

Solvent Replacement Applicability of Organic SEC (GPC) Columns

Solvent	Product Name									
	Shipping Solvent : THF						Shipping Solvent : DMF			
	KF-801	KF-802 KF-802.5 KF-803L KF-804L	KF-803	KF-804 KF-805 KF-805L KF-806M KF-806L KF-807L	KF-401HQ KF-402HQ KF-402.5HQ	KF-403HQ	LF-804 LF-604 LF-404	KD-801 KD-802 KD-802.5	KD-803	KD-804 KD-805 KD-806 KD-807 KD-806M
Tetrahydrofuran (THF)	✓	✓	✓	✓	✓	✓	✓	×	×	✓
Chloroform	✓	✓	✓	✓	✓	✓	✓	×	×	✓
Carbon tetrachloride	×	✓	✓	✓			✓	×	×	✓
Benzene	✓	✓	✓	✓	✓	✓		×	✓	✓
Toluene	✓	✓	✓	✓	✓	✓	✓	×	✓	✓
p-Xylene	×	✓	✓	✓	✓	✓		×	✓	✓
o-Dichlorobenzene (ODCB)	×	×	✓	✓	✓	✓		×	✓	✓
1,2,4-Trichlorobenzene (TCB)	×	×	✓	✓	✓	✓		×	✓	✓
Dioxane	×	✓	✓	✓				×	✓	✓
Diethyl ether	×	×	✓	✓				×	✓	✓
Ethyl acetate	×	×	✓	✓				×	×	✓
Acetone	×	×	✓	✓	✓	✓		×	✓	✓
Methyl ethyl ketone	×	×	✓	✓	✓	✓	✓	×	✓	✓
N,N-Dimethylformamide (DMF)	×	×	✓	✓	✓*	✓*	✓*	✓	✓	✓
N,N-Dimethylacetamide (DMAc)	×	×	✓	✓	✓*	✓*	✓*	×	✓	✓
Hexafluoroisopropanol (HFIP)	×	×	×	✓	×	△*	✓*	×	✓	✓
m-Cresol	×	×	✓	✓				×	✓	✓
o-Chlorophenol	×	×	✓	✓				×	✓	✓
Quinoline	×	×	✓	✓				×	✓	✓
N-Methyl-2-pyrrolidone (NMP)	×	×	✓	✓	✓*	✓*	✓*	×	✓	✓
Dimethyl sulfoxide (DMSO)	×	×	×	×	△*	✓*	✓*	×	×	✓
30 % m-Cresol/Chloroform	×	✓	✓	✓			✓	×	✓	✓
30 % o-Chlorophenol/Chloroform	×	✓	✓	✓			✓	×	✓	✓
30 % HFIP/Chloroform	×	✓	✓	✓				×	✓	✓
Hexane	×	×	×	×	×	×	×	×	×	×
Acetonitrile	×	×	×	×	×	×	×	×	×	×
Methanol	×	×	×	×	×	×	×	×	×	×
Water	×	×	×	×	×	×	×	×	×	×

✓ : Solvent replacement possible

△ : Solvent replacement possible, but this may cause column performance to deteriorate slightly

* : Usable at 40 °C or higher

× : Solvent replacement not possible

See page 66 for solvent replacement method for Organic SEC (GPC) columns.

Solvent Replacement Method for Organic SEC (GPC) Columns

Size exclusion chromatography (SEC) is a chromatography that separates the analytes based on the size of sample (polymer) molecules. The samples are generally prepared in a solvent that dissolves the target polymer well. Therefore, some target polymers may be dissolved in a solvent different from the shipping solution of SEC columns. In such cases, the column solvent needs to be replaced with the solvent to be used.

Shodex offers several SEC column series such as Asahipak GF series and OHPak series. Here, the GPC series, organic SEC (GPC) columns, are used as an example to explain the solvent replacement method.

Please consider this page as a general guideline and make sure to read the column-specific operation manual before replacing the solvents in your columns.

■ Before Starting: Check List

Incorrect solvent replacement method may damage the column. Please make sure to check the followings before replacing the solvents (the details follow after the list).

1. Applicable Solvents
2. Solvent Miscibility
3. Solvent Boiling Point
4. Column Specifications (maximum allowable pressure, flow rate, column volume, maximum temperature)
5. Setting the Pump Limiter's Maximum Pressure
6. HPLC System's Solvent Compatibility

■ Applicable Solvents

Check solvent replacement applicability of the column. Please refer to page 60: Solvent Replacement Applicability of Organic SEC (GPC) columns.

Please pay an extra attention as different pore-size columns even within the same column series may have different solvent replacement applicability. You may also check the solvent replacement applicability in the column-specific operation manual and Shodex website (<https://www.shodex.com/en/dc/06/03/09.html>).

■ Solvent Miscibility

Check miscibility/solubility of the desired new solvent and the solvent currently filled in the column. Please refer to Shodex website (<https://www.shodex.com/en/dc/06/0115.html>) for the miscibility of the solvents.

THF, chloroform, DMF, and HFIP are miscible with each other. On the other hand, sodium trifluoroacetate is soluble in HFIP but difficult to dissolve in THF. Thus, if HFIP with sodium trifluoroacetate and THF are mixed, sodium trifluoroacetate will precipitate. Precipitation of salt in the HPLC system or column may damage the system and/or the column. Therefore, it is important to check solubility of the salt in addition to checking the miscibility of solvents.

■ Solvent Boiling Point

The column pressure sometimes increases during the solvent replacement. Increasing the column oven temperature is an effective way of reducing the column pressure.

However, setting the column oven temperature higher than the boiling point of the solvent makes the solvent to generate air bubbles. Since the bubbles may affect the filling condition of the packing material, please pay an attention when increasing the column oven temperature.

■ Column Specifications

Please refer to the below table for the maximum pressure allowed per column and the recommended flow rate. Also, please check details of specifications described in the column-specific operation manual and Shodex website.

< Column Specifications >

Product Series	Flow Rate (mL/min)	Maximum Pressure (MPa/column)	Maximum Temperature (°C)
GPC KF-800 series	0.5 ~ 1.0	3.5	60
GPC KD-800 series	0.5 ~ 1.0	3.5	60
GPC KF-400HQ series	0.3	7	45
GPC HK-400 series (for GPC HK-402)	0.3 ~ 1.0	25 (20)	60
GPC LF-804	1	3.5	60
GPC LF-604	0.5	3.5	60
GPC LF-404	0.3	3.5	60

Column volume of a column can be calculated using the below equation.

$$[\text{Column Volume}] = \left(\frac{[\text{Inner Diameter (I.D.)}]^2}{2} \right) \pi \times [\text{Length}]$$

The below table lists the column volumes of different-size columns.

< Column Volume (mL) >

Column Length (mm)	I.D. (mm)		
	4.6	6.0	8.0
10	0.2	-	-
50	-	-	2.5
100	-	-	5.0
150	2.5	4.2	-
250	4.2	-	12.6
300	-	-	15.1

■ Setting the Pump Limiter's Maximum Pressure

The pressure applied to the entire HPLC system (system pressure) and the pressure applied to the column (column pressure) are influenced by eluent type, flow rate, and temperature. Thus, the column pressure may increase during the solvent replacement process. To prevent applying a pressure above the maximum allowable pressure of a column, use a pump limiter's maximum pressure setting.

The pressure displayed on the HPLC system is a sum of system pressure and column pressure. Therefore, generally the pump limiter's maximum pressure is set at "the sum of system and column's maximum allowable pressures". However, low flow rate used during the solvent replacement generates a negligible system pressure, thus using the column's maximum allowable pressure as the pump limiter's maximum pressure is a practical choice.

If multiple columns are used together, add maximum allowable pressure of all columns when setting the limit.

e.g. 1 When using one column with maximum allowable pressure = 3.5 MPa.

➔ Set the pump limiter's maximum pressure at 3.5 MPa.

e.g. 2 When using two columns with both maximum allowable pressures = 3.5 MPa.

➔ Set the pump limiter's maximum pressure at 7.0 MPa.

■ HPLC System's Solvent Compatibility

Some materials used in HPLC system may have low chemical compatibility to organic solvents to be used in SEC analysis. To avoid damaging the HPLC system, please make sure that the chosen solvent is applicable for all HPLC system parts that have contact with the solvent.

Please note that there are tubes and fittings made of various materials. Among them, stainless steel types are recommended because of their high durability against various organic solvents.

■ Solvent Replacement Steps

1. Reduce the flow rate to the half of the regular flow rate and increase the column oven temperature while keep introducing the current solvent (the temperature required depends on the column and the solvent used).

2. Move to next step once the column temperature reaches to the set temperature.

Case 1. When replacing the current solvent with a miscible solvent.

First introduce about 3 - 5 column volumes of an intermediate solvent (current solvent : new solvent = 1:1 mixture). Then, introduce another 3 - 5 column volumes of 100 % new solvent.

e.g. To replace THF to chloroform in KF-803 (8.0 mm I.D., 300 mm L)

First introduce 45 - 75 mL of an intermediate solvent (THF : chloroform = 1:1 mixture). Then, introduce 45 - 75 mL of 100 % chloroform.

Case 2. When replacing the current solvent with a solvent with low miscibility/solubility to the current solvent.

First introduce about 3 - 5 column volumes of a solvent that is miscible/soluble to both solvents. Then, follow the steps in Case 1.

e.g. To replace DMF with LiBr to THF in KD-806M (8.0 mm I.D., 300 mm L)

First introduce 45 - 75 mL of intermediate solvent 1 (DMF) to wash-out LiBr. Then, introduce 45 - 75 mL of intermediate solvent 2 (DMF : THF = 1 : 1 mixture), followed by 45 - 75 mL of 100 % THF.

3. Set the flow rate and the temperature to the desired. Start the analysis once a stable baseline is obtained.

■ Additional Recommendations

Frequent eluent replacement may damage the column, and thus not recommended.

If analyses using different eluents are frequently expected, it is recommended to have dedicated columns for each solvent.