Identification and Determination of Caffeine Contents in Soft Drinks Commercially Available in Bangladesh Market By Using Reserve Phase HPLC Method.

A dissertation submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University in the partial fulfillment of the requirement for the degree of Master's of pharmacy.

Submitted by

Student ID: 183-46-222

Spring 2019

Batch: 7th

Department of Pharmacy

Faculty of Allied Health Sciences

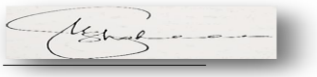
Daffodil International University

September, 2019

APPROVAL

This Project report entitled, **Identification and Determination of Caffeine Contents in Soft Drinks Commercially Available in Bangladesh Market By Using Reserve Phase HPLC Method**, Submitted by ID: 183-46-222 to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirement for the degree of Master's of Pharmacy and approved as to its style and contents.

BOARD OF EXAMINERS



Dr. Sharif Mohammad Shaheen

Professor

Head of the Department

Department of Pharmacy

Faculty of Allied Health Sciences

Daffodil International University

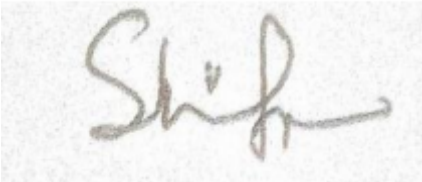
---------- External Examiner-1

---------- Internal Examine

Declaration

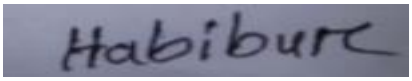
I hereby declare that, this project report is done by me under the supervision of Dr Sharifa Sultana**,** Assistant Professor, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, impartial fulfillment of the requirement for the degree of Master's of Pharmacy. I am declaring that this project is my original work. I am also declaring that project nor has any part thereof been submitted elsewhere for the award of Master's or any degree.

Supervised By



Dr. Sharifa Sultana
Assistant Professor
Department of Pharmacy
Faculty of allied health science
Daffodil International University

Submitted By



Md Habibur Rahman
ID: 183-46-222
Department of Pharmacy
Faculty of Allied Health Sciences
Daffodil International University

ACKNOWLEDGEMENT

I wish to take this excellent opportunity to thank a lot of people who have assisted and inspired me in the completion of my project.

At first I would like to give my profound thanks to Almighty God for giving me the strength and capability to complete my project successfully in time.

I would like to express my deep and sincere gratitude to Dr Sharifa Sultana, My supervisor, to whom I am extremely indebted for his tremendous support and guidance throughout my project work and writing of this report. Being working with him, I have not only earned valuable knowledge but was also inspired by his innovativeness which has helped enrich my experience to a greater extent. His ideas and way of working was truly remarkable.

My deepest gratitude and gratefulness will also go to my friends who are currently studying in Daffodil International University in Pharmacy Department for giving their valuable time in the design and graphical works of my project report.

Last but not least, heartiest thanks go to my precious family for their never ending support and love in journey of my life which has motivated me to such work.

Dedicate to.....

I dedicate this work first and foremost to almighty God and secondly to my family and to my teachers and my friends.

ABSTRACT

Caffeine is a central nervous system (CNS) stimulant. It is also called psychoactive drug but it is legal and unregulated in nearly all parts of the world. Caffeine is a bitter, white crystalline purine and a methyxanthine alkaloid. The purpose of this study is to identify and determine the amount of caffeine in soft drinks by reverse phase HPLC method. Six branded soft drinks was taken which include two of top brand samples. Quantitative analysis was done by reverse phase HPLC method with methanol:water (40:60v/v) as mobile phase and C_{18} as stationary phase with flow rate 1 ml/min and maximum UV 254 nm as the detector. The minimum caffeine level of soft drinks was observed in Mojo (23 mg/per 250 ml bottle), while sample Current showed the highest caffeine content (199 mg/per 250 ml bottle). The levels of caffeine in all energy drinks samples are well below the maximum allowable limits set by the food regulatory bodies because regulatory bodies suggest that the daily intake of caffeine for healthy adults with no medical issues is 300 mg-400 mg can be safe without any adverse effects but it is not recommended to take more than 200 mg caffeine for pregnant or lactating women and children.

CONTENTS

LIST OF FIGURE

LIST OF TABLE

LIST OF CHROMATOGRAM

CHAPTER 01: INTRODUCTION

CHAPTER 02: LITERATURE REVIEW

CHAPTER 03: METHODOLOGY

CHAPTER 04: RESULT & DISCUSSION

CHAPTER 05: CONCLUSION

CHAPTCHAPTER 06: REFERENCES

CHAPTER ONE
INTRODUCTION

1.1 Introduction

Caffeine is a naturally arising alkaloid which is bitter, white crystalline purine. It is an alkaloid of methylxanthine family. It is not found in almost all countries. The main source country for caffeine are Africa, East Asia and South America. Caffeine found at large amount in coffee bean. The beverage products also contain caffeine. Caffeine has various medicinal effects like it can prevent drowsiness and can increase working performance. Coffee, tea, soft drink and or energy drinks contain caffeine at large amount and they are popular source for caffeine. Its molecular weight and melting point are very high and its molecular weight and melting point are accordingly 119.9 gram and 236°C, pH is 6.900, specific gravity is 1.20, volatility is 0.50%, vapor pressure is 760 mmHg at 178.11°C, solubility in water is 2.170%, vapor density 6.70. Caffeine was thought most important chemical in the past decades because caffeine has physiological effects and stimulatory effect. (18)The Food and Drug Administration (FDA) says in a report that caffeine is a safe substance. Although, FDA also said in a report that carbonated beverages can cantain caffeine 0.02% .

1.2 Caffeine

Caffeine is considered as the psychoactive drug in the past time and naturally in the leaves and seeds of many plants contain caffeine and it is bitter in taste. Many compounds or the product contain caffeine like coffee, tea, energy drinks or soft drinks are many of them. Caffeine has also a property that can increase stimulant properties in the products, but in the the present day, caffeine is mixed with various type of foods related products items to produce energy and elevate mood. Now caffeine mixing with food like coca cola, cake etc are increasing gradually which has bad effect on human body. This negative effect of caffeine on the human body may become serious like death. Regulatory authorities of each country should be concerned about the bad effect in case of pregnant and lactating women, children, people with heart and other health problem. (2)

1.3 Caffeine Chemistry Information

The chemical name of caffeine has various name like it is called as theine, mateine, guaranine or methyl theobromine. Naturally, plants are the main source for caffeine harvesting or collecting.

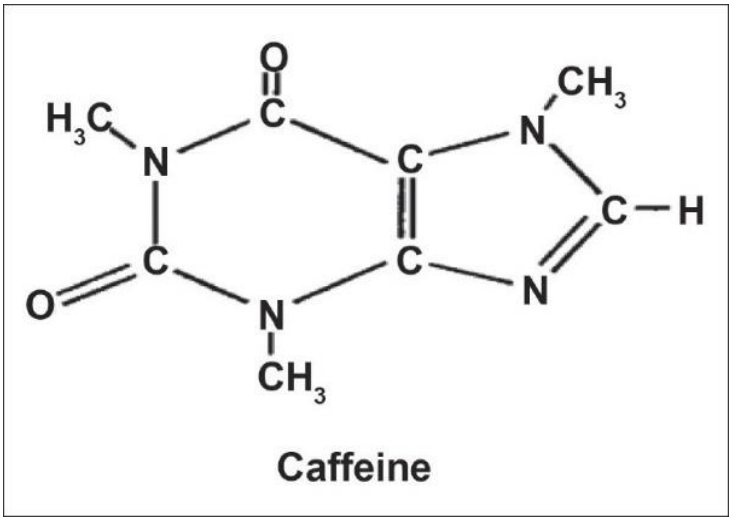


Figure 1.1: Structure of caffeine

Molecular Formula: C8H10N4O2

Molecular Weight: 194.19

Molar mass: 194.19 g/mol

IUPAC ID: 1, 3, 7-Trimethylpurine-2, 6-Dione

Melting point: 455°F (235°C)

Density: 1.23 g/cm

Caffeine is collected from various plants source as a powder form. Caffeine has a taste which is like bitter. Caffeine is polar that's why caffeine is soluble in water. Caffeine can effect on nervous system which can stimulant the brain. Caffeine has drowsiness removing property and can recovering alertness of any type of persons. (3)

1.4 Health benefit of caffeine

a) Caffeine has effect on Kidney and caffeine is very useful in reducing kidney Stone Risk. One study showed that people who take caffeine at greater extent those people will have greater chance to be affected of kidney stone.

b) Caffeine has drowsiness inducing property.

c) People who take t caffeine, there is less chance to occur asthmatic problem. It works like medicine.

d) Caffeine helps in reducing driving errors and it help the driver to make smooth driving.

e) Caffeine is good for those patients which have hepatitis C like disease.

1.5 Pharmacodynamics and Pharmacokinetics of Caffeine

Pharmacodynamics of caffeine

Adenosine can be produced in the human body and this adenosine goes to the synapse and then this adenosine bind with adenosine receptor. When adenosine binds with adenosine receptor, cellular response will be produced and it will be induced the drowsiness. So when a person takes caffeine, this caffeine will be acted as antagonist to adenosine. So adenosine will be not binded with adenosine receptor and there will be no produced cellular response. So drowsiness will not be produced.

1.6 Metabolism of Caffeine

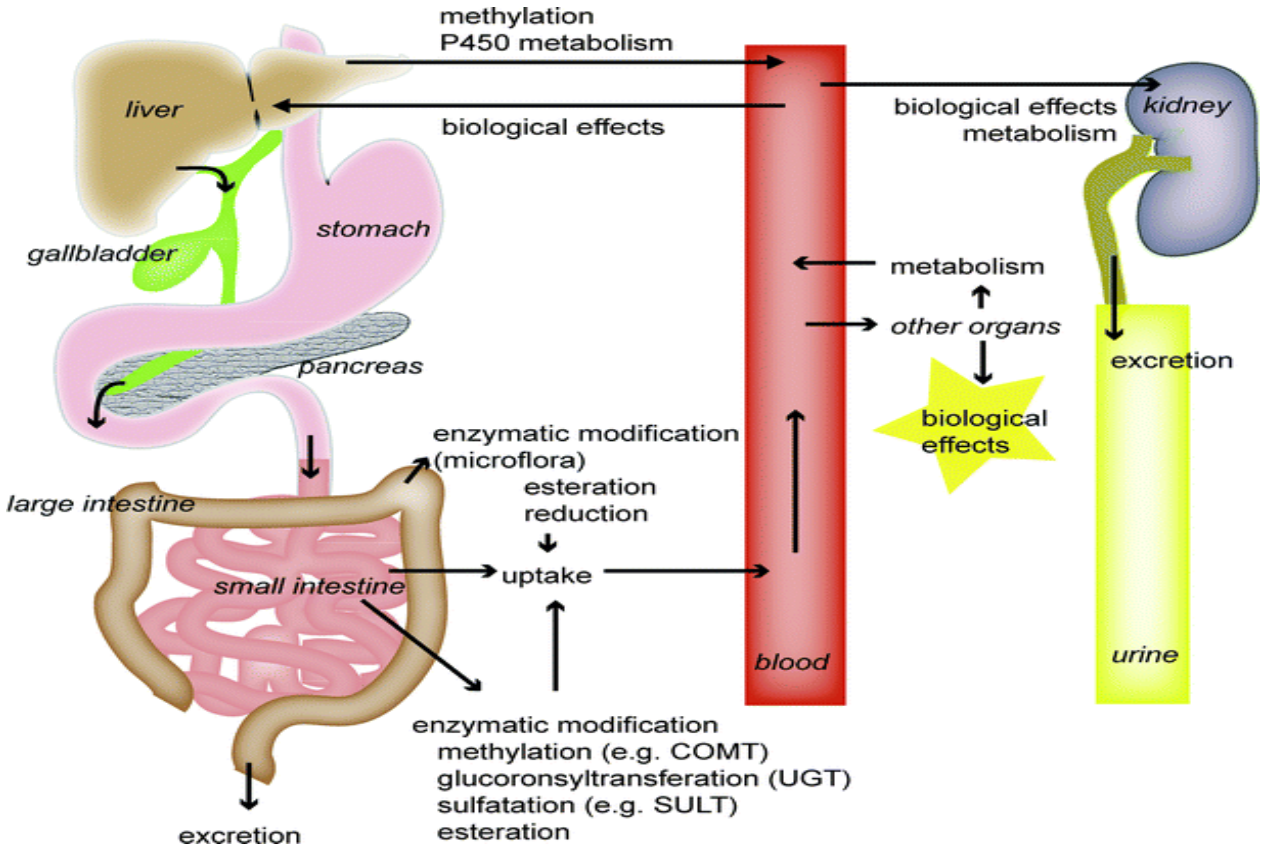


Fig 1.2: Metabolism of Caffeine

1.7 Dependence of caffeine

Caffeine withdrawal can affects in daily working performance of people. So we can say caffeine can produce dependence which effect on people body. Mainly dependence is also a psychological problem. For example when people take caffeine with tea or coffee, they become defences on caffeine mixing tea or coffee and finally they falling various physiological problem when they do not caffeine mixing food like tea or coffee. Although Caffeine withdrawal problems are physiological problem but it can affect the human body.

like fatigue, headache, irritability, depressed mood etc can produce due to withdrawal of caffeine. (6)

1.8 Mechanism of Action

Caffeine has various mechanism, drowsiness reduce is one of them. Now I will describe how caffeine reduces drowsiness. Adenosine can be produced in the human body and this adenosine goes to the synapse and then this adenosine bind with adenosine receptor. When adenosine bind with adenosine receptor cellular response will be produced and it will be induced the drowsiness. So when a person takes caffeine, this caffeine will be acted as antagonist to adenosine. So adenosine will be not bonded with adenosine receptor and there will be no produced cellular response. So drowsiness will not be produced.

Caffeine help in releasing of nor-adrenaline neurons and this nor-adrenaline help in producing or releasing of dopamine neurotransmitter. Mainly caffeine works on nervous system and it also help in releasing of serotonin. (8)

1.9 Side Effects

a) We know caffeine has various property, creating alertness is one of them. Mainly caffeine inhibits the work of the adenosine neurotransmitter, Now I will describe how caffeine inhibit the work of adenosine. Adenosine first produce in the human body then adenosine goes to the adenosine receptor and adenosine receptor produce cellular response and produce drowsiness but when a people take caffeine, caffeine will inhibit the adenosine to bind with adenosine receptor. So cellular response will not produce and by this process caffeine reduce drowsiness.

One study show that the people who take 1000 mg caffeine or more per day those fall in various physiological problems. Nervousness, jitteriness are one of them or more serious problem can also occur.

b) Caffeine has great effect on blood pressure. Blood pressure may produce due to take caffeine. There are some conditions to occur blood pressure which are described in bellow:

Hypertensive patients has greater chance if they take caffeine with tea or coffee at large amount and blood pressure occur due to consume caffeine incase of those people who normally everyday not take caffeine. People who have hypertension, in this case if this hypertensive patient take caffeine more than 250 mg with food or without food, then this patients will face blood pressure consistently.

c) Addiction

Although caffeine has health benefits, there is no denying that it may become habit-forming. A detailed study advise that although caffeine triggers certain brain chemicals similarly to the way cocaine and amphetamines do, it does not cause any classic addiction the way these drugs do. Although it may lead to psychological or physical dependency, in case of high dosages.

d) Rapid Heart Rate

Caffeine has also effect on heart rate. We know there are two types of heart beat. Mainly caffeine causes bradycardia in this case heart beat increase more 100 times per minutes. Heart beat rhythm like problem also occur in those of people whose age less than 18 due to take energy drinks or soft drinks at large amount.

Slow metabolism type problem, kidney failure like serious health disease also occur if people take high amount of caffeine. Heart rate problem do not occur in all kinds of person, mainly heat rate problem can vary from person to person physiological condition. If any person feel that hate rate is increasing due to consume of high amount of caffeine with food or without food then this person should take step to control rate .(9)

e) Fatigue

Caffeinated soft drinks or energy drinks can produce energy in the human body and by producing energy, people can remove fatigue. However, caffeine has another effect in case of producing fatigue. Now I will describe the process. When a person take energy drinks they got energy but when this energy drinks are removed from the body, then the people face fatigue like problem. (9)

f) Frequent Urination and Urgency

Caffeine has various side effect but frequent urination is big problem that's why people can not do their daily work frequently. So people face various type of problem. Now i will explain it with a example. when a person consume tea or coffee then this person do urination more than normal due to this tea or coffee contain caffeine.(9)

1.10 Caffeine Overdose

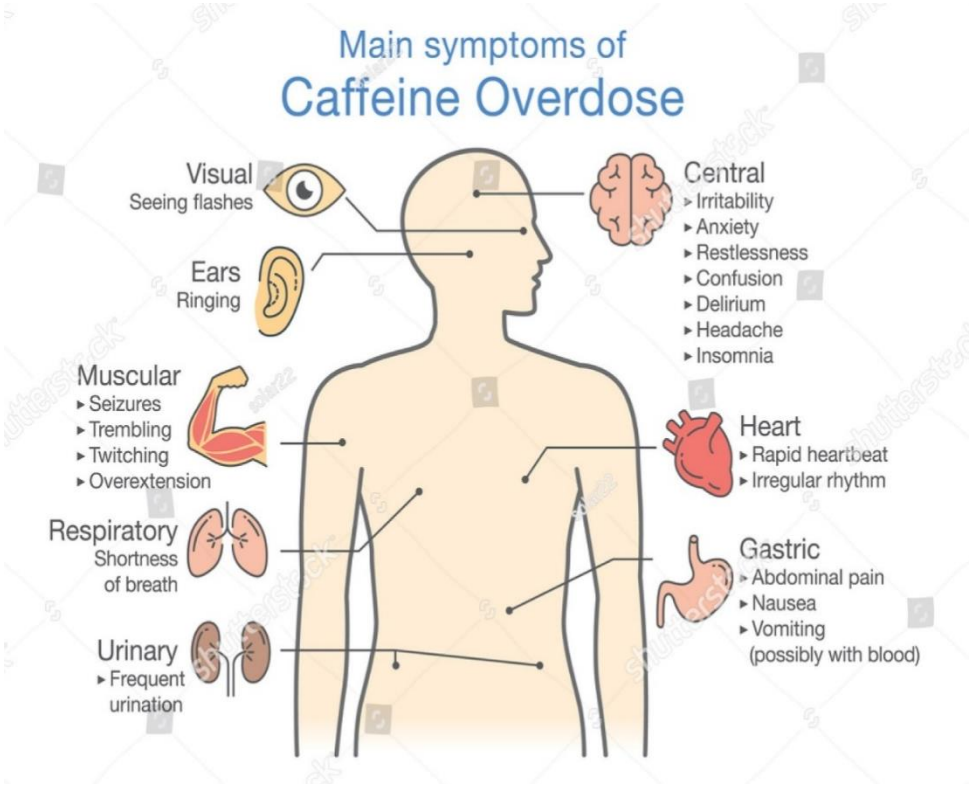


Figure 1.3: Caffeine Overdose (10)

1.11 Caffeine Interaction

Caffeine interaction with Alcohol

We know that caffeine make alertness in the human body but when caffeine is taken with alcohol then alcohol acts as inhibitory. when alcohol is taken with caffeine then alcohol inhibits the alertness effect of caffeine.

Tobacco

When tobacco is taken with caffeine then tobacco increase clearance of caffeine.

Birth control

When Birth control pills are taken with caffeine then birth control pill increases renal and half-life of caffeine (7)

1.12 Soft drinks and energy drinks

Soft drinks and energy drinks

When a drink contains carbonated water then this drink is called soft drinks, mainly soft drinks do not contain alcohol and they are usually sweet product. There are many soft drinks in the world and the purpose of this soft drinks to help in food digestion of people. Soft drinks contain various ingredients. Suger, colorings, preservatives are many of them.

1.13 Bottom Line of energy drinks

- Alcohol is not mandatory for soft drinks or energy drinks but some kinds of soft drinks contain little amount of alcohol which do not causes serious problem. For example, soft drinks can contain 0.5% alcohol of the total volume.

1.14 Regulations for Caffeine in Energy drinks

The consumption of high caffeine containing drinks are increasing day by day among the people especially the young generation. In Bangladesh, there are many beverage company which do not follow the regulation in case of maintaining caffeine content in their products that's why various kinds of disease risk is increasing day by day. Among the six item energy drinks, maximum company follow the FDA regulation in manufacturing their products.

The scientific reviews and clinical data showed that up to 500 mg of caffeine per day in any kinds of products is safe for most healthy adults or any kinds of persons. So from my result we can say that one should avoid regular uptake of caffeine containing drinks for better healthy life & should fully avoid in children and pregnant women. (12)

1.15 PURPOSE OF THE STUDY

The use of energy drinks or soft drinks is high among the general population and military personnel.

The main purposes of this study are given below:

- Most of the soft drinks or energy drinks which are available in Bangladesh contain caffeine in significant amount. But they have not mentioned the actual amount or sometimes don't contain the caffeine in the label. Our aim is to find out which sample contain the caffeine compound and in what amount.
- Second purpose is to determine the actual amount of caffeine compound in each bottle or per ml.
- The effect of the caffeine in our body especially in each organ, its benefit. Side effect, adverse effects find out by this study.
- Is those soft drinks and energy drinks consumption harmful for our body is another aim of this study.

1.16 High Performance Liquid Chromatography

HPLC or high performance liquid chromatography is a machine which is used to identify and determine the amount of content in any kind of products. This machine is mainly playing a great role in separating of substances. High performance liquid chromatography is similar in principle with column chromatography. In high performance liquid chromatography, there is a pump which work is to turn mobile phase in the column but in column chromatography there is no pump. In high performance liquid chromatography, there is a injector by which standard or samples are injected. But in column chromatography, there is no injector. In high performance liquid chromatography, there is a column mainly this column help to separates the substances. Like the HPLC, column chromatography also has a column which help to separate the substances. In case of normal phase HPLC stationary phase is polar and mobile phase is non polar and stationary polar part retain the polar part of sample. High performance liquid chromatography has various parts. The parts are given bellow:

a) The pump
b) Solvent Reservoir
c) Column
d) Injector
e) Detector
f) Digital display

Parts of HPLC

The pump

There is a pump in the high performance liquid chromatography which is use in HPLC to do a particular work. The work has been described in the bellow:

- Pump in the HPLC produce proper pressure.This pressure help the HPLC machine to create proper flow of mobile phase in column. That's why machine works properly and finally the separation of chemical substances occur.
- Pump in the HPLC, also has another work. It also maintain proper flow of mobile phase.(18)

There are two types of pump:

- Single pump
- Double pump

Figure 1.4: Pump of HPLC

The Injector

Injector is the important part of HPLC by which standard or sample are injected. This injected sample or standard then goes to the column.[15)]

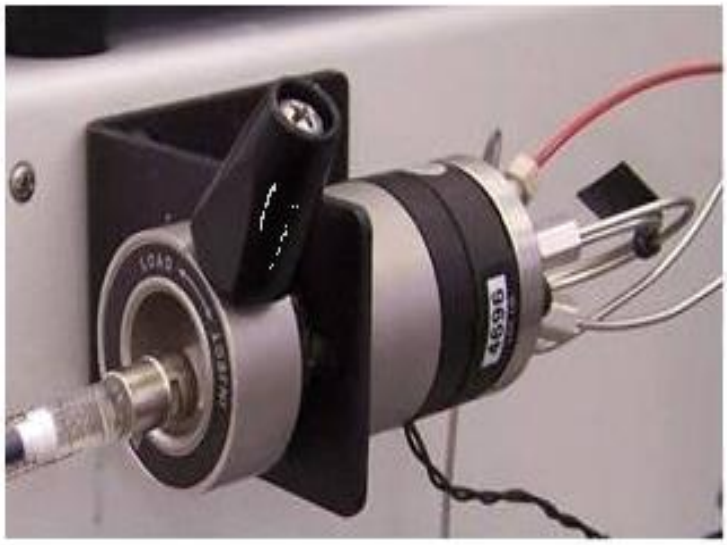


Figure 1.5: Injector of HPLC

The column

The column are important part of HPLC machine which is called heart of machine which main work is to separate chemical substances of mixture. In column, there has been replaced a stationary phase. In normal phase HPLC machine, this stationary phase is polar and this phase retains the polar part of sample mixture and by this way separation occur.

Figure 1.6: Column of HPLC

Detector

Without detector in HPLC machine, no result will be found. SO we can say detector is the necessary part of the HPLC machine.

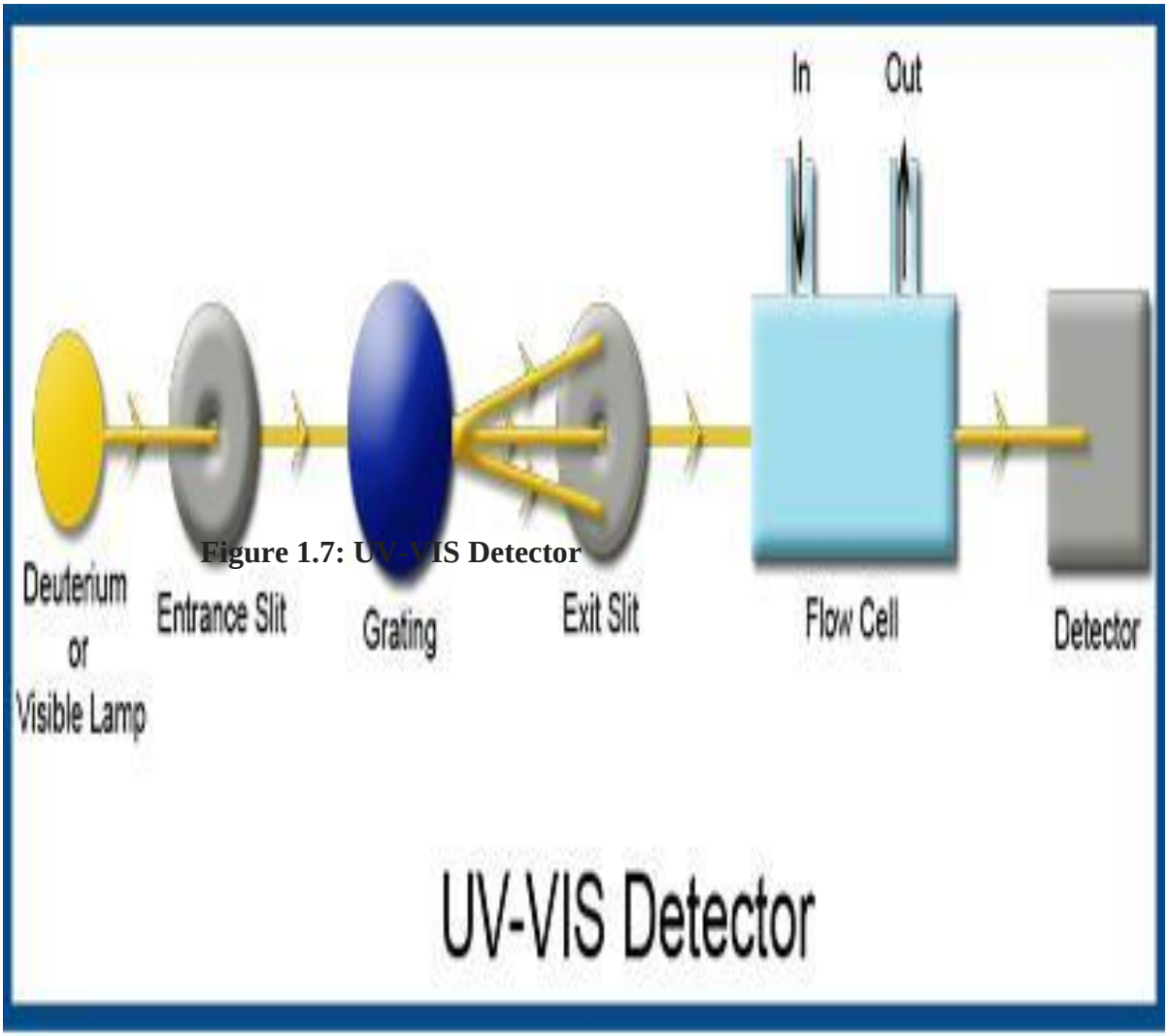


Figure 1.7: UV-VIS Detector

Figure 1.7: UV-VIS Detector

There are various types of detector in the HPLC machine. Some detectors are given bellow:

- **Photo Diode Array Detector**
- **Fluorescence Detector**
- **Refractive Index Detector**

Reversed-phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC) is a form of HPLC machine, in this machine has similar part like both machine has a pump, a injector, a column and a detector. But in this two machine has some basic different like in normal phase HPLC, stationary phase is polar and mobile phase is non polar and stationary phase retain polar part of sample mixture during separation process, but in case of revered phase HPLC machine, stationary phase is non polar and mobile phase is polar and stationary phase retain non polar part of sample mixture during separation.

CHAPTER TWO
LITERATURE REVIEW

2.1 **Literature review**

Title: Quantitative determination of caffeine in tea, coffee and soft drinks and its importance.

Author: Abdul Mumin,
The University of Western Ontario

Caffeine is a substance which is found in naturally but sometime caffeine is found by synthetic process. Plants are the main source for collect caffeine but sometimes animal source also be used for collect caffeine. Plants source like coffee, cocoa, tea leaves are the main source for collect caffeine. The main purpose for this study is to determine caffeine identification of caffeine and to determine the amount of caffeine in tea, coffee and soft drinks or energy drinks by high-performance liquid chromatography (HPLC). Now caffeine containing food like tea, coffee etc are consumed by people at large extent. Due to take high amount of coffee and tea, people are facing various health issue. People should concerned about taking of tea and coffee specially of pregnant and lactating women, children should concerned about it (15)

Title: Significance of Determining the Caffeine Content in Energy Drinks

Author: Jasmina dedibegovic and Elma omeragic

Faculty of Pharmacy, University of sarajevo

In the present time, there are many soft drinks company in Bangladesh. This company contains various ingredients in their products. Caffeine is one of their ingredients that is used in those soft drinks or energy drinks. People in Bangladesh are taking this soft drinks at large extent but they do not know how much caffeine they should take or consume or how much caffeine is good for their body. For this reason, people in Bangladesh are facing various health issue. So people should have sufficient knowledge about it. The soft drinks company should concerned about it and they should aware people who taking this soft drinks or energy drinks about rule and regulation in case of taking

this soft drinks (16)

CHAPTER THREE
METHODOLOGY

3.1 MATERIALS AND METHODS

Table 3.1.1: Reagent and solvent details

SI. No	Name	Source	Country ofOrigin
1	HPLC grade water	Fisher Chemical	Uk
2	HPLC grade Methanol	Merck KGaA	Germany
3	Coca-Cola	Local Market	Bangladesh
4	Speed	Local Market	Bangladesh
5	Mojo	Local Market	Bangladesh
6	Pepsi	Local Market	Bangladesh
7	Current	Local Market	Bangladesh
8	Coca-Cola	Local Market	Bangladesh

Table 3.1.2: Instrumental details

SI. No	Name	Source	Country of origin
1	An ODS reverse phase column [Promosil C18 (250 mm × 4.6 mm, 5µm)]	Technologics Ltd.	India
2	LC-20 AT Isocratic Single Pump (P 3000)	Technologics Ltd.	India
3	Analytical Balance	OHAUS	Switzerland

3.2 Chromatographic condition

- ❖ Mobile phase
  - HPLC grade Water
  - Methanol
- ❖ Flow rate
  - 1 ml per minutes
- ❖ Run time
  - 15 minutes
- ❖ Injection volume
  - 25 µl
- ❖ Column temperature:
  - 25 °C
- ❖ Detection
  - Standard : 254 nm
  - Sample : 254 nm

3.3 Method preparation

Preparation of mobile phase

Methanol and HPLC grade water was mixed accurately and sonicated. Then filtered for preparing the mobile phase. The ratio of the mobile phase was methanol 40%, water 60 %.

Preparation of caffeine standard

First weight 20.0 mg of caffeine then dissolve upto 100 ml with HPLC grade water in a volumetric flask. Then I took about 1 ml from this solution and diluted with appropriately 9

ml HPLC grade water to fill up 10 ml (Ten time dilution). Then filter the standard with .45 µ filter before inject.

Preparation of sample

Tiger:

Firstly Degased soft drink by sonication in a sonicator .I transfered exactly 5 mL of Tiger sample to a volumetric flask and again I added accurately 5 ml water which is HPLC grade to fill up 10 ml. This 10 ml sample then filtered with .45µm filter.

Speed:

Firstly Degased soft drink by sonication in a sonicator .Then I transfer exactly 5 ml of the 0.45 µm filtered Speed sample to a volumetric flask and again added 5 ml water which is HPLC grade to fill up 10 ml. This 10 ml sample then filtered with .45µm filter.

Mojo:

Firstly Degased soft drink by sonication in a sonicator .Transfer exactly 5 ml of the 0.45 µm filtered Mojo sample to a volumetric flask and again added 5 ml water which is HPLC grade to fill up 10 ml. This 10 ml sample then filtered with .45µm filter.

Mountain Dew

Firstly Degased soft drink by sonication in a sonicator .Transfer exactly 5 ml of the 0.45 µm filtered Tiger sample to 50 ml volumetric flask and again added 5 ml water which is HPLC grade to fill up 10 ml. This 10 ml sample then filtered with .45µm filter.

Current:

Firstly Degased soft drink by sonication in a sonicator .Transfer exactly 5 ml of the 0.45 µm filtered Tiger sample to a volumetric flask and again added 5 ml water which is HPLC grade to fill up 10 ml. This 10 ml sample then filtered with .45µm filter.

Coca-Cola :

Firstly Degased soft drink by sonication in a sonicator .Transfer exactly 5 mL of the 0.45 µm filtered Tiger sample to a volumetric flask and again added 5 ml water whis is HPLC grade to fill up 10 ml. This 10 ml sample then filtered with .45µm filter.

3.4 Operation

1. First I started HPLC machine, and then I set various parameters of machine which will help the mechine to perform very well such as I selected wavelength 254 nm.

2. Then I flushed the column at 1.0 ml per minute with the methanol which is mobile phase that will help the machine to get a stable base line

3. After baseline is satisfactory, then I injected 25 µl standards and I got a chromatograph and then I recorded the standard chromatograph.

4. Then I injected my samples like Tiger,speed , and I got various chromatogram for various samples and I recorded the samples chromatogram..

5. From this chromatographs I determined the caffeine contents in sample and by the chromatography equation and from this graphs I find out the caffeine in the sample. (21)

CHAPTER FOUR
RESULT & DISCUSSION

4.1 DATA ANALYSIS

Table 4.1.1: Color of the sample

Sample name	Color
Tiger	**Straw**
Speed	**Orange**
Mojo	**Dark brown**
Mountain Dew	**Green**
Current	**Straw**
Coca-Cola	**Dark brown**

Table 4.1.2: weight/ml of the sample

Sample name	weight/ml per 5 ml
Tiger	**0.836**
Speed	**1.05**
Mojo	**0.890**
Mountain Dew	**0.860**
Coca Cola	**0.850**
Current	**1.02**

Chromatogram of Standard

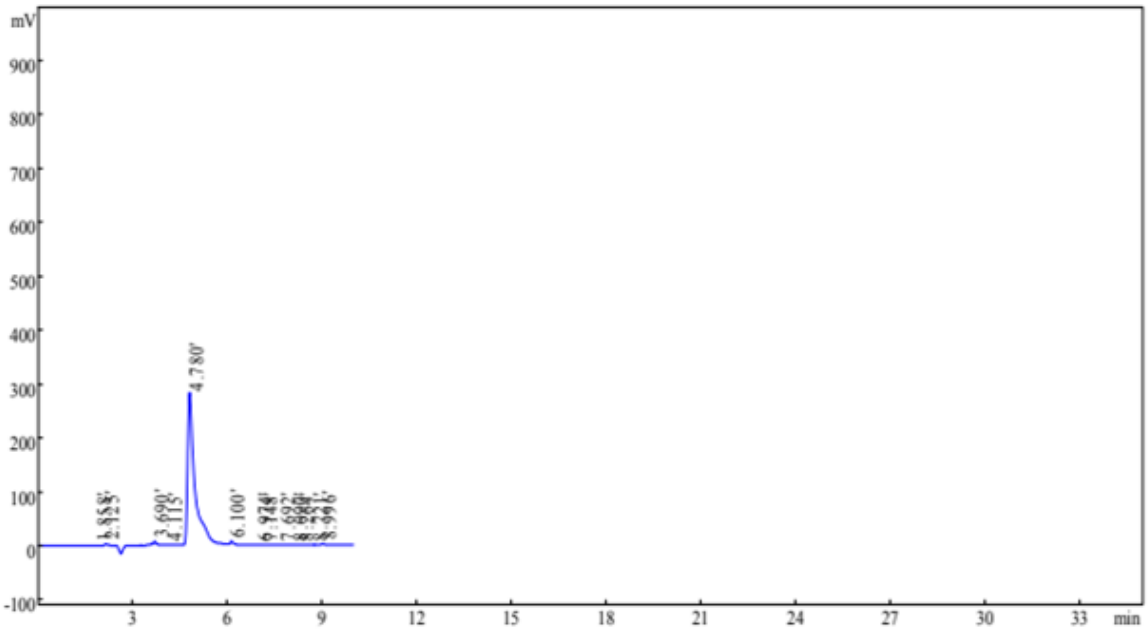


Figure 4.1: Chromatogram of Standard

Table 4.1.3: Peak Area of Standard

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	1.858		3.923	354333	9988	64	0.67
2	2.125		4.979	449747	15734	129	2.77
3	3.690		14.16	1278876	22774	101	1.00
4	4.115		4.516	407900	14145	475	1.45
5	4.780		66.38	5996365	297917	1316	2.98
6	6.100		0.3447	31139	15530	215966	3.07
7	6.974		0.8628	77937	6998	9151	0.80
8	7.148		1.885	170250	7635	2397	2.27
9	7.692		0.6721	60707	5042	9523	0.75
10	8.090		0.9497	85790	3987	3299	0.56
11	8.264		0.2778	25095	3140	24950	0.70
12	8.721		0.7355	66433	1989	1591	0.56
13	8.996		0.314	28363	4397	45383	1.09

Chromatogram of sample & its calculation

Chromatogram of Tiger

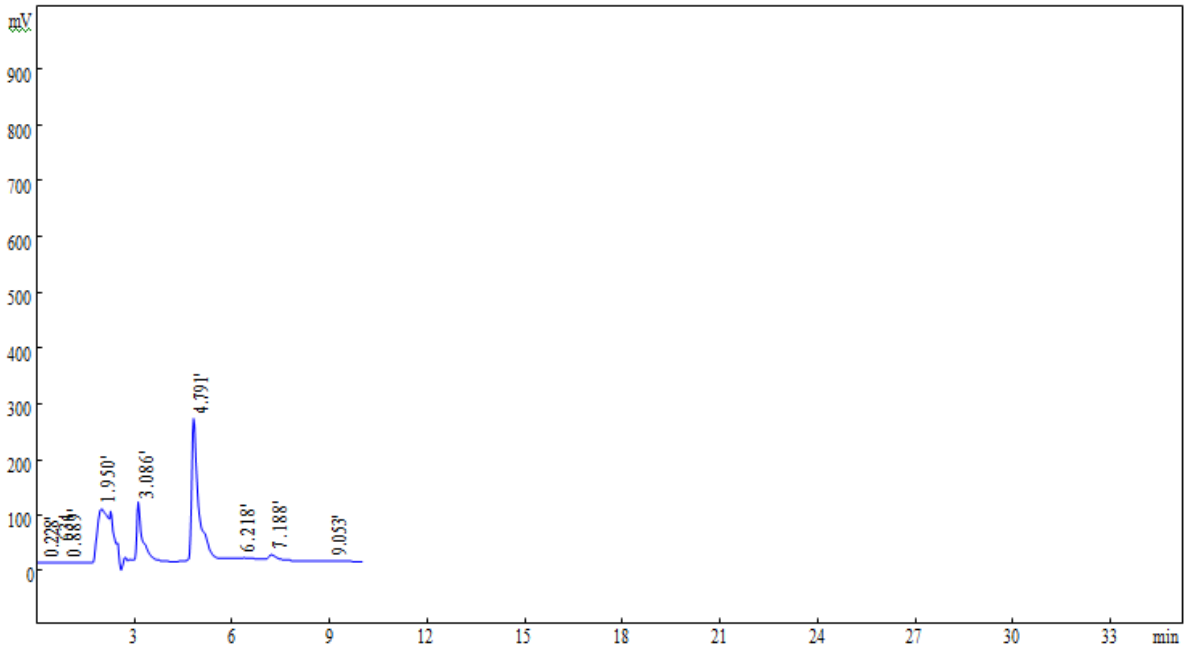


Figure 4.2: Chromatogram of Tiger

Table 4.1.4: Peak Area of Tiger

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	0.228		0.1381	14264	1457	13	0.54
2	0.634		0.6357	65662	3799	31	0.56
3	0.889		0.8897	91896	5796	73	0.66
4	1.950		39.3	4059870	108993	64	1.74
5	3.086		12.26	1266316	107470	1600	3.07
6	4.791		43.44	4486268	259009	1785	2.60
7	6.218		1.206	124546	2394	333	0.99
8	7.188		1.842	190255	9850	3231	1.23
9	9.053		0.2901	29966	789	1326	1.21

The Amount of the six samples is determined by the following equation:

Caffeine content (mg) = (Peak Area of Sample/Peak Area of Standard) x (Weight of Standard/Weight of Sample) x Dilution Factor x Potency of Caffeine x Wt./ml

Caffeine content (mg) in Tiger = ((4486268/5996365) x (20/5) x (1/100 × 1/ 10 ×10/1) × (99.9)×(0.836))

=2.49

Chromatogram of Speed

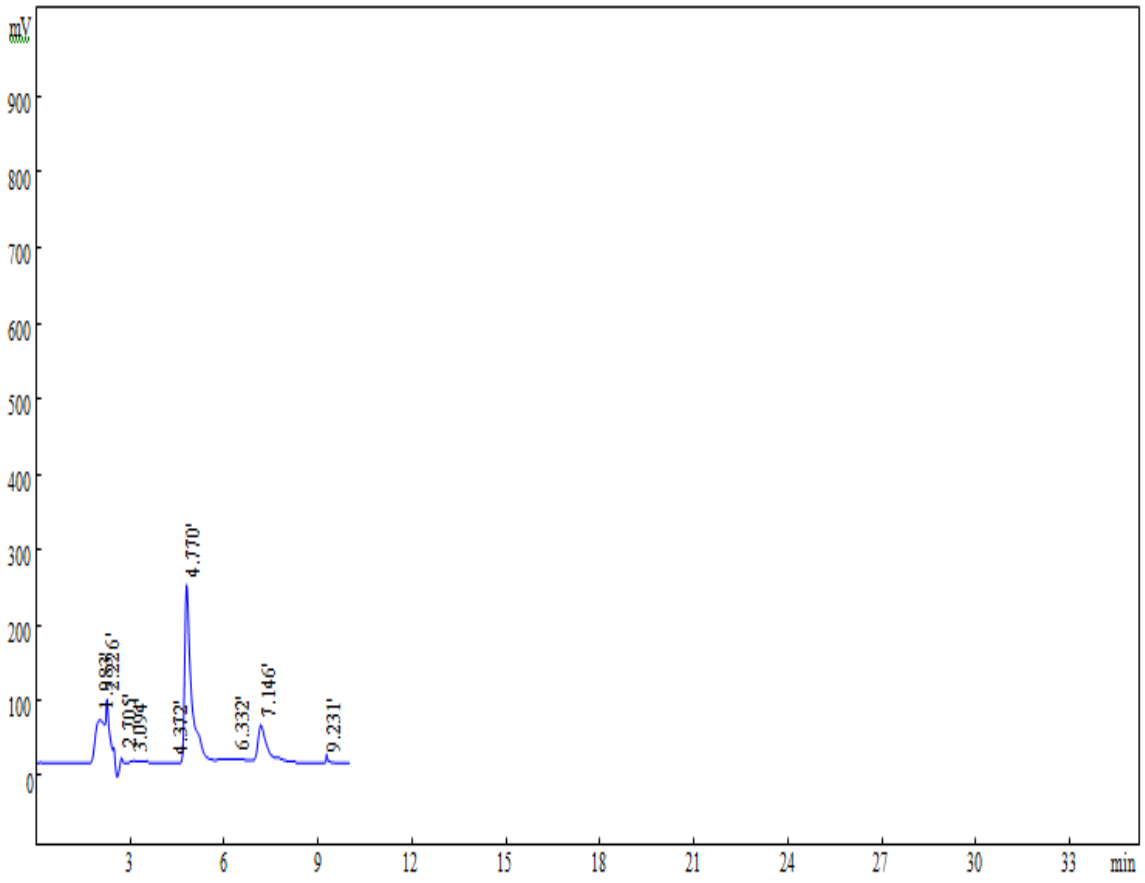


Figure 4.3: Chromatogram of Speed

Table 4.1.5: Peak Area of Speed

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	1.983		12.59	1551402	68788	180	1.65
2	2.226		8.817	1086884	105776	1095	1.29
3	2.705		2.073	255576	27326	1952	1.34
4	3.094		13.21	1628591	22498	43	2.47
5	4.372		1.384	170578	14163	3074	0.84
6	4.770		45.88	5657299	253219	1064	2.62
7	6.332		0.4193	51688	1328	618	0.76
8	7.146		14.14	1742517	57550	1300	2.04
9	9.231		1.488	183462	17274	17627	5.94

Caffeine content (mg) = (Peak Area of Sample/Peak Area of Standard) x (Weight of Standard/Weight of Sample) x Dilution Factor x Potency of Caffeine x Wt. /ml

Caffeine content (mg) in Speed = ((5657299/5996365) x (20/5) x (1/100 × 1/ 10 ×10/1) × (99.9)×(1.05))

=3.95

Chromatogram of Mojo

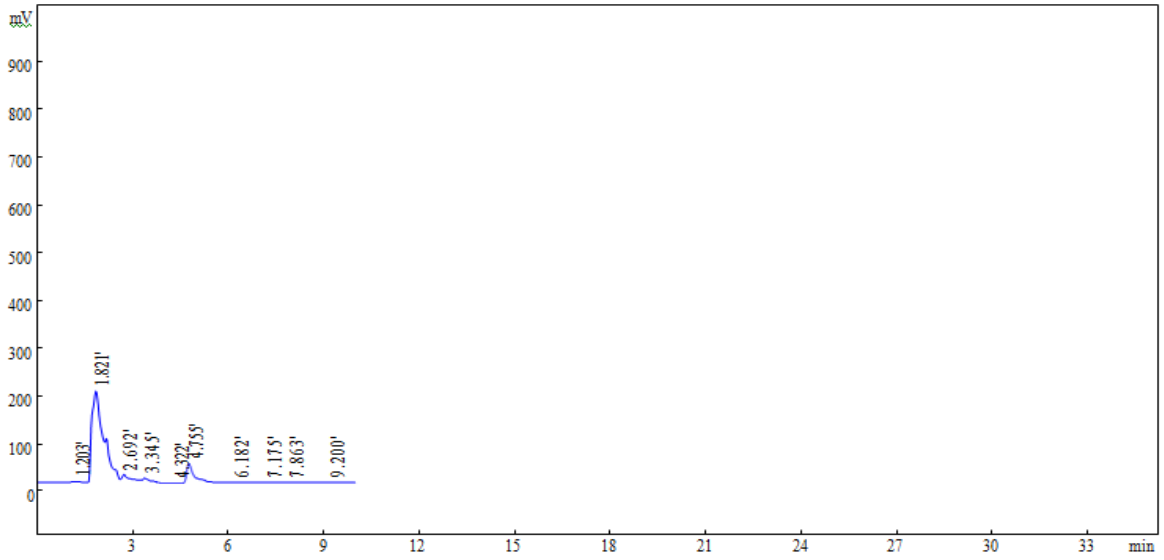


Figure 4.4: Chromatogram of Mojo

Table 4.1.6: Peak Area of Mojo

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	0.085		0.03058	2030	111	1	0.98
2	1.203		1.287	85460	3362	52	1.02
3	1.821		82.68	5488583	193665	96	2.13
4	2.692		2.327	154458	11144	880	2.11
5	3.345		1.236	82063	6748	1765	1.41
6	4.322		0.1681	11156	924	2990	
7	4.755		11.77	781039	41567	1494	2.76
8	6.182		0.2527	16775	872	2409	0.62
9	7.175		0.09913	6580	300	2497	0.92
10	7.863		0.03227	2142	1407	622253	2.14
11	9.200		0.1159	7693	727	17640	1.57

Caffeine content (mg) = (Peak Area of Sample/Peak Area of Standard) x (Weight of Standard/Weight of Sample) x Dilution Factor x Potency of Caffeine x Wt. /ml

Caffeine content (mg) in Mojo = ((781039/5996365) x (20/5) x (1/100 × 1/ 10 ×10/1) × (99.9)×(0.890))

=0.46

Chromatogram of Mountain Dew

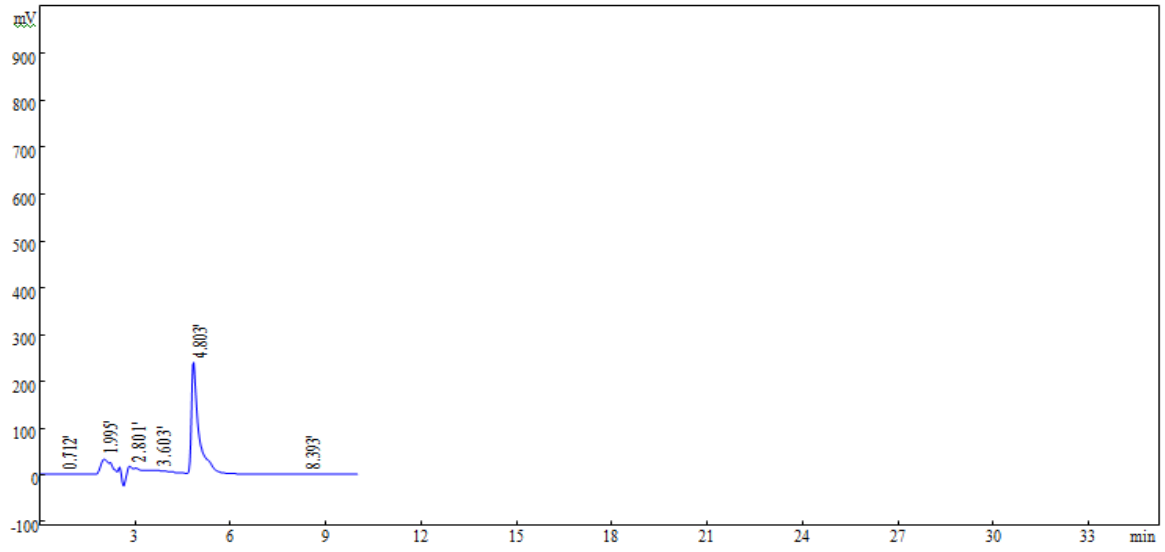


Figure 4.5: Chromatogram of Mountain Dew

Table 4.1.7: Peak Area of Mountain Dew

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	0.712		2.527	311152	7921	8	0.63
2	1.995		20.27	2496051	52356	41	1.69
3	2.801		10.95	1348563	42899	185	3.68
4	3.603		16.77	2064833	30648	67	0.70
5	4.803		49.38	6081216	258950	976	2.95
6	8.393		0.1041	12815	1712	29331	1.33

Caffeine content (mg) = (Peak Area of Sample/Peak Area of Standard) x (Weight of Standard/Weight of Sample) x Dilution Factor x Potency of Caffeine x Wt. /ml

Caffeine content (mg) in Mountain Dew = ((6081216/5996365) x (20/5) x (1/100 × 1/ 10 ×10/1) × (99.9)×(0.860))

=3.48

Chromatogram of Coca Cola

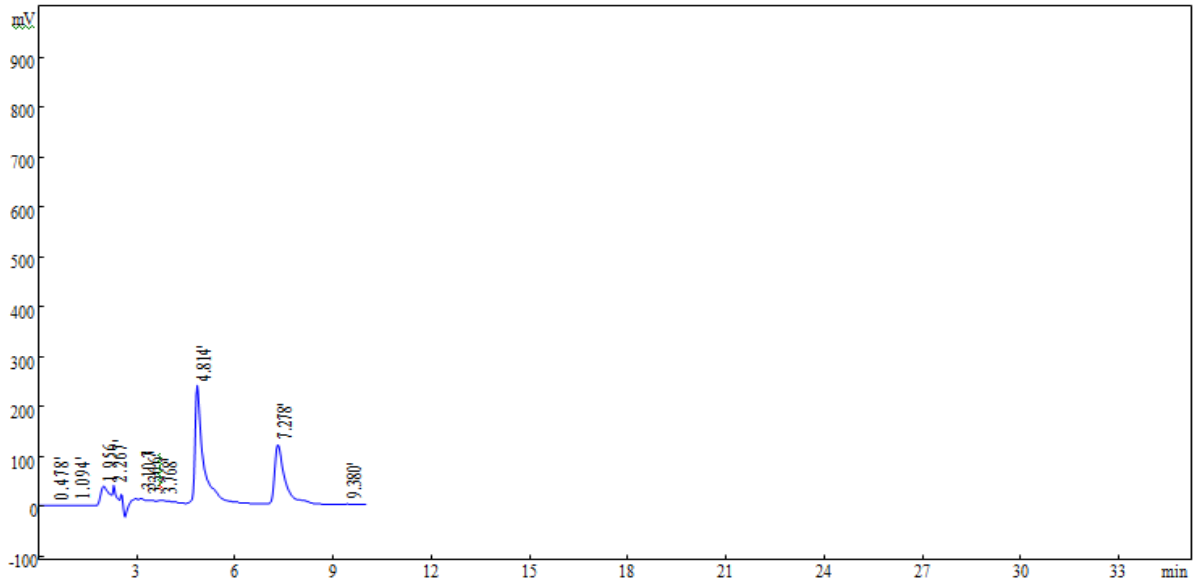


Figure 4.6: Chromatogram of Coca Cola

Table 4.1.8: Peak Area of Coca Cola

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	0.478		0.8541	144306	6401	10	0.56
2	1.094		1.491	251902	11618	59	0.53
3	1.956		9.845	1663353	57802	108	1.66
4	2.267		5.662	956694	64404	543	1.18
5	3.107		7.582	1281097	37493	193	0.77
6	3.366		2.954	499061	33670	1203	1.46
7	3.768		9.212	1556519	31283	134	0.70
8	4.814		40.09	6772967	258488	788	2.97
9	7.278		21.78	3679494	131106	1569	2.60
10	9.380		0.5321	89908	3125	2480	1.39

Caffeine content (mg) = (Peak Area of Sample/Peak Area of Standard) x (Weight of Standard/Weight of Sample) x Dilution Factor x Potency of Caffeine x Wt. /ml

Caffeine content (mg) in Coca Cola = ((6772967/5996365) x (20/5) x (1/100 × 1/ 10 ×10/1) × (99.9)×(0.850))

=3.83

Chromatogram of Current

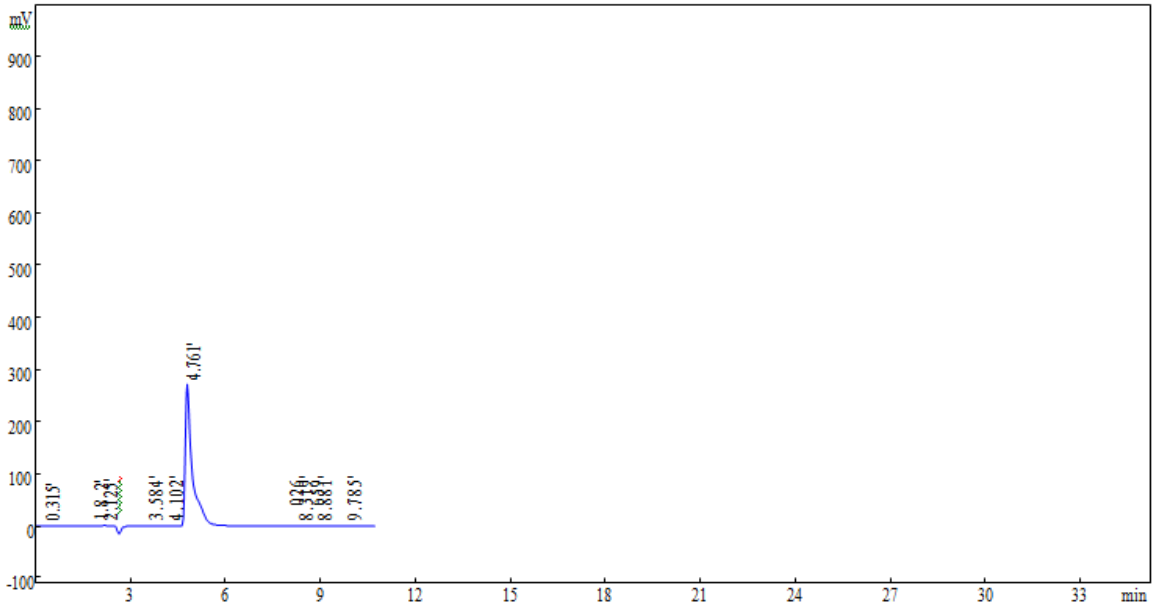


Figure 4.7: Chromatogram of Current

Table 4.1.9: Peak Area of Current

Rank	Time	Name	Conc.	Area	Height	T.PlateNum	Asymmet
1	0.315		0.1359	10851	573	6	0.55
2	1.822		0.4191	33471	1049	76	0.58
3	2.125		3.138	250626	6577	73	2.99
4	3.584		12.56	1003158	14206	60	0.83
5	4.102		6.242	498546	13140	273	1.00
6	4.761		73.46	5867623	286220	1258	2.93
7	8.026		1.959	156479	3832	901	0.52
8	8.319		1.099	87794	3065	1968	0.54
9	8.659		0.507	40491	2588	7148	0.56
10	8.881		0.4615	36857	1696	3897	2.28
11	9.785		0.01597	1275	76	7935	0.87

Caffeine content (mg) = (Peak Area of Sample/Peak Area of Standard) x (Weight of Standard/Weight of Sample) x Dilution Factor x Potency of Caffeine x Wt. /ml

Caffeine content (mg) in Current= ((5867623/5996365) x (20/5) x (1/100 × 1/ 10 ×10/1) × (99.9)×(1.02)) = 3.98

4.2 RESULT

Table 4.2.1: Caffeine Content in Soft Drinks

Sample	Caffeine Content / 5 ml
Tiger	2.49 mg
Speed	3.95 mg
Mojo	0.46 mg
Mountain Dew	3.48 mg
Coca-Cola	3.83mg
Current	3.98 mg

Table 4.2.2: Caffeine Content per bottle

Sample	Caffeine Content(per 250 ml bottle)
Tiger	124 mg
Speed	197 mg
Mojo	23 mg
Mountain Dew	174 mg
Coca Cola	191 mg
Current	199 mg

4.3 Discussion

Six Samples were tested and and it was observed that 5 ml sample contain 2.49 mg caffeine in Tiger, 3.95 mg caffeine in Speed, 0.46 mg caffeine in Mojo, 3.48 mg caffeine in Mountain Dew, 3.83 mg caffeine in Coca Cola, 3.98 mg caffeine in Current and amount of caffeine in 250 ml bottle of Tiger-124 mg, Speed -197 mg, Mojo -23 mg, Mountain Dew -174 mg, Coca Cola-191 mg, Current-199 mg. From those tested samples found that the amount of caffeine in Current (soft drink) has highest caffeine than the other soft drinks and Mojo (soft drink) has lowest amount of caffeine in 250 ml bottle. Ranking in terms of caffeine content per bottle, the ranking is as follows:

Current>Speed> Coca-Cola > Mountain Dew > Tiger > Mojo

CHAPTER FIVE
CONCLUSION

Conclusion

From 1987, there are increasing the various kinds of soft and energy drink in the market. But beverage producing companys are not following the rule and regulation. The beverage producing company authority should have concerned about regulation of caffeine contents in soft drinks. Due to caffeine intake caffeine dependence is increasing day by day which is not good for pregnant women, children and adolescents. Caffeine mixed with foods can also be dangerous. This caffeine containing food can produce drowsiness that's why people fall in various health issue.

The consumption of high caffeine containing drinks are increasing day by day among the people especially the young generation.In Bangladesh, there are many beverage company which do not follow the regulation in case of maintaining caffeine content in their products that's why various kinds of disease risk is increasing day by day. Among the six item energy drinks, maximum company t follow the FDA regulation in manufacturing their products.

The scientific reviews and clinical data showed that up to 500 mg of caffeine per day in any kinds of products is safe for most healthy adults or any kinds of persons. So from my result we can say that one should avoid regular uptake of caffeine containing drinks for better healthy life & should fully avoid in children and pregnant women.

CHAPTER SIX
REFERENCES

REFERENCES

1) Srdjenovic, B., Djordjevic-Milic, V., Grujic, N., Injac, R., & Lepojevic, Z. (2008). Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. Journal of chromatographic science, 46(2), 144-149.
2) De Camargo, M. C. R., & Toledo, M. C. F. (1999). HPLC determination of caffeine in tea, chocolate products and carbonated beverages. Journal of the Science of Food and Agriculture, 79(13), 1861-1864.
3. https://www.researchgate.net/profile/Andrew_Smith24/publication/11540186_Factorsassociated_with_caffeine_consumption/links/557eaf2408ae26eada8de044.pdf
4. https://www.caffeineinformer.com/top-10-caffeine-health-benefits
5. Quantitative thin-layer chromatography/mass spectrometry analysis of caffeine using a surface sampling probe electrospray ionization tandem mass spectrometry system Analytical chemistry, 77(14), 4385-4389.
6. https://www.coffeeandhealth.org/topic-overview/caffeine-and-dependence-2/
7. https://www.drugs.com/drug-interactions/caffeine.html
8. https://pubs.acs.org/doi/abs/10.1021/ac050488s
9. http://site.iugaza.edu.ps/mlatif/files/2015/02/Experiment-6.pdf (45-47)
10. https://www.shutterstock.com/image-vector/main-symptoms-caffeine-overdose-illustration-about-746526538 (15)
11. Chad J. Reissig,a Eric C. Strain,a and Roland R. Griffithsb
12. Caffeinated Energy Drinks -- A Growing Problem
13. http://chem-net.blogspot.com/2013/10/pump-column-detector-injector-hplc.html
14. http://chemnet.blogspot.com/2013/10/pump-column-detector-injector-hplc.html (21-27)
15. http://site.iugaza.edu.ps/mlatif/files/2015/02/Experiment-6.pdf (45-47)
16. https://www.researchgate.net/publication/308889296_Significance_of_Determining the Caffeine Content_in_Energy_Drinks

17. https://www.ncbi.nlm.nih.gov/pubmed/125 19715

18. https://m.scirp.org/papers/91016

19. https://www.ncbi.nlm.nih.gov/pubmed/28438661

20. https://www.ncbi.nlm.nih.gov/pubmed/29981767

21. http://site.iugaza.edu.ps/mlatif/files/2015/02/Experiment-6.pdf

22. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-311X2005000600014

23.https://www.healthline.com/health/caffeine-effects-on-body