



HPLC Determination of Benzoic Acid, Saccharin, and Caffeine in Carbonated Soft Drinks

Doris O. Seyinde¹, Ikechukwu P. Ejidike^{1*}, Segun Ayejuyo²

¹Department of Chemical Sciences, Faculty of Science and Science Education, Anchor University, Lagos, Nigeria

²Department of Chemistry, Faculty of Science, University of Lagos, Akoka, Nigeria

Abstract : With increase in worlds populace, the demand for soft drinks is increasing, hence, additives utilized during drinks preparations have become a global health challenge as a result of adverse health effectson consumers. In this paper, a reverse phase high performance liquid chromatographic method that could simultaneously and quantitatively determine the amount of saccharin, caffeine and benzoic acid in soft drinks samples was utilized. It involved the use of ahypercil C-18 column and a binary eluent consisting of 10% acetic acid in ultra pure water. The analysis of the results showed the regular cola caffeine content was $0.13\pm 0.03\text{mg/mL}$ and benzoic acid content of fanta $0.67\pm 0.01\text{ mg/mL}$. The linearity of the calibration curve for all additives gave $R^2 > 0.99$. Relative standard deviations of 0.68, 0.08 and 1.44% were found for the qualification of saccharin, caffeine and benzoic acid confirming good precision. The results, therefore, emphasized the significance of a robust monitoring procedures for these additives by the public and food health establishments in Nigeria.

Keywords : HPLC, Food Additives, Benzoic Acid, Retention Times, Saccharin, Caffeine.

1. Introduction

Artificial sweeteners, preservatives and dyes arewidely added to soft drinks either to enhance the flavor and appearance or to extend their shelf life by preventing biological degradation[1-3]. Soft drinks have become part of the global lifestyle since the nineteenth century and many of the soft drinksbeing consumedat present are the same as those first enjoyed centuries ago. The ingredients used in soft drink beverages are approved and closely regulated by bodies such as the US food and Drug Administrator or the food standard agencies in the UK [4,5]. Since several of these additives are prepared by chemical synthesis, their presence in food is the cause of extensive consumers mistrust, in that they may exhibit adverse health effects [6, 7]. Consequently, many governmentsentities have set threshold value for the acceptable daily intake (ADI) varying from country to country. In particular, the European community has set legal limits for the maximum content of these additives in many foodstuffs [8,9].

Benzoic acid is often added to drinks as a preservative in form of its salts, sodium benzoate, potassium benzoate, calcium benzoate, benzene carboxylic acid and phenilcarboxylic acid[5, 10]. Under acidic conditions,

benzoates are converted to undissociated benzoic acid [11]. Benzoates are often preferred to benzoic acid due to their improved solubility [12,13]. Concern has been expressed that benzoic acid and its salt may react with ascorbic acid (Vitamin C) in some soft drinks forming small quantities of benzene. Health Canada conducted a survey of benzene in fruits and soft drinks and revealed that the level was below 5µg/L which is considered to be of very low concern to human health [14].

Benzoic acid and its salts are permitted food additives by international laws in restricted amounts, but their content must be declared and not exceed the established limits of 150ppm stipulated by the Nigeria and European legislation [15]. Benzoates are considered innocuous for humans at an allowed concentration levels, but it was reported to be linked to DNA damage in yeast cells, hyperactivity in children and adverse carcinogenic consequence for its short- and long-term exposures [16,17].

Caffeine, a mildly addictive stimulant drug is added as part of the overall profile of soft drinks, which consumers enjoy for refreshment, taste and hydration. Caffeine's addictiveness is one reason why most soft drinks contain caffeine [16, 18]. Caffeine has the ability to stimulate mental awareness, but excessive doses cause aggression or restlessness [17]. Consistent with federal regulations, beverages companies list caffeine on product labels when it is added as an ingredient [19].

Saccharin (1,2-benzisothiazol-3(2H)-one-1,1-dioxide) used in the form of sodium or calcium salt, is a non-glucose, low calorie product with a highly sweet taste. Saccharin is characterized by rapid onset, a short persistence with bitter and metallic after taste [17]. In order to reduce or eliminate this after taste via synergistic effect, saccharin is usually blended with other sweeteners such as cyclamate, aspartame or sucralose [20, 21]. Saccharin provides no energy because it is not metabolized by humans but rather is excreted via the kidney, around 80% as saccharin and the rest as hydrolysis product. Saccharin, although innocuous for humans, has been reported to have potential toxicity and thus, have been prohibited in some countries considering possible carcinogenic effect [22,23].

However, excessive consumption of carbonated soft drinks is usually associated with several health concerns, such as obesity, developing non-communicable diseases and even death. The convention use of food additives in beverages has been believed to be linked to risk factor for the development of many health complications. Therefore, the study seeks to investigate the level of benzoic acid, saccharin, and caffeine soft drinks samples by high performance liquid chromatographic method.

2. Materials and Methods

2.1. Materials

All reagents, sodium saccharin, caffeine, sodium benzoate was of USP grade. Ultra-pure water was utilized for this experiment. The stock solution and the corresponding dilutions were made with ultra-pure water. HPLC was performed on an Hypersil C-18 using a Hitachi Lachrom L-2130 pump, L-2200 autosampler, L-2420 UV detector, (254nm wavelength) sonicator for degassing, Hypersil C-18 UV Detector and EZ Chrom Elite software.

2.2. Pre-Procedure

The determinations were made under isocratic conditions by using a mobile phase of 10% acetic acid. The samples were degassed with a sonicator, filtered and diluted in ultra pure water. The test solutions were filtered through 0.45 µm pore diameter membrane before injection. The volume injected was 10 µl and the flow rate of the mobile phase was 0.7 ml/min at a pressure of 879 psi.

2.3. Procedure

1.0mg/mL stock solution each of sodium saccharin, caffeine and sodium benzoate standard solutions were prepared by accurately weighing 10mg each of sodium saccharin, caffeine and sodium benzoate into a 10mL volumetric flask, dissolved and made up to mark with ultra-pure water. 0.286mg/mL working solutions were prepared from the stock and 10mL of each working solution was injected separately into the liquid chromatograph to establish the retention times of each; saccharin, caffeine and benzoic acid concentrations. 0.02

and 0.30mg/mL were prepared by serial dilution method and 10ml of each solution injected. A graph of peak area response was plotted against concentration in order to obtain the calibration curves.

2.4. Mixed Standards

0.5mg/ml mixed standard solution was prepared by weighing 125mg each caffeine, sodium saccharin and sodium benzoate into a 250mL volumetric flask. 6 μ L of the mixed standard solution was injected into the instrument and peak areas of the additives measured. In the sample preparation, soft drinks were decarbonated with the aid of a sonicator. 2-5mL aliquot was filtered through 0.45 μ m pore diameter membrane filter and 10 μ L was immediately injected into the HPLC system. Analyses were performed in triplicate.

3. Result and Discussions

3.1 Analysis of standard Solutions

A number of additives are frequently added into soft drinks that affect the beverage's taste and characteristics. An example such popular ingredient is caffeine, natural xanthine alkaloid stimulant that occurs in several plants as a natural insecticide, such as kola nuts, cacao beans, coffee beans, and tea leaves. Also, benzoic acid is added to various soft drinks and other foods as a protective against microbial growth [16, 18, 24]. The responses of the additives: saccharin, caffeine, and benzoic acid showed excellent linearity over approximately five concentrations ranging from 0.02 to 0.30mg/mL. It was determined using the equation of the calibration curves and correlation coefficients for the additives are shown in the Table 1.

The linearity of calibration curves for all compounds was very good ($R^2 > 0.99$). In order to test peak area and reproducibility of retention time, the EZChrom Elite software was used for the calculation of the relative standard deviations (RSD) for the retention time of the three analytes at all levels of the calibration curve and for peak area at each calibration level. The retention times of saccharin, caffeine and benzoic acid were 0.93 ± 0.00 min, 2.37 ± 0.00 min and 4.78 ± 0.01 min respectively with relative standard deviation (RSD) of 0.46%, 0.09% and 0.10% respectively, indicating that the proposed method provides stable retention times [17, 25]. The retention times and relative standard deviations (RSD) of each standard are shown in Table 2. In this study, saccharin, caffeine, and benzoic acid were analyzed in soft drinks and Table 3 gives chemical information on these compounds.

Table 1: Standardization curves and correlation coefficients (r^2) for the three analytes

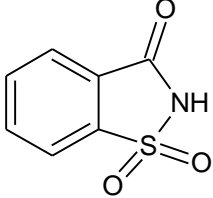
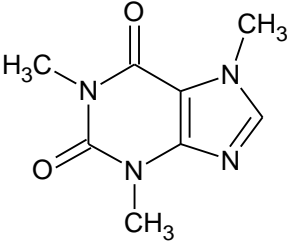
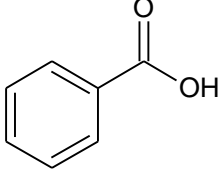
Standards	Calibration curves	Correlation coefficients (r^2)
Saccharin	$Y = 6.2576 \times 10^6 x + 0.0000$	0.9972
Caffeine	$Y = 3.6398 \times 10^7 x + 0.0000$	0.9998
Benzoic Acid	$Y = 4.3434 \times 10^6 x + 0.0000$	0.9947

Table 2: Characteristics of standard retention times

Standards	Retention time (mins)	Relative Standard Deviation (%)
Saccharin	0.93 ± 0.00	0.46
Caffeine	2.37 ± 0.00	0.09
Benzoic Acid	4.78 ± 0.01	0.10

This shows that in standard solutions, the HPLC method provides stable retention times. Moreover, the calculated relative standard deviations also prove stability in terms of peak area, peak height and peak asymmetry.

Table 3: IUPAC names and structures of the analyzed additives in Soft drinks

Common names	IUPAC name	M.W.	Structure
Saccharin (Sweet'N Low®)	1,1-Dioxo-1,2-benzothiazol -3-one	183.18	
Caffeine	1,3,7-trimethyl-1H-purine- 2,6(3H,7H)-dione	194.19	
Benzoic Acid	benzoic acid	122.12	

3.2. Analysis of Soft Drink Samples

In order to establish the method applicability on real samples, a bottle of regular cola, Fanta and fayrouz available on sale were degassed, filtered, diluted in ultra-pure water and analyzed using the proposed method. The final concentrations of the additives were calculated according to the following equation.

$$\frac{V_{sa} \times P_{asa} \times C_{st}}{P_{ast} \times C_{sa}}$$

Where C_{st} = Concentration in mg/ml of relevant compound in standard solution
 C_{sa} = Concentration in mg/ml of relevant compound in sample.
 P_{asa} = Peak area of same compound in the sample
 P_{ast} = Peak area of same compound in the standard
 V_{sa} = Volume of sample per bottle

The precision of the proposed method was determined from the peak area of each of the additive in the mixed external standard solution analyzed. Based on calibration curves and the dilutions made during the analysis, the saccharin, caffeine and benzoic acid content for all soft drink samples was established. The amounts of additives in the samples analyzed are displayed in Table 4. Good precision was confirmed for saccharin, caffeine and benzoic acid with the relative standard deviation (RSD) of 0.68%, 0.08% and 1.44% for peak areas respectively, demonstrating constancy in terms of peak height and asymmetry, this is in correlation with a study in Romania reported by Trandafir et al. [18]. The regular coke had a caffeine concentration (mg/ml) of 0.13 ± 0.00 , fanta a benzoic acid concentration (mg/ml) of 0.67 ± 0.91 , whereas the fayrouz had a saccharin concentration of 1.23 ± 0.03 mg/ml (Table 4). Consequently, the saccharin, caffeine and benzoic acid concentrations in three (3) samples showed low standard deviation (SD) signifying the analytical method for the sample contents gave a high precision and reproducibility [17].

It is interesting to note that saccharin content was observed only in Fayrouz, caffeine content only in regular coke, and benzoic acid content only in Fanta. The benzoic acid and saccharin contents (mg/ml) found in the samples analyzed were found to be higher than that of caffeine (0.13 ± 0.00 mg/ml). This can be attributed to the fact that benzoic acid and saccharin are weak acids that partially dissociate in water with comparable water solubility. This declaration is supported by the fact that water solubility of saccharin was 3.4 g/L at ambient temperature as reported by O'Donnell and Kearsley[23] while Mahindru[1] reported that the water solubility of benzoic acid was 3.44 g/L at ambient temperature, while caffeine is being soluble in water approx. 16 g/L at room temperature. Saccharin was detected only in Fayrouz beverages, benzoic acid only in Fanta soft drinks, and in regular cola was detected with caffeine. Similar occurrences have been reported by Sik[21] during a study for the detection of any artificial sweeteners in 56 soft drink samples possessing 12 different trademarks sold in Turkey. The author reported the presence of saccharin detected only in two out of the six analyzed diet cola beverage products ranging from 25.47 ± 0.14 ppm to 78.85 ± 0.19 ppm. In like manners, regular cola beverage was not detected with any artificial sweeteners, such as saccharin, acesulfame-K and aspartame.

In another investigation by Oroian and co-workers [26] in which 30 samples of carbonated drinks from the Romanian market were analyzed, it was observed that the concentration of saccharin ranged between 0 and 83.75 ppm with the mean value of 9.72 ppm. In 2011, Ree and Stoa[16] reported the presence of benzoic acid level in the canned diet cola to be 150.45 ppm for the quantitative analysis of food additives in sugar free beverages. Furthermore, it is interesting to note that the concentration of artificial sweeteners such as benzoic acid varies significantly between different brands of beverage samples. This is noted from reports by several researchers who have conducted study on the quantitative determination of benzoic acid in soft drinks. Kusi and Acquah[27] reported the benzoic acid level in the analysis of 34 soft drinks samples within Ghana to be ranged from not detected to an extreme level of 564 ppm with the mean concentration of 70.2 ppm. In the analysis of 25 samples of traditional soft drinks by Lino and Pena [22], the authors reported the benzoic acid mean concentration of 158 ppm ranging from 91 ppm to 172 ppm. In a study to determine the levels of aspartame, benzoic acid, caffeine, and saccharin in sugar-free beverages using HPLC, Ree and Stoa[16] reported the diet coke from a can to have 131.925 ppm of caffeine, coke zero contained 100.250 ppm of caffeine, diet Nestea had 37.0 ppm of caffeine, while the levels of benzoic acid was found to be 150.45 ppm, 123.875 ppm, and 246.125 ppm in canned diet coke, coke zero, and diet Nestea respectively.

Javeed et al. [28] reported the concentration of saccharin to range from 46.8 ppm to 62.9 ppm in different cola brands from a local market in Pakistan. The authors also confirmed saccharin levels within a range of 82.9 to 95.7 ppm for soda lemon flavoured beverages and 93.2 to 134.1 ppm in soda orange flavoured soft drinks. Chua and Teo[17] in an investigation into the saccharin and benzoic acid levels in regular and diet cola-flavoured carbonated soft drinks in Malaysia, revealed that regular cola canned drink contained 13.89 ± 4.74 ppm of saccharin and 226.6 ± 14.1 ppm of benzoic acid, and the diet cola canned drink contained 31.39 ± 2.10 and 206.8 ± 28.2 ppm in saccharin and benzoic acid respectively.

To evaluate the accuracy of the proposed method, the analyzed results were compared to the HPLC method proposed by the health protection branch laboratories, Ontario Regional laboratory, Scarborough [29] for the determination of saccharin, sodium benzoate and caffeine in beverages. The obtained results were subjected to F-test and student t-test to determine any significant difference between the methods used for this study as shown in Table 5.

Table 4: : Benzoic acid, caffeine and saccharin contents in the samples analyzed						
Soft						
Drink	Content (mg/330 ml)			Content (mg/ml)		
	Benzoic Acid	Caffeine	Saccharin	Benzoic Acid	Caffeine	Saccharin
Regular	ND	44.41± 0.98	ND	ND	0.13± 0.00	ND
coke						
Fanta	220.37 ± 2.09	ND	ND	0.67± 0.91	ND	ND
Fayrouz	ND	ND	407.07± 8.53	ND	ND	1.23 ±0.03
ND- Not Detected						
Table 5: Comparison of the results obtained by both methods						
Amount						
(mg/330 ml)	Reference method			Proposed method		
	Fanta (benzoic acid)	Regular cola (caffeine)	Fayrouz (saccharin)	Fanta (benzoic acid)	Regular cola (caffeine)	Fayrouz (saccharin)
Injection 1	220.13	43.34	414.75	219.38	43.43	416.32
Injection 2	222.47	45.38	401.35	222.77	45.39	399.52
Injection 3	219.45	44.41	407.63	218.97	44.40	405.35
Average	220.69 ± 1.59	44.38 ± 1.02	407.91 ± 6.70	220.37 ± 2.09	44.41 ± 0.98	407.07 ± 8.53
F-calculated				1.73	1.09	1.62
t-calculated				0.21	0.04	0.114
n = 3						

From the F-test, the F-calculated values for saccharin, caffeine and benzoic acid were 1.62, 1.09 and 1.73 respectively. Since the F-calculated was less than the F-tabulated value of 19.0, it was, therefore, concluded that there was no significant difference in the precision of the proposed and the official method. The student t-test revealed values of 0.14, 0.4 and 0.21 for saccharin, caffeine and benzoic acid respectively which were less than the t-tabulated value of 4.303 at 95% confidence level. This shows that there is no statistical difference in the results of the proposed and the official method. The proposed method could compare favorably with the established one.

The results obtained in this work showed the usefulness of the method to quantitatively determine the presence of saccharin, caffeine and benzoic acid in soft drink. In spite of the complex composition of the sample analyzed, the method can be applied without any previous step unlike AOAC procedures for saccharin, benzoic acid and caffeine that are time consuming, nonspecific and of relatively low sensitivities [30, 31].

4. Conclusion

In summary, the HPLC method presented here is simple, fast, precise, and accurate technique for simultaneously and quantitatively determining the contents of the additives in soft drinks. The accuracy and precision of data are considered sufficiently reliable to warrant the use of this method as a rapid screening procedure for these additives in soft drinks. This is due to the rapid consumption of carbonated soft drinks accompanied with fast foods especially young children and adolescents. Sweeteners and preservatives are some of the chief food additives added into soft drinks as a means of significantly improving the taste and shelf life. Although, the usage of these additives remains significant to the production of these beverages, their usage should be controlled in various food products owing to the emerging health concerns associated with them. Such as weight gain, increased risks of preterm delivery, hypersensitivity, adverse carcinogenic effect for its short- and long-term exposures and cancer during long term exposure.

The caffeine content of regular cola was found to be 0.13 ± 0.00 mg/ml, the saccharin content of Fayrouz was found to be 1.23 ± 0.03 mg/ml, while the benzoic acid content of Fanta was 0.67 ± 0.01 mg/ml. The linearity, precision and accuracy of the proposed method were found to be analytically acceptable. Also, the F-calculated was less than the F-tabulated value of 19.0, meaning that there was no significant difference in the precision of the proposed and the official method used in this study. Therefore, it is important to set up a robust monitoring measures of preservatives in beverages by the health authorities in Nigeria.

Acknowledgements

The authors acknowledge the assistance received from NAFDAC Yaba Unit, Lagos, Nigeria during the analysis.

Conflicts of Interests

The authors declare that they have no conflict of interest.

References

1. Mahindru S., Food science and technology. New Delhi: APH Publishing Corp, 2009, 21- 29.
2. Mephram B., Food additives: an ethical evaluation, Br. Med. Bull. 2011, 99(1), 7-23.
3. Shukla P., Akshay S. and Ashok S., Food additives from an organic chemistry perspective, MOJ Biorg. Org. Chem., 2017, 1(3), 70-79.
4. Sharma L.L, Teret S.P. and Brownell K.D., The food industry and self-regulation: standards to promote success and to avoid public health failures, Am. J. Public Health, 2010, 100(2), 240-246.
5. Chaleshtori F.S., Arian. A. and Chaleshtori R.S., Assessment of sodium benzoate and potassium sorbate preservatives in some products in Kashan, Iran with estimation of human health risk, Food Chem. Toxicol., 2018, 120, 634-638.
6. Carochi M., Barreiro M.F., Morales P. and Ferreira I.C.F.R., Adding molecules to food, pros and cons : A review on synthetic and natural food additives, Compr. Rev. Food Sci. Food Saf., 2014, 13 (4), 377-399.

7. Pressman P., Clemens R., Hayes W., Reddy C., Food additive safety: A review of toxicologic and regulatory issues, *Toxicology Research and Application*, 2017, 1, 1-22.
8. Dossi N., Toniolo R., Susmel S., Pizzariello A. and Bontempelli G., Simultaneous RP-LC determination of additives in soft drinks, *Chromatographia*, 2006, 63(11-12), 557-562.
9. Lehto S., Buchweitz M., Klimm A., Straßburger R., Bechtold C. and Ulberth F., Comparison of food colour regulations in the EU and the US: a review of current provisions, *Food Addit. Contam. Part A*, 2017, 34(3), 335-355.
10. Mazdeh F.Z., Moradi Z., Moghaddam G., Moradi-Khatoonabadi Z., Aftabdari F.E., Badaei P. and Hajimahmoodi M., Determination of synthetic food colors, caffeine, sodium benzoate and potassium sorbate in sports drinks, *Trop. J. Pharm. Res.*, 2016, 15(1), 183-188.
11. Doležal P., Comparison of the effect of benzoic acid addition on the fermentation process quality with untreated silages, *Acta univ. agric. et silvic. Mendel. Brun.*, 2004, LII (2), 15-22.
12. Ene C.P. and Diacu, E., High performance Liquid Chromatography method for the determination of benzoic acid in beverages, *UPB Sci. Bull. Series B*, 2009, 71, 81-88.
13. Khosrokhavar R., Sadeghzadeh N., Amini M., Ghazi-Khansari M., Hajiaghvaei R., Ejtemaei M.Sh., Simultaneous determination of preservatives (sodium benzoate and potassium sorbate) in soft drinks and herbal extracts using High Performance Liquid Chromatography (HPLC), *Journal of Medicinal Plants*, 2010, 9(35), 80-87.
14. Kongo J.M., Partidário A., Duthoit I., Moniz C. and Ponte D., Assessment of benzoic acid, and benzene in "Pimenta-Da-Terra" red hot pepper (*Capsicum Sp*) processed traditionally, *J. Nutr. Food Sci.*, 2013, 3, 242.
15. Akter S., Zubair A., Khan S.H. Bari L., Huq A. and Rashid M.A. Identification and quantification of sodium benzoate in different brands of mango juices available in Tangail region, Bangladesh. *Bangladesh Pharm. J.*, 2017, 20(1), 20-26.
16. Ree M. and Stoa E., Simultaneous determination of aspartame, benzoic acid, caffeine, and saccharin in sugar-free beverages using HPLC. *Concordia College Journal of Analytical Chemistry*, 2011, 1, 71-77.
17. Chua S.L. and Teo S.S., Analysis of saccharin and benzoic acid in regular and diet Cola-flavoured carbonated soft drinks, *Int. J. Food Sci. Nutr.*, 2017, 2(4), 58-64.
18. Trandafir I., Nour V. and Ionică, M.E., Development and validation of an HPLC method for simultaneous quantification of acesulfame-K, saccharin, aspartame, caffeine and benzoic acid in cola soft drinks. *Scientific Study & Research*, 2009, X (2), 185-194.
19. Kole J. and Barnhill A., Caffeine content labeling: A missed opportunity for promoting personal and public health, *J. Caffeine Res.*, 2013, 3(3), 108-113.
20. Hyvonen L., Koivistoinen P. and Ratilainen A., Sweetening of soft drinks with mixtures of sugars and saccharin, *J. Food Sci.*, 2006, 43(5), 1580-1584.
21. Sik B., Development and Validation of a Green High Performance Liquid Chromatographic Method for the Determination of Some Artificial Sweeteners and Caffeine in Soft Drinks, *Food Anal. Methods*, 2012, 5(6), 1443-1452.
22. Lino C. and Pena A., Occurrence of caffeine, saccharin, benzoic acid and sorbic acid in soft drinks and nectars in Portugal and subsequent exposure assessment, *Food Chem.*, 2010, 121(2), 503-508.
23. O'Donnell J. and Kearsley M.W., *Sweeteners and Sugar Alternatives in Food Technology*, Second Edition, John Wiley & Sons, 2012.
24. Mumin A., Akhter K.F., Abedin Z. and Hossain Z., Determination and characterization of caffeine in tea, coffee and soft drinks by solid phase extraction and high-performance liquid chromatography (SPE-HPLC), *Malaysian Journal of Chemistry*, 2006, 8(1), 045-051.
25. Torbeck L., When to use percent relative standard deviation—and how to do so correctly. *Pharm. Technol.*, 2010, 34(1), 263.
26. Oroian M., Amariei S., Leahu A. and Gutt G., Acesulfame K, aspartame and saccharin occurrence in carbonated drinks from the Romanian market. [ebook] *Romania: Recent Researches in Medicine, Biology and Bioscience*, 2013, 25-28. Available from: <http://www.wseas.us/e-library/conferences/2013/Chania/BIOMED/BIOMED-02.pdf> [Accessed 12 March 2019].
27. Kusi J. and Acquah S., Levels of benzoic acid in soft drinks and fruit juices in Ghana. *IOSR J. Environ. Sci. Toxicol. Food Technol.*, 2014, 8(12), 36-39.
28. Javeed M., Ikhtlaq N., Ahmad S. and Ahmad I., Estimation of saccharin in soda beverages syrups, kulfies and candies, *Journal of Applied Sciences*. 2001, 1(4), 486-488.

29. Smyly D.S., Woodward B.B. and Conrad E.C., Determination of saccharin, sodium benzoate and caffeine in beverages by reversed phase High-pressure Liquid Chromatography. *J. Assoc Off Anal Chem.*, 1976, 59(1):14-19.
30. McDevitt V.L., Rodríguez A. and Williams K.R. (1998). Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, 1998, *J. Chem. Educ.*, 75(5), 625-629.
