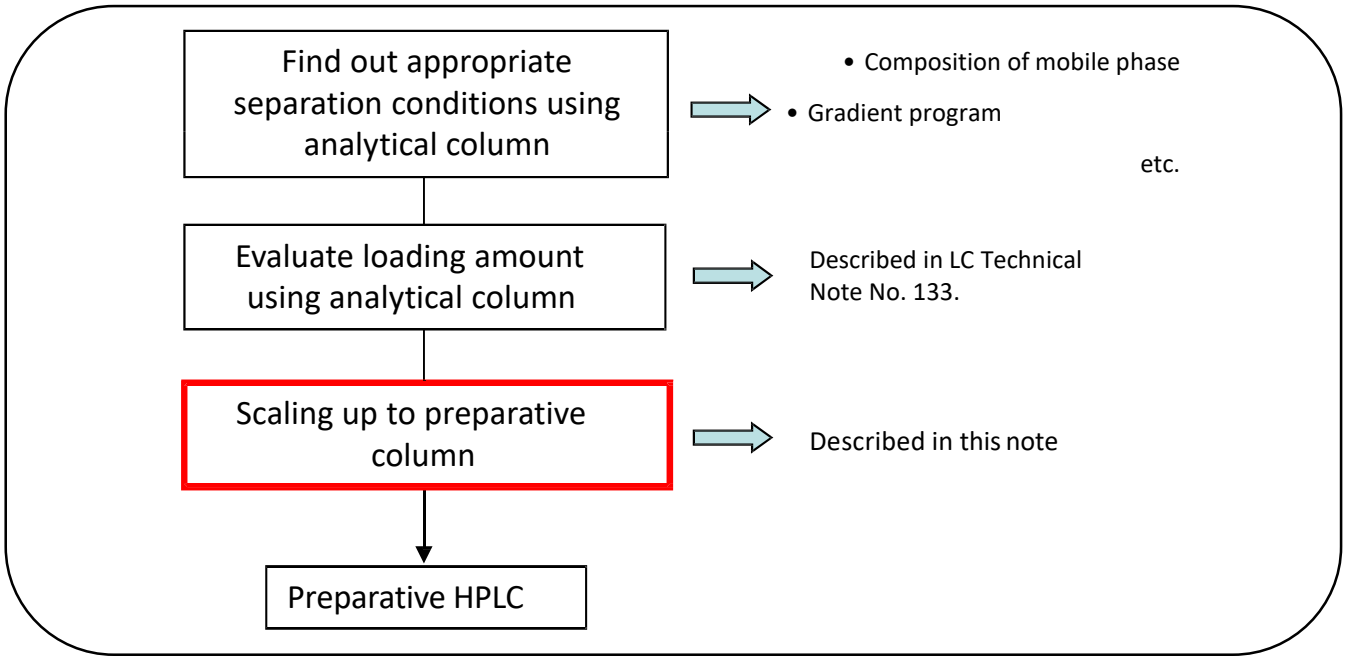
How to Use Preparative HPLC - Part 2
Scaling up from Analytical HPLC

It is not easy to find out optimal condition for preparative HPLC. Not only large volume of solvent but also substantial amount of precious sample may be required for the evaluation of separation conditions, particularly in preparative HPLC. Consequently, we recommend that the evaluation should be carried out using analytical column (4.6 mm I.D.) in the beginning. Condition for preparative HPLC can be investigated efficiently by using analytical column packed with the same gel as in preparative HPLC column.

In this note, Inertsil ODS-3 was taken as an example, and how to scaling up from analytical column to preparative column is described.

(K. Kanno)

Conditions of preparative HPLC are generally optimized as the following flowchart

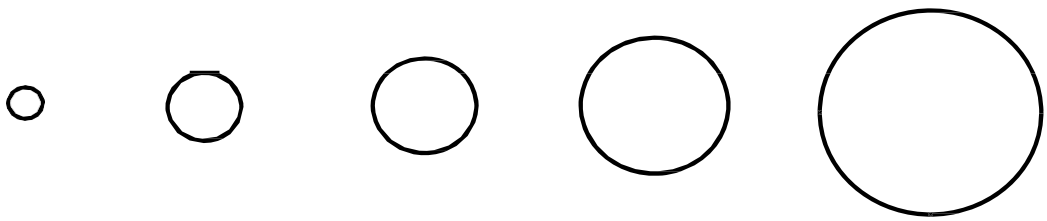


＜What is important to scale up＞

It is important to calculate ratio of cross-sectional area of preparative column to that of analytical column. The ratio can be used as follows;

1)Increase flow rate in proportion to cross-sectional area of column

2)Increase loading amount (injection volume) in proportion to cross-sectional area of column

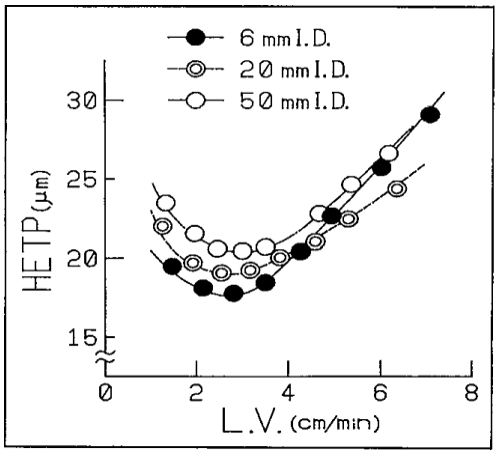


Inner diameter	4.6 mm	10 mm	14 mm	20 mm	30 mm
Cross-sectional area	16.6 mm^2	78.5 mm^2	154 mm^2	314 mm^2	707 mm^2
Ratio of the area※1	1	4.7	9.3	18.9	42.5

※1 Ratio of cross-sectional to that of 4.6-mm I.D. column was calculated.

1) Increase flow rate in proportion to cross-sectional area of column

The figure shown right indicates relation between linear velocity of mobile phase and height equivalent to theoretical plate (HETP) obtained with three columns packed with 10 µm particles.



Optimum flow rate, at which the lowest HETP is obtained, is 3.0 cm/min (0.5 mm/sec) for all the columns. Therefore, it can be said that flow rate should be changed to maintain optimum linear velocity of 3.0 cm/min in case of scaling up from 10 µm particle packed analytical column to 10 µm particle packed reparative one. It is important that particle size of the two columns is same because optimum flow rate changes also depending on particle size.

2) Increase loading amount (injection volume) in proportion to cross-sectional area of column

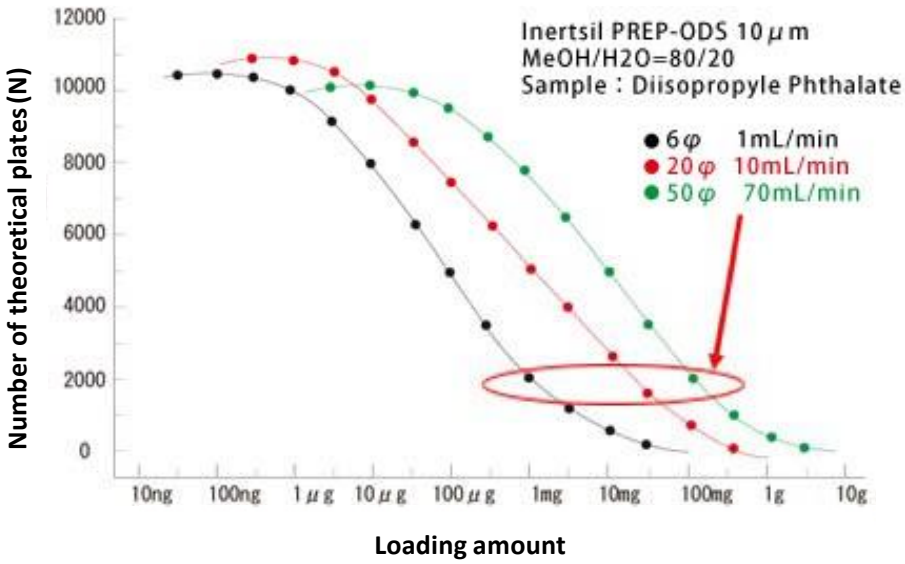
The figure shown below indicates relation between loading amount and number of theoretical plates (N). Three columns with different inner diameters were used and compared. For example, maximum loading amount for each column at which N above 2000 can be obtained is follows;

6 mm I.D. approx. 1 mg

20 mm I.D. approx. 10 mg

50 mm I.D. approx. 70 mg

Since the maximum loading amount is proportional to cross-sectional area of column, it can be said that similar separation should be achieved with preparative column as with analytical column by increasing injection volume in proportion to cross-sectional area.



＜Parameters to be changed for scaling up＞

In case of scaling up from a 4.6 mm I.D. analytical column to a 20 mm I.D. preparative column, cross- sectional area of the column is approximately 19 times enlarged. Therefore, scaling up can be achieved by increasing flow rate and loading amount (injection volume) 19 times. Red letters represent parameters to be changed for scaling up.

Column	: Inertsil ODS-3 (10 µm, 4.6 mm I.D. × 250 mm)	**Scale up** → Increase flow rate and injection volume 19 times. Keep other parameters unchanged	**Column**	: Inertsil ODS-3 (10 µm, 20 mm I.D. × 250 mm)
Eluent	: A) CH_3CN B) H_2O A/B = 40/60, v/v		**Eluent**	: A) CH_3CN B) H_2O A/B = 40/60, v/v
Flow rate	: 500 µL/min		**Flow rate**	: 9.5 mL/min (9500 µL/min)
Column Temp.	: 40 ℃		**Column Temp.**	: 40 ℃
Detection	: UV 270 nm		**Detection**	: UV 270 nm
Injection Vol.*	: 500 µL		**Injection Vol.***	: 9.5 mL (9500 µL)

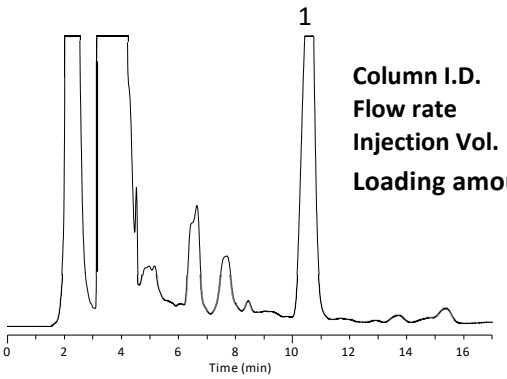
* Concentration of the sample solution is same

＜An example of scaling up＞

Chromatograms before and after scaling up are shown below

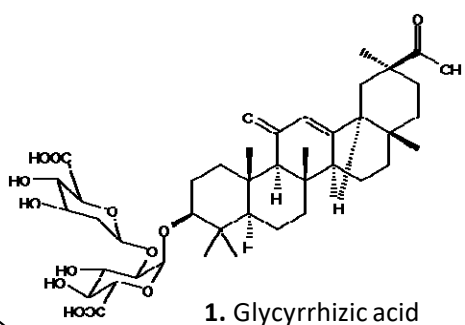
HPLC conditions

Column	: Inertsil ODS-3 (5 µm, 250 mm length)
Column I.D.	: Discribed in each chromatogram
Eluent	: A) CH_3CN B) 0.1 % TFA A/B = 40/60, v/v
Flow rate	: Discribed in each chromatogram
Column Temp.	: 40 ℃
Detection	: UV 270 nm
Sample	: Commercially available licorice extract (2.0 mg/mL)



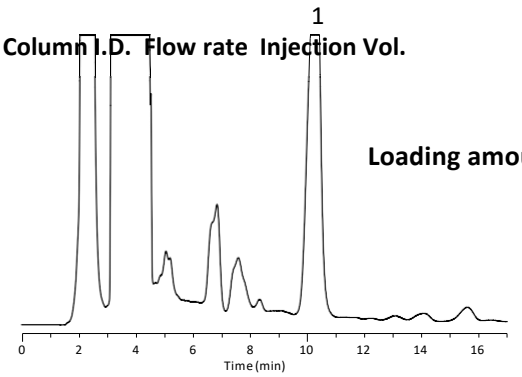
Column I.D. : 4.6 mm
Flow rate : 1.0 mL/min
Injection Vol. : 160 µL
Loading amount : 0.32 mg

Loading amount was 18.9 times increased.



1. Glycyrrhizic acid

Column I.D. : 20 mm
Flow rate : 18.9 mL/min
Injection Vol. : 3.0 mL
Loading amount : 6.0 mg



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