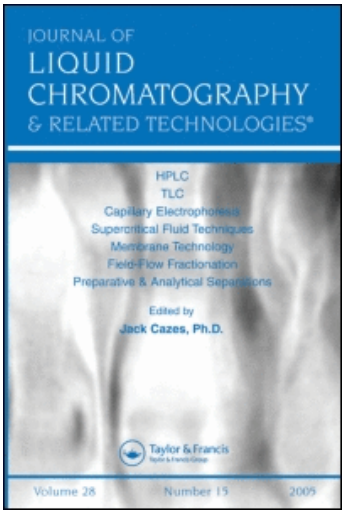

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Validated Reversed Phase HPLC Method for the Analysis of the Food Additive, Sodium Benzoate, in Soft Drinks and Jams

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Validated Reversed Phase HPLC Method for the Analysis of the Food Additive, Sodium Benzoate, in Soft Drinks and Jams

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Abstract: A rapid, simple, and sensitive method is described for the determination of the food preservative sodium benzoate in soft drinks, various jams, and ketchup. The method utilizes high performance liquid chromatography followed by diode array detection. Chromatographic separation was achieved using a C_{18} reversed phase column and methanol: water (70:30, v/v) adjusted to pH 3.45 with glacial acetic acid as mobile phase, 0.45 mL . min^{-1} flow rate, and UV detection at 245 nm. Amoxicillin was used as the internal standard. The retention time observed for sodium benzoate and amoxicillin were 5.01 and 12.07 min, respectively. The method is selective, reliable, and reproducible with a relative standard deviation of 0.66 and linear in the range of 50–450 ng/mL sodium benzoate concentration. The limit of detection and limit of quantification concentrations were 61 pg/mL and 203 pg/mL, respectively. The proposed method can be used for the routine analysis of sodium benzoate in soft drinks and jams.

Keywords: Sodium benzoate, Food additive, Food analysis, High performance liquid chromatography

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INTRODUCTION

Processed foods, such as fruit juices, ketchup, jams, and many other products are generally sold with food additives to prevent chemical changes or spoilage of food during its shelf life. Sodium benzoate (BZT), as one of the most common used food additives, is the sodium salt of benzoic acid and works well in acidic media to inhibit yeasts, molds, and bacterial growth. It is used in a variety of products, such as cosmetics and pharmaceuticals, but more commonly in food like jams and fruit juices to preserve freshness. Chemical structures of benzoic acid and BZT are given in Figure 1.

As a preservative, BZT is most effective in acidic media (pH $\leq$4.5) and is not recommended for use at higher acidic environments. Physiologically it stimulates bronchoalveolar secretion directly or reflexively, irritating gastric mucosa. Sodium benzoate rapidly metabolizes and excretes via a common pathway within 24 hrs. Systemic toxic effects on liver and kidney were observed. Sodium benzoate has low acute oral and dermal toxicity with LD_{50} values $>$2000 mg/kg.

Like every food additive, BZT also has limitations of usage to protect human life. The use of food additives in different countries is limited by specific regulations. The Food and Drug Administration (FDA) regulates the uses of sodium benzoate as a preservative in the USA. The FDA lists sodium benzoate as a substance that is generally recognized as safe (GRAS), with a maximum permitted concentration of 0.1%, in accordance with good manufacturing or feeding practices.[1] Similarly, BZT is regulated in Europe by the European Commission Health & Consumer Protection Directorate, with a limit of 150 mg/L in non alcoholic drinks and up to 500 mg/kg in jams and jellies. If higher concentrations of benzoate are used ($\approx$0.1%), then alterations in taste may occur in soft drinks. BZT maximums in non alcoholic drinks and jams are stated as 150 mg/L and 500 mg/kg, respectively, in the Turkish Food Codex.[2,3]

In this paper, we report a simple, specific, sensitive, and reproducible high performance liquid chromatography (HPLC) method for the determination of BZT in soft drinks and jams, using diode array detection in which amoxicillin tri hydrate was used as the internal standard.

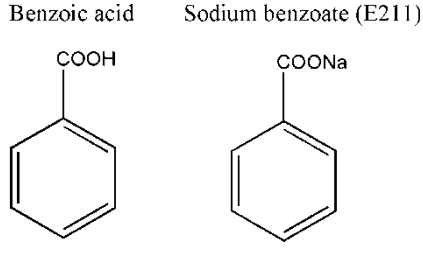

Figure 1. Chemical structure of benzoic acid and sodium benzoate.

Several studies have been reported for the determination of sodium benzoate. Methods used for the analysis of BZT in pharmaceuticals and food products have been HPLC,[4–22] gas chromatography,[23] capillary electrophoresis,[24] Uv-visible spectrophotometry,[25–30] ion selective electrodes,[31] and the P-matrix method.[32]

The aim of this study is to investigate BZT levels in various food products, such as soft drinks, jams, and ketchups produced in Turkey. Fifty three different food products were analyzed towards this purpose. Linearity, accuracy, and precision of the methodology were examined, and recovery studies were conducted for their validation. The superiority of this assay is the utilization of an internal standard, which enhances the accuracy of results and the reproducibility of the method.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Spectra System SCM 1000 degasser, Spectra System P1000 isocratic pump, Spectra System SN4000 connector, Spectra System UV6000LP diode array detector (all Thermo-Finnigan, USA). The analyte peaks were resolved at the ambient temperature on a Phenomenex Luna C_{18} (150 × 4.6 mm I.D.; particle size 5 μm) column. The volume of the injection loop was 20 μL. The data were collected and analyzed with a Chrom Quest™ 4.0 HPLC database program installed on an IBM computer. A model UV2401PC spectrophotometer (Shimadzu, Japan) and quartz cells for the measurement of the absorbance were used.

Reagents and Standards

Acetonitrile (LiChrosolv® for chromatography), glacial acetic acid, and standard sodium benzoate (99.9%) were purchased from Merck KGaA (Darmstadt, Germany). Standard amoxicillin as internal standard (IS, 99.8%) were kindly supplied from Fako Ilaçları A.S. (Istanbul, Turkey). The water needed in the experiments was double distilled. All solutions were protected from light and used within 24 h, in order to avoid decomposition.

Chromatographic Conditions

The proposed method was conducted using the reversed phase technique with UV monitoring at 245 nm, and employing amoxicillin as IS. A simple mobile phase without the need of buffer was chosen, which involves no complex procedure to prepare sample solutions. The mobile phase consisting of methanol:water (70:30, v/v, adjusted to pH 3.45 with glacial acetic acid) was filtered

through a 0.45 μm membrane filter, degassed for 20 min before use, and pumped from reservoir to the column at the rate of 0.45 mL . min^{-1}.

Sample Preparation

All samples were obtained from local food stores and chosen to be representative of what a consumer would find in a market basket study.

The sample preparation was carried out using the methods mentioned in the literature.[4] Beverage samples were prepared by diluting 1.0 mL of sample with 10.0 mL of mobile phase. Carbonated samples were degassed in an ultrasonic bath prior to dilution. Solid samples (jams and ketchups) were prepared by blending 10 g of the sample with 50 mL of mobile phase for 2 min. The sample blend was centrifuged at 3 g for 5 min and 1.0 mL of the supernatant liquid was diluted 1:10 with mobile phase, just like the liquid samples. After dilution, all samples were filtered through a micro filter to remove insoluble particles.

RESULTS AND DISCUSSION

Several compounds were examined and chromatographically tested to find a suitable IS for the assay. As a result of these studies, amoxicillin was chosen to be used as an internal standard (IS) since it has similar solubility properties and retention behaviors to those of sodium benzoate.

The elution of BZT and IS together, was achieved using a highly methanolic mobile phase. It was observed, that highly aqueous mobile phases did not elute the analytes off the column. Methanol was chosen because of low cost and conventional usage in reversed phase chromatographic separations.

The optimum elution of sodium benzoate was improved by regulating the pH of the mobile phase. Significant variations in the retention time of analytes, along with changes in resolution, were observed as the pH of the mobile phase was increased or decreased. Since BZT has a pKa of about 4.20, the increase of pH of the mobile phase caused a relatively fast elution that can not be acceptable for complex matrix assays. IS, having the advantage of two pKa points (2.8 and 7.2), did not cause a chromatographic problem and eluted easily in acidic and basic media. IS was detected in an acceptable time of 12 minutes. The effect of mobile phase pH to the chromatography was investigated by pH scanning between 2 to 7. The instrument was calibrated for the separation of the compounds between pH 3.0 and 4.0 and a pH value of 3.45 was found to be optimum for the separation of sodium benzoate. The main reason for this selection was the stability and conventional usage of benzoate in acidic media.

The HPLC system was also optimized for peak morphology by varying flow rate from 0.2 to 0.8 mL . min^{-1}. The analysis was run at a flow rate of 0.45 mL . min^{-1}.

The detection wavelength was chosen, according to preliminary studies which were performed using an additional UV-visible spectrophotometric method. BZT and IS were separately dissolved in the mobile phase mixture, which was used in HPLC analysis and, using the same solution as a blank, the spectrum of the analytes were recorded separately and together. Evaluation of the peaks and valleys pointed the wavelength of 245 nm as the preferable detection wavelength, giving acceptably high absorbance for both BZT and IS. Therefore, the detector was operated at the wavelength of 245 nm.

Standard solutions of BZT were prepared in mobile phase between 50 and 450 ng/mL concentrations. The system was calibrated between these ranges to cover the expected range of sodium benzoate in diluted samples. Linearity between the concentration of BZT and the UV absorbance at 245 nm was recorded and the correlation coefficient for each standard curve exceeded 0.9998.

The HPLC system presented optimum separation with minimal bandwidth and short elution time. The retention time for sodium benzoate and IS were 5.01 and 12.07 min, respectively. The methodology provided relatively fast elution, so that the total chromatographic analysis required only 14 min to complete. A representative chromatogram for separation of sodium benzoate and IS is shown in Figure 2.

The chromatographic procedure was validated for linearity, accuracy, and precision. The detector responses of sodium benzoate and IS were evaluated together by applying peak normalization. Peak normalization value (PN) of a signal was calculated by dividing the area of the signal to the retention time. The validation of the method was evaluated by using different concentrations of sodium benzoate versus a standard concentration of IS, employing the [BZT PN]/[IS PN] ratio for every concentration as the analytical response.

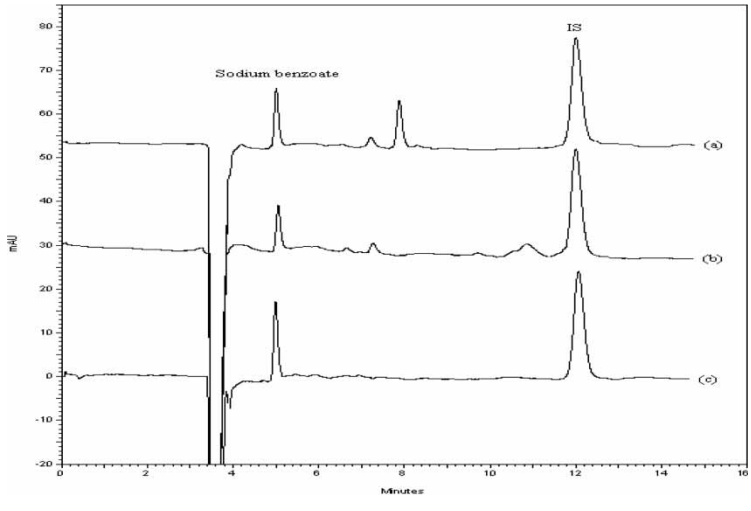

Figure 2. Typical chromatogram of the assay. a: ketchup b: jam c: soft drink.

Table 1. The statistical evaluation of the calibration studies

	Intra-day calibration (k = 1, n = 6)			Inter-day calibration (k = 3, n = 18)
	First-day	Second-day	Third-day	
Slope ± SD	$0.0026 \pm 4.80 \times 10^{-5}$	$0.0027 \pm 6.27 \times 10^{-5}$	$0.0027 \pm 6.08 \times 10^{-5}$	$0.0026 \pm 8.92 \times 10^{-5}$
Intercept	0.032	0.024	0.031	0.029
r	0.9998	0.9997	0.9997	0.9994
Slope ± CL (p = 0.05)	$0.0026 \pm 5.97 \times 10^{-5}$	$0.0027 \pm 7.80 \times 10^{-5}$	$0.0027 \pm 7.55 \times 10^{-5}$	$0.0026 \pm 4.95 \times 10^{-5}$

SD: Standard deviation.
r: Correlation coefficient.
CL: Confidence limit.
k: Number of set.
n: Number of sample.

Table 2. Repeatability of the chromatographic assay

	Inter-day precision (k = 1, n = 8)			Intra-day precision (k = 3, n = 24)
	First-day	Second-day	Third-day	
Mean	0.652	0.671	0.671	0.665
SD	0.0044	0.0057	0.0049	0.0010
RSD%	0.67	0.86	0.74	1.53
±CL (p = 0.05)	±0.0037	±0.0048	±0.0041	±0.0043

SD: Standard deviation.
RSD %: Relative standard deviation.
CL: Confidence limit.
k: Number of set.
n: Number of sample.

The signal normalization values were chosen instead of area response, to minimize the external or internal factors that affect the analysis. Such as variation of ambient temperature, mobile phase composition, pH, etc. The data are given in Table 1.

Precision of the method was assessed by performing replicate analyses of a standard BZT solution. For this purpose, the solution prepared at the concentration of 250 ng/mL was consequently injected to the HPLC eight times per set (fully 3 sets) at intra and inter-day intervals. The results were tabulated in Table 2.

The limit of detection (S/N = 3.3) and limit of quantification (S/N = 10) concentrations were calculated to be 61 pg/mL and 203 pg/mL, respectively.

Table 3. Results of the precision studies of the assay

	Nominal concentration added ($ng \cdot mL^{-1}$)	Concentration found ($ng \cdot mL^{-1}$)	n	Average recovery (%)	RSD (%)
Ketchup	50.00	49.75	5	99.50	0.57
	250.00	249.26	5	99.70	0.69
	450.00	449.77	5	99.95	0.72
Jam	50.00	49.44	5	98.88	0.54
	250.00	244.41	5	97.76	0.66
	450.00	445.21	5	98.94	0.54
Soft drink	50.00	49.22	5	98.43	0.61
	250.00	248.66	5	99.46	0.78
	450.00	437.57	5	97.24	0.70

n: Number of sample.
RSD (%): Relative standard deviation.

Table 4. Assay results of the foodstuffs

Sample (n = 3)	Concentration found ($\mu g \cdot mL^{-1}$)	RSD %	CL (p = 0.05)
Soft drink No 1	11.68	1,56	0,23
Soft drink No 2	61.44	2,25	0,55
Soft drink No 3	22,25	2,36	0,80
Soft drink No 4	48,30	2,04	1,50
Soft drink No 5	39,99	2,15	1,30
Soft drink No 6	36,29	2,83	1,56
Soft drink No 7	45,27	2,62	1,80
Soft drink No 8	47,14	1,85	1,33
Soft drink No 9	21,40	0,70	2,26
Soft drink No 10	52,89	2,36	1,90
Soft drink No 11	53,72	2,89	2,36
Soft drink No 12	59,29	1,69	1,53
Soft drink No 13	52,84	2,61	2,10
Soft drink No 14	18,63	3,09	0,88
Soft drink No 15	20,16	1,76	0,54
Soft drink No 16	91,51	2,07	2,88
Soft drink No 17	85,08	2,28	2,94
Soft drink No 18	12,50	5,67	1,08
Soft drink No 19	20,75	3,79	1,20
Soft drink No 20	85,88	2,33	3,05
Jam No 1	29,19	3,14	1,40
Jam No 2	61,84	1,87	1,76
Jam No 3	35,16	3,94	2,10
Jam No 4	49,97	2,34	1,78
Jam No 5	53,33	2,59	2,10
Jam No 6	46,22	2,31	1,62
Jam No 7	21,50	3,52	1,15
Jam No 8	56,47	2,11	1,81
Jam No 9	24,38	4,26	1,58
Jam No 10	52,89	2,36	1,90
Jam No 11	53,72	2,89	2,36
Jam No 12	59,28	1,69	1,53
Jam No 13	33,50	3,34	1,70
Jam No 14	17,26	3,83	1,00
Jam No 15	31,15	3,86	1,83
Jam No 16	67,16	1,79	1,83
Jam No 17	19,25	3,39	0,99
Jam No 18	73,16	1,78	2,00
Jam No 19	28,05	4,05	1,73
Ketchup No 1	82,03	1,57	1,96
Ketchup No 2	55,82	1,89	1,61
Ketchup No 3	71,94	2,01	2,19

(*continued*)

Table 4. Continued

Sample (n = 3)	Concentration found ($\mu g \cdot mL^{-1}$)	RSD %	CL (p = 0.05)
Ketchup No 4	43,49	2,31	1,53
Ketchup No 5	35,94	3,40	1,86
Ketchup No 6	52,15	2,14	2,70
Ketchup No 7	31,27	4,47	1,85
Ketchup No 8	58,82	3,02	2,40
Ketchup No 9	49,84	2,30	1,74
Ketchup No 10	58,54	3,43	3,05
Ketchup No 11	62,54	2,14	1,45
Ketchup No 12	87,12	3,87	2,61
Ketchup No 13	45,17	2,51	2,66
Ketchup No 14	65,88	1,63	1,50
Ketchup No 15	32,56	4,11	2,08

n: Number of sample.
RSD %: Relative standard deviation.
CL: Confidence limit.

The possibility of interference originating from ingredients of the foodstuffs is an important fact and always needs to be investigated. This was carried out by performing recovery studies. The extraction efficiency (recovery) was determined by calculating the relative ratio of the amount of extracted compound from beverage, jam, and ketchup samples spiked with known amounts of BZT, to the amount of compound added at the same concentrations just prior to HPLC injection. The recovery results were given in Table 3.

Recovery values of sodium benzoate from spiked jam, beverage, and ketchup samples were between 97.24 and 99.95%. It can be concluded that the ingredients had no effect in the determination.

The developed method was applied to the determination of 53 foodstuffs, comprising soft drinks, jams, and ketchups. BZT content of target foodstuffs are given in Table 4.

According to the results, BZT content was between the limitations stated by FDA, EU, and the Turkish Food Codex.

CONCLUSION

A validated HPLC method for the analysis of sodium benzoate is developed using diode array detection. The method described is selective, reproducible, simple, and reliable for the determination of sodium benzoate in food products using a high capacity reverse phase system. The extraction procedure with methanol is economical, time saving, and easy to carry out for the routine

analyses of sodium benzoate. The diode array detection is a powerful tool for testing the specificity of the HPLC method herein presented, but a conventional UV detector is sufficient for routine quantitative analyses of BZT. Generally, the levels of BZT tested were in compliance with the regulations stated by FDA, EU, and Turkish Food Codex.

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