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Determination of sodium benzoate in different brands of orange juices available in Bangladesh by high-performance liquid chromatography

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Abstract

Sodium benzoates as a chemical preservative are common practice in modern food technology. It is permitted by international laws in food as a food additive with a restricted amount. But it is highly used in different food products like fruit juices. Therefore, an experimental study was conducted to determine the concentration of sodium benzoate in different brands of orange juices available in the market Tangail region, Bangladesh using High Performance Liquid Chromatography (HPLC). Chromatographic analysis was performed by a column (250 × 4.6 mm) with an isocratic solvent system at 40°C. The mobile phase consisted of sodium acetate and acetic acid buffer (pH was 4). The correlation of coefficient was 0.9999 from the standards curves and the variety of external standard absorptions was 0.93 to 20.05 µg/mL. Analysis of four brands of orange juice samples showed that the concentration of sodium benzoate was within the FDA standard, but two brand samples slightly exceeded the Bangladesh Standard and Testing Institute (BSTI) standard. Brand 1 and Brand 4 samples contained sodium benzoate 14.61 and 9.59 mg/100 mL respectively which were within the level BSTI standard range whereas Brand 2 and Brand 3 samples contained 16.11 and 16.33 mg/100 mL respectively which exceeded the level of BSTI standard.

1. Introduction

Fruit juices are called nutritious beverages and day by day it is becoming an important drink in the modern diet. Fresh fruit juices are a natural, nutrient-dense and fat-free beverage that can play a significant role in preparing a healthy diet (Franke *et al.,* 2005). Orange juice is a popular type and most frequently consumed fruit juice among children and adolescents. Orange juices contain a high amount of vitamin C, provide potassium, riboflavin, niacin and folate as well as excellent source of natural antioxidants (Rampersaud and Valim, 2017). Regular consumption of orange juice beneficially affects anthropometric indices, lipid profile, inflammatory markers and insulin resistance status (Rangel-Huerta *et al.,* 2015; Motallaei *et al.,* 2021). Orange juices are available in their natural concentrations or in processed forms in the global market. But orange juices are highly perishable and easily decayed because of the propagation of bacteria and other microorganisms (Rodríguez *et al.,* 2017). Numerous preservation methods like high temperature and pressure are used for the reduction of microbes but they may reduce the freshness of juice and show significant changes in nutrients (Keyser *et al.,* 2008). Therefore, non-thermal processes like chemical preservatives have emerged as preservation methods in modern food technology (Baert *et al.,* 2009). Chemical preservatives prolong the shelf life of orange juice by inhibiting microbial deterioration. Commercial orange juice often contains sodium benzoate and/or potassium sorbate as a chemical preservative. Sodium benzoate (C_6H_5COONa) is mostly used in acidic foods and under certain conditions, it is converted into benzoic acid, which has bacteriostatic and fungistatic properties. Sodium benzoate is one of the most acceptable food preservatives that belong to a category of products generally recognized as safe (GRAS) and allowed by the Bangladesh Standard and Testing Authority (BSTI) in Bangladesh. However excessive addition of sodium benzoate in any beverage may present adverse health effects like allergic reactions, metabolic acidosis, hyperpnoea, urticaria, asthma to consumers (Tfouni and Toledo, 2002; Lino and Pena, 2010). Therefore, the use

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of sodium benzoate as a preservative should not exceed its permitted level of 0.1% (Code of Federal Regulations (CFR), 2023). The standard value for sodium benzoate in Bangladesh is 150.0 mg/kg (Kayshar *et al.,* 2014). This necessitates the need for regular monitoring of the use of sodium benzoate in orange juice in order to ensure quality and safety for human consumption. Therefore, this study aimed to develop a simple method to investigate the levels of sodium benzoate in different brands of orange juice by using high-performance liquid chromatography.

2. Materials and methods

2.1 Chemicals

HPLC grade acetonitrile, 97% sodium acetate were procured from Merck (Darmstadt, Germany). Glacial acetic acid and standard sodium benzoate were purchased from Sigma Chemical Co. (Darmstadt, Germany). Barnstead Nano pure water purification system (Barnstead, USA) was used to get deionized water used in this study. A total of 36 samples from four brands of orange juice were procured from the local market of Bangladesh after getting verbal consent from shopkeepers. The expiry date of all orange juice samples was within the study period. After collecting orange juice samples were preserved an appropriate temperature in a refrigerator until analysis.

2.2 Instrumentation and operating condition

The determination of sodium benzoate in the orange juice sample was determined by High Performance Liquid Chromatography (HPLC). The chromatography system consists of a Shimadzu isocratic pump (10 Avp), column, a degasser, UV-Vis detector, oven, a LC Workstation Class-VP for data analysis. Chromatographic separation with a Luna 5 µ C18 (2) 100Å analytical column (250 × 4.6 mm) with an isocratic solvent system was used separation at 37°C temperature. The mobile phase consisted of sodium acetic acid buffer whose pH was 4. The flow rate of solvent was 1 mL/min. The wavelength of the detector was noted at 254 nm.

2.3 Preparation of mobile phase

The mobile phase was prepared by modifying the method of Pylypiw and Grether (2000) that consists of 80% acetate buffer with 20% HPLC grade acetonitrile. At first 1 g of sodium acetate was mixed with 1 mL glacial acetic acid in a volumetric flask that contains 1000 mL de-ionized water and shaken well. Then 200 mL of acetonitrile was added to 800 mL of the acetate buffer solution. After mixing the solution was then filtered with a nylon-66 filter membrane, pore size 0.2 µm.

2.4 Standard solution preparation

Fifty mg sodium benzoate and 49 mL deionized water were taken in a 50 mL volumetric flask then shaken and filtered, making the concentration of the stock solution 1 mg/mL. After that, 1 mL solution from the stock solution was taken in another 50 mL volumetric flask and diluted with deionized water up to the mark. Now, the concentration of the second solution was 20 µg/mL. In the same way, 0.0, 62.5, 125, 250 and 500 µg/mL of each standard solution was prepared, filtered and finally injected into the column. The solutions were filtered through sample filters (pore size 0.2 µm) prior to injection into the column.

2.5 Sample preparation

Approximately 5 mL of orange juice was taken in a 50 mL flask and then added 50% acetonitrile for dilution. From this solution, 10 mL was taken in another flask and the same solvent up to the mark (50 mL mark). After filtering, it was injected into the column.

2.6 Experimental analysis of sodium benzoate

Initially, each sample was five times diluted with the mobile phase. The diluted sample was again ten times diluted with the mobile phase. The HPLC operation denoted the lowest point of the detection of a sample known as LOD and the lowest point of quantification known as LOQ (Akbari-Adergani *et al.,* 2018).

2.7 Recovery study

At least three serially diluted concentrations of a sample (2.5 mg/mL, 5 mg/mL and 7.5 mg/mL) were prepared by spiking of known standard sodium benzoate. The % of recoveries was calculated by dividing the observed concentration and the known concentration by multiplication with 100.

2.8 Statistical analysis

A descriptive analysis was analyzed by using Statistical Package for the Social Science software (SPSS) package version 16.0 (SPSS Inc., Chicago, IL, USA) for all variables. A one-way analysis of variance (ANOVA) was carried out and differences between mean values were considered to be significant at $p< 0.05$. All of the values are expressed as the mean±standard error mean (SEM).

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3. Results

3.1 Analysis of chromatogram

An HPLC method has been developed and validated as per ICH, USP and FDA guidelines for the determination of the sodium benzoate by using the mobile phase comprising of sodium acetate buffer (pH = 4) and acetonitrile in the ratio of 80:20 (v/v) over C-18 column at 40°C. The flow rate was at 1.0 mL/min and the eluent was monitored by a UV detector at 254 nm. The retention time of sodium benzoate was 8.1585±0.012 min (Figures 1 and 2). The calibration curve (Figure 3) for sodium benzoate was obtained by plotting the peak areas of different concentrations of working standard solutions prepared from the stock solutions. A very good linearity for sodium benzoate was obtained as it is presented in Figure 3 with an excellent regression factor (0.9999). The linear regression line was obtained y = 0.773x.

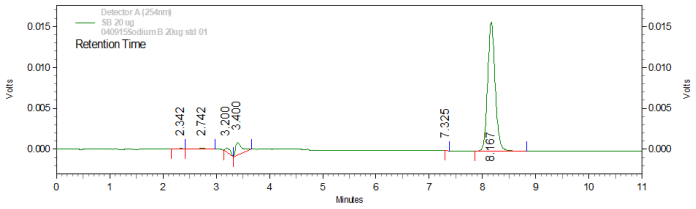

Figure 1. Chromatogram of 20 µl/L sodium benzoate standard solution.

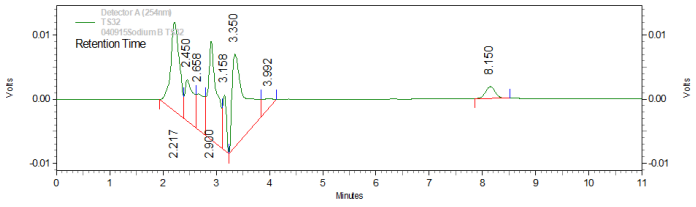

Figure 2. Chromatogram of brand 1 orange juice containing sodium benzoate.

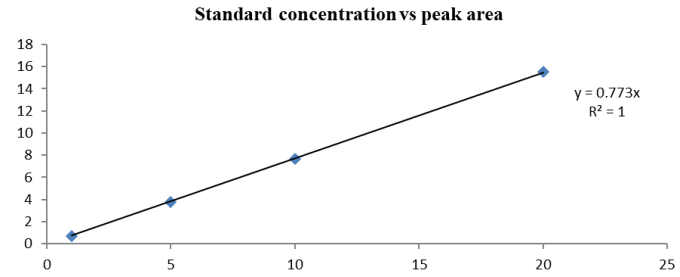

Figure 3. Calibration curve for the sodium benzoate standard.

In order to obtain greater sensitivity, the identification of sample peaks was confirmed by their UV spectra (254 nm) and by comparing their retention time with those of standard solutions of compounds. The limit of detection (LOD) and limit of quantification (LOQ) for sodium benzoate were calculated in accordance with ICH 1996 and obtained 0.0051 mg/100 mL and 0.0153 mg/100 mL respectively (Table 1).

Table 1. Analytical characteristics of HPLC method.

Parameter	Value
Accuracy	97.39±3.35
Slope	0.773
Intercept	0.00
Linearity range	0.93 µg/mL to 20.02 µg/mL
Correlation coefficient	0.9999
SE of intercept	9.15
SD of intercept	11.84
LOD	0.0051 mg/100 mL
LOQ	0.0153 mg/100 mL

3.2 Analysis of sodium benzoate in orange juice sample

Table 2 shows the concentration of sodium benzoate in all orange juice content. There was a significant difference between the labels of sodium benzoate in Brand 1, Brand 2, Brand 3 and Brand 4 which were 14.61±0.31, 16.11±0.28, 16.33±0.06 and 9.59±0.15 mg/100 mL respectively. Table 3 shows that sodium benzoate concentration in orange juice samples which were within the range of US FDA standard range of 0.1% (100 mg/100 mL). But based on BSTI standard range (15 mg/100 mL) brand 2 and brand 3 orange juice exceeds the level of sodium benzoate (16.11 and 16.33 mg/100 mL respectively) (Figure 4).

Table 4 shows the percentage recovery of brand 3 orange juice. The known amount of sodium benzoate was added to Brand 3 orange juice at three different levels of concentration considered: low (2.5 µg/mL), medium (5.0 µg/mL) and high (7.5 µg/mL). The percentage recovery of three concentrations was 98.16±0.60, 99.20±0.04 and 98.11±1.03 respectively.

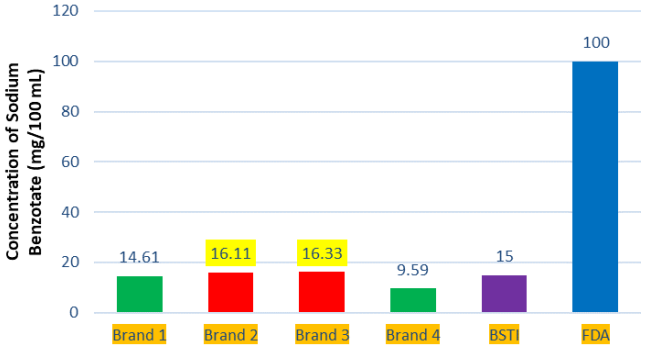

Figure 4. Overall comparison of sodium benzoate concentration among different brands of orange juices with standard range.

4. Discussion

The analytical determination of these preservatives is not only important for quality assurance purposes but also for consumer interest and protection. Since the maximum permitted concentrations of preservatives in each type of food are controlled by legislation, their

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Table 2. Concentration of sodium benzoate from different brands of orange juices.

	Shop	Concentration of Sodium Benzoate (mg/100 mL)			
		Sample 1	Sample 2	Sample 3	Mean±SD (mg/100 mL)
Brand 1	1	14.37	14.05	14.47	14.30±0.22
	2	14.67	14.37	14.77	14.61±0.21
	3	14.63	15.05	15.07	14.92±0.25
Brand 2	1	16.05	17.70	15.62	16.42±1.09
	2	15.97	16.17	15.7	15.95±0.24
	3	16.05	15.95	15.9	15.92±0.76
Brand 3	1	16.27	16.3	16.22	16.26±0.04
	2	16.47	16.3	16.37	16.38±0.09
	3	16.27	16.12	16.65	16.35±0.27
Brand 4	1	9.70	9.65	9.65	9.67±0.03
	2	9.60	9.37	9.27	9.42±0.17
	3	9.62	9.67	9.80	9.70±0.09

Table 3. Concentration of sodium benzoate from different brands of orange juices in comparison with BSTI and FDA.

	Concentration of sodium benzoate (mg/100 mL)			Mean±SD (mg/100 mL)	P value	BSTI standard (mg/100 mL)	FDA standard (mg/100 mL)
	Shop 1	Shop 2	Shop 3				
Brand 1	14.3	14.61	14.92	14.61±0.31	$P^a = 0.000$; $P^b = 0.000$	15	100
Brand 2	16.42	15.95	15.92	16.11±0.28	$P^a = 0.000$; $P^b = 0.000$		
Brand 3	16.26	16.38	16.35	16.33±0.06	$P^a = 0.000$; $P^b = 0.000$		
Brand 4	9.67	9.42	9.70	9.59±0.15	$P^a = 0.000$; $P^b = 0.000$		

P^a : value compared with BSTI, P^b : value compared with FDA

Table 4. Percentage recovery of sodium benzoate from spiked sample.

Sample	Concentration before spike (mg/100 mL)	Spiked level (µg/mL)	% Recovery (Mean±SD)
Brand 3	16.26	2.50	98.16±0.60
	16.38	5.00	99.20±0.04
	16.35	7.50	98.11±1.03

determination is a mandatory step in routine food analyses. Sodium benzoate was analyzed by the HPLC identification of sample peaks confirmed by their UV spectra (254 nm) and by comparing their retention time with those of standard solutions of compounds. The limit of detection (LOD) and limit of quantification (LOQ) for sodium benzoate were calculated in accordance with ICH 1996 and obtained 0.0051 mg/100 mL and 0.0153 mg/100 mL respectively. The thirty-six samples of orange juices from four different brands available in the market were analyzed for the levels of sodium benzoate. All the brands selected for this study had declared to contain sodium benzoate as a preservative. The concentration of the samples ranged from 9.15 mg/100 mL to 17.8 mg/100 mL. Brand 2 recorded the highest concentration of 17.8 mg/100 mL and brand 4 recorded the lowest concentration of 9.15 mg/100 mL. All of the four brands contain sodium benzoate of 14.61, 16.11, 16.33 and 9.59 mg/100 mL, respectively. These findings are similar to the findings of Akbari-Adergani *et al*. (2018) who found 5.84±0.05 to 23.12±0.19 mg/100 mL sodium benzoate in orange juice samples. The mean concentration of all the samples was 14.16 mg/100 mL. The coefficient variation observed was not high, although the samples were produced from different companies and therefore each company has its own production practices. Brand 1 and Brand 4 samples contained sodium benzoate 14.61 and 9.59 mg/100 mL respectively which were within the level BSTI standard range whereas Brand 2 and Brand 3 samples contained 16.11 and 16.33 mg/100 mL respectively which exceeded the level of BSTI standard (150 mg/kg) (Kayshar *et al.,* 2014). The FDA has limited usage of sodium benzoate to 0.1% of a product by weight (Shahmohammadi *et al.,* 2016). Sodium benzoate is a widely used preservative found in many foods and soft drinks. It is metabolized within mitochondria to produce hippurate, which is then cleared by the kidneys. Ingestion of sodium benzoate at the generally regarded as safe (GRAS) dose leads to a robust excursion in the plasma hippurate level (Lennerz *et al.,* 2015). Although these preservatives are frequently used in various food

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products, they are harmful at higher than permitted safety levels. Sodium benzoate has the chemical formula $NaC_7H_5O_2$; it is a widely used food preservative, with E number E211. It is used to prevent food from molding. It is especially used to preserve acidic foods and beverages such as pickles, salad dressings, fruit juices, and soft drinks. It is the sodium salt of benzoic acid and exists in this form when dissolved in water. When sodium benzoate combines with vitamin C in foods, it can create benzene, a carcinogen that causes leukemia and other cancers (Mirza *et al.,* 2017). The risk is low, but it's there. Those with food allergies may also experience a negative reaction, asthma or hives after ingesting, and it may increase hyperactivity in children. The name of the preservatives used in juice products and their specific quantity should be declared on the label. Government agencies such as Bangladesh Council of Scientific and Industrial Research and Bangladesh Standard and Testing Institute should take control and regular monitoring to check the level of sodium benzoate in all brands of juices.

5. Conclusion

The result showed all the selected brands of orange juices used sodium benzoate as a preservative. The concentration of sodium benzoate varied a little between the different brands of orange juices and no brands used excess amount of sodium benzoate. The extraction procedures described were economical, time-saving and easy to carry out. The general detection of sodium benzoate in all the samples implies that this is the most frequently used preservative by the manufacturers.

Conflict of interest

The authors declare that no competing interests exist.

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References

Akbari-Adergani, B., Poorasad, M. and Esfandiari, Z. (2018). Sunset yellow, tartrazine and sodium benzoate in orange juice distributed in Iranian market and subsequent exposure assessment. *International Food Research Journal*, 25(3), 975-981.

Baert, L., Debevere, J. and Uyttendaele, M. (2009). The efficacy of preservation methods to inactivate foodborne viruses. *International Journal of Food Microbiology,* 131(2-3), 83-94. https://doi.org/10.1016/j.ijfoodmicro.2009.03.007

Code of Federal Regulations (CFR). (2023). Potassium sorbate. 21CFR182.3640. Retrieved from website: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr = 182.3640

Franke, A.A., Cooney, R.V., Henning, S.M. and Custer, L.J. (2005). Bioavailability and antioxidant effects of orange juice components in humans. *Journal of Agricultural Food Chemistry*, 53(13), 5170-5178. https://doi.org/10.1021/jf050054y

Kayshar, M.S., Saifullah, M., Rahman, A. and Uddin, M.B. (2014). An overview of quality status of selected commercial brands of juices and jams based on public perception and laboratory analysis. *Journal of Bangladesh Agricultural University*, 12, 183-188. https://doi.org/10.3329/jbau.v12i1.21410

Keyser, M., Műller, I.A., Cilliers, F.P., Nel, W. and Gouws, P.A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science and Emerging Technologies*, 9(3), 348-354. https://doi.org/10.1016/j.ifset.2007.09.002

Lennerz, B.S., Vafai, S.B., Delaney, N.F., Clish, C.B., Deik, A.A., Pierce, K.A. and Mootha, V.K. (2015). Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. *Molecular Genetics and Metabolism*, 114(1), 73-79. https://doi.org/10.1016/j.ymgme.2014.11.010

Lino, C.M. and Pena, A. (2010). Occurrence of caffeine, Saccharin, benzoic acid and sorbic acid in soft drinks and nectars in Portugal and subsequent exposure assessment. *Food Chemistry,* 121(2), 503-508. https://doi.org/10.1016/j.foodchem.2009.12.073

Mirza, S.K., Asema, U.K. and Kasim, S.S. (2017). To study the harmful effects of food preservatives on human health. *Journal of Medicinal Chemistry and Drug Discovery*, 2, 610-616.

Motallaei, M., Ramezani-Jolfaie, N., Mohammadi, M., Shams-Rad, S., Jahanlou, A.S. and Salehi-Abargouei, A. (2021). Effects of orange juice intake on cardiovascular risk factors: A systematic review and meta-analysis of randomized controlled clinical trials. *Phytotherapy Research*, 35(10), 5427-5439. https://doi.org/10.1002/ptr.7173

Pylypiw, H.M.J and Grether, M.T. (2002). Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods. *Journal of Chromatography A,* 883(1-2), 299-304. https://doi.org/10.1016/S0021-9673(00)

00404-0

Rampersaud, G.C. and Valim, M.F. (2017). 100% citrus juice: nutritional contribution, dietary benefits, and association with anthropometric measures. *Critical Reviews in Food Science and Nutrition,* 57(1), 129-140. https://doi.org/10.1080/10408398.2013.862611

Rangel-Huerta, O.D., Aguilera, C.M., Martin, M.V., Soto, M.J. and Rico, M.C. (2015). Normal or high polyphenol concentration in Orange juice affects antioxidant activity, blood pressure, and body weight in obese or overweight adults. *The Journal of Nutrition*, 145(8), 1808–1816. https://doi.org/10.3945/jn.115.213660

Rodríguez, M., Oteiza, J., Giannuzzi, L. and Zaritzky, N. (2017). Evaluation of mutagenicity associated with *Escherichia coli* inactivation in UV-treated orange juice. *Toxicological and Environmental Chemistry*, 99(2), 315-330. https://doi.org/10.1080/02772248.2016.1178752

Shahmohammadi, M., Javadi, M. and Nassiri-Asl, M. (2016). An overview on the effects of sodium benzoate as a preservative in food products. *Biotechnology and Health Sciences*, 3(3), 7-11. https://doi.org/10.17795/bhs-35084

Tfouni, S.A.V. and Toledo, C.F. (2002). Determination of benzoic and sorbic acids in Brazilian foods. *Food Control,* 13(2), 117-123. https://doi.org/10.1016/S0956-7135(01)00084-6

