Caffeine content of Energy Drinks

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DATE

July 2011

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1. Pollution Monitoring Laboratory of CSE

The Centre for Science and Environment (CSE), a non-governmental organization based in New Delhi, has set up the Pollution Monitoring Laboratory (PML) to monitor environmental pollution. PML is an ISO 9001:2008 certified laboratory accredited by SWISO, CH-5610, Wohlen, Switzerland, conducting Pollution Monitoring and Scientific Studies on Environmental Samples. The Lab has highly qualified and experienced staff that exercise Analytical Quality Control (AQC) and meticulously follow what is called Good Laboratory Practices (GLP). It is equipped with most sophisticated state-of-the-art equipments for monitoring and analysis of air, water and food contamination, including Gas Chromatograph with Mass Detector (GC-MS), Gas Chromatograph (GC) with ECD, NPD, FID and other detectors, High Performance Liquid Chromatograph (HPLC), Atomic Absorption Spectrometer (AAS), UV-VIS Spectrophotometer, Mercury Analyzer, Respirable Dust Sampler etc. Its main aim is to undertake scientific studies to generate public awareness about food, water and air contamination. It provides scientific services at nominal cost to communities that cannot obtain scientific evidence against polluters in their area. This is an effort to use science to achieve ecological security.

2. Introduction

Caffeine, a representative of the group of three methylxanthine compounds, acts as a stimulant on the central nervous system. Energy drinks are non-alcoholic beverages containing caffeine, guarana, glucuronolactone, taurine, ginseng, inositol, carnitine, B-vitamins etc. as main ingredients that act as stimulants. In recent years, a number of different energy drinks have been introduced in the Indian market to provide an energy boost or as dietary supplements. These drinks contain high levels of caffeine which stimulates the nervous system. Energy drinks are heavily marketed to young adults and others and manufacturers compare the effects of the drinks to the use of drugs like cocaine. Many of these drinks are heavily promoted in bars or for use in combination with alcohol, which could further increase the health risk to consumers. There are a number of scientific reports on the adverse consequences of excessive consumption of caffeine. The main sources of caffeine are tea, coffee and soft drinks. In energy drinks, caffeine is added at levels of up to 80 mg per serve. The drinks usually have a number of added water soluble vitamins such as, niacin, pantothenic acid, vitamin B6 and vitamin B12 and other substances, such as amino acids. There are, at present, no standards for caffeine in energy drinks. The present study was undertaken to determine the caffeine content of energy drinks.

3. Caffeine

Caffeine ($C_8H_{10}N_4O_2$) is the common name for trimethylxanthine (systematic name is 1,3,7-trimethylxanthine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione) and is an alkaloid that occurs naturally in the leaves, seeds and fruits of tea coffee, cacao, kola trees an many other plants(Reid, 2005). The chemical is also known as coffeine, theine, mateine, guaranine, or methyltheobromine. The molecule was first isolated by the German chemist Friedrich Ferdinand Runge in 1819. When purified, caffeine is an intensely bitter white powder. It is added to colas and other soft drinks to impart a pleasing bitter note. However, caffeine is also an addictive stimulant. When ingested, it is a stimulant of the central nervous system and can temporarily increase blood pressure and heart rate(Hartley *et al*, 2004). A normal dose of caffeine is generally considered to be 100 mg, which is roughly the amount found in a cup of coffee. Caffeine is quickly and completely removed from the brain. Its effects are short-lived and it tends not to negatively affect concentration or higher brain functions. However, continued exposure to caffeine leads to developing a tolerance to it. Too much caffeine can result in caffeine intoxication, which is characterized by nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations(Leson, 1988)

4. Regulations for Caffeine in Energy drinks

The regulation of beverages to which caffeine is added has been challenging, partly because of the widespread and long-term use of beverages such as coffee and tea in which caffeine is a natural constituent. Nonetheless, several countries have enacted measures to regulate the labeling, distribution, and sale of energy drinks that contain significant quantities of caffeine. US FDA regulates caffeine content in soft drinks but does not regulate caffeine contained in energy drinks. Caffeine is considered ‘generally regarded as safe’ under US Code of Federal Regulations.The European Food Safety Authority mandates that energy drinks with over 150 parts per million (ppm) or 150 mg per litre caffeine content should be labeled as ones with “high caffeine content” and the exact amount should be indicated; Australia has banned energy drinks with over 320 ppm caffeine level and proposes to classify them as pharma products. Countries like Denmark, Uruguay and Turkey have banned energy drinks altogether while Sweden has banned its sale among children The health implications of caffeine have also been enquired into by several countries.

In India, there are no standards for energy drinks under PFA Act, 1954. The standards of carbonated water under PFA Rules, 1955 specify the maximum limits of caffeine of 200ppm which subsequently on recommendations by Central Committee on Food Standards were

reduced to maximum level of 145ppm and notified vide notification GSR 431(E) dated 19.06.2009.

5. Health impacts

Caffeine is a stimulant, which has different health effects concerning stimulation of the central nervous system and can produce restlessness, headaches, and irritability. Large amount of caffeine consumption can cause physiological and psychiatrical dependence. Research has shown links between heavy caffeine consumption and osteoporosis, high blood pressure, heart disease, heart burn, ulcers, severe insomnia and infertility. Caffeine has diuretic properties when administered in sufficient doses to subjects who do not have a tolerance for it.The diuretic effect of coffee causes excretion of fluid through the kidneys, which may lead to dehydration (Maughan & Griffin, 2003). Pregnant women who consume high amounts of caffeine have increased risk of miscarriage, difficult birth and delivery of low-weight babies (Weng *et al*, 2008). The major behavioral problems associated with heavy caffeine use are anxiety and depression. Over the long-term as the body gets used to caffeine, it requires higher amounts to get the same effects.Common features of caffeine intoxication include nervousness, anxiety, restlessness, insomnia, gastrointestinal upset, tremors, tachycardia, psychomotor agitation (American Psychiatric Association, 1994) and in rare cases, death (Kerrigan and Lindsey, 2005).

6. Objective of the Study

In view of the importance of Caffeine in energy drinks, the Caffeine content of Energy Drinks by HPLC-DAD

7. Materials and Methods

Sampling

16 Energy drink samples - 2 samples each of 8 brands –XXX(Rejuve , Nicofix, Minus), Burn, Redbull, Cloud 9 Monster, Tzinga were randomly purchased from a local market in June 2011(Annexure 1). All samples were analysed for caffeine and kept sealed in the refrigerator until analysed.

Caffeine content of Energy Drinks

Chemicals

Caffeine (1,3,7-trimethylmethylxanthine, anhydrous) – purity (>99%) was obtained from Fluka Chemie GmbH (Buchs, Switzerland). Acetonitrile (HPLC grade) was purchased from Waligens (Mumbai, India) and water (HPLC grade) was obtained from Merck Limited (Mumbai, India).

Equipment

High Performance Liquid Chromatograph of Agilent 1100 series equiped with Diode Array Detector and HP Chemstation software, Zorbax ODS, 4.6mmID x 250mm (5µm) column and 50µL Hamilton syringe.

Standard preparation:

1000 µg/mL (ppm) stock solution of caffeine standard was prepared by dissolving solid caffeine in HPLC grade water. Working solutions ranging from 1-500ppm were prepared from the stock solution of caffeine and filtered through a 0.45µm syringe filter.

Sample preparation and analysis

The determination of caffeine was performed by an Agilent 1100 series high performance liquid chromatograph equipped with DAD detector and HP chemstation software following AOAC official method 979.08 for the simultaneous determination of benzoate, caffeine and saccharin in soda beverage.The method after validation was applied to quantify samples (16) of 8 different popular energy drinks XXX(Rejuve , Nicofix, Minus), Burn, Redbull, Cloud 9 ,Monster and Tzinga. Mobile phase used was acetonitrile/water mixure, 20/80 (v/v) with a flow rate of 1ml/min. Retention time of Caffeine was 3.6 minutes (Figure 1)

Approximately 100mL of sample of carbonated beverage were degassed in ultrasonic bath and filtered through a 0.45µm syringe filter. There was no further clean up procedure.20µL of filtered sample were injected directly into HPLC. Every sample was injected in triplicate (n=3). The retention times of the peaks for the sample were compared with the retention time of the reference standard and confirmed when the retention time of the sample was identical to those of the pure standards.

8. Result and Discussion

The standard curve was linear between 1- 500 µg/ml (ppm) and the detection limit was 0.05 µg/ml. The mean correlation coefficient was 0.999±0.0004 (n=5). Results for caffeine analyzed in triplicate for two different samples of 8 brands of energy drinks have been summarized in ppm (Annexure 2). Caffeine was detected in the range from 112.23- 314.45 ppm.Chromatograms from two typical products (one with a lower caffeine level and one with a higher caffeine level) are shown in Figure 2 and 3. The mean amount of caffeine in energy drinks was XXX (Rejuve) - 117.14 ppm, XXX (Nicofix) -121.71 ppm, Redbull - 310.08 ppm, Burn -291.73ppm, XXX (Minus)- 153.30ppm, Cloud 9 - 142.25 ppm, Monster - Ripper - 314.09 ppm and Tzinga was 258.37ppm. Caffeine was also detected in samples which did not mention on the label that it had caffeine. 38% of the samples breached the limit mentioned on the label by up to 24%. 38% of the samples were within the limit mentioned on the label. 25% of the samples i. e 4 out of 12 samples did not mention the caffeine content on the label. 44% of the samples breached the limit set by Central Committee on Food Standards limit of 145 ppm.

9. Conclusions

The results showed that the caffeine levels in most brands exceeded 145 ppm. Only two brands—XXX Rejuve and XXX Nicofix— stuck to the 145 ppm limit XXX Minus and Cloud 9 flouted labeling rules. The former did not mention the caffeine levels and the latter did not mention that it had caffeine. The tests showed they had 153.30 ppm and 142.25 ppm caffeine, respectively. The warning that lactating mothers should not consume energy drinks is missing from Cloud 9, Burn and XXX Minus labels. The label on XXX Minus was not legible. Though caffeine levels in Tzinga and Red Bull vary, both claim to have caffeine content equivalent to that in an average cup of coffee—80 mg (320 ppm) in a 250 ml can.

10. References

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Caffeine content of Energy Drinks

Annexure 1 Details of the energy drink samples purchased from Delhi and analysed for Caffeine

S.No.	Na me	Manufacturer	Date of manufacture	Expiry date	Batch Number	Cost	Other Information
1	XXX-2(Rejuve)	Union Beverages factory, P. O.Box 804, Jebel Ali Industrial Area 2, Dubai, UAE	05.12.2009	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.		75	Made in Dubai, Marketed by GT& T India, JMJ House Orchard Avenue Hirandani Gardens, Powai, Mumbai-400076
2	XXX-1(Rejuve)	Union Beverages factory, P. O.Box 804, Jebel Ali Industrial Area 2, Dubai, UAE	27.04.2010	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	3	75	Made in Dubai, Marketed by GT& T India, JMJ House Orchard Avenue Hirandani Gardens, Powai, Mumbai-400077
3	XXX-1(Nicofix)	Union Beverages factory, P. O.Box 804, Jebel Ali Industrial Area 2, Dubai, UAE	12.12.2009	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	Not given	75	Made in Dubai , Marketed by Goa technology and trade Pvt Limited India, JMU House Orchard Avenue Hirandani Gardens, Powai, Mumbai-400076
4	XXX-2(Nicofix)	Union Beverages factory, P. O.Box 804, Jebel Ali Industrial Area 2, Dubai, UAE	08.09.2010	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	108101	75	Made in Dubai , Marketed by Goa technology and trade Pvt Limited India, JMU House Orchard Avenue Hirandani Gardens, Powai, Mumbai-400077
5	Red Bull 1	Rauche Fruchtsafte GmbH &Co OG, Kuhbruckweg 2, 6714 Nuziders by Red Bull Asia FZE Dubai Airport free Zone, UAE	6.10.2010 19:02	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	1168989C3	75/-	Product of Austria , Imported for India by Red Bull India Pvt Ltd, Kakad Chambers Annex, 132 Annie Besant Rd, Worli , Mumbai, 40018
6	Red Bull 2	Rauche Fruchtsafte GmbH &Co OG, Kuhbruckweg 2, 6714 Nuziders by Red Bull Asia FZE Dubai Airport free Zone, UAE	6.10.2010 10:02	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	1168989C3	75/-	Product of Austria , Imported for India by Red Bull India Pvt Ltd, Kakad Chambers Annex, 132 Annie Besant Rd, Worli , Mumbai, 40019
7	Burn	Kian Joo Canpack DDN BHD, 107, Jalan Permata 1, Kawasan Industri Arab Malaysian , 71800 Nilai , Negeri Sembilan , Malaysia	26.10.2010 14:37	Best before9 months from manufacture when stored in cool and dry place away from direct sunlight.	BN 01	75/-	Made in Malaysia Imported by Hindustan Coca Cola Beverages Pvt Ltd, 13, Abul Fazal Road Bengali Market, New Delhi 110001
8	Burn	Kian Joo Canpack DDN BHD, 107, Jalan Permata 1, Kawasan Industri Arab Malaysian , 71800 Nilai , Negeri Sembilan , Malaysia	28.02.2011 10:02	Best before 9months from manufacture when stored in cool and dry place away from direct sunlight.	BN 01	75/-	Made in Malaysia Imported by Hindustan Coca Cola Beverages Pvt Ltd, 13, Abul Fazal Road Bengali Market, New Delhi 110001

Caffeine content of Energy Drinks

S.No.	Name	Manufacturer	Date of manufacture	Expiry date	Batch Number	Cost	Other Information
9	XXX-1(Minus)	Pushpam Food and beverages Pvt Ltd Gat No 104/2A, Barote Vasti (Nandur) Tal Daund, District Pune 412202	27.11.2010 5:18	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	MO910	100/-	Made in India , Market ed by XXX Energy drinks Pvt Ltd JMJ House , Orchard Avenue, Hirandani Gardens, Powai, Mumbai-400076by
10	XXX-2(Minus)	Pushpam Food and beverages Pvt Ltd Gat No 104/2A, Barote Vasti (Nandur) Tal Daund, District Pune 412202	27.11.2010 5:53	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	MO810	100/-	Made in India , Market ed by XXX Energy drinks Pvt Ltd JMJ House , Orchard Avenue, Hirandani Gardens, Powai, Mumbai-400076by
11	Cloud 9(Wild Berry)	Pushpam Food and beverages Pvt Ltd Gat No 104/2A, Barote Vasti (Nandur) Tal Daund, District Pune 412202	21.02.2010	Best before 18 months from manufacture when stored in cool and dry place away from direct sunlight.	W0710	75/-	Made in indiaGoldwin Health Care Pvt Ltd.ISO 9001 &140001:2004 Certified company 7, Zaver arcade , Zaver road Mulund (W) Mumbai-400080
12	Cloud 9(Premium)	Pushpam Food and beverages Pvt Ltd Gat No 104/2A, Barote Vasti (Nandur) Tal Daund, District Pune 412202	23.02.2010	Best before 18 months from manufacture when stored in cool and dry place away from direct sunlight.	A1610	75/-	Made in indiaGoldwin Health Care Pvt Ltd.ISO 9001 &140001:2004 Certified company 7, Zaver arcade , Zaver road Mulund (W) Mumbai-400081
13	Monster(Ripper)	Monster Energy Limited South Bank House Barrow street, Dublin 4, Ireland	10:00	Best before end 04.2012 when stored in cool and dry place away from direct sunlight.	D10060		Manufactured in EU
14	Monster(Ripper)	Monster Energy Limited South Bank House Barrow street, Dublin 4, Ireland	12:47	Best before end 04.2012 when stored in cool and dry place away from direct sunlight.	D10060		Manufactured in EU
15	Tzinga	Hector Beverages Pvt Ltd 132, sector 3 Manesar , Gurgaon 122050 Haryana	13.04.2011	Best before 3 months from manufacture when stored in cool and dry place away from direct sunlight.	B2823	20/-	
16	Tzinga	Hector Beverages Pvt Ltd 132, sector 3 Manesar , Gurgaon 122050 Haryana	30.04.2011	Best before 3 months from manufacture when stored in cool and dry place away from direct sunlight.	BN-2816	20/-	

Caffeine content of Energy Drinks

Annexure 2 Caffeine Content of Energy drink samples

S. No.	Brand Name	Caffeine Concentration detected * (ppm)	Concentration on the label (ppm)	Percent Deviation from the claim on the label	No of times above PFA standard of 145ppm
1	XXX-1(Rejuve)	112.23	100	12.23	within limit
2	XXX-2(Rejuve)	122.05	100	22.05	within limit
3	XXX-1(Nicofix)	119.48	100	19.48	within limit
4	XXX-2(Nicofix)	123.94	100	23.94	within limit
5	Red Bull 1	308.80	320	within limit	2.1
6	Red Bull 2	311.35	320	within limit	2.1
7	Burn-1	294.34	300	within limit	2.0
8	Burn-2	289.12	300	within limit	within limit
9	XXX-1(Minus)	153.69	not given	--	within limit
10	XXX-2(Minus)	152.90	not given	--	within limit
11	Cloud 9(Wild Berry)	148.05	not given	--	within limit
12	Cloud 9(Premium)	136.44	not given	--	within limit
13	Monster-1(Ripper)	314.45	300	14.45	2.2
14	Monster-2(Ripper)	313.72	300	13.72	2.2
15	Tzinga-1	259.68	300	within limit	1.8
16	Tzinga-2	257.05	300	within limit	1.8

*Average of triplicate run

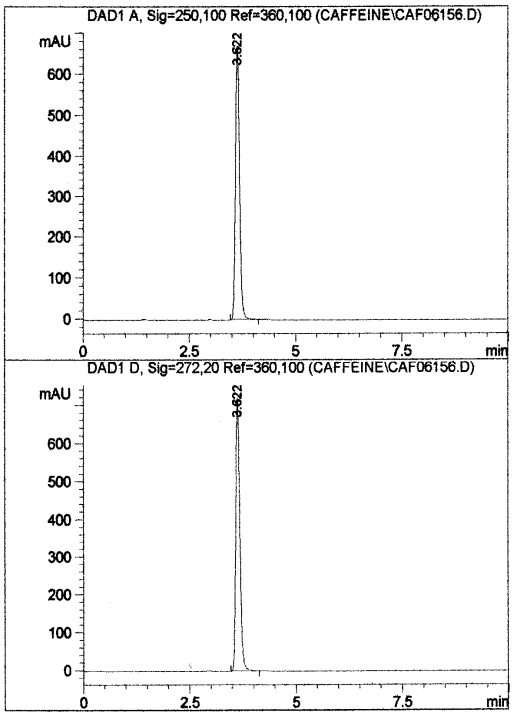

Figure 1: HPLC Chromatogram of Caffeine Standard(100ppm)

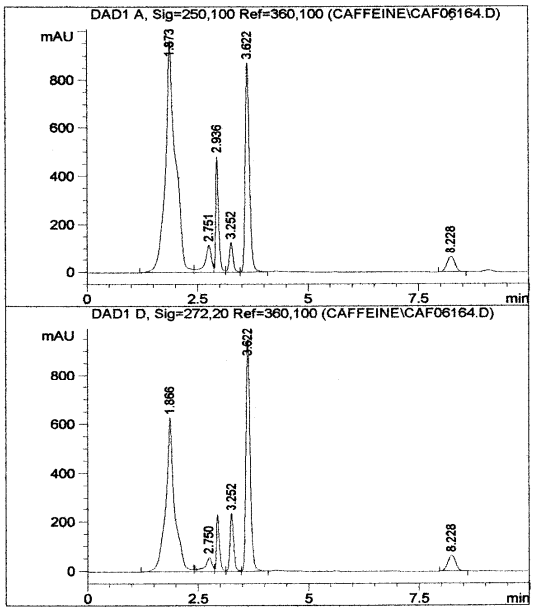

Figure 2: HPLC Chromatogram of Sample 03 with low level of Caffeine, retention time 3.62 min)

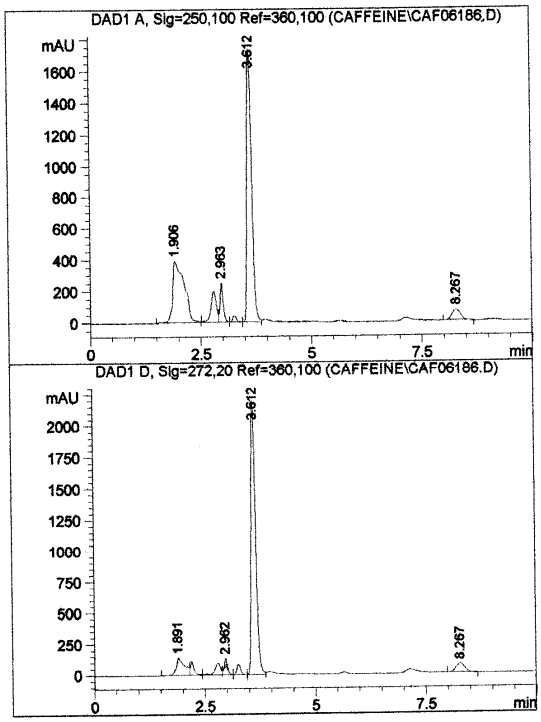

Figure 3: HPLC Chromatogram of Sample 05 with higher level of Caffeine, retention time 3.62 min)