

Improvement of purification using HPLC column

~ Comparison between column chromatography and preparative HPLC ~

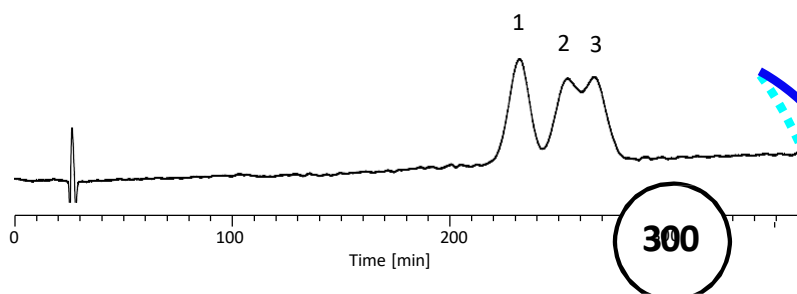
To purify crude product of organic synthesis or to isolate some compounds from natural product, column chromatography with silica gel of 50 μm - 200 μm particle is generally used. However, separation efficiency can be improved by use of 5 μm - 10 μm particle for HPLC column. Moreover, separating time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

Separation using 50 μm particle

(Flow rate: 0.1 mL/min)



Conditions:

Column : ODS column (250 × 4.6 mm I.D.)
Eluent : A) CH₃CN
 B) 0.1% HCOOH in H₂O A/B = 40/60, v/v
Column Temperature : 40 °C
Detection : UV 270 nm
Injection Volume : 10 μL

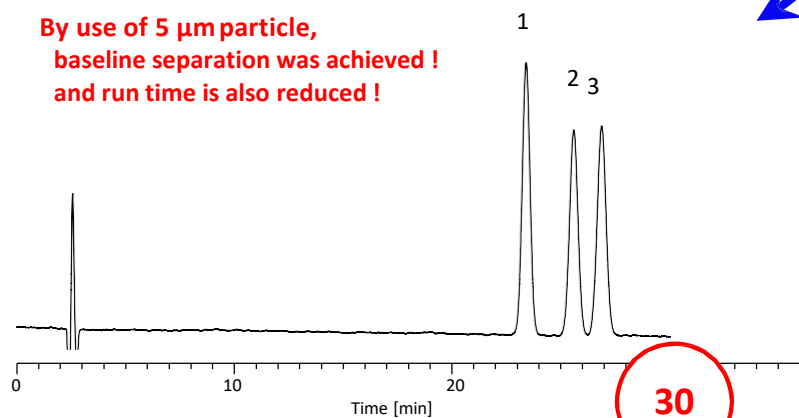
1. *sec*-Butyl *p*-Hydroxybenzoate
2. *iso*-Butyl *p*-Hydroxybenzoate
3. *n*-Butyl *p*-Hydroxybenzoate

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!

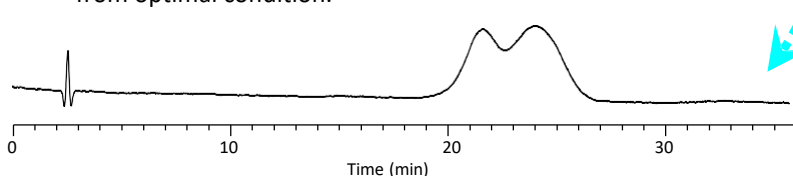


If flow rate was increased as the particle size was unchanged, ...

Separation with 50 μm particle and 1.0 mL/min

1 2+3

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.

An Example of Scale-up using HPLC column

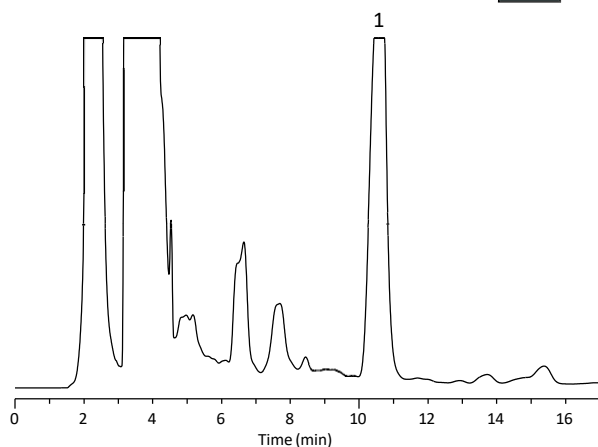
If inner diameter of HPLC column is changed to another, almost the same elution order and retention time should be obtained by adjusting flow rate depending on the inner diameter. Therefore, solvent usage and sample amount can be saved by using columns of small inner diameter for investigation of HPLC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

Conditions:

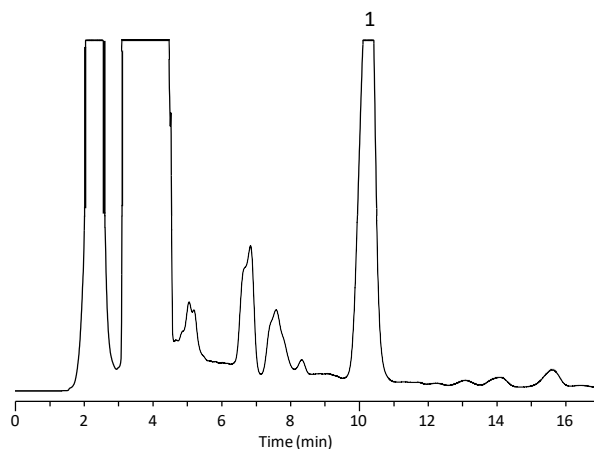
Column : Inertsil ODS-3 (5 μ m, 250 mm length)
 Eluent : A) CH₃CN B) 0.1 % CF₃COOH in H₂O
 A/B = 40/60, v/v
 Temp. : 40 °C
 Detection : UV 270 nm
 Sample : Glycyrrhizae radix extract (2.0 mg/mL)

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

1. Glycyrrhizic acid



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



Inner diameter : 20 mm
 Flow rate : 18.9 mL/min
 Injection volume : 3.0 mL
Loading amount : 6.0 mg

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