ejpmr, 2019,6(11), 496-499

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

QUANTITATIVE ESTIMATION OF CAFFEINE IN SOFT DRINKS USING UV-VISIBLE SPECTROPHOTOMETRIC METHOD

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Article Received on 20/09/2019 **Article Revised on 10/10/2019** **Article Accepted on 30/10/2019**

ABSTRACT

Caffeine is a well-known stimulant which is added as an ingredient to various carbonated soft drinks. Caffeine has drawn more attention due to its physiological effects beyond that of its stimulatory effect. Consumers are interested in knowing the exact amounts of caffeine existing in beverages. It is important to obtain information about the drinks because they are widely consumed all over the world. Various analytical techniques are used for tea analysis like HPLC because these techniques have high accuracy and precision but UV-Vis spectrophotometry is mostly used because it is cheap and easily available in laboratories. Results showed the largest amount of caffeine was present in Brand X and the smallest amount - Brand-Z. as per the US FDA the daily intake of the caffeine content not more than the 200-ppm concentration.

KEYWORDS: Caffeine, accuracy, precision.

INTRODUCTION

Caffeine is a naturally occurring alkaloid which is found in the leaves, seeds or fruits of over 63 plants species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves and the worldwide consumption of products derived from these natural materials means that caffeine is one of the most popular and commonly consumed drugs in the world. Caffeine's popularity stems mainly from the fact that it is a pharmacologically active substance and a mild central nervous system stimulant. It is generally agreed that there is little risk of harm when a person consumes less than 300 mg of caffeine a day.[1,2] However at times of anxiety or stress, or during pregnancy, the FSA recommends consumption of less than 200 mg a day.[3] While there are no regulatory requirements to control or label food products with their caffeine content, numerous studies have been carried out to determine the typical caffeine content of commonly consumed beverages. A wide variety of methods have been employed with High Pressure Liquid Chromatography (HPLC) being the method of choice in many analytical studies as it commonly is subject to fewer interferences than alternative methods.

The Food and Drug Administration (FDA) defines caffeine as a generally recognized as safe (GRAS) substance. However, FDA specifies that the maximum amount in carbonated beverages is limited to 0.02% (FDA 2006). Therefore, the highest legal amount of caffeine allowed in a 355 mL (12oz) can of soft drink is about 71mg. Caffeine has attracted the interest of consumers and health professionals alike due to its wide consumption in the diet by a large percentage of the population and its pharmacological effects in humans (Mandel 2002). The human's saliva caffeine level, which demonstrates the extent of absorption, peaks around 40 minutes after caffeine consumption (Liguori*et al* 1997). Its physiological effects on many body systems have been reported by researchers, including the central nervous, cardiovascular, gastrointestinal, respiratory, and renal systems (Nehlig*et al* 1992). The International Olympic Committee (IOC) defined caffeine as a drug and abuse is indicated when athletes have urine caffeine concentrations higher than 12µg/mL (de Aragao*et al* 2005).

Caffeine Chemistry and General Information

Caffeine (1, 3, 7-trimethyxanthine), theophylline (3, 7-dimethylxanthine), and theobromine (1, 3-dimethylxanthine) are in the family of alkaloid methylxanthines.

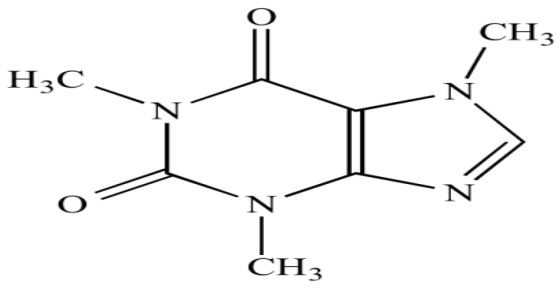


Figure 1: General Structure of Caffeine.

Caffeine (1, 3, 7-trimethylxanthine) Caffeine is an odorless, white solid that has the form of needles or powder. Caffeine has a bitter taste. The molar mass of caffeine is 194.19 g/mol. Caffeine is slightly soluble in water due to its moderate polarity. Caffeine is a natural central nervous system stimulant, having the effects of reducing drowsiness and recovering alertness. Since it is widely consumed by humans, caffeine is considered the most frequently used psychoactive substance in the world (Ligouri*et al* 1997).

Physiological effects of caffeine to human Caffeine has numerous physiological effects on major organ systems, including the nervous system, cardiovascular system, digestive system, and respiratory system. Renal function and skeletal muscles are also affected by caffeine. Numerous studies have proven caffeine to be a stimulant to human's central nervous system (Spiller, 1998). It is also increase heartbeat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in plasma. 1 g of caffeine leads to insomnia, nervousness, nausea, ear ringing, flashing of light derillum and tremulosness. In cases of overdosing and in combination with alcohol, narcotics and some other drugs, these compounds produce a toxic effect, sometimes with lethal outcome (Mamina and Pershin, 2002; Ben Yuhas, 2002; Wanyika*et al*., 2010; James *et al*., 1990; Tavallali and Sheikhaei, 2009). Caffeine facilitates the conduction velocity in the heart and directly affects the contractility of the heart and blood vessels. Nevertheless, caffeine may significantly reduce cerebral blood flow by constricting of cerebral blood vessels. Caffeine provides a diuretic effect due to elevating the blood flow and glomerular filtration rate of the kidneys. Heartburn is an issue for some subjects' gastrointestinal system after consuming caffeine. The effects of caffeine to skeletal muscles are mainly the increasing occurrence of tremors (James 1991; Spiller 1998).

MATERIAL AND METHODS

Instrument

UV/VIS spectrometer Perkin Elmer lambda35.

The double beam spectrophotometer having the range 190-1100 nm and bandwidth: 0.4-4 nm (variable).
pH determination Beverages pH was determined by using Sartorius pH meter.

Preparation of stock solution

All glassware was washed with distilled water. Then glassware was dried in oven at 105ºC.

A 100 ppm stock standard of caffeine was prepared by dissolving 20 mg caffeine in 250 ml carbon tetra chloride in 200 ml volumetric flask.

Preparation of standard solution

Working standards were prepared by pipetting 0.1, 0.15, 0.2, 0.25, 0.3ml respectively aliquots of stock standard solution into a separate volumetric flasks of 10 ml and dilute it with carbon tetra chloride and forms 1, 1.5, 2, 2.5, 3 mg/L standards solution. The absorbance of each solution was measured at absorption maximum of 270 nm using 10 mm quartz cuvettes.

Caffeine extraction procedure

The brands different soft drinks were taken by different shops. Then the sodium carbonate solution is prepared by dissolving 20g sodium carbonate into distilled water in 25ml volumetric flask. Then separating funnel was taken and adjusts it in the stands with beakers. Then 5ml of drink sample was drawn in the separating funnel by addition of distilled water and add 1ml of sodium carbonate solution in the separating funnel and add 20ml of carbon tetra chloride in it. The caffeine was extracted by inverting funnel at least three times venting the funnel aier each inversion. The non-aqueous carbon tetra chloride layer was removed to a clean 50ml volumetric flask. Another 20ml portion of carbon tetra chloride was added to aqueous solution in separating funnel and extraction procedure was repeated twice and carbon tetra chloride layers combined. This procedure was repeated for all drink samples the absorbance of resulting solutions was measured on UV/Vis Spectrophotometer at 270 nm using 10 mm quartz cuvette.

RESULTS AND DISCUSSION

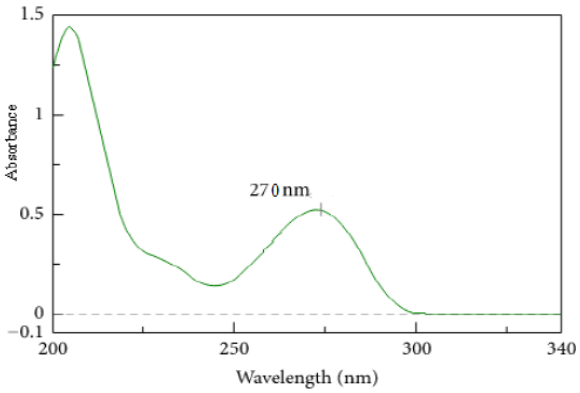


Figure 1: UV Spectrum of caffeine at 270nm.

Calibration line preparation for caffeine analysis

The caffeine spectrum was taken at UV range between 200-400nM and the maximum absorption spectrum were occurred 270 nM which indicates that its maximum absorption occurs at 274nm. So further photometric analysis of all samples was carried out at 270 nm.

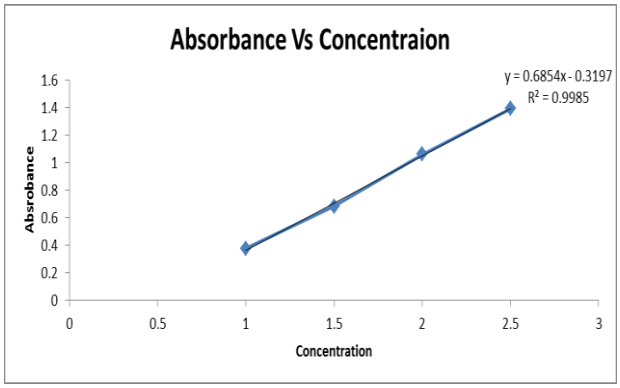
The calibration curve for caffeine was drawn by using calibration solutions of caffeine in the concentration range of 1-3 ppm and absorbance was measured on a UV/Vis spectrometer at wavelength 270 nm, as shown in Table 1.

Calibration curve for the caffeine standard

S. No	Volume (ml)	Absorbance at 270nm
1	1	0.378
2	1.5	0.684
3	2	1.063
4	2.5	1.394
5	3	1.845

Absorbance value for the different soft drink beverages

S.No	Sample Code	Absorbance at 270 nM
1	X	1.52
2	Y	0.89
3	Z	0.437



Calibration curve for the caffeine standards

The absorbance values of the six working standard solutions were measured;

A linear regression of absorbance versus standard concentration, forced through the origin, gave equation 1. y = 0.0181x…… [1]

A linear regression of concentration vs absorbance allowed the factor of 55.358, included in equation 2, to be determined.

Equation 2 was then used to calculate the concentration of caffeine in the extracted sample solution, from the solutions measured absorbance value.

Conc (ppm) = 55.358 x Abs…… [2]

The final caffeine content of the beverage under test is then calculated from the extracted sample solution's concentration using equation 3. Dividing this value by the volume of the drink gives the caffeine content per ml.

$$\text{Caffeine content mg} = \text{Conc (ppm)} \times \frac{(\text{Total Sample Vol [ml]})^2}{(\text{Measured Sample Vol[ml]}) \times 1000} \quad \text{.....[3]}$$

Sample No	Total volume (ml)	Measured Volume (ml)	Caffeine content
X	330	50	0.56
Y	330	50	0.33
Z	330	50	0.16

DISCUSSION

The analysis of the caffeine in the different soft beverages measured the UV Visible spectroscopy .and the caffeine spectrum was run at 200-400nm and spectrum shows the absorption Max at 270nm. The standard concentration was prepared at ration of 1-3 µg/ml and the calibration curve was made the linear equation was found to be y = 0.685x - 0.319.

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Conc (ppm) = 55.358 x Abs…… [2]

The final caffeine content of the beverage under test is then calculated from the extracted sample solution's concentration using equation 3. Dividing this value by the volume of the drink gives the caffeine content per ml.

The caffeine content in the different brands were calculated and found to be for Brand-X, Brand-Y, Brand-Z was found to be 0.56, 0.33,0.16 mg respectively.

CONCLUSION

UV/Vis spectrophotometry was used for the determination of caffeine in beverages. Caffeine is the world's most widely consumed psychoactive drug. It is important to obtain information about the drinks because they are widely consumed all over the world. Various analytical techniques are used for tea analysis like HPLC because these technique has high accuracy and precision but UV-Vis spectrophotometry is mostly used because it is cheap and easily available in laboratories. Results showed that in case of tea samples the largest amount of caffeine was present in Brand X and the smallest amount - Brand-Z. As per the US FDA the daily intake of the caffeine content not more than the 200 ppm concentration.

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