

COLUMN AND SOLVENT SWITCHING AND PACIFICATION

Purpose

1. Study the effect changing the stronger solvent or the column.
2. Do a column washout and QC check
3. Do a system pacification using a column blank.

Equipment and Reagent

1. Gradient HPLC system
2. Back-pressure device on the detector outlet
3. C18 column(5 μ m, 15-25 cm)
4. C8 column (5 μ m, 15-25 cm)
5. Column Blank (5 ft of 0.01-in tubings, fittings, and unions)
6. Four-component test mixture (P.J Colbert Cat. No. 962202)
7. Seven-Component test mixture (P.J Colbert Cat. No. 962202)
8. HPLC-grade acetonitrile, methanol, and water
9. Concentrated nitric acid

METHOD

1. Purge line A with water and line B with MeOH. Dial-a-mix 70% MeOH and equilibrate the C18 column at 1.0 mL/min, When stable, inject 15 μ L of the seven-component test mixture and annotate the chromatogram's start. Run an isocratic chromatogram.
2. Reduce the flow to 0.1 mL/min, remove the C18 column, and replace it with the C8 column (do not connect to the detector). Increase the flow to 1.2 mL/min and wash with six column volumes of mobile phase. Connect the column to the detector inlet and run until base line is flat.
3. Inject 15 μ L of the seven-component standards test mixture. Annotate and run an isocratic chromatogram.

4. Dial-a-mix 60% methanol/water. Equilibrate the C8 column. Inject 15 μL of the seven-component standards mixture. Annotate and run an isocratic chromatogram.
5. Put acetonitrile in B reservoir. Purge the pump inlet line acetonitrile, Dial-a-mix 60% acetonitrile/water. Reconnect the C18 column at 0.1 ML/min and equilibrate the column. Inject 15 μL of the seven component test mixture. Annotate and run the chromatogram.
6. Make up 100mL of 20% nitric acid (1 part acid added to 4 part water).
7. Very important Lab note: Remove the column! Replace the column with the column blank. Put water in the reservoir and wash the system at 2 mL/min with water.
8. Check your HPLC system manual it make to make system sure that compatible with nitric acid washing Make sure the reservoir sinker is made of stainless steel and not Monel metal. Replace the water in solvent reservoir with 20% nitric acid, Stop! *Note: Make sure the column has been replaced with the column bridge. Do not pump nitric acid through a boned-phase column. Discard the wash carefully.*
9. Wash the system with water (2 mL/min) (set UV detector at 230 nm, 2.0 AUfS) and monitor Baseline for disappearance of nitric acid. When baseline is flate or your class time has elapsed, collect effluent and check pH against laboratory water.

RESULT

10. Examine chromatograms of standard run on the C8 and C18 columns for peak shifting. Examine chromatograms run in methanol/water and acetonitrile/water for peak shifting. Measure last peak retention times in all three chromatograms. Look for peak switching by looking at peak height and positions.
11. Pacification of column-less HPLC with nitric acid: Observe the length of time necessary to wash all the nitric acid out of the HPLC system.