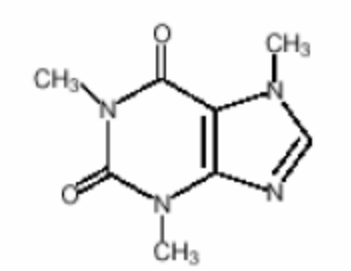
Determination of Caffeine in Beverages

Introduction



Caffeine

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.

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 containing 1% acetic acid. 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

25 – 50 µL syringe
0.45µm syringe filters with 5 mL syringes
Beverage samples
Small vials

Caffeine standards (50, 100, 150, 200 ppm)
50:50 Methanol:water
Ultra-pure or HPLC grade water
HPLC grade methanol
Caffeine stock solution - 1000 ppm
or Funnels with #5 filter paper
Small beakers
Disposable pipettes
Balance

Procedure

Preparation of samples

1. Obtain 1-2 beverage samples.
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 or use a #5 filter paper with gravity filtration. Transfer the filtered solution into a sample vial.

Preparation of caffeine standards

1. Prepare a 1000 ppm stock solution of caffeine in 1 liter of water.
1. Prepare the following solutions from the 1000 ppm stock solution using 250mL or 100mLvolumetric glassware.

Caffeine Stock Solution	mL 1000 ppm stock solution	Dilute to volume with 50:50 Methanol Water	Caffeine (mg/L) [ppm]
1			50
2			100
3			150
4			200

2. Use a pipette to deliver the stock solution to the volumetric flask and dilute to the final mass with the methanol/water solution.

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. Clean the syringe by rinsing several times with the solution to be injected. With the injector lever in the LOAD position, inject the sample.
5. Beverages with a high caffeine content 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 or 1:10 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. 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 and then calculate the caffeine content based on the calibration curve.

Analysis of data

1. Using the data obtained for the standard solutions, prepare a *calibration curve* by graphing peak area versus concentration of caffeine.

2. Use the calibration curve to determine the amount of caffeine in your samples. Suggested unit are ppm, mg/mL, and/or 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.

Questions to answer in you Lab report, either in the introduction, discussion, or results section.

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. Caffeine Content of Food & Drugs, http://www.cspinet.org/new/cafchart.htm

	Serving Size	**Caffeine (mg)**
Coffee, generic brewed	8 oz.	133 (range: 102-200) (16 oz. = 266)
Starbucks Brewed Coffee (Grande)	16 oz.	320
Coffee, generic decaffeinated	8 oz.	5 (range: 3-12)
Espresso, generic	1 oz.	40 (range: 30-90)
Starbucks Espresso decaffeinated	1 oz.	4
Tea, brewed	8 oz.	53 (range: 40-120)
Snapple, Lemon (and diet version)	16 oz.	42
Arizona Iced Tea, black	16 oz.	32
Nestea	12 oz.	26
Snapple, Just Plain Unsweetened	16 oz.	18

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	Serving Size	Caffeine (mg)
Arizona Iced Tea, green	16 oz.	15
Vault	12 oz.	71 (20 oz. = 118)
Jolt Cola	12 oz.	72
Mountain Dew MDX, regular or diet	12 oz.	71 (20 oz. = 118)
Coke Blak	12 oz.	69 (20 oz. = 115)
Code Red, regular or diet	12 oz.	54 (20 oz. = 90)
Mountain Dew, regular or diet	12 oz.	54 (20 oz. = 90)
Pepsi One	12 oz.	54 (20 oz. = 90)
Diet Coke	12 oz.	47 (20 oz. = 78)
TAB	12 oz.	46.5
Pibb Xtra, Diet Mr. Pibb, Pibb Zero	12 oz.	41 (20 oz. = 68)
Dr. Pepper	12 oz.	42 (20 oz. = 68)
Dr. Pepper diet	12 oz.	44 (20 oz. = 68)
Pepsi	12 oz.	38 (20 oz. = 63)
Diet Pepsi	12 oz.	36 (20 oz. = 60)
Coca-Cola Classic	12 oz.	35 (20 oz. = 58)
Coke Cherry, regular or diet	12 oz.	35 (20 oz. = 58)
Coke Zero	12 oz.	35 (20 oz. = 58)
Barq's Root Beer	12 oz.	23 (20 oz. = 38)
7-Up, regular or diet	12 oz.	0
Fanta, all flavors	12 oz.	0
Mug Root Beer, regular or diet	12 oz.	0
Sierra Mist, regular or Free	12 oz.	0
Sprite, regular or diet	12 oz.	0
Spike Shooter	8.4 oz.	300
Cocaine	8.4 oz.	280
Monster Energy	16 oz.	160
Full Throttle	16 oz.	144
Rip It, all varieties	8 oz.	100
Enviga	12 oz.	100
Tab Energy	10.5 oz.	95
SoBe No Fear	8 oz.	83
Red Bull	8.3 oz.	80
Red Bull Sugarfree	8.3 oz.	80
Rockstar Energy Drink	8 oz.	80
SoBe Adrenaline Rush	8.3 oz.	79
Amp	8.4 oz.	74
Glaceau Vitamin Water Energy Citrus	20 oz.	50
SoBe Essential Energy, Berry or Orange	8 oz.	48