Simultaneous determination of caffeine and theobromine in local area coffee brews

Kyle Czech, Alex Johnson, and Nathan Rodeberg
Department of Chemistry, Concordia College, 901 8th S, Moorhead, MN 56562

Abstract

The concentrations of caffeine and theobromine were determined using the method of external standards and high-performance liquid chromatography (HPLC). House brews from Atomic Coffee, Babs, Coffee Stop (Caribou Coffee), Dunn Bros., Concordia’s Maize (Minnesota Mudd and Nicaraguan), Moxie Java, and Starbucks were collected and analyzed. Dunn Bros. reported the highest amount of caffeine per ounce, while the Maize’s Minnesota Mudd showed the lowest concentration. Theobromine concentration was minimal in all samples of coffee.

Introduction

Coffee is one of the most consumed beverages around the world today, often to provide a burst of energy when needed. A chief ingredient of coffee is caffeine. Caffeine is a methylxanthine found in plants that increases the excitability of the central nervous system. On average, 90% of adults consume caffeine on a daily basis from beverages ranging from coffee, soda, tea, and others. There are numerous benefits and drawbacks to caffeine consumption. Because caffeine is a stimulant, it can provide energy, decrease fatigue, and enhance motor performance. Additionally, caffeine can help maintain attention when needed (not a characteristic lost on college students!). Coffee is often consumed by college students and other adults for this increase in energy and attention, so the amount of caffeine in various types of coffee is certainly of interest to the community.

However, there are drawbacks to caffeine as well. Caffeine can lead to addiction. Although it may not be as serious as an addiction to more harmful substances, withdrawal symptoms can occur after prolonged use, including headaches, fatigue, decreased attention, and general irritability. The U.S. Food and Drug Administration has declared a low to moderate intake of caffeine to be 130-300 milligrams a day, a high intake to be above 400 milligrams a day, and heavy and dangerous caffeine consumption to be 6,000 milligrams a day. Consumption at a high or heavy level for a prolonged amount of time can lead to chemical dependence.

According to literature sources, a plain, brewed coffee has a range of 102-200 milligrams per eight ounces (12.75 to 25 milligrams per fluid ounce). The amount of caffeine contained in a typical coffee depends on the type of coffee bean used to brew the coffee and how well the caffeine was extracted from the beans upon grinding.

Theobromine is also a methylxanthine like caffeine, and thus shows similar stimulating effects, though to a lesser degree, as it does not affect the central nervous system. Theobromine is highly prevalent in chocolate, as well as present in teas and cola nuts.
The most popular method for determining the levels of caffeine and theobromine in beverages is high-performance liquid chromatography (HPLC), which will be used for this particular experiment.\(^6\) It is also possible to use gas chromatography/mass spectroscopy (GC-MS) or simple UV/VIS spectroscopy.\(^4\)

**Experimental**

**Preparation of Mobile Phase**

A buffer for use as a mobile phase was prepared by dissolving 1.540 g of ammonium acetate in ultra pure water and diluted to 2.00 L. The pH of the buffer was then adjusted to 7.5 by adding 1.00 M NH\(_3\) solution drop-wise and monitoring with a calibrated handheld pH meter. Once the buffer solution had been adjusted to pH 7.5, the buffer was filtered with a Millipore 0.45-μm glass frit filtration system coupled with a 0.20-μm nylon filtration membrane. This filtered solution constituted the 20 mM ammonium acetate buffer that was employed in conjunction with HPLC grade methanol as the mobile phase.

**Sample Preparation**

Several 12-oz house brews were purchased from Atomic Coffee, Babbs, Coffee Stop (Caribou Coffee), Dunn Bros., Concordia’s Maize (Minnesota Mudd brew and Nicaraguan brew), Moxie Java, and Starbucks. When given the choice, the darker roast house brew was chosen for consistency. Each brew was poured into a separate 125-mL Erlenmeyer flask along with a magnetic stirring bar and stirred on a stirring plate. After being sufficiently stirred, 5 mL of each sample were pipetted into 100-mL volumetric flasks and diluted to the mark with distilled water. Eight separate 0.45-μm syringe filter were used to filter the sample solutions into the HPLC vials.

**Preparation of Standards**

To prepare the standard stock solution for caffeine, 125 mg of caffeine was transferred to a 250-mL volumetric flask. Approximately 150 mL of distilled water was added, and the flask was sonicated until the caffeine was fully dissolved. The solution was then diluted to the mark. Last, 10 mL were pipetted from this solution to a 100-mL volumetric flask and diluted to the mark to finish our standard stock solution of caffeine.

To prepare the standard stock solution for theobromine, 125.6 milligrams of theobromine was placed in a 1-L Erlenmeyer flask. Approximately 500 mL of distilled water was added to the flask along with a magnetic stirring bar. The flask was then heated and stirred until the theobromine fully dissolved. The solution was then transferred to a 1-L volumetric flask and diluted to the mark to finish our standard stock solution of theobromine.

Last, the five standards were prepared by pipetting 2 mL, 5 mL, 10 mL, 15 mL, and 25 mL caffeine into separate 100-mL volumetric flasks. The process was repeated for theobromine into the same respective volumetric flasks. Each of the five flasks was then diluted to the mark to finish preparing the standards. The concentrations of each compound in the standard solutions are shown in Table 1.
Table 1. Concentration of standard solutions (ppm)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Standard 1</th>
<th>Standard 2</th>
<th>Standard 3</th>
<th>Standard 4</th>
<th>Standard 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>1.00</td>
<td>2.50</td>
<td>5.00</td>
<td>7.50</td>
<td>12.50</td>
</tr>
<tr>
<td>Theobromine</td>
<td>2.512</td>
<td>6.28</td>
<td>12.56</td>
<td>18.84</td>
<td>31.40</td>
</tr>
</tbody>
</table>

**Instrument Preparation and Analysis**

An isocratic elution was carried out on the HPLC. The mobile phase ratio was a 20:80 MeOH:20 mM ammonium acetate buffer at pH 7.5. The flowrate of the system was 1.00 mL/min, with an injection volume of 20 μL. The column employed in this separation was a Phenomenex Kinetex 2.6 um XB C-18 and was kept in an external temperature control column oven at 35°C.

The HPLC system was a Varian modular Analytical/Preparative system controlled by Galaxie software. The Integration Events used to quantify peak areas were: Minimum Peak Height of 3.00 mAU and the baseline of each peak was defined by initiating Backward Horizontal Baseline by Peak. The wavelength under investigation was 272 nm. Calibration plots were produced within the Galaxie software as part of the method file. From the standards, the retention times of theobromine and caffeine were determined and used to identify each of the components of interest within the unknown coffee samples. The relationship between concentration and the peak area of theobromine and caffeine was determined through the production of calibration plots for each component.

**Results and Discussion**

**Calibration Plots**

The dependence of peak area on concentration of caffeine theobromine was determined by the Galaxie software included with the HPLC. The two line equations for theobromine and caffeine respectively are shown below.

\[
\text{Peak Area (mAU*min) = 3.32374[Theobromine] - 0.06981, } R^2 = 0.99999 \quad \text{Eq. 1}
\]

\[
\text{Peak Area (mAU*min) = 2.88371[Caffeine] + 0.21072, } R^2 = 0.9988 \quad \text{Eq. 2}
\]

![](image1.png)

Figure 1. Calibration plots of theobromine and caffeine.
Figure 2. Theobromine and Caffeine standard chromatograms reveal an overlaid depiction of each of the standard samples used to compose the calibration plots. The two components were confirmed to exhibit distinct retention times with exceptional resolution.

Figure 3. The overlay of the two Coffee Stop confirmed reproducibility regarding both peak area magnitude and peak retention times.
Table 2. Concentration of caffeine and theobromine in coffee (mg/oz). Concentrations were calculated using the linear regression equations from the calibration plots.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Caff. (Run 2)</td>
<td>12.86</td>
<td>-</td>
<td>13.00</td>
<td>16.09</td>
<td>10.20</td>
<td>12.37</td>
<td>-</td>
<td>15.95</td>
</tr>
<tr>
<td>Std. Dev</td>
<td>1.19</td>
<td>-</td>
<td>0.30</td>
<td>0.94</td>
<td>0.59</td>
<td>0.55</td>
<td>-</td>
<td>0.35</td>
</tr>
<tr>
<td>Theo (Run 1)</td>
<td>0.82</td>
<td>0.82</td>
<td>0.82</td>
<td>1.00</td>
<td>0.63</td>
<td>0.69</td>
<td>-</td>
<td>0.94</td>
</tr>
<tr>
<td>Theo (Run 2)</td>
<td>0.76</td>
<td>-</td>
<td>0.82</td>
<td>1.00</td>
<td>0.69</td>
<td>0.76</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Theo (Avg.)</td>
<td>0.79</td>
<td>-</td>
<td>0.82</td>
<td>1.00</td>
<td>0.67</td>
<td>0.73</td>
<td>-</td>
<td>0.97</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.04</td>
<td>0.04</td>
<td>-</td>
<td>0.04</td>
</tr>
</tbody>
</table>

A possible explanation for the variance of caffeine concentration between runs is different preparation of the coffee at the respective coffee shops. The way the coffee beans are grated produces higher or lower extraction of caffeine. These variables cannot be controlled unless the beans are bought from the coffee shops themselves and grated by the experimenters. The assumption is also made that each set of beans used to make each batch of coffee had roughly the same amount of caffeine. This assumption is a possible source of error, as batches of coffee can be made with varying amounts of beans. However, these assumptions were necessary to make because we didn’t buy the beans and brew ourselves. Variance in the preparation of coffee at these shops is natural, and anyone reading these results should be aware of the fact that the caffeine per ounce of each batch of brew will likely vary due to these variables.

As can be seen, the concentrations of theobromine were very low in each sample. We expect that concentrations in tea and chocolate would be much higher and show more significant result, as according to literature, theobromine is considered an important ingredient in tea and chocolate, while it is not mentioned in coffee to a similar degree.

Conclusions

This experiment utilized external standards and high-performance liquid chromatography to determine the amount of caffeine and theobromine in various coffee brews. The experiment produced reproducible data. Dunn Bros. had the highest average concentration of caffeine per ounce, followed closely by Starbucks. The Maize coffees exhibited smaller caffeine concentrations than the typical coffee brew, and had concentrations lower than the expected 12.75 mg/oz. All coffee brews showed negligible concentrations of theobromine. For more quantitative comparisons of theobromine concentrations, it is suggested that a different matrix, such as tea or chocolate, should be used.

References


3) Van der Stelt, O. Caffeine and Attention. 

   http://www.cord.edu/dept/chemistry/analyticallabmanual/
