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Analysis of caffeine contents in commercial beverages and tea samples of Pakistan using UV/Visible spectrometry

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In this study, caffeine contents in tea samples and beverages were determined photometrically using UV/Visible spectrometry which is a rapid, precise and accurate method. Calibration solutions were prepared in the concentration range of 1-25 ppm from a 100 ppm stock solution. Absorbance of all calibration solutions was measured at the absorption maximum at 274 nm. Caffeine was extracted from tea samples and beverages by chloroform and reading was performed against chloroform as a reference. The concentration of unknown samples was read from the calibration graph. Various concentrations of caffeine were present in the tea samples and beverages. Results showed that in case of tea samples the highest amount of caffeine (16.111 mg/g) was present in Tetley and the lowest amount (0.251 mg/g) - in Ghatnctr. In case of beverages the highest amount of caffeine (222.3 mg/L) was present in Boost and the lowest (0.101 mg/L) - in 7-up.

Keywords: Beverages, tea samples, UV/Vis spectrometry, caffeine.

INTRODUCTION

Caffeine is an alkaloid belonging to the methylxanthines family. pH of a 1% solution of caffeine in water is 6.9. It is a stimulant drug having bitter taste in its pure state and it is white crystalline xanthine [1]. 1,3,7-trimethylxanthine is the systematic name of caffeine. Other names of caffeine are trimethylxanthine, theine, mateine, guaranine and methyl theobromine. Formula of caffeine is given in Fig. 1 and molar mass is 194.19 g/mol. It is an odorless white powder or needles. Density of caffeine is 1.2 g/cm³. Caffeine solubility is very low in water, slight in ethyl acetate, pyrimidine, pyrrole, acetone, and very high in petroleum ether, ether, benzene and chloroform. The melting point of caffeine is 237 °C and the boiling point is 178 C (sublimation) [2].

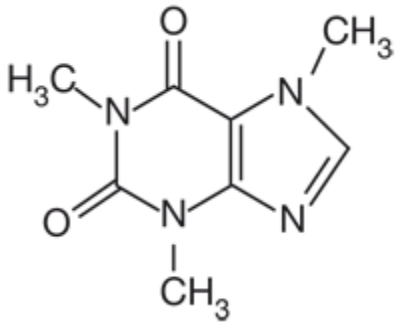

Fig. 1. Caffein structure.

Acidity of caffeine is 10.4. At room temperature caffeine solubility in water is moderate (2 g/100 mL), but very high in boiling water (66 g/100 mL). It is also moderately soluble in ethanol (1.5 g/100 ml) [3]. Caffeine is weakly basic having a pK_a value of 0.6; a strong acid is required to protonate it. Caffeine is included in achiral molecule [4] because it contains no stereogenic centres. Methylation of theobromine forms caffeine. Caffeine and related compounds contain an imidazole ring fused to a pyrimidine ring [5]. The metabolic product of caffeine is divided into three primary metabolites (paraxanthine 84%, theobromine 12% and theophylline 4%) - metabolic products formed in the liver. Caffeine is an antagonist of adenosine. It possesses diuretic action (favors urination) [6].

There are many sources of caffeine including tea, coffee, soft drinks, energy drinks, chocolates, cocoa beans, etc. Tea is a common major source of caffeine. Certain types of tea such as black tea and oolong tea contain a higher concentration of caffeine, as compared to other types of tea, such as green tea. Small amounts of theobromine are present in tea. The latter also contains theophylline at a slightly higher level as compared to coffee. The colour of tea is not a good indicator of caffeine amount because the colour of caffeine is white [5].

Tea is included in aromatic beverages; preparation of tea is by pouring hot or boiling water over leaves of the tea plant. China is the origin of tea as a medicinal drink [7]. Tea is derived from a plant called *Camellia sinensis*, which is a shrub native to China and India (Asian countries) but its cultivation also occurs in tropical and subtropical areas. Tee contains unique antioxidants called flavonoids. During the seventeen century it became popular in British countries. The British are responsible for the introduction of tea in India. Anti-inflammatory and neuroprotective properties have been known in tea catechins, helping to regulate food consumption [1].

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The risk of diseases such as stroke, cognitive impairment, and osteoporosis is lower for elderly people consuming green tea. Many other nutrients including anti-oxidants, minerals, caffeine, vitamins, amino-acids, theobromine and theophylline which have strong beneficial effects on human health are also present in tea. The tea differences arise from geography, growing conditions, method of processing and time of vintage. White, green, black and oolong tea include herbal infusions, also called herbal tea. Each type of tea has specific characteristics including different taste and varyous benefits to health. There are five basic types of tea: black, oolong, green, white, and puer.

The purest form of tea includes white tea and has light flavor and color, the most delicate form of all teas is white tea. Green tea is the most favorite choice of beverage in Asia. It is made of steamed tea leaves having high amount of epigallocatechin Gallate (EGCG). Green tea has a subtle flavor. Non-fermented tea is green tea having catechins in a large amount as compared to black tea or oolong tea. Wu long tea is another name of Oolong tea, having a flavor full aroma. Oolong tea is undergoing partial oxidation. The taste and fragrance of fresh flowers are often compared to Oolongs. Oolong tea contains large amounts of antioxidants. Black tea has a robust flavor. Aged black tea is imported from China. It is incredibly deep and rich in flavor without bitterness. It is made of fermented tea leaves; black tea has the greatest caffeine content and a high quantity of polyphenols such as flavonoids. These flavonoids work against harmful agents [8]. Y
The most common component of soft drinks is caffeine, such as Coca-Cola, which is derived from kola nuts. 10 to 50 mg of caffeine per serving are present in soft drinks but the energy drink Bull contains 80 mg of caffeine per serving [9].

There are many advantages of caffeine like: it is used to reduce physical fatigue due to its medicinal properties [6]. Caffeine can induce hair growth. Dopamine concentration increases in the brain by caffeine which helps to ease depression. Glutamate and dopamine concentrations enhance by caffeine in shell of nucleus accumbens [10]. Caffeine increases stamina, protects the person from eyelid spasms, cataracts, prevents skin cancer. Caffeine can decrease the risk of several types of cancer including liver and colorectal cancer. These cancer risks are reduced by drinking coffee. Caffeine reduces the risk of type 2 diabetes and Parkinson's disease. In type 2 diabetes, the ability to use insulin regulates blood sugar effectively in the body [11].

There are many disadvantages of caffeine, e.g., it can cause anxiety if high doses of 300 mg or higher are taken [12], accelerates bone loss at the spine in elderly postmenopausal women and causes an increase in the likelihood of experiencing auditory hallucinations. The central and sympathetic nervous systems are stimulated by caffeine which results in an elevation of hormones which causes the body to enter in a state similar to that of the fight or flight response [11]. Excess or higher dosages of caffeine can cause several problems that include high blood pressure, restlessness, irritability, anxiety, heartburn, headache (sometimes severe), sleeplessness, increased heartbeat, nausea, enhanced urination, heart palpitations, gastrointestinal disturbance (diarrhea), dizziness, nervousness and jitters.

The absorbance of calcium, magnesium, zinc, chloride and sodium in the body is reduced by using caffeine. Osteoporosis is caused by caffeine. Iron present in blood is oxidized by caffeine as much as 75%. Sugar and fatty acid concentrations in blood and homocysteine level increase by caffeine. Melatonin formation is reduced by caffeine [13].

Beverages are drinks containing caffeine mainly for human use [14]. There are two types of beverages: alcoholic and non-alcoholic beverages. Non-alcoholic drinks include those beverages that have a small quantity of alcohol or alcohol may not be present. Examples of non-alcoholic beverages are non-alcoholic wine, energy drinks including Red Bull, Monster and Power House. Alcoholic beverages are those which contain alcohol in varying amounts. Examples of alcoholic drinks are cider, wine, etc.

There are many advantages of drinking tea. Antioxidants present in green tea affect the growth of bladder, breast, stomach, pancreatic, lungs and colorectal cancers. The antioxidant EGCG is present in green tea, which can treat various diseases. Green tea has health enhancing benefits as compared to black and oolong tea [15]. White tea can help you look younger, white tea helps to inhibit wrinkle production by strengthening the collagen and elastin. Antioxidants found in oolong tea decrease the bad cholesterol levels. Wuvi is a variety of oolong tea used as a weight loss diet; green tea also reduces the bad cholesterol amount and prevents platelet clumping [16].

There are many disadvantages of drinking tea. Fluoride is present in all tea leaves, mature leaves contain a higher amount of fluorides as compared to young leaves from the same plant [17]; overconsumption of tea can cause fluorosis in humans [18]. Tea leaves contain some amount of aluminium [19]. The levels are safe but sometime that level of aluminium becomes the cause of

Alzheimer's disease [20]. The symptoms of Alzheimer's disorder are lack of learning skills, loss of memory, lack of imagining power and reasoning skills in acute cases. Oxalates are also present in tea, kidney stone are formed by oxalates**.** Oxalates are accumulated in the kidney tissue [21]. Large amounts of black tea use by men, enhance risk of prostate cancer by 50% [22]. Uv/Vis spectrometry is used for the determination of caffeine in tea, coffee and beverages [23].

EXPERIMENTAL WORK

Chemicals

The chemicals used in this study include hydrochloric acid (HCl), chloroform ($CHCl_3$) obtained from Friend's laboratory chemical, sodium carbonate (Na_2CO_3) obtained from Riedel-de Haen and caffeine obtained from AppliChem.

Sample collection

Different samples of tea and beverages were purchased from different markets of Kasur (Pakistan). Different types of energy drinks and soft drinks including Coca-Cola, Pepsi, diet cola, mountain dew, Gourmet cola, Boost, Panda, Red bull, Sting, Mask, Pepsi diet, Power house were purchased from the local markets in Pakistan.

Instrument

The UV/vis spectrometer Labomed UVD-3500 was used for the analysis of caffeine in different samples of tea and beverages.

Calibration solutions preparation

Caffeine stock solution of 1000 ppm was prepared by dissolving 0.1 g of pure caffeine in 100 mL chloroform. It was analyzed by UV/Vis spectrometry for determining λ_{max} and the resulting spectrum is shown in Fig. 2. Further dilutions were prepared in the range of 1-25 ppm and their absorbances were measured at λ_{max} 274 nm as shown in Fig. 2. The resulting values are given in Table 1. They were used to draw the calibration line for caffeine analysis as shown in Fig. 3. Cuvettes of plastic were not used because chloroform dissolved plastic.

Extraction of caffeine from tea

All glass apparatus was rinsed with chromic acid and distilled water before use. 2 g of dried tea powder was taken in a beaker and 20 mL of distilled water was added to it and boiled. After boiling, 2 g of sodium carbonate was added for precipitating tannins, then filtered. The filtrate was heated and concentrated to 5 mL. Then 5 mL of chloroform was added for extraction of caffeine using a separatory funnel. The extract was analyzed for caffeine contents and the average values are reported in Table 2.

Extraction of caffeine from beverages

A beverage portion was drawn by a 10 mL pipette and poured directly in a separatory funnel, then 1 mL of 20% (w/v) sodium carbonate solution and 5 mL of chloroform were added and shaken for few minutes. The lower (organic) layer containing caffeine was taken in a sample cell [6].

Preparation of a sample solution of tea

The best solvent used for the direct UV-Vis spectrophotometric determination of caffeine in tea and beverages was chloroform, as shown by Maidon [1]. He compared four solvents for caffeine analysis including chloroform, methanol, ethyl acetate and water. Among them, chloroform displayed th best ability to dissolve tea leaves [1]. Initially 0.1 mL of the extract of tea, present in the sample cell was taken to the test tube and 5 mL of chloroform was added to it. Then the absorbance of the solutions was measured at 274 nm. Three samples of each brand were used for caffeine analysis and the average values are given in Table 2.

Preparation of a sample solution of beverages

0.1 mL of the extract of beverages, present in the sample cell, was dissolved in 5 mL of chloroform to form the sample solution. Reading was performed at a wavelength of 274 nm. Three samples of each brand were used for caffeine analysis and the average values are given in Table 3.

RESULTS AND DISCUSSION

Calibration line preparation for caffeine analysis

In Fig. 2 the caffeine spectrum is given which indicates that its maximum absorption occurs at 274 nm. So further photometric analysis of all samples was carried out at 274 nm.

In Fig. 3 the calibration curve for caffeine was drawn by using calibration solutions of caffeine in the concentration range of 1-25 ppm and absorbance was measured on a UV/Vis spectrometer at wavelength 274 nm, as shown in Table 1.

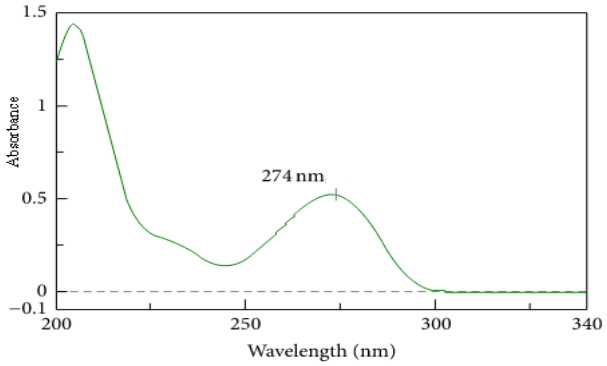

Fig. 2. Caffeine spectrum

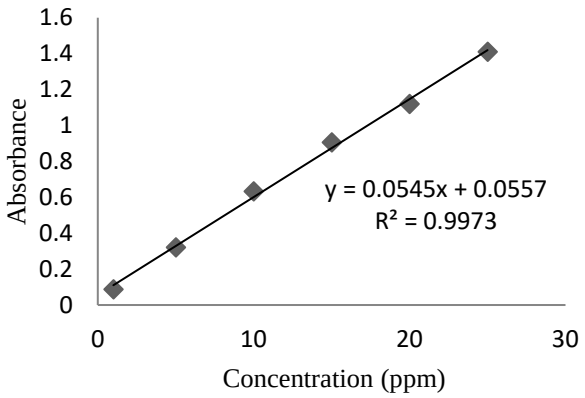

Fig. 3. Calibration curve of caffeine.

Table 1. Absorbance of the calibration solutions of caffeine

No.	Concentration (ppm)	Absorbance
1	1	0.088
2	5	0.322
3	10	0.633
4	15	0.906
5	20	1.12
6	25	1.411

Determination of caffeine in different tea samples

The UV/Vis spectrometric method was used for the quantitative analysis of caffeine in different samples of tea and beverages. This method is rapid, cheap, simple, reproducible, accurate and precise. The concentration of caffeine in the tea samples was determined in triplicate and the average values are given in Table 2. Some tea samples contain large amounts of caffeine, and others less. Thirty two different samples of tea were analyzed. The level of caffeine in Tetley, Lipton, Alkozoay, Doctor, Utility and Vital tea samples lies in the range of 16.111-11.371 mg/g, whereas in Tezdam, A1 Kark, Danadar, Supreme samples, it was in the range of 7.622-6.44 mg/g. In Khuli Kenya, Qamar, Bangladesh Kenya, Kenya, Islam Brand Kenya, Golden Kenya and Green leaf samples, the concentration of caffeine was in the range of 5.744-3.262 mg/g. The lowest range was found in the tea samples of Sapna, Thomata, Malnya 8, Sea Shad 49, Kanya F1, Kagra, Kateve F1, Malnya F1, Kagway, Tameta, Manga, Sea shad 55 and Ghatnctr. The Tetley sample had the highest amount of caffeine and the Ghatnctr – the lowest one. Al Rasbi analyzed different tea samples and according to his result Lipton tea contained 22 mg/g of caffeine but in this analysis Lipton tea contained 13.672 mg/g of caffeine [24].

Table 2. Determination of caffeine in different tea samples available in Pakistan.

Sample	Caffeine Concentration (mg/g)	Sample	Caffeine Concentration (mg/g)
Ghatnctr	0.251	Green leaf	3.262
Kanya F1	0.312	Kenya	3.421
Sea shad 55	0.363	Golden Kenya	3.791
Malnya 8	0.422	Bangladesh Kenya	4.353
Kagway	0.421	Qamar	5.712
Seashad49	0.430	Khuli Kenya	5.744
Kagra	0.430	Supreme	6.442
Malnya F1	0.433	Danadar	6.821
Kateve F1	0.511	A1 kark	6.991
Thomata	0.582	Tezdam	7.622
Manga	0.591	Vital	11.371
Tameta	1.011	Doctor	11.532
Sapna	2.221	Utility	12.071
Lajpal	2.273	Alkozoay	12.892
Islam brand Kenya	2.952	Lipton	13.672
Alaichee tea	3.006	Tetley	16.111

Result obtained in these analyses were different from the reported one, maybe due to the different place, laboratory conditions and most importantly different tea brewing method. Tea brewing conditions and time are mainly responsible for caffeine contents determination by UV/Visible spectrometry.

Analysis of caffeine in different beverages

In beverages different concentrations of caffeine were found. Various analytical techniques are used for the analysis of caffeine in different beverages, like: high performance liquid chromatography, derivative spectrophotometry, ion chromatography, Fourier transform infrared spectrophotometry, partial least squares method, solid phase Fourier transform-Raman spectrometry, a novel auto analyzer method, continuous-flow solid-phase spectrophotometric sensing method, GC/MC method, sample pre-treatment method, solid phase

extraction method, multivariate method, reversed phase ultraviolet visible high performance liquid chromatography method, etc. In this study, UV/Vis spectrometric method was used. In table 3 the concentration of caffeine in different beverages is reported as an average of three replicates.

Fourteen different beverages were analyzed. The level of caffeine in Coca Cola, Pepsi, 7-up, Gourmet Cola, Mountain Dew, Big Apple, Diet Cola, and Pepsi Diet was found to be in the range of 0.101-96.1 mg/L. The concentration of caffeine in Boost, Power Horse, Panda, Red Bull, Sting, Mask was in the range of 130.2-222.3 mg/L. In all of these samples, Boost had the largest amount of caffeine (222.3mg/L), whereas the smallest amount (0.101 mg/L) was found in 7-up. Carbonated soft drinks contain lower amounts of caffeine as compared to energy drinks available in the commercial market.

Tauta *et al*. [6], also worked on carbonated soft drinks and energy drinks. Tauta used a UV-Vis spectrometric method for the determination of caffeine content in different beverages. The result obtained by this method was that carbonated Coca cola had small amount of caffeine and Red Bull had large amount of caffeine. Kalra also worked on beverages and showed that the highest concentration of caffeine was present in Power-ex (46 µg/mL) and the lowest concentration - in XXX (19.5 µg/mL) [25].

Musa Ali also worked on beverages. The result obtained was that energy drinks contained large amount of caffeine as compared to carbonated soft drinks [2]. Results obtained in these analyses were not similar, maybe due to the different place, seasonal changes and different laboratory conditions.

MgO supported catalysts with 5 wt. % Co_3O_4 or $CoFe_2O_4$ loading were prepared by incipient wetness impregnation [15]. Bulk Co_3O_4 and $CoFe_2O_4$ were prepared by the precipitation/co-precipitation method.

XRD patterns of the samples were obtained on a TUR M62 powder X-ray diffractometer (XRD) using Co-Kα radiation (λ= 1.789 Å) at 40 kV and 20 mA. The morphology of the catalysts was characterized by a JEOL JEM 2100 high resolution transmission electron microscope (TEM) using an accelerating voltage of 200 kV. Two basic regimes of microscope mode were used – bright field transmission microscopy (TEM) and selected area electron diffraction (SAED). The pH of the point of zero charge (pH_{PZC}) of the catalysts was determined by the pH drift method [17]. The amount of cobalt and iron in the prepared samples as well as the concentrations of leached Co and Fe in the solution were measured by atomic absorption spectroscopy (AAS, Perkin-Elmer).

Catalytic oxidation of RhB with PMS was carried out in a 400 cm^3 glass reactor at 293 K with constant stirring at around 400 rpm. In a typical experimental procedure, a fixed amount of catalyst was added to a 200 cm^3 solution containing 50 mg dm^{-3} of RhB and the suspension was stirred for 30 min to achieve adsorption-desorption equilibrium. The reaction was initiated by addition of oxidant to attain the predefined PMS/RhB molar ratio. Aliquots of 4.0 cm^3 withdrawn from the mixture at given time intervals were immediately mixed with 1 mL methanol to quench the reaction. The RhB concentration in aqueous solution was determined by means of UV/Vis spectrophotometry (Cintra 101, GBS) at 554 nm. All tests were conducted in triplicate to ensure reproducibility of experimental results.

CONCLUSIONS

UV/Vis spectrophotometry was used for the determination of caffeine in tea samples and beverages. Caffeine is the world's most widely consumed psychoactive drug. It is important to obtain information about the drinks because they

Table 3. Determination of caffeine in different beverages available in Pakistan.

Sample	Caffeine Concentration (mg/10mL)	Caffeine Concentration (mg/L)	Sample	Caffeine Concentration (mg/10mL)	Caffeine Concentration (mg/L)
7 up	0.001	0.101	Gourmet cola	0.381	38.1
Panda	0.091	9.1	Diet cola	0.812	81.2
Big Apple	0.102	10.2	Coca cola	0.961	96.1
Mountain dew	0.105	10.5	Power horse	1.302	130.2
Mask	0.17	17	Sting	1.643	164.3
Pepsi	0.199	19.9	Red bull	1.914	191.4
Pepsi diet	0.319	31.9	Boost	2.223	222.3

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 Tetley and the smallest amount - in Ghatnctr. In case of beverages the largest amount of caffeine was present in Boost and the smallest amount - in 7-up.

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АНАЛИЗ НА СЪДЪРЖАНИЕТО НА КОФЕИН В ТЪРГОВСКИ НАПИТКИ И ПРОБИ ОТ ЧАЙ С ПОМОЩТА НА УВ-СПЕКТРОМЕТРИЯ

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(Резюме)

В това изследване се определя фотометрично съдържането на кофеин в чаени проби и в напитки с помощта UV/Vis-спектрофотометрия. Методът е бърз и точен. Калибровката е направена в интервал от концентрации 1-25 ppm от изходен разтвор от 100 ppm. Абсорбцията на всички калибрационни разтвори при абсорбционен максимум при 274 nm. Кофеинът се екстрахира от пробите с хлороформ, който се използва като фон при фотометрията. Резултатите показват, че в случая на чаени проби кофеинът е в по-голямо количество на кофеин (16.111 mg/g) в чая „Tetley“, а най-ниско – в Ghatnctr (0.251 mg/g). При напитките най-високо е съдържанието в Boost (222.3 mg/L), а най-ниско - 7-up (0.101 mg/L).