Determination of the concentration of caffeine, theobromine, and gallic acid in commercial tea samples

Janna Erickson

Department of Chemistry, Concordia College, 901 8th St S, Moorhead, MN 56562

Abstract

Green tea, one of the most widely consumed beverage in the world, has many health benefits due to compounds such as caffeine, theobromine, and gallic acid. High-performance-liquid chromatography was used to determine the concentration of caffeine in commercial green tea samples.

Introduction

Green tea is the most commonly consumed beverage in the world after water.[1] It is derived from the tea plant *Camellia sinensis*. The extent of fermentation of the tea plant determines the type of tea. Oolong and paochong teas are partially fermented; black and red teas are fully fermented; and green teas are nonfermented.[2] The nonfermented green tea supposedly contains the highest concentration of polyphenols.[3] Polyphenols are powerful antioxidants which are compounds that may protect cells from damage by breaking down free radicals. Green tea has many different compounds, including proteins, carbohydrates, xanthic bases, pigments, volatile compounds, minerals and trace elements, and polyphenols and phenolic acids.[1] Some important compounds, such as those analyzed by Fernández et al. include caffeine, theobromine, and catechins such as gallic acid. Caffeine and theobromine are alkaloids that share a similar structure. The compounds also have similar effects as nervous system stimulants and diuretics. Gallic acid is a phenolic acid and an antioxidant. The structures of these three compounds are shown in Figure 1.
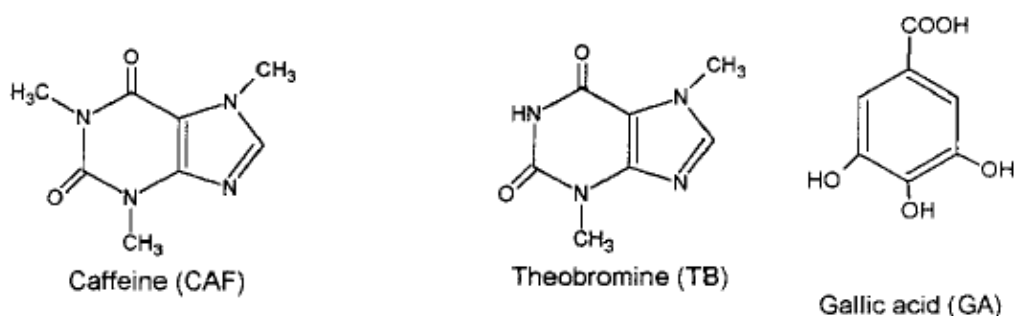

Figure 1. Structures of Caffeine, theobromine, and gallic acid

Green tea is growing in popularity in the United States because of its health benefits. It has been used as a medicine since ancient times. Chinese medicine has used it as treatment for headaches, body aches and pains, digestion, depression, and an energizer.[1] Green tea is also thought to have antimutagenic and anticarcinogenic potential, due to the large number of antioxidants. For that reason, the health benefits of green tea have been researched. It is important to determine the concentrations of such helpful compounds in

different tea samples. College students in particular are consuming more and more tea as its availability increases. Green tea is available in supermarkets, either in bag form or bottled, or in cafés and coffee shops. The composition of these teas may or may not be similar. It is important to know the relative concentrations of beneficial compounds such as caffeine, gallic acid, and theobromine. In a method based off of Fernández et al.[2], caffeine, theobromine, and gallic acid concentrations were attempted to be determined using HPLC analysis.

Experimental

Materials and reagents

Caffeine, HPLC-grade acetonitrile, acetonitrile, and formic acid were in stock. Theobromine and gallic acid were purchased from Sigma-Aldrich. Four brands of teas were analyzed. Three were in tea bags; one was a liquid. The samples were Bigelow Green Tea, Bigelow Organic Green Tea, and Stash Premium Green Tea. The liquid sample was Lipton Green Tea with Citrus.

Mobile Phase

The first mobile phase was ultra-pure filtered water. The second mobile phase was HPLC-grade acetonitrile.

Standard Preparation

Caffeine, gallic acid, and theobromine standards (200 ppm for caffeine and gallic acid and 100 ppm for theobromine) were prepared by dissolving 0.0557 g, 0.0534 g, and 0.0260 g, respectively, in a final volume of 250 mL of acetonitrile. The theobromine stock solution had to be heated to dissolve. A second caffeine standard was made by dissolving 0.0638 g of caffeine in 250 mL of distilled water. A standard of each stock solution was prepared by diluting 5-mL portions to 50 mL in a volumetric flask. Mixtures of 2 mL, 5 mL, 10 mL, and 15 mL portions of each stock solution were prepared in separate 100-mL volumetric flasks and diluted with acetonitrile for the first set of standards. The second set of standards was diluted with water.

Sample Preparation

The green tea leaves from the tea bags were weighed out (0.5 g). The samples were extracted in 125-mL Erlenmeyer flasks in 60 mL of acetonitrile and 40 mL of distilled water for about an hour with constant stirring. The extracted solutions were then filtered and diluted to 100-mL with distilled water. A 20-mL portion of each sample was diluted to 50 mL with distilled water. The liquid Lipton tea was sonicated, and a 20-mL portion was then diluted to 50 mL with distilled water. The sample solutions were filtered through a 0.45-μm syringe filter into auto-sampler vials.

Instrumentation

A Varian Prostar HPLC with a Varian Prostar 325 LCD detector was used for the HPLC analysis. The column used was a Kinetex 2.6u XB-C18 100A column at a temperature of 35°C. Mobile phase flow rate was 1 mL/min at a wavelength of 265 nm with an injection volume of 10 μL. The data was collected using a 90:10 mobile phase ratios.

Results

Chromatograms

Figure 2 displays examples of the chromatograms from the HPLC analysis. Figure 2A shows the 5-mL standard of caffeine, gallic acid, and theobromine. Figure 2B shows the chromatogram of sample 3, the Stash Premier green tea.
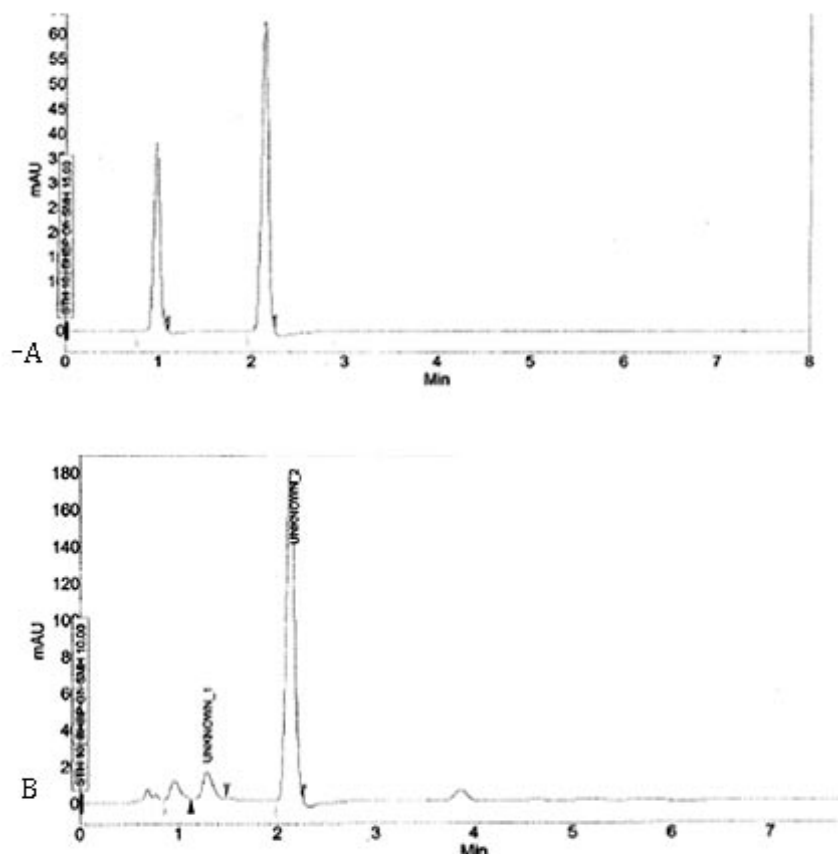

Figure 2. Samples of chromatograms. A) 5-mL Standard Chromatogram. B) Sample 3 chromatogram

The caffeine peak appeared around 2 minutes. The theobromine and gallic acid peaks both appeared around 1 minute. Different gradients were attempted in order to separate the two peaks, but due to time restrictions, these compounds were not quantified.

Calibration Plot

The caffeine standards were used to construct a calibration plot. It is shown in Fig. 3.

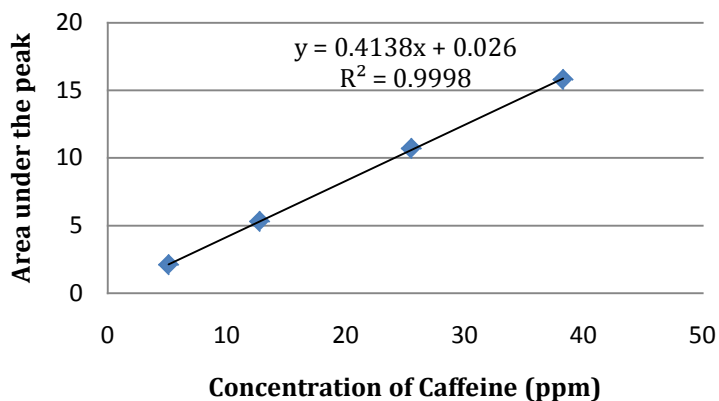| Table 1. Peak areas for caffeine standards	
Concentration in ppm	peak area (mAu)
5.104	2.1
12.76	5.3
25.52	10.7
38.28	15.8

Figure 3: Calibration plot of caffeine standards

Caffeine concentrations of the Samples

Using the regression equation from the calibration plot, the concentrations of caffeine in the four samples were calculated. The percent dry weight for the tea leaf samples were also calculated. The results are shown in Table 2.

Table 2. Concentration of Caffeine in Green Tea Samples

Sample Number	Sample Name	Concentration of Caffeine	Percent dry weight
1	Bigelow Green Tea	101.341	1.95%
2	Bigelow Organic	108.591	2.08%
3	Stash Premium	109.195	2.00%
4	Lipton Green Tea	41.5298 (in 16 oz)	NA

Using the stated concentration of caffeine in the Lipton Green Tea, a percent error was calculated. It was rather large: 32.18% error.

Discussion

Originally, a gradient elution was to be used for the HPLC analysis. However, this elution had a duration of about 20 minutes. It was time consuming. The column that was being used for this first set of HPLC runs was longer. However, the peaks of gallic acid and theobromine were too broad and small to be integrated. In addition, the caffeine standard also resulted in broad, overlapping peaks, indicating contamination of the sample. This was the reason the standards were remade.

A smaller column was installed for the next HPLC analysis, in hopes of attaining more defined peaks. The run-time was shortened to 8 minutes. Acetonitrile, gallic acid, and theobromine had retention times of around one minute. Caffeine had a retention time of about 2 minutes. The peaks due to acetonitrile, gallic acid, and theobromine overlapped. Both gradient and isocratic elutions were attempted. Also, the mobile phases were changed

from two different mixtures of water, acetonitrile, and formic acid to ultra-pure water and acetonitrile, in order to try to separate the compounds. However, the separation of gallic acid and theobromine did not work. Only caffeine concentrations were analyzed.

To improve upon this experiment, it would be necessary to develop a method of separating gallic acid and theobromine. The longer column worked well for separating; gallic acid appeared around four minutes, and theobromine around six minutes. However, the resolution and peak height would need to be improved. This could be done by finding a better detection wavelength or using more concentrated samples.

Another area to improve on would be the sample method. Only one chromatogram for four samples was obtained. More data is needed to perform statistical analyses. This could be why the percent error of the Lipton Green Tea was rather large; if there were more samples, the variance would be lower. Different samples should be analyzed as well, such as those from tea shops or other commercial teas like Peace Tea.

Conclusions

This experiment used HPLC to quantify the concentration of caffeine in commercial tea samples. Although theobromine and gallic acid were also used as standards, the retention times of the peaks were too close together. The percent dry weight of caffeine for the tea leaf samples were 1.95%, 2.08%, and 2.00%, for samples 1, 2, and 3, respectively. The percent error for the caffeine concentration in the liquid sample was 32.18%, which could be due to the small sample size. For future study, a method to separate the theobromine and gallic acid peaks should be used. Also, more samples could be used as green tea is becoming more and more popular in the United States.

Acknowledgments

References

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3) University of Maryland Medical Center. Complementary Medicine: Green Tea. http://www.umm.edu/altmed/articles/green-tea-000255.htm (accessed April 17, 2011).