

**Packed Columns for Organic Solvent
High Performance GPC
TSK_{gel} H Type**

INSTRUCTION MANUAL



TOSOH CORPORATION

Safety Precautions

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

[Notational Conventions]

Notation	Meaning
 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.**
When using with flammable solvents, it can cause fire, explosion, or poisoning.
- **Use only in well ventilated areas.**
In case of insufficient ventilation, flammable and toxic solvents can cause fire, explosion, or poisoning.

CAUTION

- **Do not spill solvents.**
Spillage and leakage can cause fire, electric shorts, poisoning, injury, and corrosion.
When cleaning up the spill, wear suitable protective equipment.
- **Wear eye protection and protective gloves.**
Organic solvents or acid is harmful in contact with skin.
- **Handle at appropriate temperatures.**
Inappropriate handling can degrade performance of the TSK – GEL column.
- **Do not use for unintended use.**
This product is for separation and purification, do not use for any other purpose.
- **When packing the columns, keep appropriate pressure.**
Overpressure can cause rupture and spattering.
- **Dispose of used and unused solvent in an authorised way.**
Dispose of in the conventional procedures in compliance with local, state and federal regulations.

NOTE

- **If the name plate attached to the product is stained or unreadable, contact your service representative for a new label.**
- **Keep this manual with the product.**

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1. Introduction

Polystyrene-based H type TSKgel is a unique packed column for high performance GPC in organic solvent developed by TOSOH.

It is suitable for measuring high molecular weight distribution of polymers and separating low molecular weight compounds. Several column sizes of H types (Both preparative and analytical) are available to match user's needs.

Please read this Instruction Manual carefully prior to operation and use these columns correctly in order to effectively utilize their high performance.

2. Unpackage

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



Fig.1 Appearance of the package

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

- 1 copy Instruction Manual
- 1 copy Inspection Data

3. Column Parts

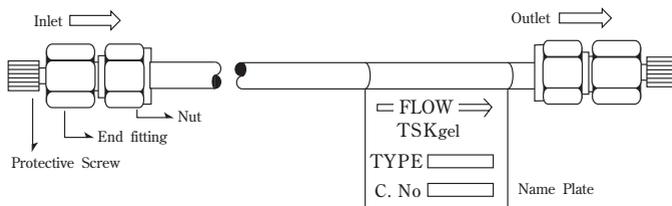


Fig.2 Column Parts(H, HXL, and HHR series)

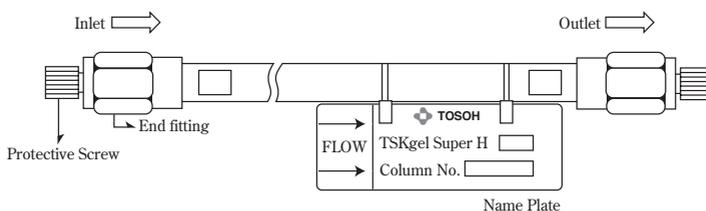


Fig.3 Column Parts(SuperH series)

4. Installation

4-1 Connections

All connections are of the swage lock type and of inch dimensions..

4-2 Flow Direction

Use the column in the direction shown with an arrow on the tag in Fig.2. and Fig.3. Flowing in the reverse direction for a long time will cause degradation of column performance.

4-3 Prevention of Bubble Entrance into Column

Be careful not to admit any bubble into the column at the time of its installation or removal from the equipment. Install the column only after removing bubbles from all pipings. Admitting bubbles into the column will cause degradation of its performance through occurrence of channeling, etc.

4-4 Installation

When solvent oozes from the end fitting at the time of removing the cap on the

inlet side of the column. connect the column to the equipment carefully as mentioned above so that no bubble will be brought into the column.

When no solvent oozes from the inlet side of the column. connect the outlet side to the equipment and feed solvent with the feed pump in order to expel air.

Feed the solvent slowly. since rapid pressurization or solvent feeding may cause degradation of the column performance. After confirming solvent leakage without any bubble at the inlet side of the column. arrange the column in the direction of normal flow and connect the inlet side to the injector.

4-5 Connection of Columns in Series

When a number of columns are connected in series. connect them as described above in sequence of descending pore size in order to separate first the higher molecules which tend to cause overloading Interconnecting tubing should be inserted fully into the compressor fittings before tightening in order to minimize dead volume. Finally. connect the outlet end of the last column to the detector.

4-6 Prior to Measurement

Rapid pressurization or solvent feeding must be avoided as mentioned above. since it may cause degradation of column performance. Be careful especially when a feed pump showing rapid pressure rise is applied.

4-7 Prevention of Pulsatory Flow

This type of column is easily affected by pulsatory flow of the solvent. A pulseless pump with no fluctuation should be used. If applying a pump with pulsation, connect a pulse damper (accumulator) to the outlet side of the pump in order to compensate for the pulsation.

4-8 Column connector and HPLC systems

The H type column is ferrule position of column connector shown in Fig.4.

The SuperH series column should be used following HPLC systems.

1) Connecting tube

The use of a 0.1mm(ID) stainless steel tube is recommendable. The void volumes between an injector and a column. between a column and a detector cell must be less than $5 \mu\text{L}$.

2) Time constant

The smallest time constant (less than 50 ms) is needed to maintain high column performance.

3) Sample volume

The sample volume should be less than $10 \mu\text{L}$.

4) Sampling pitch

The sampling pitch of an integrator should be set at minimum value.
(less than 100ms).

4-9 Column washing

When the detector will be used lightscattering detector (LS).

Before connect columns to detector, you must wash the column sufficiently and removedust in your columns.

1) H_{HR} and SuperH series

Flow eluent for overnight (8~10h) at the same flow rate as analysis.

It is not always necessary to filtrate solvent when you use special grade or HPLC grade solvent.

When you use different solvent from packing solvent, change solvent completely at lower flow rate (generally $< 0.5\text{ml/min}$) and wash as the same way above. In this case it is necessary both solvents are miscible.

2) H_{XL} series

Flow eluent for longer than H_{HR} series (usually one or two days).

Other procedures are the same as H_{HR} series.

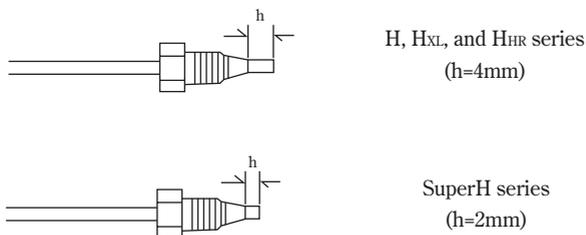


Fig.4 Ferrule Position of Column connector

5. Maintenance

5-1 Measurement at higher Temperature than Room Temperature

Do not stop the pump immediately after finishing the measurement, but continue to feed solvent until the column temperature lowers to the room temperature. If the pump is stopped while the column is hot, air may be sucked into the column by

contraction of the solvent.

5-2 Routine Daily Use

If the column is to be used in routine daily operation, it is permissible to leave solvent in the column overnight.

5-3 Storage

When the columns will not be used within a few days, remove the columns from the equipment being careful that no bubble is brought into the column, and seal them with the protective screws supplied with the columns.

5-4 Storage Temperature

Store columns at room temperature, preferably at constant temperature. Do not store columns at a temperature at which the solvent in the columns may freeze.

5-5 Exposure to Direct Sunlight

Avoid exposure to direct sunlight.

5-6 Corrosive Gases

Store at a place safe from corrosive gases.

6. Solvents

6-1 Delivery Solvent

Since frequent and repeated solvent replacement should be avoided as much as possible on H type columns, it is very important to select the appropriate solvent for delivery.

Please select your solvent correctly before placing your order.

Standard solvents for H type columns on delivery are tetrahydrofuran (THF) and chloroform, respectively. Columns packed with a special solvent such as dimethyl formamide (DMF), acetone, methyl ethyl ketone (MEK) and o-dichlorobenzene (ODCB) are also available. It should be noted that although the H type gel (polystyrene-divinylbenzene bead) is very stable with various organic solvents, solvent replacement must be restricted to avoid loss of its high efficiency caused by shrinking or swelling of the gel. It is recommended to use H type columns with the delivery solvent as long as possible.

6-2 Features of Typical Solvent

THF is the most popular solvent owing to its high dissolving power with various polymers and oligomers. One disadvantage of THF is that it usually contains a fairly large amount of high boiling impurities.

Chloroform is usually used for preparative application, because it can easily be removed after fractionation.

ODCB is a special solvent for high temperature GPC. DMF is often used for polymers (e.g. polyamide) soluble in DMF and insoluble in THF.

6-3 Solvent Compatibility

Solvent compatibility depends on the delivery solvent. Columns packed with a standard solvent (THF or chloroform) can be replaced with solvents shown in Table 1.

On the other hand, columns packed on delivery with a special solvent cannot be switched to any other solvents.

Table 1 Solvent Compatibility

H6, H8 and HXL series	HHR and SuperH series
Toluene	Toluene
Benzene	Benzene
Xylene	Xylene
Chloroform	Chloroform
Dichloromethane*	Dichloromethane
Dichloroethane*	Dichloroethane
* These solvents cannot be used for G1000H column	Dimethylformamide
	Dimethylsulfoxide
	Dioxane
	n-Hexane
	Cyclohexane
	Dodecane
	N-Methylpyrrolidone
	Quinoline
	m-Cresol/Chloroform
	Methylethylketone
	o-Dichlorobenzene
	Trichlorobenzene
	Hexafluoroisopropanol
	Hexafluoroisopropanol/Chloroform
	Pyridine
	o-Chlorophenol/Chloroform
	Carbontetrachloride
	Ethylacetate
	Methanol/Chloroform
	Acetone
	Ethanol
	Dimethylacetamide
	1-Chloronaphthalene
	Trichloroethane

6-4 Solvent Replacement

Solvent exchange should be done carefully. Particularly, the following points should be taken into consideration.

i) The flow rate should be lower than that of shown in Table 2 and Table 3.

Table 2 Flow Rate for Solvent Replacement

Type	Column size mm(ID) × cm(L)	Flow Rate(ml/min)
HXL series	7.8×30	0.5
H series	7.5×60,7.5×30	0.5
H series	21.5×60	3.0

Table 3 Flow Rate for Solvent Replacement

Solvent	Flow Rate(ml/min)		
	HHR series		SuperH series
	7.8mm(ID)×30cm(L)	21.5mm(ID)×30cm(L)	
Toluene	0.5	3.0	0.3
Dichloromethane	0.6	3.5	0.35
Chloroform	0.5	—	0.3
Dimethylformamide	0.4	2.5	0.2
Dimethylsulfoxide	0.2	1.0	0.1
Dioxane	0.2	1.0	0.1
n-Hexane	0.9	5.0	0.5
N-Methylpyrrolidone	0.2	1.0	0.1
Quinoline	0.1	0.5	0.05
Methylethylketone	0.7	4.0	0.4
o-Dichlorobenzene	0.2	1.0	0.1
Hexafluoroisopropanol	0.1	0.5	0.05
Pyridine	0.3	2.0	0.15
Carbontetrachloride	0.3	2.0	0.15
Ethylacetate	0.6	3.5	0.35
Ethanol	0.2	1.0	0.1
1-Chloronaphthalene	0.1	0.5	0.05
Tetrahydrofuran	—	3.0	—

ii) The volume of new solvent should be more than 3 times the column volume.

iii) Never let bubbles into the column. It is better to introduce new solvent as a gradient.

7. Flow Rate

7-1 Flow Rate Selection

The flow rate should be selected with consideration to resolution, analytical time, column life, etc. Although the analytical time becomes shorter with higher flow rate, column efficiency improves with lower flow rate.

Furthermore, lower flow rate is preferable from the viewpoint of column life and also has the advantage that top-off (the phenomenon in which a gap is generated on the inlet side of the column) does not readily occur.

7-2 Suitable Flow Rate

Apply flow rates on the basis of Table 4 and Table 5. Even in the case of an urgent measurement, be sure to use the column within the maximum range. Using a column at too high flow rate for a long time may accelerate degradation of their performance.

7-3 Solvent Viscosity

With a lower solvent viscosity higher flow rate can be used. The higher the viscosity of the solvent, keep the flow rate at a lower level.

8 Temperature

8-1 Temperature Range

It is recommended to use a H type column at temperature above room temperature. And TSKgel G1000H, G2000H, G2500H, G3000H and MultiporeHXL-M can be used up to 60°C, G4000H, G5000H, G6000H, G7000H and GMH can be used up to 80°C. Other columns can be used up to 140°C, if the temperature is below the boiling point of solvent for delivery.

Please refer to section 6 when you need to replace the solvent in your column with another one.

8-2 Measurement at High Temperature

Use the solvent only after sufficient degassing. After finishing the measurement at high temperature, follow the instructions of item 5-1.

Table 4 Flow Rate

Types	Column Size mm(ID)×cm(L)	Suitable flow rates (ml/min)	Max. Flow rates (ml/min)	Max. Pressure-drops /column (MPa)		
TSKgel G1000H _{XL} TSKgel G2000H _{XL} TSKgel G2500H _{XL} TSKgel G3000H _{XL} TSKgel G4000H _{XL} TSKgel G5000H _{XL} TSKgel G6000H _{XL} TSKgel G7000H _{XL} TSKgel GMH _{XL} TSKgel GMH _{XL} -HT TSKgel GMH _{XL} -L	7.8×30	0.5~1.0	1.0	5.0		
1.2			3.5			
1.5			5.0			
1.5						
1.5						
TSKgel G1000H _s TSKgel G2000H _s TSKgel G2500H _s TSKgel G3000H _s TSKgel G4000H _s			7.5×30	0.8~1.2	1.6	3.0
2.0					2.0	
2.4					3.0	
3.0						
3.0						
TSKgel G1000H ₆ TSKgel G2000H ₆ TSKgel G2500H ₆ TSKgel G3000H ₆ TSKgel G4000H ₆ TSKgel G5000H ₆ TSKgel G6000H ₆ TSKgel G7000H ₆ TSKgel GMH ₆	7.5×60	0.8~1.5	2.0	4.0		
2.0			4.0			
TSKgel G2000H _s TSKgel G2500H _s TSKgel G3000H _s TSKgel G4000H _s	21.5×60	3.5~6.0	7.0	4.0		
7.2						
4.0~6.0		9.0	3.0			
9.0						
9.0						
9.0						
9.0						
9.0						
9.0						
9.0						

Note : For 7.8×30, 7.5×60 Columns

Flow rate for THF solvent at room temperature.

(TSKgel GMH-HT : ODCB solvent)

For 21.5×60 Columns

Flow rate for Chloroform solvent at room temperature.

Table 5 Flow Rates

Types	Column Size mm(ID)×cm(L)	Suitable flow rates (ml/min)	Max. Flow rates (ml/min)	Max. Pressure-drops /column (MPa)		
TSKgel SuperH1000	6.0×15	0.3~0.6	0.8	7.0		
TSKgel SuperH2000				6.0		
TSKgel SuperH2500				4.0		
TSKgel SuperH3000				3.0		
TSKgel SuperH4000				4.0		
TSKgel SuperH5000						
TSKgel SuperH6000						
TSKgel SuperH7000						
TSKgel SuperHM-L				4.0		
TSKgel SuperHM-N						
TSKgel SuperHM-M						
TSKgel SuperHM-H						
TSKgel G1000 ^{HHR}	7.8×30	0.5~1.0	2.0	5.0		
TSKgel G2000 ^{HHR}						
TSKgel G2500 ^{HHR}						
TSKgel G3000 ^{HHR}						
TSKgel G4000 ^{HHR}						
TSKgel G5000 ^{HHR}						
TSKgel G6000 ^{HHR}						
TSKgel G7000 ^{HHR}						
TSKgel GMH ^{HHR} -L						
TSKgel GMH ^{HHR} -N						
TSKgel GMH ^{HHR} -M						
TSKgel GMH ^{HHR} -H						
TSKgel G5000 ^{HHR} (S)					2.5	2.0
TSKgel G6000 ^{HHR} (S)						
TSKgel G7000 ^{HHR} (S)						
TSKgel GMH ^{HHR} -M(S)						
TSKgel GMH ^{HHR} -H(S)	7.8×30	0.5~1.0	3.0	1.5		
TSKgel GMH ^{HHR} -H(30)						
TSKgel GMH ^{HHR} -H(20)						
TSKgel G2000 ^{HHR} -H(30)						
TSKgel G2000 ^{HHR} -H(20)	7.8×30	0.5~1.0	3.0	1.5		
TSKgel GMH ^{HHR} -H(30)HT			2.5	2.0		
TSKgel GMH ^{HHR} -H(20)HT			2.0	3.5		
TSKgel GMH ^{HHR} -H(S)HT			3.0	1.5		
TSKgel GMH ^{HHR} -H HT						
TSKgel G2000 ^{HHR} (30)HT						
TSKgel G2000 ^{HHR} (20)HT			3.0	1.5		
TSKgel MultiporeHXL-M	7.8×30	0.5~1.0	3.0	1.5		
TSKgel G1000 ^{HHR}	21.5×30	6.0~8.0	14.0	5.0		
TSKgel G2000 ^{HHR}						
TSKgel G2500 ^{HHR}						
TSKgel G3000 ^{HHR}						
TSKgel G4000 ^{HHR}						
TSKgel GMH ^{HHR} -H						

Note : For 6.0×15, 7.8×30 Columns

Flow rates for THF solvent at room temperature.

(TSKgel GMH-HT : OCDB solvent)

For 21.5×30 Columns

Flow rates for Chloroform solvent at room temperature.

8-3 Advantages of Measurement at High Temperature

The advantages of measurement at high temperature consist of the following points:

- i) The viscosity can be reduced by elevating temperature.
- ii) The number of theoretical plates increases and resolution improves in comparison with measurement at room temperature.

8-4 Measurement at Temperature below Room Temperature

In this case disadvantages appear contrary to the advantages mentioned above. Furthermore, since the viscosity of the solvent or sample becomes higher, it is necessary to keep the flow rate lower than in operation at room temperature.

9 Sample Preparation

9-1 Preparation of a Sample Solution

Prepare a sample solution just before injection by dissolving the sample into the solvent to be used as an eluent.

9-2 Filtration of Insoluble Particles

Filter the sample solution with a micropore-filter ($0.5 \mu\text{m}$). Even if nothing can be seen, insoluble particles often exist in the sample solution.

9-3 Sample Loading

Typical sample sizes are shown in Table 6 with column size and purposes.

Table 6 Sample Size

Column size mm(ID) × cm(L)	Purpose	Sample size (mg)
6.0 × 15	Analytical	0.0005 ~ 0.2
7.5 × 30	Analytical	0.001 ~ 0.5
	Semipreparative	0.5 ~ 5
7.5 × 60	Analytical	0.001 ~ 1
	Semipreparative	1 ~ 10
7.8 × 30	Analytical	0.001 ~ 0.5
	Semipreparative	0.5 ~ 5
21.5 × 30	Preparative	5 ~ 50
21.5 × 60	Preparative	10 ~ 100

10 Calculation of the Number of Theoretical Plates and Asymmetry Factor

The number of theoretical plates (N), the Asymmetry factor (As) and their measurement conditions are as shown in the inspection Data.

10-1 Calculating Method for the Number of Theoretical plates

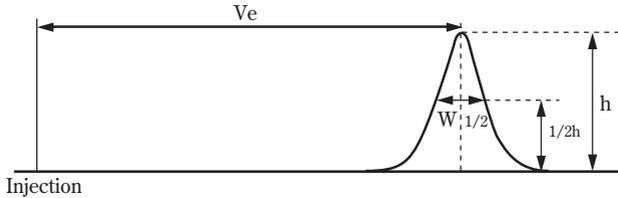


Fig. 5 Calculating method for the number of theoretical plates

The number of theoretical plates a column is calculated by the half peak width method as shown in Fig.5 and the following equation.

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

V_e : Elution time

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

h : peak height

N : Number of theoretical plates/column

10-2 Calculation Method for Asymmetry Factor

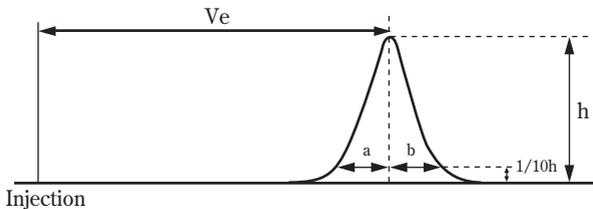


Fig. 6 Calculating method for Asymmetry factor

The Asymmetry factor of a column is calculated by the 1/10h method.

$$As=b/a$$

10-3 Dead Volume

N and As should be measured with an instrument of sufficiently small dead volume. Indication of a lower number of theoretical plates than the standard value

may be caused by too large a dead volume or the instrument or increased injection volume.

11 Guard Column

Fundamental keys for preventing trouble are listed in items 4 to 9. But when impurities tending to be adsorbed by the packing material are present in a sample, they are adsorbed on the inlet side of the column and accumulate gradually, causing reduction of the number of theoretical plates and degradation of column efficiency. In such cases it is possible to restore original column efficiency by connecting a guard column before the column and replacing it in the case of efficiency degradation by adsorbed material. Utilize a guard column as long as possible in order to prevent trouble more surely.

However, the guard column is not for analysis. No improvement of resolution can be expected by connecting a guard column. Utilize it only for preventing trouble.

11-1 Effects of Guard Column Installation

- 1) Prevention of the top-off trouble due to pump pulsation, abnormal flow rate and pressure rise.
- 2) Prevention of contamination of the main column by cutting off adsorptive material
- 3) Protection of the main column by cutting off insoluble substances.

11-2 Kind and Selection of Guard Column

Specifications for the guard columns are shown in Table 7. There are 18 kinds of guard column for the H type.

11-3 Guard Column Replacement

Since the guard column has a limit of corresponding adsorbing capacity, it has a definite life. The guard column must be replaced before contamination extends to the main column.

Replacement frequency can not be standardized because it depends on various factors such as application (analysis or preparative separation), sample properties (properties of principal components, properties and concentrations of impurities, etc.), sample loading amount, solvent, flow rate, etc.

Since the pressure rise during operation means clogging at the end fitting of guard column or contamination of the gel, it is good to replace the guard column when the pressure rises to some extent. In general, when some change in measured data is observed, replace the guard column immediately.

Table 7 Kinds and Applications of Guard Column

Cat.No.	Types	Column Sizes mm(ID)×cm (L)	Applied Column mm(ID)×cm (L)
05157	TSKguardcolumn H ₆	(7.5×7.5)	H ₆ Types (7.5×30)
05156	TSKguardcolumn H ₈	(7.5×7.5)	H ₈ Types (7.5×30)
05158	TSKguardcolumn HM	(7.5×7.5)	GMH & GMH-HT Types (7.5×30,7.5×60)
07113	TSKguardcolumn H _{XL} -L	(6.0×4.0)	G1000H _{XL} ~G4000H _{XL} Types (7.8×30)
13727	TSKguardcolumn H _{XL} -H	(6.0×4.0)	G5000H _{XL} ~G7000H _{XL} & GMH _{XL} Types (7.8×30)
05159	TSKguardcolumn H	(7.5×7.5)	H ₆ & H ₈ Types (21.5×60)
18271	TSKguardcolumn H _{HR} -L	(7.5×7.5)	G1000H _{HR} ~G4000H _{HR} Types (21.5×30)
18272	TSKguardcolumn H _{HR} -H	(7.5×7.5)	GMH _{HR} -H Type (21.5×30)
17368	TSKguardcolumn H _{HR} -L	(6.0×4.0)	G1000H _{HR} ~G4000H _{HR} Types (7.8×30)
17369	TSKguardcolumn H _{HR} -H	(6.0×4.0)	G5000H _{HR} ~G7000H _{HR} & GMH _{HR} -L,N,M,H Types (7.8×30)
17367	TSKguardcolumn H _{HR} -(S)	(7.5×7.5)	G5000H _{HR} (S)~G7000H _{HR} (S) & GMH _{HR} -M (S), H(S) Types (7.8×30)
18002	TSKguardcolumn SuperH-L	(4.6×3.5)	SuperH1000~SuperH4000 Types (6.0×15)
18003	TSKguardcolumn SuperH-H	(4.6×3.5)	SyperH5000~SuperH7000 & SuperHM-L, N, M, H Types (6.0×15)
18402	TSKguardcolumn H _{HR} (30)	(7.5×7.5)	G2000H _{HR} (30),(20) & GMH _{HR} -H(30), (20) Types (7.8×30)
18396	TSKguardcolumn H _{HR} (30)HT	(7.5×7.5)	G2000H _{HR} (30) HT,(20) HT & GMH _{HR} -H (30) HT,(20) HT Types (7.8×30)
18397	TSKguardcolumn H _{HR} (S)HT	(7.5×7.5)	GMH _{HR} (S) HT Types (7.8×30)
18404	TSKguardcolumn MP(XL)	(6.0×4.0)	MultiporeH _{XL} -M Types (7.8×30)

12 Troubleshooting

Most trouble can be avoided by carefully following the instructions in items 4~9 and 11. Particularly, appropriate utilization of a precolumn is very effective. However, if trouble happens, follow the procedure described below.

12-1 Clogging of Inlet Filter

This is evidenced by a decline in pressure or flow rate. In this case, clean the fitting by reversing flow through the column (The flow rate must be kept below 0.5ml/min). If the clogging can not be removed, replace the end fitting as follows:

- a) Prepare a new end fitting and carefully remove the clogged end fitting from the column.
- b) Be careful not to lose the gel. Transfer gel remaining in the old end fitting into the new one.
- c) Attach the new end fitting to the column.
- d) Expel air from the inlet side by reversely connecting the column to the pumping system (Refer to item 4-4).
- e) Connect the column in the normal direction and test efficiency by measuring the number of theoretical plates and asymmetry factor.

13 Quality Specifications and Warranty

13-1 Inspection Data

Inspection conditions and results are shown in the Inspection Data. The number of theoretical plates is expressed that per column.

13-2 Quality Specifications

The H type packed columns are delivered according to the specifications below.

13-3 Warranty

Immediately after receipt, check the appearance of the column and test its performance according to the conditions shown in the inspection data and calculate the performance according to the procedure shown in section 10.

If the guaranteed specifications in Table 8 can not be obtained or the column has been damaged during the transportation, contact TOSOH, representative within two weeks. TOSOH will replace the column with free of charge.

No column should be returned to TOSOH without its prior authorization.

The specifications of these columns may change without notice for their improvement.



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