## (2) Scale Up and Scale Down

### 1) Inner Diameter of Column

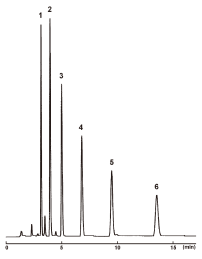
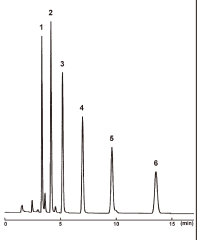
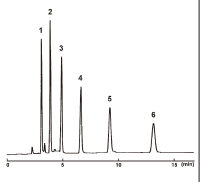
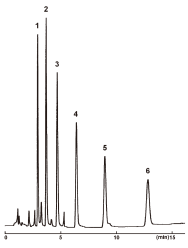
#### Introduction

The table below shows general parameters for 1.0 mm to 50 mm I.D. COSMOSIL columns. It may help to scale up or down from the most commonly used 4.6 mm I.D. columns, depending on your application.

| Inner Diameter (mm I.D.) | 1.0 | 2.0 | 3.0 | 4.6 | 10 | 20 | 28 | 50 |
|---|---|---|---|---|---|---|---|---|
| Flow Rate (ml/min) | 0.05 | 0.2 | 0.4 | 1.0 | 5.0 | 19 | 37 | 70 |
| Detector Cell, Injector | Semi-Micro | | Analytical | | | Preparative | | |
| Tubing Internal Diameter (mm) | 0.05 | 0.1 | 0.2–0.3 | | | 1.0 | | |
| Application | LC–MS<br>Solvent use reduction | | Solvent use reduction with conventional system | Standard | Preparative (small scale) | Preparative (medium scale) | Preparative (large scale) | Preparative (very large scale) |
| Cross-Sectional Area (4.6 mm I.D. = 1) | 0.05 | 0.19 | 0.43 | 1.00 | 4.73 | 18.90 | 37.05 | 118.15 |
| Particle Size (µm) | 5 or lower | | | | 5 | | 15 or higher | |

#### Scale Down

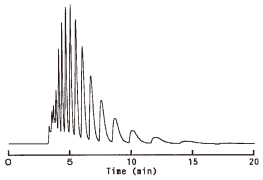
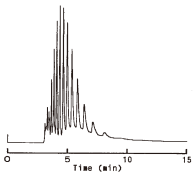
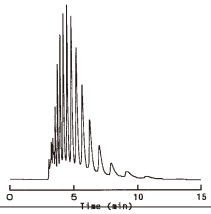
When scaling down from a 4.6 mm I.D analytical column to a 3.0 mm I.D. or lower column of the same column length, sample loading is proportional to the cross-sectional area of column. 3.0 mm I.D. columns provide good sensitivity and reduce solvent use without the need to change existing equipment. Semi-micro columns (2.0 and 1.0 mm I.D.) provide higher sensitivity for analysis of trace components, but necessitate changing the tubing, injector, and detector cell.

| Column Size | 4.6 mm I.D. x 150 mm | 3.0 mm I.D. x 150 mm | 2.0 mm I.D. x 150 mm | 1.0 mm 1.D. x 150 mm |
|---|---|---|---|---|
| Chromatogram |  |  |  |  |
| Flow Rate (ml/min) | 1.0 | 0.4 | 0.2 | 0.05 |
| Pressure (MPa) | 3.4 | 3.6 | 3.8 | 3.6 |
| Injection Volume (µl) | 1.0 | 0.4 | 0.2 | 0.05 |
| Detector Cell・Injector | Analytical | | Semi-Micro | |
| Detector sensitivity (AUFS) | 0.08 | | 0.04 | |
| Tubing Internal Diameter (mm) | 0.25 | | 0.10 | 0.05 |

| | | | |
|---|---|---|---|
| Column | COSMOSIL 5C$_{18}$-MS- II | Sample | |
| Mobile Phase | Acetonitrile : Water = 70 : 30 | 1. Benzene | 4. Propylbenzene |
| Flow Rate | 1.0 ml/min | 2. Toluene | 5. Butylbenzene |
| Temperature | 30°C | 3. Ethylbenzene | 6. Amylbenzene |
| Detection | UV 254 nm | | |

## Scale Up

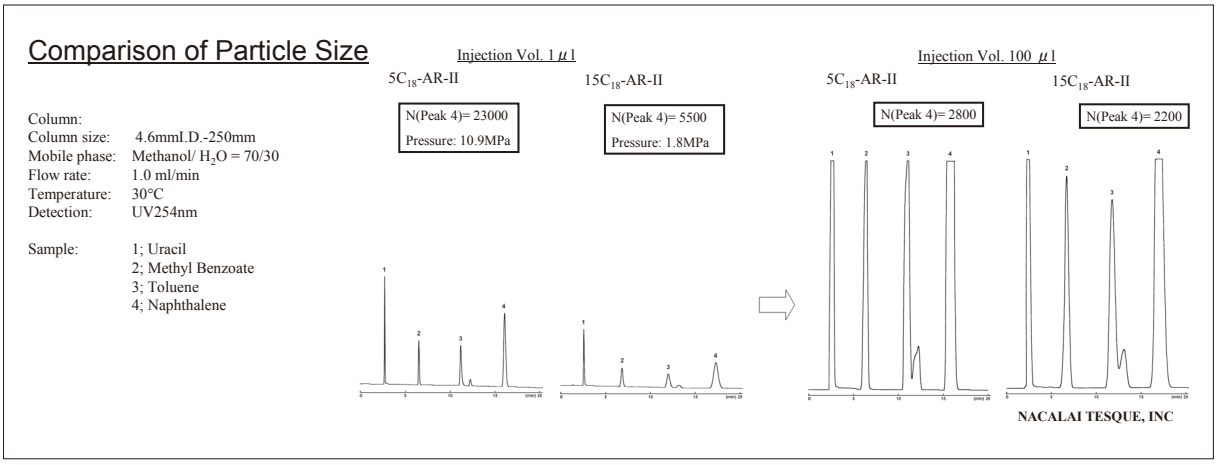
When scaling up from an analytical column to a preparative column of the same packing material (particle size) and length, sample loading capacity is proportional to the cross-sectional area of the column.

| Column Size | 4.6 mm I.D. x 250 mm | 10 mm I.D. x 250 mm | 20 mm I.D. x 250 mm |
|---|---|---|---|
| Chromatogram |  |  |  |
| Flow Rate (ml/min) | 1.0 | 5.0 | 18.9 |
| Pressure (MPa) | 5.5 | 5.9 | 5.8 |
| Injection Volume (µl) | 125 | 625 | 2,500 |
| Detector Cell・Injector | Analytical | | Preparative |
| Tubing Internal Diameter (mm) | 0.25 | | 1.0 |

Column COSMOSIL 5SL-II
Mobile Phase Ethyl Acetate : Ethanol = 4 : 1
Temperature 30°C
Detection UV 254 nm
Sample Triton X-100

## Changing Particle Size of Packing Material

When changing the particle size of the packing material from 5 µm to 15 µm, the number of theoretical plate (N) is reduced to one-third, and the pressure is reduced to one-ninth. As shown in the figure below, when a small amount of sample is injected, there is a big difference in the number of theoretical plates. However, when a large amount of sample is injected, there is not much difference between the two. Therefore, the low-pressure packing material (particle size 15 µm) is recommended for preparative columns (28 mm I.D. or larger).



## 2) Core-Shell and Fully Porous Particles

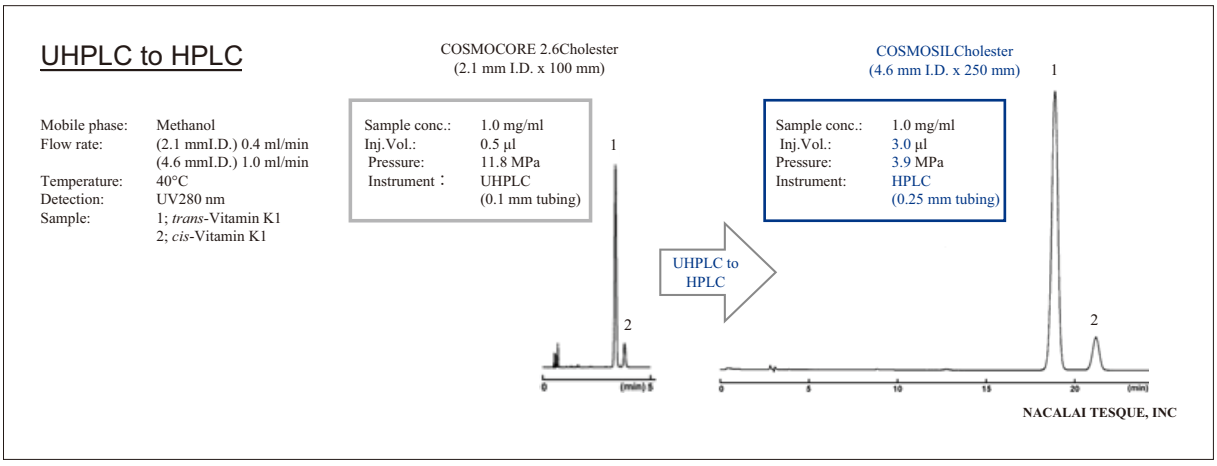
### Scaling up from COSMOCORE 2.6Cholester to COSMOSIL Cholester

Changing conditions from UHPLC to HPLC

| Column | COSMOCORE 2.6Cholester | COSMOSIL Cholester |
|---|---|---|
| Silica Gel | Core-shell silica gel | Fully porous silica gel |
| Average Particle Size (μm) | 2.6 | 5 |
| Average Pore Size (Å) | 90 | 120 |
| Surface Area ($m^2$/g) | 150 | 300 |
| Bonded Group | Cholesteryl | |
| Column Size | 2.1 mm I.D. x 100 mm | 4.6 mm I.D. x 250 mm |
| Instrument | UHPLC | HPLC |
| Flow Rate (ml/min) | 0.4 | **1** |
| Tubing Internal Diameter (mm) | 0.1 | **0.25** |

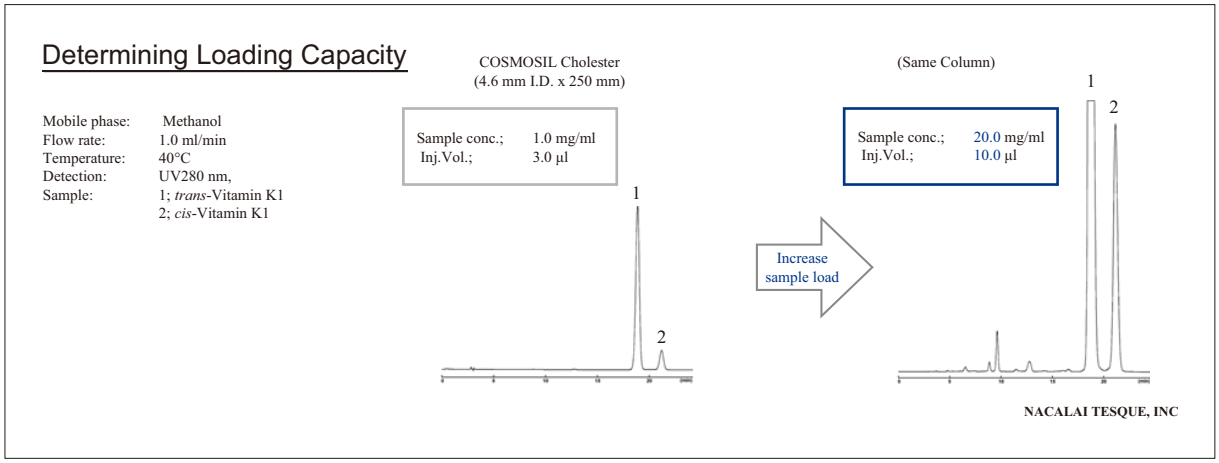
UHPLC to HPLC

By adjusting the analysis conditions, similar separation is achievable on both HPLC and UHPLC. When changing from COSMOCORE 2.6Cholester (2.1 mm I.D.) to COSMOSIL Cholester (4.6 mm I.D.), the injection volume was increased by a factor of more than 5.




### Determining Loading Capacity

Before scaling up to a preparative column, gradually increase the sample load on an analytical column to determine the optimal sample load.



# Transferring a Method to a Preparative Column

## Recommended conditions for different column diameters

| Internal diameter (mm I.D.) | 4.6 | 10.0 | 20.0 | 28.0 | 50.0 |
|---|---|---|---|---|---|
| Standard flow rate (ml/min) | 1 | 5 | 19 | 37 | 70 |
| Detector cell, injector | Analytical | | Preparative | | |
| Tubing Internal Diameter (mm) | 0.2–0.3 | | 1.0 | | |
| Application | Analytical | Preparative (small–scale) | Preparative (medium–scale) | Preparative (large–scale) | Preparative (very large–scale) |
| Cross–sectional area relative to 4.6mm I.D. column | 1 | 4.73 | 18.9 | 37.05 | 118.15 |

## Moving from analytical to preparative

When moving from a 4.6 mm I.D. column to a 10 mm I.D. column (same length), similar separation can be achieved by increasing both the mobile phase flow rate and the injection volume by a factor of about 5. For other diameters, the flow rate and sample load should be increased by the ratio of the cross–sectional areas (see table above).