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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DEVELOPMENT OF A GREEN HPLC METHOD USING ETHANOL IN THE MOBILE PHASE COMPOSITION FOR THE DETERMINATION OF SODIUM BENZOATE AND POTASSIUM SORBATE IN BEVERAGES

İÇECEKLERDE SODYUM BENZOAT VE POTASYUM SORBAT TAYİNİ İÇİN HAREKETLİ FAZ BİLEŞİMİNDE ETANOL KULLANILAN YEŞİL HPLC YÖNTEMİNİN GELİŞTİRİLMESİ

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ABSTRACT

Objective: *This work aims to develop a novel and green high-performance liquid chromatography (HPLC) method for determining sodium benzoate (Na-BZT) and potassium sorbate (K-SBT) in beverages using ethanol as an environmentally friendly solvent in the mobile phase.*
Material and Method: *The chromatographic parameters were optimized using the Box-Behnken design. Validation studies were carried out in accordance with international guidelines.*
Result and Discussion: *The developed method displayed high accuracy (98.54-106.3%), precision (RSD≤5%), and specificity, with a total run time of 7 minutes. The limit of detection values for Na-BZT and K-SBT were 0.06 and 0.14 µg/ml, respectively. The use of ethanol, a less toxic solvent, minimized environmental impact compared to traditional solvents. The method's applicability was confirmed by analyzing ten different beverage samples. The results demonstrate the potential for broader application of ethanol-based HPLC methods in the beverage industry.*
Keywords: *Design of experiments, food analysis, HPLC, potassium sorbate, sodium benzoate*

ÖZ

Amaç: *Bu çalışma, hareketli fazda çevre dostu bir çözücü olarak etanol kullanarak içeceklerde sodyum benzoat (Na-BZT) ve potasyum sorbat (K-SBT) tayini için yeni ve çevreci bir yüksek performanslı sıvı kromatografi (HPLC) yöntemi geliştirmeyi amaçlamaktadır.*
Gereç ve Yöntem: *Kromatografik parametreler Box-Behnken tasarımı kullanılarak optimize edilmiştir. Validasyon çalışmaları uluslararası kılavuzlara uygun olarak gerçekleştirilmiştir.*
Sonuç ve Tartışma: *Geliştirilen yöntem, 7 dakikalık çalışma süresiyle yüksek doğruluk (%98.54-106.3), kesinlik (RSD≤%5) ve spesifiklik sergilemiştir. Na-BZT ve K-SBT için tespit limiti değerleri sırasıyla 0.06 ve 0.14 µg/ml idi. Daha az toksik bir çözücü olan etanol kullanımı, geleneksel çözücülere kıyasla çevresel etkiyi en aza indirmiştir. Yöntemin uygulanabilirliği on farklı içecek örneğinin analiz edilmesiyle doğrulanmıştır. Sonuçlar, içecek endüstrisinde etanol bazlı HPLC yöntemlerinin daha geniş uygulama potansiyelini göstermektedir.*
Anahtar Kelimeler: *Deneysel tasarım, gıda analizi, HPLC, potasyum sorbat, sodyum benzoat*

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INTRODUCTION

Na-BZT and K-SBT are commonly preferred preservatives in the food and beverage industry. The growth of bacteria, yeast, and fungi can be inhibited by Na-BZT in acidic settings. K-SBT is also capable of preventing the growth of molds and yeasts in food [1]. These preservatives are regulated by various health authorities, including the United States Food and Drug Administration and the European Food Safety Authority, which set maximum allowable concentrations to ensure consumer safety [2]. In Türkiye, the regulation and monitoring of these compounds are overseen by the Ministry of Agriculture and Forestry. According to the Turkish Food Codex Regulation on Food Additives, the maximum concentrations of Na-BZT and K-SBT can vary depending on the type of beverage, generally ranging from 150 to 200 mg/l for Na-BZT and from 250 to 2000 mg/l for K-SBT [3].

Although K-SBT and Na-BZT are considered safe, excessive use of these preservatives may lead to genotoxicity, causing DNA damage and chromosomal aberrations in various cell types. Studies also suggest that Na-BZT can lead to general genomic injuries, particularly in pregnant and their fetuses, emphasizing the potential health risks associated with the consumption of these additives. In this manner, accurate quantification of Na-BZT and K-SBT in beverages is necessary not only to ensure compliance with regulations but also to prevent potential adverse health effects [4].

A number of analytical methods based on spectrophotometry [5], gas chromatography [6], capillary electrophoresis [7], and liquid chromatography [1,2,8-12] have been developed for the determination of Na-BZT and K-SBT in food and beverages. Among these, HPLC is the most commonly utilized owing to its improved sensitivity, accuracy, and ability to handle complex matrices [10,11].
Lately, there has been a growing interest in developing "green" HPLC methods that minimize environmental impact. The idea of green chemistry (GrC) was introduced in the 1990s, which involves creating chemical products and procedures that minimize or eradicate the production and utilization of harmful substances. [13]. A roadmap for attaining sustainability in chemical procedures is presented through the twelve principles of GrC. Some of the fundamental principles involve the utilization of more secure substances and processes, boosting energy effectiveness, and the design of products for easy degradation. The importance of GrC lies in its potential to protect human health and the environment, reduce costs, and improve safety in chemical manufacturing [14].

Green analytical chemistry (GAC) extends the principles of GrC to chemical analysis. It aims to develop analytical methods that are environmentally benign, consume fewer resources, and generate less waste [15]. This shift towards greener practices is particularly relevant in chromatography, a widely used technique in analytical chemistry. Green chromatography focuses on minimizing the environmental impact of chromatographic methods at all stages, from sample collection and pretreatment to final instrumental analysis. It emphasizes the use of solventless extraction techniques, reducing solvent consumption and waste generation, and replacing toxic solvents with more environmentally benign alternatives [16]. Traditional HPLC methods often use acetonitrile or methanol as solvents, which are toxic and generate significant hazardous waste. In contrast, ethanol, a less toxic and more environmentally friendly solvent, offers a safer alternative and provides comparable performance in terms of solubility and elution strength for various analytes. The implementation of ethanol in the mobile phase aligns with the principles of GrC, aiming to moderate the utilization of harmful substances and generate less waste [17-20].

The optimization of HPLC methods using chemometric tools such as experimental design can significantly enhance the efficiency and robustness of the analytical process. Traditional one-factor-at-a-time (OFAT) approaches to method optimization can be time-consuming and labor-intensive, as they involve varying one parameter while keeping others constant. Furthermore, the OFAT approach often fails to identify optimal conditions due to the interaction effects between variables [21,22]. Chemometric tools, such as experimental design, address these limitations by allowing simultaneous variation of multiple parameters. This approach saves time and effort and provides a more comprehensive understanding of the effects and interactions of different factors on the analytical response. A design frequently utilized in chromatographic method optimization is the Box-Behnken design (BBD), which is advantageous as it requires fewer experiments than full factorial designs while still providing sufficient information to model quadratic response surfaces. It is particularly effective for identifying

optimal conditions and understanding the interactions between variables in HPLC method development [23,24].

In this study, a novel HPLC method was introduced using eco-friendly ethanol in the mobile phase to analyze Na-BZT and K-SBT in beverages. The optimization approach was based on experimental design, ensuring optimal separation and detection of the preservatives. This approach addresses the need for safer and more sustainable analytical practices and maintains the analytical performance required for accurate food preservative determination. To the best of our knowledge, the use of ethanol in the mobile phase for the separation of Na-BZT and K-SBT was reported for the first time in this work.

MATERIAL AND METHOD

Chemicals and Materials

Ethanol was purchased from ISOLAB Laborgeräte GmbH (Eschau, Germany). Sodium acetate trihydrate was obtained from Tekkim (İstanbul, Türkiye). Na-BZT, K-SBT, hydrochloric acid (HCl), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), and methanol were sourced from Sigma Aldrich (St. Louis, USA). Purified water from a Sartorius Stedim Arium pro UV instrument (Göttingen, Germany) was used throughout the study.

Instrumentation

A Prominence-20 HPLC system From Shimadzu (Kyoto, Japan) equipped with an SPDM20A diode array detector (DAD) was used. The operation of the system was overseen by Lcsolutions 1.25 software. An MX-S vortex from Isolab Laborgerte (Wertheim, Germany), an NF 615 centrifuge from Nüve (Ankara, Türkiye), An HI 2211 pH meter from Hanna Instruments (Woonsocket, United States), and an ultrasonic bath from Thermomac (İstanbul, Türkiye) were utilized for sample and standard preparation.

Preparation of Standard Solutions

Stock solutions (1000 µg/ml) of Na-BZT and K-SBT were prepared in methanol. Stock solutions were diluted with deionized water to prepare calibration and quality control (QC) solutions. All solutions were kept in the dark and at +4ºC until use.

Chromatographic Conditions

The chromatographic analyses were carried out using a Chromolith HighResolution RP18e monolithic column (100 × 4.6 mm). The mobile phase consisted of ethanol and pH 4.3, 20 mM acetate buffer in a ratio of 17.5:82.5 (v/v), delivered isocratically at a flow rate of 1.1 ml/min. The column temperature was adjusted to 25ºC. The DAD detector was set at 235 nm. A 10 µL of sample or standard solution was injected into the system.

Sample Preparation

Beverage samples, including two kinds of orange-flavored carbonated drinks, blackberry-, pineapple-, ginger-flavored drinks, lemon-flavored mineral water, tonic water, fizzy drink, energy drink, and coke, were purchased from a local market in Trabzon, Türkiye. Before HPLC analysis, the liquid sample was transferred to a beaker and sonicated for 5 min to remove the dissolved CO_2. Then, the tube was centrifuged at 5000 rpm for 3 min. The supernatant obtained was filtered through a syringe filter (0.45 µm). The final filtrate was diluted 10-fold with water before being injected into the system.

Method Validation

For the system suitability test (SST), a 25 µg/ml standard solution of Na-BZT and K-SBT was analyzed six times. Linearity was assessed by analyzing standard solutions at six different concentrations (0.5, 1, 2.5, 5, 10, 25, and 50 µg/ml) in triplicate. To evaluate the intra- and inter-day accuracy and repeatability of the method, quality control (QC) solutions at three concentrations (1, 25, and 40 µg/ml) were analyzed. Intra-day experiments involved three analyses at each level within the

same day, while inter-day experiments were performed over three consecutive days with seven analyses. Relative standard deviation (RSD) and % accuracy were used to represent repeatability and accuracy, respectively. The recovery of analytes following the dilute-and-shoot approach was also examined. For this, pre-analyzed beverages (blackberry flavored drink, coke, orange-flavored carbonated drink) were spiked with Na-BZT and K-SBT at 20 µg/ml level. Theoretical and experimental results were compared to estimate recovery. The limits of detection (LOD) and quantification (LOQ) were statistically estimated as previously described [25]. The method's selectivity was evaluated by checking the peak purity index values obtained for analytes during sample analysis.

Experimental Design

The influence of three factors, including ethanol ratio (%) in the mobile phase, pH, and flow rate, on the capacity factor (*k*) of the first peak and resolution of (*Rs*) Na-BZT and K-SBT was examined by BBD. The desirability approach was used to optimize several responses simultaneously [26]. Design Expert 11.1.2 was utilized to construct the BBD matrix and evaluate the model. The accuracy of the results was ensured by duplicating the measurements for each run.

RESULT AND DISCUSSION

Optimization of Chromatographic Conditions

Traditionally, chromatographic method optimization is based on the OFAT approach, which is time-consuming, involves many experiments, and cannot identify interactions among factors. On the other hand, by examining the interaction between critical factors and their combined influence on the response, experimental design facilitates the efficient manipulation of various variables. Therefore, optimization of chromatographic conditions was performed based on an experimental design aiming to obtain acceptable retention for the first-eluting peak and a satisfactory *Rs* between K-SBT and Na-BZT.

Chromatographic optimization is generally performed by response surface designs such as BBD and central composite design (CCD) [27]. Compared to CCD, BBD is a more straightforward approach since it involves conducting fewer experiments and eliminates the need for simultaneous experimental runs with all factors at extreme levels [28]. Consequently, BDD was utilized to optimize the chromatographic conditions for separating K-SBT and Na-BZT.

Since the initial screening runs demonstrated that analytes could be separated by isocratic elution, the first parameter was chosen as the ethanol ratio (%). The other two parameters were determined to be the pH and flow rate. The *k* of the first peak and *Rs* between K-SBT and Na-BZT were selected as responses to be optimized. Parameter ranges for ethanol ratio (%), pH, and flow rate were determined as 15-25%, 2.5-7, and 0.8-1.2 ml/min according to initial screening experiments. The design matrix and experimental results are given in Table 1.

Following the implementation of multiple regression analysis, the second-order polynomial equations were employed to represent the effects of factors on responses. All variables were subjected to a log10 transformation to enhance the models' capacity to interpret the data. Below are the mathematical expressions for the two responses:

$$\text{Log}_{10}(Rs) = 0.695774 - 0.151219A - 0.134066B - 0.0189029C - 0.0340106AB + 0.00785617AC + 0.00120551BC - 0.578631A^2 - 0.0186688B^2 + 0.0152894C^2$$

$$\text{Log}_{10}(k \text{ of first peak}) = - 0.0328285 - 0.55373A - 0.125051B - 0.00851598C + 0.0645791AB + 0.00637101AC - 0.0110173BC + 0.0481244A^2 - 0.011943B^2 + 0.0148512C^2$$

Where A, B, and C are pH, ethanol ratio (%), and flow rate, respectively. ANOVA was used to evaluate the models statistically, with the findings shown in Tables S1 and S2. The statistical significance of both models was confirmed by ANOVA ($p<0.0001$). The models effectively captured the relationships between factors and responses as indicated by the determination coefficient (R^2), adjusted R^2, and predicted R^2 values exceeding 0.96.

Table 1. The BBD matrix and experimental results for two responses

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	**Run**	**A: pH**	**B: Ethanol Ratio**	**C: Flow rate**	**Resolution**	***k* of first peak**
			%	**ml/min**		
4	1	7	25	1	0.642	0.242
5	2	2.5	20	0.8	2.09	3.78
8	3	7	20	1.2	0.909	0.313
17	4	4.75	20	1	5.00	0.922
11	5	4.75	15	1.2	6.70	1.28
9	6	4.75	15	0.8	7.30	1.28
15	7	4.75	20	1	4.96	0.928
10	8	4.75	25	0.8	3.60	0.715
12	9	4.75	25	1.2	3.34	0.645
1	10	2.5	15	1	2.09	5.65
13	11	4.75	20	1	4.92	0.929
7	12	2.5	20	1.2	1.84	3.57
3	13	2.5	25	1	1.44	2.50
6	14	7	20	0.8	0.963	0.312
16	15	4.75	20	1	4.95	0.930
14	16	4.75	20	1	4.98	0.927
2	17	7	15	1	1.28	0.301

In experimental design, lack-of-fit (LOF) serves as a statistical tool to evaluate the adequacy of a model in representing the collected data. LOF can be utilized to assess if there is a notable difference between the model predictions and the actual data. The LOF F-value is calculated by comparing the difference between the actual and predicted values from the model with the variation in repeated measurements. Therefore, a statistically significant LOF may occur due to the high precision at the central points of the design and the presence of error at axial points [21]. RSD values for two responses at the central point were all below 0.62% for five repeated measurements. In this context, a significant LOF was attributed to the limited variation at the central point.

Response surfaces obtained by the selected regression models are presented in Figure 1. pH was found to be the most significant factor for the *k* of the first peak. Retention of the analytes increased with the decrease of pH as both are found in neutral form under acidic conditions (Figure 1A). It should be noted that the elution order of the analytes changed with the increase in pH. K-SBT eluted earlier than Na-BZT at pH 2.5, while Na-BZT was the first-eluting analyte for pH 4.75 and 7. Retention of analytes increased with the decrease in ethanol ratio due to the decrease in elution strength of the mobile phase with a low organic solvent ratio. As expected, the flow rate did not change the *k* of analytes significantly. pH was also found to be the most significant parameter for the *Rs* of Na-BZT and K-SBT. The *Rs* of Na-BZT and K-SBT initially increased with pH from 2.5 to 4-5, then decreased with a further increase in pH to 7 (Figure 1B). The ethanol ratio had a negative effect on *Rs* due to decreased retention of analytes. A decrease in *Rs* was observed with the increase in flow rate, which can be attributed to the decline in column performance at elevated flow rates.

The optimum chromatographic condition was determined by Design-Expert software based on the desirability function [26]. The studies aimed to achieve a *k* value exceeding 1 for the first-eluting peak, i.e., Na-BZT, and an *Rs* better than 2.5. The optimal pH, ethanol ratio (%), and flow rate values were estimated as 4.3, 17.5%, and 1.1 ml/min. The chromatogram recorded under these conditions is given in Figure 2. The selected models proved reliable as the variations between predicted and actual values were under 4%. Na-BZT and K-SBT were eluted from the column at 3.82 and 5.05 min with a run-to-run analysis time of 7 min.

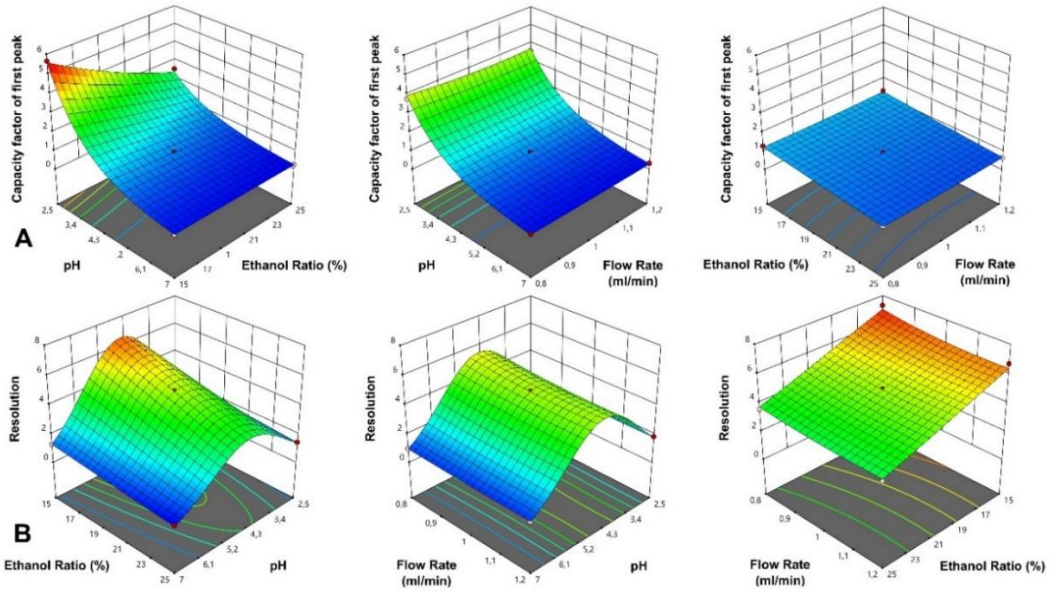

Figure 1. Response surfaces for (A) *k* of the first peak and (B) *Rs* of K-SBT and Na-BZT

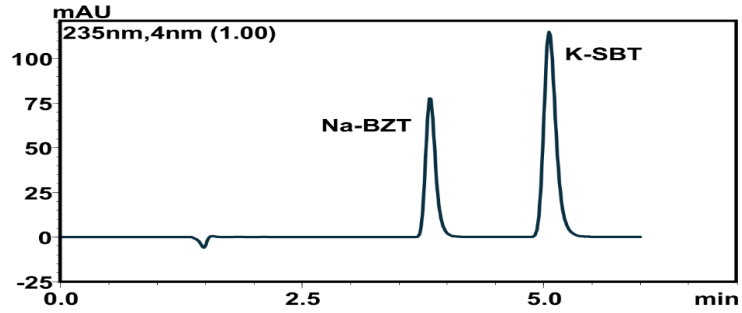

Figure 2. Chromatogram obtained at 235 nm under optimized conditions for a standard mixture (25µg/ml) of Na-BZT and K-SBT

Method Validation

Validation studies followed ICH guidelines to evaluate the method's reliability [29]. Before validation experiments, an SST was performed to ensure the chromatographic system's performance [2,20]. The results of the SST are presented in Table 2. The tailing factors were 1.29 for Na-BZT and 1.27 for K-SBT, both well within the acceptable limit of less than 2. The *k* values were 1.45 for Na-BZT and 2.24 for K-SBT, demonstrating the adequate retention of both analytes. The *Rs* between the two peaks was 5.46. Theoretical plates were higher than the minimum requirement of 2000. The selectivity factor was 1.54. The RSDs for retention time and peak area were less than 0.2% for both analytes.

Calibration graphs were plotted for Na-BZT and K-SBT within the 0.5-50 µg/ml concentration range. (Table 3). A correlation coefficient (*r*) of 0.9999 was observed for both analytes, confirming the linearity of the method. The LOD was determined to be 0.06 µg/ml for Na-BZT and 0.14 µg/ml for K-SBT. LOQ values were 0.18 µg/ml and 0.43 µg/ml for Na-BZT and K-SBT, respectively (Table 3).

The accuracy and precision of the method were assessed by evaluating the intra-day (n=3) and inter-day (n=7) variations at three concentration levels (1, 25, and 40 µg/ml) for both Na-BZT and K-SBT (Table 4). The method showed an intra-day accuracy range of 98.74%-106.3%, with RSD values ranging from 0.02% to 4.2%. Inter-day accuracies ranged from 98.54% to 105.3%, with RSD values ranging from 0.10% to 3.3%. Results demonstrate that the developed method displays acceptable accuracy and precision for determining Na-BZT and K-SBT. Furthermore, excellent mean recoveries in the ranges of 100.3-101.1% and 99.65-101.8% were obtained for Na-BZT and K-SBT, respectively,

which can be attributed to the direct injection of samples following the simple dilution with water (Table 5).

Table 2. Results of SST experiments (n=6)

	Na-BZT	K-SBT	Recommended value
Retention time (min)	3.82	5.05	-
Tailing factor (T)	1.29	1.27	<2
Capacity factor (*k*)	1.45	2.24	>1
Resolution (Rs)	-	5.46	>1.5
Theoretical plates (N)	5524	6685	>2000
Selectivity factor (*a*)	-	1.54	>1.05
RSD% of retention time	0.12	0.086	<1
RSD% of peak area	0.15	0.13	<1

Table 3. Results of validation experiments related to linearity and sensitivity

	Na-BZT	K-SBT
Linear range (µg/ml)	0.5-50	0.5-50
Slope	22557	41876
Intercept	948.2	-1990.4
SE of slope	18.96	84.23
SE of intercept	409	1817
Correlation coefficient (*r*)	0.9999	0.9999
LOD (µg/ml)	0.06	0.14
LOQ (µg/ml)	0.18	0.43

Table 4. Results of accuracy and precision experiments conducted at three quality control levels (1, 25, and 40 µg/ml)

		Intra-day*		Inter-day*	
Analyte	**Concentration level (µg/ml)**	**Accuracy (%)**	**RSD (%)**	**Accuracy (%)**	**RSD (%)**
Na-BZT	1	100.3	4.2	98.54	3.3
	25	99.89	0.019	99.88	0.11
	40	100.5	0.058	100.5	0.11
K-SBT	1	106.3	2.2	105.3	1.8
	25	98.74	0.029	98.66	0.10
	40	100.8	0.072	100.7	0.14

*The number of experiments is 3 and 7 for intra- and inter-day experiments, respectively.

Table 5. Results of recovery experiment performed on three different beverage samples (n=3)

Analyte	Sample	Added (µg/ml)	Found ±SD (µg/ml)	Recovery (%)
Na-BZT	Blackberry-flavored drink	20	20.08±0.02	100.4
	Coke	20	20.07±0.02	100.3
	Orange-flavored carbonated drink	20	20.22±0.02	101.1
K-SBT	Blackberry-flavored drink	20	19.93±0.03	99.65
	Coke	20	20.10±0.04	100.5
	Orange-flavored carbonated drink	20	20.36±0.05	101.8

Analysis of Food Samples

The feasibility of the proposed technique was proved by analyzing ten different beverages.

Representative chromatograms of 6 selected samples are given in Figure 3.

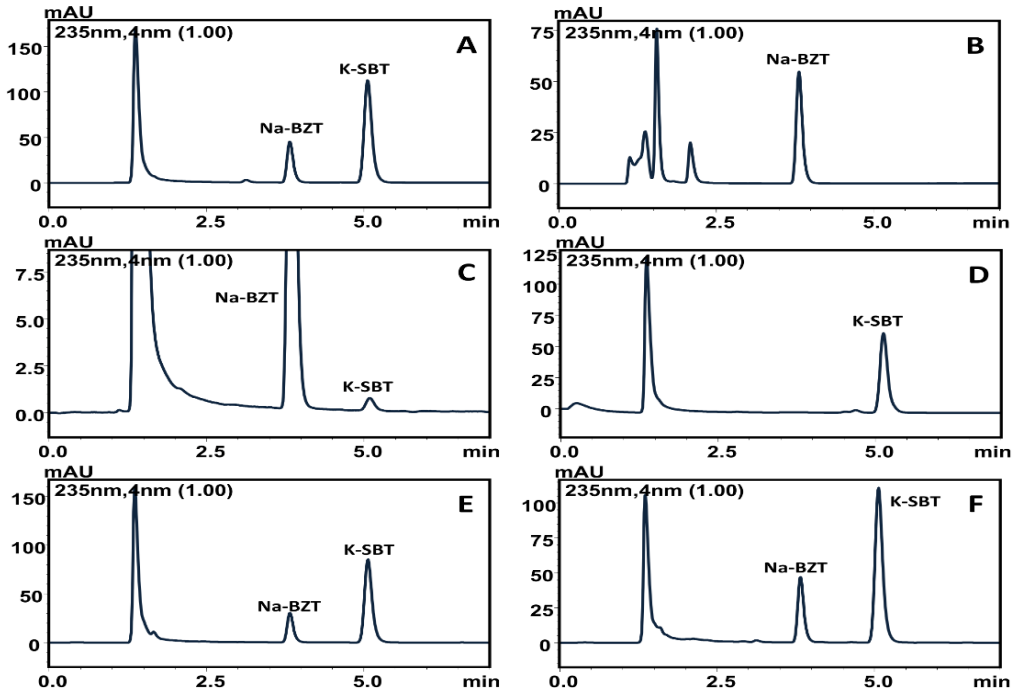

Figure 3. Representative HPLC chromatograms of beverages. (A) Pineapple-flavored drink, (B) Coke, (C) Lemon-flavored mineral water, (D) Tonic water, (E) Orange-flavored carbonated drink, and (F) Blackberry-flavored drink

The peaks were identified by comparing the retention times and absorption spectra of unknown peaks with Na-BZT and K-SBT standards. As can be seen, no interference from sample matrices was noticeable. This observation was further confirmed by the obtained peak purity index values higher than 0.9999 for all samples, indicating the selectivity of the proposed method. The amounts of Na-BZT and K-SBT in beverages are given in Table 6. Concentrations of preservatives ranged from 1.83 to 171.2 for Na-BZT and from 2.03 to 236.7 for K-SBT. Notably, the concentrations of Na-BZT and K-SBT in all samples were within the maximum limits set by the Turkish Food Codex Regulation. On the other hand, despite the absence of any mention on the label, orange-flavored carbonated drink-2 and lemon-flavored mineral water showed the presence of Na-BZT and K-SBT traces.

Table 6. Contents (mean ± SD) of Na-BZT and K-SBT in beverage samples (n = 3)

Sample	Na-BZT (µg/ml)	K-SBT (µg/ml)	Na-BZT declared	K-SBT declared
Blackberry-flavored drink	142.0±0.19	229.6±0.27	Yes	Yes
Coke	171.2±0.16	-	Yes	No
Orange-flavored carbonated drink	93.0±0.15	176.9±0.54	Yes	Yes
Orange-flavored carbonated drink-2	1.83±0.08	230.5±0.18	No	Yes
Pineapple-flavored drink	140.0±0.20	236.7±0.16	Yes	Yes
Ginger-flavored drink	141.2±0.32	223.4±0.21	Yes	Yes
Tonic water	-	135.6±0.13	No	Yes
Fizzy drink	166.8±0.01	-	Yes	No
Energy drink	-	215.9±0.20	No	Yes
Lemon-flavored mineral water	142.01±0.24	2.03±0.02	Yes	No

Evaluation of Method's Greenness

AGREE (Analytical GREEnness Metric Approach) is a comprehensive tool designed to evaluate the environmental greenness of analytical methodologies [30]. It is based on the 12 principles of GAC [31] and provides a unified 0-1 scale to assess various criteria, including sample preparation, reagent toxicity, waste generation, energy consumption, and operator safety. The AGREE tool generates a pictogram with a score in the center, where a score closer to 1 indicates a greener method. Each segment around the perimeter of the pictogram represents one of the GAC principles, using colors (green, yellow, red) to indicate the method's environmental performance in each area visually.

The method's greenness is assessed using the AGREE software, achieving a score of 0.77 (Figure 4), which indicates significant adherence to GrC principles. The sample preparation process involves minimal steps, including sonication, centrifugation, and filtration, which reduces chemical and energy usage. The method employs a small sample amount (e.g., 0.1 ml sample was enough), minimizing resource consumption and waste generation. Although the device positioning is marked in red due to offline sample analysis, this aspect often depends on laboratory setups and may not be easily modified. The method avoids derivatization, reducing the need for additional chemicals and associated waste. Waste management is enhanced by using ethanol, a less toxic and more environmentally friendly solvent compared to traditional solvents, such as acetonitrile and methanol. The method demonstrates high throughput with a total run time of 7 min. Energy consumption is generally optimized in HPLC systems, but further improvements could involve using more energy-efficient equipment such as UPLC. The reagents used, particularly ethanol, are sourced to minimize environmental impact. Overall, the developed method aligns well with GrC principles, offering a sustainable solution for analyzing Na-BZT and K-SBT.

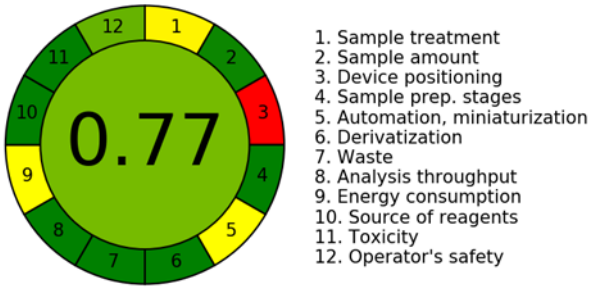

Figure 4. Greenness assessment of the HPLC method using the AGREE metric

Conclusion

A green HPLC method was presented to determine Na-BZT and K-SBT in beverages, utilizing ethanol in the mobile phase as an eco-friendly alternative. Chromatographic conditions were optimized via a chemometric approach using BBD to ensure optimum separation and detection of these preservatives. The proposed method aligns with the principles of GrC by minimizing environmental impact and maintains high analytical performance. Using ethanol to separate Na-BZT and K-SBT proved to be an effective and environmentally benign alternative to traditional solvents such as acetonitrile and methanol. The proposed method demonstrated high accuracy, precision, and specificity. The technique's feasibility was realized by analyzing ten beverages with excellent recoveries. Overall, this study highlights the feasibility of incorporating GrC principles into routine analytical methods, offering a sustainable solution for food preservative analysis. The findings emphasize the potential for broader application of ethanol-based HPLC methods in food safety monitoring and regulatory compliance.

AUTHOR CONTRIBUTIONS

Concept: S.Y.; Design: S.Y.; Control: S.Y.; Sources: S.Y.; Materials: S.Y.; Data Collection

and/or Processing: S.Y.; Analysis and/or Interpretation: S.Y.; Literature Review: S.Y.; Manuscript Writing: S.Y.; Critical Review: S.Y.; Other: -

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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