

Constant Pressure Method on two Instruments Producing Different Flow Rates- Troubleshooting

Most HPLC methods specify a constant flow rate, not constant pressure, because volumetric flow is directly tied to retention behavior. When a method is run under constant pressure, two instruments programmed to use the same pressure may still deliver different flow rates, which can significantly affect chromatographic performance.

This variability occurs because every LC system has its own unique hydraulic characteristics. Differences in system components—such as the internal diameter of tubing, detector cell geometry, solvent-line path length, and other mechanical factors—will change the system backpressure. Even small differences can cause one instrument to generate a higher or lower flow rate at the same pressure setting.

Other variables can also lead to pressure-related inconsistencies:

- **Particulate contamination** or buildup within the LC system can artificially raise pressure, reducing the actual flow rate.
- **Pump wear, seals, and check valves** can alter pressure-flow behavior.
- **Column-to-column variation**, even when dimensions are identical, can cause different pressures because stationary phases have different permeabilities.

Because flow rate directly affects the column void time (t_0), retention times, separation selectivity, and gradient behavior, these differences can cause significant changes in chromatographic results. For this reason, using constant pressure is generally not recommended, especially for methods intended to be reproduced on multiple systems or transferred between laboratories.

In contrast, constant flow rate operation ensures consistent chromatographic behavior, making methods more robust, transferable, and reliable across instruments and column lots.

