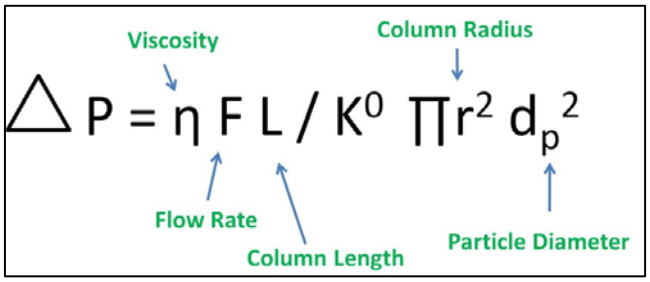
LC Troubleshooting Series
Pressure Changes

Ron Majors is a Senior Scientist and HPLC Ambassador at Agilent. John Henderson is an Applications Chemist working with Chemistries and Supplies.

Changing pressure is a symptom of something that is going on in your system or your column.

The Pressure Equation

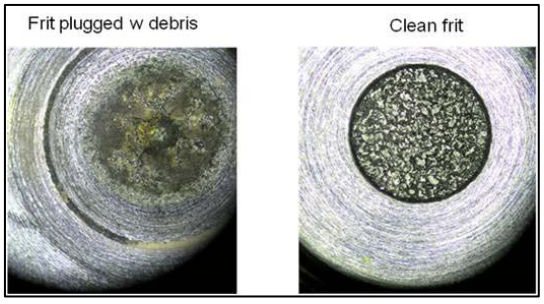
The Pressure Equation identifies five key factors that affect system pressure: solvent viscosity, flow rate, column length, column radius, and particle diameter.



Finding the Source of Your Pressure Issue

HPLC System causes of increased pressure can include obstructions in the flow path, from the pump outlet to the mobile phase waste tube after the detector outlet.

Column frits are a common point of clogging. The impact of this type of clogging will be gradual, not sudden.

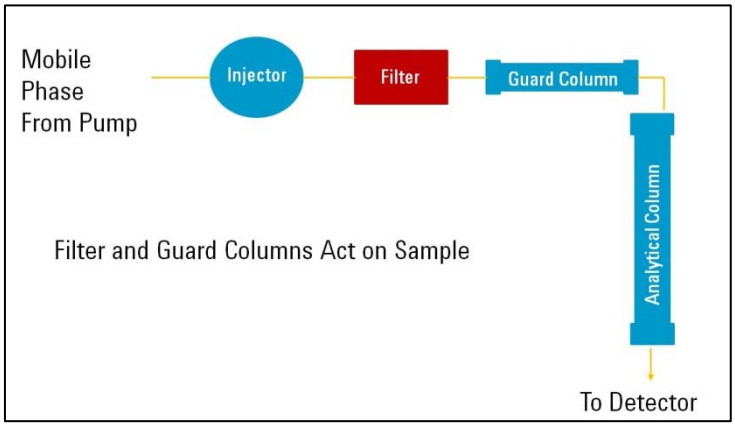


The source of column clogging comes from particulates in a number of places:

- the sample
- the wearing of instrument parts (such as rotor seals and pump seals)
- septa from worn vial caps
- solvents and buffers.

It's important to reduce extracolumn volume, by using narrow internal diameter (ID) tubing. Use the least amount of tubing necessary to make connections.

The easiest way to source your issue is to disconnect the column from the instrument and see if this solves the problem. You can work upstream, removing filters, guard columns, and perhaps instrument components (e.g. autosampler) one by one, to isolate the point where the pressure issue stops. You should also look at capillary tubing connections as a possible point of blockage.



A blockage can occur in any spot, from the pump to the detector outlet. Your system should be plumbed to prevent particulates from getting to your column inlet.

Backflushing Columns

For contamination that comes from particulates, you may be able to backflush the column to address it.

Some, but not all columns can be backflushed. Be sure to check the specifications of your column, or contact your column manufacturer if you are not sure.

To backflush a column, take the column, remove it from the system, and connect the outlet to the inlet tubing from the pump. Pump water, acetonitrile or your mobile phase through the column to dislodge the particles that are blocking the frit.

Do not connect your column to the detector when you are backflushing. Instead, run the inlet end into a beaker to capture the solvent or water and any particulates that are flushed from the inlet frit.

Do not run a high flow rate. Start out slow and build to a higher flow rate as needed to dislodge the particulates.

Tips For Preventing Column Clogging

In-line filters can help trap particulates. Guard columns are also important tools to prevent blockage in your analytical column.

There are many other filters and buffers available to help reduce particulates that cause clogging:

1) Syringe filters of varying diameters and various materials of construction;
2) The mini-uniprep filter that permits a filtration in an autosampler vial;
3) Individual membrane filters that are placed in a reusable filtration setup and
4) Glass, paper and ceramic filters, which are used less frequently.

When using membrane filters, make sure that the polymeric material is compatible with the mobile phase and sample solvents.

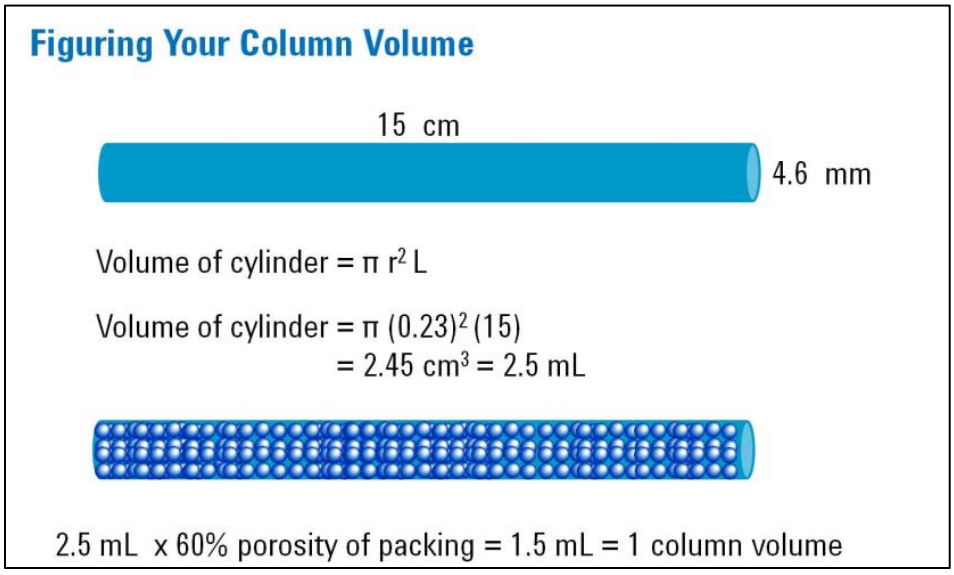
Centrifugation is another way to remove particulate matter from samples.

Chemical Contamination

Chemical contamination of your column packing is different than particulate contamination – it will be more difficult to remove. Most often, you will have chemical contamination on the front end of your column.

The best way to deal with this type of contamination is to find a solvent that will dissolve it without damaging your column packing.

If your column *can* be backflushed, it is worth trying first to backflush your chemical contamination from the **head** of the column. You should flush an amount that is sufficient to clean the column. We measure this in "column volumes", which can be determined using a formula. Generally, we recommend 10 column volumes for a starting point.



Tips for cleaning columns:

Flush with stronger solvents than your mobile phase. Make sure the detector is taken out of the flow path. Use a beaker to collect the liquid as it passes through the column.

Do not add your organic solvent directly to the buffer, as this may cause the buffer salts to precipitate out and lead to more clogging, and backpressure.

For Reversed Phase:

Use at least 10 column volumes of each solvent for analytical columns.

1. Start with your mobile phase without buffer salts (water/organic)
2. Next, use 100% Organic (MeOH or ACN)
3. Check pressure to see if this has returned it to normal; if not, then
4. Discard column or consider more drastic conditions: 75% Acetonitrile / 25% Isopropanol
5. Try 100% Isopropanol
6. Try 100% Methylene Chloride*
7. Try 100% Hexane*

* When using either Hexane or Methylene Chloride, the column must be flushed with Isopropanol before returning to your reversed-phase mobile phase

Buffer salts can precipitate out and cause backpressure buildup inside the column. If this occurs, run water slowly through the column to remove them.

Normal Phase

For Normal Phase, column cleaning can only be done with organic solvents. The options, in order of increasing strength, include:

- 50% Methanol: 50% Chloroform
- 100% Ethyl Acetate

Summary:

- First, familiarize yourself with the pressure equation to understand the key contributors to system pressure: column length, radius, particle size, flow rate, viscosity.
- For blockages, find the source by disconnecting your column from the flowpath, and working upstream with other system components (guard columns, capillaries, etc.) to find the source.
- Once located, you can replace the part, or clean it.
- Some columns can be backflushed as a way to remove particulates. Be sure to check with your manufacturer if you are unsure if your column can be backflushed. For chemical contamination which has dissolved into your column, you'll possibly need to use stronger solvents to remove it, as discussed.
- For a build-up of buffer salts, run 100% water through your column.
- Use preventive measures to protect your column from clogging. These include guard columns, inline filters and other filtration devices for solvents and samples. Sample preparation can also help reduce clogging. You can filter or centrifuge your sample.
-

If you have additional questions, please visit us on the web at www.agilent.com/chem/contactus to connect with an Agilent Technical Support Representative.