# 1. 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, pathothenic acid, vitamin B6 and vitamin B12 and other substances, such as amino acids. There are, at present, no Codex Standards for soft drinks or nonalcoholic carbonated beverages. Several countries have approved energy drinks as dietary supplements. 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. The present study was undertaken to find out the caffeine content in energy drinks.

# 2. Objective of the study:

Caffeine content in major Energy drink samples

# 3. Materials and methods:

#### Sampling

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

# 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 (5 $\mu$ m) column and 50 $\mu$ L 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.

#### Calibration:

A linear response was obtained over the range of 1ppm-500ppm standard solution by injecting  $20\mu L$  of each standard. Regression analysis of the data indicated linearity,  $R^2$  =0.9996. The quantitative amount of caffeine in samples was determined using this curve.

# Sample preparation and analysis

The determination of caffeine was performed at an Agilent 1100 series high performance liquid chromatograph equiped with DAD detector and HP chemstation software following AOAC official method 979.08 for the simultaneous determination of benzoate, caffeine and saccharin in soda beverages with some modifications. The wavelength was set at 272nm. Mobile phase used was acetonitrile/water mixure, 20/80 (v/v) with a flow rate of 1ml/min.

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.

# 4. Result and Discussion:

S. No.	Sample Name	Caffeine Concentration detected * (ppm)	Concentration on the label (ppm)	Percent Deviation from the claim on the label	PFA Standards for carbonated beverages 145ppm (no. of time above)
1	XXX-1(Rejuve)	112.23	100.00	12.23	Within Limit
2	XXX-2(Rejuve)	122.05	100.00	22.05	Within Limit
3	XXX-1(Nicofix)	119.48	100.00	19.48	Within Limit
4	XXX-2(Nicofix)	123.94	100.00	23.94	Within Limit
5	Red Bull 1	308.80	320.00	Within Limit	2.1
6	Red Bull 2	311.35	320.00	Within Limit	2.1
7	Burn	294.34	300.00	Within Limit	2.0
8	Burn	289.12	300.00	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	300.00	14.45	2.2
14	Monster-2(Ripper)	313.72	300.00	13.72	2.2
15	Tzinga-1	259.68	300.00	Within Limit	1.8
16	Zinga-2	257.05	300.00	Within Limit	1.8

# \*Average of triplicate run

In all the 16 out of 16 i.e 100% samples tested, caffeine was detected.

38% of the samples breached the limit mentioned on the label by upto 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 is 145ppm** 

Caffeine is a stimulant, which has different health effects concerning stimulation of the central nervous system. Large amount of caffeine consumption can cause physiologically and psychiatrically dependence. There are many effects from the consumption of caffeine including a raised blood pressure, incidence of bladder and stomach cancer and increased heart rate. In 1981, the U.S. FDA warned that pregnant women should avoid the consumption of caffeine containing food and drugs.

Table 2. Details of the energy drink samples purchased from Delhi and analysed for Caffeine

S.N o.	Name	Manufacturer	Date of manufact ure	Expiry date	Batch Numbe r	Cost	Other Information
1	XXX- 2(Rejuve)	Union Beverages factory, P. O.Box 804, Jebel Ali Industrial Area 2, Dubai, UAE	05.12.200 9	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	27.04.201	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	12.12.200 9	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	08.09.201	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.	116898 9C3	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	6.10.2010 10:02	Best before 24 months from manufacture when stored in cool and dry place away from direct sunlight.	116898 9C3	75/-	Product of Austria , Imported for India by Red Bull India Pvt Ltd, Kakad Chambers Annex, 132 Annie Besant Rd, Worli , Mumbai, 40019

		free Zone, UAE					
7	Burn	Kian Joo Canpack DDN BHD, 107, Jalan Permata 1, Kawasan Industri Arab Malaysian , 71800 Nilai , Negeri Sembilan , Malaysia	26.10.201 0 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	28.02.201 1 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
S.N o.	Name	Manufacturer	Date of manufact ure	Expiry date	Batch Numbe r	Cost	Other Information
9	XXX-1(Minus)	Pushpam Food and	27.11.201	Best before 24 months from	MO910	100/-	Made in India , Market ed by
		beverages Pvt Ltd Gat No 104/2A, Barote Vasti (Nandur) Tal Daund, District Pune 412202	0 5:18	manufacture when stored in cool and dry place away from direct sunlight.			XXX Energy drinks Pvt Ltd JMJ House , Orchard Avenue, Hirandani Gardens, Powai, Mumbai-400076by
10	XXX-2(Minus)  Cloud 9(Wild	No 104/2A, Barote Vasti (Nandur) Tal Daund,	•	cool and dry place away from	MO810	100/-	House , Orchard Avenue, Hirandani Gardens, Powai,

12	Cloud 9(Premium)	Pushpam Food and beverages Pvt Ltd Gat No 104/2A, Barote Vasti (Nandur) Tal Daund, District Pune 412202	23.02.201	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(Ripp er)	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(Ripp er)	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	13.04.201 1	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	30.04.201 1	Best before 3 months from manufacture when stored in cool and dry place away from direct sunlight.	BN- 2816	20/-	