LT087
GL Sciences Inc.

How to Save Acetonitrile – Part 2

In this note, a simple way to reduce usage of acetonitrile, of which a world-wide shortage has occurred in 2008, is described.

Columns with 4.6 mm inner diameter (I.D.) are most widely used. Only to decrease column I.D. enables us to save the usage of mobile phase. Scaling down from 4.6 mm I.D. to 4.0 mm I.D. provides 25% reduction of mobile phase, whereas that from 4.6 mm I.D. to 3.0 mm I.D. results in 60% cut.

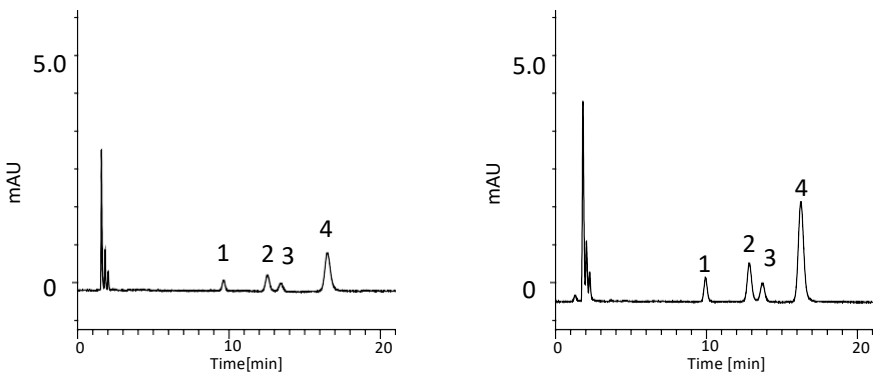
Downsizing of columns reduces not only running cost but also amount of waste solution. Switching to small I.D. columns is good for the ecology.

(K.Suzuki)

An example of downsizing

Similar retention times can be obtained by adjusting the mobile phase flow rate in proportion to the cross-sectional area of the column .

In case switching from 4.6 mm I.D. to 3.0 mm I.D., the flow rate should be decreased to about 0.4 times.



I.D.: 4.6 mm
Flow rate: 1.0 mL/min

→

I.D.: 3.0 mm
Flow rate: 0.4 mL/min

60 % cut of solvent usage

10

Ratio of column cross-sectional area

4

Conditions

System : GL-7400 HPLC system
Column : Inertsil ODS-3 (5 µm, length: 150 mm)
Eluent : A) CH_3CN
B) H_2O
A/B = 80/20, v/v
Col. Temp. : 40 ℃
Detection : UV 254 nm
Inj. Vol. : 10 µL

1. *n*-Butylbenzene
2. *o*-Terphenyl
3. *n*-Amylbenzene
4. Triphenylene

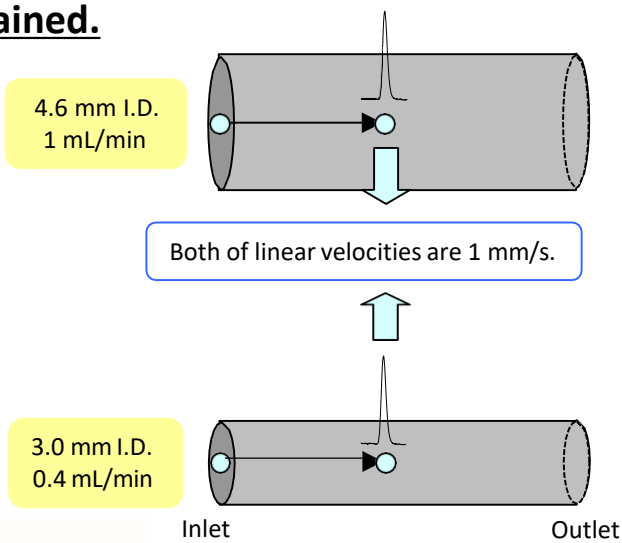
Equalize the linear velocity of the mobile phase

⇒ Similar retention times should be obtained.

Linear velocity is longitudinal flow rate of mobile phase in column. It is often defined as follows;

$$\text{Linear velocity (mm/s)} = \frac{\text{Flow rate of mobile phase (mm}^3\text{/s)}}{\text{Cross-sectional area of column (mm}^2\text{)}}$$

Therefore, linear velocity can be adjusted by changing flow rate of mobile phase. The interaction behavior between mobile phase and stationary phase in column can be almost recovered by equalizing the linear velocity. In isocratic elution, the same linear velocity often enables us to obtain the similar chromatogram.



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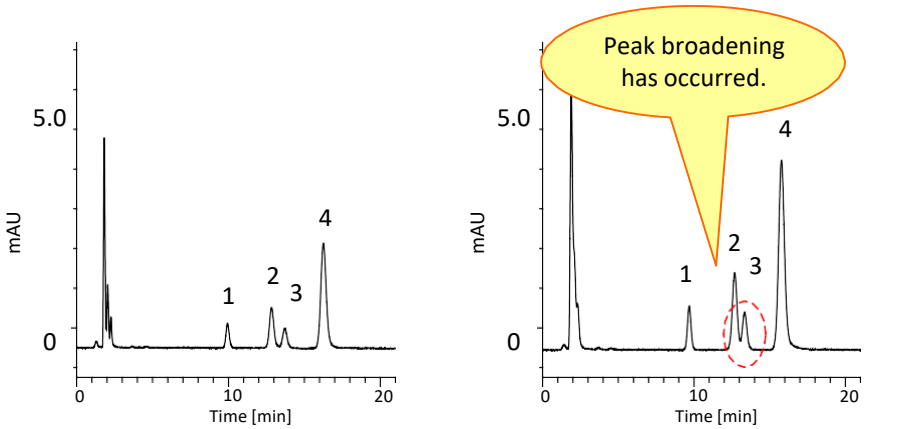
An example of further downsizing

In downsizing from 3.0 mm I.D. to 2.1 mm I.D., the flow rate should be reduced to half. If good separation is achieved with 3.0 mm I.D. column, it is possible to scale down using conventional HPLC systems. However, if not, peak broadening often occurs in low flow rate because of the sample diffusion caused by dead volume. To solve this problem, it is necessary to use semi-micro HPLC systems or capillary LC systems.



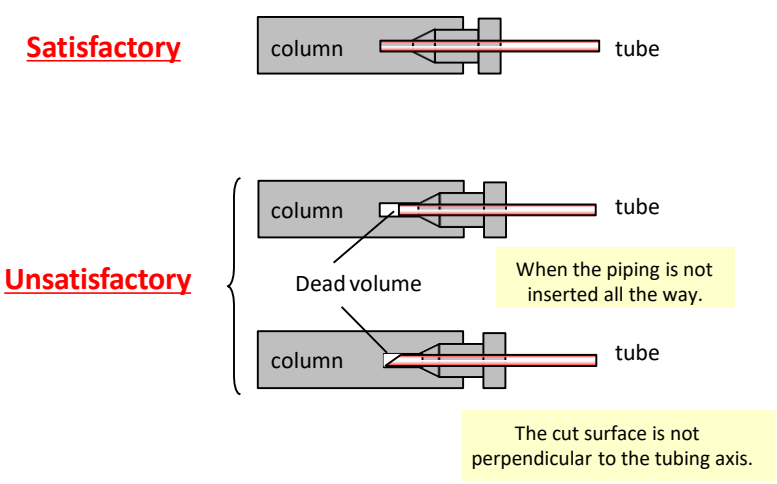
Conditions

System : GL-7400 HPLC system
Column : Inertsil ODS-3 (5µm, length: 150 mm)
Eluent : A) CH_3CN
B) H_2O
A/B = 80/20, v/v
Col. Temp. : 40 ℃
Detection : UV 254 nm
Inj. Vol. : 10µL

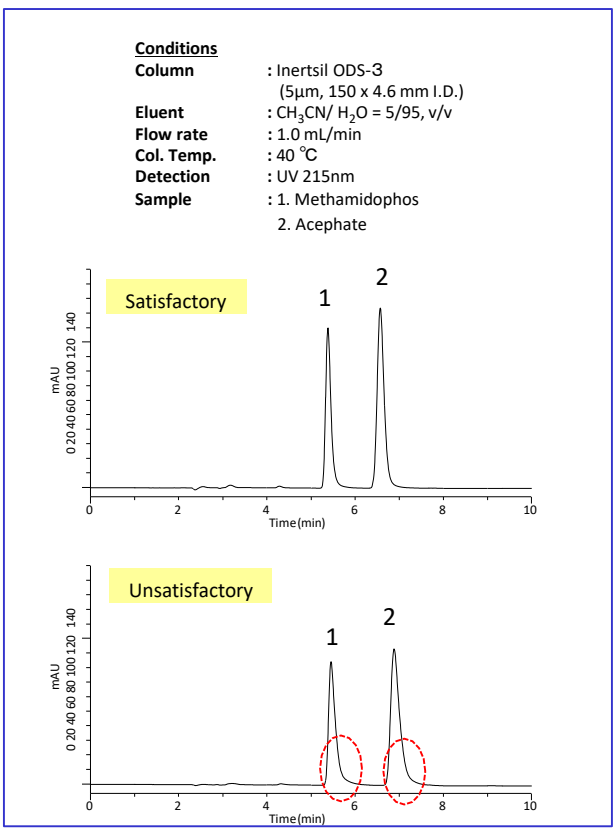
1. n-Butylbenzene
2. o-Terphenyl
3. n-Amylbenzene
4. Triphenylene

< Caution 1: Diffusion by dead volume >

Please take care to minimize dead volume associated with column and tubing connection as shown below. Otherwise, peak broadening may occur even with 4.6 mm I.D. columns.



Conditions
Column : Inertsil ODS-3 (5µm, 150 x 4.6 mm I.D.)
Eluent : CH_3CN/ H_2O = 5/95, v/v
Flow rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 215nm
Sample : 1. Methamidophos
2. Acephate



An advantage of downsizing

The chromatogram shown below was cited from a previous report (LC technical notes, No.8). Even in this analysis, equalizing linear velocity provides similar retention times. Moreover, peak heights increased more than twice. This phenomenon is often obtained when concentration-dependent detectors (e.g. UV-VIS detector and fluorescence detector) are used.

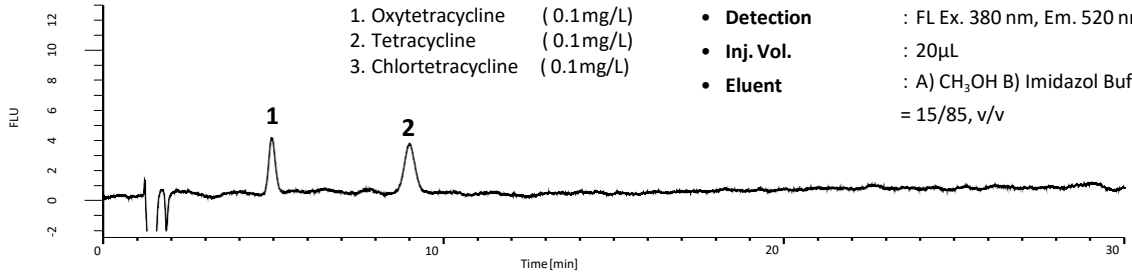
- 4.6 mm I.D. column

(150 x 4.6 mm I.D., particle size: 5 µm, flow rate: 1.2 mL/min)

1. Oxytetracycline (0.1mg/L)
2. Tetracycline (0.1mg/L)
3. Chlortetracycline (0.1mg/L)

Conditions

- **Column** : Inertsil ODS-3
- **Column Temp.** : 40 ℃
- **Detection** : FL Ex. 380 nm, Em. 520 nm
- **Inj. Vol.** : 20µL
- **Eluent** : A) CH_3OH B) Imidazol Buffer A/B = 15/85, v/v

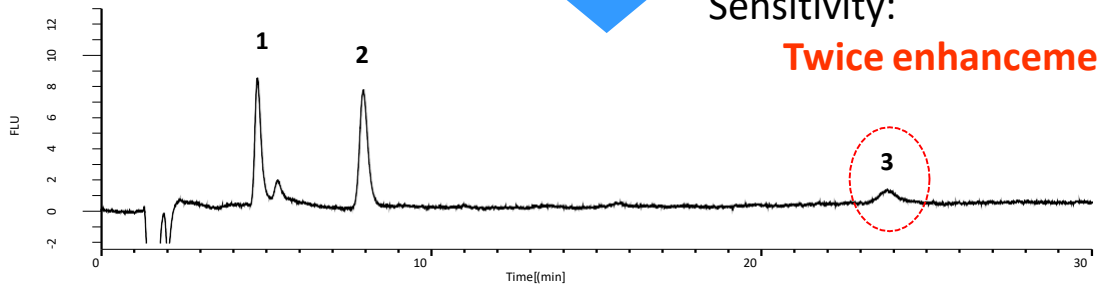


- 3.0 mm I.D. column

(150 x 3.0 mm I.D., particle size: 3µm, flow rate: 0.5 mL/min※2)

Solvent usage: **60 % cut**

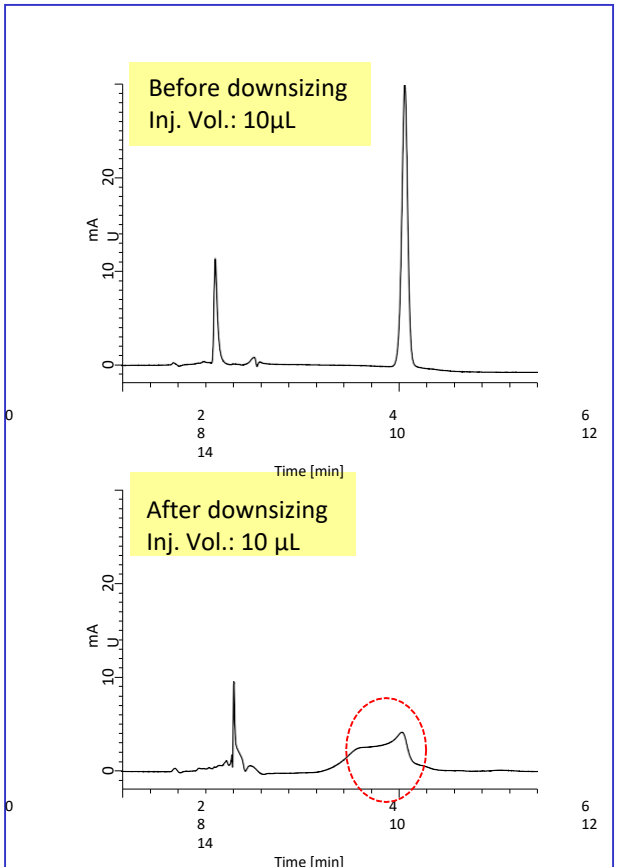
Sensitivity: **Twice enhancement**



< Caution 2: Injection volume >

With concentration-dependent detectors, downsizing can be expected to improve the sensitivity as described above. However, if polarity of the sample solvent was different from that of mobile phase, peak shapes sometimes get worse as you can see in the chromatograms on the right. In such case, column lifetime also becomes shorter.

To prevent this problem, injection volume should be reduced in proportion to the flow rate or diluted with mobile phase. When the injection volume is the same, use a larger I.D. column at first to confirm if the peak shapes are not deteriorated by increasing the injection volume. After confirming the peak shapes are not deteriorated , then use a smaller I.D. column.



Flow rates which offer 1 mm/s in columns with various I.D.

I.D. of column [mm]	7.6	6.0	4.6	4.0	3.0	2.1	1.5
Flow rate [mL/min]	2.7	1.7	1.0	0.8	0.4	0.2	0.1

If less than 3 mm I.D. columns are utilized, it is recommended to use, not conventional, but semi-micro HPLC system.

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