

Why do I see large intermittent pressure spikes in HPLC runs and can it be a defective column - FAQ

Two scenarios are most likely to be the cause of the pressure spikes, but it is <u>not</u> likely due to a defective column that operates normally at any time:

1. An air bubble is present in the system. You can narrow the problem down to the system by trying your column on another HPLC. If you don't see the pressure spikes on the different HPLC, you know it is a problem with your system. If you don't have another instrument, you can try a different column on the suspect instrument and see if the pressure spikes are still there. If you still see the pressure spikes, it suggests that it is a system issue.

Be sure to wait a while to see if the pressure spikes show up again on the new column. It is not always immediate and if you don't see them right away you may mistakenly attribute it to a column issue. The air bubbles are often produced in either the pump head, check valves, or on the detector flow cell. To remove an air bubble, you can try several things. First, if you momentarily block the tubing outlet such that extra back pressure is applied to the system, this can remove the air bubble. If the air bubble is in the pump head, you will need to purge the pump.

2. There is immiscible solvent in the column. If you are using your column in reversed phase for instance and some normal phase solvent ended up in the column *(e.g. hexane)* you will see pressure spikes. In this case you must flush the column with a mutually miscible solvent, then with a Normal Phase *(non polar)* HPLC solvent such as ethyl acetate, then back to the mutually miscible HPLC solvent. Be sure that the solvents are thoroughly degassed and filtered.

A higher than normal pressure in HPLC can be caused by many things such as particulate matter at the frit inlets, a blockage somewhere in the system such as crimped tubing, and so on. However, in all these cases the pressure will remain high and will not "spike", so a pressure that intermittently runs high or spikes to a high value and then returns to normal is certainly not caused by any of these.



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