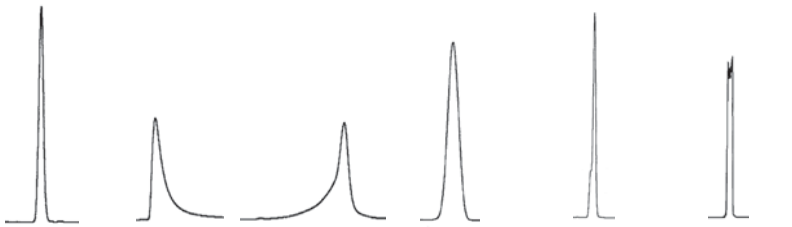
(3) Troubleshooting

T1. Poor peak shape



Normal Peak　Tailing Peak　Fronting Peak　Broad Peak　Peak Spike　Peak Splitting

Symptom	Cause	Solution
Particular sample is tailing	Undesirable ion exchange interaction between basic compounds and packing material.	Replace the column with less residual silanols (C_{18}-MS-II). Or add 0.1–1% of acid to mobile phase.
	Undesirable coordinate interaction between metal coordination compound and packing material.	Add 5mmol/l of di-sodium dihydrogen ethylenediaminetetraacetate dihydrate (EDTA・2Na) to mobile phase.
	Undesirable hydrogen bonding interaction between sample and packing material.	Change the organic solvent (e.g., acetonitrile to methanol).
All samples are tailing	Have spaces in packing material. Or column may have deteriorated.	Replace the column.
	(If the tailing does not improve after replacing the column) Sample is spreading out side of the column.	Reduce dead volume (Refer to Q25 on page 172 for more information on dead volume).
Fronting	Inject large volume of sample solvent that is significantly different in elution properties or pH comparing to mobile phase.	Dissolve sample in mobile phase. If the sample does not dissolve, dissolve in soluble solvent first, then dilute in mobile phase.
		Reduce injection volume to 1/2–1/10 Attention; Spikes or peak broadening may also occur.
Broad peaks 1 Sample has high molecular weight (MW: 2,000 or more).	Protein with high molecular weight cannot go into pores of packing material.	Use wide pore (pore size: 300A) column for reversed phase chromatography, COSMOSIL Protein-R. Refer to page 42 for more information.
	Sample volume is too large.	Reduce inject volume to 1/2–1/10. Attention; Tailing peaks may also occur.
	In case of broad tailing of a particular sample, compound may be adsorbed onto packing material.	Use COSMOSIL Protein-R, it has high recovery rate. Refer to page 42 for more information.
	In case of broad tailing of all samples, column may deteriorate.	Replace the column.
	Concentration of ammonium sulfate in sample solution is too low on hydrophobic chromatography (HIC).	Adjust concentration of ammonium sulfate to 1 mol/l or more.

Symptom	Cause	Solution
Broad peaks 2 Sample has low molecular weight (MW: 2,000 or less).	Sample volume is too large.	Reduce sample amount from 1/2 to 1/10. Caution; Tailing peaks may occur instead of broad peaks.
	In case of broad peak of a particular sample, compound may be adsorbed onto packing material.	Replace with a column that has a different packing material. (COSMOSIL 5C$_{18}$-MS-II is recommended for basic sample. The column has less adsorption to basic compounds. Please refer to page 14 for more information.)
	In case of broad peaks for all samples, the column may deteriorate.	Replace the column.
Peak spikes or peak splitting may occur for certain sample.	More than 2 samples are contained, and slightly separated.	Find the condition which enables separation of the two samples.
	Mobile phase and sample solvent are significantly different in their separation properties.	Dissolve sample in mobile phase. If sample does not dissolve, dissolve sample in sample soluble solvent first before mixing it with the mobile phase.
		Reduce loading capacity to 1/2 to 1/10.
	Mixed dissociated and non-dissociated ionic sample.	Adjust pH of mobile phase to pKa ± 2 or more of the ionic sample.
Peak spikes or peak splitting occur for all samples	Mobile phase and sample solvent are significantly different in their separation properties.	Dissolve sample in mobile phase. If sample does not dissolve, dissolve sample in sample soluble solvent first before mixing it with the mobile phase.
		Reduce loading capacity to 1/2 to 1/10.
	The column may have deteriorated.	Replace the column.

T2. Ghost peaks

Separation Mode	Cause	Solution
<Reversed Phase Chromatography> Use gradient elution method	Peaks from water impurities	Use new HPLC grade distilled water.
		Use a pre-column. Please refer to page 198 for more information.
<Reversed Phase Chromatography> Protein samples	Sample on previous analysis may be adsorbed onto the column, and elute on the next analysis.	Wash column, Please refer to page 170 for more information on washing methods.
		COSMOSIL Protein-R, which has high recovery rate for protein separations, is recommended. Please refer to page 42 for more information.
<All Separation Mode> Sample solvent and mobile phase are significantly different.	Sample solvent has peaks.	Dissolve samples in the same solvent as the mobile phase.
		Dissolve sample in mobile phase. If sample does not dissolve, dissolve sample in sample soluble solvent first before mixing it with the mobile phase.
<All Separation Mode> Mobile phase have peaks in blank analysis. (Peak area decreases with each injection.)	Injector is polluted	Wash column by injecting with a syringe of 20 ml solvent, e.g., methanol that can dissolve the pollutants
	Micro-syringe is polluted	Wash column with solvent e.g., methanol, chloroform or water to dissolve the pollutants. Ultrasonic cleaning is effective.
Others	Contamination or deterioration of samples	Adjust the sample again.
	Stabilizers in the mobile phase	Use HPLC grade solvent without stabilizers.

T3. No peaks

[How to confirm cause] Check t_0 first.

Analysis result of t_0	Cause
t_0 is not detected.	Detector may be defective.
Retention time of t_0 shifted.	Pump may be defective.
Retention time of t_0 is the same as usual.	Column may be defective.

● Solution for Each Column Type

Column Type	Cause	Solution
Reversed phase chromatography column	Sample is still in the column due to its high hydrophobicity.	Increase elution power of mobile phase until the sample elutes. e.g., 1. Increase concentration of methanol or acetonitrile (maximum 100%). 2. If the sample still does not elute, add 10–30% of higher elution organic solvent (e.g., tetrahydrofuran or chloroform) in methanol or acetonitrile (e.g., Tetrahydrofuran : methanol = 30 : 70).
	Metal coordination or basic compounds may be adsorbed onto the column.	Basic compounds may interact with residual silanols in the packing material. Use COSMOSIL 5C_{18}–MS–II, which has less residual silanols. Or add 0.1–1% of acid (e.g., trifluoroacetic acid, acetic acid) to the mobile phase.
		Metal coordination compounds may interact with a small amount of metal in the packing material. Add 5 mmol/l di–sodium dihydrogen ethylenediaminetetraacetate dihydrate (EDTA・2Na) to mobile phase.
Normal phase chromatography column	Hydrophilicity of sample is too strong so that sample is still inside of column.	Increase elution power of mobile phase until the sample elutes. Replace with strong eluting solvent e.g., ethanol, or increase the concentration
	Metal coordination or basic compounds may be adsorbed onto the column.	Add 0.1–1% of acid (e.g., trifluoroacetic acid, acetic acid) to mobile phase.
Column for saccharides analysis (Sugar–D, NH_2–MS) or Hydrophilic chromatography column (HILIC)	For COSMOSIL 5NH_2–MS, sample is adsorbed to amino groups.	Use COSMOSIL Sugar–D with less undesirable adsorption.
	Sample is still in column because it is higly hydrophilic.	Increase concentration of water in mobile phase until the sample elutes. COSMOSIL Sugar–D or COSMOSIL HILIC is compatible with 100% aqueous mobile phase. COSMOSIL 5NH_2–MS is compatible with 50% water (e.g., acetonitrile : water = 50 : 50).
Gel filtration chromatography column (Diol)	Sample has ionic effect on silanol group.	To increase ionic strength in mobile phase, add approx. 0.3 mol/l of salt, e.g., sodium chloride
		Adjust mobile phase pH to 5.5 or less to prevent ionic interaction.
	Sample may be adsorbed due to hydrophobic interaction.	Add 10–50% of organic solvent (e.g.,acetonitrile) to mobile phase.
Hydrophobic chromatography column (HIC)	Hydrophobicity of sample is too strong	Add 5% of organic solvent (e.g., methanol or acetonitrile).
	Sample may have ammonium sulfate precipitation before injection.	Decrease concentration of ammonium sulfate to 0.5mol/l or less until no precipitation is observed.

● Defective Pump

Cause	Solution
Bubbles are generated in a pump.	Collapse bubbles (Please refer to T7 on page 180.).
Solvent leaking	Tighten connectors or replace tubings.

● Defective Detector

Cause	Solution
Detector is not connected correctly.	Follow the detector user's manual to connect correctly.
Defective signal from the detector	Contact detector manufacturer
UV adsorption range is not suitable for your sample.	Analyze at suitable UV adsorption for a sample. If the sample has no or little UV adsorption, use refractive index detector (RI detector) or evaporative light scattering detector (ELSD), or labeling the sample.

T4. Unstable base line

Cause	Solution
Impurities adsorbed onto a column previously are eluting out now.	Wash with strong eluting solvents (Please refer to Q13 on page 170.).
COSMOSIL PE-MS • π NAP • PYE • NPE • PBB-R • Cholester have UV absorption on stationary phase, and base line is not stable due to slight shedding (the detachment of stationary phase that has UV absorption.).	Wash with strong eluting solvents (Please refer to Q13 on page 170.).
Sudden change in the pump pressure may create bubbles.	Degas (Please refer to T7 on page 180.).
When using refractive index detector (RI detector)	
large temperature variations	Use thermostatic bath to keep a constant temperature. Beware of the air conditioner blowing on the RI detector or tubing. Caution; Cover equipment or tubing to avoid temperature fluctuation from an air conditioner.
Residual gas in mobile phase changes.	Degas (by ultrasonic wave or aspirator) a mobile phase.
Have needle-like peaks from an ultra violet visible detector	
Bubbles may be mixed in the column or detector.	Increase pressure to remove bubbles by blocking exit of the detector. Caution; Too much pressure may break the detector cell. If the problem persists, run thick solvent (e.g., 2-propanol) through for 15 min, disconnect the column from the detector.
Column temperature may be above the boiling point of mobile phase, creating bubbles in a column.	Analyze at suitable temperature. Basically, 20-50°C is suitable temperature for a column. Caution; To get the best result, analyze at 20-50°C lower than the boiling point of mobile phase (e.g., in case of Methanol [boiling point: 64.7°C], analyze at 45°C or less.).
When using ion-pair reagent for mobile phase or buffer.	
Inadequate equilibration of a column	Make equilibration time longer. When using ion-pair reagent for mobile phase or buffer, longer equilibration time is required compare to mobile phases without salt.
Salt may precipitate in the mobile phase and the mobile phase reservoir may become cloudy.	(a) Decrease concentration of buffer. (e.g., 100 mmol/l → 20 mmol/l) (b) Replace with a different buffer solution. (e.g., phosphoric acid buffer → acetic acid buffer) (c) Reduce concentration of organic solvent. (e.g., 70% acetonitrile : water = 70 : 30 → 50 : 50) (d) Replace with a different organic solvent. (e.g., acetonitrile → methanol)

T5. Unstable retention time

● Cause by Equipments

Equipment	Cause	Solution
Pump	Bubbles in the check valve of pump.	Degas (Please refer to T7 on page 180.).Normal phase solvents have lower boiling point, so bobbles are easily created. Furthermore, its low-viscosity prevents bubbles from eluting.
	Solvent leaking	Tighten the leaking part. If the problem does not solve, replace it.
Thermostatic Bath (for adjusting temperature)	Column temperature may vary by season or the time of day without the use of thermostatic bath or column oven.	Use thermostatic bath or column oven to keep consistent column temperature. Caution; Set thermostatic bath or column oven to 5°C above the room temperature when doing room-temperature analyses.

● Cause by Columns

Column Type	Cause	Solution
Reversed Phase Column	Inadequate column equilibration when using ion paring reagents.	Make equilibration time longer. When using ion-pair reagents, longer equilibration time is often required.
	If 100% water is used as mobile phase on C_{18} column, phase collapse may occur.	COSMOSIL C_{18}-PAQ is compatible with 100% aqueous mobile phase (Please refer to page 18.). Caution; For unstable retention time, wash the column with high organic solvents (e.g., methanol : water = 70 : 30) to recover.
Normal Phase Column	A small amount of water in organic solvent may affect retention time.	Replace with mobile phase without water. If sample solvent has water, change sample solvent or decrease the injection volume. If water is trapped, wash with ethanol to recover.
Columns for Saccharide Analysis (Sugar-D, NH_2-MS) or Hydrophilic Chromatography column(HILIC)	A small amount of stationary phasedetached.	COSMOSIL Sugar-D or COSMOSIL HILIC can be recovered by washing with 100% water for 15 minutes. COSMOSIL 5NH_2-MS may be recovered by washing with 50%water (e.g., acetonitrile : water = 50 : 50) for 15 minutes.

T6. Increased column pressure

Please refer to Technical Information 3 on page 191.

T7. Unstable pump pressure

Cause	Solution
Bubbles in the check valve of a pump.	Degas the check valve (open drain valve, and let mobile phase through) according to the pump instruction. If the problem persists, wash check valve, e.g., ultrasonic cleaning in water.

Caution;

1. If the bubbles occur often in normal phase chromatography, connect pre-column to increase pressure, and let bubbles elute.
2. Degas a mobile phase by a ultrasonic or an aspirator.

T8. Poor resolution on C_{18} columns

Solution	Features
Use a longer column	A longer column enables sharper peaks. The pressure will increase and the retention time will be longer.
Change mobile phase condition (e.g., pH, type or concentration of organic solvent)	Experience or knowledge is required. Do not set complicated condition as it lacks repeatability.
Use packing material with non-hydrophobic interactions.	Separate sample by molecular shape selectivity or π-πinteraction (except for hydrophobic interaction). Please refer to Technical Information 7 on page 201 for more information on special column with various interactions.

T9. No retention on reversed phase columns

Solution	Features
Use ion pair reagents	Ion pair reagents enable separation by forming ion pairs with the sample to increase hydrophobicity. Therefore, it is not applicable for non-dissociative samples.
Use hydrophilic columns (HILIC). Please refer to page 36.	Less hydrophobic samples are retained longer.

T10. Excessive retention time is long on reversed phase columns

Solution	Features
Use the gradient elusion method	Gradient elution method shortens analysis time by changing organic solvent concentration during analysis. Disadvantages are having a capable equipment, increasing baseline, and the need for equilibration time between each runs.
Use UHPLC Columns	Please refer to page 62, for more information.
Change mobile phase condition	Problem may be solved by changing pH, type or concentration of organic solvent.
Use column with small hydrophobicity	COSMOSIL CN-MS is recommended. Please refer to page 31, for more information.

T11. Different separation performance compare to the past

Symptom	Cause	Solution
Decreased theoretical plates	Natural deterioration of the packing material	No method to recover column.
Decrease of retention time or separation.	Impurities may be adsorbed onto the packing material.	The column can be recovered by washing
	Stationary phase shedding.	No method to recover column.

T12. Different separation performance with a new column

Cause	Solution
Analytical condition does not suit to sample	Adjust pH of mobile phase to pKa ±2 or more.
	Use mobile phase with high repeatability.
Column may deteriorate	If the column deteriorate, decrease of retention time, change of peak shape may occur. Replace the column.
Variation from lot to lot	Contact us with the column name, product no, present separation status. (a) Evaluate columns with 3 different packing material lots. We provide validated column with 3 different packing material lots for the following columns (Size 4.6 x 150 mm, 3 pkg set). Contact us for more information. Packing material / Product No. COSMOSIL 5C_{18}-MS-II / 09397-73 COSMOSIL 5C_{18}-AR-II / 09396-83 COSMOSIL Cholester / 07970-03 COSMOSIL HILIC / 09385-23 (b) Find an analysis condition with less influence from lot-to-lot variation.
Cause is not column (e.g., mobile phase, flow rate, temperature)	Find the cause.

T13. Colored elute from columns (colorless sample)

Cause	Solution
A small amount of shedding, impurities or previous samples.	Wash column with strong elution solvent (e.g., methanol) or use cleaning solution kit for reversed phase HPLC columns (Product No. 08966-30)

Caution;
A small amount of stationary phase does not affect retention time.

T14. Air got inside the column (dried out)

Pump solvent with low viscosity (e.g., methanol) through at half of analysis flow rate for 1 hour.

Caution;

Store it tightly plugged in a cool dark place.