(2) FAQ

Q1. What is the pressure limit of column?

Column		Pressure Limit
UHPLC Column	COSMOSIL 2.5C_{18}-MS-II COSMOSIL 2.5Cholester COSMOSIL 2.5πNAP	30 MPa
CNT Column	COSMOSIL CNT Series	15 MPa or less
Other Column	Analysis Column (I.D.1.0–7.5 mm)	20 MPa or less
	Analysis Column (I.D.10.0 mm or more)	15 MPa or less

Attention;
A large pressure change may deteriorate columns even within the recommended pressure range.

Q2. What is the flow rate limit?

You can raise the flow rate under the pressure limits stated on Q1

Attention;

We recommend using standard flow rate described in the technical information 2 on page 189. Generally, higher column pressure corresponds to shorter column lifetime.

Q3. What is the recommended pH range?

Column		Recommended pH Range
COSMOSIL (silica gel base) Series	COSMOSIL C_{18}-MS-II	pH 2–10
	COSMOSIL C_{18}-AR-II	pH 1.5–7.5
	Other Columns	pH 2–7.5
COSMOGEL (polymer base) Series	COSMOGEL IEX Series	pH 2–12

Attention;
Table above shows tolerant range for the packing material. Adjust the pH for ionic samples.

Q4. What is the concentration of buffer and salt?

Column		Buffer and Salt Concentration
Reversed Phase, Normal Phase, HILIC		Buffer concentration: 0.005–0.1 mol/l Additive concentration (trifluoroacetic acid, formic acid or acetic acid)：0.1–1.0%
Ion Exchange	COSMOGEL IEX Series	Buffer concentration: 0.02–0.1 mol/l Water miscible organic solvent concentration limit (e.g., methanol): 20% or less
Gel Filtration	COSMOSIL Diol Series	Buffer concentration limit: 0.5 mol/l or less Salt concentration limit: 0.5 mmol/l or less
Hydrophobic Interaction	COSMOSIL HIC	Buffer concentration limit: 0.5 mol/l or less Salt concentration limit: 2 mmol/l or less

Attention;

1. Insoluble compounds may clog columns. Filter buffers or salt solution before using.
2. Deposition of salt during analysis may deteriorate columns or equipments. Use the column under the concentration which salt does not precipitate.
3. Salt often precipitates when the organic solvent is mixed with the solution. Be careful when mixing mobile phases.
4. When equipment or a column contains organic solvent, replace mobile phase with salt-free mobile phase first before using salt-containing mobile phase.

Q5. How do I adjust mobile phase?

Please refer to Technical Information 1 on page 187.

Attention;

1. Adjust mobile phase ratio and buffer concentration exactly each time because concentration and pH may affect separation performance.
2. Degas the solvent after mixing.

Q6. What solvent grade should I use for the mobile phase?

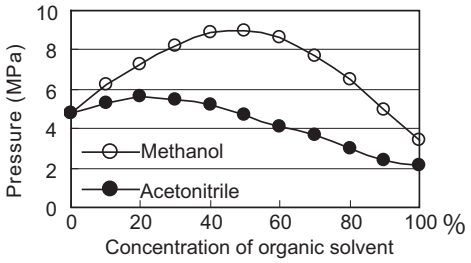
We recommend using HPLC grade solvents, please refer to page 76.

Attention;

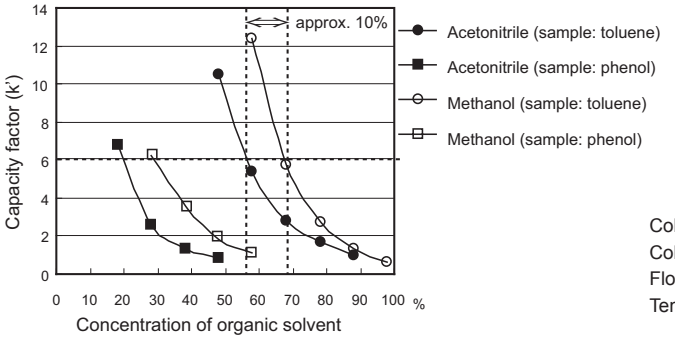
GR grade solvents are not suitable for gradient analysis or micro-scale analysis because they contain impurities that have ultraviolet adsorption causing unstable baseline or inaccurate detection. This is especially problematic in short wavelength (210-220nm). Antioxidant containing GR grade solvents (e.g.. tetrahydrofuran, chloroform) may produce ghost peaks. GR grade trifluoroacetic acid may be chemically unstable and not recommended for HPLC.

Q7. What is the difference between acetonitrile and methanol?

Table below shows difference between acetonitrile and methanol

<table>
<tr><th></th><th>Acetonitrile (for HPLC)</th><th>Methanol (for HPLC)</th></tr>
<tr><td>Pressure</td><td colspan="2"> 

Column 5C18-MS-II
Column size 4.6 mm I.D. x 150 mm
Flow rate 1.0ml/min
Temperature 30°C

Back pressure differs depending on species of organic solvents and mixing ratio. Back pressure of acetonitrile is lower than that of methanol at the same concentration.</td></tr>
<tr><td>Elution Strength</td><td colspan="2"> 

Column 5C18-MS-II
Column size 4.6mmI.D.-150mm
Flow rate 1.0ml/min
Temperature 30°C

Elution strength of acetonitrile/water is stronger than methanol/ water at the same concentration. Within the range of about 30-80% in the concentration of the organic solvent, elution strength of acetonitrile/water is almost equivalent to that of methanol/water which was raised methanol concentration about 10% (e.g., acetonitrile : water = 60 : 40 → methanol : water = 70 : 30)</td></tr>
<tr><td>Absorbance</td><td>Acetonitrile has a lower UV absorbance in far UV region (less than 250 nm) .</td><td>Methanol has a higher UV absorbance than acetonitrile in far UV region (less than 250 nm).</td></tr>
<tr><td>Degas of Mobile Phase</td><td>When acetonitrile is mixed with water, it is endothermic. It is difficult to degas.</td><td>When methanol is mixed with water, it is exothermic. It is easy to degas.</td></tr>
</table>

Q8. Which mobile phase can be used for LC/MS or ELSD detector?

Volatile solvent should be used for LC/MS detector or ELSD. Phosphoric acid buffer can not be used.

Regent	Usable solvent / Additive
Solvent	Methanol, ethanol, acetonitrile, water and others
pH Adjusting Reagent	Acetic acid, formic acid, trifluoroacetic acid, ammonia water, ammonium acetate, ammonium formate and others
Ion Pair Reagent	Dibutylamine, triethylamine and others

Attention;
A small amount of non-volatile solvent, such as DMSO (dimethylsulfoxide) or DMF (dimethylformamide) can be used if they are mixed with methanol or acetonitrile. However, if the concentration becomes higher, detection sensitivity may decrease.

Q9. What should I pay attention to when I use ion-pairing reagents?

- Concentration of ion–pairing reagent should be 5–10 mmol/l.
- Use mobile phase of pH7 for acidic ion–pairing reagents and pH2.5 for basic ion–pairing reagents.
- Have enough equilibration time.
- Please refer to page 78 for choosing conditions.

Attention;

1. Higher ion pair concentration will result longer retention time.
2. Adjust pH of mobile phase so that sample is well ionized.
3. Need longer equilibration time compared to mobile phase without ion–pairing reagent.
4. Use a column exclusively with ion–pairing reagents since it is difficult to eliminate it from the column.

Q10. What flow direction should I use for the mobile phase?

Pump mobile phase through the direction specified on the column label.

Attention;

Pumping mobile phase in reverse direction may deteriorate packing material and decrease theoretical plates. Furthermore, impurities previously adsorbed on the column tip may loosen, causing contaminated detector, tubing, and noise.

Q11. What is the recommended temperature of columns?

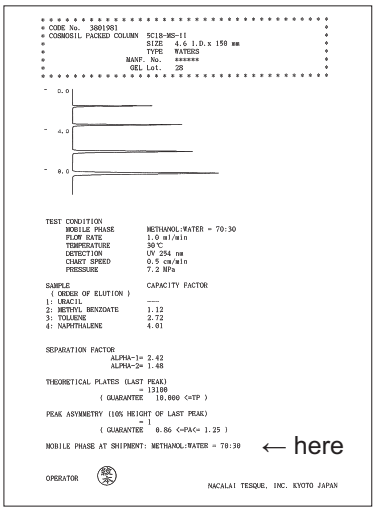
The maximum temperature is 60°C, but the recommended temperaturerange is 20–50°C.

Attention;

1. Operating analysis at high temperature under alkaline or acidic condition may shorten column lifetime.
2. Maintain constant temperature during analysis as column temperature affects retention time. Generally, higher temperature lowers column pressure and shortens retention time.

Q12. What is the shipping solvent?

It depends on the column type. Please refer to the certificate of analysis inside the column box.



Q13. How do I wash columns?

1. How to eliminate buffer, salt or acid from a column?
   Wash the column with solvent without buffer, salt or acid for 10–15 min. and store.
   (E.g., When methanol : 20 mmol/l phosphoric acid buffer = 50 : 50 is used, wash with methanol : water = 50 : 50)
   Attention;
   If ratio of organic solvent and water is changed, salt may precipitate.

2. How to wash impurities in a column to achieve a stable base line?
   Dissolve sample well, and use solvent with strong elution properties.

Type	Usable Solvent / Additive
Reversed Phase	(1) Sample is not protein Methanol or tetrahydrofuran Cleaning Solution Kit for Reversed Phase HPLC Columns (Product No. 08966–30) (2) Sample is protein 50–70% acetonitrile : water containing 0.1% trifluoroacetic acid. Attention; Some proteins may precipitate when concentration of organic solvent is high.
Normal Phase	Methanol, tetrahydrofuran, ethanol
Column for Fullerene Separation	*1,2,4*–trichlorobenzene and others
Sugar–D, NH_2, HILIC	Acetonitrile : water = 50 : 50 *Sugar–D and HILIC can be washed with 100% water.

Attention;
Do not use alkali solution (pH 7.5 or higher), which dissolve silica gel. Do not use strong acid solution (pH 1.5 or less) that may cleave bonded stationary phase.

3. How to eliminate impurities clogging the column entry end?
   Pumping solvent through in a reversed direction at half the speed of ordinal analysis.
   Attention;
   Disconnect column from the detector during washing.
4. If column pressure is high, please refer to Technical Information 3 on page 191.

Q14. How do I store columns?

1. Store for short time (a few days)
   Wash the column with solvent without buffer, salt or acid for 10–15 min. and store
   (E.g., When methanol : 20 mmol/l phosphoric acid buffer = 50 : 50 is used, wash with methanol : water = 50 : 50)
2. Store for long time (one month or more)
   Replace solvent with the following solvent to avoid fungus, dry and deterioration of column.

Column	Concentration of Buffer or Base
Reversed Phase	Storage Solution for Reversed Phase HPLC Columns (Product No. 08967–20) Methanol : water = 70 : 30, Acetonitrile : water = 70 : 30
Normal Phase	Halogen or acid–free organic solvent (hexane : ethanol = 90 : 10)
Ion Exchange Gel Permeation Hydrophobic Interaction	0.05% sodium azide solution and others
Column for Fullerene Separaton	Toluene and others
Sugar–D, NH_2, HILIC	Acetonitrile : water = 70 : 30

Attention;
Store tightly plugged columns in a vibration-free, cool dark place.

Q15. How long does a column last?

Column life time may change depending on the operation condition (sample, concentration, salt, acid, pH of organic solvent)
Attention;
The most common cause of a short column lifetime is inadequate sample treatment. Please refer to Technical Information 4 on page 193.

Q16. What happens when a column deteriorates?

Common symptoms of column deterioration include increasing column pressure, decreasing theoretical plates, shortening retention time, worsening of peak shape and decreasing resolution.

Attention;

Please refer to Troubleshooting on page 174.

Q17. How can I confirm the deterioration of column?

Evaluate deterioration of column under the same condition as the attached “certificate of analysis that comes with the column”. Use the same instrument every time to record the performance of the column. Record standard values over time and change to a new column if they are off.

Valued Item	Contents of Value
Capacity Factor (k ’)	If stationary phase is stripped, retention time may shorten.
Theoretical Plates (N)	It may decrease due to impurities or the state of packing material.
Peak Asymmetry (S)	It may decrease due to deterioration of packing material or adsorption of impurities.
Pressure	It may increase due to clogging of column filter, sample adsorption to packing material, compression of packing material and others.

(Reference value)

The following is reference value for 5C$_{18}$-MS-II (4.6 mm I.D. × 150 mm)

- Capacity factor (k ’) ：90% or less of Naphthalene’s k’
- Theoretical plates (N) ：9000 or less
- Peak asymmetry (S) ：out of range of 0.86–1.25
- Pressure ：20 MPa or more

Q18. What should I pay attention to when I use semi-micro columns?

Use injector, tubing and detector cell designed specifically for semi–micro column.

Attention;

Confirm linear mobile phase flow rate is in proportion to the column sectional area. For more information, please refer to Technical Information 2 on page 189.

Q19. What should I pay attention to when I use UHPLC columns?

- Use equipment for UHPLC.
- Shorten response of detector (e.g.,0.02 sec) when you use standard HPLC equipment.
- Use injector, tubing and detector cell designed for UHPLC columns.
- Column pressure should be 30 MPa or less.

Q20. How much sample can be loaded in a preparative column?

The sample load may differ depending on the sample resolution or solubility in mobile phase. Optimize maximum loading capacity by an analytical column, and determine maximum purified value in proportion to sectional area of column inner diameter.

Attention;

For scaling up from analytical column to preparative separation, please refer to page 35. For column inner diameter, flow rate and pipe, please refer to Technical Information 2 on page 189.

Q21. What should I pay attention to when I use both reversed phase and normal phase in the same instrument?

Replace solvent which both mobile phase can be mixed such as ethanol, then replace to new mobile phase.

Attention;

Mobile phase for reversed phase (e.g., methanol, water) and for normal phase (e.g., hexane) cannot be mixed.

Q22. What is the connection type of column?

Connection type of COSMOSIL and COSMOGEL is Waters type.

Attention;

Generally, waters type can be connected to most of instruments, but confirm the connection type with the manufacturer before using.

Q23. Which detection methods should I use?

Proper selection of a detector depends on the sample or the purpose of the experiment.

Detector	Feature	
Ultra Violet Visible Detector (UV/VIS detector)	[How to detect]	Detect sample absorbance.
	[Sample]	High sensitivity for compounds which have UV absorbance. Can not be used for compounds which do not have UV absorbance.
	[Feature]	Easy, widely used.
Fluorescence Detector (FLD)	[How to detect]	Detect fluorescence of photon excited sample.
	[Sample]	Fluorescent sample
	[Feature]	For sample which has little or no UV absorbance. High detection sensitivity enables trace component analysis.
Refractive Index Detector (RI detector)	[How to detect]	Detect difference in index of refraction between sample and mobile phase.
	[Sample]	All sample
	[Feature]	For sample which has little or no UV absorbance (e.g., saccharides, alcohols, amino acids). Its disadvantages include sensitive to change of temperature, component of mobile phase and flow rate.
Electro Chemical Detector (ECD)	[How to detect]	Detect electrochemically-active compounds such as oxidized or reducing compounds.
Evaporative Light Scattering Detector(ELSD)	[How to detect]	Detect scattering light of microparticulated target compound by evaporating mobile phase.
	[Sample]	Sample which has little or no UV absorbance (e.g., saccharides, alcohols, amino acids).
	[Feature]	For sample which has little or no UV absorbance (e.g., saccharides, alcohols, amino acids). It is not applicable for low-boiling compounds.
Mass Spectrometric Detector (MS Detector)	[Feature]	Able to measure MS spectrum of separated components for qualitative analyses. Disadvantage is compatible mobile phases are limited.

Q24. What is the common pressure unit?

Most of unit is in SI unit, MPa

Attention;

Old equipment sometimes have different unit (conversion：1 MPa = 10.197 kgf/cm^2= 145.0 psi = 10 bar).

Q25. What is dead volume?

Dead volume is the follow path volume from injector to detector not relevant to resolution.

Attention;

1. If dead volume is large, sample may spread and have poor peak shape.
2. Choose proper injector, detector cell and tubing , and column inner diameter to minimize dead volume.

For inner diameter of tubing or cell, refer to Technical Information 2 on page 189.