How to use preparative HPLC - Part 1 Evaluation of loading amount

In preparative HPLC, it is quite important to investigate loading amount per one injection because required times of injection and suitable column dimension can be estimated from loading amount.

In this note, how to evaluate loading amount using analytical column (4.6 mm I.D.) is described.

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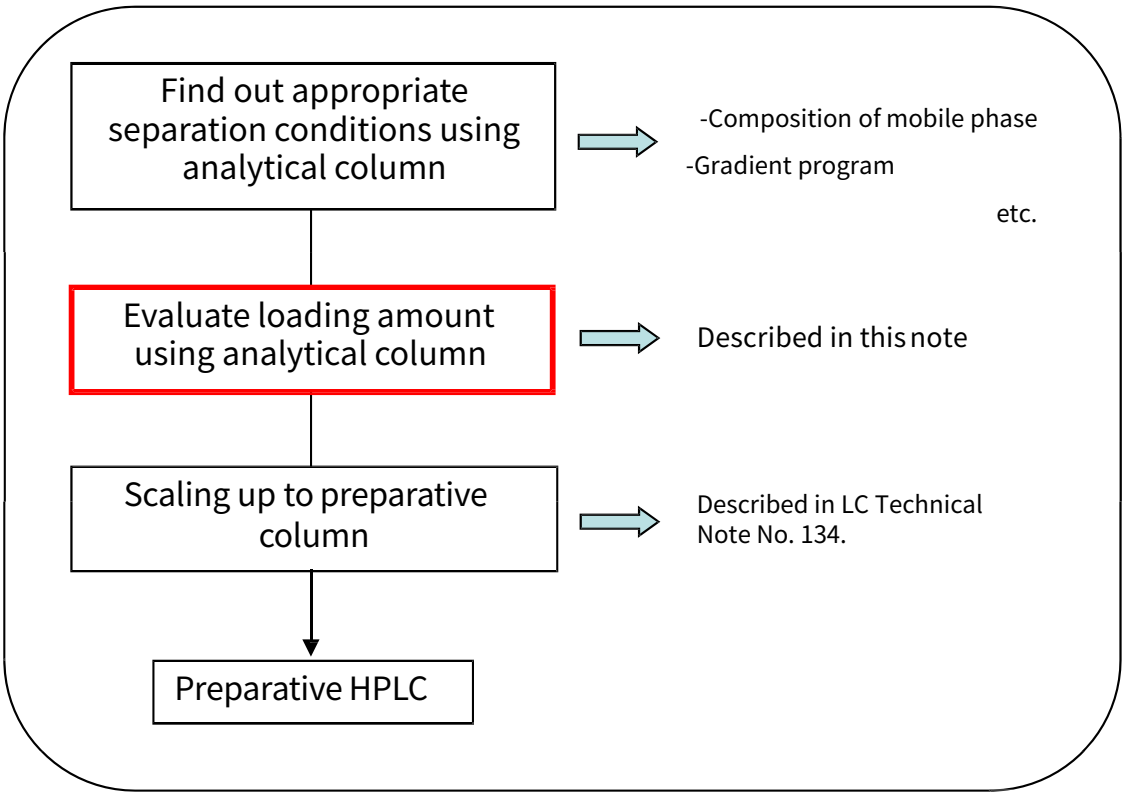
Conditions of preparative HPLC are generally optimized as the following flowchart.



＜What is important to evaluate loading amount＞

Main purpose of preparative HPLC is not quantitation but purification of sample. Therefore, good peak shape is not necessarily required. It dose not matter , either, if injected amount exceeds detector’ s upper limit of linearity. Following two points are important to evaluate loading amount;

1)All amount of the target compound is well retained on column

2)Target compound is successfully separated from other peaks

1) All amount of the target compound is well retained on column

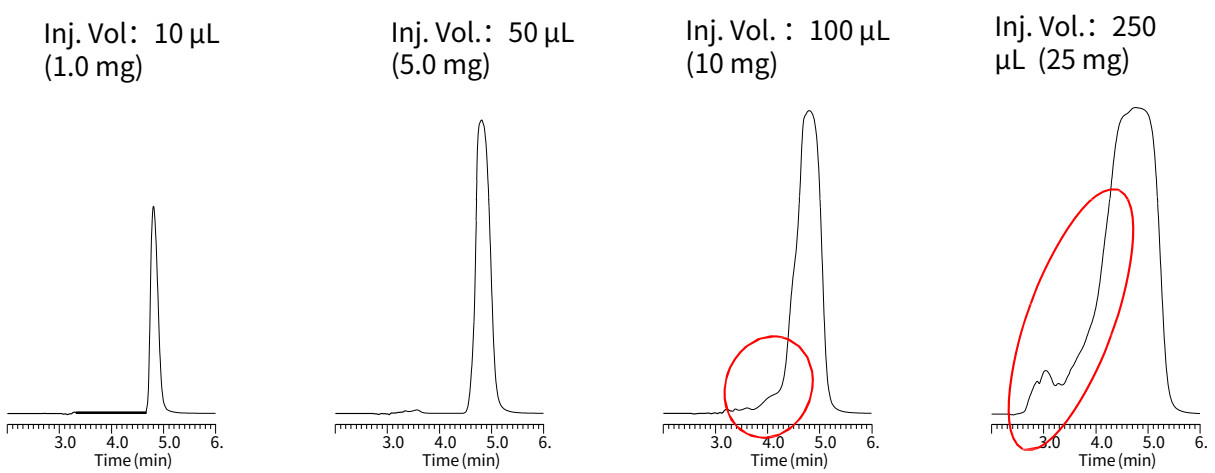
① Prepare sample solution at as high concentration as possible.

② Begin with small amount, and then increase injection volume step-by-step.

In the figure shown below, injection volume was changed as follows; 10 µL → 50 µL → 100 µL → 250 µL. It should be efficient to evaluate peak shape every time increasing injection volume from 2 to 10 times.

③ Peak shape gets worse as injection volume is increased. Injection volume at which peak shape begins to be deteriorated is the maximum amount under the conditions.

In the figure shown below, the peak began to lose its shape when 100 µL of sample solution was injected. Peak was split into two with 250 µL injection. Therefore, it can be said that upper limit of injection volume (amount) under the condition should be around 100 µL (10 mg).



Column : Inertsil WP300 C18 (5 µm, 250 x 4.6 mm I.D.)
Eluent Flow : CH_3OH/H_2O = 90/10, v/v (gradient mixer)
Rate : 1.0 mL/min
Temperature : 40 °C
Detection : UV 270 nm
Sample : *tert*-Buthylbenzene (approx. 100 mg/mL) dissolved in eluent

In case the maximum injection volume obtained with 4.6-mm I.D. column is 100 µL as the figure shown above, the maximum injection volume of preparative column is estimated as follows. Detail of scaling up is described in LC Technical Note No.134.

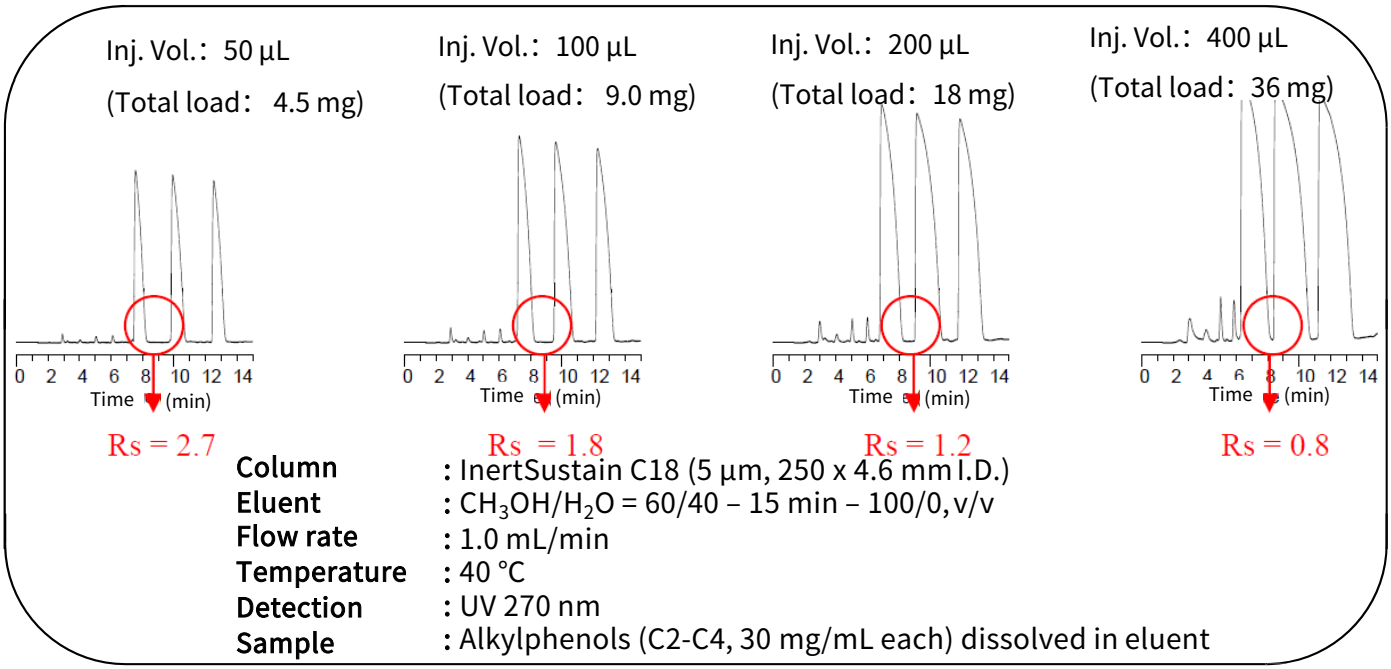
Inner diameter (I.D.) of column	4.6 mm	10 mm	14 mm	20 mm	30 mm
Relative cross-sectional area	1	4.7	9.3	18.9	42.5
The maximum injection volume	100 µL	470 µL	930 µL	1.89 mL	4.25 mL

2) Target compound is successfully separated from other peaks

① Prepare sample solution at as high concentration as possible.
② Begin with small amount, and then increase injection volume step-by-step.

In the figure shown below, injection volume was changed as follows; 50 µL → 100 µL → 200 µL → 400 µL. It should be efficient to evaluate separation of compounds every time increasing injection volume from 2 times to 10 times.
③ The separation gets worse as injection volume is increased. Peak resolution (Rs) is quite useful to evaluate whether sufficient separation is achieved. Target value of Rs varies depending on required purity or how to fractionate the peak, while baseline separation (Rs > 1.5) is ideal. Rs value of between 1.2 and 1.5 is often sufficient when fractionation is carried by detecting peak height or peak slope.

In the figure shown below, it can be said that upper limit of injection volume (total loading amount) should be around 200 µL (18 mg) in case the target value of Rs is set at 1.2.



Column : InertSustain C18 (5 µm, 250 x 4.6 mm I.D.)
Eluent : CH_3OH/H_2O = 60/40 – 15 min – 100/0, v/v
Flow rate : 1.0 mL/min
Temperature : 40 °C
Detection : UV 270 nm
Sample : Alkylphenols (C2-C4, 30 mg/mL each) dissolved in eluent

In case the maximum injection volume obtained with 4.6 mm I.D. column is 200 µL as the figure shown above, the maximum injection volume of preparative column is estimated as follows. Detail of scaling up is described in LC Technical Note No.134.

Inner diameter (I.D.) of column	4.6 mm	10 mm	14 mm	20 mm	30 mm
Relative cross-sectional area	1	4.7	9.3	18.9	42.5
The maximum injection volume	200 µL	940 µL	1.86 mL	3.78 mL	8.5 mL

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