Determination of Caffeine in Soda

Introduction:
High performance liquid chromatography (HPLC) is a type of chromatography which is very similar to liquid chromatography. In HPLC, however, the stationary phase column is more tightly packed than in other types of liquid chromatography. In this lab, the column is packed with C_{18} particles that are less than 10 μm in diameter. The small diameter of the particles allows unprecedented resolution and high efficiency. Since the particles in the column are so small, it is necessary to pump the mobile phase through the column at a very high pressure. The pump keeps a precise flow rate so that the positions of the peaks in time can be used to identify the species in a sample. This is done by comparing the chromatographs of prepared standards of the particular species to be determined. The common peak is an indication of the standard.

A small sample (20 μL) is injected into the injector port where the mobile phase moves it through the column. Each component, being different in physical composition, will move at a different rate through the C_{18} column. Thus, the components will be separated according to the size and shape of the molecules. The smallest and least hindered molecules will be eluted first since it is easiest for them to pass through the finely packed column. As each set of molecules elutes from the column, a detector (most often UV) recognizes it and records a peak. The area of this peak (in relation to the area of other peaks) is proportional to the concentration of that particular species in the sample. The identity can also be found by comparing the sample peaks to standards. Identical substances (peaks) will have identical retention times.

Purpose:
The purpose of this lab is to determine the amount of caffeine in a sample of soda.

Materials:

100 μL syringes	vacuum
254 nm UV detector	
60:40 methanol : water (HPLC grade,degassed)	
60 mL syringe isocratic HPLC system	
C_{18} column	caffeine standards
integrator	
	distilled water
	HPLC grade methanol
	soft drink sample
computer	printer

Safety:
- Always wear safety glasses in the lab.

Procedure:
Preparation of soda samples

1. Obtain a soft drink sample.

2. Degas the sample by placing it in a vacuum flask and connecting the flask to a vacuum pump or water aspirator. Leave it under vacuum until no more bubbles appear in the soda sample. (If no vacuum is available, allow the soda to stand open overnight.)

Preparation of caffeine standards

1. Prepare a 1000 ppm solution of caffeine.
2. Prepare standard caffeine samples of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm by diluting portions of the 10000 ppm solution with distilled water.

Instrument set-up

1. Obtain the degassed 60:40 methanol : water solvent for the mobile phase.
2. Place solvent tube with attached metal filter into the solvent bottle – the filter must be submerged. Place the thin tube with attached black trap into the waste bottle. Place the short thin tube with no attachment into another waste bottle.
3. Turn on the HPLC. (The switch is at the right rear above the power cord.) Prime the pump by attaching a 60 mL plastic syringe to the nozzle at the right front of the prime/purge valve. Open the valve by turning the knob to the left. Bring the flow of solvent up to 1.00 mL/min by using the up/down arrows on the front display. **Make sure the valve is open before pushing the prime button. If it is not, the column could be damaged.** Push the prime button and pull solvent into the syringe until no air bubbles are observed in the solvent tubing or the syringe. Stop the pump, close the valve, and remove the syringe. Press the run button and let the HPLC run approximately 15 minutes before injecting your sample. (Make certain that waste is collecting in the waste bottle.) Discard the solvent in the syringe in the waste bottle.
4. Turn on the computer and double click on the Peak Simple program. Once the computer and HPLC have communicated for awhile, Standby will appear in the upper right corner of the display.
5. Set the range to 0.020 absorbance by pressing up/down range arrows.
6. Press the Autozero button.
7. Make sure all bubbles are removed from the tubing and detector.
8. Clean the syringe by rinsing several times with the solution to be injected.
9. Rinse the 100μL syringe several times with your sample. Draw up sample to the 100 μL mark, and expell down to 80 μL; make sure that there is no air in the syringe.
10. Make sure the injector lever is in the LOAD position, and insert the sample with the syringe.
11. Move injector lever to inject position. The display in the upper right corner of the computer screen will change from standby to running in red letters. If it doesn't, press the spacebar, or click on acquisition, then run.
12. The run may be stopped by pressing the end key on the keyboard or clicking on acquisition, then stop and postrun. **Be sure to note the number of your run.** This can be observed by looking at the upper left line of the computer.
13. Return injector lever to LOAD position.
14. Print or save any runs if instructed to do so.
15. Repeat steps 7 - 13 for each sample (standards and sodas).

Analysis of data

1. Use standard caffeine samples to identify the caffeine peak and record the retention time of caffeine. The peak will increase from 20 to 100 ppm and will be after the small solvent peak.
2. Use the retention time to determine if caffeine is present in the soda sample.
3. To quantitatively determine the amount of caffeine in the sample, measure the caffeine peaks of the standards, and construct a standard curve.
4. Measure the caffeine peak in the soda sample chromatograph, and use the concentration to peak area relationship to determine the concentration of caffeine in the soda sample.

To shut down HPLC

1. Clean syringe with methanol (HPLC grade).
2. Run methanol as a sample a few times.
2. Run 100% methanol (HPLC grade) as a solvent for 5-10 minutes.
3. Turn off pump, detector, and computer.

Note: Failure to clean the column may result in a clogged column. Please clean after each class.

Data/Calculations:

Retention of caffeine in standards: _____

Standard (ppm)	Area
50	
100	
150	
200	
250	

List retention times and areas for the peaks in your soda sample(s). Use retention time to determine if caffeine was present, and use area to determine the concentration of the caffeine.

Questions:

1. Briefly explain how HPLC is used as a separation technique.

2. What is the purpose of the mobile phase? Of the stationary phase?

3. What is the purpose of the caffeine standards?

4. Why does the syringe have to be carefully rinsed before each use?

5. How could you be certain a peak in the soda was caffeine and not another substance with a similar retention time?