Use a linear flow rate test to check back pressure on an HPLC column - Tech Information

Date: 17-FEBRUARY-2013 Last Updated: 5-JULY-2025

If you're noticing unusually high pressure when using an HPLC column—you may be wondering whether the column is blocked or not functioning properly. One simple and effective way to check this is by comparing it to a known good analytical column using a linear flow rate test.

This test helps determine whether the column is causing abnormal back pressure or if the system is functioning as expected.

🧪 What You'll Need

- A working analytical column with the same stationary phase (same particle size, pore size, and length) as the column you're testing.
- The test column. *This can be a semi-prep or another analytical column.*
- Acetone (or uracil) standard – used as a void volume marker.
- A mobile phase of 75:25 Acetonitrile:Water (v/v).
- Your HPLC system.

✅ Step-by-Step Procedure

1. Install your working Analytical Column (the known good one).
2. Prepare your mobile phase (75:25 Acetonitrile:Water) and set the flow rate to 1.0 mL/min.
3. Inject a small amount of acetone. Acetone doesn't retain on reversed-phase columns, so it will come out quickly and mark the void volume.
4. Record the retention time of the acetone peak and the system pressure during the run.
5. Remove the workling analytical column and install the column in question. (This can be a semi-prep column or the column you're testing the functionality on.)

    - If the test column dimensions are the same, start the pump and gradually increase the flow rate to 1.0 mL/min.
    - If the test column dimensions are larger, (i.e. semi-prep) gradually increase the flow rate until the system pressure matches what you recorded with the analytical column.

6 .Inject the same acetone standard and record the retention time.

🔍 How to Interpret the Results

- If the acetone retention time is the same as with the working analytical column, your test column is likely functioning properly.
- If the retention time is longer, it may indicate partial blockage or restricted flow in the column.

- If the pressure is much higher than expected even at lower flow rates, the column may be fully or severely blocked.

💡 Helpful Tips for Beginners

- Always filter your mobile phase and samples to prevent particles from clogging the column. Keep a log of pressure readings for each column over time to spot trends.
- If you're unsure about matching column specs (like particle size or length), check the manufacturer’s datasheet or contact technical support.
- Use acetone or uracil because they are non-retained compounds—they help you measure flow behavior without interference from retention.

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