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DETERMINATION OF CAFFEINE CONTENT IN COMMERCIAL ENERGY BEVERAGES

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TATSVAREYI CAROLINE

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DEDICATION

This research is dedicated to my family

ABBEVIATIONS AND ACRONYMS:

FDA ....United States Food and Drug Adminisration

ABSTRACT

This study is going to determine the caffeine content of four commercial energy drinks sold in Zimbabwe.Pursposive and convenience sampling were used to select three energy drinks for each brand ,a total of 12 energy drinks were used. Two methods, iodometry and spectrophotometry were used to analyze caffeine in the samples. The comparative results of the study showed that, there were significant differences in levels of caffeine in drinks. The study also established that spectrophotometry results were higher while iodemetry results were lower, but all values were close range of the labeled container. Therefore the researcher recommend the need to quantify caffeine in large sample of energy drink formulations including coffee and tea brands. Furthermore there is need to compare methods used with chromagraphic technique.

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CHAPTER 1

1.2 Background

The basis of sound health is rooted in good nutrition practices. Beverages that are rich in caffeine have a long time captured humankind owing to their enticing savor and reviving taste (Khalid et al ,2016) The need for determination of caffeine content in beverages and product cannot be under estimated because the humankind has dependent on caffeinated beverages(Barbra et al ,2018) According to Idumah et al., (2020) a deficit of reliable drinking water supplies and change in climate particularly hot climate that has been prevailing lately has aggravated consumption of energy and soft drinks. Besides, soft drinks such sodas, energy drinks, chocolate, ice-cream and fruit drinks equally hydrate, quench thirst and cool the body in the same way drinking water does (Chaudhary, 2018).Basic ingredients that confer favorable taste which is enjoyable in the formulations of soft drinks and energy drinks are sugar, caffeine, coloring, dilute phosphoric acid and flavoring agents (Damle et al., 2011). However, some of these ingredients if excessive taken in may be harmful to human health. There is extensive disquiet in medical and consumers owing to the negative health issues related to preservatives, sweeteners and caffeine (Kregiel, 2014). Caffeine (1, 3, 7-trimethylxanthine) is a natural crystalline active alkaloid used in mild psychoactive therapy and has no taste. It is legally not regulated and consumed to activate pulse, brain as well as stimulating the body. (Gerald et al, 2014) With the current of commercial beverages containing caffeine, such as of coffee, tea, energy drinks and soft drinks, it is important to determine caffeine levels. Such information is necessary to consumers, quality control, regulatory compliance as well as health considerations.

To date, there are different types of soft drinks and non-alcoholic beverages that found in the market which are believed to have high sugar and caffeine content (Ohilebo and Blessing, 2020). Moreover, they may be led to increase in psychoactive behavior exhibited by people of all ages nowadays. As such, reliable and sensitive method for determining caffeine in selected soft drinks, within the shortest timeframe is no longer a choice, but a must to ensure food security. Therefore, this study aims focus is to determine caffeine content of four selected commercial energy drink and soft drink samples sold in Zimbabwe using UV/Visible spectrophotometric assay.

1.3 Aim

This main focus of the study is to develop and validate a spectrophotometric method for determining caffeine levels in four commercially available beverages in Zimbabwe.

1.4 Objectives

The objectives of the study are

1. To determine content of caffeine in four energy beverages available in market in Zimbabwe
2. To assess difference in caffeine levels in energy beverages and ascertain level of consistence in caffeine content
3. To determine deviation of caffeine content of different type of commercially available energy drinks from purported content on label.

1.5 Research questions

1. How does caffeine content vary among different types of commercially available energy drinks?
2. What is the level of variability in caffeine content within brands of energy drinks?
3. How does the caffeine content in different commercial energy drinks compare to the labeled caffeine content?

1.6 Significance of the study

The study is going to equip consumers with accurate information with regards to level of caffeine in energy beverages available in the market and this will help consumers to regulate caffeine intake. The regulation of caffeine intake promotes personal health and well-being of consumers. With reference to the beverage producers, the study aim to offer valuable contribution in relation to product quality control and consistency. Reliable and validated spectrophotometric method for determining caffeine level in beverage products will help the manufacturers to uphold consistence in their product thereby ensuring consumer satisfaction. In this regard ,manufacturers can be proudly stand behind quality and reliability of their products and take pride in their commitment to delivering energy beverages that have consistent levels of caffeine .Furthermore the finding of the study will contribute to establishment of accurate and effective regulations. Potential health risk can be eliminated through setting relevant limits on caffeine levels in energy beverages. Strict adherence to established regulation confers pride in regulatory bodies in their effort to protect public health and standards of safety. Moreover this study fosters transparency and trust in industry through enforcement of correct and precise labels of caffeine level on energy beverages as well as compliance to set standards .As a result the manufacturer's reputation and credibility becomes undoubted.

1.7 Limitations of the study

The limitation of the study is number and variety of samples. Shortage of resources it was not possible to obtain samples all brands sold in Zimbabwe hence the study fall short in presenting all commercial energy beverage in Zimbabwe. The study is also eliminated by time, there was no time to store samples and evaluate them over a period of time thereby affecting assessment of the consistency of caffeine level in different brands and batches over longer periods of time. Moreover the accuracy of caffeine determination may be impacted on by interference of other additives that are soluble in extracting solvent used the study.

1.8 Delimitation of the study

The study mainly focuses on analysis of caffeine content in commercially beverages such as energy drinks .Other beverage type as sports drinks, herbal tea or specialty beverages are not included in this study. Therefore, the study may not be applicable to these specific beverage categories. The study was limited to specific limited sampling location due to practical constraints. This could have an impact on the findings to a broader population or different region with varying brewing practices or ingredient sources. While the study focuses to develop a reliable and variable spectrophotometric method for caffeine determination the focus is limited to one technique.

1.9 Hypotheses

H_0: There is no significant difference in content of caffeine labelled on energy drink and ones determined spectrphotometrically for all energy beverages

H_1: There is a significant difference between the labeled or advertised caffeine content and the measured caffeine content in commercially available beverages.

CHAPTER 2

LITERATURE REVIEW

2.1 Introductory note

By year 2016, intake of energy drinks globally had increased by 17% United States was leading in terms of volumes of sales while Thailand was toping in terms of consumption of drinks per individual (McClellan et al , 2016) Energy drink first appeared in Eurasia around 1960 and market grew exponent tacky since then and more than 500 new brands have been introduced the world over (Stephens et al,2014)Caffeine content and concentrations varies per can or bottle and per ounce for different brands available on the market .The active ingredient in energy drinks is mainly Caffeine , although there are some that contain riboflavin,nicotinamide,herbal derivatives.taurine ,pyndoxine and other vitamins. However long term and acute effects of excessive consumption of these additives are not yet fully understood despite increase in consumption worldwide (Chaudhary, 2018; Barbra et al, 2016, Damle et al 2011)

2.2 Regulation relating to caffeine content in food and beverages

It has being difficult to regulate caffeine added to beverages owing to long term and global consumption of coffee and tea that naturally contain caffeine .Despite that countries have put in place measures that regulate how energy drinks that contain substantial amounts of caffeine are labelled distributed and sold, For example . In Canada it is prerequisite that labels must indicate that Red Bull must not be mixed with alcohol. Energy drinks .according to European Union must carry the label 'high caffeine content' (European Union, 2007; Health Canada, 2005) In Zimbabwe the food and Allied industries Act of Zimbabwe primarily governs food safety and standards in the country. It also establishes the Zimbabwe Food Standards Advisory Board (ZFSAB), which sets and enforces food safety standards, including those relating to the use of additives such as caffeine .Under the primary legislation, food and beverage manufacturers are required to comply with standards and regulations set by ZFSAB (Ministry of Health and Child Care) The standard include guidelines for use of additives such as caffeine in beverages. The ZFSAB sets limits on permissible concentration of caffeine in different types of beverages. The ZFSAB ensure consumer safety .In addition .labeling requirements, to provide consumers with information about the presence and quantity of caffeine in beverages are put in place by ZFSAB.

The labels must be clear and accurate with regards to caffeine presence and content (Food and Foods Standards Act, Zimbabwe).

The United States Food and Drug Administration (FDA) once proposed the elimination of caffeine from drinks, a decision that was later reversed after manufacturers had argued to that caffeine is used to enhance flavor (Food and Drug Administration, 1980; PepsiCo Inc., 1981) despite the fact that the basis of the claim has been scientifically contested.

2.3 Advertisement of energy drinks

Promotion of energy drinks on the market is based on the stimulating effect and the assertion that they offer variable benefits which include loss of weight enhanced performance, endurance and attention albeit pending substantiation of some of the claims. Caffeine is known to counter reduced performance due to abridge alertness as a result of fatigue or deprivation of sleep. There is a debate whether caffeine has net positive enhancing effects because of punch-drunk caffeine withdrawal effects or the effect are a result of reversal of caffeine withdrawal symptoms (Haskell et al ,2005;Heatherley et al .2005; James and Rogers, 2005)

The advertisement of energy drinks is targeted mainly to young people using attractive names such as ‘STING’,’DRAGON’ and ‘SWITCH ‘among others. The campaigns advance the stimulating effect, performance enhancement and psycho-activity of the drinks and seemingly exalt the use of drugs .There is a marked contrast between marketing of energy drinks as stimulants and recreational products and marketing of such soft drinks which has been limited to rather inoffensive and relatively cryptic declarations akin to those used to promote brands such as Coca Cola and Fanta. As previously discussed about the FDA’s notion of limiting caffeine from soft drinks versus justification of flavor-enhancement as put forward by manufacturers, there is great likelihood that manufacturers are unwilling to portray their products as stimulants for their regulatory explanation for adding caffeine becomes baseless and invalid.

2.4 Overdose and toxicity of caffeine

There are issues pertaining to content of caffeine in energy drinks due to probable adverse effects related to its use .For example, caffeine intoxication is a clinical group of signs and symptoms that emerge as forthright consequence of consuming caffeine. This entails tremor, insomnia, nervousness, restlessness, anxiety, psychomotor agitation, tachycardia and upset stomach. (World Organization, 1992a, b).

In this regard, consuming energy drinks increase the peril for overdosing with caffeine in those abstaining from caffeine and those who are habitual takers of caffeine in tea, soft drinks or coffee. It is an established fact that the risk of caffeine intoxication through soft drinks is more significant compared to that resulting from other dietary sources of caffeine because of a number of reasons such as inadequate labelling (because it is not requirement) so consumers are ignorant of the amount of caffeine they ingest. Moreover, advertisement of energy drinks purport that that the drink have various desirable effects but as a matter of fact, the effects are debatable with reference to empirical evidence. For instance, Red Bull advert portrays an array of benefits of the energy drink which may compel consumers to take more servings at a time anticipating better results thereby risking themselves. Sale of energy drinks has no age restriction, therefore, children and adolescents are more prone to caffeine intoxication.

Research has shown that caffeine intoxication and rapid ingestion of energy drinks is related to seizures, stroke and acute main (Nehling 2016).In Australia, Sweden and Ireland, there has being reports of deaths linked to intake of energy drinks but there were speculations whether energy drinks directly led to death. Despite the exclusion of caffeine from diagnostic schema of substance dependence their arguments with regards to magnitude of reinforcement effects and potential abuse of caffeine because there is overwhelming pointing towards caffeine as a substance that is capable of producing substance dependence syndrome in some people. Some studies has proved failure to quit using caffeine regardless of its harm and withdrawal. Close to 30% of study population met the diagnostic criteria of dependence on caffeine (Barbra et al, 2016; Greald et al, 2014)

The frequency of dependence on caffeine is aggravated by marketing campaigns that encourage use of energy drinks among young people. Medical literature has described symptoms related to caffeine withdrawal such as headache within 12 to 24 hours past the last caffeine intake.

Furthermore, fatigue, drowsiness, depression, muscle, stiffness, being irritable, dysphonic, mood and vomiting are indicators of caffeine withdrawal. Severity of withdrawal symptoms may vary and in some cases, they are quite intense. Combined intake of energy drinks and alcohol has increased greatly in last couple of years. There are reports that symptoms of alcohol intoxication are reduced when alcohol is taken as a combination with energy drinks. Such activities aggravate the possibility of injuries related to alcohol. People who took alcohol with energy drinks exhibit marked incidence of ability to take advantage of other or being taken advantage of sexually and getting injured or hurt (FDA 2003;Chaudhary 2018;FDA 2007 a,b).

CHAPTER 3

3.1 Methods

3.1.1 Chemicals and materials

Caffeine standard, chloroform, sodium carbonate, distilled water,glassware,wash bottles,potassiumiodate,potassium iodide, hydrochloric acid, sodium thiosulphate,sulphuric acid. Analytical grade reagents were used. Aqueous reagent solutions were prepared using distilled water.

3.1.2 1Instrumentation

Geneys 10S UV-Visible Spectrophotometer (Thermoscientific), 1 cm quartz cuvettes.

3.1.3 Collection of samples

Sampling was purposeful. A total of 12 energy drinks (3 drink per band for 4 brands) were bought from vendors and shops in town of Bindura, Mashonaland Central Province, Zimbabwe. The sellers were asked to select samples of energy beverages that appeared to be most preferable ones to consumers. All of the sampled energy drinks are registered and licensed in Zimbabwe by their respective companies. The caffeine content of each of sample beverages was obtained from label and recorded.

3.1.4 Wavelength selection

1 00ppm caffeine solution was used to determine maximum absorption wavelength. The maximum wavelength of adsorption for caffeine was determined by scanning in the range 200 to 800nm on spectrophotometer.

3.1.5 Preparation of standard solutions

250ml of 100ppm caffeine standard stock solution was prepared using chloroform and serially diluted to make calibration standards with concentration 25, 12.5, 6.25, 3.125, and 1.5625mg/l.A calibration curve was prepared using these standards based on maximum absorption wavelength obtained from scanning.

3.1.5 Treatment of samples and spectrophotometric determination of caffeine content in energy drink samples

All samples were degassed by sonication until no more bubbles no gas were produced.5ml of energy beverage sample were pipetted into a 125ml separatory funnel.10ml of distilled water were added to the sample followed by 1ml of 20% sodium carbonate and 10ml chloroform. The contents of the separatory funnel were vigorously shaken for 60 seconds. The mixture was allowed to stand for 10 minutes and decanted. The procedure was repeated thrice for each sample with the same volume of chloroform.(as shown in fig 1,2 and 3) The organic layer was decanted into a volumetric flask. All samples were treated in the same manner, bulking after each extraction done on a sample. The absorbance of the resulting solution were measured in triplicate using a UV-Vis spectrophotometer at the absorption wavelength obtained from scanning. The percent deviation from the amount on the label was completed.

fig 1

Fig 2

Fig 3

3.1.6 Iodometric of caffeine content in energy drinks (Back titration)

The method used was obtained from Michael et al., (2018) with modifications.

Standardization of sodium thiosulphate

To 10ml 0.1 N potassium iodate solution in conical flask,10ml of 10% potassium iodide solution and 2.5ml ,4.0 N hydrochloric acid were added and the resultant solution was titrated with 0.1N sodium thiosulphate solution to pale-yellow color.3 drops of freshly prepared starch indicator were added and titration was continued until the blue color disappeared. The procedure was repeated thrice.

Determination of caffeine content in energy drinks.

25ml of energy drink, 20ml of standardized iodine solution and 5ml sulphuric acid (10%) were placed in conical flask and swirled to mix. The solution was incubated at room temperature for 15 minutes after which the precipitate was removed by filtration on a whatman No. 1 filter paper. The filtrate was immediately titrated with standard sodium thiosulphate solution to pale-yellow color then 3 drops of freshly prepared starch indicate were added. Titration was continued until the blue color disappear or until the blue color of solution was gone. The

procedure was repeated thrice for each sample. The stoichiometric equations used in calculations are as follows:

$$C_8H_{10}N_4O_2 + 2I_2 + KI + H_2SO_4 \rightarrow C_8H_{10}N_4O_2.HI.I_4 + KHSO_4 \ldots\ldots\text{..Equation 1}$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 3NaI + Na_2S_4O_6 \ldots\ldots\ldots\ldots\text{Equation 2}$$

3.1.7 Date analysis

Date was analyzed using Microsoft Excel and IBM SPSS Version 26.All values were reported as mean ± standard deviation.

CHAPTER 4

RESULTS

4.1 Calibration and spectrophotometry

Figure illustrates the calibration curve obtained using caffeine standards with concentrations 25, 12.5, 6.25, 3.125 and 1.5625 mg/l.

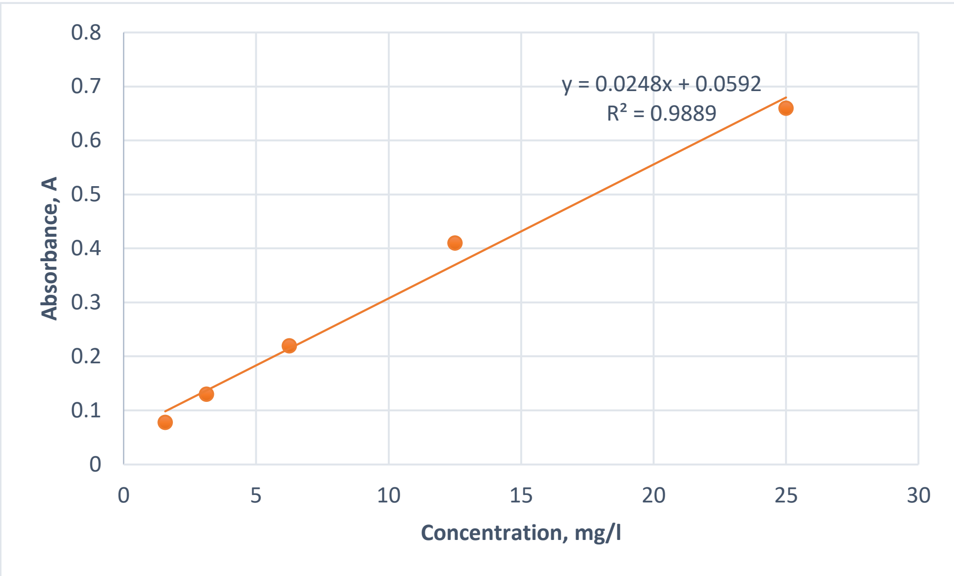

Figure 4: Calibration curve of caffeine standards

Table 1 shows the mean concentrations of caffeine in energy drinks that were under study as determined by spectrophotometry. All values were statistically different at $p \leq 0.05$ across brands. The largest caffeine content was observed in Switch blue (0.38 mg/ml); followed by Dragon (0.37). both are South African products. Sting and Red Bull had 0.35 mg/ml and 0.33 mg/ml caffeine respectively. The former is produced in Austria and imported into South Africa while the latter is a Zimbabwean product.

Table 1: Experimental caffeine content in energy drinks

Energy drink sample	Mean caffeine concentration, mg/l
Dragon	0.37±0.01
Red bull	0.33±0.02
Switch blue	0.38±0.04
Sting	0.35±0.03

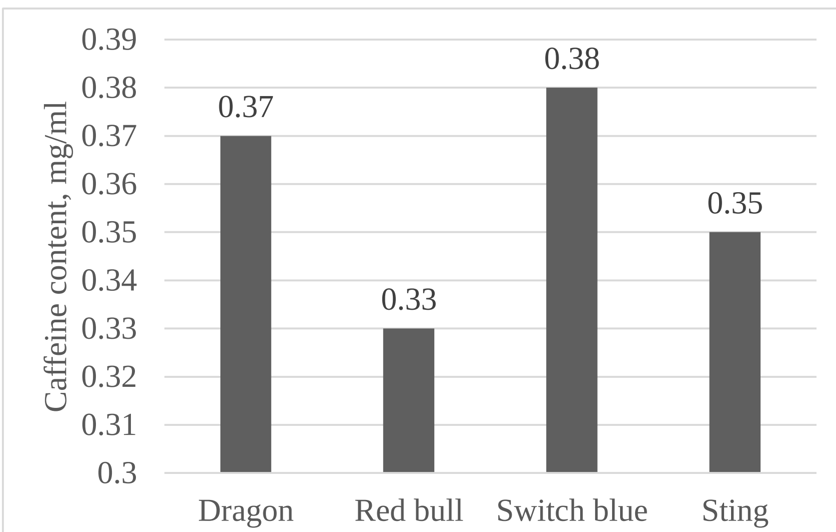

Figure 5: Bar graph of caffeine content in energy drinks

4.2 Iodometric determination of caffeine content in energy drinks

Iodemetry is a simple technique for determination of caffeine. The results obtained using this technique are presented in table 2 and figure 5.

Table 2: Tabulation of iodometric caffeine levels in energy drink

Energy drink sample	Mean caffeine concentration, mg/l
Dragon	0.27±0.03
Red bull	0.28±0.01
Switch blue	0.29±0.02
Sting	0.25±0.04

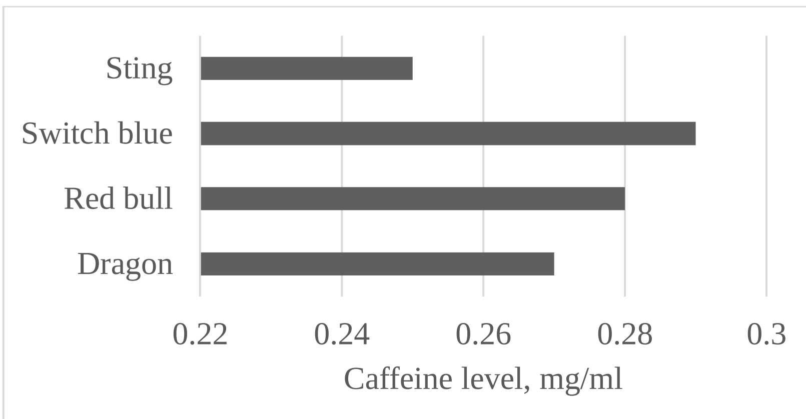

Figure 6: Comparison of iodometric values of caffeine levels in drink samples

4.3 Presentation of caffeine levels obtained from containers the samples

It is a requirement to place the caffeine content on energy drink containers. This section presents and compares caffeine levels obtained from containers per 100 ml, 250 ml and 500 ml serving.

Energy drink samplex	Caffeine content	
	mg/100 ml	mg per serving
Dragon	30.00	150.00*
Red bull	32.00	80.00ʿ
Switch blue	32.00	160.00*

Sting	28.00	140.00*

[s]means serving is 250 ml; *means serving is 500 ml

4.4 Comparison of spectrophotometric, iodometric and labeled caffeine content

Table 3: Comparison standard label and experimental values

Energy drink sample	Caffeine content mg/100ml		
	On container	Spectrophotometric	Iodometric
Dragon	30.00	37±1.00	27±3.00
Red bull	32.00	33±2.00	28±1.00
Switch blue	32.00	38±4.00	29±2.00
Sting	28.00	35±3.00	25±4.00

Table 3 and figure 7 compare label and experimental values of caffeine levels in energy drink samples.

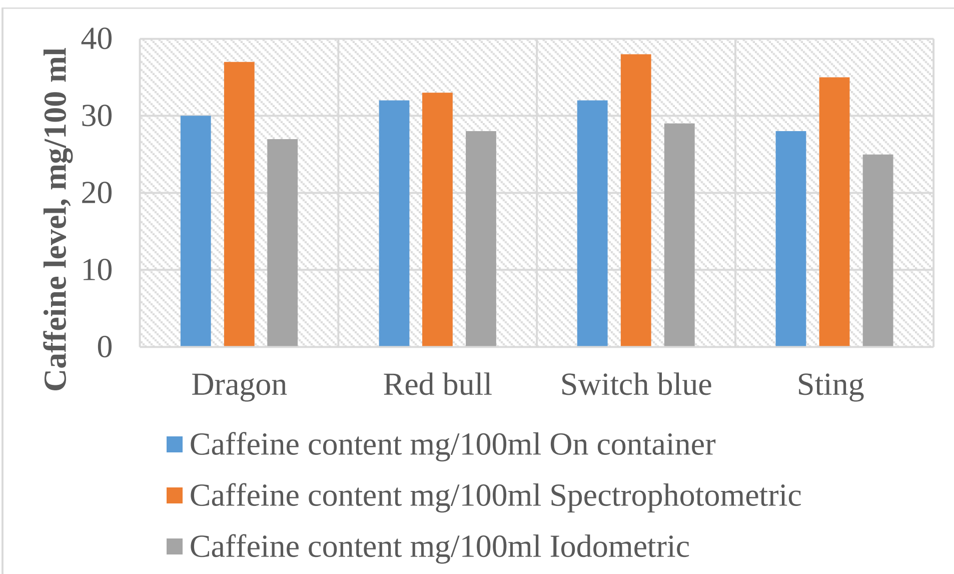

Figure 7: A comparison of label and experimental caffeine levels

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This work analyzed content of caffeine in energy beverages that are sold in Zimbabwe. They are sought-after particularly by youths. University or college students, and bus crews. Two methods were used iodometry and spectrophotometry is widely used owing to its robustness, efficiency in terms of time and excellent precision. The result were obtained in study using spectrophotometry are shown in table 1and figure 2.The maximum wavelength of absorption of caffeine was obtained by scanning standard caffeine at 200 to 400 nm and it was found to 277nm.This was the wavelength which was used in all the analyses. Calibration was done using standards concentrations ranging from 25, 12.5, 6.25, 3.125 and 1.5625mg/g.The curve followed Beer-Lambert law with a correlation coefficient of 0.9889 and the equation was y=0.0248x+ 0.0592(figure1).Acorrelation coefficient that is close toast possible to 1 is indication of excellent correlation between concentration of caffeine in the standards and absorbance such that regression equation can be used to determine concentration accurately.

Energy beverages that were used had labels values of 32, 28 and 30 mg/100ml serving. The estimated caffeine levels using spectrophotometry in Switch, Dragon, Red Bull and Sting were 29±2.00, 27±3.00, 28±1.00 and 25±4.00mg/100ml in respective order while using iodometric titration, values were 29±2.00, 27±3.00, 28±1.00 and 25±4.00mg/100ml respectively. Values obtained in iodometric titration were lower than the labels one, it is likely that presence of other compounds in samples may have caused interference. In the spectrophotometry, interferences were reduced by partitioning caffeine in chloroform. Addition of sodium carbonate ensures caffeine remains deprotonated and is more soluble in organic solvent. Iodometric back titration is very simple and accurate in determining levels of caffeine in sample as there are no complicated required. Under acidic conditions, caffeine reacts with iodine and precipitate. Excess iodine then reacts with standard sodium thiosulphate.From this reaction, amount of caffeine can be determined by stoichiometric calculation.

The comparative results of this study are presented in Figure 4 and table 3.There were significant differences in level of caffeine in all the drinks for both methods except for Red Bull although all values were within close range of values on containers Spectrophotometry results were higher while iodometry results were lower that the label values across all brands.

5.2 Conclusion and recommendations

The focus of the study was to determine caffeine content in four energy drinks using iodometry and spectrophotometry. Both methods produced results that were within expected and acceptable ranges. Iodometry proved to economical, simple and appreciably accurate in determination of caffeine levels in energy drinks. Spectrophotometry had superior sensitivity and precision in quantifying caffeine in energy drinks. This method allowed accurate measurement of caffeine through obtaing maximum wavelength of absorption. The results of both methods were agreeing therefore both methods were valid as reliable techniques of analysis.

There is need to quantify caffeine in a larger sample of energy drink formulations including tea and coffee brands across various geographical areas.Comparisonof said methods with chromatographic techniques is another area which can be explored besides investigating possible effects of other excipients present in drinks on accuracy of analytical methods.

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