LC Technical Note
LT114
GL Sciences Inc.

Improvement of purification using HPLC column ～ Comparison between column chromatography and preparative HPLC ～

To purify crude product of organic synthesis or to isolate some compounds from natural product, column chromatography with silica gel of 50 µm - 200 µm particle is generally used. However, separation efficiency can be improved by use of 5 µm - 10 µm particle for HPLC column. Moreover, separating time can be reduced because optimal flow rate for 5 µm - 10 µm particle is faster than that for 50 µm - 200 µm particle.

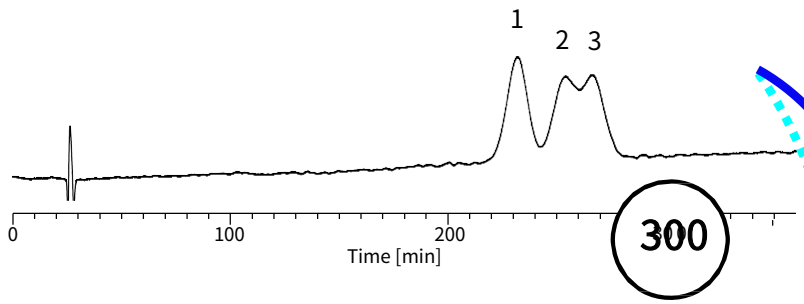
We recommend purification using HPLC when sufficient performance is not offered with conventional column chromatography or flash column chromatography. (C. Aoyama)

Conditions;

Column : ODS column (250×4.6 mm I.D.)
Eluent : A) CH_3CN
B) 0.1% HCOOH in H_2O
A/B = 40/60, v/v
Column Temperature : 40 °C
Detection : UV 270 nm
Inj. Volume : 10 µL

Separation using 50 µm particle

(Flow rate: 0.1 mL/min)



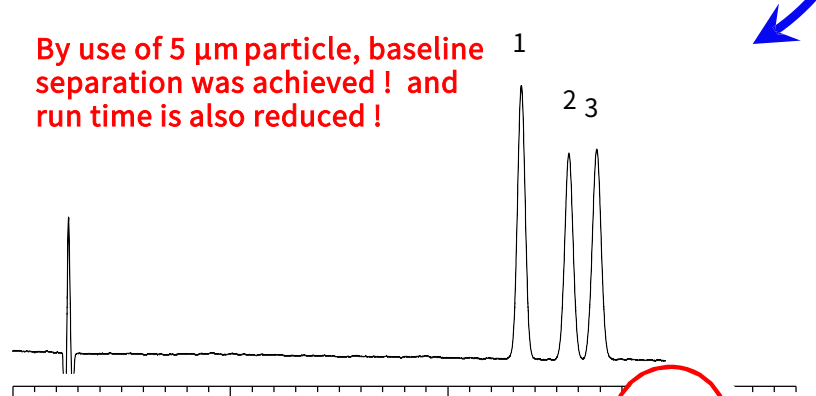
1. sec-Butyl *p*-Hydroxybenzoate
2. iso-Butyl *p*-Hydroxybenzoate
3. n-Butyl *p*-Hydroxybenzoate

Even when separation is difficult with conventional column chromatography, …

Separation using 5 µm particle

(Flow rate: 1.0 mL/min)

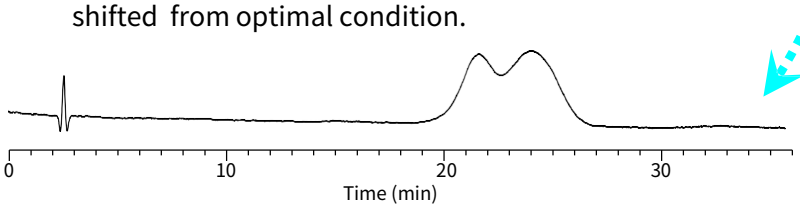
By use of 5 µm particle, baseline separation was achieved ! and run time is also reduced !



If flow rate was increased as the particle size was unchanged, …

Separation with 50 µm particle and 1.0 mL/min

Separation got worse because the flow rate was shifted from optimal condition.



In general, optimal flow rate bears an inverse relation to particle size.

Particle size [µm]	Typical flow rate* [mL/min]
2	2.5
3	1.7
5	1.0
10	0.5
20	0.2
50	0.1

* Optimal value for ODS column of 4.6 mm inner diameter.

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An Example of Scale-up using HPLC column

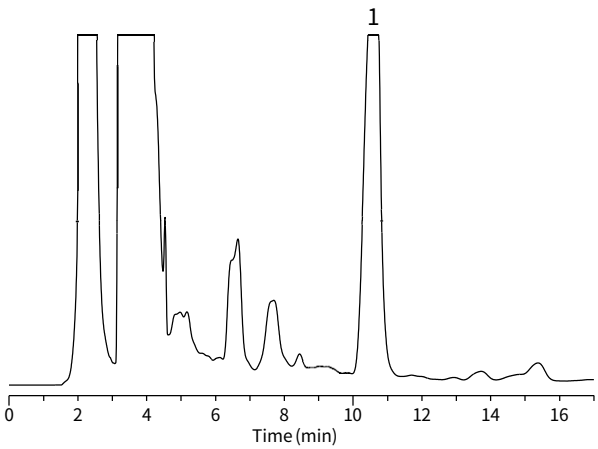
If inner diameter of HPLC column is changed to another, almost the same elution order and retention time should be obtained by adjusting flow rate depending on the inner diameter. Therefore, solvent usage and sample amount can be saved by using columns of small inner diameter for investigation of HPLC condition. (Detail information about relation between inner diameter of column and flow rate is described in LC technical note No.87.)

Conditions;

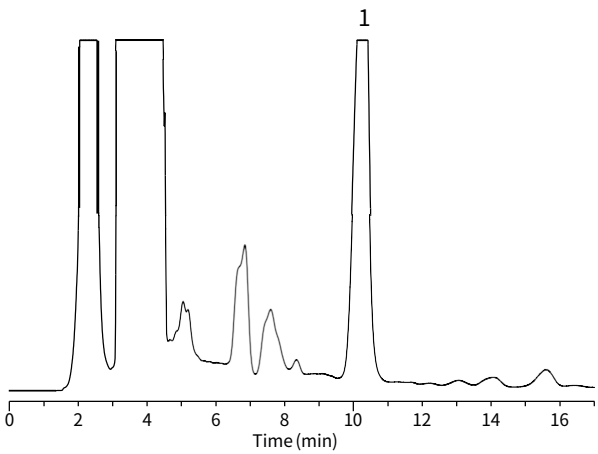
Column	: Inertsil ODS-3 (5 µm, 250 mm length)
Eluent	: A) CH_3CN B) 0.1 % CF_3COOH in H_2O A/B = 40/60, v/v
Temp.	: 40 °C
Detection	: UV 270 nm
Sample	: Glycyrrhizae radix extract (2.0 mg/mL)

By switching inner diameter of column, 20 times increase of loading amount was achieved without changing the elution order.

1. Glycyrrhizic acid



Inner diameter : 4.6 mm
Flow rate : 1.0 mL/min
Injection volume : 160 µL
Loading amount : 0.32 mg



Inner diameter : 20 mm
Flow rate : 18.9 mL/min
Injection volume : 30 mL
Loading amount : 6.0 mg

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