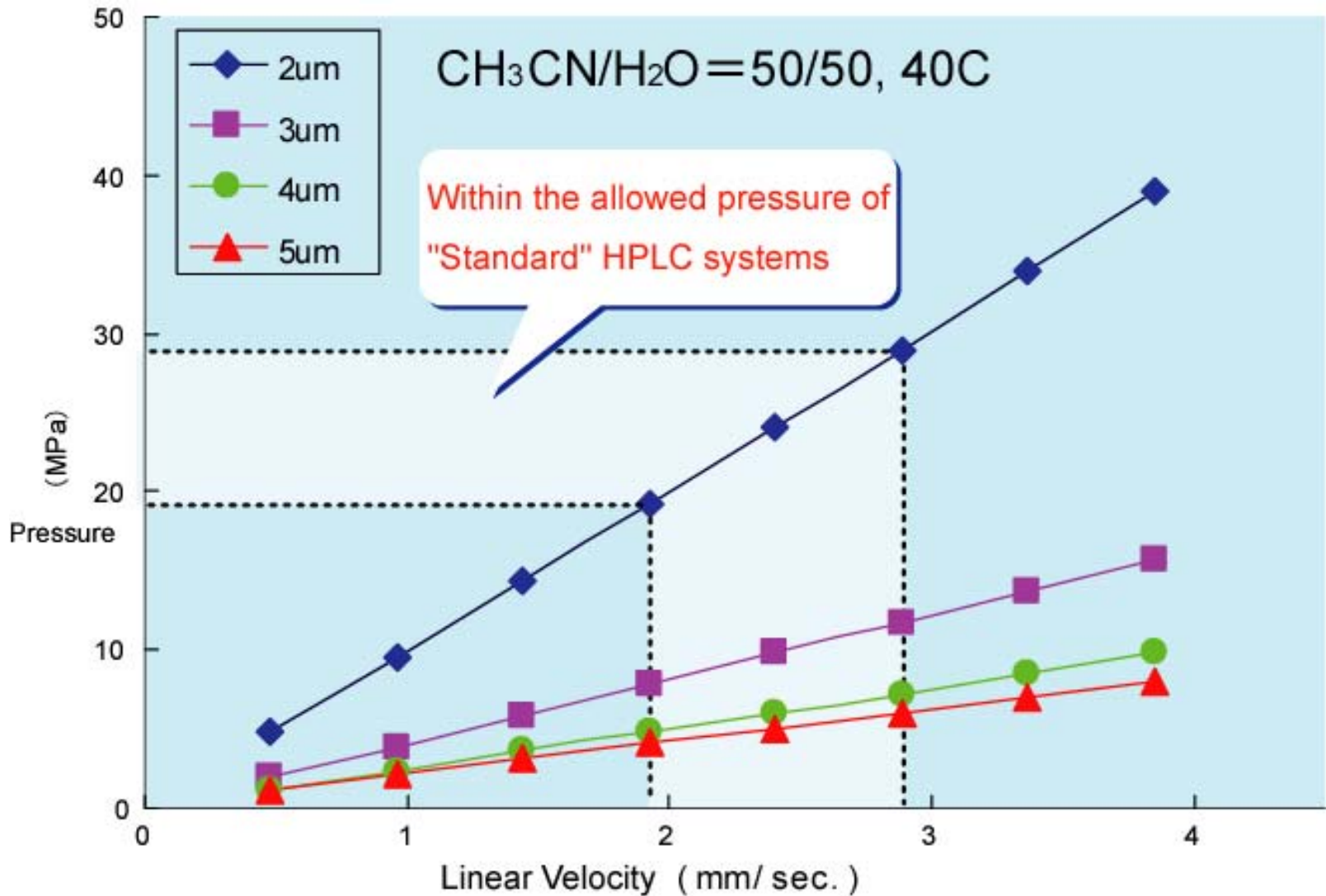
Mobile Phase: CH₃CN/H₂O = 65/35
Column Temp.: 40C

5 μm



2 μm vs 5 μm

Pressure versus Flow Rate



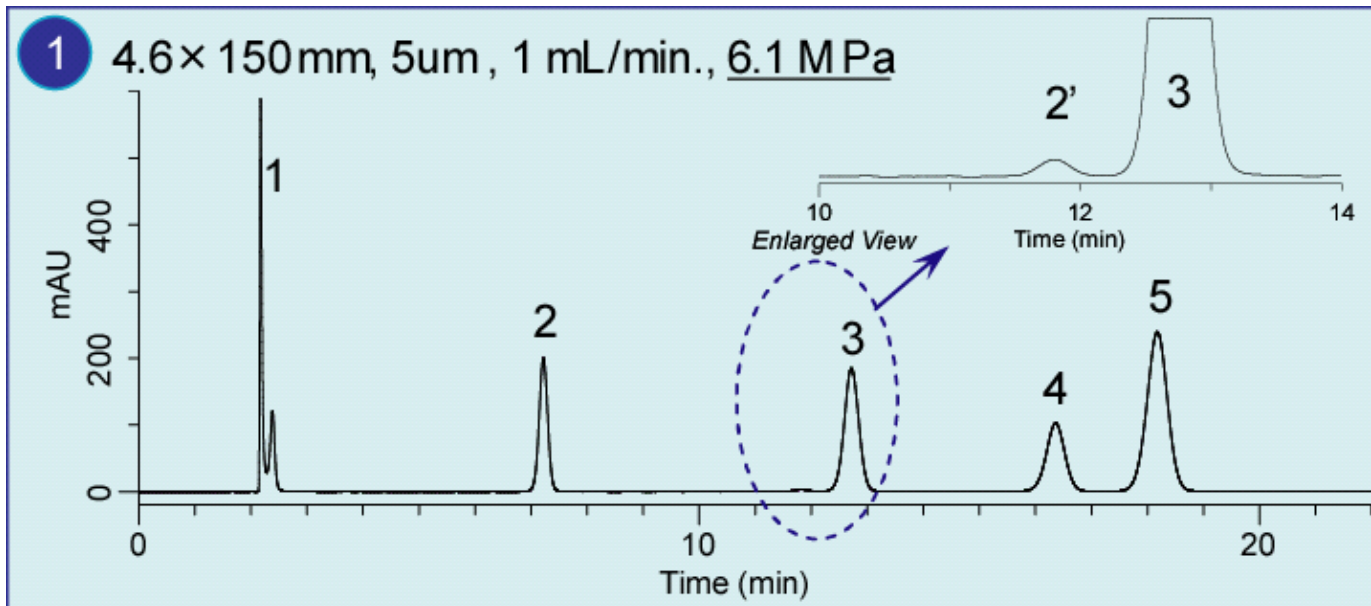
Example of High-Speed Separation

Analytical Condition

Column: Inertsil ODS-3
Eluent: A: Acetonitrile
B: 0.1% Phosphoric Acid
A/B=15/85
Flow Rate: 0.5 ml/min., 1.0 ml/min.
Column Temperature: 40 ° C
Detector: PDA 275 nm
Injection Volume: 10 µl (4.6 mm I.D.), 5 µl (3.0 mm I.D.)

Sample

1. Gallic acid (0.17 mg/ml)
2. Vanillic acid (0.17 mg/ml)
2'. Vanillin (Impurity of Vanillic acid)
3. p-Coumaric acid (0.17 mg/ml)
4. Ferulic acid (0.17 mg/ml)
5. m-Coumaric acid (0.17 mg/ml)



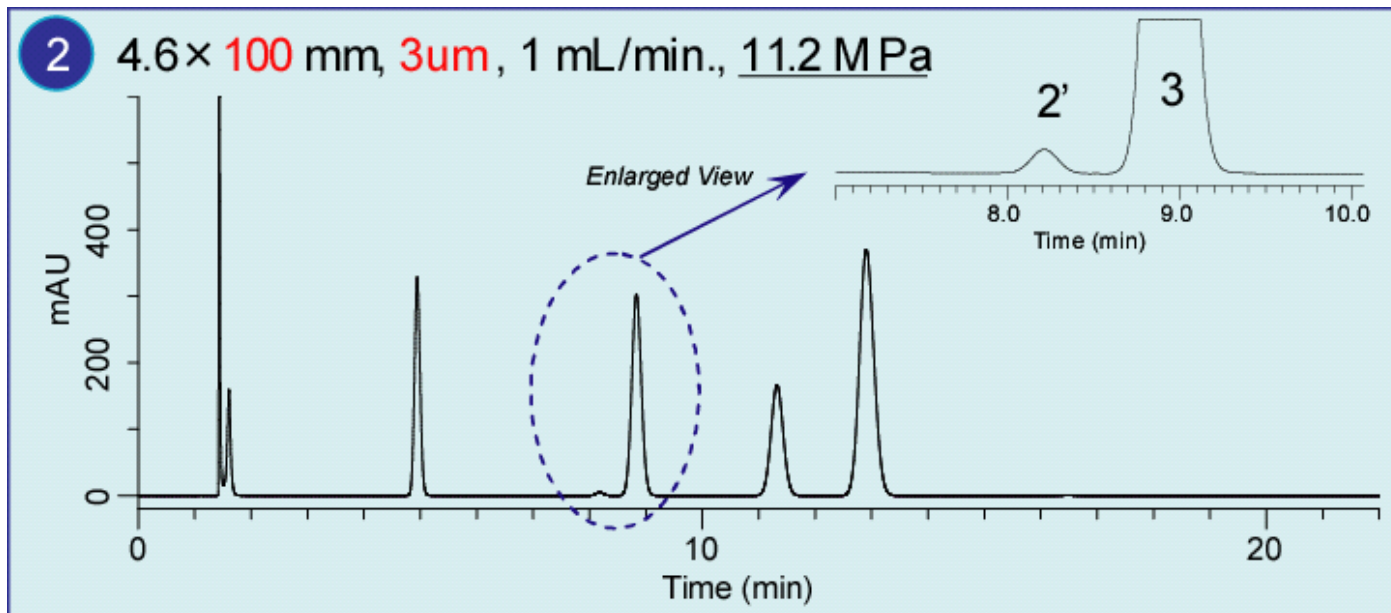
**Analysis Time:
19 minutes**

Results:

All peaks were well separated.

Example of High-Speed Separation

- Shorten the column length
- Changing the packing material to a 3 μm



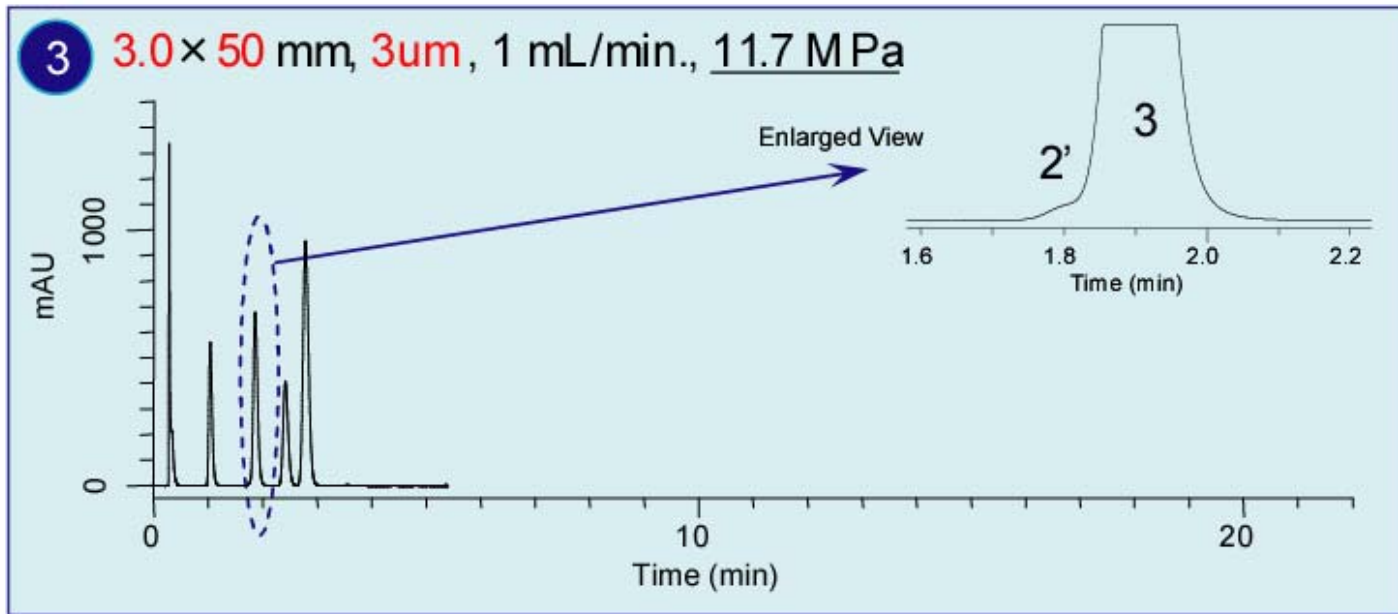
**Analysis Time:
14 minutes**

Results:

Obtained the same resolution with shorter analysis time.

Example of High-Speed Separation

- Shorten the column length
- Using the same flow rate



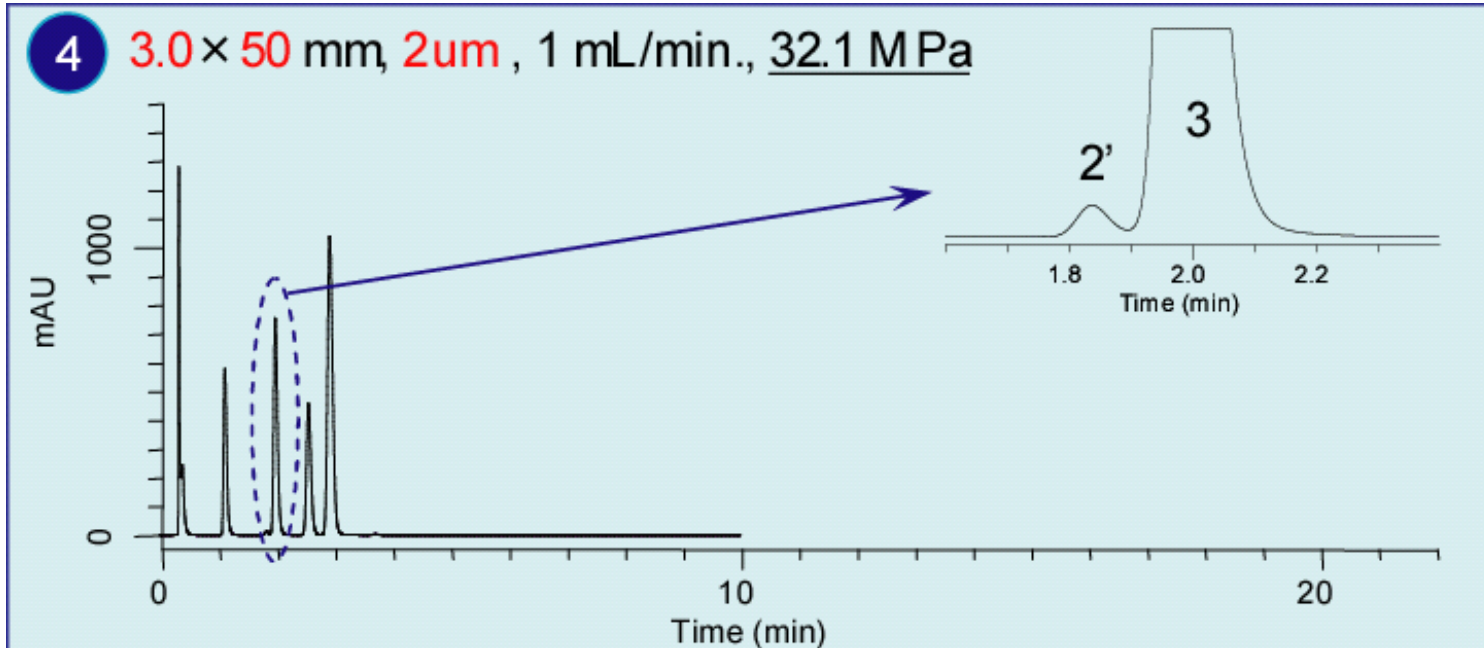
**Analysis Time:
3.5 minutes**

Results:

The analysis time was shortened to 3.5 minutes.
However, peak 2' and 3 were not separated.

Example of High-Speed Separation

-Changing the packing material to a 2 μm



**Analysis Time:
3.5 minutes**

Results:

The analysis time was shortened to 3.5 minutes.

In addition, complete separation between peak 2' and 3 is obtained.

Features of Inertsil ODS-3 2 μ m

- **Advanced classification for sizing silica gels realized an ideal balance between theoretical plate number and column back pressure, enabling high throughput analysis in the existing standard HPLC system.**
 - **Fine particles are accurately eliminated and proper mesh size is employed, which result in less column clogging problems.**
 - **Customers who are currently using Inertsil ODS-3 5 μ m, 4 μ m or 3 μ m, can simply achieve Higher Speed Separation without changing the analytical conditions.**
 - **A newly developed column joint is used to minimize the dead volume. It has an ideal design for high throughput analyses, which result in high/sharp peak shapes for those fast eluted samples.**
- * Maximal performance can be obtained at the following flow rates:**

2.1 mm I.D.: From 0.4 to 0.6 ml/min

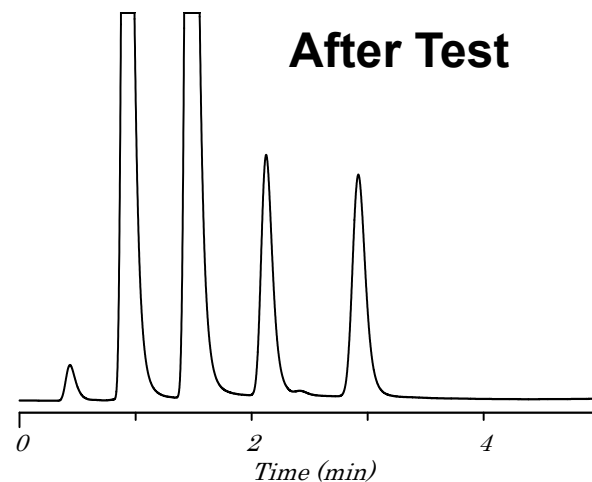
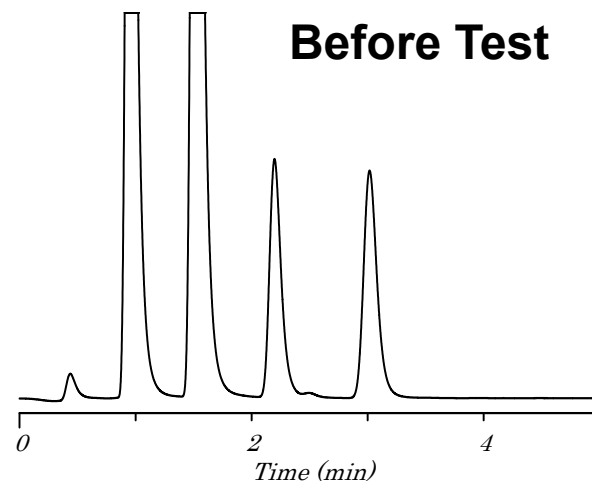
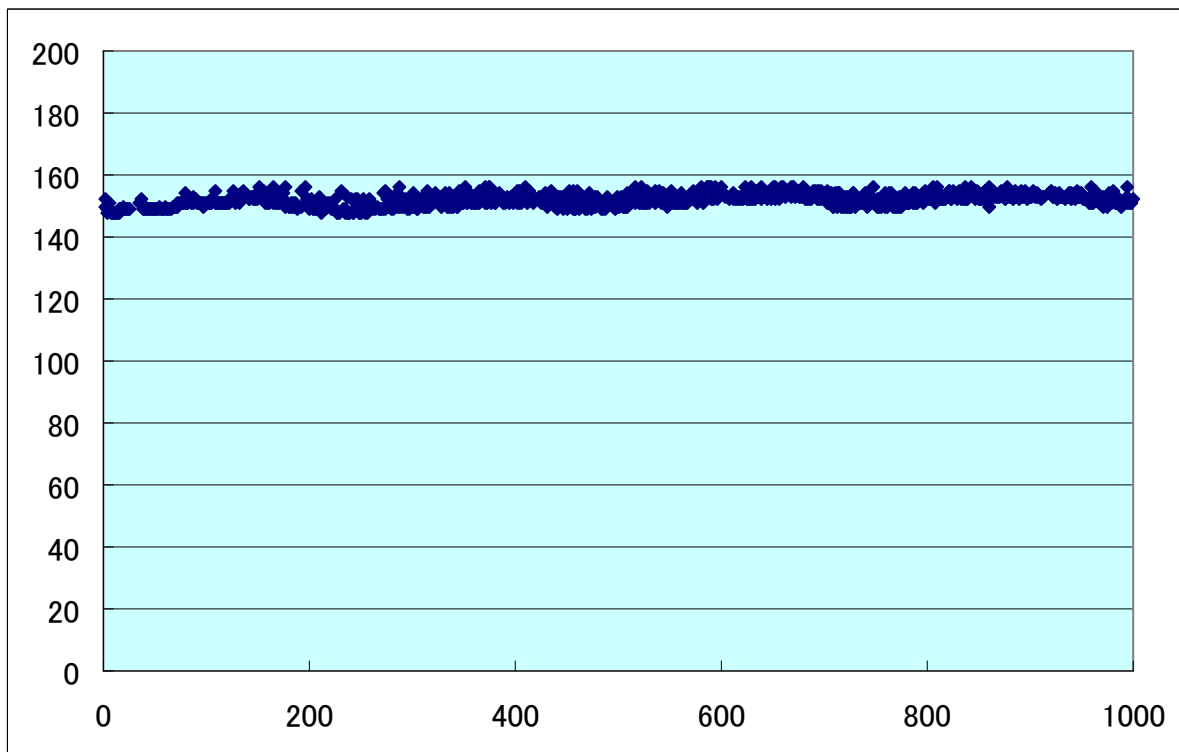
3.0 mm I.D.: From 0.8 to 1.2 ml/min

Durability Test – Column Clogging

Confirmed, no column clogging or increase of column back pressure after a 1,000 injections of pretreated 5 μ l **serum**.

Pretreated serum: Using an human serum supernatant, which was centrifuge mixed with commercially available human serum adding equal volumes of methanol.

The pressure fluctuation of the column

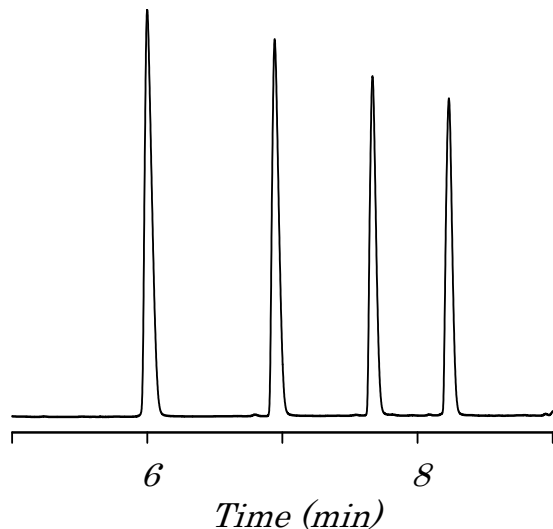


Durability Test – Pressure Durability

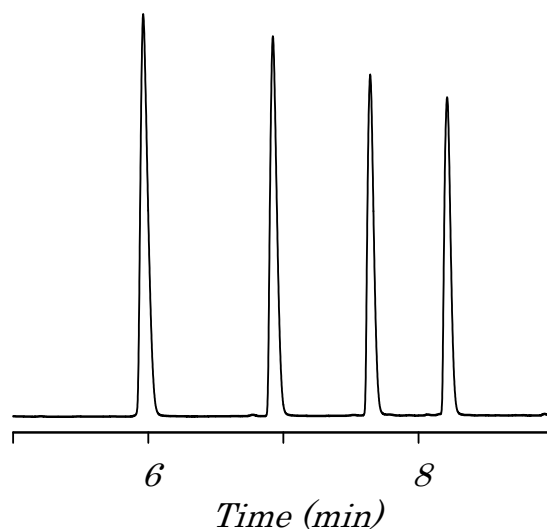
- The analytical condition was set to have the maximum pressure reach at 50 MPa.
- The test was done many times and there where no variation in retention and peak shape.
- Instead of an isocratic, a gradient condition was used which generates more pressure fluctuation resulting in more demanding durability test.

Analytical Conditions

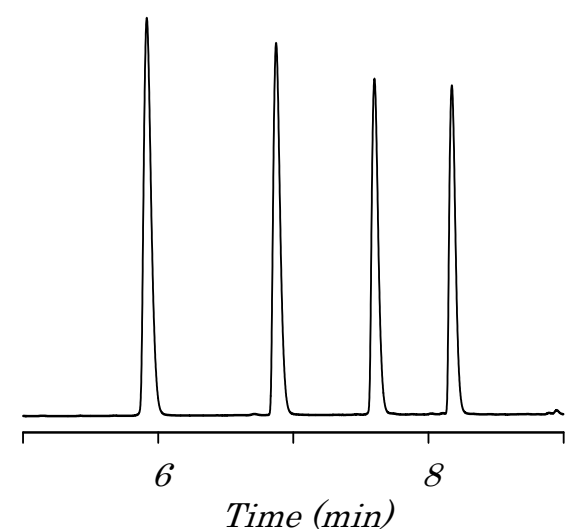
Mobile Phase: Water 100% → 10 min → Methanol 100%
Flow Rate: 0.5 ml/min
Maximum Pressure: 50 MPa (At Water/Methanol =50/50)
Sample: Alkylphenol



1st Injection



After 100 Injections



After 500 Injections

Inertsil ODS-3 2 μ m

Instruction for Use

- **Please be careful with the fittings**, when using an ultra high pressure specification instrument .
- **Operate at pressures within 50 MPa (approx. 7,200 PSI)**, to maximize column life.
- **Extreme care should be taken on the internal diameter of tubing and detector cell**, to maximize the performance of the column,
- The below mentioned Seal Tight Fitting (Upchurch) can be tightened by hand, however, leakage may be observed due to tolerance or worn tubing.
Use a stainless steel male nut and ferrule when analyzing low trace samples.
- Please be careful with setting the response speed of detector and retrieving interval on the data processor in a high throughput analysis.

Partnumber	Description, number of pieces in package
6010-72304	F-195X (upchurch), Seal Tight Fitting, Short Nut, Pk/10
6010-72305	F-196X (upchurch), Seal Tight Fitting, Long Nut, Pk/10

* For a quotation, please contact our local representative

- PEEK (2 piece fittings ; Seal Tight Ferrules (Comes with F-192X)
- O.D. 1/16" Tube (Nut Standard: No. 10-32UNF)
- Resistance to Pressure

When using Stainless Steel Piping:	9,000psi (Approx. 62 Mpa)
When using PEEK Piping:	7,000psi (Approx. 48 Mpa)

