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 (100 μLx2) 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:

- isocratic HPLC system
- 254 nm UV detector
- C_{18} column
- integrator
- 100 μL syringes
- #42 filter paper
- funnel
- computer
- vacuum
- 60 mL syringe
- caffeine standards
- 60:40 methanol : water (HPLC grade,degassed)
- distilled water
- HPLC grade methanol
- soft drink sample
- 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.)
3. Filter the degassed soda through #42 filter paper.

Preparation of caffeine standards

1. Prepare a 1000 ppm solution of caffeine.
2. Prepare standard caffeine samples of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm by diluting portions of the 1000 ppm solution with distilled water.

Determination of caffeine content

1. Obtain the degassed 60:40 methanol : water solvent for the mobile phase.
2. Place solvent tube into the solvent and secure. Place the other line into the waste bottle.
3. Turn on the HPLC. Prime the pump using one of the 60 mL syringes. **Make sure the valve is open before pushing the prime button. If it is not, the column could be damaged.** Bring the flow of solvent up to 1.00 mL/min. Let the HPLC run approximately 15 minutes before injecting your sample. (Make certain that waste is collecting in the waste bottle.)
4. Set the range to 0.020 absorbance by pressing up/dn range arrows.
5. Press the Autozero button.
6. Make sure all bubbles are removed from the tubing and detector.
7. Clean the syringe by rinsing several times with the solution to be injected.
8. Draw up sample to the 100 μL mark. Make sure that there is no air in the syringe.
9. Make sure the injector lever is in the LOAD position, and inject two samples.
10. 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.
11. 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.
12. Return injector lever to LOAD position.
13. Print any runs if instructed to do so.
14. 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 100% methanol (HPLC grade) as a solvent for 5-10 minutes.
3. Turn off pump, detector, and computer.

Data:

Retention of caffeine in standards: _____

Soda	Retention Times	Caffeine present	Concentration

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?

Teacher Notes

Lab Time: Each group - 20 minutes for standards + 1 sample; 4 minutes for each additional sample.

Preparations:

Time: 30 minutes

Check mobile phase. If less than 300 mL prepare more. For each 100 mL to be prepared, pour 30 mL HPLC methanol and 70 mL HPLC water into a vaccuum flask. Put in a stir bar. Stopper, connect to vaccuum, and stir rapidly under vaccuum for five minutes.

Have several soda samples ready (with and without caffeine).

Ansers to Questions:

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

   HPLC uses a pump to deliver a mobile phase solvent at a uniform rate at an assigned pressure through a stationary phase column. The sample is injected into the column; components of the sample move through the column at different rates due to polarity. More polar components move more quickly than non-polar components.

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

   The mobile phase moves particles through the column while the stationary phase allows particles to remain in the column.

3. What is the purpose of the caffeine standards?

   The standards provide a frame of reference to which the data obtained from the soft drinks can be compared. A calibration curve for peak area vs. concentration is made. One can determine the amount of caffeine in the soft drinks by plotting data on this curve.

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

   Contaminents must be removed from the syringe.

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

   Change the mobile phase and inject the caffeine and then the sample. Both peaks should be affected similarly if they are both due to the presence of caffeine.

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