Research Article

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Environmentally sustainable analytical quality by design aided RP-HPLC method for the estimation of brilliant blue in commercial food samples employing a green-ultrasound-assisted extraction technique

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Abstract: Brilliant blue FCF (E133) is a commonly employed azo synthetic dye in the food industry owing to its visually appealing color and widespread consumer acceptance. The health risks associated with the excessive use of brilliant blue necessitate prioritizing eco-friendly methods for its quantification. The purpose of this study is to develop and validate an analytical quality by design (AQbD) based eco-friendly high-performance liquid chromatography method adhering to the 12 principles of green chemistry followed by ultrasound-assisted extraction of food samples and confirming them using Fourier transform infrared spectroscopy. Rotatable central composite designs (CCDs) were utilized to optimize the chromatographic parameters. The separation was achieved on a Phenomenex column (Luna C_{18}, 250 mm × 4.6 mm, i.d. 5 µm) employing ethanol and acetate buffer as a mobile phase in the ratio 25:75 (v/v) at a flow rate of 1 mL·min^{-1} with detection at 626 nm, which resulted in elution of brilliant blue at 2.692 min. The developed method fulfills the predetermined requirements of linearity, accuracy, sensitivity, and reproducibility at the specified working point. The green analytical procedure index, analytical eco scale, and analytical greenness metrics were used for assessing greenness, which yielded the most benign outcomes. In the future, this research could lead to the adoption and enhancement of eco-friendly robust AQbD methodologies for evaluating various food colorants, utilizing green solvents.

Keywords: brilliant blue, RP-HPLC, AQbD, UAE, Green chemistry

1 Introduction

Food safety management is a prevailing and relevant phenomenon in current society, as it pertains to the pursuit of a fulfilling and well-being-oriented lifestyle. The utilization of synthetic dyes has garnered significant attention due to the substantiated impact of color on customer perception and acceptance of food products. Various ingredients, such as spices and seasonings, have traditionally been used as food dyes; however, they have now been substituted by other compounds that possess similar coloring properties [1]. The food industry has widely adopted the use of synthetic dyes, particularly in large-scale production, for their cost-effectiveness, ability to maintain food uniformity, vibrant appearance, and high stability to external factors such as light, oxygen, heat, and pH. Additionally, synthetic dyes exhibit a wider spectrum of colors with increased intensity and have enhanced stability and accessibility compared to natural dyes [2]. Although synthetic food coloring offers certain advantages, excessive consumption has been associated with various health issues, particularly among children including allergies, food intolerance, cancer, multiple sclerosis, attention deficit hyperactivity disorder (ADHD), brain damage, nausea, and even heart disease [3]. According to the Prevention of Food Adulteration Act in India, only eight food colors have been officially authorized which include sunset yellow FCF, brilliant blue FCF, carmoisine, erythrosine, fast green FCF, indigo carmine, ponceau 4R, and tartrazine [4].

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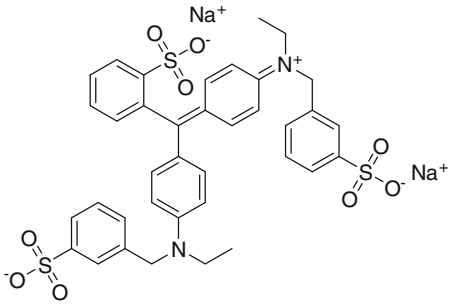


Figure 1: Chemical structure of brilliant blue.

Brilliant blue (BB) (E133), also known as FD&C Blue No. 1 and Food Blue No. 1 (Figure 1), is one of the most common, extensively used, and consumed water-soluble synthetic food colors. BB has been authorized for use as a food additive in multiple countries, specifically for dairy products, chocolates, cereals, cheese, toppings, jellies, liquors, and soft beverages [5]. The utilization of this dye is rationalized based on its favorable cost–benefit ratio, as the color blue is not naturally present in body fluids [6]. The daily consumption of BB has been determined as 6 $mg \cdot kg^{-1}$ by the World Health Organization, taking into consideration the weight of the human body. According to the evaluations conducted by JECFA in 1970 and the SCF in 1975, the acceptable daily intake (ADI) of BB was 12.5 $mg \cdot kg^{-1}$ $bw \cdot day^{-1}$. However, in 1984, the SCF reduced the ADI to 10 $mg \cdot kg^{-1}$ $bw \cdot day^{-1}$ based on new long-term research [7]. Several studies have documented the adverse effects of BB on both animals and humans, including convulsions, gastrointestinal tumors, neurotoxicity, inflammation, neuropathic pain, cancer, ADHD in children, etc. [8–11]. Hence, it is crucial to establish a prompt, precise, and reliable sustainable methodology to evaluate the safety of the BB colorant before its authorized incorporation in commercially accessible commodities.

The emerging discipline of green chemistry is currently witnessing significant expansion due to the increasing recognition of the mandatory safeguarding of the environment, mitigating pollution, establishing a more sustainable industrial ecosystem, and adopting cleaner production methods. The aforementioned phrases are utilized to characterize techniques that have the objective of reducing the consumption of feedstock, reagents, and energy [12], implementing waste management systems [13], and improving safety for analysts and the environment [14]. It is essential to find and investigate potential sources for extraction as well as to build sustainable ways for recovery in order to guarantee the continuation of environmentally friendly and sustainable chemistry operations. Ultrasound-assisted extraction (UAE) has become a significant technology in the field of sustainable "green" chemistry and extraction. It has proven to be more time-efficient, consume less solvent, and give higher extraction results compared to conventional heating methods [15,16].

High-performance liquid chromatography (HPLC) is commonly used to detect synthetic food dyes because it offers reliable repeatability and satisfactory sensitivity when combined with conventional UV-Vis detection. Consequently, the analysis of food color becomes more intricate when it must adhere to the principles of green analytical chemistry (GAC). The majority of compounds employed in liquid chromatography (LC) methodologies possess high volatility, hence facilitating rapid dispersion and posing potential risks to the environment. Several of these substances have the potential to induce both acute and chronic toxicity. Analytical chemists have to prevent or at least mitigate any negative environmental repercussions of what they do and to guarantee that there is no excessive pollution. The primary objective in achieving sustainability throughout developed procedures is the selection of suitable green solvents. The study conducted by Larsen et al. [17] introduced a novel chemometric tool to identify environmentally friendly and sustainable solvents. This tool evaluates the greenness score outlined in the GSK Solvent Sustainability Guidelines, which offers a quantitative assessment of solvents using a comprehensive set of criteria represented by the composite score (G). According to Eq. 1, G indicates the numerical value of the fourth root obtained by multiplying four crucial sustainable factors: health (H), safety (S), environment (E), and waste disposal (W):

$$G = \sqrt[4]{(H \times S \times E \times W)} \tag{1}$$

The interpretation of all values, which range from 1 to 10, is that the lower score value denotes characteristics that make a certain solvent unsustainable, while the higher score value denotes an ideal sustainable and green solvent. Methanol and acetonitrile are commonly employed solvents; nevertheless, despite their excellent elution properties, these solvents pose considerable safety and health concerns. As a result, it has been established that ethanol, due to its qualities as a safe and environmentally friendly solvent, as well as its effectiveness as a replacement for hazardous substances like methanol and acetonitrile, is deemed the most appropriate choice [13,18].

The application of analytical quality by design (AQbD) principles offers a promising strategy to improve the assurance, regulation, and standardization of analytical methods, while also facilitating the efficient communication of analytical findings in the domain of food analysis. The significance of the HPLC method has been amplified within the context of quality by design (QbD) because of its influence on the assurance of product quality. QbD strategy is a very effective approach that overcomes the limits of the conventional

one-factor-at-a-time approach by allowing for comprehensive consideration of multiple variables simultaneously in relation to method response [19–23]. According to the recommendations established by the International Council for Harmonization (ICH) in Q14, the use of AQbD has become mandatory [24,25].

Despite the recent advancements made in the field of food analysis, the absence of efficient control, standardization, and harmonization of analytical methods poses challenges in ensuring the integrity, risk mitigation, production, safety, and authentication of food products. The Fourier transform infrared (FTIR) technique is extensively utilized in qualitative chemical analysis due to its environmentally conscious, non-invasive, and expeditious analytical capabilities. This technique is particularly advantageous in identifying specific and functional group regions of a sample, as well as offering insights into the stretching motion of chemical bonds, which are observable in the fingerprint region [26,27].

In today's world, the escalating phenomenon of globalization and the concurrent expansion of international trade requires the use of methodologies that exhibit enhanced reliability, efficiency, and expediency in the detection, identification, and quantification of dyes. HPLC is widely acknowledged as a very efficient and appropriate method for the quantification of synthetic colors in samples of food and beverages. Through a thorough review of existing research articles, numerous HPLC techniques have been established to accurately quantify the presence of BB in different matrices, even when additional colorants are present. These methods utilize either UV/Visible or photodiode array (PDA) detection [28–33]. A comparison of the analytical performance of previously published literature and the proposed method is represented in Table 1. Nevertheless, a significant proportion of HPLC techniques devised for colorant analysis encounter restrictions regarding their capacity to achieve effective separation. Moreover, these methodologies tend to consume a significant amount of time, especially when endeavoring to concurrently ascertain a diverse array of food color additives. All existing reported methods are ineffective in optimizing performance-related factors, and the few that do attempt optimization employ a univariate approach. Likewise, the adequate validation of methods is frequently disregarded. Consequently, the present work employed the AQbD approach to produce a robust separation performance. The mobile phase in all documented methods has typically consisted of either acetonitrile or methanol, both of which are known to be very toxic and do not align with the concepts of green chemistry. However, the proposed method utilizes ethanol as an alternative, which is considered to be more environmentally friendly.

Table 1: Comparison of the analytical performance of the proposed method with previously published methods

Sl. No.	Solvent used	Mobile phase used	Time of analysis (min)	Linearity range	LOD	LOQ	Ref
1.	Acetonitrile	0.02 M ammonium acetate/acetonitrile in gradient ratio	20	0.125–4.0 $mg{\cdot}L^{-1}$	0.06 $mg{\cdot}L^{-1}$	0.10 $mg{\cdot}L^{-1}$	[28]
2.	Acetonitrile	Acetonitrile/100 mM sodium acetate buffer pH 7 in gradient ratio	35	1–100 $mg{\cdot}L^{-1}$	NA	NA	[29]
3.	Acetonitrile	100 mM ammonium acetate/acetonitrile in gradient ratio	7	50–10,000 $ng{\cdot}mL^{-1}$	NA	LLOQ-52.4 ULOQ-10480	[30]
4.	Methanol	0.02 $mol{\cdot}L^{-1}$ ammonium acetate aqueous solution (pH 4.5 adjusted by glacial acetic acid)/methanol in gradient ratio	15	0.81–2,000 $ng{\cdot}mL^{-1}$	0.063 $ng{\cdot}mL^{-1}$	NA	[31]
5.	Methanol	Aqueous ammonium acetate solution (0.02 M)/methanol	8	NA	0.104 $ng{\cdot}mL^{-1}$	NA	[32]
6.	Acetonitrile	Water containing 1% ammonium acetate (with pH 6.8, adjusted with ammonium hydroxide)/acetonitrile	12	0.5–50 $\mu g{\cdot}mL^{-1}$	0.067 $\mu g{\cdot}mL^{-1}$	0.223 $\mu g{\cdot}mL^{-1}$	[33]
7.	Ethanol	(Ethanol/ acetate buffer of pH 5) ratio (25:75, v/v)	5	8–12 $\mu g{\cdot}mL^{-1}$	0.42 $\mu g{\cdot}mL^{-1}$	1.28 $\mu g{\cdot}mL^{-1}$	Proposed method

NA: not available.

The integration of GAC principles into the AQbD framework enables the successful validation of intended analytical method performances through risk assessment and compliance with environmentally sustainable criteria [34–39]. The current approach for the method development has been designed to minimize potential risks to both the analyst and the environment. Consequently, our objective was to create and verify a new AQbD-supported environmentally friendly HPLC method for quantifying the food colorant, BB, in various ready-to-eat liquid and solid food samples using the green UAE approach. The present study provides a novel and innovative contribution to the field by being the first to detect the presence of BB in food samples obtained from the Indian market using an AQbD-assisted green HPLC methodology. The proposed methodology presents numerous benefits, such as its simplicity, convenience, environmental sustainability, and cost-effectiveness, for the extraction of BB from food matrices by utilizing the green UAE technique which enhances the overall value of the method and contributes additional advantages in terms of extraction yield, selectivity, extraction time, as well as the quality and safety of the extracted compounds. FTIR spectra confirm the successful extraction of BB from the food samples. The proposed approach has been evaluated in accordance with the (ICH) Q14 recommendation.

2 Materials and methods

2.1 Drugs, chemicals, solvents, and food samples

The standard dye BB (E133) (98.1% purity) was procured as gift samples from Redner Pharmaceuticals, Chennai, India. Ethanol (HPLC grade 100%) was procured from Hayman Group Ltd, UK. Analytical reagent grade sodium acetate anhydrous of purity 99% and sodium hydroxide (NaOH) of purity 98% were obtained from SRL, Maharashtra, India. Analytical reagent grade hydrochloric acid (HCl), glacial acetic acid (CH_3COOH), and hydrogen peroxide (H_2O_2) 3% (Rankem, New Delhi, India) were used. Food samples were purchased from a local supermarket in Chennai, Tamil Nadu. Milli Q filter system (ELGA Lab Water, Lane End, High Wycombe, UK) was utilized to produce HPLC-grade water, filtered using a membrane filter with a pore size of 0.45 µm, and then subjected to degassing for 30 min in an ultrasonic bath (Labman, Chennai, India).

2.2 Instrument and software

HPLC Agilent 1220 infinity II equipped with a binary solvent delivery pump, autosampler injector, and diode array detector was used for separation analysis and method development. Agilent Open Lab CDS chemstation (version 2.6) was utilized for data collection and data processing. In experimental design, the software Design-Expert® trailed version 12 (Stat-Ease Inc., Minneapolis, USA) was utilized for optimization of various chromatographic parameters and the development of design space (DS).

2.3 Preparation of solutions

2.3.1 Preparation of buffer solution (acetate buffer, pH 5)

A total of 13.6 g of sodium acetate anhydrous and 6 mL of glacial acetic acid were dissolved in water to form 1,000 mL. If necessary, pH was adjusted. A sonicator was used to degas the filtered buffer solution.

2.3.2 Preparation of standard stock solution

By dissolving 10 mg of BB with 10 mL of ethanol, a stock solution of 1,000 $\mu g \cdot mL^{-1}$ was produced. From the filtered stock, 0.1 mL of the solution was taken out and diluted in 10 mL of ethanol to obtain 10 $\mu g \cdot mL^{-1}$ of the working standard solution.

2.3.3 Preparation of food samples

The study used a total of ten samples that were obtained from a supermarket in Chennai. These included a range of samples that were categorized as chocolate candies (A1, A2), lollipop (A3), carbonated beverages (B4, B5), fruit jelly (C6), ice cream (D7), electrolyte sports drinks (E8, E9), and sugar confectionary (F10).

2.3.3.1 Preparation of liquid food samples

The liquid samples, such as carbonated beverages and electrolyte sports drinks, were subjected to degassing in a sonicator for 20 min. A total of 1 mL of the sample was

measured and subsequently spiked with a series of standard solutions of BB, ranging from 8 to 12 μg·mL^{-1}, using the standard addition method and was diluted to a final volume of 10 mL using ethanol. The extraction process involved subjecting samples to a UV probe sonicator.

2.3.3.2 Preparation of solid food samples

The solid samples, such as candy, lollipop, jelly, and confectionery, were subjected to pulverization using a mortar and pestle, and 2 g of the resulting powder was dissolved in 50 mL of ultrapure water. The samples were subjected to heating in a heating mantle at 50°C for 30 min until complete dissolution was achieved. Then, the samples were subjected to centrifugation at a speed of 3,500 rpm (REMI Centrifuge, Mumbai, India) for 10 min followed by decantation of the liquid part. About 1 mL of the sample was measured and subsequently spiked with a range of standard solutions of BB, with concentrations ranging from 8 to 12 μg·mL^{-1}, using the standard addition method, and the total volume was made up to 10 mL using ethanol. The resulting mixture was subjected to extraction using a UV probe sonicator.

2.3.3.3 UAE of food samples

The UAE of food samples was carried out according to Shen et al. [40]with minor modifications. The sample mixture underwent UAE utilizing an ultrasonic probe sonicator (Rivotek, Mumbai, Maharashtra) for 15 min. The supernatant layer was separated by centrifugation, collected, and later transferred to a distinct test tube. The ethanol extraction method was performed sequentially and repeated three times. The supernatant layers were collected and subjected to filtration using a 0.45 μm microporous film. The filtered solution was then transferred to an HPLC vial. Figure 2 shows the green UAE technique for sample extraction.

Figure 2: The green UAE technique for sample extraction.

2.4 Optimized HPLC conditions

In order to develop an appropriate HPLC method for quantifying BB, preliminary trials were carried out using the reported literature. All of them used organic solvent systems (i.e., acetonitrile, methanol) with acetate buffers in the pH range of 3.0–7.0 (adjusted with glacial acetic acid) at variable flow rate(s). As the primary objective of this study is to develop an environmentally sustainable and economically feasible method for estimating BB, ethanol is used as a safe and environmentally friendly solvent in place of toxic acetonitrile or methanol. This study investigates a causal relationship between experimental conditions, leading to a decrease in the amount of time and effort required. The main objective of separation is to obtain a specific mobile phase combination that yields an optimal retention time (RT) with exceptional peak symmetry, reduced tailing, and increased theoretical plates, while also enabling the rapid elution of analytes from the stationary phase with a minimum amount of solvent and time. The use of AQbD employing CCD coupled with risk assessment facilitated the judicious identification of the influential factors, as well as the most suitable chromatographic conditions for the analysis of BB.

Separation was achieved using a Phenomenex column (Luna C_{18}, 250 mm × 4.6 mm i.d., 5 μm). The pH adjustment of the buffer solution was performed using the Eutech instruments pH 700 (India). Filtration was carried out using 0.45 μm nylon membranes. The solvents were degassed using an ultrasonicator. The optimum mobile phase comprised ethanol and sodium acetate buffer (pH 5) in the proportion of 25:75 v/v, a flow rate of 1 mL·min^{-1}, injection volume of 10 μL, detection wavelength at 626 nm, and run time of 5 min. All the conditions mentioned were tested and optimized following the ICH Q14 guidelines.

2.5 System suitability

In order to assess the system suitability parameters, a standard solution of 10 μg·mL^{-1} was injected six times in a replicate. The % relative standard deviations (RSDs) were

computed for RT, peak area, theoretical plates, and tailing factor. Figure 3 shows the standard chromatogram for BB.

2.6 Stress degradation study

BB (10 μg·mL^{-1}) was exposed to various stress conditions including acid, alkali, oxidation, and heat treatments in order to evaluate its degradation processes. Degradation was operationally defined as a reduction in the magnitude of peak area or the appearance of supplementary peaks. The quantification of the degradation level was accomplished by employing % recovery.

Acid degradation: Hydrochloric acid (HCl) at a concentration of 0.001 N was utilized to execute acid degradation: 1 mL aliquot of a standard solution was introduced into a 10 mL volumetric flask, followed by the addition of 1 mL of 0.001 N HCl. The flask was then filled to the required volume using ethanol.

Base degradation: BB was subjected to alkali degradation by treatment with 0.001 N sodium hydroxide (NaOH). A 10 mL standard flask was utilized to contain 1 mL of standard solution. Subsequently, 0.001 N NaOH was added to the flask, followed by the addition of ethanol until the flask was filled to the mark.

Oxidation with hydrogen peroxide (H_2O_2): 3% v/v hydrogen peroxide was used for peroxide degradation. In a 10 mL volumetric flask, 1 mL of standard solutions followed by 1 mL of 3% v/v peroxide solution was added, and then the final step involved adjusting the solution volume to the appropriate level using ethanol.

Thermal degradation: The standard solution of BB was kept in an oven heated to 60°C for 1 h. The prepared solutions were injected into the HPLC system at 30 min and 1 h under ideal chromatographic conditions. The food samples are also kept for 1 h and injected in HPLC subjected to degradation.

2.7 Method validation

The suggested technique was validated as per ICH Q14 recommendations.

2.7.1 Linearity, LOD, and LOQ

In a 10 mL standard flask, aliquots of BB were obtained from stock solutions. The diluent was then used to produce successive dilutions, resulting in BB concentrations ranging from 8 and 12 μg·mL^{-1}. The calibration curve was generated by plotting concentration versus peak area. The regression correlation coefficient and intercept value were used to evaluate linearity. The method's sensitivity was assessed using the limit of detection (LOD) and limit of quantification (LOQ).

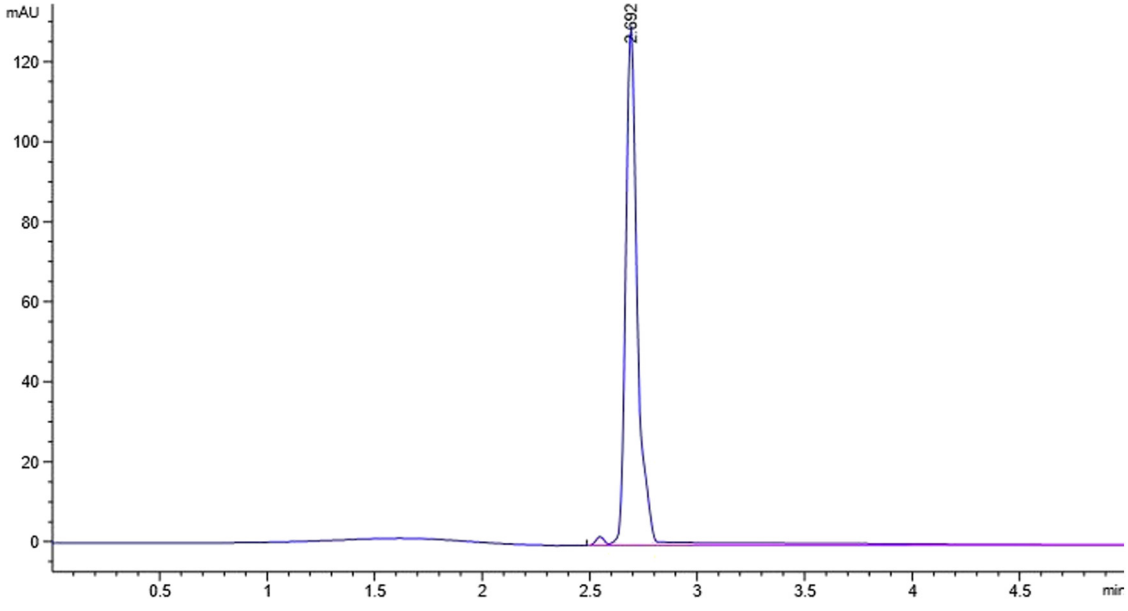


Figure 3: Standard chromatogram of brilliant blue.

3 Results and discussion

Within the field of pharmaceutical analysis, it is feasible to develop analytical methodologies that possess environmentally friendly attributes, characterized by the absence of hazardous constituents and a diminished ecological footprint. This study offers a thorough elucidation of the AQbD approach, utilizing the concepts of GAC, to methodically construct an analytical method. To achieve the necessary separation, an optimization of the HPLC procedure was conducted employing a CCD, allowing for the modification of many factors. The value of analytical advancement is therefore amplified by this tri-combination, which is seen to be mutually reinforcing.

3.1 Selection of the mobile phase

The selection of the mobile phase posed a significant difficulty in the field of analytical chemistry when it was integrated with the concepts of GAC. This challenge arose due to the limited availability of environmentally friendly organic solvents, as well as the considerable difficulties in achieving compatibility between the mobile phase and the analytical system. BB demonstrates solubility in a wide variety of solvents routinely utilized in sample preparation for HPLC, including methanol and acetonitrile. However, the use of these hazardous solvents leads to complex environmental issues and undermines the principles of green chemistry. The organic phase for this study was chosen exclusively according to the solvent selection guide provided by GSK. A significant proportion of the ecologically friendly solvents mentioned in the solvent selection guide may exhibit limited compatibility with the LC system. Biodegradable ethanol and propylene carbonate have been identified as the most effective alternatives to toxic solvents, such as acetonitrile or methanol. Therefore, it was decided to use only ethanol, an organic solvent that is not as dangerous as some others. In the initial stages, ethanol was employed as the organic phase in conjunction with ultrapure water in a 50:50 ratio but peak splitting was observed. Following this, ethanol and phosphate buffer in a 50:50 ratio with different pH values produced asymmetric peaks, gradually increasing the amount of buffer in various ratios causes peak broadening. Finally, ethanol with acetate buffer in a 50:50 ratio produced a symmetric peak but RT and tailing were observed. By increasing the amount of buffer in the ratio of 40:60, 35:65, 30:70, and 25:75, the peak shape was nearly similar; however, a sharp peak with no tailing was seen in case of 25:75. As our goal is to reduce the amount of the organic phase, this particular combination of 25:75 facilitated the expedited elution of analytes from the stationary phase, requiring a minimal amount of solvent as well as time, while also yielding optimal RT with exceptional peak symmetry, reduced tailing, and increased theoretical plates.

3.2 AqbD-aided method development

The field of pharmaceutical analysis has experienced advancements in approaches for drug analysis in various matrices. However, one notable drawback of these methodologies is the difficulty in method transfer, which requires constant re-validation and raises issues regarding safety and environmental consequences. The AQbD framework comprises five sequential processes that aid in understanding the variables and their interactions within a method, identifying elements that significantly affect process performance, and establishing acceptable limits for their variability.

The AQbD technique comprises a set of five discrete processes that are utilized in the development of statistical and experimental designs: (i) the analytical target profile (ATP) can be characterized as the intended result of a specific methodology; (ii) the determination of critical quality attributes (CQAs) or critical analytical attributes (CAAs) entails the assessment of method attributes or critical method parameters (CMPs); (iii) the implementation of risk assessment improves the effectiveness and compliance with the ATP of the system; (iv) the process of identifying the method operable design region (MODR) or DS entails the examination of the interrelationship between several aspects that impact a given process and its resultant output; and (v) the control strategy and management of the product lifecycle are to assure the resilience and effectiveness of the approach [32]. The incorporation of the GAC principles into the AQbD approach offers a cooperative framework for the development of strategies that are both environmentally sustainable and highly efficient while also being adaptive.

3.2.1 ATP, CQAs, or CAAs

The primary objective of ATP is to provide a method that is efficient, rapid, environmentally sustainable, and economically feasible for the study of BB. The selection of the HPLC-PDA analytical technology was considered suitable for the attainment of these aims due to its numerous advantages, such as reliability, robustness, and simplicity. CQAs are quantifiable variables that effectively capture the

performance characteristics of a specific methodology. The major goal of CQAs is to decrease the amount of ethanol and provide a steady flow rate within a predetermined range. The responses were intended to maximize column efficiency by increasing the number of theoretical plates. On the contrary, the objective of minimizing peak tailing was pursued in order to achieve favorable peak shapes. Peak area, RT, theoretical plates, and tailing factor were used as factors for selecting the most important CAAs.

3.2.2 Risk assessment or scouting phase

The identification of risks was carried out by employing the Ishikawa fishbone diagram, as depicted in Figure 4 [39,41]. A risk assessment is considered to be a comprehensive framework for analyzing multiple factors and their associated outcomes, without the requirement of performing empirical analysis. To align with the tenets of GAC, ethanol was utilized as an organic modifier instead of other solvents that possess hazardous characteristics. HPLC-PDA is employed with a short run time of only 5 min. The aforementioned methodology successfully tackles the problem of excessive utilization of solvents and facilitates expeditious and ecologically conscious assessment. The compound demonstrates the most significant response when subjected to a wavelength of 626 nm. Isocratic elution was used instead of the more complex and time-consuming gradient elution method due to its relative ease of use and shorter processing times. The optimization of factors for achieving chromatographic separation of BB involved the selection of mobile phase composition (ethanol content: 25–35% v/v) and flow rate (0.8–1.2 mL·min^{-1}) at five different levels. The values of the design, along with their corresponding outcomes, are presented in Table 2.

3.2.3 Experimental design

3.2.3.1 Optimization of the method employing rotatable central composite design (rCCD)

Two CMPs were experimentally studied with four distinct CAAs in order to optimize the process utilizing the response surface approach employing rCCD. In order to get the best possible results with the chosen CAAs, appropriate levels were assigned to each parameter based on an initial risk assessment. Thus, a CCD was carried out, with 13 experimental runs covering three unique ethanol percentage ranges (25, 30, 35) and flow rate ranges (0.8, 1, 1.2). The errors were evaluated by repeatedly measuring the zero-level midway of each variable five times during the experiment. In order to examine each factor in the response, statistical tools such as analysis of variance (ANOVA) for the quadratic model, 2D contour and 3D surface plots, perturbation plots, and lack of fit (LOF) graphs were employed and that

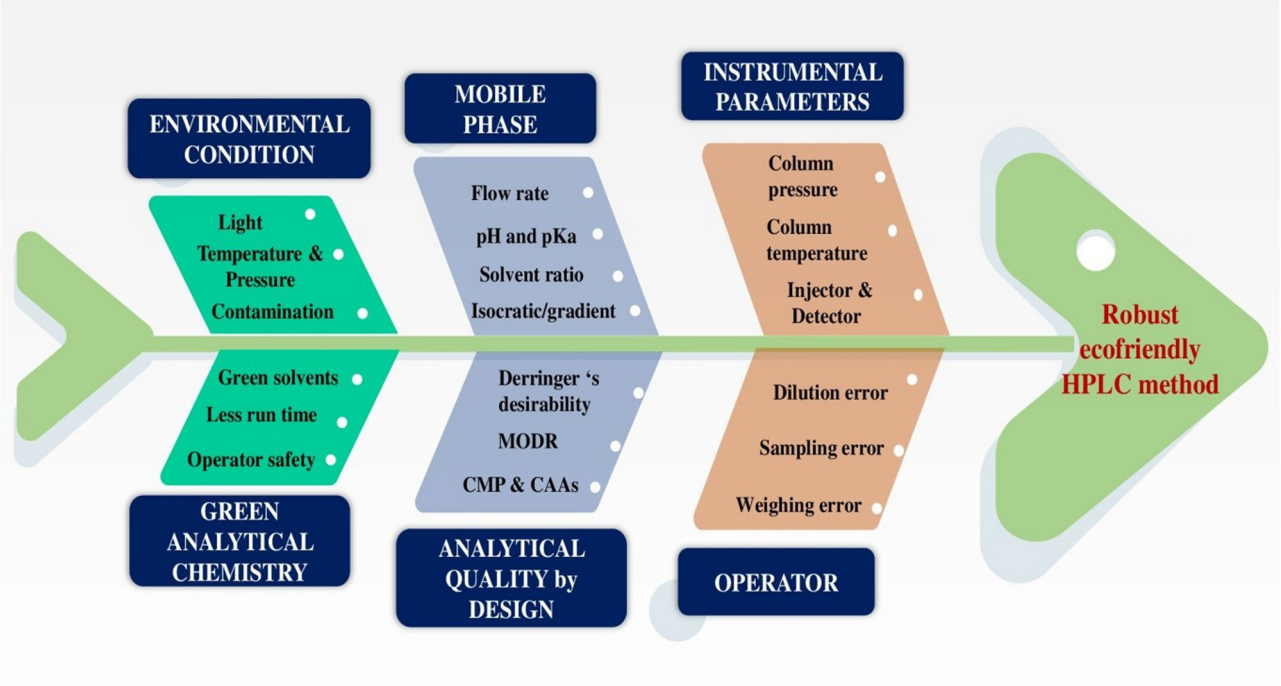


Figure 4: Ishikawa fishbone diagram.

Table 2: Experimental design: actual and measured responses

Std	Run	Factors A: mobile phase (ethanol)	Responses B: flow rate	RT (*R*1)	Area (*R*2)	Theoretical plates (*R*3)	Tailing factor (*R*4)
11	1	30	1	2.791	189	10,678	1.31
13	2	30	1	2.89	178	11,989	1.34
1	3	26.4645	0.858579	2.995	232	17,899	1.34
8	4	30	1.2	2.576	399	2,190	0.99
12	5	30	1	2.749	187	9,989	1.35
2	6	33.5355	0.858579	2.764	288	10,876	1.34
10	7	30	1	2.789	234	9,987	1.33
6	8	35	1	2.699	378	12,878	1.41
5	9	25	1	2.636	184	10,899	1.38
7	10	30	0.8	2.97	243	10,962	1.27
9	11	30	1	2.781	143	10,900	1.38
4	12	33.5355	1.14142	2.75	499	11,989	1.12
3	13	26.4645	1.14142	2.34	269.4	1,543	1.23

contribute an effective DS. All of the factors were analyzed using quadratic findings, allowing for careful selection and timely completion within the constraints. Two-factorial or linear models would have made this difficult to accomplish. This guarantees that each control variable has a significant impact on the response variable. Each response was analyzed using 2D and 3D graphs to understand how the factors played a role. The study analyzed the effects of the identified factors on the dependent variables. The results of ANOVA performed to determine the statistical significance of the experimental model is given in Table S1 (Supplementary Information). The experimental results were included into a quadratic model, and the model was shown to be statistically significant at a *p*-value of <0.0001. The statistical

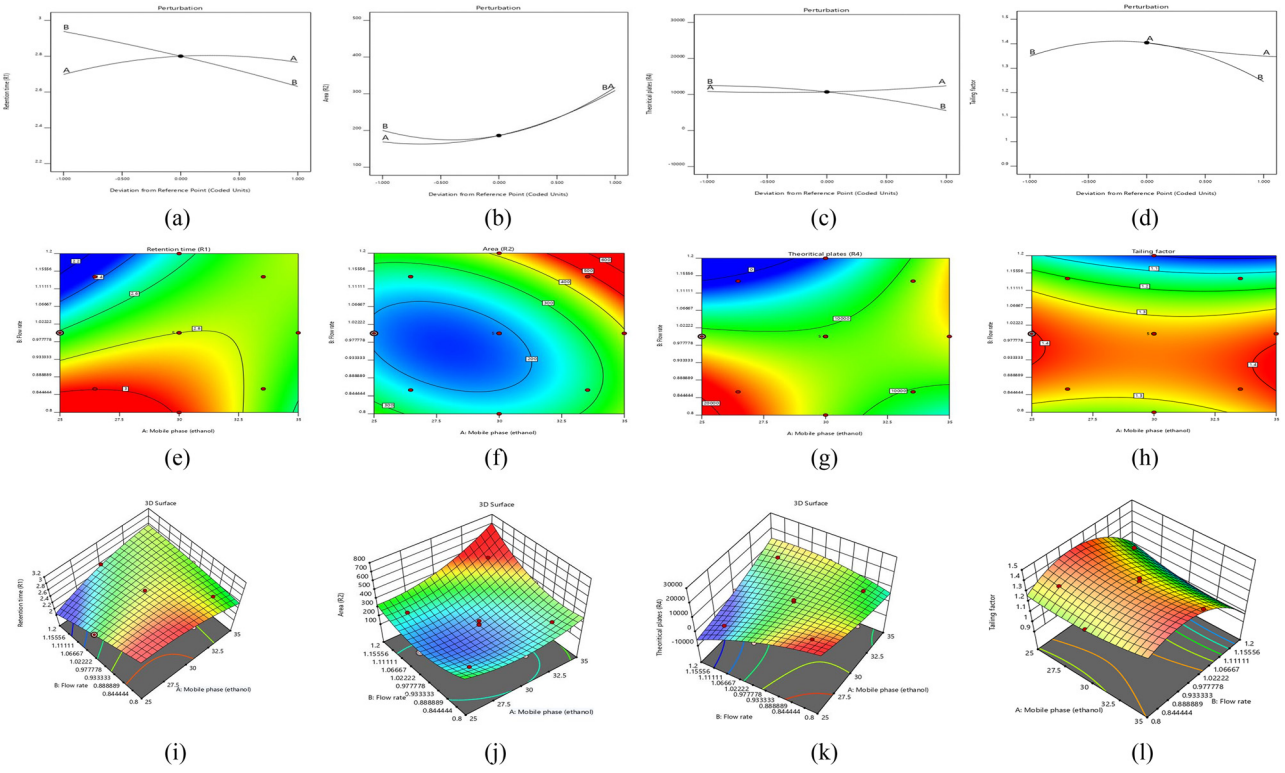


Figure 5: Perturbation plot (a)–(d), contour plot (e)–(h), and 3D response plot (i)–(l) for RT (*R*1), peak area (*R*2), theoretical plates (*R*3), and tailing factor (*R*4) of BB.

significance of each model coefficient is also illustrated by the Fischer ratios, often known as F values. Large R^2 and low LOF values suggest that the model fits the data well, while a high F ratio shows that the analytical model equation has statistical significance. Using the correlation coefficient (R^2), the adjusted correlation coefficient (Adj. R^2), and the predicted correlation coefficient (Pred. R^2), the polynomial regression equations were assessed. The determination coefficient, denoted as r^2, exhibits values that are close to 1 across all instances. This means that the regression curve has a very high degree of accuracy (not less than 99%) in fitting the data. Furthermore, the adequate precision value is greater than 4, indicating the presence of an acceptable signal.

3.2.3.2 Interpretation and derringers desirability function

Graphical data interpretation with perturbation, contour, and 3D surface plots was used to study the effects of CMPs on each CAA. Figure 5 represents perturbation (a–d), contour (e–h), and 3D plots (i–l) of BB.

3.2.3.3 Response of ethanol concentration and flow rate on RT

The perturbation (Figure 5a), contour (Figure 5e), and 3D plots (Figure 5i), along with the second-order polynomial equation for RT, indicate that an increase in the flow rate leads to a decrease in RT. Additionally, it is observed that higher ethanol concentrations have a negligible impact on RT. The polynomial equation for the RT is

$$\begin{aligned}\text{Retention time}(R1) = {} & +7.52546 + 0.016579 \\ & \times \text{Mobile phase(ethanol)} \\ & - 9.14882 \times \text{Flow rate} \\ & + 0.320500 \times \text{Mobile phase(ethanol)} \\ & \times \text{Flow rate} - 0.005460 \\ & \times \text{Mobile phase(ethanol)}^2 \\ & - 0.775000 \times \text{Flow rate}^2\end{aligned}$$

3.2.3.4 Response of ethanol concentration and flow rate on the peak area

The perturbation (Figure 5b), contour (Figures 5f), and 3D plots (Figure 5j), with the second-order polynomial equation indicate that an increase in both parameters results in a significant increase in the area of the peak. The polynomial equation for the area is

$$\begin{aligned}\text{Area}(R2) = {} & +9208.06880 - 319.84252 \\ & \times \text{Mobile phase(ethanol)} - 9456.94334 \\ & \times \text{Flow rate} + 86.80000 \\ & \times \text{Mobile phase(ethanol)} \times \text{Flow rate} \\ & + 4.21400 \times \text{Mobile phase (ethanol)}^2 \\ & + 3633.75000 \times \text{Flow rate}^2\end{aligned}$$

3.2.3.5 Response of ethanol concentration and flow rate on the theoretical plate

The perturbation (Figure 5c), contour (Figures 5g), and 3D plots (Figure 5k), with the second-order polynomial equation indicate that an increase in the flow rate results in a significant decrease in theoretical plates. Additionally, it is observed that higher ethanol concentrations have a negligible impact on theoretical plates. The polynomial equation for theoretical plates is

$$\begin{aligned}\text{Theoretical plates } (R3) = {} & +2.70751 \times 10^5 - 12959.68867 \\ & \times \text{Mobile phase(ethanol)} \\ & - 1.13456 \times 10^5 \times \text{Flow rate} \\ & + 8734.50000 \\ & \times \text{Mobile phase(ethanol)} \\ & \times \text{Flow rate} + 74.08600 \\ & \times \text{Mobile phase(ethanol)}^2 \\ & - 86508.75000 \times \text{Flow rate}^2\end{aligned}$$

3.2.3.6 Response of ethanol concentration and flow rate on the tailing factor

The perturbation (Figure 5d), contour (Figures 5h), and 3D plots (Figure 5l), with the second-order polynomial equation indicate that the flow rate has a significant effect on the tailing factor compared to an increase in higher ethanol concentrations. The polynomial equation for theoretical plates is

$$\begin{aligned}\text{Tailing factor}(R4) = {} & -3.13915 - 0.068589 \\ & \times \text{Mobile phase(ethanol)} \\ & + 11.73332 \times \text{Flow rate} \\ & - 0.055000 \times \text{Mobile phase(ethanol)} \\ & \times \text{Flow rate} + 0.002020 \\ & \times \text{Mobile phase(ethanol)}^2 \\ & - 5.36250 \times \text{Flow rate}^2\end{aligned}$$

3.2.3.7 MODR

MODR, often known as the control space, was developed utilizing robustness simulations and CMA models. The predicted solution chosen was ethanol 25% by volume, a flow rate of 1 mL·min^{-1}, an RT of 2.692 min, and a theoretical plate size of 13,779 with a desirability value of 1.000. The predicted experimental conditions determined the control strategy. The values of the system suitability parameters were then calculated, and it was found that they were within the desirable range of less than 2%. The overlay plot with the ideal DS region is shown in Figure S1.

3.2.3.8 Optimized chromatographic conditions

An ecologically friendly HPLC approach for analyzing BB has been suggested. The optimum chromatographic conditions for this method comprise a mobile phase made up of ethanol and acetate buffer in a ratio of 25:75 (v/v), with a flow rate of 1 mL·min^{-1}. This study used the Phenomenex column (Luna C_{18}, 250 mm × 4.6 mm i.d., 5 µm). The injection volume was 10 µL, and the detection was carried out for 5 min at a wavelength of 626 nm.

3.3 System suitability

Table 3 displays the outcomes of the system suitability evaluation. The obtained %RSD for parameters such as RT, peak area, and tailing factor were all below 2%. Additionally, the presence of theoretical plates exceeding 2,000 serves as excellent proof of the system's suitability.

3.4 Method validation

3.4.1 Linearity, LOD, and LOQ

A strong linear correlation (R^2 = 0.999) was observed between the concentration and peak areas of BB within the range of 8–12 µg·mL^{-1}, under optimal chromatographic conditions. The analytical results for linearity, including the slope and intercept, are presented in Table 4. The values of the LOD and LOQ were calculated using the standard deviation (SD) of the response and the slope of the regression line. This calculation highlights the high sensitivity of the proposed method. The LOD and LOQ were found to be 0.42 and 1.28, respectively.

Table 3: Results of system suitability parameters for the proposed HPLC method

System suitability parameters	Average mean ± SD	%RSD	Reference value
RT	2.632 ± 0.0193	0.7360	—
Peak area	183.6 ± 3.44	1.87	—
Theoretical plates	10,425.8 ± 113.5	1.08	NLT 2000
Tailing factor	1.32 ± 0.017	1.32	NMT 2

3.4.2 Precision and accuracy

Both intraday precision and interday precision were carried out, and the results are presented in Table 4. The measured RSD was below 2%, indicating a significant level of precision. The accuracy results produced from the developed method exhibit a good range of % recovery, hence indicating a commendable level of accuracy for the recommended approach. The results pertaining to accuracy are presented in Table 4.

3.5 Outcomes of degradation studies

Studies on forced degradation were conducted with BB. The major degradation conditions were 0.001 N NaOH, 0.001 N HCl, 3% H_2O_2, and thermal degradation at 60°C. BB degrades quickly when exposed to acid, alkali, and 3% H_2O_2. As the peak's area substantially decreased under acid, alkali, and oxidation conditions, BB is quite susceptible to these conditions. The peak was completely deformed in an acid and oxidation condition at 0 min. For alkali degradation at 0 min, BB started degrading; however, the peak shape was not favorable and it exhibited various disturbances in addition to the movement of the RT. After

Table 4: Results of validation parameters for BB

Parameters	Observations
Slope	90.5
Intercept	−569.2
SD	143.14
LOD	0.42
LOQ	1.28
Regression equation	90.5 × (−569.2)
Correlation coefficient (R^2)	0.999
Accuracy	99.1–101.2%
Interday precision (%RSD)	0.56
Intraday precision (%RSD)	0.39

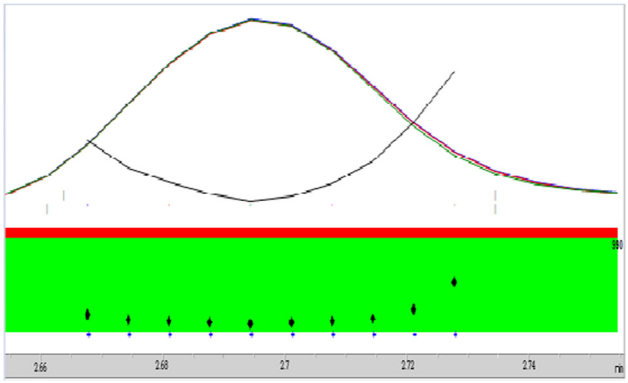


Figure 6: Peak purity plot of BB.

30 min, the standard's thermal degradation was noticed, and 91.6% of the BB was recovered. Thermal degradation was observed for the food samples A1 and B4 after 30 min, and the percentage of BB recovery was found to be 85.1% and 83.5%, respectively. The peak purity result of BB showed that the purity factor is within the threshold limit confirming that there is no coelution of other peaks, as shown in Figure 6.

The outcomes of the degradation study and their chromatograms are presented in Figure S2 and Table 5.

3.6 Application to real samples

The HPLC approach suggested in this study was effectively employed to analyze BB in ten regularly consumed food samples in India, with a specific focus on its prevalence among children. These samples included carbonated drinks, electrolyte sports drinks, fruit jelly, ice cream, chocolate candy, lollipops, and sugar confectionery. The literature assessment demonstrates that a previous study has already undertaken HPLC analysis on BB in the matrices listed above. Nevertheless, these methods possess certain limitations. The use of dangerous and deadly solvents, negligence in employing environmentally friendly extraction processes in the sample preparation, and inadequate measures to guarantee food safety have been seen in their practices. Given the paramount importance of ensuring food safety within our societies, our primary emphasis has been on developing and validating a methodology that effectively enforces safety standards at every stage, utilizing the principles of GAC. Additionally, we have utilized the UAE technique for obtaining BB from various food matrices. The quantification of BB was carried out using a standard addition method. The recovery study involved the spiking of known quantities of the standard dyes under investigation to the samples prior to processing. The resulting outcomes were then compared to those obtained from the same samples without spiking with standard dyes. The % recovery was estimated by calculating the difference in concentrations and then expressing the results as percentages. The developed method combines ease and selectivity by employing distinct cleanup/extraction steps prior to color extraction to prevent interference from complex food matrices. By injecting a lab-made placebo into the HPLC system and analyzing the chromatograms, the matrix effect was assessed. No placebo effect was identified. This study aims to analyze the amounts of BB in different food samples and subsequently compare the obtained results with international regulatory limits in an environmentally conscious manner, utilizing a method that offers high sensitivity and efficient recovery of the samples, aided using UAE.

Table 6 displays the recovery percentage in various food samples using the newly developed HPLC approach.

Table 5: Forced degradation study of BB

Conditions	% BB recovery			% BB degradation					
	Standard BB	Food sample (A1)	Food sample (B4)	Standard BB	Mass balance	Food sample (A1)	Mass balance	Food sample (B4)	Mass balance
Thermal – 30 min	91.6	85.1	83.5	8.4	99.57	14.9	96.88	16.5	95.26
Alkali degradation (0.001 N NaOH) – 0 min	80.5	78.5	85.5	19.5	98.45	21.5	97.34	14.5	95.74
Acid (0.001 N HCl) and 3% H_2O_2 degradation**									

**For acid and H_2O_2 degradation, 0.001 N HCl and 3% H_2O_2 were used, respectively; however, it was seen that in both cases, the peak shape was fully deformed with a drastic reduction of the peak area; therefore that data is not shown in the table.

The recovery of BB exhibits a range of values, ranging from 98.26% to 102.56%, across different food samples. The recovery rate of BB is higher in carbonated beverages in comparison to solid food samples. The amount of BB in all the commercial food samples is under the specified level. The overlay FTIR spectra of the standard BB and the extracted food sample A1 (Figure 7) provide evidence for the successful extraction of BB from food samples using UAE. This comparison validates the recovery of the sample using an environmentally friendly extraction technique and also improves the authenticity of the extraction method.

Figure 8 depicts the chromatogram illustrating the unspiked and 10 µg spiked chocolate candy sample (A1) and carbonated drink sample (B4).

3.7 Greenness evaluation of the developed method

In the twenty-first century, analysts are highly driven to pursue the development of innovative and ecologically sustainable methods of analysis, which necessitates the replacement of dangerous and harmful solvents with safer alternatives. To facilitate the progress of sustainable analytical procedures, GAC has put out a series of tactics that adhere to the principles of the 3Rs (replace, reduce, and reuse). In contrast to previous methods utilized for analyzing BB, the proposed method highlights the efficient usage of ecologically sustainable ethanol as a substitute for potentially harmful chemicals like methanol or acetonitrile. Furthermore, the incorporation of HPLC in this

Table 6: Recovery percentage obtained by the developed HPLC method for BB in different food samples

Sample	Spiked concentration ($mg \cdot kg^{-1}$) added to the sample	Average dye concentration in the sample ($mg \cdot kg^{-1}$)*	Average dye concentration in the spiked sample ($mg \cdot kg^{-1}$)	% Recovery
Chocolate candy (A1)	8	19.5	27.1	98.54
	10	19.3	28.9	98.63
	12	19	30.8	99.35
Chocolate candy (A2)	8	18.9	26.7	99.25
	10	19	28.9	99.65
	12	19.5	31.2	99.04
Lolipop (A3)	8	19.9	27.5	98.56
	10	18.7	28.6	99.65
	12	20.1	32	99.68
Carbonated drink (B4)	8	30.5	39	101.29
	10	30.9	41.9	102.44
	12	31	43	100
Carbonated drink (B5)	8	32	40.1	100.25
	10	31.9	42	100.23
	12	32.5	45.3	101.79
Fruit jelly (C6)	8	30	38.5	101.31
	10	30.1	40.5	100.99
	12	32	43.6	99.09
Ice cream (D7)	8	29	36.9	99.72
	10	29.9	39.8	99.74
	12	30.9	43.5	101.39
Electrolyte sports drink (E8)	8	20	27.9	99.64
	10	20.5	30.4	99.67
	12	21.8	33.5	99.11
Electrolyte sports drink (E9)	8	24.3	32.1	99.38
	10	25.1	36	102.56
	12	25	37.3	100.81
Sugar confectionary (F10)	8	15	22.6	98.26
	10	15.9	25.6	98.84
	12	16.3	27.9	98.58

*n = 3, mean average of three observations.

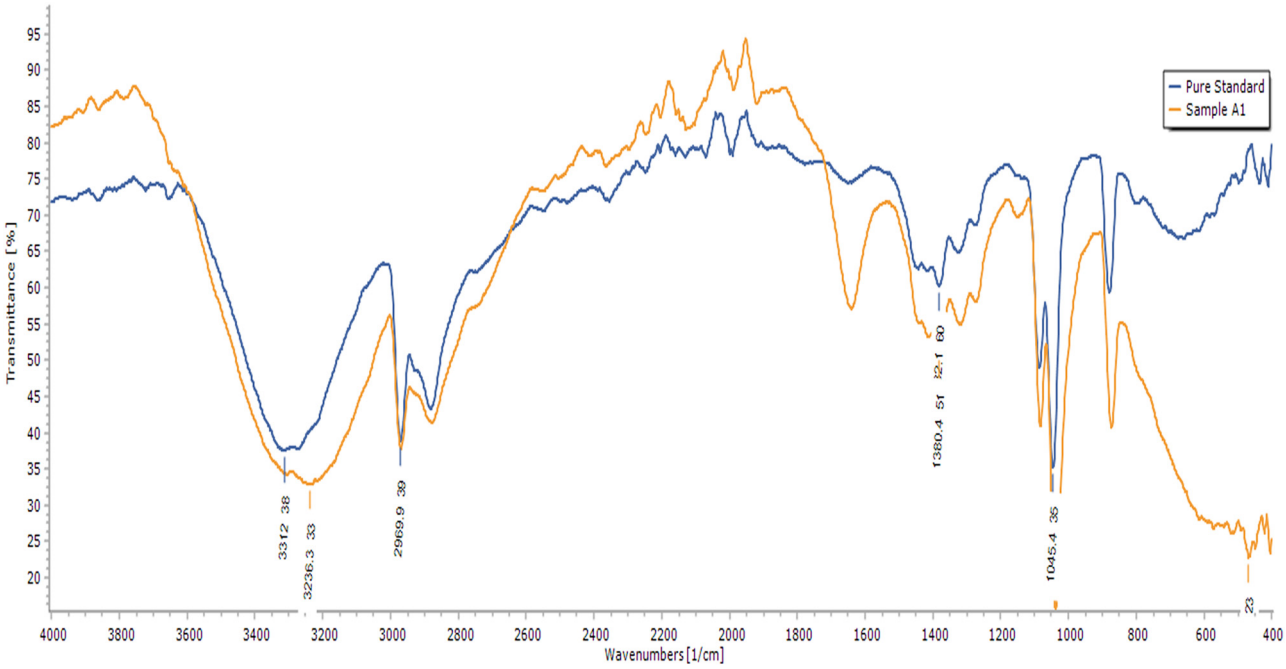


Figure 7: Overlay FTIR spectra of standard BB and the sample (A1).

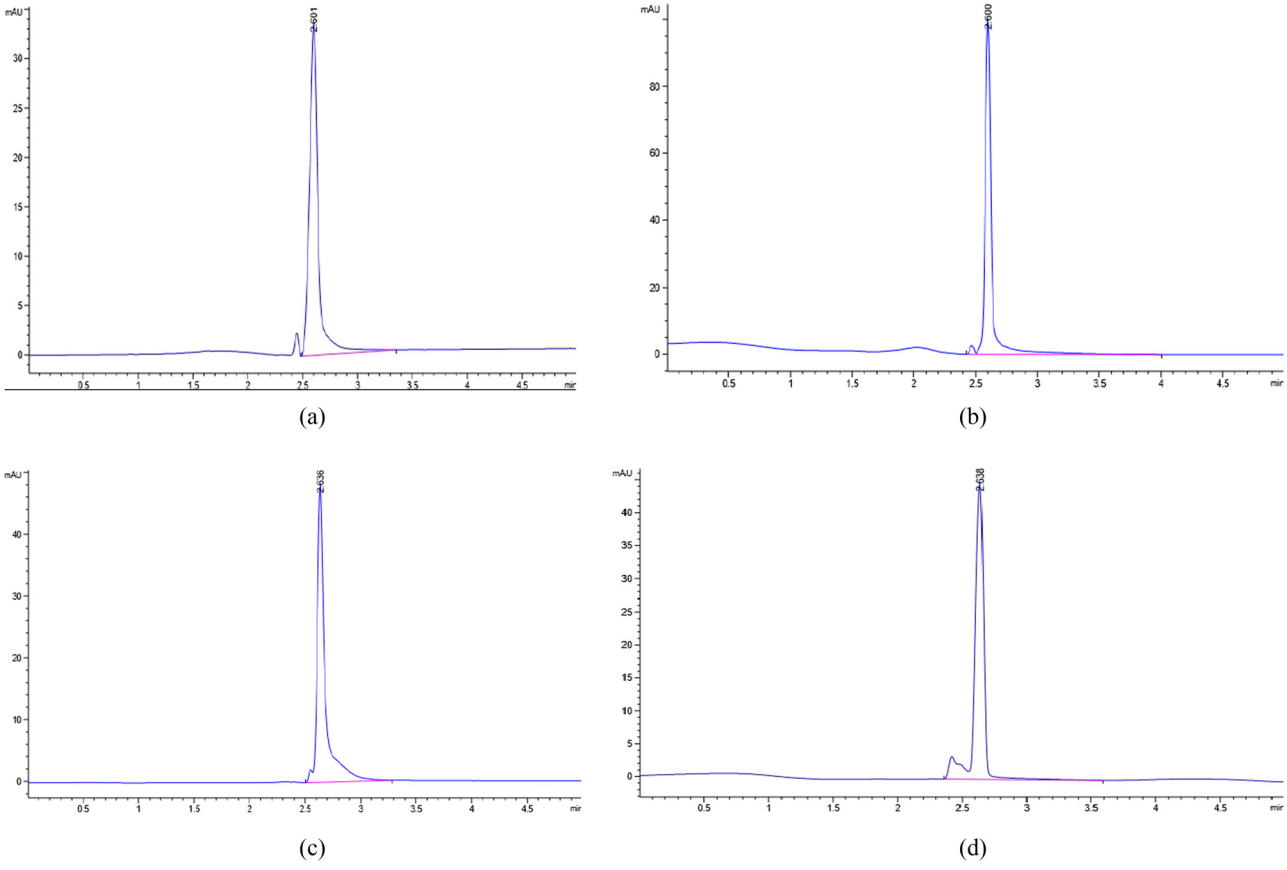


Figure 8: Chromatogram of food samples: (a) unspiked chocolate candy sample A1, (b) 10 µg of spiked chocolate candy sample A1, (c) unspiked carbonated drink sample B4, and (d) 10 µg of spiked carbonated drink sample B4.

methodology effectively decreases the necessary duration of the analysis while simultaneously minimizing the utilization of solvents and energy resources. This study aimed to evaluate the efficacy of the methodology by employing three metrics that specifically focus on environmental aspects: the analytical eco scale (AES), the green analytical procedure index (GAPI), and the software-based analytical greenness metric (AGREE) tool [42–44].

3.7.1 Green solvent selection tool (GSST)

The proposed HPLC method was carried out using ethanol instead of acetonitrile or methanol. This substitution was made after evaluating the *G* values obtained using the GSST tool, which can be accessed online at: http://green-solvent-tool.herokuapp.com/.

($G_{ethanol}$ = 6.6 with category score: W = 4.2, H = 8.9, E = 6.7, S = 7.7)

($G_{acetonitrile}$ = 5.8 with category score: W = 2.8, H = 5.9, E = 8.9, S = 7.7)

($G_{methanol}$ = 5.8 with category score: W = 4.0, H = 4.9, E = 8.4, S = 7.1)

The proposed HPLC method demonstrates an attempt to identify alternative solvents (with a high *G* value) that are more sustainable compared to the HPLC method previously published. The GSST offers a readily accessible online tool that facilitates the assessment of solvents in accordance with the GSK Solvent Sustainability Guidelines. The specifics of the Hansen space pertaining to the choosing of solvents are depicted in Figure 9.

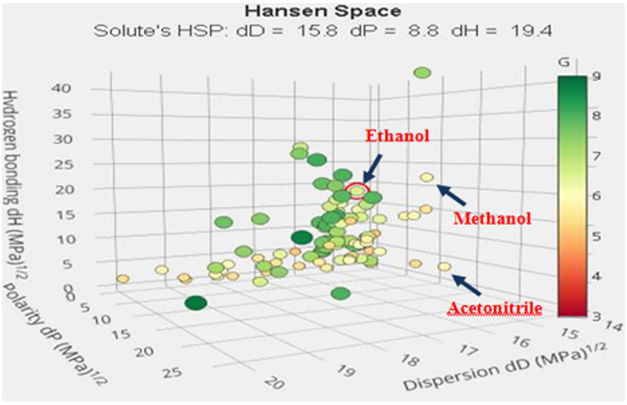


Figure 9: Hansen space shows ethanol (*G* = 6.6), methanol (*G* = 5.8), and acetonitrile (*G* = 5.8).

3.7.2 GAPI

The GAPI metric is generally recognized as one of the most commonly employed indices for assessing the level of greenness in analytical methods. The tool offers a prompt, uncomplicated, and reliable approach to assessing the environmental sustainability of the analytical procedure. The GAPI tool is well recognized as a helpful semi-quantitative tool for both laboratory practice and educational applications. The GAPI framework has the advantage of assessing an approach from both qualitative and quantitative perspectives. This is visually represented in the fifth component of the GAPI pictogram, where the presence of a ring-like structure indicates a quantitative assessment. The GAPI assessment method mainly focuses on three essential domains: (I) the preparation of samples, (II) the utilization of reagents and solvents, and (III) instrumentation assessment. The aforementioned three categories have been further divided into a cumulative total of 15 distinct subcategories. The pictogram employs a coding scheme comprising three separate colors: red, yellow, and green. The purpose of this coding system is to assess the ecological considerations of the complete analytical process, encompassing the stages of sample collection to result interpretation. Through the utilization of this technique, the pictogram provides qualitative data and simplifies the process of comparing various analytical methodologies clearly and directly. The method comprises a series of 15 steps for evaluating the outcomes, which are characterized by a semi-qualitative and intricate representation [45,13]. Therefore, the implementation of this concept is very difficult when suitable software is not available. Figure 10a depicts the graphical representation of the GAPI.

3.7.3 AES

AES tool is a semi-quantitative method for measuring greenness, which involves the calculation of penalty points (PP) from the method [46,47]. This approach provides a

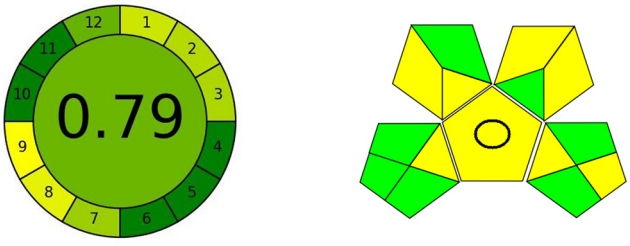
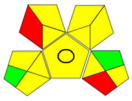
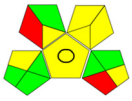
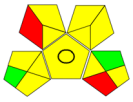
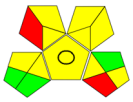


Figure 10: Pictogram represents green assessment results for the suggested method: (a) AGREE and (b) GAPI.

Table 7: Evaluation of green assessment with a comparison between suggested vs the published HPLC method

Sl no	Chromatographic method	Extraction technique	GAPI	AGREE	AES	Developed by
1.	HPLC, ammonium acetate solution (0.02 M)/methanol, gradient programming	Liquid–liquid microextraction			10 + 1 + 3 + 3 = 17, AES = 83	Zhu et al. [32]
2.	RP-HPLC, UPLC-ESI-MS/MS, acetonitrile/4 mM ammonium acetate (80:20, v/v)	Liquid sample extracted with HPLC water followed by sonication and filtration. Solid sample extracted with 1% ammonia solution			14 + 0 + 3 + 3 = 20, AES = 80	Abdel Hameed et al. [33]
3.	0.25% (v/v) Triton X-100 aqueous solution and 50 mmol·L^{-1} phosphate buffer at pH 7	Artificial juice and gelatin samples homogenized and dissolved in hot water			6 + 1 + 3 + 3 = 13, AES = 87	Vidotti et al. [51]
4.	0.25% (v/v) Triton X-100 aqueous solution and 50 mmol phosphate buffer at pH 7	Juice, colored rice, and saffron sample diluted with deionized water (1:1), followed by adding HCl. 10% solid sample mixed with 30 mL of ammonia followed by sonication and filtration			6 + 1 + 3 + 3 = 13, AES = 87	Hajimahmoodi et al. [52]
5.	Ethanol/acetate buffer (pH 5) ratio (25:75, v/v)	UAE using ethanol solvent			8 + 1 + 0 + 0 = 9, AES = 91	Proposed method

semi-quantitative method for determining the appropriate deduction of points from a given score. The evaluation of the method's ecological consequences is carried out based on four criteria, including the scoring of chemicals or reagents employed (referred to as PP), the utilization of instrumental energy, the degree of occupational hazard, and the quantity of waste produced by the entire process. In order to derive the ultimate green analysis score, it is necessary to deduct the PP acquired from the four aforementioned stages from the optimal score of 100. The outcomes of the thorough AES calculation are presented in Table S2.

3.7.4 AGREE metrics

The software-based AGREE metrics is a novel quantitative technique employed for evaluating the greenness profile [48,49]. These metrics comprehensively encompass all 12 concepts of green analysis. The use of the AGREE tool is generally considered more suitable than the semi-quantitative tool AES, primarily because of its stronger focus on quantitative outcomes [13]. Each principle or component is assigned a numerical score ranging from 0 to 1 based on the degree of risk connected with the principles of environmental sustainability. A value in proximity to 1 signifies a greater level of environmental friendliness associated with the particular method. The AGREE tool visually illustrates the concept of greenness by employing a classic clock design, where numbers 1–12 are positioned along the circumference of a circular shape. The numerical values shown herein serve as representations of the 12 fundamental principles of the GAC. According to the data presented in Figure 10b, the proposed method achieved a comprehensive green score of 0.79, signifying its compliance with all green standards. The primary aim of the research was to evaluate the sustainability of the approach by employing three distinct green assessment tools, each utilizing various approaches or procedures to analyze the environmental compatibility of the approach. Irrespective of the specific tactics employed, all of the methodologies employed in this study consistently demonstrated that the technique under investigation exhibits environmentally benign characteristics and possesses the potential for adaptability in future green assessments without encountering any significant issues [50]. The comparative analysis of the environmental sustainability of the proposed technique and the previously published method (as shown in Table 7) highlights the superior performance of the proposed method in terms of the GAPI, AES, and AGREE metrics.

4 Conclusion

The contemporary approach to promoting a balanced and satisfying way of life relies on the effective management of food safety. While BB is a synthetic dye that possesses a wide range of applications, it is crucial to acknowledge the safety considerations pertaining to its potential effects on human health. In order to guarantee the safety of food, it is vital that the color present in the food product corresponds accurately to the information provided on the label, and that the quantity of colorants used is stated clearly. Hence, the objective of this study is to maintain the sensitivity, robustness, safety, and efficacy of BB in diverse commercially available ready-to-eat food products. A novel analytical approach, distinguished by its ecological attributes and outstanding resilience, was devised by creatively combining the AQbD method and GAC principles. The statistical optimization study utilized an experimental design, rCCD. The application of AQbD in the proposed approach has substantiated the enhanced stability and efficacy of CAAs while also considering the impact of CMPs on CAAs. In pursuance of the principles of GAC, ethanol was utilized as an organic solvent substituting a potentially hazardous solvent. The green UAE approach has been utilized to extract BB from various food samples. The UAE not only enhances the rate of BB recovery from the food samples but also ensures the maintenance of extracts' quality and safety, which has been confirmed by the FTIR data. The developed method meets the established criteria for validation parameters like reproducibility, accuracy, sensibility, and linearity at the specified working point. The method was subsequently validated by the utilization of GAPI, AES, and AGREE metrics, which collectively provided evidence of its superior environmentally friendly outcomes. Ultimately, the proposed method demonstrates superiority over previously documented methods while also exhibiting minimal environmental repercussions. The potential future consequences of this research could involve the widespread adoption and advancement of environmentally friendly and robust AQbD procedures for analyzing various food colorants utilizing green solvents, which might be effectively employed in the investigation of potentially illicit products available in the market.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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