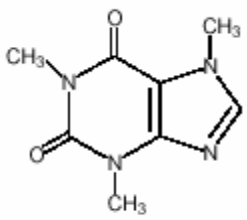
Determination of Caffeine in Beverages

HPLC-1

Introduction

This experiment provides an introduction to the application of High Performance Liquid Chromatography (HPLC) to the solution of complex analytical problems. Cola type drinks, coffee, and tea all are complex chemical systems that contain varying amounts of caffeine. The amount of caffeine present in these beverages can be determined by HPLC.



Caffeine

An isocratic HPLC using a reverse phase C_{18} column is used in this experiment. The mobile phase is 50% by volume methanol in water prepared from ultra-pure water and HPLC grade methanol. With the instrument that is being used, it is not necessary to degas the water or the methanol. Care should be taken not to aerate solutions when pouring them. The beverage samples will be filtered to remove any insoluble material.

In reverse phase HPLC the mobile phase is polar and the stationary phase in non-polar. When a sample is injected into the instrument, the mobile phase moves it through the column. Molecules in the sample that are polar interact more strongly with the mobile phase and elute from the column first. Non-polar molecules interact more strongly with the stationary phase and elute from the column last. As molecules elute from the column, they are detected, and a peak is recorded in chromatogram. The identity of a peak can be found by comparing its retention time (the time that it eluted from the column) to the retention time of known compounds. Identical substances will have identical retention times. The area of the peak is also important as peak area is proportional to the concentration of that particular species in the sample.

Purpose

To determine the amount of caffeine in various beverages.

Materials

Isocratic HPLC system
254 nm UV detector
C_{18} column
5 mL syringes
25 µL syringe
0.45µm syringe filters
Beverage samples
Small vials
Labels
Caffeine standards (50, 100, 150, 200 ppm)
50:50 Methanol:water
    Ultra-pure or HPLC grade water
    HPLC grade methanol
Kimwipes
Caffeine stock solution - 1000 ppm*
Small beakers*
Disposable pipettes*
Balances (0.001 g)*

*Required only if your class is preparing caffeine standards.

Safety

Always wear safety glasses in the lab.

Procedure

Preparation of samples

1. Obtain a beverage sample.
2. For soda samples, degas the sample by placing it in a vacuum flask and connecting the flask to a vacuum pump or water aspirator. Keep it under vacuum until no more bubbles appear in the soda sample. (If no vacuum is available, allow the soda to stand open overnight or place in a sonicator bath).
3. For instant powder beverages, prepare as indicated on the package for a normal serving using the methanol/water solution instead of just water. Any insoluble material will be removed in the filtering step.
4. Filter the beverage sample through a 0.45μm syringe filter with a 5mL syringe. Dispense the initial 1mL into waste. Dispense the remaining volume into the sample vial.
5. Rinse the syringe filter with three 2-3 mL aliquots of the methanol/water solution between each use.

Preparation of caffeine standards

Note: The caffeine standards can be prepared by your class or can be provided by SIM.

1. Prepare the following solutions from the 1000 ppm stock solution provided with this lab. These solutions will be prepared by mass.

Mass Caffeine Stock Solution (grams)	Final Mass (grams)	Caffeine (mg/L) [ppm]
1	20.0	50
2	20.0	100
3	20.0	150
4	20.0	200

2. Use a disposable pipette to deliver the stock solution to a small beaker and dilute to the final mass with the methanol/water solution.

3. Filter each standard solution through a 0.45μm syringe filter with a 5mL syringe. Dispense the initial 1mL into waste. Dispense the remaining volume into a labeled vial.

4. Rinse the syringe filter with three 2-3 mL aliquots of the methanol/water solution between each use.

At the instrument:

1. Aliquots consisting of 25-μL of the standards or samples should be injected.

2. Switch the manual injector to the "load" position and push at least three 25-μl volumes of each sample through the sample loop, making certain each volume does not contain air bubbles.

3. With the syringe still in the injector, turn to the "inject" position, at which time the instrument will automatically start data acquisition. Leave the injector in the "inject" position, and remove the syringe.

4. Rinse the syringe with at least 3 volumes of the methanol/water solution, dispensing the rinses into the waste beaker.

5. Beverages with a high caffeine content may need to be diluted. If the area of the caffeine peak for a beverage sample is greater than the area of the caffeine peak for the 200 ppm standard, dilute the beverage sample with the methanol/water solution and run again. Start with a 1:1 dilution.

Results

1. Use the caffeine standards to identify the caffeine peak.
2. Record the retention time and area of the caffeine peak for each of the standards on the Data Sheet. The peak area will increase from the lowest standard to the highest.
3. Use the retention time of the caffeine peak to determine if caffeine is present in the beverage samples.
4. If caffeine is present in a beverage sample, record the retention time and area of the caffeine peak on the Data Sheet.

Analysis of data

1. Using the data obtained for the standard solutions, prepare a *calibration curve* by graphing peak area versus concentration of caffeine in ppm.
2. Use the calibration curve to determine the amount of caffeine in your samples. Report your results as instructed by your teacher. Suggested unit are ppm, mg/mL, and mg/serving.
3. Use Table 1 to calculate the percent difference (error) between the results obtained in this experiment and the approximate amount of caffeine in your selected beverage.

Data Sheet

Name _____
Name _____
Class _____ **Period** _____
Date _____

Data (attach your calibration curve)

Standard	Retention Time	Area
50 ppm		
100 ppm		
150 ppm		
200 ppm		

Beverage	Serving Size	Caffeine (yes/no?)	Retention Time	Area	Conc. (ppm)	Conc. _____	Conc. _____

Calculations

Beverage	Experimental Conc.	Conc. from Table 1	% Difference (Error)

Questions

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

2. What is the purpose of the caffeine standards?

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

Table 1 – Approximate guide for caffeine amounts in common beverages.[1]

Beverage	Caffeine (mg/serving)	Serving Size (oz)	Caffeine (mg/mL)
Jolt	100	12	0.282
Mountain Dew	55.0	12	0.155
Diet Mountain Dew	55.0	12	0.155
Mello Yello	52.8	12	0.149
Surge	51.0	12	0.144
Coca Cola	45.6	12	0.128
Diet Coke	45.6	12	0.128
Mr. Pibb	40.8	12	0.115
Dr, Pepper	39.6	12	0.112
Pepsi	37.2	12	0.105
Diet Pepsi	35.4	12	0.100
Krank2O	100.0	16	0.211
Water Joe	100.0	16	0.211
Tea, Iced	70	12	0.197
Tea, brewed, imported	60	7	0.290
Tea, brewed, domestic	40	7	0.193
Tea, instant	30	7	0.145
Drip Coffee	80-135	7	0.652
Espresso	100.0	1.5-2	1.690–2.254

[1]National Soft Drink Association and Bunker and McWilliams, J. Am. Diet, 74:28-32, 1979.