HPLC SAMPLE PREPARATION & METHOD DEVELOPMENT

PURPOSE

1. Run a scouting gradient.
2. Select SFE cartilage column withdrawing condition from the gradient.
3. Run SFE window cuts in selected dial-a-mix conditions.

Equipment and Reagents

1. Gradient HPLC system
2. C18 HPLC column (5 µm,15-25 cm)
3. C18 SFE cartilage columns(Whatman Part No. 6804-0405)
4. 5-ml B-D disposable syringes
5. Seven- components test mixture (P.J. Colbert cat No.962201)
6. HPLC-grade methanol and water
7. 10-ml test tube

METHOD

1. Purge pumps A with water and pump B with methanol. Dial-a- mix 20% B and equilibrate the column at 1.5 mL/min (six column volumes or a stable Baseline at 254 nm, about 10 min). (Lab Note: If the gradient system is a low-pressure mixing system, solvents must be degassed by purging or running under Helium.)
2. Inject 15 µL of the seven-component standard. Run a 15-min gradient to 100% for 5 min. Watch the chromatogram during the run record %B of the first and least peaks. For a 25-cm column, deduct 10% from the first peak's %B of the first and last peaks.
3. For a 25-cm column, deduct 10% from the peak's %B and equilibrate the column with this dial-a-mix isocratic. Equilibrate the column with this mobile phase.
4. Inject standards and rub the chromatogram.
5. Pretrate a C18 cartridge column with 2 mL, of water (Lab Note: Remove plunger from a 10-mL syringe tip. 2 mL of MeOH in the syringe Barrel. Put solvent and air through the

cartridge with the plunger and collect eluent in waste test tube. Do not pull out plunger Replace cartridge on tip. Go to next wash solvent.

6. Dilute 1 mL of standard solution 3-fold with water. Put sample in syringe barrel with cartridge in place. Insert the barrel and push the sample in to the cartridge. Collect the effluent as collect the effluent as collect tube.1 Based on the scouting gradient; select a window that should leave the three washes to window off standards. Select a window that should leave the three middle compounds in the second cut(Lab Note: In windowing from a scouting gradient, start at the injection mark and move to the last peak you want to wash Off in the first cut. Find its equivalent %B on the gradient trace. Deduct 7-10% B to find its isocratic equivalent for your first wash condition. Wash the cartridge with 1 mL of this Eluent. Collect it as collect it as collect tube. 2 On the chromatogram, move to the last peak you want in your second window fraction and determine its equivalent wash off %B. Wash the cartridge with 1 mL of 100% methanol. Collect this as collect tube 4.
7. Run 15 µL of each collect tube in isocratic dial-a-mix mobile phase chosen in step 4.

RESULT

8. Examine the four chromatograms of the breakthrough and windowing cuts (collect tube 1-4).Measure retention times of the peaks in each collect tube and compare with the final method development chromatogram. If you found more or less than three peaks in collect 3, or if some of the %Bs Of The window frame to improve cut3. Do not repeat the windowing experiment to prove your point!