

TECHNICAL NOTE

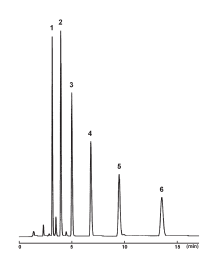
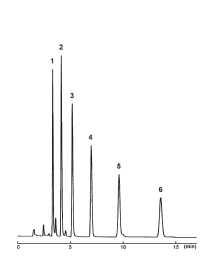
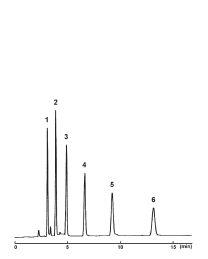
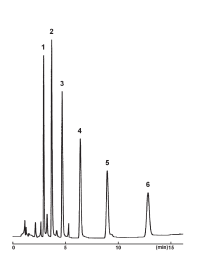
8. Inner diameter of column (scale down and scale up)

The figure below shows general parameters for 1.0 mm to 50 mm I.D. COSMOSIL columns : flow rate, equipment, inner diameter of pipe, application, surface ratio (compared with 4.6 mm I.D.) and particle size. It may help to scale up or down from the most commonly used 4.6 mm I.D. column.

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	18	37	70
Detector cell · Injector	for semi-micro		for analytical			for preparative		
Inner diameter of pipe (mm)	0.05	0.1	0.2-0.3			1.0		
Application	LC-MS solvent saving		solvent saving with standard system	standard	preparative (small scale)	preparative (medium scale)	preparative (large scale)	preparative (super large scale)
Surface ratio with 4.6 mm I.D.	0.05	0.19	0.43	1.00	4.73	18.90	37.05	118.15
Particle size (μm)	3 or 5				5		15 or more	

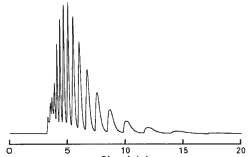
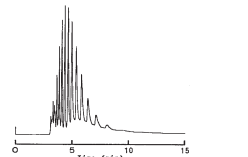
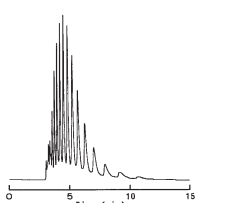
Scale down

When scaling down from the most commonly used analytical column (4.6 mm I.D.) to a semi-micro or 3.0 mm I.D. analytical HPLC column (of the same column length), sample loading dose is proportionate to the cross section of column. The 3.0 mm I.D. columns provide high sensitivity and solvent saving without the need to change the existing equipment settings. Semi-micro columns (2.0 mm I.D. and 1.0 mm I.D.) provide higher sensitivity and enable analysis of minor components, but one needs to change the piping of HPLC equipment, the injector and the detector cell for semi-micro columns.

Column size	4.6 mm I.D. × 150 mm	3.0 mm I.D. × 150 mm	2.0 mm I.D. × 150 mm	1.0 mm I.D. × 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	for analytical		for semi-micro	
Detector sensitivity(AUFS)	0.08		0.04	
Inner diameter of pipe (mm)	0.25		0.10	0.05
	Column Mobile phase Flow rate Temperature Detection	COSMOSIL 5C ₁₈ -MS-II acetonitrile : water = 70 : 30 1.0 ml/min 30°C UV 254 nm	Sample	1. Benzene 2. Toluene 3. Ethylbenzene 4. Propylbenzene 5. Butylbenzene 6. Amylbenzene

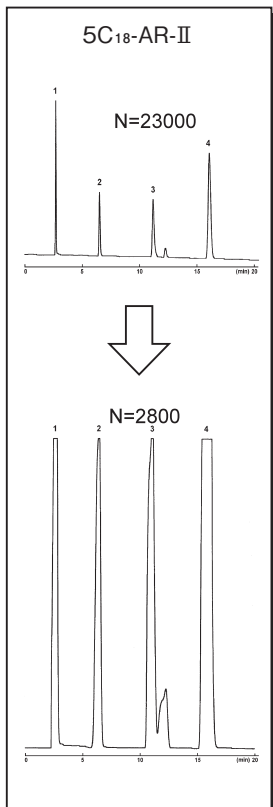
Scale up

When scaling up from analytical column (4.6 mm I.D.) to preparative HPLC (of the same packing material (particle size) and length), sample loading capacity is proportionate to the cross section of column.

Column size	4.6 mm I.D. × 250 mm	10 mm I.D. × 250 mm	20 mm I.D. × 250 mm
Chromatogram			
Flow rate (ml/min)	1.0	5.0	19.8
Pressure (MPa)	5.5	5.9	5.8
Injection volume(μg)	125	625	2,500
Detector Cell · Injector	for analytical		for preparative
Inner diameter of pipe (mm)	0.25		1.0
Column	COSMOSIL 5SL-II		Temperature 30°C
Mobile phase	acetic ether : ethanol = 4 : 1		Detection UV 254 nm
Flow rate	1.0 ml/min		Sample Triton X-100

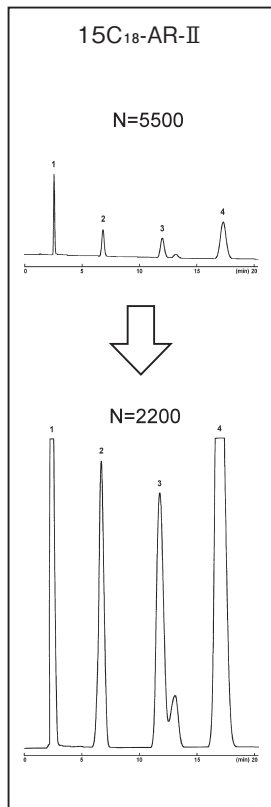
Comparison of particle size

When change particle size of packing material from 5 μm to 15 μm, the number of theoretical plate (N) is reduced by one-third, and the pressure is reduced by one-ninth. As shown in the figures below, when a small amount of sample is injected, there is a big difference in the number of theoretical plates between 5 μm and 15 μm. 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 more).



Injection volume = 1 μl

Injection volume = 100 μl



The number of theoretical plate of N=4. Naphthalene peak.

Column size 4.6 mm I.D. × 250 mm
 Mobile phase methanol : water = 70 : 30
 Flow rate 1.0 ml/min
 Temperature 30°C
 Detection UV 254 nm
 Pressure 5C₁₈-AR-II : 10.9 MPa
 15C₁₈-AR-II : 1.8 MPa
 Sample
 1. Uracil
 2. Methyl Benzoate
 3. Toluene
 4. Naphthalene