Determination of caffeine and vitamin B₆ in energy drinks by high-performance liquid chromatography (HPLC)

Kristiana Sather and Teresa Vernig
Department of Chemistry, Concordia College, 901 8th St S, Moorhead, MN 56562

Abstract
The concentrations of vitamin B₆ and caffeine in four different energy drinks: NOS, AMP, Full Throttle, and Rockstar were found by standard addition using high performance liquid chromatography. Energy drink sales have been increasing since 2006 and are especially targeted towards college students, therefore, it is important to be able to efficiently determine and know the concentrations of the ingredients within the drinks. The results indicated that the experimentally determined concentrations of vitamin B₆ and caffeine differed significantly from the amounts listed on the Nutrition Facts found on the side of the cans. Despite this discrepancy, because both of the analytes were clearly detected using HPLC and since linear standard addition curves were produced, it was determined that HPLC provides a successful method of detecting and determining concentrations of caffeine and vitamin B₆ in energy drinks.

Introduction
Vitamin B₆ and caffeine are two main ingredients found in the newly popular energy drinks consumed by college students across America. Between July 2006 and July 2007 over 200 new energy drinks were introduced to the U.S. market and the sales of these drinks have continued to increase.¹ The popularity of these drinks is due to the fast-acting energy boost it gives consumers through caffeine, vitamins, carbohydrates, and other ingredients such as taurine. Although the consumption of caffeine and vitamins are recommended in certain amounts, the over-consumption of these ingredients could potentially be harmful.

Vitamin B₆ is a water-soluble vitamin that passes through the body more quickly than lipophilic vitamins by circulating in the bloodstream and being excreted in the urine.² Formally known as pyridoxine, Vitamin B₆ is present as pyridoxine hydrochloride in the multi-vitamin pill and energy drink form. Pyridoxine is a cofactor of several enzymes that catalyze decarboxylations, transaminations and racemizations of amino acids in the human body. Humans must acquire Vitamin B₆ from nutrient intake. In order to meet the normal/acceptable range of 100 mg of pyridoxine per day as proposed by the United States Food and Nutrition Board, humans must acquire Vitamin B₆ from nutrient intake.³ An over consumption of Vitamin B₆ can lead to neurological damage and disorders.³

The other analyte that will be determined is caffeine. Caffeine is a xanthine alkaloid and is used as a diuretic and a stimulant in the central nervous system. It is absorbed and distributed throughout the body by the circulation of blood flow to a final destination within the brain.⁴ Besides being a stimulant and diuretic, there are a variety of unpleasant side effects due to the over-consumption of caffeine which include: nausea, vomiting, restlessness, anxiety, depression, tremors and difficulty sleeping.⁵ Similar to Vitamin B₆,
Caffeine is not produced in humans, therefore, must be taken up by ingesting other nutrients that contain caffeine as a byproduct. The normal/acceptable range of caffeine for the average adult is 250 mg per day.4

Three common methods of analysis used to determine the vitamin B₆ and caffeine content within energy drinks include high performance liquid chromatography (HPLC), ultraviolet visible absorption spectrometry (UV-Vis) and thin layer chromatography (TLC). A newer and less common technique used in the analysis of energy drinks to determine vitamin B₆ and caffeine content, is the use of surfactant-mediated matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). When compared to UV-Vis, MALDI-TOF-MS yielded a “marked improvement” in its results and efficiency, which showed promising future directions into the rapid screening technique for fortified drinks.5

This study utilized the method of standard addition and HPLC with UV absorption detection to determine the concentrations of caffeine and vitamin B₆ in four energy drinks: NOS, AMP, Rockstar and Full Throttle.

Experimental

Preparation of Phosphate Buffer for Mobile Phase

A 50 mM pH 3 phosphate buffer solution was made for the HPLC mobile phase by dissolving a 21.305-g sample of Na₂HPO₄ in 1000 mL of deionized (DI) water and then adding concentrated hydrochloric acid drop-wise until it reached a pH of 3. The remaining solution was then diluted with DI water to a final volume of 3 L to make a 50 mM solution. This solution was then filtered in order to obtain a HPLC-grade phosphate buffer.

Standard Solution Preparation

A standard solution was made by combining 1920 mL of the newly filtered phosphate buffer with 1280 mL HPLC-grade methanol to make a 60:40 (v/v) phosphate buffer/methanol solution. A 0.0504-g amount of pyridoxine hydrochloride and a 0.1253-g amount of caffeine were weighed and transferred to the same 100-mL volumetric flask. This flask was then diluted to the mark with the phosphate buffer/methanol solution and sonicated in order to dissolve the pyridoxine hydrochloride and caffeine. This was labeled our standard solution.

Sample Preparation

Approximately 60 mL of the energy drink NOS was poured into a 125-mL Erlenmeyer flask and degassed by sonicating for five minutes. A 10.00-mL aliquot of the degassed energy drink was placed in each of the five 100-mL volumetric flasks. Increasing amounts of the standard solution was then placed into each of the five 100-mL volumetric flasks: 0.00, 1.00, 2.00, 3.00, and 4.00mL. The five volumetric flasks were then diluted to the mark with the phosphate buffer/methanol solution and mixed thoroughly. This was then repeated with the three other energy drinks: Rockstar, Full Throttle, and AMP.
Preparation of the Samples by Standard Addition

The twenty samples of the energy drinks were too concentrated to distinguish peak areas on the HPLC instrument. Therefore, all twenty samples were diluted with the 60:40 phosphate buffer/methanol solution to create final concentrations of 0.2016, 0.4032, 0.6048 and 0.8064 ppm for Vitamin B₆ and 0.5012, 1.0024, 1.5036 and 2.0048 ppm for caffeine. These 20 samples were then analyzed using the HPLC machine.

Instrument Preparation

For this experiment a Varian ProStar HPLC system with Model 410 autosampler and reversed phase column (Varian C-18 Microsorb-MV 100 Å) with a 3-μm particle size and column dimensions of 4.6 mm x 50 mm was used. The HPLC instrument was operated at a flow rate of 1.0mL/min for all 20 samples. The first trial used an isocratic elution of 60:40 (v/v) of phosphate buffer/methanol solution while the second trial used a gradient elution method of a 90:10 (v/v) solution of phosphate buffer/methanol solution in order to distinguish peak areas more accurately. The HPLC instrument had one reservoir, which contained the mobile phase of the phosphate buffer/methanol solution. Detection wavelengths were 272nm and 290nm for caffeine and vitamin B₆, respectively. Four standard addition plots were obtained for caffeine and vitamin B₆ from the standard addition of the standard solution to the energy drinks individually.

Results and discussion

Concentrations of Standards used in the Standard Addition

The concentrations of the standards added to the energy drinks were recorded and used for later calculations in the determination of the concentrations of caffeine and Vitamin B₆ in the different energy drinks (Table 1).

<table>
<thead>
<tr>
<th>Type of Solution</th>
<th>[Caffeine] ppm added</th>
<th>[Vitamin B₆] ppm added</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mL of Standard Addition</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1mL of Standard Addition</td>
<td>0.5012</td>
<td>0.2016</td>
</tr>
<tr>
<td>2mL of Standard Addition</td>
<td>1.0024</td>
<td>0.4032</td>
</tr>
<tr>
<td>3mL of Standard Addition</td>
<td>1.5036</td>
<td>0.6048</td>
</tr>
<tr>
<td>4mL of Standard Addition</td>
<td>2.0048</td>
<td>0.8064</td>
</tr>
</tbody>
</table>

HPLC Data

The standard addition samples were analyzed using the HPLC and data was collected using the Galaxie HPLC software program. Chromatograms were then able to be created using Excel (Figs. 1-4).
Figure 1. Chromatograms of standard addition solutions of differing concentrations of Caffeine and Vitamin B₆ in Full Throttle at 272 and 290 nm.

Figure 2. Chromatograms of standard addition solutions of differing concentrations of Caffeine and Vitamin B₆ in AMP at 272 and 290 nm.

Figure 3. Chromatograms of standard addition solutions of differing concentrations of Caffeine and Vitamin B₆ in NOS at 272 and 290 nm.
Standard Additions

Separate standard addition plots for each drink and each analyte were created using the trapezoid method of calculating the area under the caffeine and Vitamin B₆ peaks from the chromatograms that had been created in Excel (Figs. 5-8). One of the standard additions of the caffeine was discarded from the Full Throttle and Rockstar standard addition plots, along with one of the standard additions of Vitamin B₆ in Rockstar. This is because these points deviated greatly from the trend lines created and from the other data presented.

Figure 5. Standard addition plots of [Caffeine] and [Vitamin B₆] in Full Throttle

Figure 6. Standard addition plots of [Caffeine] and [Vitamin B₆] in AMP
The calculated Caffeine and Vitamin B6 concentrations were compared to literature values and the percent errors were calculated and recorded (Table 2). The calculated concentrations and literature values differed significantly, especially for the Vitamin B6 data. Whereas the literature values obtained for the concentration of caffeine were in milligrams per 16 ounces, the literature values for the Vitamin B6 concentrations were found in percents of the recommended daily value. Because this data was given as a percent rather than an exact amount, this could explain the large discrepancy between the calculated and literature values of Vitamin B6.

Another possible explanation for the large discrepancy between the calculated and literature values, could be because of the presence of other compounds found within the drinks. Energy drinks contain a variety of ingredients such as taurine, sugars and other compounds which could have similar retention times as the caffeine and vitamin B6, thereby making it difficult to get distinct peaks. The other ingredients could also interact with the two analytes causing indistinct results. To obtain more defined peaks of the Vitamin B6 compound, a gradient-elution method was used rather than an isocratic method. Although it appeared defined in the chromatograms when using the gradient-elution method, there may have still been a small peak from a different compound that interfered with Vitamin B6 data. Furthermore, two caffeine peaks near each other were obtained when the gradient-elution method was used, therefore, it was difficult to calculate an exact caffeine peak area. This could have also increased the percent error.
Conclusions

The Caffeine and Vitamin B6 concentrations in four different energy drinks were determined using a standard addition method and high-performance liquid chromatography. A gradient-elution method was used in order to create separate peaks, and the wavelengths 272 and 290 nm were used in order to promote maximum absorption from the Caffeine and Vitamin B6, respectively. The Caffeine and Vitamin B6 peak areas were determined using the trapezoid method of calculating the areas under the curve, which were then used to generate standard addition plots of the data using the same software. A difference from what was expected from the literature values, was that the caffeine content was found to be the greatest in Rockstar, followed by NOS, AMP and then Full Throttle. The Vitamin B6 concentrations were more variable in comparison to the literature values and yielded large percent errors. Although the comparison of the calculated and literature values of the analyte concentrations yielded large percent errors, the standard addition plots yielded R² values close to 1, which implies that the method of standard addition was successful. By analyzing the standard addition plots that were generated, we determined that HPLC is a useful method for determining concentrations of analyte in energy drinks.

Acknowledgments

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References


