

If you are suspecting high pressure on an HPLC column in a semi-prep column for example, you can check whether it is operating properly by testing it against an analytical column of the same stationary phase material (pore size, particle size etc) and length.

***Follow this procedure to check whether the pressure of a column is operating properly:***

1. Prepare a mobile phase of 75:25 Acetonitrile : Water v/v. Using a flow rate to 1mL / minute, inject an acetone standard onto the column you know is working properly (the analytical column in this example). Acetone shows no retention under these conditions for reversed phase columns and therefore serves as a void volume marker. Uracil is another common choice.
3. Record the acetone retention time and the pressure in your system during the run.
4. Remove the first column (analytical column) and connect the other column (the semi prep column in this example) to your system.
5. Start the pump and increase the flow rate until you achieve the same pressure drop as the first column (analytical column). Under these conditions, the linear flow rates should be the same if the columns are behaving normally.
6. Inject the acetone standard on this column and record the retention time. It should be the same retention time as the analytical column. If the retention time is higher, then the column is blocked (or partially blocked). However, if the retention time is the same then the column is operating according to specifications of the first column regarding pressure and flow characteristics.

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