HPLC determination of caffeine in headache medicine: Does brand matter?

Kamiya Coles

Dr. Diane Nutbrown

Science Senior Seminar

The Neighborhood Academy

May 2021

ABSTRACT

Caffeine is one of the most consumed stimulants and 80% of the population worldwide consume caffeine daily whether it is coffee, medication or any other caffeinated products. Having the acknowledgment of people taking pills, we want to know about whether there are differences between the name brand medications and the generic brand medications that have caffeine in them. People who consume caffeine pills take them to reduce the pain they are experiencing. High-Performance liquid chromatography (HPLC) is a type of chromatography used to separate and analyze complex mixtures of different substances using a liquid mobile phase. In this case, the HPLC instrument will be used to measure the amount of caffeine in each pill. There were two different HPLC systems that were used: Agilent Eclipse Plus C18 which was used at the University of Nebraska-Omaha by Dr. Andrew Miller. The other system that was used was an Agilent 1100 HPLC with autosampler and it was used at the Regis University by Dr. Lynetta Mier. When looking at Excedrin and Walgreens Migraine relief, there was a question of whether the name of each brand had any differences. There are no differences to the medications beine name-brand and generic. They both serve the same purpose, which is to reduce the amount of pain in the head. The concentrations of both medications were tested to see if there were any differences in the amount of caffeine being included in the medication.

INTRODUCTION

Caffeine is a natural stimulant present in several different products such as coffee, medication, and other foods and beverages (*1*). Caffeine is consumed daily by up to 80% of the population worldwide whether it is the consumption of coffee, medication, or any other caffeinated products (*2*). Caffeine has the formula of $C_8H_{10}N_4O_2$ and the official name for caffeine is 1,3,7-trimethylxanthine (Figure 1) (*3*). Since caffeine absorbs UV light, several techniques that employ UV detection can be used to measure the amount of caffeine in a sample. In this proposed study, the concentration of caffeine will be determined in brand name and off-brand migraine medications using high-performance liquid chromatography (HPLC).

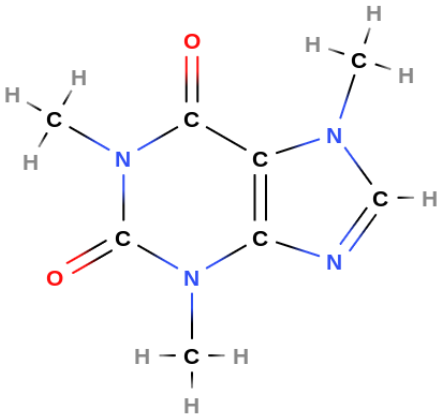

Figure 1. Structure of Caffeine (*4*)

Caffeine is the most commonly consumed stimulant in the world (*5*). Caffeine affects the human body in various ways such as changing one's mood, stamina, and wakefulness (*1*). Caffeine is used within medications to relieve and improve tension for head pain. According to the FDA (Food and Drug Administration), a low to moderate amount of caffeine is 130 mg to 300 mg per day, a moderate amount is 200 mg to 300 mg per day, high doses are above 400 mg and heavy consumption of it would be more than 6,000 mg per day (*1*). An average 8 oz cup of coffee has 95 mg of caffeine in it, in a 12 oz can of Pepsi there is 38 mg of caffeine, and in a typical dose of headache medicine like Excedrin Migraine there is 65 mg of caffeine (*1*).

Caffeine is included in other common over-the-counter (OTC) headache medicine due to the fact that caffeine enhances the efficacy of several analgesics. Lipton, et al. reviewed seven clinical trials that were placebo-controlled and focused on migraine/tension-type headaches treated only with OTC pain medicine. The key takeaway was that all major/common pain relievers ibuprofen (e.g. Advil), acetaminophen (Tylenol), acetylsalicylic acid (Aspirin), and naproxen sodium (Aleve) are more effective when combined with caffeine. The conclusions drawn by Lipton, et al. corroborate earlier findings that

suggest caffeine increases the effect of acetylsalicylic acid (aspirin) and acetaminophen (Tylenol) by 40%. (Laska Em et al 1984 - #32 from Lipton paper) A typical dosing of caffeine is 130 mg for tension-type headaches and at least 100 mg for migraines (*6*).

Whether caffeine has been a trigger or a cure for migraines and headaches, caffeine has been an ongoing topic in different researches because no one has had the answer. People consume caffeine to help control the pain of migraines and headaches but, it turns out that it can possibly be the cause of both migraines and headaches. Caffeine withdrawal and overusing it, can be one of the causes of migraines for some people (Nowaczewska 1, et al.) (*2*). Caffeine monotherapy is related to pain relief more than placebo when treating migraines and tension type headaches (Lipton, et al.) (*6*).

To streamline our literature search for methods to quantify caffeine, we focused our attention on chemical education journals that described experiments that were reasonable for an undergraduate student to complete. Several straightforward laboratory experiments have been published such as, Simultaneous Determination of Caffeine and Vitamin B6 in Energy Drinks by High-Performance Liquid Chromatography (*7*), The Determination of Caffeine in Coffee: Sense or Nonsense? (*8*), Quantitative HPLC Analysis of an Analgesic/Caffeine Formulation: Determination of Caffeine (*9*), Analysis of Soft Drinks: UV Spectrophotometry, Liquid Chromatography, and Capillary Electrophoresis (*10*), and Quantitative Determination of Caffeine in Beverages Using a combined SPME-GC/MS Method (*11*). In the paper titled Quantitative HPLC Analysis of an Analgesic/Caffeine Formulation: Determination of Caffeine, Ferguson compared a study that had been done in 1983 by Kagel and Farwell to show that her experiment is an improvement compared to the earlier study. Their goal was to separate components of Goody's Extra Strength Headache Powders (*9*). In this research study, we will be referring to the paper above and will be looking at the materials that were used.

HPLC (High-Performance Liquid Chromatography) is a type of method used to separate and analyze small compounds. Chromatography starts by dissolving the mixture in the mobile phase and then flows through the stationary phase (*12*). In the stationary phase which is also referred to as the column, a separation occurs and it is filled with beads. Chromatography works because molecules that have similar polarities will be attracted to each other, this is because of intermolecular forces which cause attraction between particles. In normal phase HPLC, the stationary phase is polar and the mobile phase is nonpolar. In reversed phase HPLC, interactions are based on compounds' lipophilicity. Compounds that are lipophilic (nonpolar) interact more with the beads that are also lipophilic. Compounds that are hydrophilic (polar) will avoid the beads and will go through the column. The different compounds in the mixture will then interact with both the beads and the mobile phase. An analyte that has minimal interaction with the stationary phase will tend to move the fastest through the column. Analytes that interact the most with the stationary phase will stay longer in the column. Hence, mixture components are separated primarily on

the basis of polarity and their retention times are related to their affinity for the stationary phase (i.e. the greater the affinity for the column, the longer the retention time). The presence of mixture components is identified by the detector of the HPLC system. The different types of detectors are UV (ultra-violet), Diode Array (DAD), and Fluorescence (*13*).

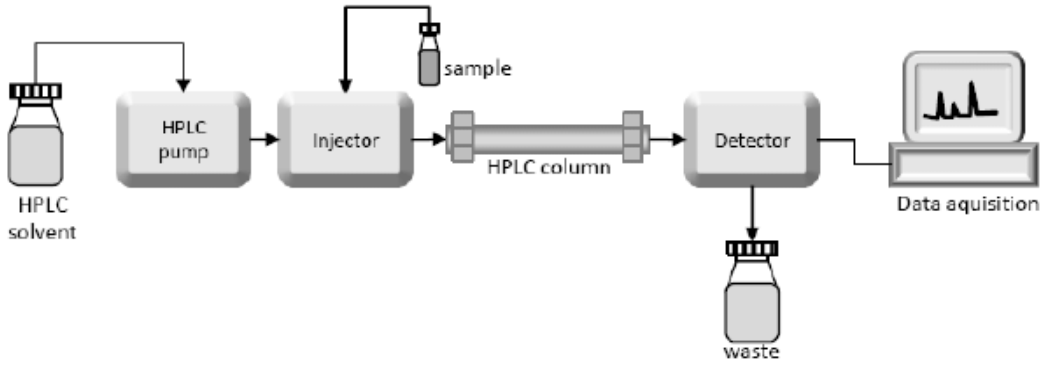

Figure 2. Figure of Schematic of HPLC system (*14*)

When looking at a brand name medication versus a generic brand of pharmaceuticals, it is expected to have differences between the two but, there are only similarities when looking at the label amounts of caffeine in Excedrin Migraine Relief and Migraine Relief medication. In both medications, there are amounts of 250 mg of acetaminophen, 250 mg of Aspirin, and 65 mg of Caffeine (*15, 16*). The only differences so far between the two medications are the price and the name, and they both serve the same purposes. In this study, the concentrations of both medications will be determined to see if there are any differences in the amounts of caffeine.

HPLC is well-established for the determination of caffeine in consumer products/foods and it was the method adopted for this study. It helped recognize the comparing agreement of the labeled quantity of caffeine to the detached amount of caffeine in the medications. Each sample of medication was measured, filtered and diluted and then injected into the HPLC. The medications will be crushed (Regis U.) and heated to a boil (UNO) to make the solutions and determine the concentrations of samples using a certain kind of standard to determine the concentrations. Excedrin Migraine relief treats all of the symptoms of migraines, reduces pain in 30 minutes, and it gives an effective relief in one dose.(N) Headache Relief medication relieves minor aches and pains, it is for headache, arthritis, and toothaches, it also gives extra strength for the pain (*15-16*).

EXPERIMENTAL

Experiments were performed on our behalf by Dr. Lynetta Mier at Regis University in Colorado and Dr. Andrew Miller at the University of Nebraska-Omaha (UNO).

Materials

Regis U.

Caffeine was purchased from Sigma Chemical company; the purity is unknown because the standard had been re-bottled in the chemical stockroom. Glacial acetic acid and HPLC grade acetonitrile were purchased from VWR. Over the counter medications for pain relief were recently purchased in pairs of brand name and generic name; Midol, Walgreens Menstrual and Exedrin and Walgreens headache. Expired Excedrin (Aug. 2004) and expired Anacin (Nov. 2015) were also tested. Deionized water was degassed by the instrument. HPLC analysis was performed using an Agilent 1100 HPLC with autosampler, multi-wavelength UV-Vis detector and fluorescence detection (DAD). The instrument was operated using ChemStation software from Agilent. The column was a Kinetex 5 µm EVO C-18 100 Å LC Column 150 x 4.6 mm, H17-395435.

UNO

Execdrin and Walgreens Migraine Relief were purchased from a local Walgreens store. An Agilent 1220 LC system was operated using LC Open Lab. The column was a Agilent Eclipse Plus C18, 5 micron particle size, 4.6 mm diameter, 150 mm long.

Preparation of Standards

Regis U.

An initial stock solution of caffeine was prepared by dissolving 0.1242 g of caffeine to a final volume of 100 mL in deionized water. This stock solution was diluted 1:10 to make a working solution from which other standards were prepared. These standards were in the range of 5×10^{-6} g/mL - 1×10^{-4} g/mL by diluting 1-10 mL of the working solution to 25 mL.

UNO

A 0.01 M solution was prepared by dissolving an appropriate amount of caffeine of 20 mL of 95% ethanol and then diluted to 100 mL with distilled water. The resultant solvent is 19% ethanol. This stock solution was then diluted with 19% ethanol to generate the following caffeine standards: 2×10^{-4}, 6×10^{-4}, 1×10^{-3}, 2×10^{-3} M. These correspond to the following concentrations of caffeine in mg/L, respectively: 38.84, 116.51, 194.19, 388. 38 mg/L.

Sample preparation

Regis U.

Excedrin Migraine Caplets and Headache Relief Capsules will be crushed and each sample of both brands of pills will be weighed 0.5 g and put into volumetric flasks, each sample will be done three times each. Each solution of pills will be diluted with the water and will be filtered with the mobile phase, which is a mixture of water, Acetonitrile, Triethylamine, and Acetic Acid (94.1:5.5:0.2:0.2).

UNO

After finding the mass of each sample, it was put into a 250 mL erlenmeyer. It then was put into 50 mL of deionized water that was heated to a boiling point for about an hour. The samples were then filtered into 100 mL volumetric flasks using a syringe because of gravity playing a part. There was some Walgreens Migraine relief solution that was lost within the process of transferring. From that 100 mL volumetric flask, 10 mL of that solution was put into a 25 mL volumetric flask. 5 mL of Ethanol was then added to it and then was diluted with water, that solution was injected into the HPLC system.

HPLC Analysis

Regis U.

The instrument that was used was an Agilent 1100 HPLC with autosampler, Multi-wavelength UV-Vis detector and fluorescence detection (DAD). For the sample injection volume, an ASI-100 automated injector was used. The mobile phase was a mixture of water:acetonitrile:triethylamine: acetic acid (94.1:5.5:0.2:0.2). A C18 column was used and it was a Kinetex 5 µm EVO C-18 100 Å LC Column 150 x 4.6 mm, H17-395435. The flow rate was 1.5 mL/min and the experiment was done at room temperature. The wavelength of the detection has been determined with UV-vis that is set at 254 nm.

UNO

The instrument that was used was an Agilent 1220 LC system. The column that was used was an Agilent Eclipse Plus C18, 5 micron particle size, 4.6 mm diameter, 150 mm long. The mobile phase was a mixture of ethanol:acetic acid:water (20:40:76 v/v)For the sample injection volume, it was 20 microliters. The flow rate was 1.00mL/ minute. The wavelength of detection was 275 nm.

RESULTS AND DISCUSSION

The following graphs show the calibration curves of caffeine from Regis University (Figure 2) and University of Nebraska-Omaha (Figure 3).

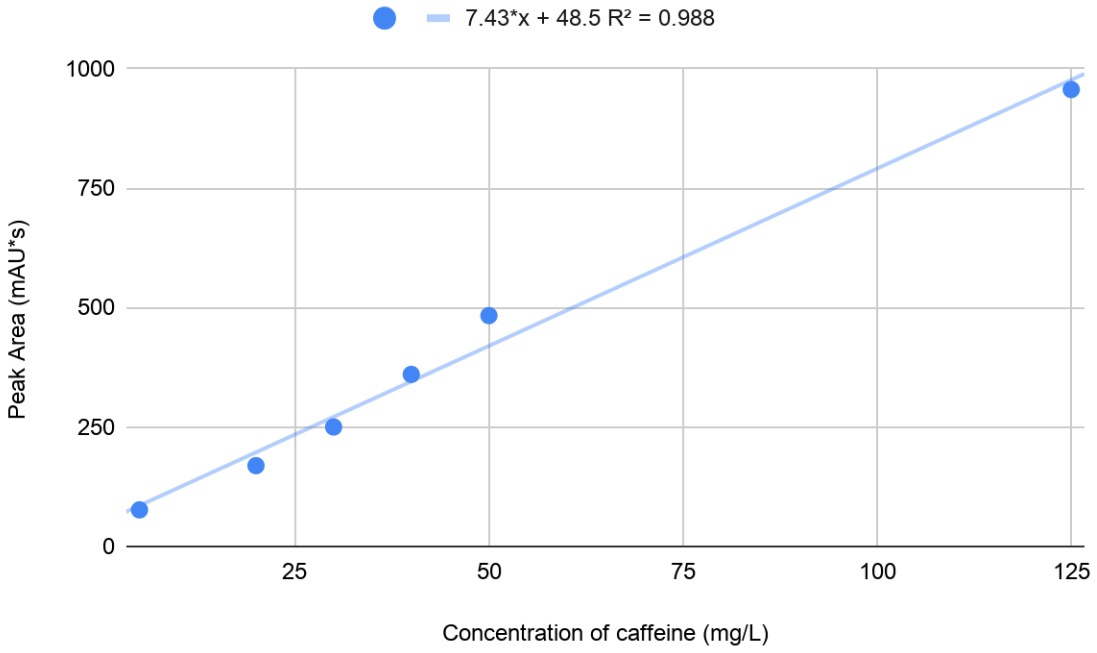

Figure 2. Calibration curve of caffeine standards from Regis University

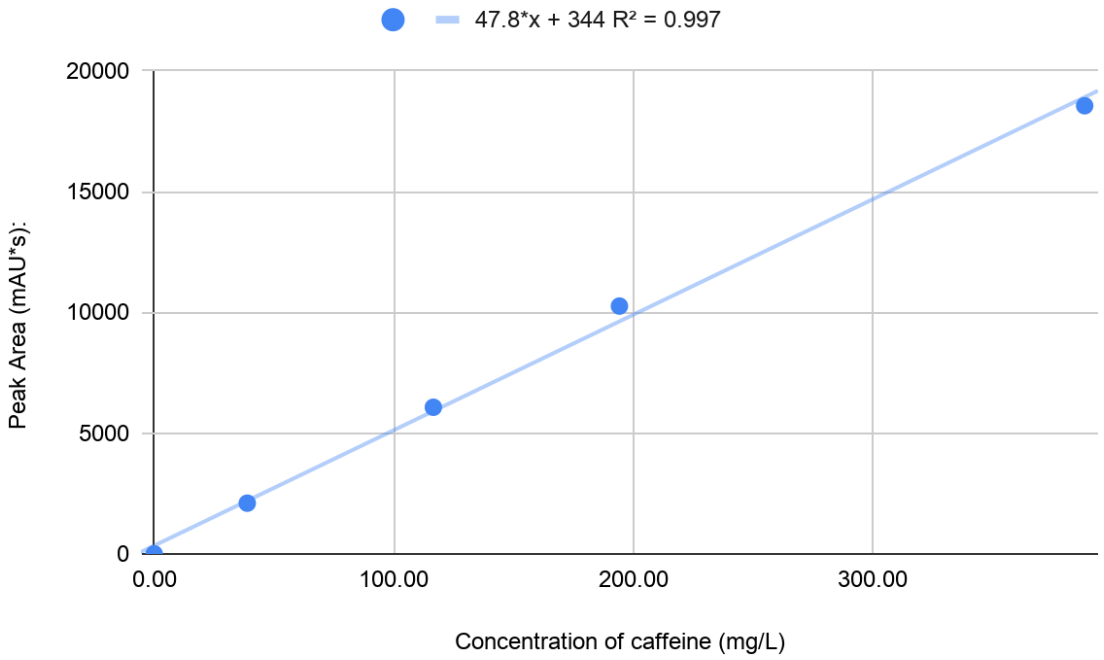

Figure 3. Calibration curve of caffeine standards from the University of Nebraska-Omaha

The HPLC data for the samples of expired Excedrin (Aug. 2004), expired Anacin (Nov. 2015), Midol, Walgreens Menstrual, Excedrin and Walgreens headache medicine from Regis is summarized in Table 1A of the Appendix and an abbreviated presentation of those results is given in Table 1 below. Table 1 also includes results from the analysis of Walgreens Migraine Relief and Exedrin conducted at UNO. A description of the relevant calculations follows. First, the concentration of caffeine in the injected sample was calculated by substituting the peak area from the chromatogram for the y-value in the line of best fit equation from the calibration curve and solving for the x-value. This step was the same for both Regis U. and UNO data.

For the Regis U. data, we calculated the concentration of the original pill solution by multiplying the injected sample concentration by 10, because the injected sample was actually a 1:10 diluted version of the pill solution. Next, the mass of caffeine in that original pill solution was determined by multiplying the volume (100.0 mL = 0.1000 L) of solution times the concentration. Finally, because the entire pill was not used in each trial, the mass in the solution needed to be converted to the mass of caffeine in the actual pill. To do this, the mass of caffeine in the solution, which was prepared using ~0.5 g of a crushed pill, was divided by the ~0.5 g mass of the sample and multiplied by the mass of the pill (~0.6 g).

For the UNO data, we calculated the concentration of the injected pill sample by taking the peak area and subtracting it from the slope coming from the calibration curve. We then took the amount of trials which was 3, and divided that by the slope once again. Next, the concentration in the original solution was calculated by taking the concentration of the injected sample and multiplying it by 25 which came from the 10 mL sample being added to a 25 mL volumetric flask. It was then divided by 10 and that came from the sample before it was added to the 25 mL flask. After calculating those values, the mass of caffeine in pills was determined by taking the concentration in the original solution and multiplied it by the volume (100.0 mL = 0.1000 L) of the solution.

Table 1. HPLC data and calculated results for caffeine in OTC medicines.

Brand	Trial	Peak Area (mAU*s)	mass of caffeine in pill (mg)	Average mass of caffeine in pills* (mg)	p-Value
Old Excedrin	1	294.7	44.68	46.08 ± 1.872	0.00007
	2	299.8	45.60		
	3	305.6	46.65		
	4	309.5	47.36		
Old Anacin	1	259.1	39.30	38.78 ± 16.83	0.02
	2	291.1	45.28		
	3	218.7	31.75		
Midol	1	376.1	56.94	56.88 ± 0.8007	0.0052
	2	375.4	56.81		
Walgreens Menstrual	1	456.4	76.24	76.44± 2.583	0.02
	2	458.6	76.64		
Excedrin	1	470.8	74.10	75.03± 11.87	0.26

	2	481.4	75.96		
Walgreens Headache	1	395.2	65.93	65.52± 5.331	0.05
	2	390.8	65.10		
Walgreens Migraine Relief[a]	1	1195	60.68	58.41 ± 5.271	0.03
	2	1145	58.07		
	3	1114	56.48		
Excedrin[a]	1	1249	63.50	64.14 ± 4.084	0.46
	2	1237	62.92		
	3	1297	66.01		

* Reported with 95% confidence interval

[a] OTC analgesics tested at UNO

For the statistical analysis, the average with its 95% confidence interval was calculated. A one-sample t-test was performed to compare the average mass of caffeine in different brands to the reported mass of caffeine of the OTC medicine labels. The p-values from these t-tests are given in Table 1. The p-values that were less than 0.05 indicate a statistically significant difference between the experimental value and the labeled value.

As a result of this study, expired medication turned out to be different from newly bought medication. For the ingredients to actively work, it is recommended to not take expired medicine. When collecting the data, there was a human error that may have affected the concentration of caffeine from the generic medication from UNO. During that transfer error that occurred, it showed a lower amount of the generic solution than the Excedrin solution. Between name brand and generic labels, there are no differences between them. On both of the labels, they have the same amounts of ingredients listed. To improve this study, more trials of each medication brand should be tested to get more data and it will be more accurate. Another way to improve this study is to test more pills that are given in each bottle so that there is more data to test and the results would be more accurate.

CONCLUSION

In conclusion, in this study we determined the mass of caffeine in a variety of different over the counter medications to determine if the experimental mass matched the label mass. Caffeine being one of the most widely consumed products around the world, it serves different purposes. People who consume caffeine for medical purposes consume it through pills. Over the counter medications such as Tylenal, Ibuprofen, and Excedrin are more accessible for people rather than prescription medications. Caffeine medication having different names does not matter when it comes to looking at what they are used for.

APPENDIX

Table 1A. Complete reporting of calculated results from HPLC analysis

Brand	Mass of pills (g)	Mass of sample (g)	Peak Area (mAU*s)	Concentration of caffeine in injected sample (mg/L)	Concentration of orig. solution (mg/L)	Mass in 0.5g sample (mg)	Mass of caffeine in pills (mg)
Old Exedrin	0.6748	0.5005	294.7	33.14	331.4	33.14	44.68
	0.6748	0.5005	299.8	33.82	338.2	33.82	45.60
	0.6748	0.5005	305.6	34.60	346.0	34.60	46.65
	0.6748	0.5005	309.5	35.13	351.3	35.13	47.36
Old Anacin	0.6898	0.4975	259.1	28.34	283.4	28.34	39.30
	0.6898	0.4975	291.1	32.65	326.5	32.65	45.28
	0.6898	0.4975	218.7	22.90	229.0	22.90	31.75
Midol	0.6458	0.5001	376.1	44.09	440.9	44.09	56.94
	0.6458	0.5001	375.4	44.00	440.0	44.00	56.81
Walgreens menstrual	0.6957	0.5010	456.4	54.90	549.0	54.90	76.24
	0.6957	0.5010	458.6	55.19	551.9	55.19	76.64
Excedrin	0.6529	0.5008	470.8	56.83	568.3	56.83	74.10

	0.6529	0.5008	481.4	58.27	582.7	58.27	75.96
Walgreens Headache	0.7065	0.5000	395.2	46.66	466.6	46.66	65.93
	0.7065	0.5000	390.8	46.07	460.7	46.07	65.10
Walgreens Headache Relief	0.6604	0.6604	1195	242.7	606.8	N/A	60.68
	0.6611	0.6611	1145	232.3	580.7	N/A	58.07
	0.6598	0.6598	1114	225.9	564.8	N/A	56.48
Excedrin	0.6650	0.6650	1249	254.0	635.0	N/A	63.50
	0.6645	0.6645	1237	251.7	629.2	N/A	62.92
	0.6632	0.6632	1297	264.1	660.1	N/A	66.012

REFERENCES

(1) Harbolic, B. Caffeine: Read About Side Effects, Addiction, and Withdrawal. *MedicineNet* [Online], Sept.10, 2020. https://www.medicinenet.com/caffeine/article.htm (accessed April 28, 2021)

(2) Nowaczewska, M.; Wiciński, M.; Kaźmierczak, W. The Ambiguous Role of Caffeine in Migraine Headache: From Trigger to Treatment. *Nutrients*. **2020**, *12*, 2259-2275

(3) National Center for Biotechnology Information. PubChem Compound Summary for CID 2519, Caffeine. pubchem.ncbi.nlm.nih.gov/compound/2519. (accessed April 29, 2021)

(4) Molview: Caffeine. https://embed.molview.org/v1/?mode=balls&cid=2519 (accessed April 30, 2021)

(5) Nichols, H. What does Caffeine do to your body?. https://www.medicalnewstoday.com/articles/285194#uses (accessed April 30, 2021)

(6) Lipton, R.B.; Diener, H-C.; Robbins, M.S.; Garas, Y. G.; Patel, K. Caffeine in the management of patients with headache. *The Journal of Headache and Pain*. **2017**, 18, 107

(7) Leacock, R. E.; Stankus, J.J.; Davis, J. M. Simultaneous Determination of Caffeine and Vitamin B6 in Energy Drinks by High-Performance Liquid Chromatography. *Journal of Chemical Education* **2011**, 88, 232-234

(8) Beckers, J. L. The Determination of Caffeine in Coffee: Sense or Nonsense?. *Journal of Chemical Education* **2004**, 81, 90-93

(9) Ferguson, G.K. Quantitative HPLC Analysis of an Analgesic/Caffeine Formulation: Determination of Caffeine. *Journal of Chemical Education* **1998**, 75, 467-469

(10) Mcdevitt, V.L.; Rodríguez, A.; Williams, K.R. Analysis of Soft Drinks: UV Spectrophotometry, Liquid Chromatography, and Capillary Electrophoresis. *Journal of Chemical Education* **1998**, 75, 625-629

(11) Yang, M.J.; Orton, M.L; Pawliszyn, J. Quantitative Determination of Caffeine in Beverages Using a combined SPME-GC/MS Method. *Journal of Chemical Education* **1997**, 74, 1130-1132

(12) What is Chromatography and How does it work? https://www.thermofisher.com/blog/ask-a-scientist/what-is-chromatography/ (accessed April 30, 2021)

(13) "Virtual Lab: HPLC Machine Virtual Lab." *Labster*, 5 Jan. 2021, www.labster.com/simulations/hplc/. (accessed on October 7, 2020)

(14) High Performance Liquid Chromatography (HPLC) : Principles, Types, Instrumentation and Applications. https://laboratoryinfo.com/hplc/ (accessed April 30, 2020)

(15) "Excedrin Migraine Pain Reliever Caplets." *Walgreens*, www.walgreens.com/store/c/excedrin-migraine-pain-reliever-caplets/ID=prod3448102-product. (accessed April 30, 2021)

(16) "Walgreens Extra Strength Headache Relief Tablets." *Walgreens*, www.walgreens.com/store/c/walgreens-extra-strength-headache-relief-tablets/ID=prod380777-product. (accessed April 30, 2021)