International Research Journal of Pure & Applied Chemistry

16(3): 1-7, 2018; Article no.IRJPAC.41441
ISSN: 2231-3443, NLM ID: 101647669

Determination and Comparison of Aspartame Levels in Some Selected Soft Drinks Consumed in Mubi, Adamawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SDY and JB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SDY and JB managed the analyses of the study. Author SDY managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2018/41441
Editor(s):
(1) Richard Sawadogo, Group Cell Death & Natural Compounds, Laboratoire de Biologie Moléculaire et -Cellulaire du Cancer Hôpital Kirchberg, Luxembourg.
Reviewers:
(1) Alok Nahata, Malaysia.
(2) Xue Xiao, Guangdong Pharmaceutical University, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/24917

Short Research Article

Received 3rd March 2018
Accepted 9th May 2018
Published 1st June 2018

ABSTRACT

Aspartame (ASP) is an Artificial Sweetener used to substitute sugar which was said to cause some health disorders. It is about 200 times sweeter than sugar, with almost the same calorie value of 17 KJ per gram; a smaller quantity then, provides the same sweetness as sugar, consequently providing lesser calorie and thereby reducing the health disorders associated with sugar intake. However, some health disorders have been said to associate with high intake of aspartame, and especially PKUs who must completely avoid sources of Phenylalanine in their diet. This research has therefore investigated the presence and concentrations of Aspartame in five soft drink samples consumed in Mubi, Adamawa State. Ninhydrin test was used for the Qualitative analysis and High Performance Liquid Chromatography (HPLC) was used for the Quantitative analysis and the results were found to be 438.45±0.03 mg/L in Zero Coke (ZRC), 365.81±0.02 mg/L in Pepsi Lite (PSL), 525.36±0.02 mg/L in Origin Zero (ORZ), 337.06±0.01 mg/L in Sans Cream Soda (SNW) and 306.13±0.02 mg/L in Malta Guinness Herbs Lite (MHL); none of these violated the standard of 3000 mg/L set by NAFDAC. PKUs, therefore, are to avoid these drinks and other consumers should not exceed the ADI recommendations.

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Keywords: Aspartame; soft drink; phenylketonuria; Mubi.

1. INTRODUCTION

Food additives are substances added with or without consent to foods; these, as a result, may be expected to directly or indirectly become components or goes to affect the characteristics of any food. These substances may be used in production; processing, treatment or any phase of food utilization such as packaging, transportation or storage [1,2] to improve and sustain some desirable qualities of the food such as colour, taste, flavour etc. Additives are used in foods to maintain product consistency, to improve or maintain nutritional value, to maintain palatability and wholesomeness, to provide leavening or control acidity/alkalinity and to enhance flavour or impart desired colour [3,4,5]. Soft drinks typically contain carbonated water, a sweetener, and a natural or artificial flavouring. The sweetener may be sugar, high-fructose corn syrup, fruit juice, sugar substitutes (in the case of diet drinks), or some combination of these. All additives in foods are regulated, evaluated and approved for consumption by food regulating agencies such as FDA, EU – SCF, JECFA, NAFDAC and EFSA in different countries.

Aspartame, (also called NutraSweet, Equal, E951, and Equal – measure) a low calorie artificial sweetener like other protein substances has a caloric value of 17 KJ per gram. It has the chemical name, 3-Amino-N-(alpha-carbomethoxy-phenethyl)-succinamic acid or N-L-alphaaspartyl-L-phenylalanine-1-methyl ester, with the chemical formula, $C_{14}H_{18}N_2O_5$ and its C.A.S number is 22839-47-0, density; 1.347 g/cm^3 molecular weight; 294.34$gmol^{-1}$. It is obtained by synthesis from two amino acids, L-phenylalanine and L-aspartic acid, which are also components of proteins in our body and in food. Approximately 50% of the Aspartame molecule is phenylalanine, 40% is aspartic acid, and 10% is methanol; it was first approved for use by FDA for dry goods in 1981 and carbonated beverages in 1983 [6]. The phenylalanine in Aspartame is slightly modified by adding a methyl group which gives Aspartame its sweet taste. Its structural formula can be seen in the figure below;

Aspartame is a white crystalline powder and is about 200 times as sweet as sucrose [7], it is widely used in food products including beverages, yoghurts, breakfast cereals and confectionary products, and in pharmaceutical industries [7,8,9]; although it was said that, high intake of Aspartame leads to various side effects such as Night vision, Headache, Dizziness, Alzheimer disease, Seizures, Insomnia, Mental depression, Anxiety, Feeling aggressive among others.

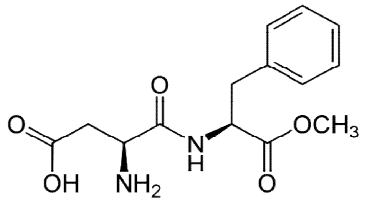

Fig. 1. Aspartame structural formula

Aspartame is fully metabolized in our gut to aspartic acid and phenylalanine, which are absorbed into the body; the methyl group modified from phenylalanine is then released to form methanol which also gets absorbed, and most of it used to produce energy. All the scientific studies to date in animals and human volunteers have shown that the breakdown of Aspartame in the gut is very rapid and complete; no Aspartame has ever been found in the blood or any organ after ingestion. Any effect reported to occur in the body following ingestion of Aspartame will be caused by one or more of the three constituents; aspartic acid, phenylalanine or methanol [10].

Phenylalanine is an essential amino acid, though cannot be biosynthesized; it is essential because it can be converted into tyrosine in the body, this act as a precursor for a variety of neurotransmitters like dopamine, noradrenalin (norepinephrine) and adrenalin (epinephrine) and for such substances as thyroid hormone and melanin [11]. Phenylalanine and its derivatives accumulate after ingestion of Aspartame in the blood, tissues and urine; this is dangerous to individuals who have the disorder called, phenylketonuria (PKU); an inborn disorder caused by lack of Phenylalanine Hydroxylase Enzyme, where phenylalanine cannot be metabolized to tyrosine [12]. Consequently, such accumulation result in mental retardation, growth retardation and hosts of other health disorders (Carol C., 2015).

To control these effects, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the EU Scientific Committee for Food (SCF) and the European Food Safety Authority (EFSA)

have evaluated and established an acceptable daily intake (ADI) for Aspartame as 40mg/kg body weight/day and that all foods containing Aspartame should indicate a warning on their label that it "Contains Aspartame, a source of Phenylalanine" [13] so as to help Phenylketonurics (PKUs) avoid such substances. In Nigeria, the NAFDAC approved concentration of Aspartame in soft drinks is 3000 mg/L.

The evaluation of the presence and amounts of Aspartame in soft drinks consumed in Mubi, Adamawa State and awareness about such has, therefore, become necessary, as many of the population consumes Aspartame drinks; which may include even ignorant Phenylketonurics, also there is tendency that some industries may have used Aspartame above the accepted level or may not have even indicated it on their label that Aspartame is contained.

2. MATERIALS AND METHODS

2.1 Study Area

Mubi metropolis is a geo-political area comprising of two local government areas; Mubi North and Mubi South. The metropolis is located between latitudes 10º05' and 10º30'N of the equator and between longitude 13º12' and 13º19'E of the Greenwich meridian. The two Local government areas occupy a land area of 192,307 Km^2 and support a total population 260,009 people (National Population Census 2006). The area shares boundary with Maiha L.G.A in the South, Hong L.G.A in the West, Michika L.G.A and the Cameroon Republic in the East.

Varieties of Business activities take place in the area to meet the demand of the population. Though no industry is located in this area, many products from industries in other parts of the country are brought for consumption. The large market; located in the heart of the town, and other large and small-scale retail businesses and shop owners have been able to accommodate the different variety of products, especially soft drinks coming into the town, and provides them readily to the consuming population.

2.2 Samples and Sampling

Five samples were collected randomly from shops in Mubi Main Market for the analysis; ZRC (Zero Coke, Coke Zero Sugar) produced by the Coca-Cola company, PSL (Pepsi Lite, Diet Pepsi) produced by PepsiCo company, ORZ (Origin Zero) produced by Guinness Nigeria Plc., SNW (Sans Cream Soda) produced by PharmaDeko Plc. and MHL (Malta Guinness Herbs Lite) produced by Guinness Nigeria Plc. Bottle (PET, Polyethylene Terephthalate) containers of ZRC, PSL, SNW and can containers of ORZ and MHL were used. The expiry date of the samples was cross-checked and those not expired were used.

2.3 Analysis

The presence (Qualitative analysis) of Aspartame in the samples collected was determined by Ninhydrin test; as was adopted by Ritu et al*.,* [14] and the amount (Quantitative analysis) of Aspartame was determined using HPLC.

2.3.1 Ninhydrin test (Qualitative analysis)

Ninhydrin Solution: 1.0 g of Ninhydrin was dissolved in 50 ml of 95% ethanol and 10 ml of glacial acetic acid. Acetonitrile Phosphate Buffer: 10 ml of acetonitrile was added to 90 ml of 6.8 g/Sol. of potassium dihydrogen phosphate and adjusted to pH 3.7 with phosphoric acid. 1.0 ml of each sample was dissolved in water, and 0.5 ml acetonitrile phosphate buffer (3.7 pH) added which was kept for five minutes, after which 1.0 ml Ninhydrin solution was added. This was warmed for seven minutes; this was done in turn for all the samples, and each of the samples gave a pink colour.

2.3.2 HPLC (Quantitative) analysis

HPLC with UV detector; 220 nm, integrator and C18 column, sampler and sampling pump cables of 1 to 3 L/min with flexible connecting tubes, syringes and syringe filters, vials, glass, scintillation, 20 mL with PTFE-lined caps, pipettes of various sizes, tweezers, ultrasonic water bath, pH meter were the equipment used and Aspartame,^{TM} 96% pure, deionized water, acetonitrile; distilled in glass, methanol; distilled in glass, 1-Heptanesulfonic acid sodium salt, monobasic potassium phosphate, phosphoric; acid reagent grade. Eluent: 2.062 g of 1-Heptanesulfonic acid sodium salt was added to 1.0L deionized water and adjusted to pH 3.0 with dilute phosphoric acid. Calibration stock solution (5 mg/mL): 0.05 g Aspartame was diluted with 10 mL of Eluent; these were the reagents used.

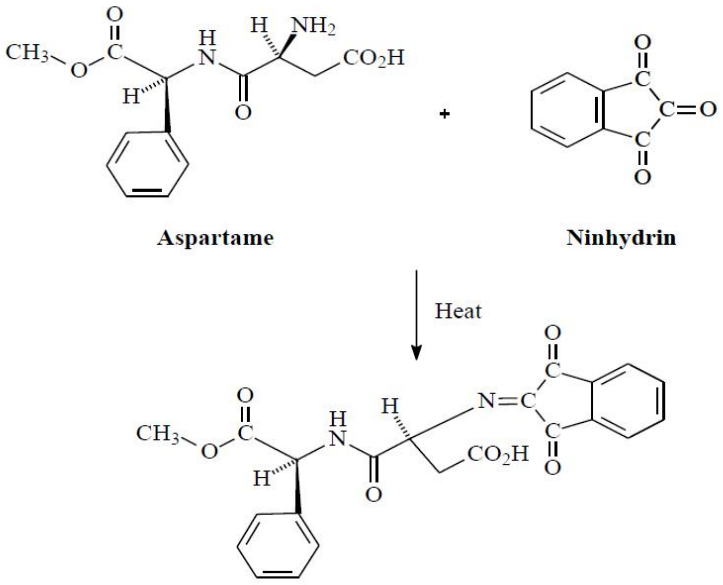

Fig. 2. The chemical reaction of Ninhydrin test

Six working standards were used for calibration; known amounts of calibration stock solutions were added to eluent using serial dilution to obtain a concentration in the ranges of 1 to 400 mg/mL and analyzed with samples and blanks. A calibration graph (peak area vs. mass of Aspartame, mg per sample) was prepared. Sample aliquot was injected and the peak areas measured.

Conditions: 0.35 ml/min Flow rate, 5 min post time and 40°C in column compartment.

3. RESULTS AND DISCUSSION

The results obtained from Ninhydrin test (Qualitative analysis) indicated that all the samples contain Aspartame, as they all gave the "pink colour" after the test.

The results from HPLC Analysis (Quantitative analysis) are on Table 1.

Concentrations of Aspartame ranged from 306.11 mg/L – 525.34 mg/L in the samples analyzed; this is also shown as: ORZ > ZRC > PSL > SNW > MHL (Decreasing magnitude of Aspartame concentration in the samples)

Ninhydrin (indane-1,2,3-trione) test has been used to test for the presence of Aspartame which contains the amino acids Phenylalanine and Aspartic acid; this is a sensitive method used for low amount sugar detection, up to 7.5 microgram. A positive test is indicated by the formation of a yellow coloured complex (Fig. 2). Thus a negative test with the absence of the yellow colour indicates the absence of Aspartame. From the Ninhydrin test carried out on the samples; the yellow colour was observed in all the samples analyzed, thereby confirming the presence of Aspartame in the soft drinks. Khesorn et al. [15] analyzed Aspartame contents of four Soft drink samples in Thailand, in the research work aimed at evaluating the identification and determination

Table 1. Concentration (mg/L) of Aspartame in soft drinks samples with Mean ± SD

Samples	Tests			Mean ± SD
	First	Second	Third	
ZRC	438.45	438.47	438.42	438.45 ± 0.03
PSL	365.81	365.83	365.80	365.81 ± 0.02
ORZ	525.34	525.36	525.37	525.36 ± 0.02
SNW	337.05	337.07	337.05	337.06 ± 0.01
MHL	306.11	306.13	306.14	306.13 ± 0.02

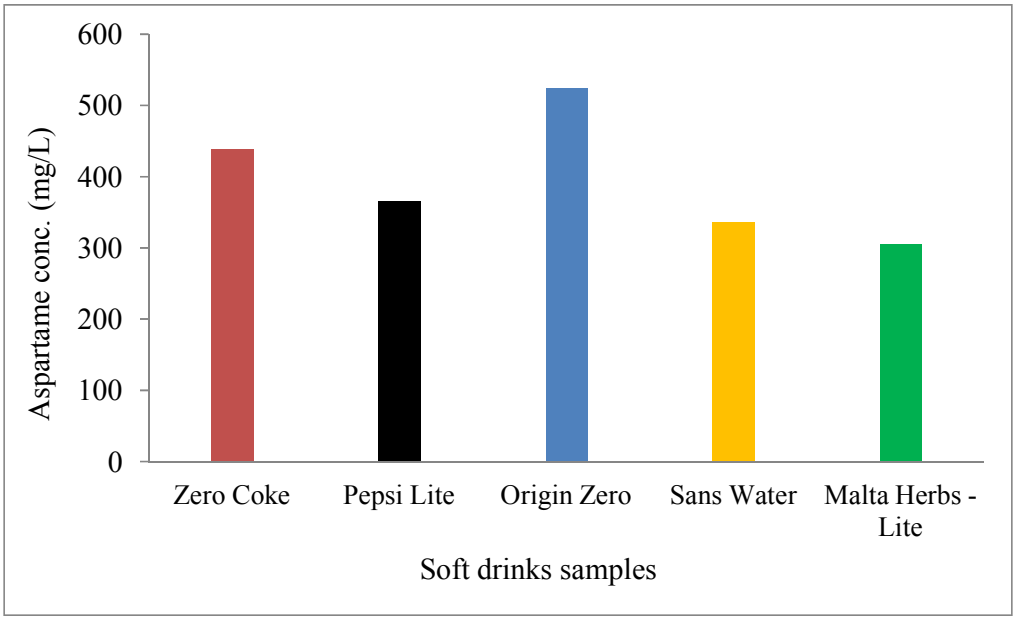

Fig. 3. The concentration of Aspartame in Soft drinks sold in Mubi

methods of Aspartame. Ritu et al. [14] also analyzed the Aspartame content of ten Soft drink samples in India; Ninhydrin test was used to confirm Aspartame in the soft drinks, and all their samples also indicated a Positive result. This then implies that ZRC, PSL, ORZ, SNW and MHL actually contains Aspartame Sweetener as indicated on their labels; this is in accordance with the law set by FDA, NAFDAC, EU – SCF etc., that all food substances containing Aspartame should indicate "Contains Aspartame a source of Phenylalanine", as it will help Phenylketonurics to avoid such food substances in their diets.

The HPLC analysis results are represented on Table 1 for ZRC (438.45 ± 0.03 mg/L), PSL (365.81 ± 0.02 mg/L), ORZ (525.36 ± 0.02 mg/L), SNW (337.06 ± 0.01 mg/L) and MHL (306.13 ± 0.02 mg/L), the results indicate that all the samples contain Aspartame amounts not more than the NAFDAC recommendation of 3000 mg/L. The analysis shows that ORZ has the highest concentration with 525.36 ± 0.02 mg/L and MHL has the least concentration with 306.13 ± 0.02 mg/L; this is in close agreement with the results obtained by [16] where the mean values for the concentrations of Aspartame for samples A, B, C, D, E and F brands in Ankara Turkey, were found as 156.81 ± 7.29 mg/L, 208.67 ± 8.97 mg/L, 236.58 ± 17.91 mg/L, 299.54 ± 26.1 mg/L, 202.39 ± 8.08 mg/L and 223.28 ± 14.08 mg/L, the data obtained from the analysis revealed that mean levels of Aspartame were found within the Turkish Food Codex (TFC) of 600mg/L in all the samples, however, some samples were not found appropriate according to the label information. The difference between the mean levels of Aspartame in samples of brands (A, B, C, D, E, F) and TFC values (600 mg/L) were statistically significant ($p<0.001$). Also, the difference between the mean levels of two different brands (A and B) of the same firm was statistically significant ($p<0.001$). However, the difference between the mean levels of two different brands (C and D) of the same firm were statistically insignificant ($p>0.05$). From the results of the analysis carried out in this research, it can also be seen then that, all samples were within the NAFDAC standard of 3000 mg/L in Nigeria.

Nurudeen [17] analyzed fourteen energy drink samples in Zaria, Nigeria for their physicochemical properties (pH, turbidity, conductivity and total dissolved solids), trace and heavy metals, Aspartame, sugar and caffeine; concentrations of Aspartame was found ranging from 6.51 mg/L – 1491.19 mg/L which were also below the NAFDAC standard of 3000mg/L as were the results of this research. Although there is wide difference between the least and highest concentrations with those of this analysis; this could be due to the fact that NAFDAC has only set a standard for the maximum amount (3000 mg/L) of Aspartame to be used. Thus any

amount can be used so long as it has not exceeded the set standard.

Casimir E [18] also found Aspartame concentrations ranging from 283.84 ppm – 956.82 ppm in ten brands of energy drinks; all these were also not more than the NAFDAC standard. The results from all these findings indicate that most Soft drinks industries comply with the standards set by the monitoring agencies.

From the analysis carried out, it can also be seen that ORZ and MHL produced by the same company; Guinness Nigeria Plc., has the highest (525.36 mg/L) and least (306.13 mg/L) concentration of Aspartame respectively, this implies that the company did not use the same concentration of Aspartame in these drinks which could mean that, the concentration of Aspartame in all their products are not consistent; this, of course, does not violet any set law. But, the company can do well to indicate the amounts of Aspartame contained in each of their products. Also considering that the highest and least level of Aspartame was contained in the soft drinks samples with can containers, while the intermediate levels were contained in samples with bottled containers; this should mean that the storage medium does not necessarily determine the Aspartame contents of soft drinks.

With regards to the ADI of 40mg/kg body weight established by the FDA, EU – SCF and other food control agencies; the results from this analysis suggest that an individual with 20 kg body weight, for instance, can take 800 mg of Aspartame daily. Such individual can therefore; take about seven bottles of 250 ml (0.25 L) ZRC which should contains 109.61 mg of Aspartame each, or about Eight bottles of 250 ml (0.25 L) PSL which should contain 91.45 mg of Aspartame each, or about Six bottles of 250 ml (0.25 L) ORZ which should contain 131.34 mg of Aspartame each, or Nine bottles of 250ml (0.25 L) SNW which should contain 84.27mg of Aspartame each, or Ten bottles of 250 ml (0.25 L) MHL which should contain 76.53 mg of Aspartame each, daily; consumption of these drinks above this rate daily may subject such individual to the health disorders said to be associated with high intake of Aspartame. Also since Approximately 50% of the Aspartame molecule is phenylalanine, a Phenylketonuric (PKUc) with 20 kg body weight for instance takes in 54.81 mg Phenylalanine per 250 ml (0.25 L) ZRC bottle, 45.73 mg Phenylalanine per 250 ml (0.25 L) PSL bottle, 65.67 mg Phenylalanine per 250 ml (0.25 L) ORZ bottle, 42.14 mg Phenylalanine per 250 ml (0.25 L) SNW bottle and 38.27 mg Phenylalanine per 250 ml (0.25 L) MHL bottle. From above it can be seen that only minute amounts of Phenylalanine are consumed in the samples, but because PKU actually arises due to accumulation of Phenylalanine in the body of Phenylketonurics (PKUs); its effect will manifest sooner or later with continuous consumption of these drinks, it is therefore rather recommended that PKUs should seize entirely not reduce, the intake of these drinks.

4. CONCLUSION

This research reveals that the amount of Aspartame sweetener in ZRC, PSL, ORZ, SNW and MHL are within the approved level in compliance with the NAFDAC standard, although the concentrations were not indicated on the labels. This research also confirms the presence of Aspartame in these samples as indicated on their label which is also by the requirement set by the food control agencies.

From the results obtained, all Phenylketonurics should, therefore, avoid these drinks as they contain Aspartame a source of Phenylalanine which their body system cannot convert to Tyrosine due to the absence of Phenylalanine Hydroxylase Enzyme, and also other consumers should adhere to the recommended amounts of these soft drinks in their daily intake in order to avoid the health disorders associated with high intake of Aspartame.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Kunkel EM, Barbara HD. Gale Nutrition and Well-being A to Z. The Gale Group

Inc. Macmillan Reference, New York, USA; 2004.
2. US. Department of Health & Human Services; 2014. Available:http://answers.hhs.gov/questions/3117
3. Korankye O. Extraction and application of plant dyes to serve as colourants for food and textiles. Masters thesis submitted to the School of Graduate Studies, Kwame Nkrumah University of Science and Technology, Kumasi. 2010;35.
4. Hui YH, Lim MH, Nip WK, Smith JS, Yu P. HF. Principles of food processing. In: Smith JS, Hui YH. eds. Food Processing: Principles and applications. Blackwell Publishing Ltd, Oxford, UK. 2004;20-21.
5. Houghton M. The American Heritage Food Science Dictionary; 2002. Available:http://www.amazon.com.Retrieved 14/12/2011
6. Carol C, Alqasim A, Mustapha Muhammed O. Aspartame food additive and its biochemical implication: A review. Food Science and Quality Management. 2015;36. ISSN 2224-6088 (Paper) ISSN 2225-0557
7. Mazurek S, Szostak R. Quantification of aspartame in commercial sweeteners by FT-Raman spectroscopy. Food Chem. 2011;125:1051-1057. Available:http://dx.doi.org/10.1016/j.foodchem.2010.09.075
8. Choudhary AK, Rathinasamy SD. Aspartame induces alteration in electrolytes homeostasis of immuneorgans in wistar albino rats. Biomed Prev Nutr. 2014;4:181-187. Available:http://dx.doi.org/10.1016/j.bionut.2013.12.006
9. Kashanian S, Khodaei MM, Kheirdoosh F. *In vitro* DNA binding studies of Aspartame, an artificial sweetener. J Photochem Photobiol B. 2013;120:104-110. Available:http://dx.doi.org/10.1016/j.jphotobiol.2013.01.001
10. Butchko H, Stargel W, Comer CP, Mayhew DA, Benninger C, Blackburn GL, De Sonneville LM, Geha RS, Hertelendy Z. "Aspartame: Review of Safety". Regulatory Toxicology and Pharmacology; 2002. PMID12180494. DOI:10.1006/rtph.2002.1542
11. Dennis Jones. Phenylalanine; an essential amino-acid with relevance to weight loss. Bariatrix Nutrition. 2006;1&2.
12. Humphries P, Pretorius E, Naude H. Direct and indirect cellular effects of aspartame on the brain. Eur J Clin Nutr. 2008. 62:451-462. Available:http://dx.doi.org/10.1038/sj.ejcn.1602866
13. EFSA. Scientific opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA Journal. 2013;11(12): 3496,1-263.
14. Ritu M, Shelly, Minakshi S. Aspartame determination in soft drinks. International Journal for Research in Emerging Science and Technology. 2015;2(4). E-ISSN: 2349-7610
15. Khesorn Nantachit, Somporn Putiyanan and Prapart Phoowiang. Identification and determination methods of aspartame. Thai Pharmaceutical and Health Science Journal. 2008;3(2).
16. Elif Ç, Buket E, Burak D, Gülderen Y. Determination of aspartame levels in soft drinks consumed in Ankara, Turkey. Journal of Food Research; 2014;3(6). ISSN 1927-0887 E-ISSN 1927-0895. Published by Canadian Center of Science and Education.
17. Nurudeen Sulaiman Abbas. (2014). "Evaluation of some chemical constituents of selected energy drinks." A thesis submitted to the school of Post Graduate studies, Ahmadu Bello University Zaria, in partial fulfillment of the requirements for the award of Master of Science degree in analytical chemistry; 2014.
18. Casimir E, Stephen E, Nurudeen S, Igelige U. Evaluation of caffeine, aspartame and sugar contents in energy drinks. Journal of Chemical and Pharmaceutical Research. 2014;6(8):39-43. ISSN: 0975-7384 CODEN (USA): JCPRC5

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24917