

Introduction

Caffeine is a natural stimulant most commonly found in tea, coffee and cacao plants. It works by stimulating the brain and central nervous system, helping you to stay alert and preventing the onset of tiredness. Is arguably the most frequently ingested pharmacologically active substance in the world. It is occur naturally in more than 60 plants .Caffeine is an organic molecule molecular formula $C_sH_mN_sO_r$.[1]



Caffeine belongs to the group of purine alkaloids, often referred to as methyl xanthine's, which include caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), and the bromine (3,7-dimethyl xanthine) more members than these three, Purine is the parent heterocyclic compound of the methyl xanthine's, which are often referred to as the purine alkaloids. [2]

Pure caffeine occurs as odorless, white, fleecy masses, glistening needles of powder. Its molecular weight is 194.19 g/mol, melting point is 236° C, point at which caffeine sublimes is 178° C at atmospheric pressure, pH is 6.9 (1% solution), specific gravity is 1.2, volatility is 0.5%, vapor pressure is 760 mmHg at 178°C, solubility in water is 2.17%, vapor density 6.7[3-6]

Caffeine is also a common ingredient of soft drinks where it is deliberately added as a flavoring agent and to make the drinks addictive. Caffeine content in soft drinks varies by brand from 10 to 50 mg of caffeine per serving [7], however the US Food and Drug Administration [8] limits the maximum amount in carbonated beverages to 6 mg/oz. Therefore caffeine content allowed in soft drinks may be in the range between 30 and 72 mg/355 mL (12 oz) or 8.45-20.28 mg/100 mL [9]

Method

A 100 ppm stock standard of caffeine was prepared by dissolving 25 mg of caffeine in 250 mL purified carbon tetrachloride (CCl₄) in a volumetric flask (250 mL). Standards were prepared by pipetting 10, 20, 30, 40, 50, 60, 70 and 80 mL, respectively aliquots of the stock standard solution into separate volumetric flasks (100 mL) and diluting to volume with purified carbon tetrachloride to produce concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 mg/L, respectively standard solution.

The absorbance of each solution was measured at absorption maximum of 280 nm using 10 mm quartz cuvette. The absorbance values were then plotted against concentrations to generate a standard calibration curve.

An aliquot (10 mL) of the drink sample was drawn with a 10 mL pipette and placed into a 125 mL separating funnel followed by the addition of distilled water (10 mL), then 20% aqueous Na₂CO₃solution (1 mL) and

analytical grade CCl4 (20 mL). The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. The non-aqueous CCl4 layer was removed to a clean 50 mL volumetric flask. Another 20 mL portion of CCl4 was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice more and the CCl4 solvent layers combined. This volume was made up to 50 mL with the solvent. This procedure was repeated for all the drink samples. The absorbance of the resulting solutions was then measured on UV/Vis spectrophotometer at 280 nm using 10 mm quartz cuvette.

Quantitative analysis of caffeine was performed by PD-303UV/Vis Spectrophotometer. The λ max was determined by scanning the standard solution from 200-600 nm and the obtained results gave an absorption spectrum, which was characterized by a single intensive absorption band located in the UV range at λ max = 325 nm. Standard linear calibration curve was run to obtain the linear range of sample analysis, and the standard calibration curve was linear over the range (10-80) ppm caffeine with equation (y = 0.0226x + 0.1443). The quantitative amount of caffeine in samples (ppm) was then determined using the standard curve.[10]

Results

The standard linear calibration curve obtained from the standard solutions analysis is presented in Fig (2). It showed a good linear relationship between the absorbance and concentrations of the standard solutions.



Fig 2: Calibration curve for standard caffeine.

Table (1): caffeine contents of beverage samples

Sample name	Caffeine conc. (ppm or mg/L)
Pepsi cola	71.01
Pepsi diet	60.11
Miranda	36.15
Mountain dew	56.02
Coca cola	70.56
Seven up	42.57
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Discussion

A moderate caffeine intake of 300 mg / day is generally safe [11]. This amount usually corresponds to 4-6 cans of soft drinks. Caffeine content in beverage beverages varies by brand from 10 to 70 mg of caffeine per meal [12]. However, the US Food and Drug Administration [8] limit the maximum amount of soft drinks to 6 mg / oz (200 ppm). Therefore, caffeine content in soft drinks can be in the range of 30 to 72 mg / 355 ml (12 oz) or 8.45-20.28 mg / 100 ml [9]. It is clear that the levels of caffeine in drink samples analyzed in this study are well below the maximum limits set by the above-mentioned food regulators.



Fig. (3): Chart showing caffeine contents in the beverage samples.

Conclusion

The results of this study showed that the concentration of caffeine in soft drinks obtained from the local market in Saudi Arabia was well below the authorized limit. The UV / VIS spectroscopy technique used in this study to measure caffeine in beverages was relatively easy and fast and cheap. The main tool required is the computerized Off-Vis / Spectrum Modern that can be obtained at an affordable price. Therefore, this analytical method for the rapid, accurate and sensitive quantity of caffeine in beverages may be recommended by any educational institution in developing countries.

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