HPLC
HIC Column
TSKgel Phenyl-5PW, Ether-5PW

INSTRUCTION MANUAL

TOSOH

TOSOH CORPORATION

Safety Precautions

To help protect your property from potential damage and ensure personal safety, please read this manual thoroughly before using the product.

[Notational Conventions]

Notation	Explanation
⚠WARNING	Alerts the user to the potential for serious injury or death.
⚠CAUTION	Alerts the user to the potential for damage to hardware or bodily harm.

⚠ WARNING

■ **Keep away from fire.**

Take proper precautions when using flammable solvents. There is the potential for fire, explosion, or poisoning.

⚠ CAUTION

■ **Use only in well ventilated areas.**

In case of insufficient ventilation, flammable and toxic solvents can cause fire, explosion, or poisoning.

■ **Do not spill solvents.**

Spillage and leakage can cause fire, electric shock, poisoning, injury, and corrosion. When cleaning up a spill, wear appropriate protective gear.

■ **Wear eye protection and protective gloves.**

Organic solvents and acids should not come in direct contact with the skin.

■ **Handle package with care.**

Inappropriate handling may cause rupturing and splattering.

■ **Only use this product as intended.**

This product is for separation and purification, do not use for any other purpose.

■ **Confirm compounds are safe.**

Check that obtained compounds and solutions after separation and purification are safe.

■ **Proper disposal.**

Dispose of in accordance with local laws and regulations.

NOTE

■ **Keep this manual for future reference.**

Table of Contents

1. Introduction

TSKgel HIC columnns are prepacked columns for the separation of sample components according to surface hydrophobicity. The separation mechanism is similar to that of reversed phase.
These columns were designed for analytical and preparative separation of proteins.
We have both steel column and glass column.
Especially glass column is biocompatible type which consists of a high precise glass tube with lastic end fittings.
This Instruction Manual contains crucial information on how to care for and use these columns in the proper manner, so as to make most effective use of their high performance capabikities.

2. Unpacking

Check that nothing is the matter with the appearance of package or the column.

Fig.1 Appearance of the package

Then check that the following documents are attached to the column :

1 copy Insruction Manual
1 copy Inspection Data

3. Installation

3-1 Connection Parts

The connections of steel column are of the swage lock type and that of glass column are 1/4"-28 UNF flange joints.

3-2 Flow Direction

Use the column in the direction shown by the arrow on the name plate in Fig.2 & 3.

Operating the column with the flow in the reverse diraction for a long time will cause degradation of column performance.

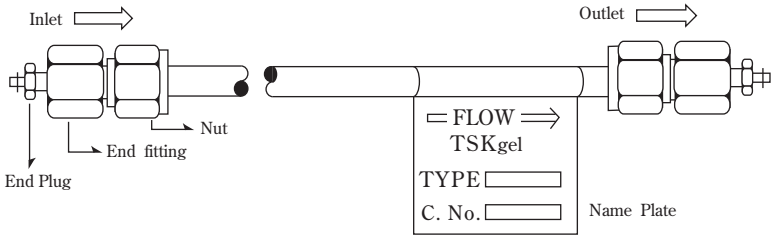

Fig. 2 Steel Column Parts

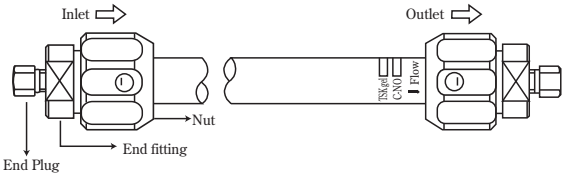

Fig. 3 Glass Column Parts

3-3 Prevention of Bubbles

Be careful not to admit any bubbles into the column during its installation or removal from the equipment. Always remove all bubbles from all pipings before installing the column.

Admitting bubbles into the column will cause degradation of its performance through the occurrence of channeling, etc.

3-4 Prevention of Pulsatory Flow

This type of column is easily affected by pulsatory flow of the solvent.

Preferably, a pump with no fluctuation should be used.

If a pump with pulsation must be used, connect a pulse damper (accumulator) to the outlet side of the pump in order to compensate for the pulsation.

The damper must be highly resistant to corrosion.

4. Column Storage

Store the columns at constant temperature in the range of 4℃ to 30℃ .
Don't store these columns below 4℃ not to freeze the solvent in the column.
Avoid exposing the columns to direct sunlight, and store the columns in a place safe from corrosive gases.
If the column is being used daily, you need not remove the column from the equipment and the buffer may be left in the column overnight, as long as the buffer salt has good solubility. When the columns are to be stored for a few days, remove the column from the instrument and seal both ends with the end plugs. For long-term storage, the column must be protected from growth of microorganisms.
Thus, aqueous buffers should be relaced with distilled and deionized water.

5. Sample and Eluent Preparation

5-1 Replacement of Solvents

HIC type columns are filled with distilled and deionized water for shipment.
Replace this solvent with a suitable solvent, using one-half of the recommended flow rate shown in Table 1.
Since frequent solvent replacement accelerates degradation of column efficiency, use the same solvent as far as possible.

5-2 pH

These columns are usable over the relatively wide pH range of 2.0 - 12.0 at room temperature.

5-3 Filtration

Use only HPLC grade solvents that have been filtered through an 0.5 μm filter.
Filter all buffered solution before using them. This reduces the problem of plugged filters and preserves column life.
Vacuum filtration or sonification may be used to remove dissolved gasses which could affect your solvent delivery system.

5-4 Salt Concentration Range

Salt concentration in the solvent is not limited up to near saturation.

5-5 Organic Solvent Concentration Range

The concentration of the organic solvent can risen up to 50% .

But be careful to avoid salt precipitation when adding salts to aqueous solution containing organic solvents.

5-6 Denaturing Agents

Denaturing agents such as urea, guanidine-HCl and detergents can be used.

5-7 Sample

The sample should be dissolved in the starting eluent and filtered through a micro-pore filter (of eg. 0.5 μ m pore size) to protect the column from accumulation of inmpurties.

6. Flow Rates

Factors such as resolution, analytical time, and column life should be carefully considered in selecting flow rates. A higher flow rate results in a shorter analyhtical time. Conversely lower flow rete results in improved column efficiency and tends to extend column life.

Standard flow rates for phosphate or other aqueous buffers are recommended between 0.4-0.8 mL ／ min. The flow rates and pressure drops for these columns are suggested below :

Table 1 Recommended flow rates

Cat. No.	Types	Column Dimensions (mmID×cm)	Max. Flow Rate (mL／min)	Recomended Flow Rate (mL／min)	Max. Pressure Drop (MPa)
	〈Steel Column〉				
07573	phenyl-5PW	7.5× 7.5	1.2	0.5—1.0	2.0
07656	phenyl-5PW	21.5×15.0	8.0	4.0—6.0	2.0
	〈Glass Column〉				
13063	phenyl-5PW Glass	5.0× 5.0	1.0	0.5—0.8	2.0
08804	phenyl-5PW Glass	8.0× 7.5	1.2	0.5—1.0	2.0
14018	phenyl-5PW Glass	20.0×15.0	8.0	4.0—6.0	2.0
	〈Steel Column〉				
08641	Ether-5PW	7.5× 7.5	1.2	0.5—1.0	2.0
08642	Ether-5PW	21.5×15.0	8.0	4.0—6.0	2.0
	〈Glass Column〉				
14013	Ether-5PW Glass	5.0× 5.0	1.0	0.5—0.8	2.0
14014	Ether-5PW Glass	8.0× 7.5	1.2	0.5—1.0	2.0
14015	Ether-5PW Glass	20.0×15.0	8.0	4.0—6.0	2.0

Caution :
On the glass column's application, the pressure at the top of the column should be less than 30 MPa to prevent the damage of the glass column and the leakage of the gel at the end fitting.

7. Temperature

The optimal operating temperature for HIC type columns is between 10-30 ℃ .
These columns should not be used above room temperature for long time.
Below 10℃ , use a lower rate to protect the columns.
For long term storage the columns should be stored at room temperature.

8. Guard Column

When impurities that tend to be adsorbed by the packing material present in the sample, they are adsorbed on the inlet side of the column and accunulate gradually, causing reduction of column efficiency.
In such case the original column efficiency can be maintained by connecting a guard column before the column and replacing it if efficiency becomes degraded by the adsorption of material to the guard column.
For mazimum insurance against such problems, a guard column should be used as much as possible.
We prepared the "Guard column kit" as the guard columns for TSKgel Phenyl-5PW and Ether-5PW.
Please refer to "INSTRUCTION MANUAL for TSKguardgel for HPLC".

Table 2 Guard Columns

Cat. No.	Types	Applied Columns
	〈TSKguardgel kit〉	
07652	TSKguardgel Phenyl-5PW kit	Phenyl-5PW (7.5×7.5)
08643	TSKguardgel Ether-5PW kit	Ether-5PW (7.5×7.5)
08808	TSKguardgel Phenyl-5PW kit Glass	Phenyl-5PW Glass (8.0×7.5)
14025	TSKguardgel Ether-5PW kit Glass	Ether-5PW Glass (8.0×7.5)
	〈TSKguardcolumn〉	
14469	TSKguardcolumn Phenyl-5PW Glass	Phenyl-5PW Glass (20×15)
14470	TSKguardcolumn Ether-5PW Glass	Ether-5PW Glass (20×15)

9. Column Efficiency

The number of theoretical plates of a column (N), the asymmetry factor (As) and their chromatographic conditions are shown in the Inspection Data.

9-1 Method of Calculating the Number of Theoretical Plates

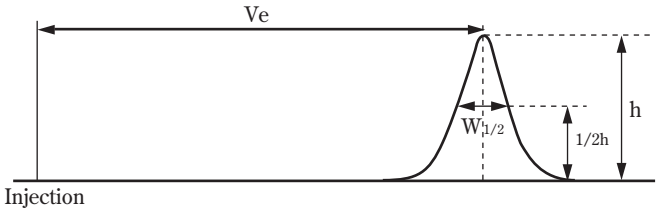

Fig. 4 Method of Calculating the Number of Theoretical Plates

The number of theoretical plates of a column (N) is calculated by the half peak width method shown in Fig.4 and the following equation.

$$N = 5.54(Ve / W_{1/2})^2$$

Ve : Elution volume

$W_{1/2}$: Half width value of peak

h : Peak height

9-2 Method of Calculating the Asymmetry Factor

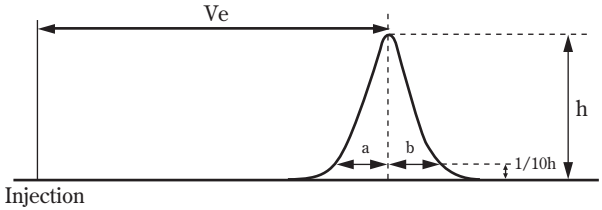

Fig. 5 Method of Calculating the Asymmetry Factor

The asymmetry factor of column (As) is calculated by the 1 / 10 h method.

$$As = b / a$$

9-3 Dead Volume

If the dead volume of the equipment or the injection volume of a sample solution becomes too large, the number of theoretical plates may decrease.

10. Troubleshooting

When using TSKgel columns, some problems can be avoided by following these instructions. But the problems (such as those due to columnn life, adsorptive materials, production of air bubbles, dried gel, or frozen solvent) can not be corrected once they occur, so care should be taken in handling these columns.

10-1 Clogging of the End Fitting

In case the pressure-drop increases or the flow rate decreases, the end fitting should be cleaned by reversing the flow through the column.

If the clog cannot be removed, prepare a new end fitting and replace the old end fitting with a new one, being very careful not to loosen any of the packed gel underneath.

10-2 Never Eluted or Eluted Late

If the elution time is extremely long, it might be caused by adsorption on the column packing.

Most problems can be avoided by washing the column.

If the column has not recovered after washing, it will not recovered anymore.

In this case you have to exchange the column with a new one.

11. Quality Specifications and Warranty

11-1 Inspection Data

The results of each inspection are described in the Inspection Data enclosed in the column package. In the Inspection Data, N is expressed as that per column.

The conditions used in determining the Inspection Data are as follows :

* Eluent : Distilled Water
* Sample : 0.5 % Acetone

* Flow Rate & Sample Volume

Types	Column Dimensions (mmID×cmL)	Flow Rates (mL/min)	Sample Volume (μL)
Steel Column	7.5× 7.5	1.0	20
Steel Column	21.5×15.0	6.0	100
Glass Column	5.0× 5.0	0.8	20
Glass Column	8.0× 7.5	1.0	20
Glass Column	20.0×15.0	6.0	100

* Detector : UV (280 nm)

11-2 Quality Specifications

Cat. No.	Types	Column Dimensions (mmID×cmL)	No. of Theoretical Plates (N/Column)	Asymmetry Factor (As)
	〈Steel Column〉			
07573	TSKgel Phenyl-5PW	7.5× 7.5	≧1,000	1.0－2.0
07656	TSKgel Phenyl-5PW	21.5×15.0	≧3,000	0.8－1.6
08641	TSKgel Ether-5PW	7.5× 7.5	≧1,000	1.0－2.0
08642	TSKgel Ether-5PW	21.5×15.0	≧3,000	0.8－1.6
	〈Glass Column〉			
13063	TSKgel Phenyl-5PW Glass	5.0× 5.0	≧ 600	1.0－2.0
08804	TSKgel Phenyl-5PW Glass	8.0× 7.5	≧1,000	1.0－2.0
14018	TSKgel Phenyl-5PW Glass	20.0×15.0	≧3,000	0.8－1.6
14013	TSKgel Ether-5PW Glass	5.0× 5.0	≧ 600	1.0－2.0
14014	TSKgel Ether-5PW Glass	8.0× 7.5	≧1,000	1.0－2.0
14015	TSKgel Ether-5PW Glass	21.5×15.0	≧3,000	1.0－2.0

11-3 Warranty

Immediately after receipt, check the appearance of the column and test its performance according to Section 9 & 11.

If the guaranteed specifications (above Table) can not be obtained, contact your TOSOH representative wihin two weeks.

Note that column lifetime is not guaranteed.

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