Journal of
Tropical Pharmacy
And Chemistry

ANALYSIS OF CAFFEINE IN TABLET DOSAGE FORM WITH SPECTROPHOTOMETRIC AND IODOMETRIC BACK TITRATION METHODS

Edy Agustian Yazid[1,*], Nuhmaan[2], Mohammad Rizal Pratama[3]

[1,3] Department of Pharmacy, Delima Husada College of Health Analyst
Jl. Arief Rahman Hakim No. 2 B, Gresik 61111, Indonesia
[2] Departement of Pharmacy, Hang Tuah University
Jl. Arief Rahman Hakim No. 150, Surabaya 60111, Indonesia
*email: estien_y@yahoo.co.id

ABSTRACT

Caffeine (1, 3, 5-trimethylxanthine) is an active ingredient that is often added to analgesics drugs to teat headaches or reduce pain. This study aims to quantitatively analyze caffeine levels in tablet dosage forms by spectrophotometric methods and iodometric back titration. In the spectrophotometric method the measurement of caffeine levels was carried out using a maximum wavelength of 272 nm.The results of caffeine levels were found for bodrex drugs (50.87 ± 0.195 mg/tab), extra panadol (64.92 ± 0.579 mg/tab), saridon (51.38 ± 0.273 mg/tab) and paramex (46.78 ± 0.072 mg/tab). The purity percentage of caffeine was found in the range between 93.57-102.76%. While the iodometric back titration method was obtained for bodrex (48.04 ± 0.889 mg/tab), extra panadol (64.45 ± 0.697 mg/tab), saridon (43.38 ± 0.756 mg/tab) and paramex (42.36 ± 0.889 mg/tab) with a purity range of 84.73-99.15%. The average caffeine content of the two method extant suitability close to the true value as stated on the label. The results of the spectrophotometric method are more accurate, while the titrimetry method is still good to use because it is cheaper and only requires simple apparatus and common chemicals.

Keywords: Caffeine, Tablet, Spectrophotometry, Back titration, Iodometry

Submitted on: 13 August 2019 Accepted on: 09 September 2019

DOI: https://doi.org/10.25026/jtpc.v4i6.214

INTRODUCTION

Caffeine is an xantin derivative alkaloid compound with the chemical name 1,3,7-trimethylxanthine has a molecular formula $C_6H_{10}N_4O_2$ (Figure 1), molecular weight 194.19 g/mol, melting point 237 ºC, density 1.05 g/cm and pKa 10.4 at 40ºC [1]. Pure caffeine takes the form of white, hexagonal crystals [2], odorless and has a bitter taste [3]. The nature of caffeine has been reported to be highly soluble in chloroform and dichloromethane compared to other organic solvents investigated, such as benzene, diethyl ether, ethyl acetate and hexane [4]. Caffeine is partly soluble in

water at room temperature (2 g/100 mL) but is very soluble in boiling water (66 g/100 mL) [5].

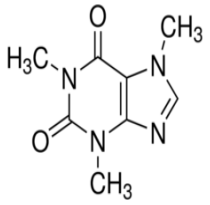


Figure 1. The chemical structure of caffeine ($C_6H_{10}N_4O_2$)

Caffeine is a natural substance found in leaves, seeds or fruits of at least 63 plant species worldwide [6]. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts, and tea leaves [7]. The amount of caffeine varies according to the species and origin of the plant [8].

Caffeine is an ingredient that is widely used as an additive added to ingredients of non-alcoholic beverages such as cola and soft drinks [9]. The caffeine content in soft drinks varies by brand, ranging from 10 to 50 mg of caffeine per serving [10]. About 120,000 tons of caffeine are consumed worldwide every year [11].

In the pharmaceutical field caffeine has extensive therapeutic uses, including being used as an analgesic drug to reduce pain and degrade fever [12]. Caffeine is one of the most commonly consumed drugs with more than 80 percent of the world's population consuming caffeine daily [13]. Caffeine in the drug combined with acetylsalicylic acid is used as an analgesic adjunct to pain reliever [14], generally was added a range of 15-65 mg per tablet [15]. Consumption of caffeine in combination with analgesic increases its effectiveness by as much as 40% depending on the specific type of pain involved [16].

Caffeine has many important physiological effects, acts as a central nervous system stimulant, increases heart rate and increases brain activity [17]. Caffeine works as a psychoactive stimulant and mild diuretic [18], medically reduces physical fatigue and to restore alertness when drowsiness occurs [17]. Excessive amounts of caffeine in the body can cause feelings of nervousness, anxiety, trembling, insomnia, nausea, seizures [19] and the effects of mutations such as DNA inhibition [20]. A fatal dose of caffeine has been evaluated to be more than 10 g (about 170 mg/kg of body weight). It is also considered to be a risk species for cardiovascular diseases, kidney damage, asthma, and may also cause hyperactivity [21].

Various methods have been developed to quantitatively determine caffeine in pharmaceutical dosage forms. The most widely used method is HPLC [15], but these sophisticated instruments have limited access, high costs and more complicated operations. Other methods are used such as GC [22], solid-phase extraction (SPE) [23], electrochemistry [20], voltammetry [24], spectrophotometry [25] and titration [26-27]. Spectrophotometry is a method that has a wide range used by many researchers and students because the cost is relatively cheap and easy to operate. While the titrimetry method is a simple technique, it has the advantage of being more efficient, cheaper and still accurate to use. This study aims to determine caffeine levels in tablet dosage forms using spectrophotometric methods and iodometric methods with back titration techniques.

MATERIALS AND METHODS

Material

All reagents and chemicals used in this study were of analytical grade unless

indicated otherwise. Pure caffeine was obtained from Sigma-Aldrich (Merck), pharmaceutical preparation tablets are taken directly from supermarkets in the city of Gresik, East Java. Other chemicals are iodine (Merck), sodium thiosulfate (Merck), potassium iodate (Merck), Potassium Iodide (Merck), sulfuric acid (Merck), hydrochloric acid (Merck), starch (Merck) and ethanol. All solutions are made in double distilled water and stored in a dark bottle container at room temperature.

Instrument

Spectrophotometer UV-1600PC (single beam), with a 10 mm matched quartz cuvette was used for absorption measurement. Other equipments are an analytical balance, hot plate, magnetic stirrer, and a set of glassware for titration purposes.

Preparation of Solutions

The starch indicator solution was prepared fresh by dissolving 1.0 g of starch into a 10 ml of double distilled water, stirring well and then being transferred into a 100 ml boiling water. The solution was stirred and boiled for one minute then left to cool at room temperature and filtered. Hydrochloric acid (4.0 M) was measured 33.3 ml of 37% HCl and poured it into a 100 ml of double distilled water. Sulfuric acid (10%) was prepared by measuring 10.2 ml of 98% H_2SO_4 and poured it into a 100 ml of double distilled water. Potassium iodide (10%) was weighed 10 g of KI and diluted in 100 ml of double distilled water. Potassium iodate (0,1000 N) was prepared by weighing 1.7833 g of KIO_3 powder and diluted in 500 ml of double distilled water. Sodium thiosulfate (0.1 N) was prepared by dissolving 24.8 g of sodium thiosulfate crystals diluted in 1000 ml of freshly boiled and cooled double distilled water. Iodine solution (0.1 N), 20 g of KI was transferred into a 100 ml beaker and 40 ml of double distilled water added with a little heating. The mixture was cooled to room temperature and 12.7 g solid iodine was dissolved in the same glass while stirring until it dissolves. The iodine solution was transferred into a 1000 ml volumetric flask, then dilute with double distilled water to the mark.

Preparation of Caffeine Standard Solution

The standard stock solution of caffeine (1000 µg/ml) was prepared by dissolving 100 mg of caffeine in 100 ml of double distilled water. Working standard solution of caffeine (100 µg/ml) was prepared by pipetting 10 ml aliquots of the caffeine stock solution into a 100 ml volumetric flask, then diluted to the mark with double distilled water.

Calibration Curve Preparation

Standard solutions of caffeine were prepared with a range concentration of 1.6-8.0 µg/ml. The caffeine working solution was taken sequentially 0.4 ml; 0.5 ml; 0.8 ml; 1.0 ml; 1.5 ml; 2.0 ml, transferred into a series 25 ml volumetric flask then diluted with double distilled water to the mark. The absorbance of each standard solution was measured at a maximum wavelength of 272 nm against double distilled water as blank using a 10 mm quartz quvet. The calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis.

Sample Preparation

Twenty tablets of pharmaceutical preparations were weighed then crushed using porcelain mortar to become a fine powder. The powder obtained was analyzed according to the method used.

Determination of Caffeine by UV Spectrophotometer

Determination of caffeine by the spectrophotometric method as proposed by

Sethuraman et al., (2013)[25]. The tablet powder was weighed accurately equivalent to 50 g of caffeine, was transferred into a 100 ml beaker and 50 ml of double distilled water was added. The solution was stirred using a magnetic stirrer for 15 minutes, then transferred into a 100 ml volumetric flask and diluted to the mark with the same solvent. The solution was then filtered using Whatman filter paper (No. 42) twice filtering with 10 ml of the first filtrate was removed. The filtrate obtained was taken 2 ml and diluted to 100 ml to obtain the sample solution. Absorbance was measured using a UV-1600PC spectrophotometer at a maximum wavelength of 272 nm.

Determination of Caffeine by Iodometric Titration

Standardize the thiosulfate solution

Taken carefully 10 ml of KIO_3 0.1000 N solution was transferred into a 100 ml Erlenmeyer flask, added 10 ml of 10% KI solution and 2.5 ml of HCL 4.0 N. This solution was immediately titrated with a standard solution of $Na_2S_2O_3$ 0.1 N to the yellow color almost gone (pale yellow). A few drops of the starch indicator were added and the titration continues until the blue color of solution was gone. All analyses were carried out in triplicate.

Determination of caffeine levels.

The estimation of caffeine by iodometric back titration using the Lok et.al., (2012) method with a slight modification [26]. Accurately weighed tablet powder equivalent to 50 g of caffeine was transferred into a 100 ml Erlenmeyer flask. An amount of ethanol (10 ml) was added, shaken for 10 minutes then added 5 ml of 10% H_2SO_4 and 20 ml of standardized iodine solution. The solution was shaken again and left at room temperature for 10 minutes to form a brown-red precipitate, then filtered with Whatman filter paper (No. 42). The filtrate obtained was immediately titrated with a standard solution of sodium thiosulfate until the yellow color was almost gone (pale yellow). A few drops of the starch indicator were added and the titration continues until the blue color of solution was gone. Titration is repeated according the above steps to three consistent results are obtained.

RESULTS AND DISCUSSION

Analysis of Spectrophotometry

The maximum wavelength (λ_{max}) of caffeine was determined by measuring the absorption of the standard caffeine solution in the wavelength range at 200-400 nm using a UV-1600PC spectrophotometer against double distilled water as blank. The determination of λ_{max} needs to be done because at this wavelength it has maximum sensitivity and absorption for caffeine. Based on the measurement results of λ_{max} for caffeine solution in a double distilled water solvent obtained 272 nm with the absorbance of 0.212. This wavelength was then used to measure the absorption of standard solutions and sample solutions of pharmaceutical preparations according to the procedure used.

The calibration curve has been carried out using a series of caffeine standard solutions has been prepared from a stock solution of caffeine 100 µg/ml. By plotting the concentration of caffeine solution versus the corresponding absorbance was obtained good a linear curve according to Lambert-Beer law in the concentration range 1,6-8,0 µg/ml. Based on the curve obtained regression equation y = 0,051x + 0,006 and the correlation coefficient value were found 0.999 (Figure 2). The correlation coefficient value approaching 1.0 indicates a very good correlation between concentration and absorbance so that the linear regression equation can be used to determine caffeine

levels in pharmaceutical preparation tablets.

This study uses samples of a pharmaceutical dosage form of tablets containing caffeine and serves as an analgesic drug. This tablet is very popular in the community and is often used as a medicine for headaches and pain relief. Analysis of caffeine in this study was determined by spectrophotometric methods and iodometric back titration. Spectrophotometry is a quantitative measurement of the reflection or transmission properties of a material as a function of wavelength [28]. This method has the advantage of using a short time, low cost, robustness and capability for high precision. The use of spectrophotometry is mainly applied widely in the analysis of pharmaceutical dosage forms.

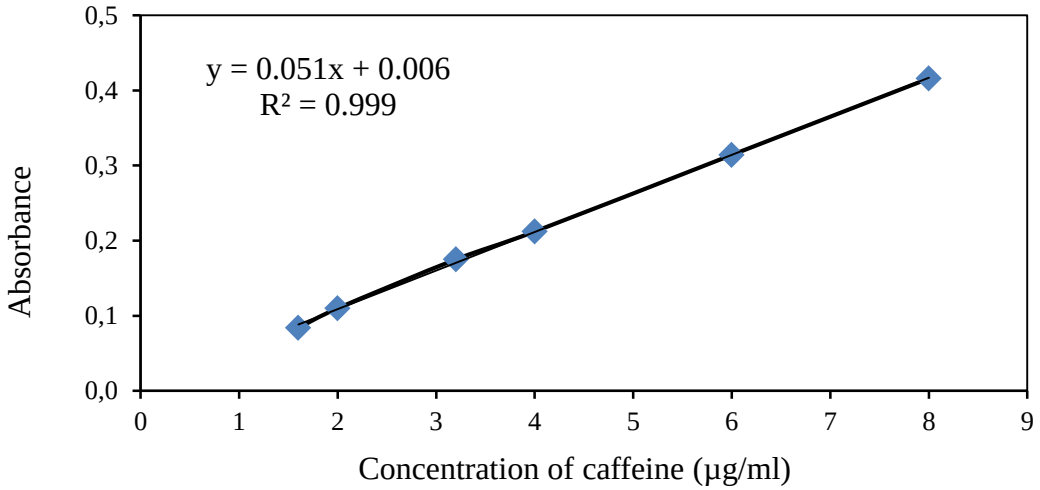
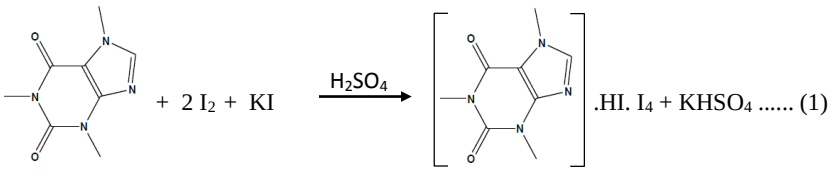


Figure 2. Calibration curve of caffeine standard solution

Table 1. Caffeine levels in tablet preparations by spectrophotometric method

Tablet samples	Labeled claim (mg/tab)	Amount found (mg/tab)	% Purity	E_{rel} %	Mean ± SD (n =3) (mg/tab)
Bodrex	50	50.755	101.510	1.735	50.87 ± 0.195
	50	50.755	101.510		
	50	51.093	102.186		
Panadol Extra	65	65.145	100.223	-0.635	64.92 ± 0.579
	65	64.267	99.884		
	65	65.361	100.555		
Saridon	50	51.537	103.074	2.759	51.38 ± 0.273
	50	51.065	102.130		
	50	51.537	103.074		
Paramex	50	46.824	93.648	-6.435	46.78 ± 0.072
	50	46.700	93.400		
	50	46.824	93.648		



Caffeine – iodine complex

$I_2 + 2\ Na_2S_2O_3 \longrightarrow 2\ NaI + Na_2S_4O_6$ ..... (2)

Figure 3. Equation of reaction to determine caffeine levels

The results of the study on average caffeine levels in analgesic drugs are shown in Table 1. Obtaining caffeine levels for bodrex drugs (50.87 ± 0.195 mg/tab), panadol extra (64.92 ± 0.579 mg/tab), saridon (51.38 ± 0.273 mg/tab) and paramex (46.78 ± 0.072 mg/tab). While the average percentage of each tablet was obtained by bodrex (101.74%), extra panodol (99.88%), saridon (102.76%) and paramex (93.57%). Overall caffeine levels still show results above 93.57% and relative errors range from -6.44% and 2.76%. The highest relative error was found in the paramex drug at -6.4%. The presence of multicomponent substances contained in the drug can be an interference affecting the results of the spectrophotometric analysis of the method used. Paramex drugs have a composition with the highest number of components among the other drugs studied. According to Wanyika, et al., (2010) within experimental errors, the value limits are generally agreed for good accuracy according to the quoted literature value is 2-5% [28].

Analysis of Iodometric Back Titration

The Iodometric back titration is a simple and accurate method for determining the amount of caffeine in an aqueous solution. This method requires a simple apparatus and common chemicals [26]. In caffeine, there are double bonds that can be added by iodine. The amount of caffeine can be determined by adding excess iodine solution which is known to be accurately concentrated.

Caffeine reacts with iodine in an acidic environment, forming insoluble precipitate then separated by filtration. The iodine contained in the filtrate is residual iodine after the addition reaction with caffeine. The remaining excess amount of iodine is then titrated with a standard sodium thiosulfate solution using the starch indicator until the solution's blue color disappears. The quantity of iodine that reacts with caffeine can be calculated by reducing the amount of iodine added with the remaining iodine. The equation for the chemical reaction that occurs is shown in Figure 3.

The results of caffeine determination in tablets with iodometric back titration are presented in Table 2. The average caffeine content for bodrex drugs was obtained (48.04 ± 0.889 mg/tab), extra panadol (64.45 ± 0.697 mg/tab), saridon (43.38 ± 0.756 mg/tab) and paramex (42.36 ± 0.889 mg/tab). The percentage of each tablet was obtained for bodrex (96.07%), panodol extra (99.15%), saridon (86.77%) and paramex (84.73%). The lowest percentage was found in paramex drugs of 84.73% and the highest in panadol drugs was 99.15%. Relative errors are obtained with a range between -15.27 and 0.85%.

The presence of multicomponents in the drugs studied can be an interference the addition reaction with caffeine. The most component was found in the paramex drug with the composition of paracetamol, propyphenazone and dexchlorpheniramine maleate. The saridon drug contains components of paracetamol and propyphenazone. The two other drugs, bodrex and extra panadol only contain paracetamol components. The highest amount of iodine is absorbed by caffeine through the addition reaction at extra bodrex and panadol drugs so that both of them get a higher percentage of 96.07% and 99.15% respectively.

In iodometric back titration, the properties of iodine solutions are volatile, some iodine can be lost during the experimental process, especially when filtration. Thus it contributes to titration errors. In addition, standard iodine solutions must be standardized with standard sodium thiosulfate solutions which have been known concentrate accurately to get the right concentration.

Table 2. Caffeine levels in tablet preparations by iodometric back titration

Tablet samples	Labeled claim (mg/tab)	Amount found (mg/tab)	% purity	E_{rel} %	Mean ± SD (n =3) (mg/tab)
Bodrex	50	48.55	97.10	-3.927	48.04 ± 0.889
	50	47.01	94.02		
	50	48.55	97.10		
Panadol Extra	65	63.72	98.03	0.851	64.45 ± 0.697
	65	64.51	99.25		
	65	65.11	100.17		
Saridon	50	42.51	85.02	-13.233	43.38 ± 0.756
	50	43.82	87.64		
	50	43.82	87.64		
Paramex	50	41.85	83.70	-15.273	42.36 ± 0.889
	50	41.85	83.70		
	50	43.39	86.78		

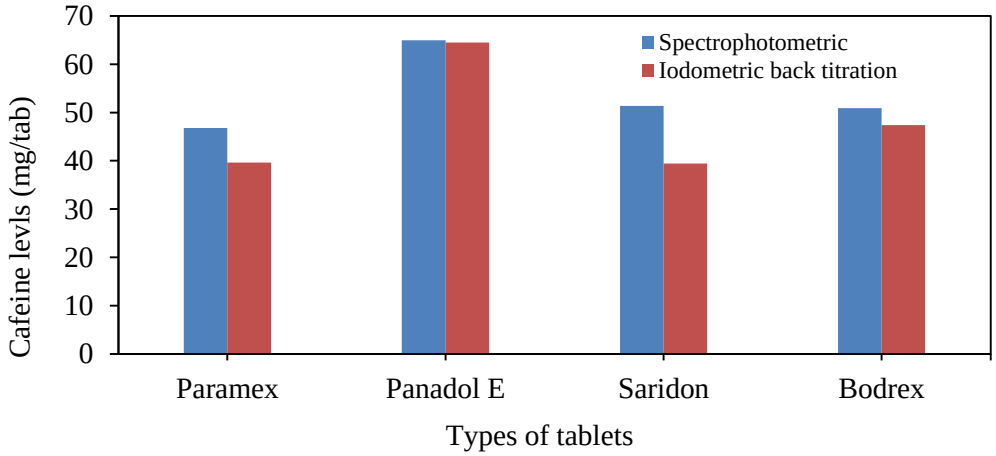


Figure 4. Comparison of caffeine levels by spectrophotometric and iodometric methods

Comparison of the two methods found that there was no large difference in caffeine levels between the two methods except in the saridon and paramex drugs, the caffeine levels were slightly lower in iodometric back titration (Figure 4). Both the spectrophotometric method and iodometric back titration extant suitability the results of the caffeine level approaching the true level as stated on the label. In the spectrophotometric method, the results are more accurate with relatively smaller errors. The titrimetry method is better if used for drug analysis with fewer components in the mixture.

CONCLUSIONS

Determination of caffeine levels in tablet dosage form by a spectrophotometric method and iodometric back titration obtained good accuracy results. Spectrophotometry is a more accurate, while the iodometric back titration method is still good enough to use, especially for tablet samples which contain fewer components. This method is cheaper, It requires simple apparatus and common chemicals only and and has a wider range of uses.

ACKNOWLEDGMENTS

Special to thank the Department of pharmacy, Delima Husada College of Health Analyst with the availability of equipment and reagents for the implementation of this study.

REFERENCES

[1] Abdalla, M..A., 2015. Determination of Caffeine, the Active Ingredient in Different Coffee Drinks and its Characterization by FTIR/ATR and TGA/DTA, *International Journal of Engineering and Applied Sciences*, 2(12), 85-89.

[2] Subila, S & Navis, M.S., 2016. Determination of Caffeine in Different Tea Samples, *Journal of Applied Chemistry*, 9(12), 75-78.

[3] Amare, M., Shimelis, A., 2012. Polymer Modified Glassy Carbon Electrode for the Electrochemical Determination of Caffeine in Coffee, *Talanta*, 93, 122–128.

[4] Shalmashi, A and Golmohammad, F., 2010. Solubility of Caffeine in Water, Ethyl Acetate, Ethanol, Carbon Tetrachloride, Methanol, Chloroform, Dichloromethane and Acetone between 298 K and 323 K., *Lat. Am. Appl. Res*., 40(3), 327-341.

[5] Budavari, S., 1996. *The Merck Index*, 2nd ed., Whitehouse Station, NJ: Merck & Co., Inc., 1674.

[6] Frary, C.D., Johnson, R.K., Wang, M.Q., 2005. Food Sources and Intakes of Caffeine in the Diets of Persons in the United States, *Journal of The American Dietetic Association*, 105(1), 110-113.

[7] Runge, F and Friedlie, F., 2014. *Latest phytochemical discoveries for the founding of a scientific phytochemistry*, Joseph publications, 20th ed. American, 144–159.

[8] Andrews, K.W., Schweitzer, A., Zhao, C., 2007. The Caffeine Contents of Dietary Supplements Commonly Purchased in the US: Analysis of 53 Products with Caffeine-Containing Ingredients, *Analytical and Bioanalytical Chemistry*, 389(1), 231–239.

[9] Bempong, D.K., Houghton, P.J., Steadman, K., 1993. The Xanthine Content of Guarana and its Preparations, *Int. J. Pharmacog*, 31 (3), 175–181.

[10] Nour, V., Trandafir, I and Ionica, M.E., 2010. Chromatographic Determination of Caffeine Contents

in Soft and Energy Drinks Available on The Romanian Market, *St. Cerc. St. CICBIA*, 11(3), 351-358.

[11] Oliveira, T.D., William S., Martini, Mellina, Santos, D.R., Matos, M.A.C and Rocha, L.LD, 2015. Caffeine Oxidation in Water by Fenton and Fenton-Like Processes: Effects of Inorganic Anions and Ecotoxicological Evaluation on Aquatic Organisms, *J. Braz. Chem. Soc*., 26(1), 178-184.

[12] Burnhan, T.A., 2001. *Drug Facy and Comparison*, St Louis: A Wolters Kluwers Company, USA.

[13] Norton, T.R., Lazev, A.B., Sullivan, M.J., 2011. The 'Buzz' on Caffeine : Patterns of Caffeine Use in a Convenience Sample of College Students. *Journal of Caffeine Research*, 1(1), 35–40.

[14] Bharate, S.S., and Bharate, S.B. 2012. Spectrophotometric and Chromatographic Determination of Acetylsalicylic Acid and Caffeine in Pure and in Tablet Dosage Form, *J. Adv. Scient .Res*. 3(1), 73- 81.

[15] Abbood, A and Aldiab, D., 2017. HPLC Determination of Caffeine in Some Beverages and Pharmaceutical Dosage Forms Available in Syrian Market, *Journal Of Chemical And Pharmaceutical Sciences*, 10(3), 1174-1179.

[16] Fiebich, B., Lieb, K., Hüll, M., Aicher, B., Ryn, J.V., Engelhardt, G., 2000. *Neuropharmacology*, 39, 2205-2213

[17] Maidon, A.B., Mansoer, A.O and Sulistyarti, H., 2012. Study of Various Solvents for Caffeine Determination Using UV Spectrophotometeric. *J. Appl. Sci. Res*., 8(5), 2439-2442.

[18] Maughan, R.J., 2003. Caffeine Ingestion and Fluid Balance: A Review, *J. Human Nutrition Dietetics*, 16, 411–20.

[19] Peri-Okonny, U. L., Wang, S. X., Stubbs, R. J., & Guzman, N.A., 2005. Determination of Caffeine and its Metabolites in Urine by Capillary Electrophoresis-mass Spectrometry, *Electrophoresis*, 26(13), 2652-2663.

[20] Švorc, L, 2013. Determination of Caffeine: A Comprehensive Review on Electrochemical Methods, *Int. J. Electrochem. Sci.,* 8, 5755 – 5773.

[21] Evans, S.M., Griffiths R.R., 1992. Caffeine Tolerance and Choice in Humans, *Psychopharmacology*, 108(1-2), 51–59.

[22] Verenitch, S.S., Lowe, C.J., Mazumder, A., 2006. Determination of Acidic Drugs and Caffeine in Municipal Wastewaters and Receiving Waters By Gas Chromatography–Ion Trap Tandem Mass Spectrometry, *Journal Of Chromatography A*, 1116(1-2), 193–203.

[23] Al-Qaim, F.F., Siti Hasmah Jusof1, S.H., Abdullah1, P., Mussa1,Z.H., Tahrim1, N.A., Khalik, W.M.A,., Othman, M.R., 2017. Determination of Caffeine in Surface Water Using Solid Phase Extraction and High Perfomance Liquid Chromatography, *Malaysian Journal of Analytical Sciences*, 21(1), 95 – 104.

[24] Zen, J.M., Ting, Y.S., and Shih, Y., 1998. Voltammetric Determination of Caffeine in Beverages Using a Chemically Modified Electrode, *Analyst*, 123, 1145–1147.

[25] Sethuraman, S., Radhakrishnan, K and Solomon, T.A, 2013. Analytical Method Development and Validation of Caffeine in Tablet Dosage Form by Using UV- Spectroscopy, *International Jornal.of Novel Trends In Pharmaceutical Sciences*, 3(4), 82-86.

[26] Lok, H.C., Kit, K.M., Tung, C.K., Ho., T.S., 2012. Chemical Test for Caffeine, *Hong Kong Chemistry Olympiad for Secondary Schools*, 1-25.

[27] Diverdi, J, 2013. *Determination of Caffeine in Coffee*, Colorado State University, Fort Collins, CO., Coloardo, 1-10.

[28] Wanyika H.N, Gatabe E.G., Gitu L.M., Ngumba E.K., Maritim C.W. 2010. Determination of Caffeine content of Tea and Instant Coffee Brands Found in The Kenyan Market. *African Journal of Food Science*. 4(6): 353 – 358.