HPLC COLUMN QUALITY CONTROL & SYSTEM START-UP

PUPOSE

1. To start up an HPLC protecting seals, plungers, and a **dry column.**
2. To run column standards.
3. To calculate plate counts/retention times.

EQUIPMENT AND REAGENTS

1. Isocratic HPLC system
2. C18 column (5 µm, 15-25 cm)
3. 25 µL injection syringe
4. HPLC-grade methanol and water
5. Column blank (5 ft of 0.010- in tubing, fitting and unions)
6. 70 psi back-pressure device on detector outlet.

METHOD

1. Prepare 200mL of 50% methanol/ Water and 100mL of 80% methanol/ water. Vacuum filter through 0.54-µm filters.
2. Remove the C18 column, cap, and set it aside. Set up the HPLC system with Column Bridge in place of the column. Prime the pumps with 50% methanol/water. Set the over pressure setting on the pump to 4000 psi. Start flow at 0.1 mL/min. Watch the pump pressure indictor for fluctuation (air bubbles? Dirty check valves?). (Lab Note: Air Bubbles can be cleared by opening the compression fitting on the outlet check valves with a wrench until solvent bubbles out, then tapping the valves housing lightly to Release bubbles. Retighten the compression fitting and move to the next check valves fitting until you have checked them all or them all or the problem has disappeared.)Stair-stepping pressure problems may indicate a dirty check valve, which should be replaced or pacified.

3. When the pressure is steady, turn the injector handle to inject (or load if it was already in the inject position) and watch the pressure. If the pressure does not jump up, the loop is not blocked. Cycle the injector handle to the inject position.
4. Watch the recorder or computer baseline. When it is stable, slow the pump flow to 0.1 mL/min, remove the column blank, and connect the C18column to the injector. Do not connect the column to the detector yet; Wash the column solvent into injector. Wash the column solvent into a breaker (start flow ramp up from 0.1 to 1.0 mL/min) for six column volumes (12-18 mL). Pressure should slowly increase to around 2000 psi at 1 mL/min due to column back-pressure. (*Lab Note:* Always hook up a column with solvent running to prevent introducing air from the column head into the column.)
5. When the pressure is stable, record column back-pressure from the pump pressure gauge in a Logbook, Connect the column to the detector inlet fitting. Turn on the detector (select 245 nm, 1.0 AUFS) and the recorder at 0.5 cm/min chart speed. Observe the baseline. Drifting indicates that the detector is still warming up or something is washing off the column. (Lab Note: The pump pressure gauge should be monitored periodically when making changes to a system; a sudden pressure increase indicates a blockage problem. Adjusting the pump over-pressure setting should prevent problems, but shut off the flow yourself and figure out what is causing the extra pressure to be sure.)
6. When the baseline is stable, inject 15 µL of column standards. (Lab Note: Inject by overfilling the syringe, point the needle up , pull the barrel back until you can see the meniscus, tap out visible bubbles in the liquid, push the plunger to the15 µL mark, wipe outside the barrel with a pulling motion. Insert into injector, Load the injector loop slowly, and leave the needle in place.) Turn the injection handle quickly. Remove the injection needle, and flush three times with solvent.
7. On the chromatogram paper, mark the inject point. Record date, time, operator names, flow rate, mobile phase, sample type, number, injection amount, column, detector wavelength, attenuation, and the chart speed so is you could duplicate this run, Record chromatogram until the baseline is reached after the four peaks.
8. Repeat standards run. Increase recorder speed to 2 cm/min. Inject standards solution. Record the four-peak chromatogram.

RESULT

9. Using the chromatogram recorded at 2 cm/min. V°, the exclusion volume of the column, V× for each peak (the solvent volume at the peak center), and W (the 5ϑ width) for peaks 1 And 4.
10. Calculate K' (peaks 1 and 4), α (peaks 1, 2), and N (1 and 4). (Lab Note: Remember

    K' (1) =V_1 - V_o/V_o, α (1, 2) = K'_2/k'_1

    $N_1 = 16(V_1/W_1)^2$, Also remember that W_1 is measured by projecting lines parallel to the sides of the peak to where they intersect the baseline. W_1 is distance between the intersection Points.)