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HPLC Caffeine Quantification of Various Energy Drinks and Caffeine Consumption Habits Survey among Young Adults

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ABSTRACT

The level of caffeine consumption has been on the rise over the past decades and a large-portion of that is due to the recent boom in energy drink popularity, especially in the adolescent and young adult populations. The caffeine content regulation of these energy drinks has become an increasingly controversial focus for the FDA, public health officials, manufacturers and consumers because of the 1994 Dietary Supplement Health and Education Act that classifies these drinks as dietary supplements rather than regulated beverages. Knowing how much caffeine is actually present in these beverages rather than relying on the labels imposed upon them is very critical. A Waters® 2695 Separations Module HPLC system and a Waters® 2487 Dual λ Absorbance Detector were used to quantify the caffeine content of six different energy drink brands. Red Bull, AMP, Rockstar and Monster all were found to have significant differences between the mean experimental concentration found and the value given on the nutritional label, with $p < 0.001$. NOS also showed a significant difference in its caffeine content with a $p < 0.02$. Full Throttle showed no significant difference between the values reported. Measuring the caffeine concentration in two portions separated by ~10 days showed a loss of caffeine. Voluntary surveys were given to college students and included a demographic questionnaire and caffeine consumption journal that showed patterns based off consumption levels. Key similarities in stimuli and effects of caffeine consumption were also identified.

Keywords: *Caffeine, HPLC, quantification, energy drink, caffeine consumption, caffeine survey, caffeine regulation, reversed phase high performance liquid chromatography*

INTRODUCTION

Caffeine is one of the most widely consumed active food ingredients throughout the world (Heckman, Weil, et al., 2010). The consumption of caffeinated products has become a widespread occurrence in everyday life for many people around the world. The regulatory aspects pertaining to the addition of caffeine to products, such as beverages, has had a challenging history and continues to be a growing problem. According to FDA, at least 80% of adults in the U.S. consume caffeine every day (Wolf 2013). Recent reports have indicated that nearly 75% of U.S. children and young adults consume at least some caffeine, mostly from soda, tea and coffee, but alarmingly enough, soda use has declined and energy drinks have become an increasingly common source of caffeine intake (Tanner, 2014). Measures have been taken around the world to regulate the labeling, distribution, and sale of energy drinks that contain significantly larger quantities of caffeine (Reisseg et al., 2009).

Caffeine is the common name for the alkaloid, 1,3,7-trimethylxanthine, that is found naturally in the leaves, seeds and fruit of tea, coffee, cacao, kola trees and more than 60 other plants (Andrews et al., 2007). Inside the body, one of caffeine's most important effects is to counteract a substance called adenosine that controls the sleep-wake cycle by releasing dopamine and regulating nerve cell activity. The metabolic products of caffeine also contribute to its whole physiological effect: paraxanthine boosts the lipolysis process for an increase of fuel for the muscles; theobromine is a vasodilator that expedites oxygen and nutrient flow to the brain and muscles; and theophylline acts as a smooth muscle relaxant that increases heart rate and force of contraction (Dews 1984).

One of the most popular techniques for the determination of caffeine in different mixtures is the use of HPLC, or high-performance liquid chromatography (Srdjenovic et al., 2008). The Reversed Phase HPLC method for the quantification of caffeine in beverages has been found to be simple, precise, sensitive and accurate and allows for the obtaining of good results (Ali et al., 2012).

Figure 1. Molecular Structure of Caffeine

The availability and consumer demand for caffeine has risen with the introduction of functional beverages, including the energy drinks category, as well as other beverages such as caffeinated sport drinks, juices, and waters (Heckman, Weil, et al., 2010). Energy drinks have had exponential growth since they arrived in the United States and the trend is expected to continue, especially because of the habits of today's society, who are always stressing over their abundant workload and their depletion of energy (Heckman, Sherry, et al., 2010).. Energy drink companies advertise the great surges of energy coupled with productivity that come with consumption of their products, yet these companies continually slide past the grasps of federal regulatory agencies. These manufacturers are not restrained by prior caffeine limits that affect beverages, such as soda pop, by appealing to their products as dietary supplements, not drugs, so that they are protected under the 1994 Dietary Supplement Health and Education Act (Reissig et al., 2009). These hidden dangers of over caffeine consumption are significant for unaware consumers.

Therefore, the study focuses on proper quantification of caffeine levels in various brands of energy drinks and the comparison to their given values in order to show any significant difference provided by energy drink labels. Successively, the study also engrosses a panel of surveys given to voluntary subjects encompassing demographics and caffeine consumption habits.

MATERIALS AND METHODS

Sample Acquisition: Six different brands of energy drinks were chosen for this experiment. They included Red Bull, Full Throttle, AMP, Rockstar, Monster, and NOS. All samples were bought from the Walmart in McPherson, KS, and their serving sizes and theoretical caffeine concentrations are recorded in Table I (Caffeine Informer, 2014). Each sample was analyzed using the Reverse Phase HPLC system and repeated nine more times for greater statistical power.

Caffeine Standard Solution Preparation: The caffeine standard solution of 500 µg/mL was prepared by dissolving 0.5000 g of Lab Grade Caffeine Standard (Sigma-Aldrich) in 1.0 L of distilled water. Constant stirring in low heat was utilized until all the caffeine had completely dissolved. Five 50-mL volumetric flasks were marked for each standard dilution. The dilutions were done with additional distilled water and included the concentrations: 500µg/mL (stock), 400µg/mL, 300 µg/mL, 200 µg/mL and 100 µg/mL. Afterwards, 1.5 mL of each solution was pipetted into a labeled Waters® Certified screw top vial, 12x32 mm, and loaded into the corresponding number carousel slot. The remaining solutions in the flasks were sealed with parafilm and stored for continued use in the HPLC.

Energy Drink Sample Preparation: Once a sample can was opened, ~50 mL of the drink was transferred into a 50-mL volumetric flask and degassed by sonication in an ultrasound bath for 30 min with occasional stirring to release any bubbles trapped on the inner wall of the flask. After which, 1.5 mL of each sample was pipetted into a labeled Waters® Certified screw top vial, 12x32 mm, and then loaded into the numbered slot in the HPLC carousel corresponding to its overall sample number. All samples were run in the RP-HPLC five times, in injection volumes of 10 µL per run, and at the experimental conditions subsequently specified. The relative integrated peak areas were determined for all replicates of each sample. The concentration of each replicate was then calculated using the caffeine calibration curve.

Experimental and Instrumentation Preparation: All the reagents used in this study were of Lab or HPLC grade and prepared with use of distilled water. The HPLC system used in this study was the Waters® 2695 Separations Module, fully equipped with a four-channel inline vacuum degasser, integral plunger seal-wash system, column & sample heater/cooler, and a Waters® 2487 Dual λ Absorbance Detector. The analytical column used was a XBridge C18 3.5 µm with an internal diameter of 4.6 mm and length of 150 mm (Waters® Corporation, Wexford, Ireland). As for experimental conditions and the solvents used, the sample and column chambers were heated and ran at a steady 40°C and the solvents included Acetonitrile, Distilled Water, and a 97:3 H_2O/Acetic Acid solution. The solvents were first run through the inline vacuum degasser in order to reduce the total amount of dissolved gas in the mobile phase. After which, the solvent management system was dry primed manually through a vent valve using a syringe to remove any bubbles that may have become trapped within the solvent loops. This was followed up by a wet prime and equilibration of the solvents that flowed each individual solvent through the system at 7.500 mL/min for 2 min in order to replace any solvents left in the path with the appropriate solvents and by equilibrating the solvents in the vacuum degasser, the initial solvent composition for runs was degassed and primed for suitable use. The initial solvent composition of the eluent used was 85% (97:3 H_2O/Acetic Acid solution), 15% Acetonitrile. The eluent flow was set at 1.2 mL/min. The pump gradient for sample runs was set for 7 min total. The first minute had a solvent composition of 85% Acetic acid solution and 15% acetonitrile, then for the next 4 minutes it adjusted into a 10% acetic acid solution and 90% acetonitrile composition before returning to its original 85% to 15% composition for the final 2 min of the run. The detection limit was set at UV 275 λ nm. The chromatographic results were processed and compiled by the MassLinks Water Software. The data was then systematized and analyzed using Microsoft Excel.

Survey Design: Subjects were chosen at random and aimed at current college students. A total of 27 surveys were taken and assessed into the study. The surveys were completely voluntary and consisted of a demographics questionnaire followed by a week-long journal tallying the individual servings of each respective caffeine source. The demographics consisted of gender, age, height, weight, ethnic background and most recent grade level. The consumption journal ran through seven consecutive days and serving sizes were based off 8 fl oz increments per tally. The weekly total caffeine consumption was summed up at the end of the week.

Table I. Theoretical Caffeine Concentrations of energy drinks

Energy Drink Brand	Serving Size per can (fl oz)	Caffeine Concentration* (µg/mL)
NOS	16.0	338.27
AMP	16.0	300.21
Full Throttle	12.0	338.98
Rockstar	16.0	338.27
Monster	16.0	338.27
Red Bull	8.4	320.00

-All samples were bought at Walmart in McPherson, KS
**Values based on information from CaffeineInformer.com, 2014*

RESULTS

Linearity: A calibration curve was generated before every set of samples ran through the HPLC. Five concentrations of caffeine, starting at 100 µg/mL and increasing by increments of 100 µg/mL up to 500 µg/mL, were measured at the previously recorded experimental conditions. Figure 2 displays the chromatogram of a caffeine standard of 500 µg/mL . The Peak areas were plotted against the concentrations of the respective standard solutions and generated the calibration curve used for the determination of caffeine content in each sample. Figure 3 shows an example of a generated calibration curve.

Caffeine Content Determination: Using the computed peak areas from the chromatograms of each sample and the regression equation, we calculated each energy drink sample concentration in µg/mL. Figure 4 displays the chromatogram obtained for a sample of Red Bull.

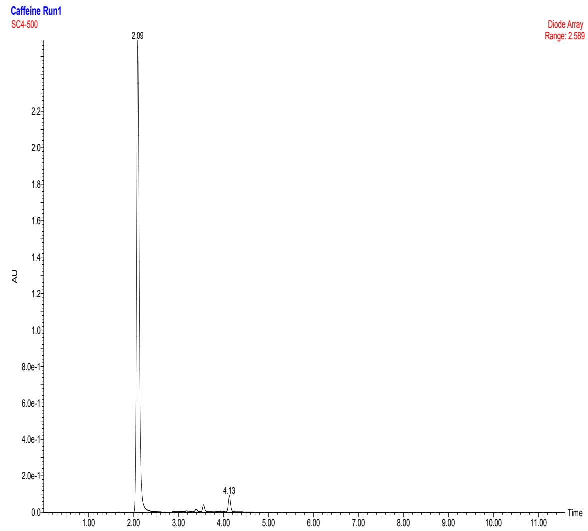

Figure 2. 500 µg/mL Caffeine Standard Chromatogram

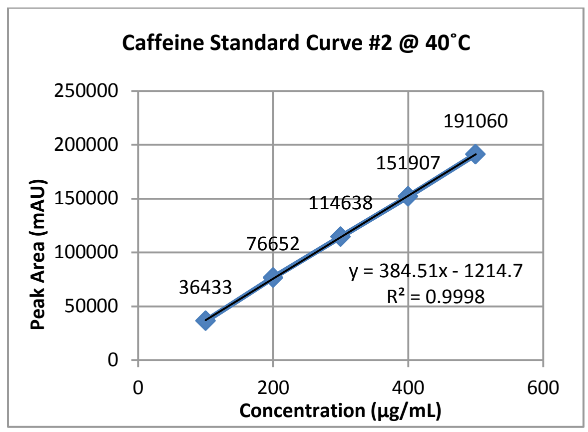

Figure 3. Example of Caffeine Standard Curve

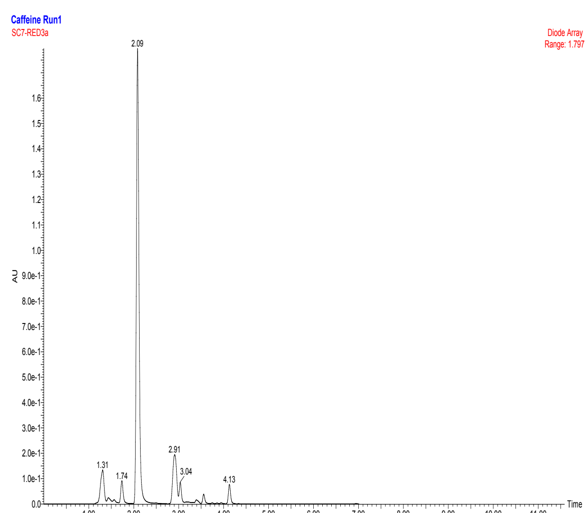

Figure 4. Red Bull Sample Chromatogram

The highest concentration was found in the Rockstar energy drink samples with a mean concentration of 364.23 μg/mL. The least concentrated was the Red Bull energy drink at 323.92 μg/mL. The RP-HPLC results for the caffeine analysis of the energy drink samples were collected and recorded in Table II. The average caffeine concentrations and standard deviations of each of the brands were calculated based off the caffeine standard curve corresponding to the RP-HPLC run on each different date. After RP-HPLC analysis of the energy drink samples, a one-sample T-test of each energy drink brand's average experimental caffeine concentration to that of the label's value was conducted and are also recorded on Table II. Samples from three different brands were measured ~10 days apart from their same brand complements and showed a loss of caffeine.

Table II. Compared Concentrations in Various Energy Drink Brands and T-test values

Brand	Theoretical Concentra-tion (μg/mL)	Caffeine Concentration Average ± SD* (μg/mL)	T-test (p-value)
Red Bull (A)	320.00	323.92 ± 1.44	p<0.001
AMP (B)	300.21	347.41 ± 3.67	p<0.001
Full Throttle (C)	338.98	337.14 ± 8.24	p=0.166
Rockstar (D)	338.27	364.23 ± 10.89	p<0.001
Monster (E)	338.27	363.07 ± 2.08	p<0.001
NOS (F)	338.27	334.35 ± 9.85	p=0.016

*SD- Standard Deviation

Survey Characteristics: Once the surveys were completed, the demographic and caffeine consumption data was compiled into table III. Each of the characteristics were computed into percentages of the total surveys taken. The mean age of the subjects was 21.81±1.84 years. The mean height and weight of the subjects were 65.81±3.95 in. and 138.78±35.23 lbs., respectively. The daily caffeine consumption ranged from 0 mg/day to 500 mg/day with a mean consumption of 194.24 mg/day.

Table III. Demographic Characteristics and Daily Caffeine Consumption of Survey Takers

Characteristic	Percent of Subjects (%)
Sex	
Female	59.3
Male	40.7
Level of Education	
Freshman	18.5
Sophomore	14.8
Junior	18.5
Senior	48.2
Ethnic Background	
Hispanic/Latino	70.3
White	22.2
Asian	3.75
Black/African-American	3.75
Mean Caffeine Intake (mg/day)	
<100	29.6
100-199	25.9
200-299	22.2
300-399	3.75
400-499	14.8
≥500	3.75

DISCUSSION

The focus of this study was to determine the caffeine concentrations in six different brands of energy drinks and compare them to their labeled values in order to determine for any significant differences between them. After using the one-sample T-tests on each brand of energy drink, significant differences were identified for Red Bull, Amp, Rockstar, Monster with values of p<0.001. Even though significant statistical differences were found in these samples, in the larger nutritional scope, the increased concentration of caffeine only accounted for 0.98 mg/can, 22.33 mg/can, 12.28 mg/can, and 11.73 mg/can, respectively. According to a research review regarding caffeine consumption, a healthy moderate daily caffeine intake would be ≤400 mg (Heckman, Weil, 2010). Therefore, such increases in caffeine concentrations may not have as dangerous implications as thought before as long as the total daily consumption is within the 400 mg parameters. NOS also showed a significant difference in its caffeine content with a value of p< 0.02. This is consistent with reports that NOS had lowered the caffeine content of their energy drinks from 260mg to 160mg per 16 fl.oz. can in order to avoid further FDA scrutiny (Caffeine Informer, 2014). Full Throttle showed no significant difference between the value reported and the experimental value found.

The second part of the study surveyed the caffeine consumption habits of young adults. Of the 27 subjects, 5 of them, or approximately 18-20%, were consuming over the 400 mg daily recommended value of caffeine. Six subjects reported no caffeine intake from any of the beverages specified. Several subjects described their caffeine consumption to be stimulated by “the need to stay awake for long hours”, “acquired taste”, “need for energy” and “it has become a habit”. Consequently, there were also reports of “feeling irritable”, “need or urge to consume caffeine", and "restlessness" when there was a lack of caffeine consumption. These specific symptoms are common features of the adverse effect of Caffeine Intoxication that is a recognized clinical syndrome included in the Diagnostic and Statistical Manual of Mental Disorders and the World Health Organization's International Classification of Diseases (Reisseg et al., 2009). Therefore, we can conclude that even though there was an observed statistical difference between some of the energy drinks, they still did not have a large enough impact on the overall recommended daily consumption value for caffeine.

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