

# Determination of Caffeine Content in Beverages with HPLC

## I. Introduction

Caffeine is a stimulant that is commonly found in many foods and drinks that we consume. Caffeine has a mildly addictive effect on the body; it is therefore interesting to know exactly how much caffeine is in certain beverages. One way to analyze caffeine content in beverages is by using high-performance liquid chromatography (HPLC). In this lab, we will prepare standard solutions of known caffeine concentration and compare them to common caffeinated beverages, such as soda, tea, and coffee.

## II. Experimental

Standard solutions of caffeine dissolved in deionized water ranging from 5-1000 ppm in concentration should be provided. For our analysis, we used four standards, at concentrations of 10, 25, 50, and 100 ppm. It should be noted that 1 ppm = 1 mg/L.

Samples are obtained by the following. A 1-ml aliquot of a standard is passed through a syringe filter and collected in a vial. 60  $\mu$ l of this filtered sample is injected into the HPLC. We used a flow rate of 2.0 ml/min; our mobile phase was a 65:35 mixture of water and methanol, run for 5 minutes. Caffeine elutes at approximately 0.9 minutes; the area of this peak is measured. Three replicates of each standard are taken, and a plot of peak area vs. concentration made.

Unknown samples are prepared according to the instructions of that type of beverage. For sodas, it is necessary to degas them by running them filter paper in a Buchner funnel under vacuum; the soda should be left for ~30 minutes after

filtration, or until no CO<sub>2</sub> bubbles are present. The beverages are filtered as before and analyzed in a similar fashion to the standards. Due to problems with the HPLC, the coffee data was taken using a flow rate of 1.0 ml/min.

### III. Data

#### Known Standards

Caffeine Concentration (ppm)	Average Peak Area (a.u.)
10	1893.225 ± 270.53
25	3576.725 ± 373.66
50	6764.05 ± 292.18
100	14240.8 ± 243.92

Slope of Best-Fit Line =  $138.404 \pm 5.361$   
Y-Intercept of Best-Fit Line =  $217.518 \pm 308.234$   
(See Appendix for calibration curve)

#### Unknown Beverages

Sample	Average Peak Area (a.u)	Caffeine Present in Sample (mg)
Lipton Decaf Tea	285.7666667 ± 10.925	0.273714392
Lipton Black Tea	2197.666667 ± 151.93	3.72719117
Safeway Green Tea	1671.233333 ± 26.417	2.776291388
Malawi Tea	963.9666667 ± 50.936	1.49875124
Coca-Cola	1385.233333 ± 13.269	2.259687822
Red Bull	3812.233333 ± 224.40	6.643592912
Folgers Decaf Coffee	262.2333333 ± 52.204	0.231205986
Folgers Regular Coffee	1203.266667 ± 156.67	1.931000308

### IV. Discussion

The results we present here are somewhat mixed in reliability. Although the HPLC is a very useful and indicative method of determining the caffeine content of certain beverages, mechanical problems affected the coffee and Malawi tea analyses. Specifically, the flow rate had to be reduced from 2.0 to 1.0 ml/min because of excessive pressure building in the column. Another factor is the high uncertainty in the calibration curve. Although each point has a relatively small uncertainty, producing a linear fit is quite difficult.

Our results show some recognizable trends. The decaffeinated coffee and tea showed very little caffeine in comparison to the regular variety. It is important to note however, that caffeine is not completely absent from decaffeinated beverages. The Lipton Decaf Tea, for example, even makes note of this on the box, although the amount of caffeine is 10 times less than the black tea variety. Our results deviate somewhat from this value.

As expected, Red Bull soda has far larger caffeine values than for regular Coca-Cola. Although we do not currently possess reference values, this large difference is not surprising. The coffee assays were marked by problems with the HPLC pressure, and I do not believe they are fully accurate as a result of this. In short, when the HPLC functions properly, it can be a very accurate, reliable method to determine the caffeine content of many popular beverages.

## Suggested Plan for Experimental

### *--Tuesday*

--Syringe filter known standards and run 3-4 replicates of each. Flow rate should be 2.0 ml/min, with a 65:35 mixture of water:MeOH. If the high flow rate is causing excessive pressure buildup, you should lower it.

--If time permits, degas any samples of soda you wish to. Let sit under vacuum for at least 30 minutes, cover, and store overnight.

### *--Thursday*

--Prepare unknown beverage servings by following package directions. You will need boiling DI water (approx. 2 L should be enough). Again, do 3-4 replicates of each. Ideally, you should be able to compare 3 teas, 3 sodas, and the 2 coffees.

### *--Data Analysis*

--For each trial, record the peak area of the caffeine peak. Average replicates, and construct a plot of peak area vs. concentration. Use this plot to determine caffeine concentration of unknown beverages.