(3) Troubleshooting for Increased Pressure

Introduction

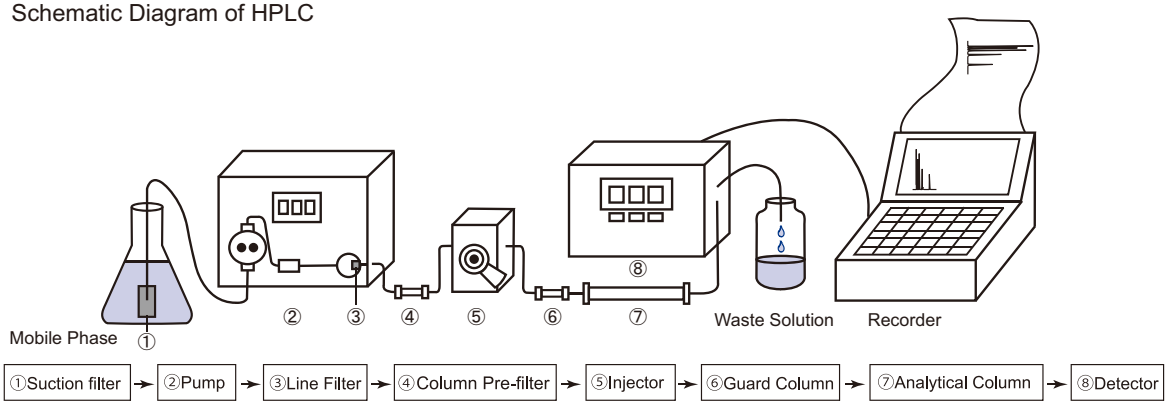
Repeated analysis may increase back pressure. Continuous use of HPLC columns under excessive pressure can cause deterioration and overload of the equipment. Therefore, it is important to monitor backpressure regularly and resolve issues before they damage the system or columns.

Identification of the Clogging Site

Backpressure increase can be due to clogging of the column or equipment. First, it is necessary to identify the clogging site.

Disconnect the system components one by one to identify the clogged component(s). Start by disconnecting the column from the system and measuring the pressure of flowing mobile phase. The pressure should be close to zero. If the pressure without a column is normal, then the pressure increase is due to clogging in the column. The column may need to be washed or replaced.

Schematic Diagram of HPLC



If elevated pressure is observed with the column disconnected, proceed to disconnect system components starting with the detector and moving backwards to the pump(s). If the pressure returns to normal, the component disconnected last can be identified as the problem.

Clogging of Equipment

Identify the specific clogging site according to the method above.

(Case 1) High pressure caused by clogged tubing

Cause: Salt deposit in tubing.

Solution: Disconnect the column and any other equipment before pumping water through the tubing. Washing in the reverse direction is also effective. If the situation does not improve, replace the tubing.

(Case 2) High pressure caused by clogged pump

Cause: Line filter of pump is clogged.

Solution: Take apart the line filter, and soak it in solvent, then clean with an ultrasonic cleaner. If the situation does not improve, replace the line filter with a new one.

(Case 3) High pressure caused by clogged manual injector

Cause: Manual injector is clogged.

Solution: Inject 20ml of a solvent that can dissolve the contaminant (e.g., methanol) by syringe. Wash both lines in LOAD and INJECT position. Disassembling and cleaning the injector in an ultrasonic bath is also effective. If solids caused the clogging, wash the injector in the reverse direction. If the situation does not improve, replace the injector with a new one.

What should I do when a clogged column causes pressure increase?

(Case 1) Salt deposit in a column caused by pumping high–organic solvent after using buffer solution

Cause: Salt deposit in a column.

Solution: Wash columns for 30 minutes at half the normal flow rate using 10% organic solvent (methanol or acetonitrile) in water to dissolve salt deposit. If the situation does not improve, wash the column with 100% water under the same conditions.

Prevention: To switch to high-organic solvent concentration after using a buffer, first wash the column with a salt-free mobile phase (with the same concentration of organic solvent as the buffer), then switch to the mobile phase of higher organic concentration.

Example : To change mobile phase from 10/90 (v/v) acetonitrile/20mmol/l phosphate buffer (pH2.5) to 50/50 (v/v) acetonitrile/water, first wash the column for 15 minutes with 10/90 (v/v) acetonitrile/water, and then switch to 50/50 (v/v) acetonitrile/water.

(Case 2) The sample is not completely dissolved or is not filtered

Cause: Column frit is clogged by insoluble sample or impurities.

Solution: Connect the column in the reverse direction, disconnect from the detector, and wash the column for 30 minutes at half of the normal flow rate with the same mobile phase used for analysis. If the situation does not improve, change the frit in the front end of the column. (We can replace end fittings as a paid service. Ask us for details.)

Prevention: We strongly recommend filtering the sample. For more information, please see page 31.

Caution: If the column is frequently washed in the reverse direction, it may deteriorate.

(Case 3) Protein samples that adsorb easily to the column or samples that are not very soluble in mobile phase

Cause: Samples have adsorbed to packing material or deposited in a column.

Solution: Wash the column for 30 minutes at half of the normal flow rate using a solvent that can dissolve the adsorbed substances.

[Reversed phase columns]

a) If the absorbed substances are not proteins, wash with methanol and/or tetrahydrofuran.

b) If the absorbed substances are proteins, wash with 50-70% acetonitrile in water (containing 0.1% trifluoroacetic acid). Proteins may precipitate in high-organic solvents, so be cautious.

[COSMOSIL SL-II] Wash with methanol, tetrahydrofuran, or ethanol.

[Fullerene columns] Wash with *o*-dichlorobenzene or *1,2,4*-trichlorobenzene.

[COSMOSIL Sugar-D/NH_2/HILIC columns] Wash with 50/50 (v/v) acetonitrile/water for NH_2-MS, or 100% water for Sugar-D and HILIC columns.

Prevention: (a) Choose appropriate pretreatment for each sample. For more information, please see page 31.

(b) We also recommend using guard columns. Please see page 37.

Notes:
- When washing columns, disconnect the column from the detector. Let the solvent flow into waste.
- Excessive washing may deteriorate the performance of columns.
- Do not use strongly alkaline solutions (greater than pH 7.5) or strongly acidic solutions (less than pH 1.5) for silica-base packing material.
- Store columns in manufacturer-recommended storage solvent after washing.
- If the column performance does not improve after washing, replace the column.

(Case 4) Gradual pressure increase over time

Cause 1: Contamination of column from normal long–term use.

Solution: Wash the column as in Case 3 above.

Cause 2: Column deterioration over time.

Solution: Replace the column.

No improvement after trying the above

You can continue to use the column at elevated pressure if peak shape is acceptable and the maximum pressure is less than the limit for the column (see the column manual for details). However, we recommend replacing the column to lessen the pressure burden on the instrument.