Selection guide of mobile phase

COMOSIL HILIC column generates retention and separation by hydrophilic interaction (mainly hydrogen bond) and anion-exchange. Refer to following recommendations to select an appropriate mobile phase condition.

(1) The effect of organic solvent type and content

- In general, acetonitrile/water is used as mobile phase.
- Retention increases as water content in the mobile phase decreased. (Fig.1)
- Use acetonitrile content in the mobile phase within the range of 0-95% (in general 50-95%).
- Methanol/water generates shorter retention than acetonitrile/water. (Fig.2)
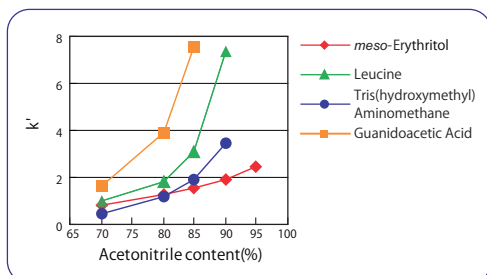- Use only HPLC grade solvent

Fig.1 The effect of acetonitrile content on retention

Column; COSMOSIL HILIC
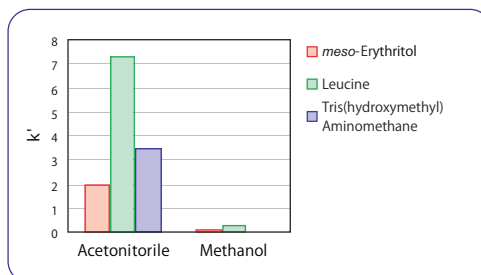Mobile phase; Acetonitrile/ 10mmol/l CH_3COONH_4

Fig.2 Difference of acetonitrile and methanol on retention

Column; COSMOSIL HILIC
Mobile phase; Organic solvent/ 10mmol/l CH_3COONH_4 = 90/10

(2) The effect of buffer pH

- Keep pH of the mobile phase within the range of 2-7.5.
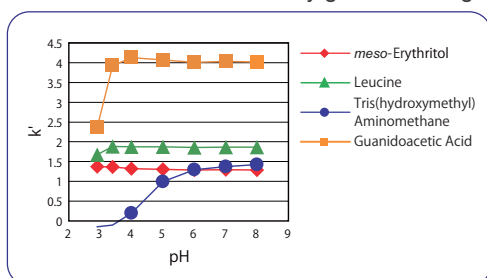- The buffer around neutrality generates larger retention. (Fig.3)

Fig.3 The effect of buffer pH on retention

Column; COSMOSIL HILIC
Mobile phase; Acetonitrile / 10mmol/l buffer = 90/10

(3) The effect of salt type and concentration

- When analyze ionic compounds, add salts or buffers in the mobile phase.
- When mobile phase has high acetonitrile content, note dissolubility of the salt. The dissolubility of phosphate buffers used widely in reversed phase chromatography is low in acetonitrile. Therefore use of phosphate buffers is not recommended. Keep the concentration of acetonitrile under 70% if use a phosphate buffer. Check that the salt does not precipitate when mixed with acetonitril before use.
- Ammonium acetate or formic acid ammonium buffers are recommended because they are soluble in high acetonitrile content.
- Use the buffer concentration within the range of 5 - 100mmol/l. Moreover, check that the salt does not precipitate after mixing buffer and acetonitrile.
- High salt concentration inhibits ion exchange capability and decreases retention. (Fig.4)
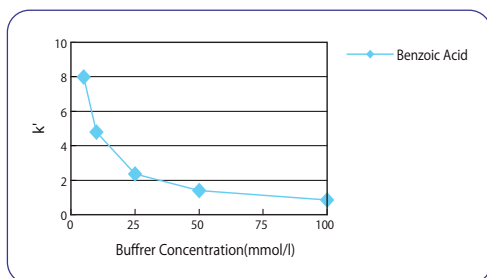- Run mobile phase through membrane filter (less than 0.45μm in pore size) before use.

Fig.4 The effect of salt concentration on retention

Column; COSMOSIL HILIC
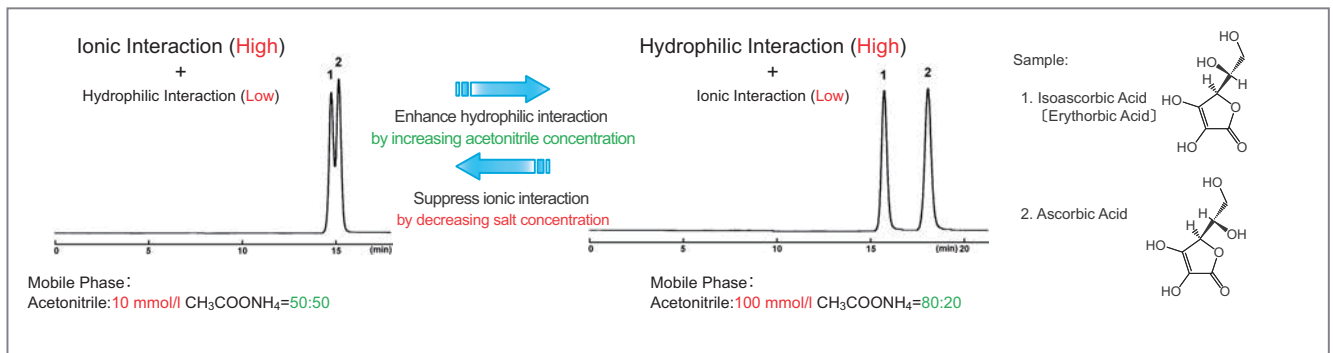Mobile phase; Acetonitrile / 10mmol/l CH_3COONH_4 = 50/50

(4) Selection of mobile phase

Following are the recommended mobile phases for different compound types.

Neutral compounds	→ Acetonitrile / Water = 90/10
Basic compounds	→ Acetonitrile / 10mmol/l CH_3COONH_4 = 90/10
Amphoteric compounds	→ Acetonitrile / 10mmol/l CH_3COONH_4 = 70/30
Acidic compounds	→ Acetonitrile / 10mmol/l CH_3COONH_4 = 50/50

↓ not eluted

Acetonitrile / 10mmol/l Phosphate buffer (pH7.0)= 50/50

(5) Two interactions (hydrophilic interaction and anion exchange capability)

The retention mechanism of COSMOSIL HILIC is the combination of hydrophilic interaction and anion-exchange, and the retention can be controlled by changing the mobile phase. More specifically, the hydrophilic interaction can be enhanced by increasing the organic solvent concentration while suppressing the ionic interaction with high salt concentration.

Ionic Interaction (High)
+
Hydrophilic Interaction (Low)

Enhance hydrophilic interaction
by increasing acetonitrile concentration

Suppress ionic interaction
by decreasing salt concentration

Mobile Phase：
Acetonitrile:10 mmol/l CH_3COONH_4=50:50

Hydrophilic Interaction (High)
+
Ionic Interaction (Low)

Mobile Phase：
Acetonitrile:100 mmol/l CH_3COONH_4=80:20

Sample:

1. Isoascorbic Acid
〔Erythorbic Acid〕

2. Ascorbic Acid

(6) Improvement of peak shape

Try following if peak shape is tailing. The peak shape might improve.

- Add 5mmol/l EDTA to mobile phase.
- Change to citrate buffer. (i. e. 10 mmol/l citrate buffer pH7.0)

80%Acetonitrile/
10mM CH_3COONH_4

80%Acetonitrile/
10mM CH_3COONH_4+5mmol/l EDTA

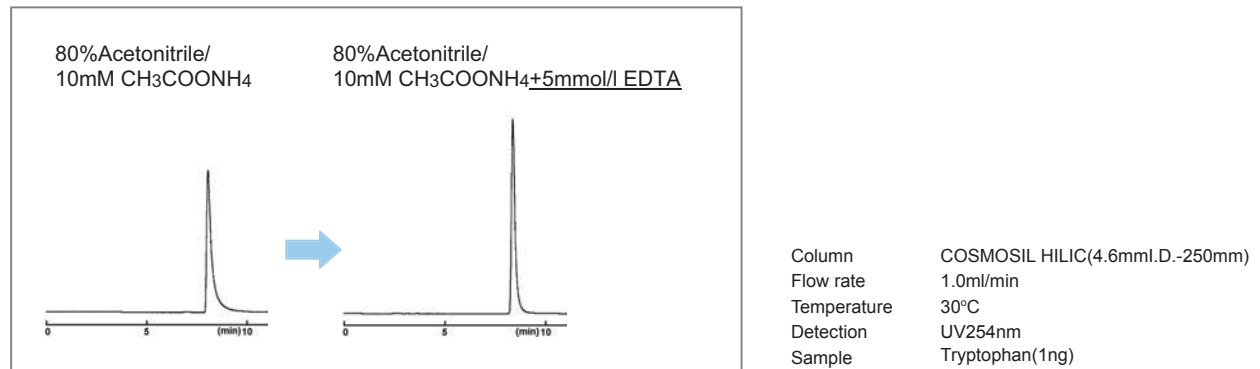|	
Column	COSMOSIL HILIC(4.6mmI.D.-250mm)
Flow rate	1.0ml/min
Temperature	30℃
Detection	UV254nm
Sample	Tryptophan(1ng)

Fig.5 Improvement of peak shape

(7) Others

- Use scrupulously degassed mobile phase. Air bubbles generate detection noise and accelerate column deterioration.
- We recommend keeping the chromatography conditions constant, since frequent changes of mobile phase shorten column life.