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Study on the identification and quantification of sodium benzoate in different brands of jelly by high performance liquid chromatography

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Abstract

A reversed-phase high-performance liquid chromatography analysis was carried out for rapid determination and quantification of sodium benzoate in different jelly products. Sodium benzoate is allowed food additive by universal laws in processing in restrictive amounts, but its content must be declared and must not exceed the established limits by legislation. An experimental study for the level of sodium benzoate in different brands of jelly available in the markets, stores and shops in the Tangail region in Bangladesh was determined by high-performance liquid chromatography. Chromatographic separation was achieved with isocratic solvent system of sodium acetate and acetic acid buffer (pH = 4.0): acetonitrile as 70:30 with a flow rate of 0.8 mL/min; chromatograms were recorded at 254 nm. The injection volume was 20 µL. The concentrations of sodium benzoate in all the samples were calculated by the external standard method with calibration of correlation coefficient 0.985 from the standards calibration curves. The limit of detection and quantification for sodium benzoate was 0.0003 mg/100 mL and 0.0009 mg/100 mL respectively. Quantification of the selected brand jellies revealed that the level of the used sodium benzoate was within the FDA standard range. But by comparing with the Bangladesh Standard and Testing Institute (BSTI), Brand-3 jelly samples were found to exceed the current legal limits.

1. Introduction

Jelly is a dessert prepared from gelatin (Yusof *et al.,* 2019). Jelly originates from the Old French gelee, "jelly" and also "frost," from the verb geler, "to congeal," with its Latin root gelare, "to freeze (Rabilloud, 2002). Using food preservation methods has been conjoint both naturally and chemically for the past 1000 to 8,000 years (Knbab *et al.,* 2016). A large number of chemical compounds are dynamic food preservatives, yet because of authoritarian laws on food safety that have been ratified by the FDA and to a lower extent due to the fact that all compounds display antimicrobial effects, adding these compounds to some food products has no upshot and only a few of them are acceptable for use in food products (Bhunia *et al.,* 2013; Makwana *et al.,* 2014). Food preservatives must be within allowable safety limits, which must not exceed the maximum allowed concentration of sodium benzoate and potassium sorbate 0.1% and 0.2%, respectively (Smith and Pell, 2003; Gören *et al.,* 2015). Although sodium benzoic is in the set of safe additives, the snags of synthetic preservatives such as sodium benzoate on human health have been earlier stated (Yolmeh *et al.,* 2014). There are many analytical methods used in qualitative and quantitative appreciation of sodium benzoate in foods such as UV-vis spectrophotometry, HPLC methods, gas chromatography, capillary electrophoresis and polarography (Michael *et al.,* 2005). Conserved foods help people to carry a variety in their diet, thereby declining nutritional inadequacies (Alghamdi, 2005). Preservatives can be used at a relatively low level to indorse that the product does well over time, which is usually one to three years (Sivakumar and Ghosh, 2017). Sodium benzoate is also trained as an animal food additive at up to 0.1%, according to AFCO's official publication (Khoshnoud *et al.,* 2018). Sodium benzoate is secondhand as an action for urea cycle complaints due to its capacity to bind amino acids (Häberle *et al.,* 2012), this clues to the flow of these amino acids and a

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reduction in ammonia levels. Sodium benzoate has been publicized to stop the progression of Parkinson's in mice (Wilcken, 2004). So, the objective of this study was to develop a simple method that provides accurate results for sodium benzoate in different brands of jelly available in Bangladesh.

2. Materials and methods

2.1 Chemicals

HPLC grade sodium acetate (97%) and acetonitrile were purchased from Merck, Darmstradt, Germany; glacial acetic acid and anhydrous sodium benzoate were purchased from Siga Chemical Co., Germany. The different brands, as well as different batches of jellies, were purchased from the market and supermarket in Tangail town. A total of twenty-seven samples were collected for the experiment of sodium benzoate analysis. The expiry date of all samples was within the study period. The volumes of the samples were 250 mL.

2.2 HPLC system

The chromatographic system consisted of a Shimadzu isocratic pump, a degasser, column, Oven; a UV-V is detector, an LC Workstation Class-VP for data acquisition and analysis (Saad *et al.,* 2005; Antakli *et al.,* 2010; Khade and Mirgane, 2014). An aliquot of 20 µL of the sample was injected into the injector. A Luna 5 µ C18 (2) 100A column (length 250×4.60 mm) was used for the chromatographic analysis and the column temperature was set at 40°C (Rana *et al.,* 2011). The sodium benzoate analysis was performed with isocratic solvent system sodium acetate and acetic acid buffer (pH= 4.0): acetonitrile- 70:30 with a flow rate of 0.8 mL/ min. chromatograms were recorded at 254 nm.

2.3 Mobile phase preparation

The mobile phase comprising 80% acetate buffer with 20% HPLC grade acetonitrile was prepared using the modified method (Pylypiw and Grether, 2000). About 1 mL of glacial acetic acid and 1000 mg of sodium acetate were 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 was added to 80 mL of the acetate buffer solution and mixed well. 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 50% aqueous acetonitrile were taken in a volumetric flask up to mark and shake 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 µg/mL standard solution). Aliquot of 0.0, 31.25, 62.5, 250 and 500 µL of each standard solution-2 was taken into Eppendorf tubes and diluted to volume 1 mL with mobile phase and mixed well with a vortex mixer.

2.5 Preparation of sample solution

Approximately 5 g of jelly was taken in a 50 mL volumetric flask. Aqueous 50% acetonitrile was added up to the mark of 50 mL volumetric flask and mixed well. 10 mL of the solution was taken in another 50 mL volumetric flask and added the same solvent up to the mark. About 5 mL of the solution was filtered with a sample filter; pore size 0.2 µm and 20 µL was injected into 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 mL was diluted 1:5 with the mobile phase. The diluted sample was again diluted 1:10 with the mobile phase. The clear aqueous solution was filtered through a PTFE syringe filter. Then the solution was transferred to the dry HPLC vials and was injected onto 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 a 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

In order 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 study, 2.0 mL of each sample mixture and 2.0 mL of 25.0 mg/L standards were taken, mixed together and injected. Due to the dilution, the

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actual concentration becomes 12.5 mg/L. The observed concentration and the known concentration are divided and then multiply by 100 to obtain the % 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 $p<0.05$.

3. Results and discussion

3.1 Analysis of chromatogram

HPLC is the most convenient and accurate technique for the analysis bulk and finished pharmaceutical products. An RP-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 70:30 (v/v) over C-18 column at 40°C. The flow rate was at 0.8 mL/min and the eluent was monitored by a UV detector at 254 nm. The retention time of sodium benzoate was 7.886±0.1025 mins (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 it is presented in Figure 3 with an excellent regression factor (0.985). A linear regression line was obtained y = 9131.3x (Table 1).

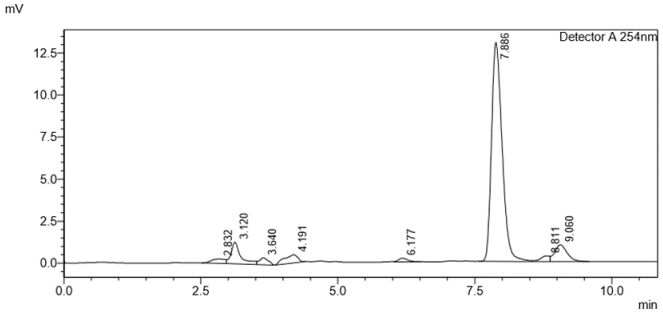

Figure 1. Chromatogram of 20 µl/L sodium benzoate standard solution

Table 1. Analytical characteristics of HPLC method

Parameter	Value
Accuracy	97.99±12.91
Slope	9131.3
Intercept	0
Linearity range	0.44 µg/mL to 19.16 µg/mL
Correlation coefficient	0.985
SE of intercept	0.83
SD of intercept	0.909
LOD	0.0003 mg/100 mL
LOQ	0.0009 mg/100 mL

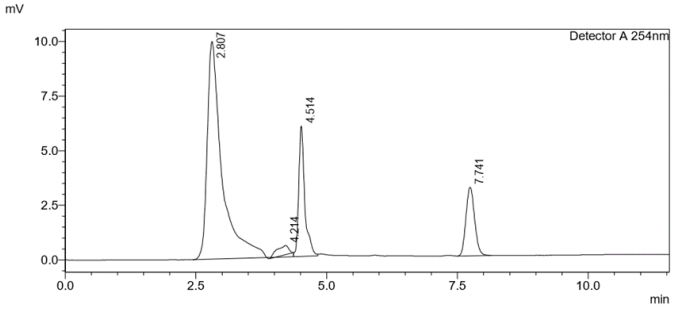

Figure 2. Chromatogram of Brand 1 jelly containing sodium benzoate

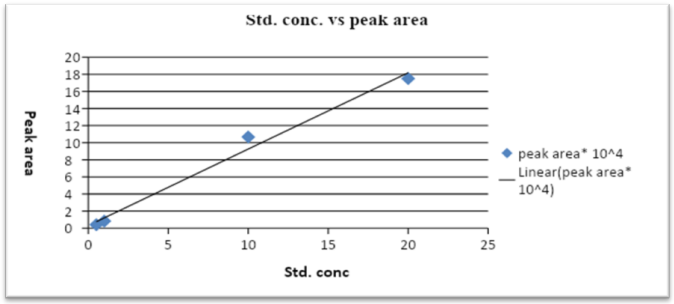

Figure 3. Calibration curve for the sodium benzoate standard

3.2 Analysis of sodium benzoate in jellies sample

Table 2 shows the concentration of sodium benzoate in all jellies content. There was a significant difference between the label of sodium benzoate in Brand 1, Brand 2 and Brand 3 which were 3.27±0.39, 11.45±1.4, and

Table 2. Concentration of sodium benzoate from different brands of jelly

	Shop	Concentration of sodium benzoate (mg/100 mL)			Mean±SD (mg/100 mL)
		Sample 1	Sample 2	Sample 3	
Brand 1	1	1.85	5.16	4.08	3.69±1.37
	2	2.47	3.54	3.71	3.24±0.67
	3	3.24	2.82	2.65	2.90±0.30
Brand 2	1	10.92	8.49	10.55	9.98±1.30
	2	10.82	11.63	11.77	11.41±0.51
	3	17.49	10.42	10.98	12.96±3.93
Brand 3	1	22.94	22.69	22.85	22.82±0.12
	2	24.23	25.80	31.33	27.12±3.72
	3	27.49	26.39	26.09	26.65±0.73

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

	Concentration of sodium benzoate (mg/100 mL)			Mean±SD (mg/100 mL)	BSTI (mg/100 mL)	FDA (mg/100 mL)	P -value
	Shop 1	Shop 2	Shop 3				
Brand 1	3.69	3.24	2.90	3.27±0.39	15	100	P^a=0.000; P^b=0.000
Brand 2	9.98	11.41	12.96	11.45±1.49			P^a=0.000; P^b=0.000
Brand 3	22.82	27.12	26.65	25.53±2.35			P^a=0.000; P^b=0.000

Values are presented as mean±SD. P^a value compared with BSTI, P^b value compared with FDA (Food and Drug Administration).

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 2	9.98	2	94.18±2.9
	11.41	4	95.17±3.9
	12.96	8	93.24±4.8

25.53±1.9 mg/100 mL respectively. This concentration is quite similar to the findings (2.6972 mg/100 mL) recorded by (Sarower *et al.,* 2015). Table 3 shows that sodium benzoate concentration from all brands of jellies was within the range according to the US FDA standard range of 0.1% (100 mg/100 mL). But according to the BSTI standard range-150 ppm (15 mg/100 mL) brand 3 jelly exceeds the level of sodium benzoate as was 25.53 mg/100 mL (Figure 4). Table 4 shows the % recovery of brand 2 jelly. The known amount of Sodium Benzoate was added to Brand 2 jelly 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 % recovery of three concentrations was 94.18±2.9, 95.17±3.9 and 93.24±4.8.

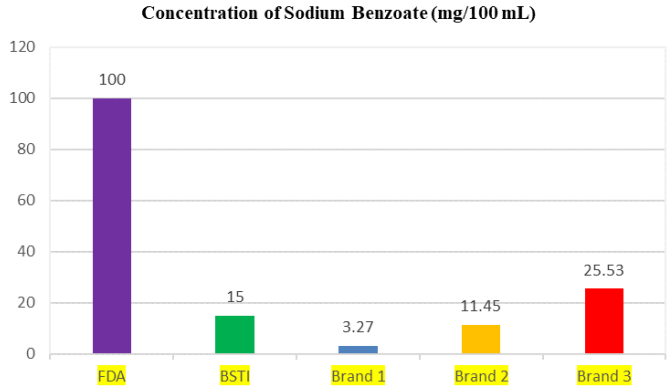

Figure 4. Overall comparison of sodium benzoate concentration among different brands of jelly with a standard range

4. Conclusion

The outcome of the present study revealed that brand 1 and brand 2 jelly contained sodium benzoate within the permitted range that is set by the international body FDA but brand 3 exceeds the permitted range of the National Authority BSTI. Government-authorized agencies such as BSTI should take control and regular monitoring to check the level of Sodium Benzoate in all brands of jelly.

Conflict of interest

The authors have declared that no competing interests exist.

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