Caffeine content analysis in energy drinks biology essay

Food & Diet

Caffeine AnalysisFinal Report

Osamudiamen Okungbowa

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CAFFEINE CONTENT ANALYSIS IN ENERGY DRINKS

INTRODUCTION:

One of the principle components in energy drinks is caffeine. Due to the energy boost that consumers get which is brought on by caffeine, it makes them like the energy drinks and therefore this gives them their popularity – which might also be a result of their rich source of nutrients especially carbohydrates and vitamins, flavour and fashion status. Too much intake of caffeine poses harm to human health thus moderation is recommended. (Sather and Vernig, 2011). Recently there has been an increase in the popularity of caffeine containing energy drinks or functional beverages. The American Dietetic Association therefore takes the position that women of childbearing potential should adopt a health promoting lifestyle. Their recommendation includes the avoidance of ingestion of > 300mg/day of caffeine, citing studies finding increased risk in spontaneous abortion and low birth weight of children born to women consuming more than 150mg/day of caffeine. (Kaiser and Allen, 2002)Determination of caffeine concentrations in foodstuff has become increasingly important in light of recent concern

about its health effects and the widespread consumption by the public. Analytical methods for quantitative determination of caffeine have been developed. These include Kjedahl method, UV spectrophotometry, gas chromatography and High performance liquid chromatography. Research has determined that caffeine concentration varies widely depending on the type of beverage and the manufactures formulation. It has further been proved that it's usually about 100µg/ml in energy drinks to over 1000µg/ml in certain types of coffee. (McCusker, Goldberger and Cone, 2006)Caffeine is absorbed and transported throughout the body by the circulation of blood to a final point within the brain. (Medline Plus 2011). Apart from caffeine being a stimulant and a diuretic, (at least at a substantial dosage), various side effects have been identified due to excessive intake of caffeine. The effects include: nausea, restlessness, vomiting, anxiety, depression, tremors and difficulty in sleeping. As the body does not usually produce caffeine on its own, it is therefore necessary to include in one's diet various nutrients that have caffeine as their by-product. 250mg is the recommended maximum daily dose of caffeine for an average adult. (Medline Plus 2011). Caffeine is normally used as a drug due to its effect on respiratory, cardiovascular and the central nervous system. When caffeine is given in conjunction with headache treatment, it reduces cerebral eye blood flow and in some anti-migraine preparations, when mixed with ergotamine, the object produces a mild positive sense of alertness. (Lawrence 1986). Caffeine can be used as a remedy for mild respiratory depression occurring as a result of the use of depressants like narcotics. Caffeine may also be used in the treatment of acute circulatory failure. In either beverage or in non-prescription tablet

form, it may be used to relieve fatigue - and it increases the amount of urine flow. There are about 3000 drugs that contain caffeine. (Jeanne 1987). Caffeine if consumed in large amounts by lactating mothers, their babies may suffer from irritability and wakefulness. Even though there are studies showing no relation between foetal growth retardation and maternal caffeine consumption, (Grosso et al., 2001) other studies have shown a positive association. One such study found that women whose caffeine intake was 71-140 mg per day had infants weighing 116 g less than those who consumed 0-10 mg per day. (Vlajinac, et al., 1997) Though the literature is inconsistent on foetal growth and spontaneous abortion as it relates to caffeine ingestion, there remains a need for caution when caffeine is consumed by pregnant women and generally by everyone in the society. One study found out that children and adolescents who take caffeine caffeine in high concentrations suffered from headaches induced by the caffeine they consumed. (Hering-Hanit and Gadoth, 2003) It was established that the subjects consumed an average of 192 mg of caffeine per day. When the subjects ceased taking the energy drinks, more than 90% of them experienced a stop in the headaches. Death due to excessive caffeine ingestion is not common. Only a few cases have been reported to have occurred. The acute lethal dose in adult humans has been estimated to be 10 g/person. This varies as death has been reported after ingestion of 6. 5g of caffeine, but survival of a patient who allegedly ingested 24g caffeine has also been reported. (Starvic 1988) People believe that continuous consumption of caffeine of 500 – 600mg every day is abuse of caffeine and potentially hazardous to one’s health. Abuse of caffeine also known as

caffeinism could result to a syndrome characterized by a range of adverse reactions such as restlessness, anxiety, irritability, agitation, muscle tremor, insomnia, headache, diuresis, sensory disturbances (e. g. tinnitus), cardiovascular symptoms (e. g. tachycardia) and gastrointestinal complaints such as nausea, vomiting and diarrhoea. (James & Paull 1985)C: UsersSGT ZEPHIEMusicAudio jobCaff. JPGThe Chemical Structure of Caffeine (Carlifonia State University, Northridge, 2009)Spectrophotometry provides a sensitive method for the detection and measurement of caffeine. The UV absorption spectrum of caffeine exhibits a pair of absorption bands peaking at 205 nm and 273 nm with a characteristic absorption shoulder between them. Typically, caffeine content is determined by measuring the absorbance at 275 nm. Soft drinks contain a wide variety of substances, many of which absorb UV light at 275 nm. Consequently, the direct measurement of the caffeine absorbance in soft drinks is not possible and one must first separate the caffeine from other components before making the absorbance measurement. (Carlifonia State University, Northridge, 2009)

AIM:

To determine the caffeine concentration in Lucozade, Mountain Dew, Pepsi Max and Red Bull energy drinks using High Pressure Liquid Chromatography by injecting caffeine standard then plotting a standard curve using their peak areas and further extrapolating the peak area of the energy drinks to find their caffeine concentration.

OBJECTIVES:

To compare the caffeine content determined against the literature value on the content of the energy drinks. Literature and/or the manufactures have documented the caffeine concentration in each energy drink hence these will be compared directly with the concentrations obtained from the project thus being an eye opener to the consumers who are not aware if what they read or what the manufacturer say is true about the drinks. This will then act as a foundation for educating consumers on the benefits and effects of caffeine to the human body. To determine the maximum allowed amount of each of the four energy drinks that can be consumed in a day by an adult. Based on previous researches that a have shown that 10g per day of caffeine can lead to death in adults and 0. 55g per day amounts to drug abuse, the amount of each drink that contains those amounts of caffeine are determined. To determine the energy drink with the highest amount of caffeine. Once the standard curve has been plotted, the concentration extrapolated for each energy drink will be used to calculate the extractable caffeine which will then be compared directly to come up with the drinks with the highest caffeine concentration. To understand the principle behind the working of High Pressure Liquid Chromatography instrument. By the end of the project the chromatographic explanations of the elution and retention of the sample, composition of the stationary phase and the characteristics of the mobile phase will be well understood.

RESOURCES / MATERIALS AND METHODS:

Material required to carry out this practical investigation are as follows:

Chromatographic column: Reverse phase ODSMobile phase: Methanol:

Water: Acetic acid (40: 59. 5: 0. 5) – approx. 600 mlVacuum degasserPure caffeine from Sigma-Aldrich (UK)4 energy drinks (Pepsi max, Lucozade, Red bull and Burn)10 ml and 100 ml volumetric flasksHPLC machineMeasuring cylinder5ml pipette50 ml beakersAnalytical weighing balanceDistilled waterWhat man No. 1 filter papersVacuum degasserThe vacuum degasser is an elevated-proficiency in-line module that evacuates gases from HPLC solvents. Its special outline guarantees dependable nonstop operation and most abnormal amount of continuous exhibition accessible without the necessity for helium degassing. Inside the unit, the solvents move through a short length Teflon tubing which is spotted in a vacuum chamber. Inside this chamber an incomplete vacuum by an unvaryingly running flat RPM vacuum pump. The dissolved gases then relocate over the tubing divider under a focus inclination processed by the vacuum as the dissolvable rushes inside the curl. Gases removed are dislodged and the chamber is supported at a consistent, pre-set vacuum level by adjusting the vacuum pump speed as required. A special port in the vacuum pump ceaselessly flushes the pump head with a modest drain of air to remove any dissolvable vapours which might drop in the pump from the vacuum chamber. This air drain disposes of the requirement for any solenoid valves inside the framework. HPLC machineThe HPLC machine, it was first turned on; computer, pumps, solvent stirrer, UV detector, RI detector, and fraction collector. The conditions for use were set as shown: Column: Reverse phase ODS 250 mm x 4. 6 mm or 150 mm x 4. 6 mmMobile phase: Mobile phase: Methanol: Water: Acetic acid (40: 59. 5: 0. 5)Flow rate: 0. 8 cm3/minDetection wavelength: 278 nmPressure: 92 kgf/cm3When the framework became stable: the force change ±2% in the

pump, the temperatures stable in the section stove and in the auto sampler, the pattern straight in the identifier and everything was prepared for the examination, the standards and samples were now ready for injection into the section using the injecting needle. The injection process was rapidly so as to avoid band broadening which is characterized by peaks that are not sharp. Preparation of the mobile phaseMethanol, water and acetic acid were mixed in the ratio 40: 59. 5: 0. 5 after which the mixture was filtered using what man filter paper No. 1to remove any minute particles that might find their way into the HPLC machine thus clogging the column. The filter paper was folded into for quarters and placed into a funnel. The filtered mobile phase was then degassed using the degassing equipment and it was then be ready for use. Preparation of stock solution standardCaffeine (0. 1 g) was accurately weighed using the analytical balance and then quantitatively transferred into a 100 ml volumetric flask and made up to the 100ml mark using the mobile phase. This produced 1000ppm caffeine stock solution. Preparation of working standardsUsing appropriate dilutions of the 1000 ppm caffeine stock solution, seven working solutions covering a concentration range 10 – 200 ppm were prepared using the formula C1V1 = C2V2. 20 µl of ach of the working standards was then inject in triplicates to produce the chromatographs. Sample preparation15 ml of the energy drink was poured into a clean beaker, dry beaker. Pouring was continued from one beaker to another back and forth until the bubbling stopped. After the drink had been sufficiently decarbonized, 5 ml of the drink was pipetted into a clean 10 ml volumetric flask and made up to the mark with the mobile phase. Analysis of samples20 µl of each of the four samples was injected into the HPLC to

produce graphs with caffeine peak areas. The peaks with the same retention time as that of the caffeine standards was identified on all the four samples. Using the graphs produced by the standards, a standard graph was then plotted using peak areas against concentration. The peak area from the graph of each sample was then extrapolated to get the concentration of the caffeine in the energy drink. The concentration of caffeine was calculated while considering the dilution factor (x2) and the initial sample volume (5ml) and the results reported in µg/ml. Extractable caffeine concentration =

COLLECTION OF DATA:

Peak AreasCaffeine sampleRun 1Run 2Run 310mg2588837232586521885322 0mg5101756392464043787584 0mg1049006593901701109815460mg1407005915297353152224998 0mg18784595196708232018137710 0mg19578673229778812365101320 0mg3856549241674267410111 94Lucozade15276812141892681352349 5Mountain dew21590318188981881881651 3Pepsi max52067195600420712146 4Red bull31909773414028414467883 5

PRESENTATION OF DATA:

Since the experiment was done in triplicate, the averages of the three runs were calculated to come up with one peak area per standard and per sample for easy calculations. Mean Peak Areas: 10ppm= 2367744. 6720ppm= 4468384. 6740ppm= 10326129. 6760ppm= 14863303. 6780ppm= 19545598. 33100ppm= 22069189200ppm= 40416984. 33Locozade= 14329858. 33Mountain dew energy= 19768339. 67Pepsi Max= 5976201Red Bull= 39330483

Summary of Peak areas of injected standards and samples

Injected SamplePeak Area10 ppm2367744. 6720 ppm4468384. 6740 ppm10326129. 6760 ppm14863303. 6780 ppm19545598. 33100 ppm22069189200 ppm40416984. 33Lucozade14329858. 33Mountain Dew19768339. 67Pepsi Max5976201Red Bull39330483

Extrapolated concentration for Lucozade

Using the equation y = 198933x + 2x106, the exact caffeine concentration in the Lucozade is therefore; 14329858. 33 = 198933x + 2x106198933x = 14329858. 33 - 2x106198933x = 12329858. 33X = 61. 98 ppm

Extrapolated concentration for Mountain Dew

Using the equation y = 198933x + 2x106, the exact caffeine concentration in the Mountain Dew is therefore; 19768339. 67 = 198933x + 2x106198933x = 19768339. 67 - 2x106198933x = 17768339. 67X = 89. 32 ppm

Extrapolated concentration for Pepsi Max

Using the equation y = 198933x + 2x106, the exact caffeine concentration in the Pepsi Max is therefore; 5976201 = 198933x + 2x106198933x = 5976201- 2x106198933x = 3976201X = 19. 89 ppm

Extrapolated concentration for Red Bull

Using the equation y = 198933x + 2x106 the exact caffeine concentration in the Red Bull is therefore; 39330483 = 198933x + 2x106198933x = 39330483 - 2x106198933x = 37330483X = 187. 65 ppm

ANALYSIS AND INTERPRATATION OF DATA:

Calculations for caffeine concentrations

Extractable caffeine concentration = Lucozade

=

= 24. 79µg/mlMountain Dew Energy

=

= 35. 73µg/mlPepsi Max

=

= 7. 96µg/mlRed Bull

=

= 75. 06µg/ml

Calculation of volumes of each energy drink which if consumed can lead to death

Consumption of approximately 10g of caffeine per day per person may cause death of the individual. Each energy drink had a specific volume that if consumed will sum up to 10g of caffeine which was calculated as shown: Lucozade24. 79 µg/ml ☾ 2. 479 x 10-5g/ml= 0. 02479g/lTherefore for one to ingest 10g of caffeine from Lucozade, the volume to be consumed is: 0. 02479g ☾ 1 L10g ☾ ?= 403. 39 LMountain Dew35. 73 µg/ml ☾ 3. 573 x 10-5g/ml= 0. 03573g/lTherefore for one to ingest 10g of caffeine from Mountain Dew, the volume to be consumed is: 0. 03573g ☾ 1 L10g ☾ ?= 279. 88LPepsi Max7. 96µg/ml ☾ 7. 96 x 10-6g/ml= 7. 96 x 10-3g/lTherefore for one to ingest 10g of caffeine from Pepsi Max, the volume to be consumed is:

7. 96 x 10-3g ☾ 1 litre10g ☾ ?= 1256. 28 LRed Bull75. 06 µg/ml ☾ 7. 506 x 10-5g/ml= 0. 07506g/lTherefore for one to ingest 10g of caffeine from Red Bull, the volume to be consumed is: 0. 07506g ☾ 1 L10g ☾ ?= 133. 23 L

Calculations of volumes of each energy drink which if consumed amounts to abuse

Consumption of approximately 0. 5 - 0. 6g of caffeine per day per person amounts to abuse by individual. Each energy drink had a specific volume that if consumed will sum up to approximately 0. 55g of caffeine which was calculated as shown: Lucozade24. 79 µg/ml ☾ 2. 479 x 10-5g/ml= 0. 02479g/lTherefore for one to ingest 0. 55g of caffeine from Lucozade, the volume to be consumed is: 0. 02479g ☾ 1 L0. 55g ☾ ?= 22. 19 LMountain Dew Energy35. 73 µg/ml ☾ 3. 573 x 10-5g/ml= 0. 03573g/lTherefore for one to ingest 0. 55g of caffeine from Mountain Dew, the volume to be consumed is: 0. 03573g ☾ 1 L0. 55g ☾ ?= 15. 39 LPepsi Max7. 96µg/ml ☾ 7. 96 x 10-6g/ml= 7. 96 x 10-3g/lTherefore for one to ingest 0. 55g of caffeine from Pepsi Max, the volume to be consumed is: 7. 96 x 10-3g ☾ 1 litre0. 55g ☾ = 69. 10 LRed Bull75. 06 µg/ml ☾ 7. 506 x 10-5g/ml= 0. 07506g/lTherefore for one to ingest 0. 55g of caffeine from Red Bull, the volume to be consumed is: 0. 07506g ☾ 1 L0. 55g ☾ ?= 7. 33 LFrom the graph and calculations done it was observed that all the energy drinks had some amount of caffeine in them though in different quantities. It was also evident that Red bull had the highest concentration of caffeine at 75. 06µg/ml followed by Mountain dew at 35. 73µg/ml, then Lucozade at 24. 79µg/ml and finally Pepsi max with the least amount of caffeine at 7. 69µg/ml. These results obtained were also used to calculate the amount of each drink that if consumed can pass the

10g caffeine amount which might cause death and the 0. 55g caffeine content that is categorized as drug abuse according to previous researches done.(James and Paull 1985). It was also observed that the concentration of caffeine in the energy drinks was below the Food and Drug Administration maximum set limit. From the chromatograms it was observed that most of the graphs had more than one peak but only one was conspicuously larger than the rest. It was also noted from the chromatograms for the four energy drinks each had two large peaks and other small ones which were also more pronounced than those seen in the standard curves. From the chromatograms it was also observed that the all the peaks were sharp and there was no band broadening or flat peaks. It was also obsereved that HPLC was an effective method for seperation of caffine from other components in energy drinks.

SOURCES OF ERROR:

In every scientific experiment there must be some possible sources of error which can be categorized as either human error or limitations of the equipment used for the lab work. Some of the special substances from the samples might have been strongly absorbed in and on the stationary phase. This might have then lead to changing the overall chromatography behaviour of the HPLC column. Incomplete separation and overlapping of peaks also influenced the project because of insufficient separation power. Working outside the detector, working range also influenced the project negatively as some of the constituents of the energy drinks might have not been detected. Many HPLC columns can only separate 50 substances in one run; it will therefore be impossible to analyse for caffeine content in an

energy drink with more than 50 compounds in it. Errors in the preparation of standard solutions might also influence the project since the end results obtained will not the accurate concentration of caffeine on the energy drinks.

CONCLUSION AND DISCUSSION:

From the project carried out to determine the caffeine content of four energy drinks in the UK market, it can be conclude that all the energy drinks analysed had some amount of caffeine in them though in different concentrations due to differences in formulation of the drinks by different manufacturers. It can also be conclude that for one to die due to the effects of consuming too much caffeine (10g per day) the individual has to consume either 380. 37 L of Lucozade or 270. 12 L of Mountain dew or 1018. 33 L of Pepsi max or 132. 28 L of Red bull. All these are practically impossible therefore; the energy drinks are generally good for health. For one to be said to have abused caffeine present in these energy drinks by intake of approximately 0. 55g of caffeine per day, they have to consume 20 L of Lucozade or 14. 86 L of Mountain dew or 56 L of Pepsi max or 7. 28 L of Red bull. The chromatograms for the energy drinks had many peaks as compared to those of standards since the standards contained purely caffeine while the energy drinks contained other compounds which were also eluted although at different time and at different quantities. All the peaks were sharp hence it can be conclude that the sensitivity of the HPLC equipment was well set and the injection process was done rapidly thus producing perfect peaks. The Food and Drug Administration (FDA) has included caffeine in its list of substances that are generally recognized as safe and has set the maximum concentration of caffeine in energy drinks at 65mg of caffeine per 360ml

which is equivalent to 1805. 56µg/ml. it can therefore be concluded that all the caffeine content of all the four energy drinks analysed were below the maximum allowable limits of caffeine concentrations as specified by FDA. It can also be concluded that HPLC is an effective separation technique that involves injection of a small volume of liquid sample into a tube packed with tiny particles called the stationary phase, where individual components of the sample are moved down the column with a liquid – mobile phase – forced through the column by pressure delivered by the pump. The components were therefore separated from one another by the column packaging that involved various chemical and/or physical interactions between their molecules and the packing material. The separated components were then detected at the exit of the column by a flow through device –detector – that measured their amount to produce liquid chromatograms. A general conclusion therefore is that the research project achieved its objectives and established that the amount of caffeine in the energy drinks analysed was so little to cause any harm to human beings under normal circumstance. I can therefore recommend that even though the caffeine concentrations in the analysed energy drinks were very minimal, care must still be taken when consuming them since the caffeine limits stated were done on adults. Care should also be taken not to give too much of these drinks to children as the levels might be high on them thus interfering with their health. Expectant and lactating mothers should also avoid energy drinks since the caffeine can find its way to the unborn child or to the lactating baby thus also impacting negatively to their health.

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