Estimation of Caffeine in Chocolates and Soft drinks by High Pressure Liquid Chromatographic technique

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Abstract:
Caffeine is a central nervous system stimulant of the methylxanthine class of psychoactive drugs. It is the world's most widely consumed psychoactive drug. High Pressure Liquid Chromatographic technique was employed to study caffeine contents in different coal drinks and chocolates. The concentration of caffeine in 1gr. Coffee bite was found to be 1887.5µg, 70.193µg in Galaxy smooth, 60.590µg in Dairy milk. We also estimate the amount of caffeine in every 50ml of soft drinks also. Results conforms that Pepsi contain 228.973µg, 85.878µg in coco cola. All the samples in the study contain high amount of caffeine. The intake of caffeine may results nurvesness, restlessness, and nervousness, increased heartbeat, nausea, anxiety, heart palpitations, insomnia, sweating, dizziness, vomiting and cardiac arrest.

Key Words: Caffeine, HPLC Estimation, coal drinks, chocolates

Introduction:
Caffeine is an alkaloid that occurs naturally in the leaves, seeds and fruit of tea, coffee, cocoa, kola trees and more than 60 other plants. The widespread occurrence of caffeine in a variety of plants played a major role in the long-standing popularity of caffeine-containing products. The most important sources of caffeine are coffee (Coffea spp.), tea (*Camellia sinensis*), guarana (*Paullinia cupana*), maté (*Ilex paraguariensis*), cola nuts (Cola vera), and cocoa (*Theobroma cacao*). The amount of caffeine found in these products varies – the highest amounts are found in guarana (4 – 7%), followed by tea leaves (3.5%), maté tea leaves (0.89 – 1.73%), coffee beans (1.1 – 2.2%), cola nuts (1.5%), and cocoa beans (0.03%).

Recreationally, caffeine is used to provide a boost of energy or a feeling of heightened alertness. It is often used to stay awake longer. The pleasant stimulant feeling, which often occurs at low doses, may be replaced by psychological symptoms, which resemble anxiety and depressive neuroses at high doses. Those with more severe psychological problems may have their symptoms exaggerated with excessive caffeine usage, or such symptoms can actually be caused by excess. Diagnosis of such conditions must take caffeine usage into account. Caffeine abuse is more prevalent than we may imagine.

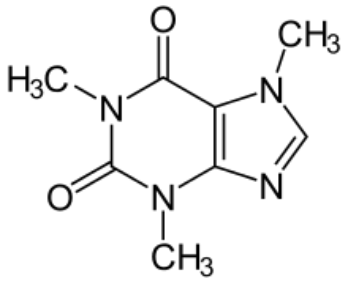

Figure 1 Molecular structure of caffeine

Caffeine is added to soft drinks as a flavoring agent, and from dietary sources is the most frequently and widely consumed central-nervous-system stimulant today. Nevertheless, it was classified as a drug of abuse by the International Olympic Committee (IOC) when present in urine at concentration levels of more than 12µg/mL. Caffeine is found in chocolate products and cocoa, and is responsible for the bitterness of these products. Increasing consumer awareness of the effects of caffeine content in chocolate and cocoa based food and beverages on their health has led to greater interest in the alkaloid.

High-pressure liquid chromatography has recently been utilized to identify and quantify caffeine levels in chocolate products and cool drinks. Hence we identify and quantitative estimation of **Caffeine** in different soft drinks and chocolates were studied using HPLC.

Materials and Methods:

Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Chemical and solvent

Caffeine: Laboratory Reagent Grade with 99% purity, NP Chemicals, Mumbai
Methanol: HPLC grade, Fisher scientific products, Mumbai
Acetonitrile: HPLC grade, Merck chemicals, Mumbai
Chloroform: Laboratory Reagent Grade, Merck chemicals, Mumbai.

3.2.3 Collection of Sample

The Chocolates and coal drinks were purchased in local market, Guntur.

3.2.4 Preparation of standard stock solution

The standard stock solution of caffeine was prepared by weighing 10mg of the standard caffeine and was dissolved in little mount of Methanol taken in 10ml volumetric flask. The solution was sonicated for 10min to complete solubility of standard caffeine in Methanol. Then make the solution up to the mark. A concentration of 1000µg/ml of standard caffeine solution was obtained. The solution was filtered using 0.45µ membrane filter paper. Then from this stock solution, 1ml was taken and further diluted to 10ml to get a stock solution of 100µg/ml. from this stock solution, required dilutions were prepared by proper dilutions.

3.2.5 Preparation of standard dilution

The standard dilution was prepared by diluting 100µg/ml of the stock solution to serially diluted the concentration was 50, 60, to 100 respectively. The resultant dilutions were used for construction of calibration curve.

3.2.6 Extraction caffeine from sample

The chocolate and coal drinks sample were extracted to collect the caffeine. 1gram of chocolate sample accurate Weighed by electronic balance. The sample was taken in 250ml beaker and was dissolved in 200ml water. 50ml each of the sample cool drink was make up to 200ml with distilled water. Both the

chocolate and cool drink samples in water was warmed in a water bath for 30minutes. After 1hrs the sample has filter by whattmen filter paper and separate the caffeine in separating funnel using 10ml of chloroform, mix well 10 minutes and keep the separating funnel on work bench to separate the organic and aqueous layers, after separated two layer chloroform layer was collected. Same procedure was repeated using with another 10ml of chloroform and the organic layer was collected. Both the organic layers were mixed and the chloroform was evaporated and the caffeine present in the sample was reconstructed with 5ml of methanol. The extracted caffeine was injected into HPLC system to determine the total amount of caffeine present in the sample.

Fig 2 Extraction of caffeine Conditions

HPLC for Estimation of Caffeine

The fallowing analytical conditions were adopted for the estimation of Caffeine in the isolated samples.

S. No	Parameter	Conditions
1	Mobile phase	Methanol and Acetonitrile (v/v ratio 3:1)
2	Pump mode	Isocratic
3	Stationary phase	C18, 250 x 4.6 and 5µm particle size
4	Flow rate	1ml/ minutes
5	Wave length	284nm
6	Retention time	5.3min
7	Run time	10 minutes
8	Injection Volume	20µm

Table 1 HPLC for Estimation of Caffeine

Construction of Calibration Curve

From the prepared stock solution, a series of calibration standards were prepared at concentrations of 50, 60, 70, 80, 90 and 100µg/ml using mobile phase as solvent. Each of these standard caffeine solutions (20µl) was injected into the column, the peak area and retention times were recorded. The calibration curve for caffeine was constructed by plotting the mean peak area against the standard caffeine concentration.

Estimation of sample

Different samples extracted from chocolates were injected into the HPLC system having the same chromatographic conditions selected for the analysis of the standard for preparing calibration curve and the retention time of the samples were compared with the standard and the amount of the caffeine

present in the samples were analyzed by substituting in the area values in the equation and the amount of caffeine found in the sample can be determined.

Result and Discussion

The caffeine contained in chocolate and coal drinks will give you a significant boost of energy. It usually provides you with an increase in performance for more monotonous tasks like filing or simple bookkeeping, and it can also help you stay awake during drawn out tasks such as long distance driving or working as a security guard.

Calibration curve was obtained with in a concentration range of 50µg/ml to 100µg/ml. regression equation was found to Y = 9647.7X – 86236 with correlation of 0.9973. The amount of caffeine found in different chocolates can be shown in table. Linearity results were presented in Table no.2 and calibration curve was shown in Figure no.3 all the chromatograms of standard 50µg/ml were shown in figure no. 4

Table 2 Standard caffeine Linearity

S. No	Concentration µg/ml	Area of peak
1	50	401531
2	60	481471
3	70	584579
4	80	698673
5	90	787187
6	100	870624
	Slope Intercept correlation	9647.7 -86236 0.9973

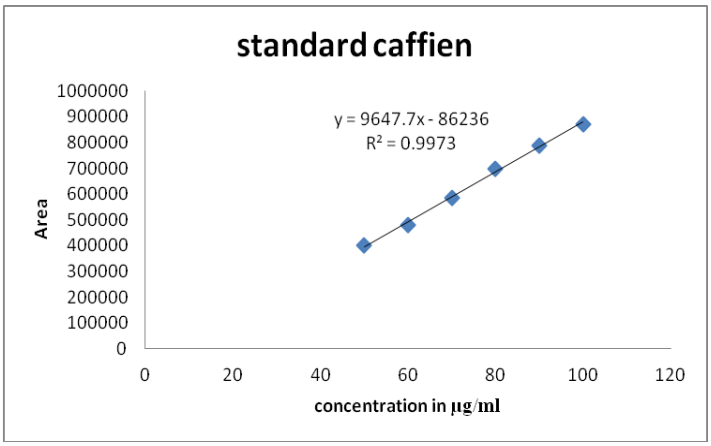

Fig.no.3 standard caffeine caibration curve

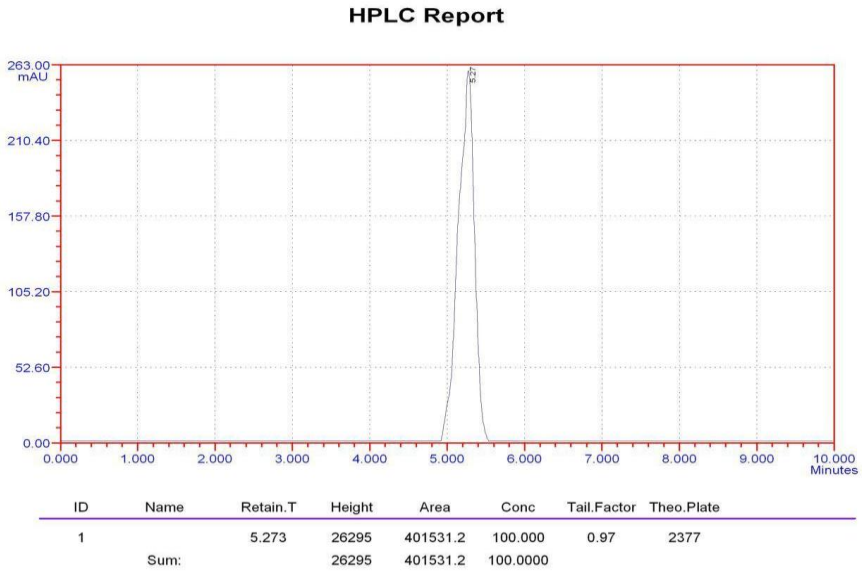

Fig. 4 Chromatograms of standard 50µg/ml caffeine solution

The results of the sample chromatograms confirm that the extracted sample contains caffeine. The retention time of the caffeine sample was found to be 5.3min. Same retention time was observed for standards. This confirms that all the samples in the study contain caffeine. The peak area at the retention time of caffeine was used for the estimation of the amount of caffeine present in the samples. The standard regression equation of Y = 9647.7X – 86236 was used for the estimation of caffeine in samples. The amount of caffeine present in the samples was given in table no.3. Among all the cool drinks in the study, Coke diet contain high amount of caffeine. An amount if 597.451µg of caffeine was present in 50ml of Coke diet. Every 100ml of Coke diet drink contain more than 1mg of caffeine. Coca cola contain comparatively less amount of 85.878µg of caffeine.

S. No	Coal drink sample	Retention time	Area of peak	Caffeine content in 50ml of Soft drink
1.	50ml Pepsi	5.33	355577.1	228.973µg
2.	50ml Thumsup	5.348	220514.2	158.976 µg
3.	50ml Coke diet	5.342	1066569.5	597.451µg
4.	50ml Coca Cola	5.348	79469.5	85.878 µg

Table no. 3 coal drink sample content caffeine

Among all the chocolates in the study, Coffee bite contains more amount of caffeine. Every one gram of Coffee bites contain1887.5µg of caffeine. Dary milk contains comparatively less amount (60.59 µg) of caffeine. Results were given in table 4.

S. No	Chocolate sample	Retention time	Area of peak	Caffeine content per 1g of sample
1.	Coffee bite	5.333	3555771.1	1887.5 µg
2.	Galaxy smooth	5.36	49204.8	70.19 µg
3.	Dary milk	5.38	30674.3	60.59 µg
4.	5 star	5.353	47672.6	69.40 µg

Table no.4 Chocolate sample

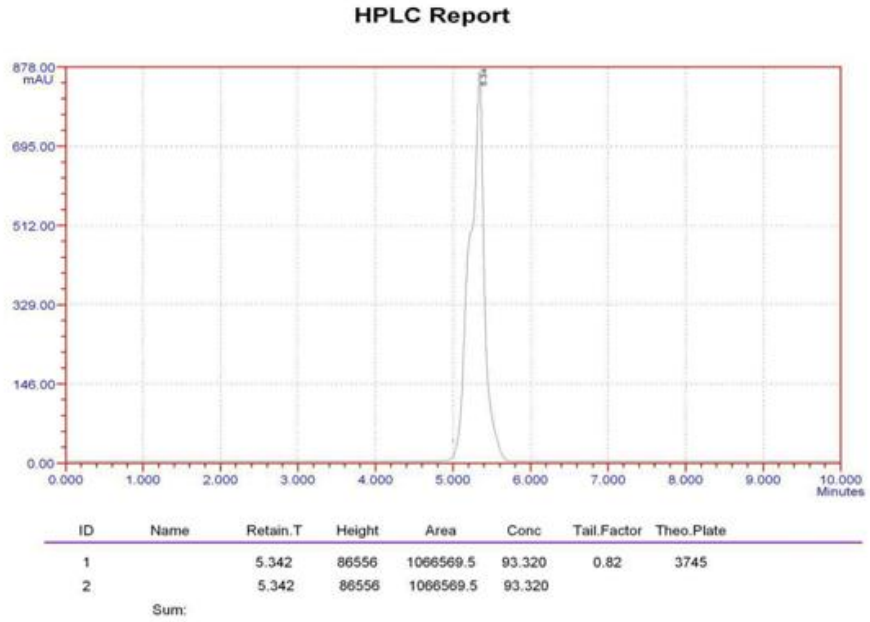

Fig. no 5 Chromatogram of coke diet

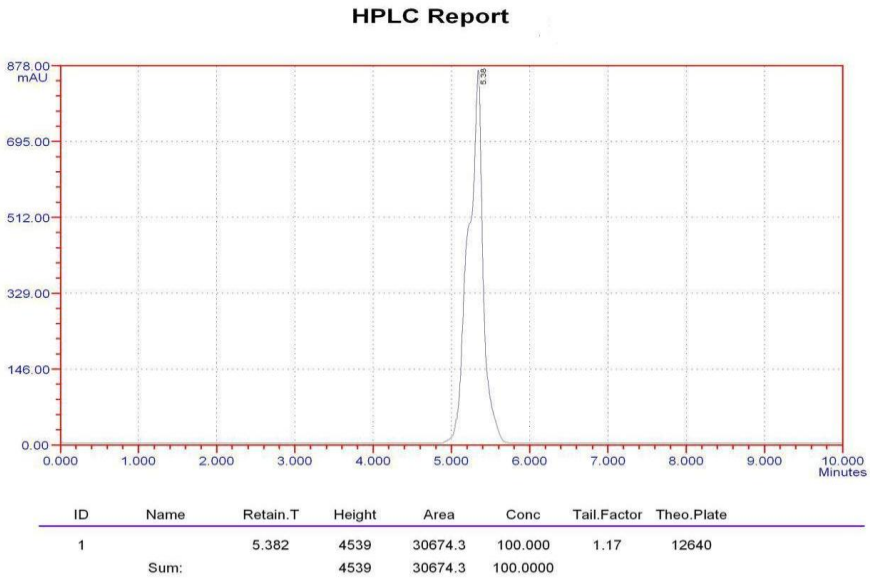

Fig. no 6 Chromatogram of dary milk chocolate

Conclusion:

Estimate the caffeine content in different soft drink and chocolate using high performance liquid chromatography was studied. a mobile phase of methanol and acetonitrile in the ratio of 75:35 (v/v) at a flow rate of 1ml/min and UV detection of 284nm was employed for the estimation of caffeine. Separation was carried using Zodiac C18 column. In the standard condition, the retention time of standard caffeine was found to be 5.3min. Liquid-liquid extraction method was fallowed for the extraction caffeine in samples and was analyzed in same standard conditions. the retention times of samples was found to be same as standard. The amount of caffeine present in samples was estimated using regression equation. 228.9732µg of caffeine was present in 50ml Pepsi. Similarly, the

concentration of caffeine in coca-cola was found to be 85.87824 µg. and the concentration of caffeine in Thumps-up was found to be 158.9758 µg, the concentration of caffeine in Coke diet was found to be 597.4509µg. The concentration of caffeine in 1gr. Coffee bite was found to be 1887.5 µg. The concentration of caffeine in 1gr. Galaxy smooth chocolate was found to be 70.19331 µg. The concentration of caffeine in 1gr. Dary milk chocolate was found to be 60.58973 µg. In different brand of soft drink the highest concentration of caffeine was found in Coffee bite and the lowest concentration of caffeine was found in dary mlk chocolate. Preliminary studies on coffee suggest that it may have some health benefits to people who suffer from Alzheimer's, Parkinson's disease, heart disease, and diabetes, among others. But the daily tolerable dosage of caffeine was 200 to 300 mg per day. The over dosage may results nurvesness, Restlessness, and Nervousness, Increased heartbeat, Nausea, Anxiety, Heart palpitations, Insomnia, Sweating, Dizziness, Vomiting and Cardiac arrest. All peoples should be aware of the effects of caffeine and avoid those chocolates and cool drinks contain high content of caffeine for healthy life otherwise their health at risk.

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