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Identification and quantification of sodium benzoate in soft drinks available in Tangail region by high-performance liquid chromatography

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Abstract

Sodium benzoate is a permitted food additive by international laws in processing restrictive amounts, but its content must be declared and must not exceed the established limits by legislation. An experimental study was performed by high-performance liquid chromatography (HPLC) for determining the level of sodium benzoate in different brands of soft drinks available in the markets, stores, and shops in the Tangail region of Bangladesh. A Luna 5 µ C18 (2) 100A column (250×4.6 mm) was used for the chromatographic analysis. Chromatographic separation was achieved with an isocratic solvent system comprising sodium acetate and acetic acid buffer (pH = 4.0)/acetonitrile in the ratio of 80:20 (1 mL/min) at 37°C and the chromatograms were recorded at 254 nm. The limit of detection and quantification for sodium benzoate was 0.0000493 mg/100 mL and 0.000149 mg/100 mL, respectively. Quantification of the selected brand soft drinks revealed that the level of the used sodium benzoate was within the FDA standard range. However, by comparing with the Bangladesh Standard and Testing Institute (BSTI), brand -2 and brand-3 soft drink samples were found to exceed the current legal limits.

1. Introduction

Soft drink consumption has become part of the modern lifestyle since the nineteenth century. Like other countries in the world, soft drink consumptions are very common in Bangladesh especially very popular among children (Doris *et al.*, 2019). Typically, it contains carbonated water, sweetener, natural or artificial flavourings, colourings, preservatives and more. The sweetener may be sugar, high-fructose corn syrup, sugar substitutes, fruit juices, or a different combination of these (Spitkovsky and Chang, 2012). The ingredients used in the soft drink as a food additive are approved and closely regulated by bodies such as the US Food and Drug Administrator and the local food standard agencies (FDA 2004). Sodium benzoate ($NaC_7H_5O_2$) is one of the most widely used food additives in soft drinks due to its bacteriostatic and fungistatic properties in acidic conditions (Dar *et al.*, 2017). It is the sodium salt of benzoic acid and exists in this form when dissolved in water (Sharif *et al.*, 2017). Benzoic acid, its salts like sodium benzoate and its esters occurs naturally in many foods (Del-Olmo *et al.*, 2017). For instance, sodium benzoate may be used as a preservative in soft drinks. However, its usage should not result in levels exceeding 0.1% (CFR, 1999). In Bangladesh, the reference value (BSTI standard) for sodium benzoate is 150.0 mg/kg (Kayshar *et al*., 2014). Daily high intake can bring adverse effects on the skin such as rash, non-immunological contact urticarial, hyperpnoea, metabolic acidosis, and asthma (Coverly, 1998). In children, it is reported to cause hyperactivity, and in those with spastic paralysis, it may cause severe side effects. Systemic toxic effects on the liver and kidney and adverse carcinogenic consequences for its short- and long-term exposures were observed (Ree and Stoa, 2011; Chua and Teo, 2017). Therefore, the analytical determination of sodium benzoate is very important for quality assurance as well as for consumer interest and protection (Shamoli *et al.,* 2017). There are various methods for the analysis of benzoates such as thin-layer chromatography, high-performance liquid chromatography (HPLC) UV spectroscopy, and gas chromatography. So, the objective of this study was to develop a simple method that provides accurate results for sodium benzoate in different brands of soft drinks available in Bangladesh.

2. Materials and methods

2.1 Chemicals and reagents

HPLC grade sodium acetate (97%) and acetonitrile

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were purchased from Merck, Darmstadt, Germany; glacial acetic acid and anhydrous sodium benzoate were purchased from Sigma Chemical Co., Germany. Different brands of soft drinks were purchased from local markets in Tangail town, Bangladesh. A total of ninety samples were collected for the experiment of sodium benzoate analysis. The expiry dates of all study samples were within the study period. The volumes of the samples were 250 mL (8 brands) and 330 mL (2 brands).

2.2 HPLC system

The chromatographic system consisted of a Shimadzu isocratic pump, column, a degasser, oven, a UV-Vis detector, and an LC workstation class-VP. About 20 ml of the sample was injected into the injector. A Luna 5m C18 (2) 100A column (250×4.6 mm) was used for the chromatographic analysis and the column temperature was set at 37°C. The sodium benzoate analysis was performed with isocratic solvent system of sodium acetate and acetonitrile (80:20) with a flow rate of 1 mL/min. and chromatograms were recorded at 254 nm.

2.3 Mobile phase preparation

The mobile phase comprising 80% acetate buffer (1 g glacial acetic acid and 1 g sodium acetate in 1 L deionized water) with 20% HPLC grade acetonitrile was prepared using the modified method of Pylypiw and Grether (2000). About 1 mL of glacial acetic acid and 1000 mg of sodium acetate was taken in a 1000 mL volumetric flask containing about 50 mL de-ionized water and shaken well. After that, the deionized water was added up to the mark to make 1000 mL and was mixed well. About 20 mL of acetonitrile were added to 80 mL of the acetate buffer solution and mixed properly. The mixture was then filtered with a nylon-66 (pore size 0.2 µm) filter membrane.

2.4 Preparation of standard solution

Approximately 50 mg of anhydrous sodium benzoate and 20 mL of 50% aqueous acetonitrile were taken in a volumetric flask up to mark and shaken well. The solution was then filtered through a syringe filter and labelled as standard stock solution-1 (1 mg/mL). After that, 1 mL of stock solution-1 was taken in a 50 mL volumetric flask and added mobile phase up to the mark and labelled as standard solution-2 (20 mg/mL standard solution). Aliquot of 0.0, 1.70, 3.13, 6.25, 12.5, and 25 µL of each standard solution-2 was taken into Eppendorf tubes and diluted to volume 1.0 mL with mobile phase and mixed well with a vortex mixer.

2.5 Preparation of sample solution

Approximately 1 mL of soft drink was added to a 50 mL volumetric flask. Aqueous 25% acetonitrile and 75% water were added up to the mark of a 50 mL volumetric flask and mixed properly. About 5mL of the solution was filtered with a sample filter; pore size 0.2 µm and 20 µg were injected onto the column.

2.6 Experimental analysis of sodium benzoate

A high-performance liquid chromatography technique was used to determine the concentrations of sodium benzoate in the samples by using the modified procedures described by Pylypiw and Grether (2000). Each of the samples of 1.0 mL was mixed with mobile phase marked up to volume 50 mL. The clear aqueous solution was filtered through a PTFE syringe filter. Then the solution was transferred to the dry HPLC vials and was injected into the column for detection and quantification.

2.7 Limit of detection and quantification

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value while the limit of quantification (LOQ) refers to the lowest level of analyte which can be determined with an acceptable degree of confidence. In this work, the detection limit (LOD) and quantification limit (LOQ) values were calculated based on the standard deviation of the response and the slope of the calibration curve (ICH, 1996). The concentration was multiplied by 3 and 10 to obtain the limit of detection and quantification, respectively.

2.8 Recovery study

To verify the accuracy and precision of the analytical procedure, recovery studies were carried out by spiking some samples with very low levels of sodium benzoate (2.0 µg/mL, 4.0 µg/mL, and 8.0 µg/mL) from a known standard. In this work, 2.0 mL of each sample mixture and 2.0 mL of 25.0 mg/L standards were taken, mixed, and injected. Due to the dilution, the actual concentration becomes 12.5 mg/L. The observed concentrations and the known concentration are divided and then multiply by 100 to obtain the percentage of recoveries.

2.9 Statistical analysis

All the sample analyses were performed in triplicate and descriptive statistics were analyzed by using SPSS software package version 16.0 (SPSS Inc., Chicago, IL, USA) for all variables. The significance of the differences between the means of the two groups was determined by independent sample Student's t-test. Differences were considered to be significant at $p<0.05$.

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3. Results and discussion

3.1 Analysis of chromatogram

HPLC is the most convenient and accurate technique for analyzing analysis bulk and finished pharmaceutical products. An RP-HPLC method has been developed and validated as per ICH, USP, and FDA guidelines for determination of the sodium benzoate by using the mobile phase comprising sodium acetate buffer (pH = 4) and acetonitrile in the ratio of 80:20 (v/v) over C-18 column at 37°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 9.41±0.13 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. Very good linearity for sodium benzoate was obtained as is presented in figure 3 with an excellent regression factor (0.9995). A linear regression line was obtained y = 808.77x-384.99 (Table 1).

Table 1. Analytical characteristics of HPLC method

Parameter	Value
Accuracy	102.4426751
Slope	808.77
Intercept	384.99
Linearity range	1.9 µg/mL to 25.1 µg/mL
Correlation coefficient	0.9995
SE of intercept	9.77
SD of intercept	0.012
LOD	0.0000493 mg/100 mL
LOQ	0.000149 mg/100 mL

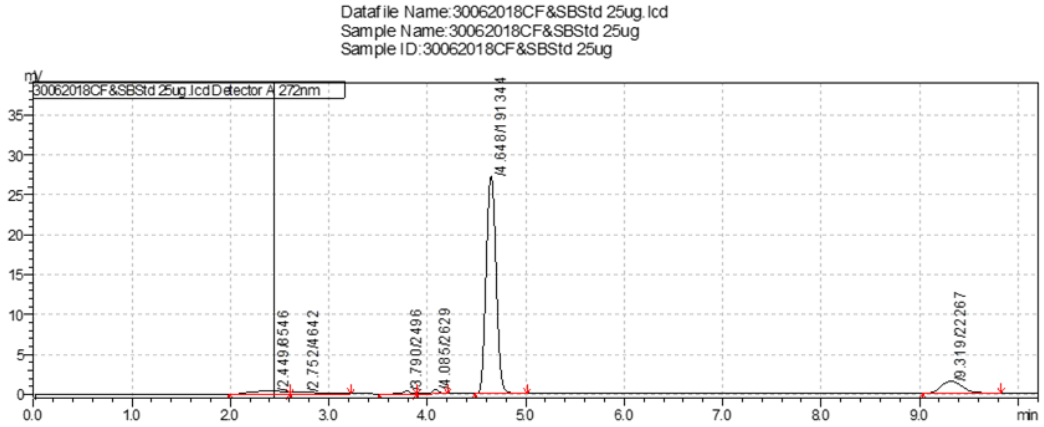

Figure 1. HPLC Chromatogram of 25 µg/L sodium benzoate standard solution

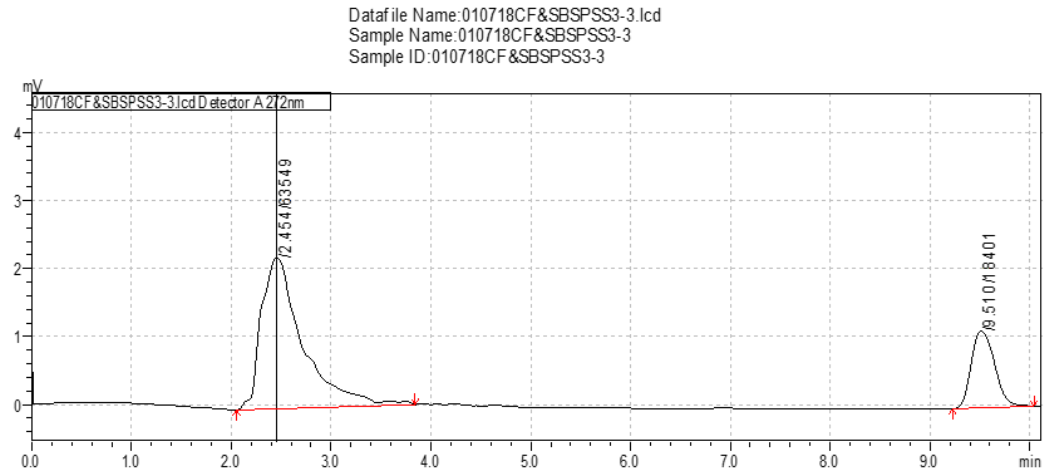

Figure 2. HPLC chromatogram of 20 µg/L sodium benzoate sample solution

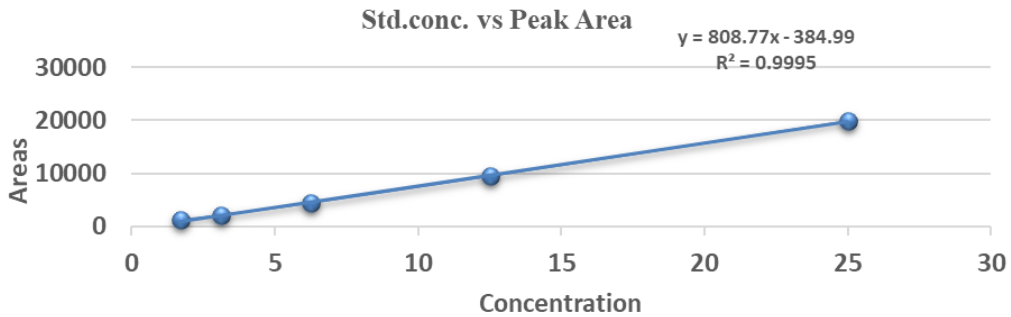

Figure 3. Calibration curve for the sodium benzoate standard solution

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3.2 Analysis of sodium benzoate in soft drinks sample

Table 2 shows the concentration of sodium benzoate in all soft drinks samples. There was no significant difference found between the label of sodium benzoate in brand-1, brand-2, brand-4 and brand-6 which was 13.44±0.26, 15.49±0.63, 14.68±0.30 and 12.72±0.28 mg/100 mL respectively. Sodium benzoate in brand-7, brand-8, brand-9 and brand-10 soft drinks was not detected. The lowest concentration of sodium benzoate (11.18 mg/100 mL) was found in the brand-5 sample whereas the highest concentration (18.64 mg/100 mL) was found in the brand-3 soft drinks sample. Another research group in Bangladesh conducted a similar type of study and found similar findings 18.1-19.1 mg/100 mL of sodium benzoate in commercial available soft drinks (Sultana *et al.,* 2016). A very recent study conducted by Azuma *et al*. (2020) sampled markets in Ghana and found similar results (13.15 mg/100 mL) in non-alcoholic carbonated (soft) drinks. Whereas concentration of sodium benzoate found in this study is quite higher than reported in Japan (2 mg/100 mL) and the Philippines (5 mg/100 mL) soft drinks sample (Villanueva *et al.*, 1994).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established daily allowable intake (0-5 mg/kg body weight per day) of benzoic acid and benzoate salts (Lazarevic *et al.,* 2011). In this study, the concentration of sodium benzoate in all brands of soft drinks was within the standard range (100 mg/100 mL) stated by US FDA but exceed the standard range (15 mg/100 mL) of BSTI (Table 3). Brand-2 and brand-3 soft drinks samples contained sodium benzoate of 15.49 mg and 18.64 mg/100 mL respectively which is quite high compared to the BSTI standard. This concentration is quite similar to the findings (18.1-19.1 mg/100 mg) recorded by Sultana *et al.* (2016) in the soft drinks sample. Concentration in brand-1, brand-4, brand-5 and brand-6 were within the limit of BSTI standard.

Table 4 shows the per cent recovery of brand-2 soft drinks. The known amount of sodium benzoate was added to Brand-2 soft drinks at three different levels of concentration considered as low (2.0 µg/mL), medium (4.0 µg/mL) and high (8.0 µg/mL). The per cent recovery of three concentrations was 90.18±2.9, 90.17±3.9.98 and 93.24±4.8.

Table 2. Concentration of sodium benzoate in soft drink sample

Sample	Shop	Concentration of Sodium Benzoate (mg/100 mL)			Mean ±SD
		Sample 1	Sample 2	Sample 3	(mg/100 mL)
Brand 1	1	13.50	12.20	13.90	13.19±0.88
	2	12.90	13.90	14.24	13.71±0.64
	3	13.20	13.60	13.48	13.41±0.20
Brand 2	1	15.65	15.05	15.54	15.42±0.32
	2	14.50	15.10	15.10	14.90±0.34
	3	16.25	15.98	16.25	16.16±0.15
Brand 3	1	20.57	20.45	20.14	20.39±0.22
	2	19.36	19.91	20.00	19.76±0.34
	3	14.93	15.89	16.49	15.77±0.78
Brand 4	1	14.97	14.67	14.49	14.71±0.24
	2	15.00	15.22	14.68	14.97±0.27
	3	14.36	14.50	14.25	14.37±0.12
Brand 5	1	10.01	10.59	11.01	10.54±0.50
	2	11.12	10.67	12.31	11.37±0.84
	3	10.23	12.34	12.30	11.63±1.21
Brand 6	1	13.23	11.88	13.93	13.02±1.04
	2	12.23	12.34	13.53	12.70±0.72
	3	13.09	11.12	13.14	12.45±1.15
Brand 7	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
Brand 8	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
Brand 9	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND
Brand 10	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	ND	ND	ND	ND

Values are presented as mean±SD. ND: not detected

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Table 3. Summary of the concentration of sodium benzoate in soft drinks sample

Sample	Concentration of sodium benzoate (mg/100 mL)			Mean ± SD	BSTI	FDA	P -value
	Sample 1	Sample 2	Sample 3	(mg/100 mL)	(mg/100 mL)	(mg/100 mL)	
Brand 1	13.19	13.71	13.41	13.44±0.26			$P^a = 0.000$; $P^b = 0.000$
Brand 2	15.42	14.9	16.16	15.49±0.63			$P^a = 0.000$; $P^b = 0.000$
Brand 3	20.39	19.77	15.77	18.64±2.50			$P^a = 0.000$; $P^b = 0.000$
Brand 4	14.71	14.97	14.37	14.68±0.30			$P^a = 0.000$; $P^b = 0.000$
Brand 5	10.54	11.37	11.63	11.18±0.56			$P^a = 0.000$; $P^b = 0.000$
Brand 6	13.02	12.7	12.45	12.72±0.28			$P^a = 0.000$;$P^b = 0.000$
Brand 7	ND	ND	ND	ND	15	100	
Brand 8	ND	ND	ND	ND			
Brand 9	ND	ND	ND	ND			
Brand 10	ND	ND	ND	ND			

Values are presented as mean±SD. ND: not detected, P^a: value compared with BSTI, P^b: value compared with FDA.

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

Sample	Concentration before spike (mg/100 mL)	Spiked level (µg/mL)	% Recovery (Mean±SD)
Brand-2	15.42	2	90.18±2.9
	14.90	4	90.17±3.9
	16.16	8	93.24±4.8

4. Conclusion

The outcome of the present study revealed that all soft drinks samples contained sodium benzoate within the permitted range set by the international body FDA but two out of six exceed the permitted range of the national authority set by BSTI. Thus, the government authorized agencies should take control and regular monitoring to check the level of sodium benzoate in all soft drinks available in the Bangladeshi market.

Conflict of interest

The authors declare no conflict of interest.

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