

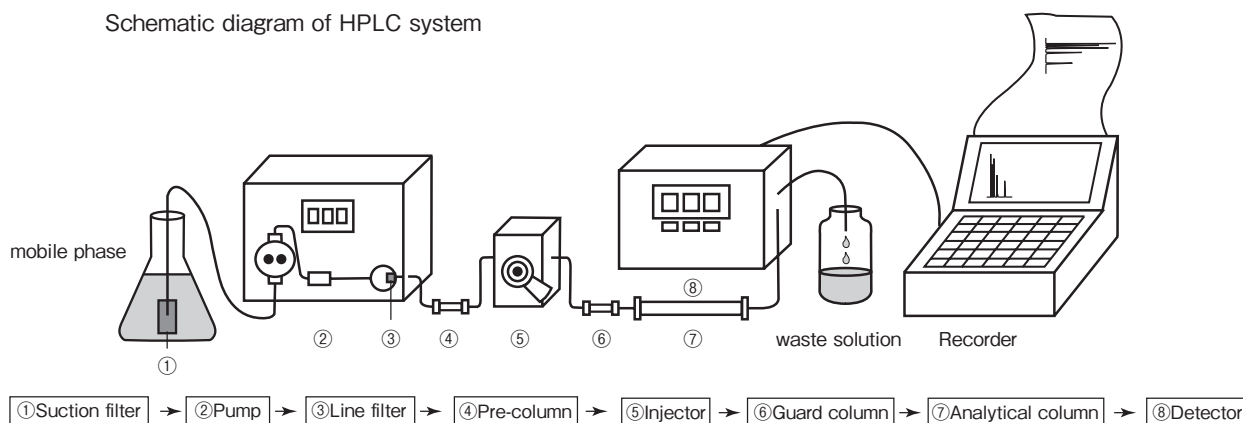
TECHNICAL NOTE

5. Troubleshooting for increased pressure

Repeated analysis may increase back pressure. Continuous use of HPLC columns under high pressure can cause deterioration and overload of the equipment. Therefore, it is important to monitor column back pressure regularly and solve the problem timely.

The back pressure increase can be due to clogging of a column or clogging of the equipment. First of all, identify the clogging site.

Schematic diagram of HPLC system



Remove analytical column first and connect the plumbing from the back of HPLC system directly to the detector. Measure the pressure of flowing mobile phase without an HPLC column. Generally equipment should hardly generate any pressure. If there is significant flow pressure, disconnect the system components one by one to identify the clogged component(s). Possible causes and solutions of clogged equipment are discussed in section II below.

If the flow pressure without a column is normal, then pressure increase is due to clogging of a column. In this case, one needs to determine the causes and whether it is time to replace the column. Possible causes and solutions of clogged column are discussed in greater details in sections I and III.

Symptom

Possible Cause

Pressure increase rapidly in short-term use	→	Flow pressure without a column is 0-0.3 MPa	→	Clogging of column Refer to section I
	Yes	Flow pressure with a column is 0.3 MPa or higher	→	Clogging of equipment Refer to section II
Pressure increase gradually in long-term use	→	Deterioration of column due to long-term use		
	Yes	Refer to section III		

I. Solution in case an HPLC column is clogged in short-term use.

Select the possible cause of clogging according to the following flow chart.

Step1	<ul style="list-style-type: none"> • Salt deposition • Use mobile phase of high concentration organic solvent right after using buffer 	→	Yes	Cause 1
↓ NO				
Step2	<ul style="list-style-type: none"> • Forget to filter mobile phase • Sample is not dissolved enough 	→	Yes	Cause 2
↓ NO				
Step3	<ul style="list-style-type: none"> • Analyzing samples which tend to absorb to a column (i.e. protein samples) • Sample deposition in column 	→	Yes	Cause 3

Cause 1 Salt is deposited on a column.

Solution : Wash columns for 30 minutes at half of the analytical flow rate with 10% organic solvent (methanol or acetonitrile) in water to dissolve deposited salt. If the situation is not improved, wash with 100% water under the same condition.

Prevention : To switch to high concentration organic solvent after using a buffer, first wash a column with a mobile phase not containing salt (with the same concentration of organic solvent as the buffer), then switch to the mobile phase of higher organic solvent concentration. Example : To change mobile phase from 10/90 (v/v) acetonitrile/20mmol/l phosphate buffer (pH2.5) to 90/10 (v/v) acetonitrile/water, first wash for 15 minutes with 10/90 (v/v) acetonitrile/water, and then switch to 90/10 (v/v) acetonitrile/water.

Cause 2 Column filter is clogged by sample or impurities.

Solution : Connect the column in reverse direction, and then wash the column for 30 minutes at half of the usual analytical flow rate with the mobile phase used for analysis. If the situation is not improved, change the end fitting in the front of column. (We can replace end fittings with a paid service fee.)

Prevention : We recommend filtering sample and/or mobile phase. For more information, please see page 88 TECHNICAL NOTE 3. Sample pretreatment for HPLC 1) filtration.

Cause 3 Sample may be adsorbed to packing material or deposited in a column.

Solution : Wash for 30 minutes at half of analytical flow rates with a solvent which adsorbed substances are dissolved in. The followings are how to wash each type of columns.

[Reversed phase columns]

a) When an adsorbed substance is not protein, wash with methanol or tetrahydrofuran.

b) When an adsorbed substance is protein, wash with 50-70% of acetonitrile/water (containing 0.1% of trifluoroacetic acid). However proteins may be deposited in high concentration of organic solvent depending on varieties.

[COSMOSIL Sugar-D/NH₂/HILIC columns]

Wash with 50/50 (v/v) acetonitrile/water for NH₂-MS and 100% water for Sugar-D and HILIC columns.

[COSMOSIL SL-II]

Wash with methanol, tetrahydrofuran or ethanol.

Prevention : Choose appropriate pretreatment for each sample. For more information, please see page 88 TECHNICAL NOTE 3. Sample pretreatment for HPLC 1) filtration. We also recommend using guard column. For more information for guard columns, please see page 96 TECHNICAL NOTE 6. Effect of guard columns.

Caution :
 • When wash columns, do not connect column exit and let the solution through.
 • Long term of washing may deteriorate the performance of columns.
 • Do not use strongly alkaline solution (more than pH 7.5) or strongly acidic solution (less than pH 1.5) for silica gel base packing material.
 • Store columns with manufacturer recommended storage solvent after washing,
 When the situation is not improved, replace the column.

II. Solutions in case pressure is too high because of clogged equipment.

First off, identify the specific clogging site by disconnecting the components in the system one by one and checking the flow pressure. The followings are possible common causes.

Cause 1 Salt is deposited in plumbing.

Solution : Flow water to the plumbing without connecting a column and any other equipment. Washing out the plumbing in a reversing connection is also an effective way. If the situation is not improved, replace it with a new one.

Cause 2 Check-valve of pump is clogged by stain

Solution : Wash the check-valve with a stain dissolving solvent. Take apart the washable part, soak it in the solvent, then clean in an ultrasonic cleaner.

Cause 3 Manual injector is clogged with stain

Solution : Wash with a stain dissolving solvent. Soak rotor seal and line filter in water and clean them in an ultrasonic cleaner. If the situation is not improved, replace the injector.

Prevention : It extends the life time of an HPLC system to maintain regular wash of the system. Wash the

system the same as wash an HPLC column. When the mobile phase contains salt, wash for 10-15 minutes with a mobile phase which has the same composition but not containing salt. For example, when using 50/50 (v/v) methanol/20mmol/l phosphate buffer, wash with 50/50 (v/v) methanol/water. When the mobile phase contains halogen, acid and/or base, wash for 10-15 minutes with mobile phase which has the same composition but not containing halogen, acid and/or base.

III. Solutions in case a column is damaged from long term use

Every column will have to be replaced eventually. Performance of a column is expected to deteriorate slowly after long term use. One has to decide whether it is time to replace the column.

Cause 1 Column deterioration result from long term use.

Solution : Wash according to Solution to Section I , Cause 3.

Prevention : Same as Prevention in Section I , Cause 3. When the column condition is not improved, you could continue to use the column if peak shapes do not change and the maximum pressure is less than 20 MPa. However, we recommend replacing the column because it place extra burden on the equipment.

Cause 2 Silica gel in the column may be cracked because of long term use.

Solution : Replace the columns.