Estimation of Caffeine present in various soft drinks.

By:

Bharat Adhikari

Department of Chemistry

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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Estimation of Caffeine present in various soft drinks.

A project work submitted

To

Department of Chemistry

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For the fulfillment of project work of fourth year of Bachelor's Degree of Science (B.Sc.)

By:

Bharat Adhikari

Department of Chemistry

Central Campus of Technology

Institute of Science and Technology

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Board of examiner and certificate of Approval

This project work entitled “Estimation of Caffeine present in various soft drink samples ”, by Bharat Adhikari (Roll No.:), T.U Regd. No.:), under the supervision of Mr. Netra Prasad Subedi, Central Campus of Technology, Hattisar, Dharan, Department of Chemistry, Nepal, is hereby submitted for the partial fulfillment of Bachelor of Science (B.Sc.) Degree in Chemistry. This project work had not been submitted in any other university or institution previously and has been approved for the award of Bachelors of Degree.

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Supervisor

Mr. Netra Prasad Subedi

Department of Chemistry

C.C.T., Dharan

……. ……

Internal Examiner External Examiner

…….. ……

Department of Chemistry

C.C.T., Hattisar, Dharan

Tribhuvan University

…….

Lalita Shrestha

Chairman

(Department of Chemistry)

Central Campus of Technology, Hattisar, Dharan

Date

Recommendation

This is recommended that **Bharat Adhikari** (Roll No.: ; Tribhuvan University Registration Number:) has carried out project work entitled "**Estimation of Caffeine present in various soft drinks**" as a partial fulfillment of 4 years Bachelor degree of 4th year in chemistry under my supervision. To my knowledge, this work has not been submitted for any other degree.

…….....

Mr. Netra Prasad Subedi

Supervisor

Department of Chemistry

Central Campus of Technology Hattisar, Dharan, Sunsari

Declaration

I hereby declare that the project work entitled “Estimation of Caffeine present in various soft drinks” which is being submitted to the Department of Chemistry, Central Campus of Technology, Dharan, Tribhuvan University, Nepal for the partial fulfillment of degree of Bachelor of Science in Chemistry is my original work carried out by me under the supervision of Mrs. Netra Prasad Subedi, Department of Chemistry, Central Campus of Technology, Dharan, Tribhuvan University.

……

Bharat Adhikari
Central Campus of Technology
Hattisar, Dharan

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August, 2019 ..…….

(Bharat Adhikari)

Abstract

This study was carried out to determine caffeine in fourteen brands of carbonated and energy drinks available in local market in Dharan, Nepal. Quantitative analysis of caffeine was performed by a simple and fast standard UV spectrophotometric method. Among all the soft drinks highest amount of caffeine was found in XL(I) (520µg/ml) and lowest was found in Tender coconut drink 52.37µg/ml. In a comparision between caffeine content in same soft drink prepared in Nepal and imported from foreign nation, it was found caffeine content in imported drink was higher. Similarly taking Recommended Daily Intake value as a reference maximum volume of soft drink a person can consume is calculated.

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List of Abbreviations

Abbreviation	Full Forms
Fig	Figure
Ml	Mili liter
µg	Microgram
TDS	Total dissolved solute
AOAC	

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Part-I

Introduction

1.1 General introduction

Caffeine, 1,3,7-trimethylxanthine, is an odorless, slightly bitter substance found in numerous plant species (Chou et al., 2007). It is a naturally occurring substance found in the leaves, seed or fruits of over 63 plants species worldwide. The most important natural 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%) (Komes et al., 2009). . It is found in common beverages including coffee, tea and soft drinks, as well as products containing cocoa or chocolate, and a variety of medications and dietary supplements (Heckman et al., 2010). Caffeine is added in soft drink like cola and energy drink intentionally as a flavoring agent inorder to make drink addictive (Tautua et al., 2014).

Sweetened water-based beverages with balanced acidity are called soft drink. The term "soft drink" specifies the absence of alcohol in contrast to "hard drink". The term drink is neutral but often denotes alcoholic content. But nowadays small amount of alcohol may be present in a soft drink, but the content must be less than 0.5% of the total volume if the drink is to be considered non alcoholic. Soft drinks are refreshing beverages that constitutes of 10-11% sugar content 0.3-0.4% acid (usually citric) and flavoring, coloring and chemical preservatives. It may contain caffeine, fruit juice, or both. Various other additional can be made including vitamins, minerals, clouding agents and foaming agents and plant exacts (Sayed and Abdellati., 2018)

Caffeine content in soft drinks varies by brand from 10 to 50 mg of caffeine per serving, however the US Food and Drug Administration (FDA, 2006) 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 (Tautua et al., 2014). Caffeine acts as a stimulant to Central Nervous System and has ability to put off sleep, thus provide alertness in the study (Bhawani et al., 2015) Dopamine concentration increases in the brain by caffeine which helps to ease depression(Rehman and Ashraf, 2017).However, too much of caffeine can lead to insomnia, nervousness, muscle twitching, headache, respiratory alkalosis, heart palitapation(Pradhan et al., 2017).

1.2 Statement of problem

Caffeine provides no nutritional value on its own. Caffeine is tasteless, so it is difficult to know if it is in our food or not. Even some medications may contain caffeine without our knowledge. Caffeine has various effects on human health. Consumption of caffeine in excess amount can cause general toxicity, cardiovascular effects, effects on bone status and calcium balance, confusion, hallucinations, vomiting, changes in adult behavior, increased incidence of cancer and effects on male fertility. As we consume the same amount of caffeine on a daily basis, our body develops tolerance to it and slowly it leads to addiction. So, if we suddenly stop consuming caffeine, it can cause health problem. The problem is that we don't know how much caffeine we are consuming.

Due to such effects of caffeine in human health, its study is very important.

1.3 Objective:

1.3.1 General objective

a. To estimate the amount of caffeine in soft drinks Coca Cola(Nepal) ,Sprite(Nepal), Mountain dew(Nepal), Fanta(Nepal), Pepsi(Nepal), Red Bull(Nepal), Coca Cola (imported), Pepsi (imported), Mountain dew (imported), Red Bull (imported), XL(imported), Bullet(imported), litchi juice(Nepal).

1.3.2 Specific objective

b. To determine the amount of caffeine present in various soft drinks. (Coca Cola, Sprite, Mountain dew, Fanta, Pepsi, Red Bull, Coca Cola (imported), Pepsi (imported), Mountain dew (imported), Red Bull (imported), XL, Bullet, litchi juice.

c. To Perform comparative study of caffeine present in soft drinks prepared in Nepal and imported from foreign Nation.

d. To find out pH, total dissolve solute (TDS) of soft drink.

1.4 Limitation:

There are various types of soft drink available in local market. But my study will be based on few selective soft drink samples. This is due to limitation of time and budget.

Part-II

Literature Review

2.1 Soft drink

Sugary drinks (also categorized as sugar-sweetened beverages or “soft” drinks) refer to any beverage with added sugar or other sweeteners (high fructose corn syrup, sucrose, fruit juice concentrates, and more). This includes soda, pop, cola, tonic, fruit punch, lemonade (and other “ades”), sweetened powdered drinks, as well as sports and energy drinks (“Sugary Drinks",2019). Soft drink are type of beverage categorized under non alcoholic, carbonated drinks. It includes juices, necters and carbonated drinks. Soft drinks are called soft due to less percentage of alcohol in contrast to hard drink or alcoholic beverages. Alcoholic percentage in soft drink should not exceed 0.5% of total volume (Mishra et al., 2013). Soft drinks can be divided into carbonated and non-carbonated drinks. Examples of carbonated drinks are Cola, lemon and oranges and non-carbonated drinks include mango drinks. Soft drinks can also be divided into cola products and non-cola products. Cola products like Pepsi, Coca-Cola, Thumps Up, and Diet Coke, Diet Pepsi etc. account for nearly 61-62% of the total soft drinks market. Non-Cola products constitute 36%, and based on the types of flavors available can be divided into Orange, Cloudy Lime, Clear Lime and Mango (Gerald et al., 2014).

Soft drinks are commonly consumed by youth not for nutritional benefit but to quench thirst during the hot summer or may be because of belief that soft drink will help them in digestion, while the athletes use energy drinks to keep up their energy during intense physical activities and competition (Mohammed et al., 2012)

Table 2.1 Ingredient of soft drink with their limit (Sayed and Abdellati.,2018).

Ingredient	Limit
Water	Upto 98% v/v
Sugars	(7-12%)m/v
Fruit Juices	(Upto 10%)
High Intensity Sweetner	Used based upon sucrose equivalence
Carbon dioxide	(0.3-60%m/v)
Acid	(0.03-0.05% m/v)
Flavors	Artificial 0.1-28%m/m and natural upto o.5m/m
Emulsion	0.1% m/v
Colors(natural or synthetic)	0-70ppm
Preservative	Statutory limits apply (eg Sorbic acid upto 250ppm in EU
Antioxidants (eg. BHA, ascorbic acid)	Less than 100ppm
Quillaia extract	Upto 200mg/L (EU), upto 95mg/L (USA)
Hydrocolloids	0.1-0.2% per GMP, minimum amount require to creat effect
Vitamins/Minerals	Allowed daily intake applies

2.1.1 Water

Diet soft drink may contain up to 99% water whereas conventional soft drink may contain 90% water. Generally softened water is used to prevent off taste due to chlorine residue. Water for soft drink should fulfill the physical, chemical, and microbial criteria for drinking water according to European Directive EC 98/1983, US Environment Protection Agency(EPA) and WHO standard.

2.1.2 Sugar and sweetener

Soft drink usually contains 1%-12% sugar (w/w) except zero calorie product. Often, sucrose, fructose, glucose is used as natural carbohydrate sweeteners. Apart from them Acesulfam (E950),Sucralose (E955), Saccharine (E954),Thaumatin (E957), Stevioside (E960) are also used as sweetener.

2.1.3 Acidity regulator and carbon dioxide

In soft drink carbonation varies from 1.5 to 5 g/L. It is used to sharpen and flavor the test and preserve soft drink for longer time. In soft drink mostly citric(E330) is first choice as it has additional benefit such as enhancing activity of beneficial antioxidants and adding aroma. Apart from it malic acid, succinic acid, phosphoric acid are used to make beverage acidic and all are subjected to EC regulation 1333/2008 on food additives.

2.1.4 Flavoring and colorings

The use of soft drink is to make product aesthetically appealing, helping to correct for natural variations in color or for change during processing and contributing to maintain the quality by which drink is recognized. Generally three basic categories of colour found are natural colors, artificial colors and caramel.

2.1.5 Preservatives

Chemicals preservative are used to improve the microbiological stability of soft drink. The chemical and physical properties of both the preservatives and soft drink determine the type of chemical to be used. Sorbates and benzoates are often used in combination in highly acidic drink.

Other ingredients: various hydrocolloids such as locust gum, pectin, and xanthan are used as stabilizers and thickeness in diet drink and fruit juice drink. The most common active ingredients of energy drink are taurine (average 3180mg/L), caffeine (360-630mg/L) . Energy drink also contain B-vitamins (B3,B6 and B12) (Kregiel, 2015)

Several kinds of beverages are consumed not for food value but rather for thirst - quenching properties or for stimulating. Soft drinks are an essential vehicle for hydration. Soft drinks are usually absorbed more readily than water (because of their osmolality), can replace lost salts and energy quickly and are rapidly thirst quenching. According to researches these beverages are in the top ten contributing foods for several nutrients, including carbohydrates, vitamins, minerals

as well as energy. There are three main areas of particular nutritional significance for soft drinks. (Troiano et al.,2015) reported that 20 - 24% of energy intake came from beverages. Some soft drinks are formulated to deliver a rapidly assimilated energy boost to the consumer Soft drinks generally contain soluble sugars, which are easy to administer. The second area of nutritional significance is that of the so-called isotonic drinks, which are of equivalent osmolality to body fluids. They promote extremely rapid uptake of body salts and water, and are very important products for sports people and others requiring almost instant hydration. Third, soft drinks have been widely formulated to low-calorie forms and these are now available for those who wish to enjoy such beverages and yet minimize their calorific intake. Other nutritional benefits that are claimed by some producers include the delivery of essential vitamins and minerals, especially to children

Soft drinks are bad for health as well. Sugary drinks can cause weight gain as it contains lots of energy. Soda eats up and dissolves the tooth enamel. According to researcher soft drink are responsible for doubling or tripling the incidence if tooth decay. Similarly experts have research, to believe that consumption of fructose particularly in the form of soft drink leads to increase in blood pressure. It is also absorbed that calcium is urinated along with phosphoric acid, by consumption of soft drink. This leads to osteoporosis (Johagirdas et al.,)

2.2 Caffeine

Caffeine, the common name for 1,3,7-trimethylxanthine, was derived from the German word *kaffee* and the French word *café*, each meaning coffee. Historians suggest that caffeine was consumed as far back as 2737 BC when Chinese Emperor Shen Nung boiled drinking water and leaves from a nearby bush, creating a pleasant aroma and the first pot of tea. It was only on late 1800's that those caffeinated soft drinks began appearing with the introduction of Dr. Pepper, followed by Coca Cola and then Pepsi Cola. The caffeinated soft drink market grew enormously during the 2nd half of the 20th century with increased popularity occurring among the beverages containing higher amounts of caffeine (Heckman et al., 2010). In 1819, the German chemist Friedrich Ferdinand Runge first time isolated pure caffeine in laboratory (Khalid et al., 2016).

Caffeine is formed when three methyl group are substituted on the parents compound xanthine. Caffeine chemical formula is $C_8H_{10}N_40_2$. Pure caffeine occurs as odorless, white, fleecy masses glistening needles of powder. Its molecular weight is 194.19 g, melting point is 236, 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 mm Hg at 178 c, vapor density is 6.7(Ali et al., 2012). . It is moderately soluble in water at room temperature (2 g/100 mL) but very soluble in boiling water (66 g/100mL). It is a drug and shares a number of traits with more notorious drugs such as cocaine and heroin. Caffeine uses the same biochemical mechanisms as those other drugs to stimulate central nervous system (CNS) (Mufakkar et al., 2014). IUPAC name of caffeine is 1,3,7 Trimethylpurine-2,6-dione (Shar et al., 2017). It is weakly basic (pKa = ~0.6) requiring strong acid to protonate it. Caffeine does not contain any stereogenic centers and hence is classified as an achiral molecule.The xanthine core of caffeine contains two fused rings, a pyrimidinedione and imidazole. The pyrimidinedione in turn contains two amide functional groups that exist predominately in a zwitter ionic resonance the location from which the nitrogen atoms are double bonded to their adjacent amide carbons atoms. Hence all six of the atoms within the pyrimidinedione ring system are sp2 hybridized and planar. Therefore, the fused 5,6 ring core of caffeine contains a total ten pi electrons and hence according to Hückel's rule is aromatic.

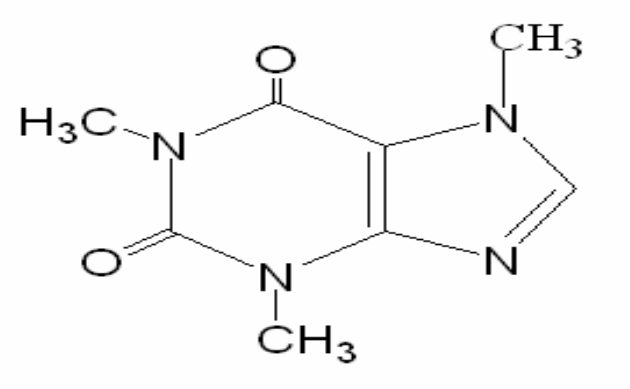

Fig. 2.1 Chemical structure of caffeine (Gerald et al., 2014).

Caffeine has been used for thousands of years and is one of the most widely consumed active food ingredient throughout the world. It is found in common beverages including coffee, tea and soft drinks, as well as products containing cocoa or chocolate, and a variety of medications and

dietary supplements (Heckman et al., 2010). According to (Mahoney et al., 2018) coffee (18%), soft drink (18%) and tea (16%) are the main source of caffeine in the diet. Caffeine is the world's most widely consumed psychoactive substance. Adult receive three quarter of their daily caffeine from coffee whereas children receive one half of their caffeine from soft drinks, energy drinks (Sohil et al., 2016). The highest legal amount of caffeine allowed in a 355 mL (12oz) can of soft drink is about 71mg. The absence of regulatory oversight has resulted in aggressive marketing of energy drinks with higher amount of caffeine, targeted primarily toward young males, for psychoactive, performance enhancing and stimulant purposes. There are increasing reports of caffeine intoxication from energy drinks, and it seems likely that problem with caffeine dependence increasing day by day. In children and adolescents who are not habitual caffeine users, vulnerability to caffeine intoxication may be markedly increased due to an absence of pharmacological tolerance. Several studies suggest that energy drinks may serve as a gateway to other forms drug dependence. Due to its toxicological effects, caffeine regulation in soft drinks is gaining importance and many countries have already set the maximum limits in different kinds of beverages (shar et al., 2017).

Table 2.2 Regulation of caffeine in different countries (Shar et al.,2017)

Country	**Beverage**	**Maximum limit**
USA	Cola type beverage	200 mg/L
Australia	Cola type beverage and flavoured syrups	145mg/L
New Zealand	Cola type beverage	200mg/Kg
Canada	Cola type beverage	200mg/L
European Union	Cola type beverage	150mg/L
South Korea	Cola type beverage	Less than 400 mg/L
Taiwan	Cola type beverage	320mg/ L
Mexico	Cola type beverage	200mg/L
Brazil	Cola type beverage	350 mg/L
Chili	Cola type beverage	500mg/L
Pakistan	Cola type beverage	No regulation

2.2.1 Health effects of Caffeine

Caffeine is usually ingested. Caffeine is soluble in water and lipids, easily crosses the blood–brain barrier, and can be found in all body fluids, including saliva and cerebrospinal fluid. Importantly, caffeine ingested by women perinatally will be present in the umbilical cord and breast milk. Hence, it will also be present in the fetus and in breastfed infants. Caffeine is absorbed rapidly and totally in the small intestine in less than 1 h and diffuses rapidly in other tissues. Absorption by the small intestine does not seem to vary by sex, genetic background, environmental factors, or other variables, although specific studies are still needed to confirm this premise. Caffeine concentrations peak in saliva 45 min after ingestion and in serum after about 2 h. Caffeine has a relatively long half-life of 3–7 h in adults. In neonates, the half-life is even longer—between 65 and 130 h—because of their immature kidneys and liver. Peak concentrations are important because the effects of caffeine depend in part on the length of time it remains in tissues. Clearly, the effects are age dependent and depend on complex genetic and environmental interactions (Temple et al., 2017). Caffeine is metabolized in the liver into three primary metabolites: Para xanthine (84%), Theo bromine (12%), and theophylline (4%).Para xanthine increases free fatty acid level in the blood plasma. Theo bromine increases urine volume. Similarly, theophylline relaxes smooth muscles of the bronchi and is used to treat asthma.

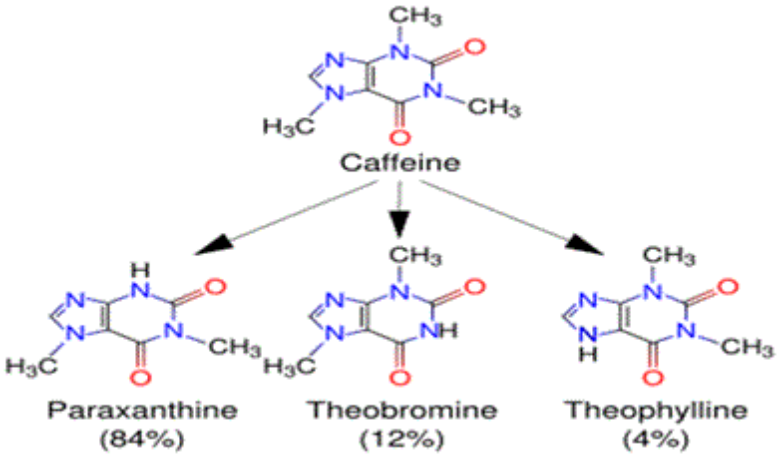

Fig 2.2 Caffeine and its metabolites (Tautua et.al., 2014)

In students, it prevents drowsiness and increase alertness which is why students take caffeinated food stuff during late night study. In sports man, it can improve sprints, endurance and team

sports performance Caffeine in anaemic patient inhibits suicidal erythrocyte death and thus helps to prevent the disease of the erythrocyte count (Jochebed et al). It enhances consolidation of long term memories (Daniel et al., 2014).According to the study conducted in Duke University by (Sinha et al., 2013) caffeine may reduce fatty liver in those with non- alcoholic related fatty liver disease.It also reduces liver fibrosis risk in patients with hepatitis C. caffeine with dose about 100 mg per day is believed to have protective benefits (Khalaf et al., 2015).Similarly, research carried out of Germany showed that weight loss study participants who drank 2-4 cups of caffeinated coffee a day were more likely to be successful at keeping the weight off than those who did not consume caffeine (Icken et al., 2016).Caffeine also stimulates brown fat cell causing them to burn more calories which ultimately increases overall metabolic rate . It also helps to keep us alert while driving at the time when a person is having less sleep (Biggs et al., 2007). Caffeinated coffee drinker have less risk of developing melanoma. People who consume caffeine have a lower risk of suicide (lucas et al., 2013). Research show that those who consume coffee are at less risk of developing Parkison's disease and it even reduces the risk of those genetically more likely develop the condition(kumar et al., 2015). In a large 217,883 person study, those that consumed caffeine from any source had less kidney stone formation than those that did not consume caffeine. The researchers believe that this is because caffeine makes urine more dilute (Ferraro et al., 2014). Another study in 2018 by (Bigotte et al., 2018) also confirmed that caffeine consumption reduces the mortality rate for those living with kidney disease.

For those who are highly sensitive to caffeine it is recommended to consume no more than 400 mg/d to avoid adverse effects, such as headache, drowsiness, anxiety, and nausea(Heckman et al., 2010). If caffeine is consumed in high amount by pregnant women then it can lead to miscarriage, difficult birth and delivery of low weight babies. Food stuffs containing more than 150 mg/L of caffeine must provide a warning message on the label (Pradhan et al, 2017). It is now widely believed that habitual daily use of caffeine >500–600mg represents a significant health risk and may therefore be regarded as ‘abuse’. Sustained abuse may in turn result in ‘caffeinism’, which refers to a syndrome characterized by a range of adverse reactions. Excessive caffeine intake (>400mg/day) may increase the risk of detrusor instability (unstable bladder) development in women. For women with preexisting bladder symptoms, even moderate caffeine intake (200–400mg/day) may result in an increased risk for detrusor instability (Arya et al., 2000).

Excessive caffeine ingestion is not common and only a few cases have been reported in the literature. The acute lethal dose in adult humans has been estimated to be 10 g/person. Death has been reported after ingestion of 6.5g caffeine but survival of a patient who allegedly ingested 24g caffeine was also reported. The most important mechanism of action of caffeine is the antagonism of adenosine receptors. Following ingestion, caffeine is rapidly and essentially completely absorbed from the gastrointestinal tract into the bloodstream. Maximum caffeine concentrations in blood are reached within 1–1.5h following ingestion. Absorbed caffeine is readily distributed throughout the entire body. It passes across the blood–brain barrier, through the placenta into amniotic fluid and the foetus, and into breast milk. Caffeine has also been detected in semen. Only 1–5% of ingested caffeine is recovered unchanged in the urine. Infants up to the age of 8–9 months have a greatly reduced ability to metabolize caffeine, excreting about 85% of the administered caffeine in the urine unchanged (P. Nawrot et al, 2002).

According to research conducted by (Mufakkar et al.,2014) in 8 brands of soft drinks of Pakistan Pepsi contain 255.27µg/mL, Coke 211.69 µg/mL, Fanta 357 µg/mL, Mountain dew 119.42 µg/mL, Sprite 30.99 µg/mL amount of caffeine.

2.2.2 Methods of estimation of Caffeine

Many methods exist for determining the methylxanthine contents of food and beverages. Some of these methods include UV-Visible spectrophotometry, potentiometry, high performance liquid chromatography (HPLC), ion chromatography, high performance thin layer chromatography (HPTLC), capillary electrophoresis, micellar capillary electrophoresis, gas chromatography, and solid-phase microextraction gas chromatography(Gerald et al., 2014)

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers.

It is easy to operate, available in college lab so I am about to perform the experiment with the help of uv-spectrophotometers.

Part III

Materials and Methods

3.1 Laboratory Setup

The entire work has been performed in the laboratories of Central Campus of Technology, Dharan.

3.2 Research Design

The project is a laboratory based experimental design.

3.3 Experimental Materials

3.3.1 Sample

Soft drink

3.3.2 Chemicals

Anhydrous Caffeine powder (Assay: 98-101.5%)

Buffer tablets of pH 4 and pH 7

Distilled water

3.3.3 Glassware

Volumetric flask

Beaker

Pipette

Measuring cylinder

Watch glass

Glass rod

Funnel

3.3.4 Instrument

UV-Vis Spectrophotometer (Labtronics, Model: LT-291)

pH meter

Digital balance

Condoctometer

3.4 Sampling

Soft drink sample was collected from Gorkha Departmental Store of Dharan.

Table 3.1 Soft drink sample specifications

Sample Name	Type	Status
coke(I)	Carbonated	Imported
Red Bull (I)	Energy drink	Imported
Pepsi (I)	Carbonated	Imported
Red Bull(N)	Energy drink	Nepal
Bullet(I)	Energy drink	Imported
XL(I)	Energy drink	Imported
Litchi juice(N)	Fruit juice	Nepal
Coke(N)	Carbonated	Nepal
Tender(I)	Fruit juice	Imported
Dew (I)	Carbonated	Imported
Dew(N)	Carbonated	Nepal
Fanta(N)	Carbonated	Nepal
Pepsi (N)	Carbonated	Nepal
Sprite (N)	Carbonated	Nepal

3.5 Methodology

3.5.1 Raw material collection

Total fourteen different types of soft drink were collected from Gorkha Departmental store of Dharan. Sample size was 100 ml for each sample and simple random sampling technique was used for sampling.

3.5.2 Determination of total dissolved solid (TDS) (AOAC, 2000)

Total dissolved solid was measured by using conductivity meter. About 10 ml of soft drink was kept in a beaker and the measuring lead was lowered in the beaker.

3.5.3 Determination of pH (AOAC, 2000)

pH was used to measure pH of soft drink. About 5ml of soft drink was kept in a beaker and by using pH meter pH was measured which was by calibrating in the buffer 4 and 7.

3.5.4 Estimation of Caffeine

Estimation of caffeine is as per the method described by Muffakar (2014) with slight modification.

3.5.4.1 Wavelength selection

Wavelength was obtained by scanning the range of 120-400 nm. Wavelength of 272 nm was used for analysis of caffeine which is described by Muffakar (2014).

3.5.4.2 Preparation of standards

Caffeine stock standard solution of 400µg/ml was prepared by dissolving 0.04 gram of caffeine in 100 ml of distilled water in 100 ml volumetric flask. The stock solution was stored in dark place at +4ºC. Working standard solutions of 0.01, 1 , 5, 10, 15 and 20 µg/ml was prepared by suitable dilution of stock solution.

3.5.4.3 Preparation of sample

1 ml of sample will be taken in 100 ml volumetric flask and distilled water will be added up to the mark. Absorbance of the samples will be measured by using Uv-spectrophotometer. Concentration corresponding to sample absorbance will be obtained by using calibration curve.

3.5.4.4 Standard calibration curve

Absorbance of working standard solutions of caffeine was measured at 271 nm using UV-Vis Spectrophotometer (Labtronics, Model: LTetric-291). Absorbance vs Concentration was plotted in graph to obtain standard calibration curve.

3.5.4.5 Calculation of Caffeine

Absorbance of different soft drink samples at 272 nm was measured and corresponding caffeine concentration was calculated using standard calibration curve.

Part IV

Result and discussion

This research was carried out for the estimation of caffeine in various soft drinks. In this research amount of Caffeine present in fourteen different soft drink samples was estimated. Along with caffeine concentration pH and Total dissolved solute of the soft drink samples was also estimated. Following results were obtained by the laboratory analysis

Table 4.1 pH and TDS value of soft drinks

Sample	**pH**	**TDS (in ppt)**
coke(I)	2.42±0.037	0.532±0.0010
Red Bull (I)	3.42±0.067	0.979±0.0020
Pepsi (I)	2.50±0.041	0.575±0.0307
Red Bull(N)	3.64±0.051	1.123±0.0025
Bullet(I)	3.68±0.054	1.122±0.0035
XL(I)	3.39±0.045	0.814±0.0035
Litchi juice(N)	3.20±0.034	0.324±0.0035
Coke(N)	2.36±0.050	0.685±0.0030
Tender(I)	5.49±0.012	4.930±0.0458
Dew (I)	3.07±0.056	0.284±0.0020
Dew(N)	3.02±0.059	0.205±0.0056
Fanta(N)	3.30±0.013	0.320±0.0047
Pepsi (N)	2.55±0.021	0.532±0.0031
Sprite (N)	3.33±0.033	0.438±0.0035

4.1 pH

The pH of soft drink was found in the range of 2.42-5.49. Among all the soft drink the most acidic soft drink among the sample soft drink was coke that was imported. Similarly, the soft drink that has least acidity is Tender coconut. The low pH values could be as a result of presence of carbon dioxide, phosphoric. acids, malic acid, tartaric acid used as preservatives by manufactures of these beverages (Khalid et al., 2016).These acids inhibits the growth of microorganism such as bacteria, mould and fungi which may contaminate beverage.

4.2 Total dissolve solute (TDS)

It represents the total concentration of dissolved solids in water. According to Bureau of Indian Standard (BIS) the maximum desirable TDS is 0.5 ppt and maximum permissible level in the absence of a better source of water is 2 ppt (Jahagirdar et al., 2016).

The highest and lowest TDS was found in Tender (4.930 ppt) made in India and dew made in Nepal by bottlers Nepal (0.205ppt). A high concentration of dissolved solids is usually not a health hazard. However, a low concentration of TDS has been found to give water a flate taste which is undesirable to many people

4.3 Standard calibration curve

The standard calibration curve for caffeine obtain by UV-Vis spectrophotometer is as shown in the figure

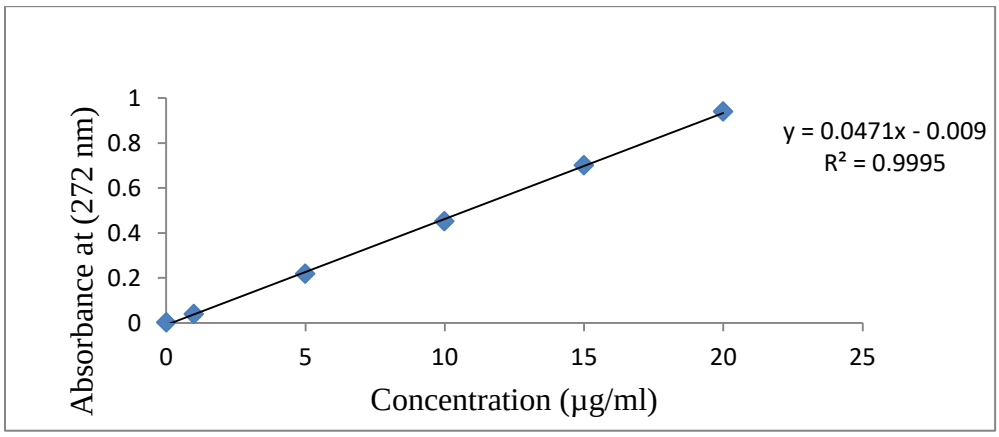

Fig 4.1 Calibration curve

The standard curve was found to show a linear increase in absorbance with an increase in concentration of caffeine solution. The equation from absorbance and concentration was found to be y = 0.047x - 0.009, which is a linear equation. The fitting of linear trend of linear trend line in the data set shows a high goodness of fit (R^2) value of 0.999.

Table 4.2 Absorbance and corresponding concentration in µg/ml

Sample	Absorbance	Concentration(µg/ml)
coke(I)	0.132±0.002	298.66
Red Bull (I)	0.223±0.002	492.56
Pepsi (I)	0.141±0.003	318.47
Red Bull(N)	0.171±0.002	381.46
Bullet(I)	0.221±0.003	488.32
XL(I)	0.236±0.003	520.88
Litchi juice(N)	0.032±0.002	87.05
Coke(N)	0.125±0.001	285.21
Tender coconut drink(I)	0.016±0.002	52.37
Dew (I)	0.15±0.002	263.27
Dew (N)	0.094±0.002	220.1
Fanta(N)	0.048±0.001	121.02
pepsi (N)	0.128±0.001	291.58
Sprite(N)	0.032±0.002	87.05

Caffeine concentration ranges in soft drink was found from 52.37 µg/ml to 520.88µg/ml. Caffeine concentration in coke (I) was found to be 298.66 µg/ml, in Red bull (I) was found to be 492.56µg/ml, in pepsi (I) was 318.47µg/ml, in red bull(N) was 318.46 µg/ml, in Bullet(I) was 488.32µg/ml, in XL(I) was 520.88 µg/ml, in Litchi juice was 87.05 µg/ml, in coke was 285.21 µg/ml, in Tender juice was 52.37 µg/ml, in dew was 220.10 µg/ml, in dew (I) was 263.27 µg/ml, in Fanta was 121.02 µg/ml, in Pepsi was 291.58 µg/ml and sprite was 87.05 µg/ml. So among all the sample the highest amount of caffeine was found in XL (520.88 µg/ml), and least amount

of caffeine was found in litchi juice and sprite (87.05 µg/ml).The above result is in accordance with the research carried by (Mufakkar et al., 2014).

Table 4.3 Recommended Daily Intake (RDI) and total volume that can be consumed in a day.

Sample	Concentration of caffeine(µg/ml)	RDI(mg/day)	Maximum limit consumption(mL)
coke(I)	298.66	400	1339.32
Red Bull (I)	492.56	400	812.08
Pepsi (I)	318.47	400	1256.01
Red Bull(N)	381.46	400	1048.60
Bullet(I)	488.32	400	819.13
XL(I)	520.88	400	767.93
Litchi juice	87.05	400	4595.06
Coke(N)	285.21	400	1402.48
Tender(I)	52.37	400	7637.96
Dew (I)	263.27	400	1519.35
Dew (N)	220.1	400	1817.36
Fanta(N)	121.02	400	3305.24
Pepsi(N)	291.58	400	1371.84
Sprite(N)	87.05	400	4595.06

The above table gives the data regarding volume of different soft drink people can consume so one does not take more than recommended caffeine. Soft drinks (Energy drink) like Bullet (I), XL (I), Red Bull (I) contain more caffeine in it so it is better not to consume it more than 819.13ml, 767.93ml, 812.08ml of respective drink. Similarly, it is better to take soft drinks like coke (I), Pepsi(I), coke (N), Pepsi (N) within 1339.32ml, 1256.01ml,1402.48ml,1371.84ml. In

soft drink like litchi juice, sprite and Fanta less amount of caffeine is found so more volume can be consumed.

4.5 Comparative study of soft drink

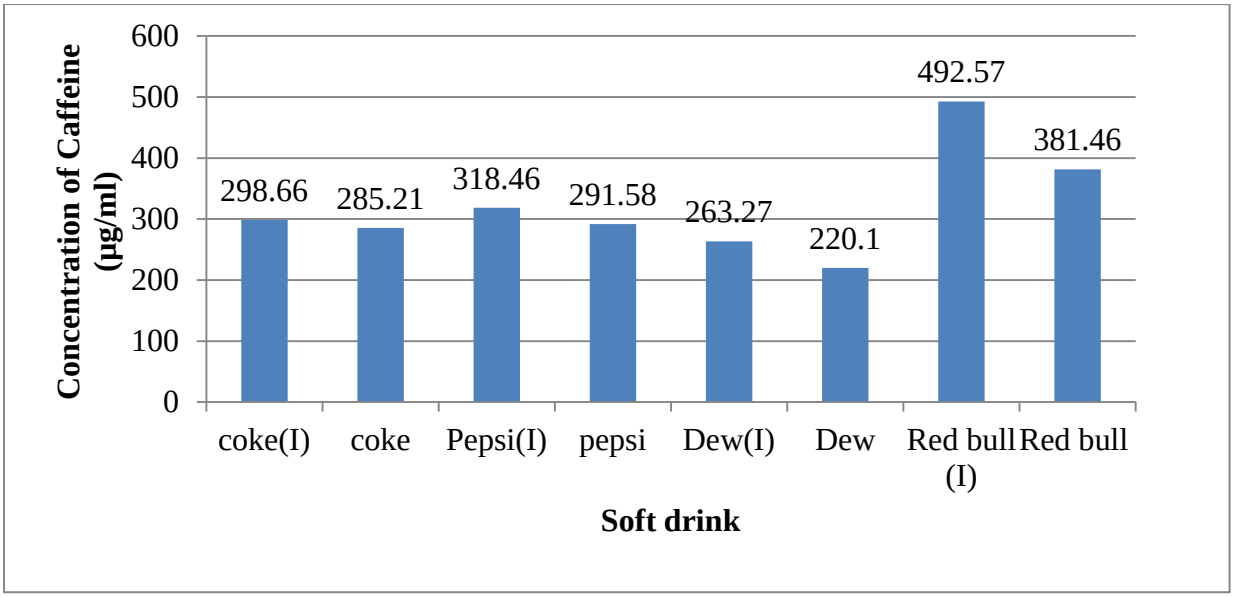

Fig 4.2 Concentration of caffeine in soft drinks (Comparative study)

Analysis is carried out between soft drink which is directly imported from foreign nation and soft drinks prepared in Nepal..The coke which is imported contain 298.66µg/ml of caffeine where as coke prepared with in Nepal contain 285.21 µg/ml of caffeine. Similarly, imported Pepsi contain 318.47 µg/ml of caffeine where as Pepsi made in Nepal contain291.58 µg/ml of caffeine. Also caffeine contained in imported Dew is 263.27µg/ml where as in Dew prepared in Nepal contain only 220.1 µg/ml. This difference may be due to different processing plant. In Red Bull prepared in Nepal the amount of caffeine was found to be 381.46µg/ml and in imported red bull caffeine contained in it was 492 µg/ml. From the above data it is found that in all sample caffeine contained in it was greater in foreign nation then it was prepared in Nepal.

Part V

Conclusion and recommendations

5.1 Conclusion

- XL contain highest caffeine concentration of 520.88µg/ml while Litchi juice and Sprite contain lowest caffeine concentration of 87.05.
- Imported soft drinks was found to have higher caffeine concentration than soft drink manufactured in Nepal.

5.2 Recommendations

- Estimation of caffeine in other many soft drinks can be carried out.
- Along with caffeine other various compositions of soft drinks like phosphoric acid and other chemical components can be estimated.
- Presence and absence of heavy metal in soft drinks can be checked

Part VI

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Part VII

Photo Gallery

Fig 1: Soft drink samples

Fig 2: Standard caffeine solutions

Fig: 3 Soft drink samples