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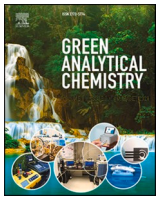

First-order derivative spectrophotometric method for simultaneous determination of brinzolamide and timolol maleate in ophthalmic formulation

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ABSTRACT

Multi-analyte determination techniques are favored over those that can only determine a single analyte at a time because they enable resource savings (cost, time, and solvents) and reduce generated waste and the number of samples required for analysis. This research presents a novel method for the simultaneous determination of brinzolamide (BRZ) and timolol maleate (TML) in pharmaceutical ophthalmic preparations using first-order derivative UV–Visible spectroscopy. The proposed approach is environmentally friendly and yet to be previously reported in the literature. In this approach, the peak amplitude of BRZ was quantified at the zero-crossing point of TML, *i.e.*, 248.80 nm, whereas TML was determined by measuring absorbance at 297.60 nm. The developed method was validated according to the International Council for Harmonisation guidelines Q2(R1). The developed method was linear with excellent correlation coefficient values ($R^2 > 0.9998$) in the range of 4 – 24 μg/ml for BRZ and 5 – 25 μg/ml for TML. The accuracy and precision results were within limits for both the analytes (%Relative standard deviation, RSD <2 %). The proposed method demonstrated LOD = 0.38 μg/mL for BRZ and 0.98 μg/mL for TML; LOQ = 0.91 μg/mL for BRZ and 2.99 μg/mL for TML), and was found to be selective, and robust. Lastly, the novel Analytical GREEnness (AGREE) metrics, the analytical eco-scale, and the green analytical procedure index (GAPI) were utilized to evaluate the developed technique's environmental sustainability and compare it to the HPLC method.

1. Introduction

The development of analytical techniques deemed sustainable today heavily relies on green analytical chemistry (GAC). One of its primary objectives is to curtail or eliminate the usage of hazardous chemicals for the environment and human health [1–3]. To accomplish this, improved analytical methods have been developed that allow for a reduction in the amount of reagents and energy consumption, cost-effectiveness, automation, reduced waste production, and lesser sampling steps while still offering high levels of sensitivity, precision, and accuracy, which are crucial for the validation of analytical techniques [4,5]. Liquid chromatography is the most popular analytical method for analyzing multicomponent formulations, but it generates much waste due to the mobile phase and hazardous solvents such as acetonitrile and hexane. Chromatographic instruments also require high costs due to expensive columns and spare parts [6]. The UV spectrophotometric approach is an alternative to this methodology, but due to the overlapping spectra of the analytes, direct analysis of multicomponent samples is challenging. However, with the use of mathematically manipulated UV spectra, such as derivatization and ratio spectra, multicomponent formulations can be analyzed without being first isolated [7]. Additionally, the UV spectroscopy approach provides several benefits, including shorter analysis

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times, reduced costs, non-toxic, less hazardous reagents for the analysis, and less waste formation [8,9].

Glaucoma is a chronic irreversible, and progressive optic neuropathy characterized by an accelerated degeneration rate of retinal ganglion cells [10]. In countries with limited resources, the prevalence of glaucoma is rising along with life expectancies, and by 2040, almost 112 million people are anticipated to be affected [11]. The main objective of glaucoma management is the prevention or control of increased intraocular pressure (IOP). Thus, to retain visual performance in glaucoma patients and reach the final aim of treatment, modern medicine focuses on three distinct targets: IOP, outflow facility, and retinal ganglion cell [12]. The European Medicines Agency (EMEA) has authorized a fixed combination of brinzolamide (BRZ) and timolol (TML), which is now accessible in many European countries [13]. The Azarga®, containing BRZ (10 mg/mL) and TML (5 mg/mL), is an ophthalmic suspension manufactured by Alcon Laboratories Ltd, Hemel Hempstead, UK [13]. BRZ is a sulfonamide carbonic anhydrase inhibitor that is heterocyclic and non-bacteriostatic. TML is a nonspecific β-adrenergic blocker [13]. Fig. 1 depicts the chemical structures of BRZ and TML.

IOP lowering in adult patients with ocular hypertension or open-angle glaucoma is the principal indication for the brinzolamide/timolol fixed combination [13]. The BRZ/TML fixed combination decreases IOP by lowering aqueous humor secretion. Brinzolamide's primary mode of action is the reversible suppression of carbonic anhydrase II in the eye's ciliary processes [13].

There are several assay techniques for measuring BRZ and TML in pharmaceutical formulations, both as individuals and in conjunction with other anti-glaucoma drugs. In the literature, there are several reports on the determination of BRZ in combination with brimonidine tartrate by UV [14–16] and HPLC [14]. Moreover, there are reports on the simultaneous estimation of BRZ and TML by ratio derivative spectroscopy [17] as well. However, there are no reports on simultaneously estimating BRZ and TML fixed-dose combinations using first-order derivative UV–Vis spectroscopy.

In this research work, we report for the first time a rapid, uncomplicated, robust, and green approach for the simultaneous quantification of BRZ and TML in methanol by first-order derivative UV spectroscopy for quantification in bulk and pharmaceutical preparations. The advantage of this approach is that it offers simplicity, high sensitivity, rapidity, selectivity, elimination of pre-treatment steps and complex calculations or equations, cost-effectiveness, reduced solvent wastage, and eco-friendliness (greenness).

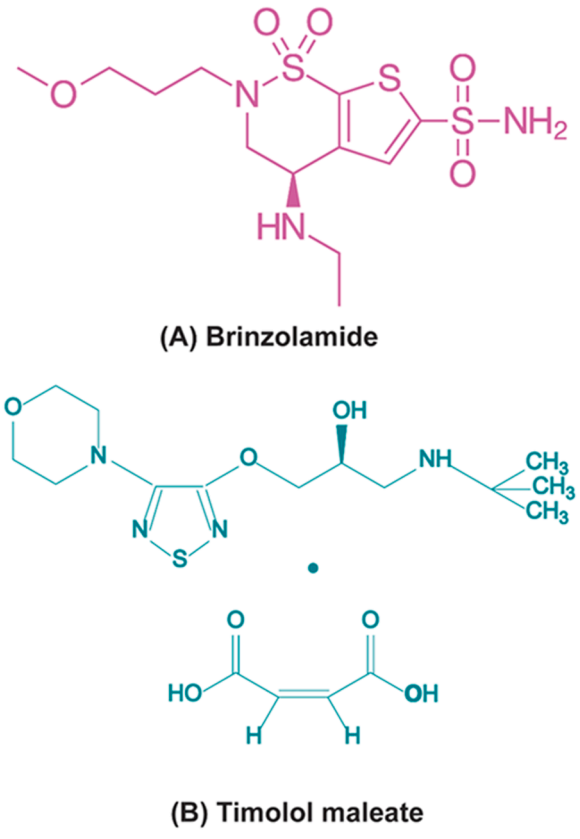

Fig. 1. The chemical structures of (A) brinzolamide (BRZ) and (B) timolol (TML).

2. Materials and methods

2.1. Materials

Analytically pure standards of Brinzolamide (BRZ) and Timolol (TML) were procured from Cipla Ltd. as gift samples. Methanol (MeOH) was purchased from Rankem. Carbopol® 980 NF, Kollidon® 30LP (polyvinyl pyrrolidone, low peroxide grade) were kindly gifted by Lubrizol India Pvt. Ltd, and BASF India Pvt. Ltd., respectively. DMSO was purchased from Rankem Chemicals. Hydroxypropyl methyl cellulose (HPMC) and Pearlitol® 50C (Mannitol) wetr obtained as gift sample from Colorcon India Pvt. Ltd. and Roquette India Pvt. Ltd., respectively. Ethylenediamine tetraacetic acid (EDTA) and Benzalkonium chloride (BKC) were procured from Loba Chemie Pvt. Ltd. and Somu Organo Chem Pvt. Ltd., respectively.

2.2. Drug formulation (marketed)

AZARGA® [10 mg/ml (BRZ) + 5 mg/ml (TML)] eye drops suspension (Novartis Pharmaceuticals UK Ltd.) (S1), Brinzotim [10 mg/ml (BRZ) + 5 mg/ml (TML)] eye drops suspension (Sun Pharmaceutical Industries Ltd.) (S2), Brinzox-T [10 mg/ml (BRZ) + 5 mg/ml (TML)] eye drops suspension (Ajanta Pharma Ltd.) (S3) were provided as samples by Dr. Sachin Dharwadkar (Glaucoma specialist).

2.3. Preparation of standard stock solutions and standard working solutions in methanol

Individual stock solutions of 1 mg/mL of BRZ and TML were prepared using analytical grade MeOH. Fresh working standard solutions were prepared each day of the experiment by diluting the stock mentioned above in MeOH.

3. Methodology

3.1. Analytical method development and validation

The developed analytical method for quantifying BRZ and TML simultaneously was validated as per ICH Q2 (R1) guidelines [18]. The current investigation used a UV double-beam spectrophotometer with a narrow-slit width of 2 mm, model number Shimadzu 2600, for spectrophotometric examination. It was used to process data and measure the peak amplitudes and absorbances.

3.1.1. Linearity range and calibration curves

The linearity ranges of BRZ (4.0–24.0 μg/mL) and TML (5.0–25.0 μg/mL) in MeOH were determined. The absorbance values that ranged between 0.20 and 0.80 were used to determine the linearity range. It is well known that readings within this absorbance range give the highest precision [19]. The statistical linearity parameters were computed. At an interval of $\Delta\lambda = 4$ nm, the first-order derivative spectrum of BRZ was traced. The calibration curve was analyzed using the least-squares linear regression approach. A one-way analysis of variance (ANOVA) test was utilized to establish the significance of linear regression, followed by Tukey's multiple comparison test. In addition to this, a normality plot,

also known as a Quantile-Quartile plot or simply a Q-Q plot, was created to ensure consistent dispersion in the data. In ordinary linear squares regression, the regression's residuals must always adhere to normal distribution [20,21]. Various test methods, such as the Anderson-Darling test, the Shapiro-Wilk test, the D'Agostino-Pearson omnibus test, and the Kolmogorov-Smirnov test, were used to verify that the distribution was normal [21,22].

3.1.2. Accuracy and precision

The accuracy of the established approach by the standard sample spiking method was evaluated using a standard addition method. The samples were prepared in triplicate and spiked with 80, 100, and 120 percent. The recovery (percent) of the added pure APIs was determined using the following equation:

$$\%\ \text{Recovery} = \frac{A_F - A_O}{A_S} \times 100$$

where A_F, A_O, and A_S represent the found, original, and added amount of APIs, respectively [23]. Using the above equation from the linear regression model, the mean accuracy (percent) was also obtained. Intra-day (repeatability/intraday precision) and inter-day (intermediate precision) variations were used to assess the precision of the proposed UV–Vis first-order derivative approach for measuring BRZ and TML. The absorbance of the three concentrations was recorded in triplicate three times a day to determine intra-day precision. Additionally, the absorbance was measured every day for three days to ensure inter-day precision [21]. The accuracy and precision of the suggested approach were expressed as a percent relative standard deviation (%RSD) and a coefficient of variation (%CV), respectively.

3.1.3. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were computed as per ICH recommendation [18,24] as 3.3 (σ /S) and 10 (σ /S), respectively, where 'σ' stands for the response's standard deviation and 'S' for the slope of the calibration curve.

3.1.4. Selectivity

By analyzing a placebo solution, the developed method's selectivity was assessed. HPMC, Mannitol, EDTA, BKC, Carbopol® 980 NF, DMSO, Kollidon® 30LP, and NaCl were all prepared in combination at amounts that are typically seen in ophthalmic preparations. These solutions were examined using the suggested approach to determine whether any formulation elements would obstruct the measurement of BRZ and TML.

3.1.5. Stability of the solution

The investigation of both the analyte's stability in MeOH was performed at 25 °C for 72 h.

3.1.6. Robustness

The primary goal of robustness evaluation was to ascertain how susceptible the findings were to any purposeful deviations that could arise when performing these analytical operations. The robustness of the developed method was demonstrated in this study by varying the wavelength by 2 nm at RT (25 ± 0.5 °C). Working concentrations of 24 μg/mL of BRZ and 25 μg/mL of TML were chosen, and RSD (%) of mean values was computed.

3.2. Drug content analysis

Three marketed ophthalmic suspensions and in-house prepared *in situ* gel containing BRZ and TML were investigated for drug content analysis using the proposed method. The *in situ* gel-forming solution (ISG) was prepared in-house by dissolving 0.2% w/w Carbopol® 980 NF (pH-responsive polymer) in sufficient deionized water. After ensuring the complete dissolution of Carbopol® 980 NF (pH-responsive polymer), 50 % DMSO (solubilizer) and 0.5 % PVP 30 LP (recrystallization inhibitor) were added to the polymeric solution. Finally, BRZ 1% w/w and TML 0.5% w/w were added to the formulation [25]. For the drug content analysis, the formulations were dissolved in MeOH and diluted appropriately to achieve the calibration range (BRZ 20 μg/mL + TML 20 μg/mL), and analyses were performed in triplicate. To ensure that the formulations and MeOH were completely homogenized, the solutions were vortexed for 5 min. The drug content of S1, S2, S3, and ISG was determined using the proposed method at 248.80 nm for BRZ and 297.60 nm for TML.

3.3. Appraisal of environmental sustainability and greenness

3.3.1. Analytical GREEnness metric approach (AGREE)

One of the techniques for evaluating the greenness of analytical methods is AGREE, which has its foundation in the twelve core concepts of GAC [26]. In the middle of the AGREE pictogram, the final output will be displayed as a graphical chart showing every vital principle and the overall score of an analytical approach [26]. A numeric value between 0 and 1 represents each principle's impact. The environmental sustainability analysis will receive a score of 1 and be colored dark green [27]. This study compared the proposed method to the previously described HPLC method and assessed its potential environmental impact [28].

3.3.2. The analytical eco-scale

The analytical eco-scale (AES) tool [29], an additional measure for the evaluation of greenness, was also implemented and compared with the reported HPMC method. Each of the chosen analytical factors, such as the quantity of hazardous reagents, energy use, and waste production, is given a penalty point (PP) value in this technique. According to this methodology, the PP for the entire procedure is summed and included in the AES calculation [30]:

$$\text{AES} = 100 - \text{total PP}$$

The computation's result is scored on a scale, with a score over 75 indicating excellent green analysis, a score between 75 and 50 indicating satisfactory green analysis, and a score below 50 indicating subpar green analysis. [3]. The tool was employed and compared with the reported HPLC method to determine the AES of the proposed method [28].

3.3.3. GAPI (Green analytical procedure index)

GAPI is made up of 15 different parameters (five pentacle shapes). It is employed for assessing any analytical method's environmental impact, every step of the technique, such as preparation of the sample, sample volume, volume, reagents and solvents associated health hazards, instrumentation, as well as quantity of the waste generated and its handling [31]. There is a color-coded system; green denotes minimal environmental impact, yellow denotes medium environmental effects, and red indicates significant environmental impact [3]. The GAPI tool was implemented to investigate the greenness of the proposed method and compare it with the reported HPLC method [28].

3.4. Statistical analysis

All investigations were conducted at least in triplicate (n = 3), and the results were reported as mean value ± SD and%RSD. Statistical data analyses were performed using one-way ANOVA and post-hoc Tukey's test. All the data with a P-value less than 0.05 was considered statistically significant. GraphPad Prism 8 (GraphPad Software, version 8.4.3, Inc., La Jolla, CA, USA) was used for statistical analysis of all the data.

4. Results and discussions

The study's main objective was to develop a simple, efficient, sensitive, and accurate analytical method for simultaneously quantifying

BRZ and TML from ocular formulations during drug content analysis. The zero-crossing technique is the most basic and widely used method for constructing analytical calibration. In MeOH, a zero-order or D^0 absorption spectrum for both the active ingredients, BRZ and TML, was scanned, and it was found that TML's absorption spectra showed a spectral zone where BRZ did not show any interference, allowing TML to be directly identified in the presence of BRZ. Conversely, the D^0 spectra of BRZ exhibited significant overlapping with TML, making it challenging to quantify BRZ in the presence of TML using standard UV spectrophotometry. This problem was effectively addressed by using first-order derivative UV spectrophotometry. Sharp bands with greater amplitudes were identified after first-order derivatization of BRZ spectra, which ensures more selective identification and simultaneous estimate of TML and BRZ. The first-order derivative amplitude of BRZ was measured at the zero-crossing of TML at 248.80 nm ($D^1_{248.80}$) with an interval of $\Delta\lambda = 4$ and a scaling factor of 4, while TML was calculated directly at 297.60 nm ($D^0_{297.60}$). These specific wavelengths were chosen because they were determined to be the most effective in eliminating interference while measuring BRZ and TML in combinations. The spectral data of both active ingredients are shown in Fig. 2.

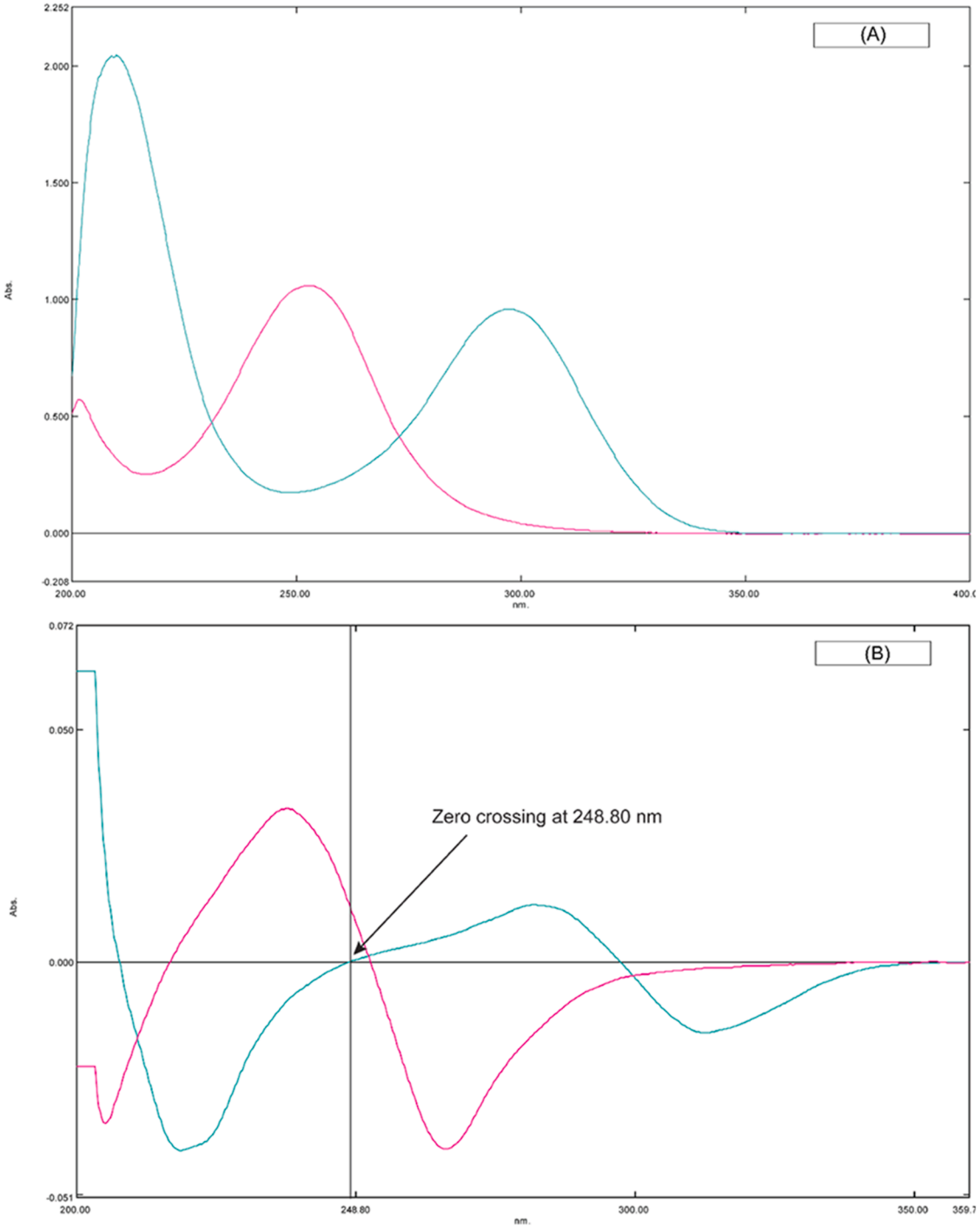

Fig. 2. (A) D^0 absorption spectrum of BRZ (Pink) (24 μg/ml) and TML (Blue) (25 μg/ml) in MeOH (B) D^1 spectrum of BRZ (24 μg/ml) and TML (25 μg/ml) in MeOH.

4.1. Analytical method validation

4.1.1. Calibration curves and linearity

According to Beer and Lambert's law, the responses were determined to be linear throughout 4.00–24.00 μg/mL and 5.00–25.00 μg/mL concentration ranges for BRZ and TML, respectively. The high correlation coefficient values confirmed the constructed calibration curve linearity. The analytical findings from the calibration curves of BRZ and TML are listed in Table 1. **Supplementary Figure S1** depicts BRZ's first-order derivative UV spectra, a calibration curve of BRZ in MeOH, zero-order spectra of TML, and a linearity curve of TML in MeOH. Linear regression analysis results for BRZ and TML confirmed that the regression model could significantly predict the outcome variable ($P < 0.05$). The findings of the one-way analysis of variance revealed no statistically significant discrepancies between the outcomes of the constructed calibration curves, shown in **Supplementary Table S1**. In addition, Tukey's multiple comparison tests ($\alpha = 0.05$) provide credence to these results by demonstrating that there is no discernible difference in the outcomes that were achieved ($P > 0.05$). It was also determined that the datasets followed the normal distribution ($P > 0.05$ at a 95.00% confidence interval), indicating no significant deviation from the normality or scatter (**Supplementary Figure S2**). The representation of the normalcy of residuals is shown in **Supplementary Table S2**.

4.1.2. Accuracy and precision

The proposed method's recovery confirmed high accuracy values greater than 99.00% and low RSD (less than 2.00 %). For BRZ and TML, the average recovery (%) varied from 96.06 to 102.21 and 101.73 to 103.34, respectively. Table 2. shows the accuracy outcomes obtained using the standard sample spiking approach. **Supplementary Table 3**. displays the precision (repeatability and intermediate precision) investigation findings. According to the RSD (%) results, which were determined to be well within the acceptable range, the proposed methodology offers outstanding repeatability and intermediate precision under the circumstances of the current experiment.

4.1.3. LOD and LOQ

The results of the LOD and LOQ assessment of the developed UV method demonstrated low LOD and LOQ values. The LOD values obtained for BRZ and TML were 0.38 μg/mL and 0.98 μg/mL, respectively. The LOQ values obtained for BRZ and TML were 0.91 μg/mL and 2.99 μg/mL, respectively (**Supplementary Figure S3**).

4.1.4. Selectivity

The developed method did not indicate any influence from the additives and excipients in the detection of BRZ and TML from the studied ophthalmic suspensions (S1, S2, and S3) and ISG thereby demonstrating the method's selectivity for the analysis.

Table 1
Results of regression analysis of the data to quantify TML and BRZ in MeOH simultaneously ($n = 3$).

Method parameters	BRZ	TML
λ_{max} in nm	248.80	297.60
Linearity range (μg/mL)	4.0 – 24.0	5.0 – 25.0
Coefficient of correlation (R^2)	0.9998	0.9999
Equation of regression		
Value of slope	4.754×10^{-4}	3.394×10^{-2}
Intercept value for Y	-6.964×10^{-5}	-1.492×10^{-3}
Intercept value for X	0.1465	0.04397
Analysis of standard error		
Standard error of the slope	3.238×10^{-6}	1.840×10^{-4}
Standard error of the Y-intercept	4.670×10^{-5}	2.786×10^{-3}
F	21,563	34,017
DFn, DFd	1, 5	1, 4
P value	<0.0001	<0.0001

4.1.5. Solution stability

The data obtained showed that the sample solutions of both analytes were stable for 72 h at 25 °C. The%recovery values obtained were within the desired range, and the computed RSD (%) did not exceed 2.0.

4.1.6. Robustness

The findings of the experiments pertaining to robustness are mentioned in **Supplementary Table S4.** The results demonstrate that small intentional alterations in wavelength did not substantially impact the mean peak amplitudes (BRZ) and absorbances (TML). This implies that the proposed method is reliable.

4.2. Drug content determination from pharmaceutical formulations

The drug content analysis from the marketed suspensions (S1, S2, and S3) and the in-house prepared *in situ* gelling system demonstrated that BRZ and TML are in agreement with their labeled amount. The results of the drug content determination are shown in Table 3. The outcomes demonstrated excellent recovery and proved that the formulation excipients did not affect the analysis. Furthermore, the normality plot of BRZ and TML drug content exhibits no significant scatter, indicating that the analytes can be measured in various ophthalmic pharmaceutical formulations (**Supplementary Figure S4**).

4.3. Appraisal of environmental sustainability and greenness

4.3.1. Analytical GREEnness metric approach (AGREE)

The proposed first-derivative UV spectroscopic method received a superior AGREE score of 0.77 compared to the HPLC method, which acquired an AGREE score of (0.62) (Fig. 3.). The inclusion of MeOH as a solvent in the proposed spectroscopic approach accounts for its noteworthy greenness compared to the published HPLC method, which used comparatively toxic chemicals such as acetonitrile as a solvent system. Additionally, much waste is produced due to the continuous running of the mobile phase for column saturation, column washing, and analysis [9]. The AGREE report sheet for both first-derivative UV spectroscopic method and HPLC method are provided as **supplementary material S1 and S2,** respectively.

4.3.2. Analytical eco-scale

The AES tool was successfully employed to analyze the greenness of the developed UV method to quantify BRZ and TML simultaneously. Due to the minimal energy footprint (0.1 Kilowatt-hour/sample) and lack of occupational risks during the analysis, UV–Visible spectrophotometers are given zero penalty points for energy consumption and occupational hazards. The reagent (type: MeOH = 2, Quantity: 1–10 ml = 1, and risks penalty points = 3) and the handling of wastes produced during the devised first-order derivative UV method (no treatment) were taken into consideration when calculating the penalty score, which was estimated to be six. Due to the little waste generated and the use of less hazardous chemicals, the suggested techniques in this study only demonstrated 12 points, which indicated excellent GAC [3]. On the other hand, the reported HPLC method employs a mixture of Acetonitrile reagent (Signal term = 2, Number of pictograms = 2, and risks penalty points = 4), MeOH, triethyl amine (Signal term = 2, Number of pictograms = 3, and risks PP = 6), and phosphate buffer as mobile phase [28]. This RP-HPLC method generates a penalty score of 23, suggesting poor greenness compared to the developed first-order derivative UV spectroscopic method [32]. The results of the AES for the developed method and the reported HPLC method are shown in Table 4.

4.3.3. GAPI assessment

Each step's environmental impact is represented by one of three colors: green, yellow, or red, which range from minimal to high impact. The most frequent hue in the pentagrams can be used to assess the procedure [33] quickly. Although there is inline sample preparation in

Table 2
Results of BRZ and TML accuracy in MeOH using the standard sample spiking procedure ($n = 3$).

		BRZ				TML			
Level of spiking (%)	Replicate no.	Qty. of standard spiked (mg)	Amt. of BRZ found (mg)	Recovery (%)	% Mean recovery (% RSD)	Qty. of standard spiked (mg)	Amt. of TML found (mg)	Recovery (%)	% Mean recovery (% RSD)
80	1	6.40	14.24	98.89	105.21 (1.66 %)	8.00	18.31	101.731	101.73 (1.60 %)
	2	6.40	14.03	97.43		8.00	18.02	100.094	
	3	6.40	13.82	95.97		8.00	18.61	103.367	
100	1	8.00	16.13	100.83	102.05 (1.57 %)	10.00	20.96	104.816	103.34 (1.42 %)
	2	8.00	15.71	98.20		10.00	20.37	101.870	
	3	8.00	16.13	100.83		10.00	20.67	103.343	
120	1	9.60	17.82	101.23	98.06 (1.72 %)	12.00	22.73	103.323	102.87 (0.75 %)
	2	9.60	18.24	103.62		12.00	22.73	103.323	
	3	9.60	18.45	104.81		12.00	22.44	101.984	

Table 3
Drug content analysis of BRZ and TML in pharmaceutical formulations.

Parameters	BRZ				TML			
	S1	S2	S3	ISG	S1	S2	S3	ISG
Label claim (mg)	10	10	10	10	6.8	6.8	6.8	10
Amount taken (μg/ml)	20	20	20	20	20	20	20	20
Amount found	20.87 ± 0.26	20.72 ± 0.40	21.08 ± 0.31	20.53 ± 0.30	21.14 ± 0.39	20.62 ± 0.38	20.50 ± 0.35	20.53 ± 0.
Mean% estimation	104.33 ± 0.26	103.59 ± 2.03	105.39 ± 1.59	102.63 ± 1.50	105.68 ± 1.96	103.12 ± 1.90	102.52 ± 1.77	102.66 ± 1.50
%RSD	1.26	1.96	1.51	1.46	1.86	1.84	1.73	1.3
N	10				10			
P-value	0.5 (Not significant)				0.5 (Not significant)			

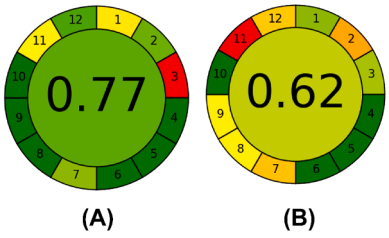

Fig. 3. Greenness evaluation results of UV method (A) and HPLC method (B) by AGREE tool.

the proposed spectroscopic methods, there was no need for storage or transportation. Additionally, the sample preparation (direct technique) involved no extraction and any further treatments. Nonetheless, the proposed method uses small amounts of solvent with little waste. Lastly, the proposed method can be used for qualification and quantification [33]. The detailed report of the GAPI assessment for each criterion is shown in Supplementary **Figure S5** and **Figure S6**. The GAPI assessment pictograms for the proposed UV method and HPLC method is shown in Fig. 4.

In summary, the high AES and AGREE score with the prevalence of green color in GAPI pictograms demonstrated the effectiveness of the suggested first-order derivative UV spectroscopic method in lowering solvent consumption, wastage, energy consumption, and usage of hazardous solvents.

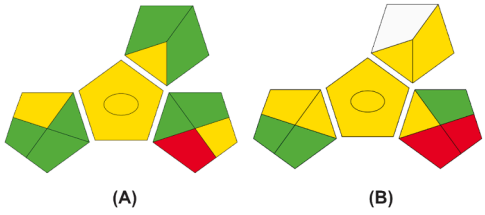

Fig. 4. Greenness evaluation results of UV method (A) and HPLC method (B) by GAPI assessment.

Table 4
The AES tool obtained penalty points for the developed analytical method.

AES tool Reagent/instrument	Proposed method			Reagent/instrument	Reported HPLC method		
	Signal word	No. of pictograms	Penalty points		Signal word	No. of pictograms	Penalty points
Methanol	2	3	6	Methanol	2	3	6
				Acetonitrile	2	2	4
				Triethylamine	2	3	6
				Phosphate buffer	0	0	0
Instrument Energy (UV/Vis spectrophotometer)			0	Instrument Energy (RP-HPLC)			1
Occupational hazards (Closed system)			0	Occupational hazards (Closed system)			0
Waste			6	Waste			6
Total Penalty points			Σ12	Total Penalty points			Σ23
Analytical eco-scale total score			88	Analytical eco-scale total score			77

5. Conclusion

A simple, direct, environment-friendly first-order derivative UV–Vis spectrophotometric method was developed to quantify BRZ and TML simultaneously. The developed method was validated for linearity, accuracy, precision, sensitivity, specificity, and robustness. Furthermore, the developed method was successfully employed to determine BRZ and TML concentration in three marketed suspensions and one in-house prepared ISG. Nonetheless, the greenness evaluation of the developed method confirmed the environmental sustainability, safety, superior cost and time efficiency as compared to HPLC method. By having access to such a reliable UV spectroscopy method, BRZ and TML in commercial pharmaceutical preparations may be estimated during routine quality control and adopted in laboratories lacking sophisticated analytical equipment.

CRediT authorship contribution statement

Srushti Tambe: Writing – original draft, Investigation, Conceptualization. **Sabya Sachi Das:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Kiran Shahane:** Writing – review & editing, Methodology, Formal analysis. **Sandeep Kumar Singh:** Writing – review & editing, Validation, Supervision, Conceptualization. **Janne Ruokolainen:** Writing – review & editing, Supervision, Formal analysis. **Purnima Amin:** Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Kavindra Kumar Kesari:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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