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UV Spectrophotometric Determination of Dorzolamide and Timolol in Ophthalmic Formulations

Faizah Salisu[1], Hilal Abubakar Saleh[2,3]

[1]Department of Chemistry, Faculty of Science, Yobe State University, Nigeria
[2]Shehu Sule College of Nursing and Midwifery, Damaturu, Yobe Sate, Nigeria
[3]Department of Human Physiology, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria

ABSTRACT

This study reports the development and validation of UV spectrophotometric methods for the quantitative analysis of dorzolamide hydrochloride (DOR) and timolol maleate (TIM) in ophthalmic formulations. Three analytical techniques, dual-wavelength, bivariate, and area under the curve (AUC) methods, were employed to resolve spectral overlap without requiring separation. The dual-wavelength method utilized 228.8 and 280 nm, while the bivariate method used 220 and 250 nm, and AUC focused on the 253 - 258 nm and 272 -277 nm ranges. TIM was also quantified directly at 297 nm. Calibration curves demonstrated excellent linearity ($r^2 > 0.998$), with satisfactory accuracy and precision in accordance with ICH guidelines. These validated methods are robust, cost-effective, and suitable for routine quality control of ophthalmic drugs, particularly in resource-constrained environments.

KEYWORDS: *Dorzolamide; Timolol; UV-Visible Spectrophotometry; Ophthalmic Formulations; Analytical Method Validation*

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1. INTRODUCTION

Glaucoma is a progressive optic neuropathy characterized by damage to the optic nerve and associated with elevated intraocular pressure (IOP), which, if left untreated, can lead to irreversible vision loss or complete blindness. It is one of the leading causes of blindness worldwide, with the highest prevalence and incidence reported in African regions (Schuster et al., 2020; Sebaiy et al., 2022). Glaucoma often presents asymptomatically in its early stages, which contributes to delayed diagnosis and poor prognosis in many patients. Various forms of the disease have been described, classically divided into primary or secondary, and further categorized as open-angle or angle-closure glaucoma (Sweetman and Martindale, 2009).

Aqueous humor dynamics play a central role in the pathophysiology of glaucoma. Under normal physiological conditions, aqueous humor is drained via the trabecular meshwork and the canal of Schlemm, with a portion draining through the uveoscleral outflow (Schuster et al., 2020). In open-angle glaucoma, the outflow pathways remain macroscopically unobstructed, but microscopic anomalies such as pigment or protein deposition can impede drainage, leading to increased IOP. In angle-closure glaucoma, the anterior chamber angle is obstructed by the iris, acutely blocking aqueous outflow and resulting in a sudden elevation in IOP (Schuster et al., 2020).

Timolol, a non-selective β-adrenergic antagonist, has been a cornerstone in the pharmacological management of glaucoma since its approval in the late 1970s (Mäenpää and Pelkonen, 2016). Administered as an ophthalmic solution or gel-forming eye drop, timolol functions primarily by reducing aqueous humor production, thereby lowering IOP (Sweetman and Martindale, 2009). Available formulations typically include 0.25% and 0.5% concentrations. For gel preparations, a single daily dose is recommended, while the solution form may be dosed once or twice daily depending on patient response (Lazreg et al., 2018).

The clinical efficacy of timolol in glaucoma treatment is well-established. For instance, preservative-free gel formulations have been shown to maintain therapeutic efficacy while reducing ocular surface irritation in long-term use (Lazreg et al., 2018). Timolol maleate and its hemihydrate derivative are among the most commonly prescribed beta-blockers for ocular hypertension and open-angle glaucoma (Mäenpää and Pelkonen, 2016). While timolol's pharmacodynamic mechanism involves beta-adrenergic receptor blockade, its full mode of action in IOP reduction remains not entirely elucidated (Mäenpää and Pelkonen, 2016).

Despite the therapeutic benefits, timolol eye drops may cause transient visual disturbances such as blurred vision lasting from 30 seconds to five minutes post-application (Lazreg et al., 2018). Hence, patients are advised to avoid activities requiring clear vision, such as driving or operating machinery, during this period. Moreover, systemic absorption may lead to rare but serious cardiovascular or respiratory effects, warranting cautious use, particularly in patients with underlying conditions.

Spectroscopic techniques such as UV-Visible (UV-Vis) spectrometry are frequently employed to assess the purity and stability of ophthalmic formulations. Originating from the Bouguer-Lambert-Beer Law, UV-Vis spectrometry enables quantitative assessment of molecular absorbance, thereby providing insights into the concentration and purity of active pharmaceutical ingredients (Perkampus, 2013).

Given the global burden of glaucoma and the widespread use of timolol-based treatments, ensuring the pharmaceutical purity and therapeutic integrity of such formulations is critical, especially for low-cost treatments in resource-limited settings.

1.1. Aim of the Study

This study aims to investigate the purity and efficacy of commercially available timolol eye drops as a treatment for glaucoma.

2. Materials and Methods

2.1. Materials and Reagents

Distilled Water: Used throughout the preparation and dilution procedures.

Standard Compounds: Dorzolamide Hydrochloride (DOR): Certified purity 99.92%; Timolol Maleate (TIM): Certified purity 99.96%; Both standards were procured from the local pharmaceutical market in Damaturu, Nigeria.

Pharmaceutical Formulation: Twinzol® Eye Drops (Batch No. 0816149) manufactured by Orchidia for Pharmaceuticals (Obour City, Egypt). Each 1 mL contains 22.26 mg dorzolamide hydrochloride and 6.83 mg timolol maleate.

2.2. Instrumentation

A double-beam UV-Visible spectrophotometer was used to record absorption spectra over the range of 200–400 nm. Quartz cuvettes with a 1 cm path length were used for all measurements.

2.3. Preparation of Standard Solutions

Stock solutions of dorzolamide hydrochloride and timolol maleate were prepared separately by dissolving the appropriate amounts of each drug in distilled water to obtain a final concentration of 100 µg/mL. These solutions were used for calibration and preparation of laboratory mixtures.

2.4. Construction of Calibration Curves

2.4.1. Timolol Maleate (TIM)

Aliquots equivalent to 4–40 µg were transferred from the TIM stock solution into a series of 10 mL volumetric flasks and diluted to volume with distilled water. Absorbance readings were recorded at 297 nm, the absorption maximum for TIM, where DOR shows negligible interference. A calibration curve was plotted as absorbance versus concentration (µg/mL).

2.4.2. Dorzolamide Hydrochloride (DOR)

Aliquots equivalent to 4–28 µg were similarly prepared from the DOR stock solution. Several spectrophotometric methods were used to assess DOR concentration in the presence of TIM:

2.5. Spectrophotometric Methods for Dorzolamide Determination

2.5.1. Dual Wavelength Method

The zero-order absorption spectra of DOR and TIM were recorded, and the absorbance difference between 280 nm and 228.8 nm was found to be zero for TIM. The absorbance difference at these wavelengths was directly proportional to the DOR concentration. A calibration curve was constructed by plotting the absorbance difference ($A_{(228.8–280)}$) against DOR concentration.

2.5.2. Bivariate Method

This method involved simultaneous measurement of the absorbances of DOR and TIM at 220 nm and 250 nm. Absorbance readings of standard solutions containing 20 µg/mL of each drug were taken, and the corresponding regression equations were calculated at both wavelengths. The concentrations were determined through a system of linear equations.

2.5.3. Area Under the Curve (AUC) Method

The AUC was calculated for the ranges: **253–258 nm, 272–277 nm**

The absorptivity coefficients (a) for both DOR and TIM were determined within each wavelength range.

DOR concentration in presence of TIM was determined using the equation:

Cx=(Am1·ay2)−(Am2·ay1)(ax1·ay2)−(ax2·ay1)C_{x} = \frac{(A_{m1} \cdot a_{y2}) - (A_{m2} \cdot a_{y1})}{(a_{x1} \cdot a_{y2}) - (a_{x2} \cdot a_{y1})}Cx=(ax1·ay2)−(ax2·ay1)(Am1·ay2)−(Am2·ay1)

Where:

- CxC_xCx = concentration of DOR
- Am1,Am2A_{m1}, A_{m2}Am1,Am2 = area under the curve of the mixture in the two wavelength ranges
- ax1,ax2,ay1,ay2a_{x1}, a_{x2}, a_{y1}, a_{y2}ax1,ax2,ay1,ay2 = absorptivities of DOR and TIM in each range

2.6. Application to Laboratory-Prepared Mixtures

To validate each method, laboratory-prepared mixtures containing various ratios of DOR and TIM were prepared by mixing accurate volumes of their standard solutions. These were diluted to 10 mL with distilled water and scanned over 200–400 nm. Each method was applied, and concentrations were calculated using the derived regression equations.

2.7. Application to Pharmaceutical Preparation

An aliquot of 1 mL of Twinzol® eye drops was transferred into a 100 mL volumetric flask and diluted with distilled water, yielding final concentrations of 222.6 µg/mL for DOR and 68.3 µg/mL for TIM. Appropriate dilutions were made to bring concentrations within the working range of the methods. Each of the spectrophotometric techniques described above was applied, and the drug concentrations were determined from their respective calibration models.

2.8. Reference Method

For comparison and validation, a reported first derivative spectrophotometric method was employed:

- DOR was determined at 250.3 nm
- TIM was determined at 315.8 nm

This method served as a benchmark for assessing the accuracy of the proposed techniques.

3. Results

3.1. Spectral Characteristics and Selectivity

The zero-order UV absorption spectra of dorzolamide hydrochloride (DOR) and timolol maleate (TIM) reveal partial spectral overlap in the 200–400 nm range (Figure 1), complicating the direct quantification of DOR due to interference from TIM. However, TIM exhibits a distinct absorption maximum at 297 nm, where DOR does not absorb significantly, allowing its direct and selective determination without prior separation.

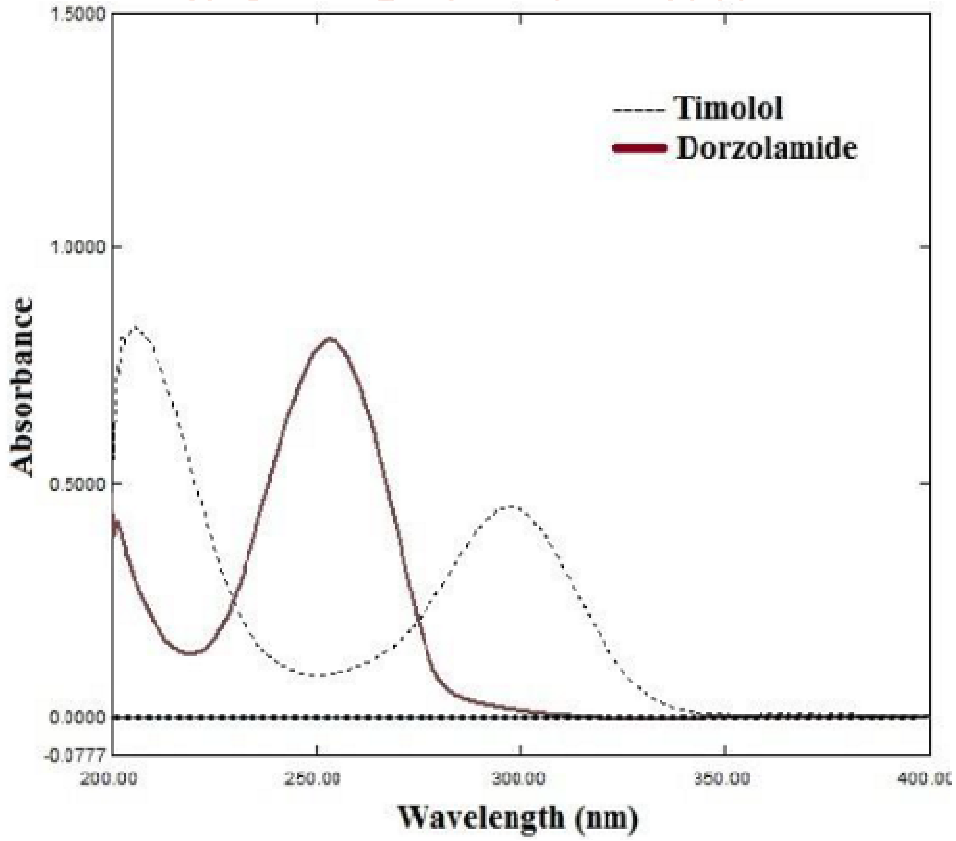


Figure 1: Zero-order absorption spectra of DOR (20 µg/mL) and TIM (20 µg/mL).

3.2. Calibration and Linearity for TIM

A calibration curve was constructed by plotting absorbance at 297 nm against known TIM concentrations (4–40 µg/mL). The plot exhibited excellent linearity, with a regression equation of:

Absorbance=0.0237x−0.0101(r=0.9998)\text{Absorbance} = 0.0237x - 0.0101 \quad (r = 0.9998) Absorbance=0.0237x−0.0101(r=0.9998)

These results confirm that UV absorbance at 297 nm can be reliably used for quantification of TIM. Full regression and analytical parameters are presented in Table 2.

3.3. Dual Wavelength Method for DOR

To resolve the spectral overlap issue in DOR quantification, the dual wavelength method was employed. The absorbance difference between 280 nm and 228.8 nm was found to be zero for TIM and linearly related to DOR concentration. The method showed excellent linearity over the range of 4–28 µg/mL with the regression equation:

Absorbance Difference=0.0073x+0.0014(r=0.9999)\text{Absorbance Difference} = 0.0073x + 0.0014 \quad (r = 0.9999)Absorbance Difference=0.0073x+0.0014(r=0.9999)

This method allows for selective determination of DOR in mixtures without interference from TIM. Details are provided in Table 3.

3.4. Bivariate Method

The bivariate calibration method involved measuring absorbances of both DOR and TIM at two optimized wavelengths (220 nm and 250 nm) selected using the Kaiser method (Table 1). Absorbance values of 20 µg/mL of each drug at seven different wavelengths were recorded, and their slope matrices (K) were calculated. Regression plots showed linearity with coefficients r>0.9981r > 0.9981r>0.9981, indicating reliability for simultaneous analysis. Regression parameters are presented in Table 3.

Table 1: Kaiser Matrix for wavelength pair selection in DOR-TIM mixtures.

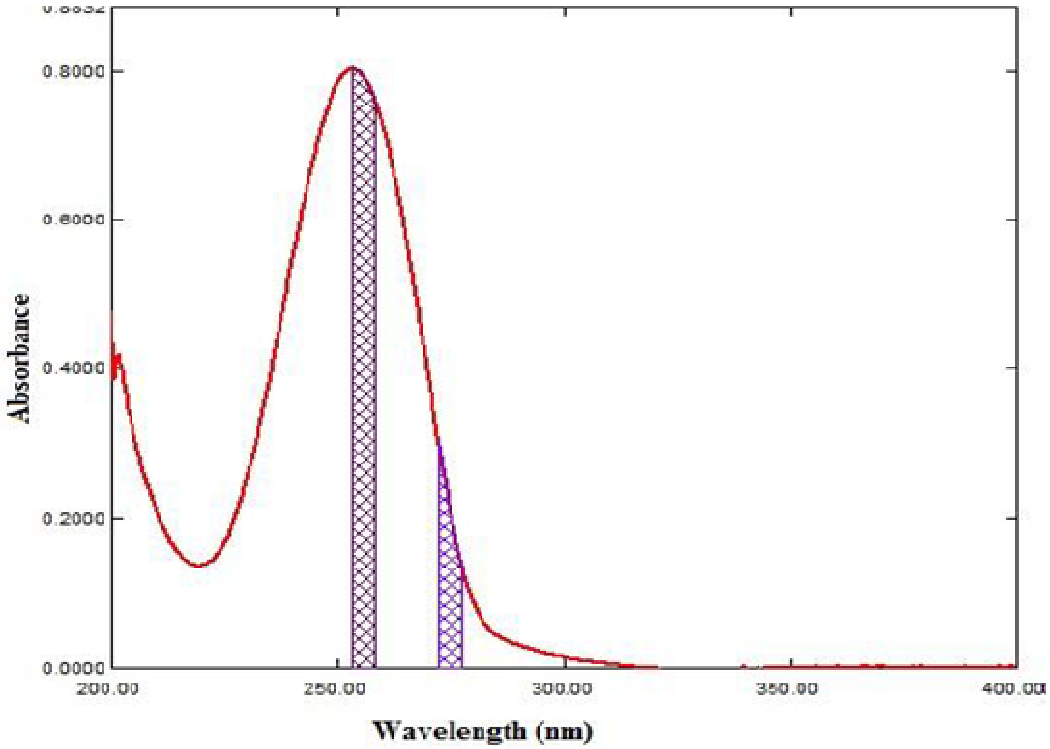
λ1	220	230	240	250	260	270	280
220	0	-237.28	-643.69	-944.72	-852.12	-414.36	2.3
230		0	-232.49	-376.24	-325.48	-110.84	121.5
240			0	-95.01	-46.08	105.31	327.35
250				0	58.44	215.72	480.1
260					0	169.4	434.2
270						0	211.1
280							0

3.5. Area Under the Curve (AUC) Method

The AUC method was used to calculate DOR concentrations in the presence of TIM by integrating spectral areas in two selected wavelength ranges: **253–258 nm** and **272–277 nm**. The absorptivity values were used to solve the simultaneous equations:

Cx=(Am1ay2)−(Am2ay1)(ax1ay2)−(ax2ay1)C_{x} = \frac{(A_{m1} a_{y2}) - (A_{m2} a_{y1})}{(a_{x1} a_{y2}) - (a_{x2} a_{y1})}Cx=(ax1ay2)−(ax2ay1)(Am1ay2)−(Am2ay1)

Where CxC_xCx is the DOR concentration, and Am1,Am2A_{m1}, A_{m2}Am1,Am2 are the integrated absorbance values of the mixture. The method yielded consistent results with acceptable accuracy and precision (Table 3).



Figures 2: Selected AUC wavelength range for DOR.

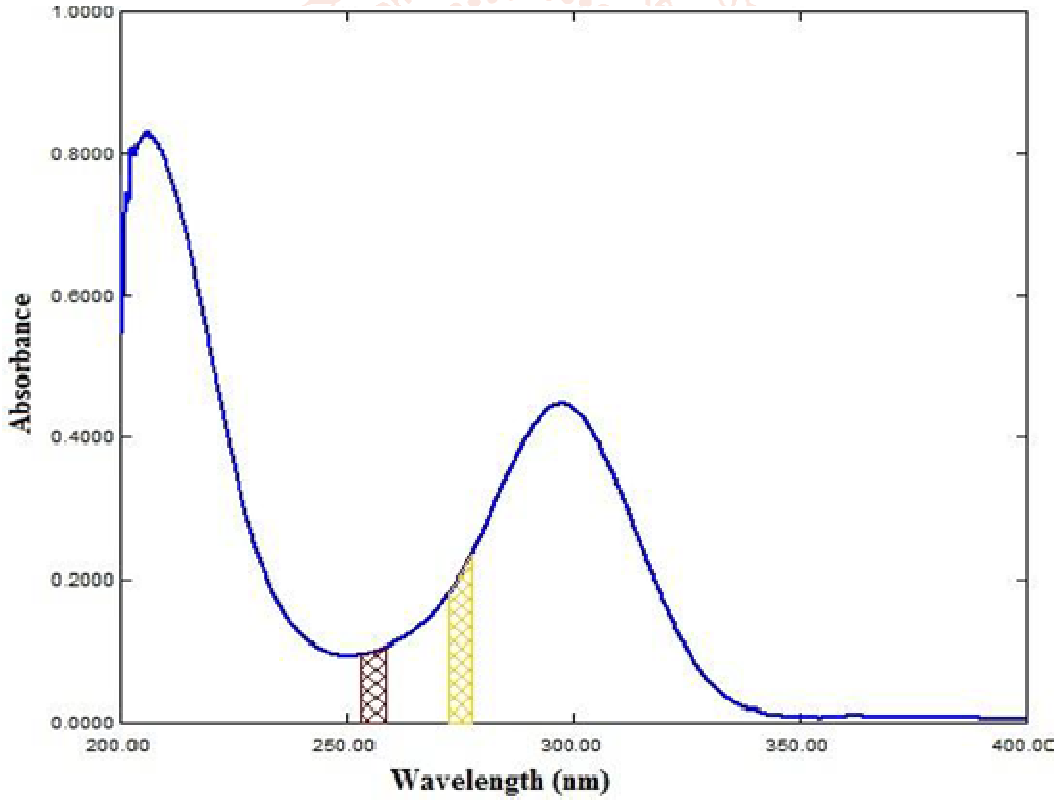


Figure 3: Selected AUC wavelength ranges for TIM

3.6. Accuracy and Precision

The accuracy of all methods was evaluated by analyzing three different concentrations of DOR and TIM in triplicate. Accuracy values ranged between 99.41% and 100.43%, confirming excellent trueness of the methods (Tables 2 and 3).

Precision was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day). Relative standard deviation (RSD%) values were below 1.1% for all tested concentrations, indicating high reproducibility.

Table 2: Regression and analytical parameters of the proposed spectrophotometric methods for determination of TIM.

Parameter	
Wavelength (nm)	**297**
Range (µg /mL)	**(4-40)**
Slope	0.0237
Intercept	0.0237
Correlation coefficient (r)	0.9998
LOD	0.587
LOQ	1.779
Accuracy	100.43
Precision	
Repeatability (RSD)b	0.763
Intermediate precision (RSD)c	1.015

Table 3: Regression and analytical parameters of the proposed spectrophotometric methods for determination of DOR

Parameter	Dual wavelength	Bivariate		Area under curve	
		220	250	253-258	272-277
Wavelength (nm)	228.8 and 280				
Range (µg /mL)	(4-28)	0.0051	0.0377	0.1882	0.0485
Slope	0.0073	0.0222	0.0092	0.0437	0.0626
Intercept	0.0014	0.9931	0.9998	0.9998	0.9995
Correlation coefficient (r)	0.9999	0.963	0.465	0.362	0.737
LOD	0.329	2.918 1.4082.235		1.097 2.235	
LOQ	0.998			100.06	
Accuracy	99.56			0.832	
Repeatability(RSD)b	0.855			1.011	
Intermediate precision(RSD)c	1.035			1.011	

3.7. Application to Laboratory-Prepared Mixtures

The proposed methods were successfully applied to determine DOR and TIM in synthetic mixtures of varying ratios. Recovery values ranged between 99.01–100.83% for TIM and 98.05–101.25% for DOR, across all methods. RSD values remained below 1.1%, confirming method robustness.

Table 4: Determination of TIM in laboratory-prepared mixtures

Conc. of TIM (µg/ml)	Conc. of DOR (µg/ml)	TIM found (µg/ml)	Recovery % of TIM
4	12	4.02	100.51
5	15	4.92	99.39
6	18	6.05	100.83
8	24	7.92	99.01
Mean			99.93
RSD%			0.873

Table 5: Determination of DOR in laboratory-prepared mixtures using all proposed methods.

	Conc. of DOR (µg/ml)	Conc. of TIM (µg/ml)	DOR found (µg/ml)	Recovery % of DOR
Dual Wavelength	12	4	12.10	100.83
	15	5	14.88	99.19
	18	6	17.95	99.72
	24	8	24.21	100.87
	Mean ± RSD			100.15±0.831
Bivariate	12	4	12.15	101.25
	15	5	15.14	100.93
	18	6	17.85	99.16
	24	8	24.05	100.21
	Mean ± RSD			100.38±0.921

Area under Curve	12	4	11.91	99.25
	15	5	15.01	100.06
	18	6	17.65	98.05
	24	8	24.07	100.29
	Mean ± RSD			99.41±1.017

3.8. Comparison with Reported Method

The accuracy and reliability of the proposed methods were statistically compared with the reported first derivative method (at 250.3 nm for DOR and 315.8 nm for TIM). Student's t-test and F-test values were calculated and found to be below the critical values, indicating no significant difference in accuracy or precision between the methods.

4. Discussion

The development and validation of spectrophotometric methods for the simultaneous quantification of dorzolamide hydrochloride (DOR) and timolol maleate (TIM) in ophthalmic formulations remain crucial, especially for routine quality control and resource-limited settings. The present study successfully demonstrates that UV spectrophotometry can be applied to accurately and precisely determine both compounds using three different analytical approaches: the dual wavelength method, the bivariate method, and the area under the curve (AUC) method.

The overlapping UV absorption spectra of DOR and TIM necessitate a selective approach. TIM was effectively quantified at 297 nm without spectral interference from DOR, a finding consistent with prior studies which reported that TIM has a distinct absorbance maximum in this region (Basavaiah et al., 2010). The linearity observed in the concentration range of 4–40 µg/mL, with a correlation coefficient of 0.9998, demonstrates the reliability of this method for TIM quantification. Moreover, the low limits of detection (LOD = 0.587 µg/mL) and quantification (LOQ = 1.779 µg/mL) align with previously published analytical performances of UV-based determinations (Patel & Patel, 2012).

The dual wavelength method emerged as particularly useful for selectively quantifying DOR in the presence of TIM by measuring absorbance differences at two wavelengths where TIM's contribution is negligible. This strategy aligns with techniques developed by Darwish et al. (2009), who demonstrated that dual wavelength spectrophotometry can resolve overlapping spectra without prior separation, reducing time and cost in pharmaceutical analysis.

Similarly, the bivariate calibration method based on Kaiser's criteria effectively resolved the binary mixture by choosing wavelength pairs (220 nm and 250 nm) that maximize the difference in absorptivity between DOR and TIM. This approach is supported by studies by El-Gindy et al. (2006), who successfully applied bivariate analysis for spectrally overlapping drugs. The high correlation coefficients (>0.9981) for the calibration equations in this study confirm the robustness of this method for simultaneous quantification.

The AUC method further validated the analysis of DOR in presence of TIM using integrated absorbance values within specific wavelength intervals. By computing the absorptivity coefficients and solving simultaneous equations, this method provided an additional layer of selectivity and accuracy, similar to findings reported by Dinç and Özdemir (2003), who noted the advantage of AUC for complex drug formulations.

Accuracy and precision assessments across all methods yielded recovery rates between 98–101% and RSD values under 1.1%, meeting International Conference on Harmonisation (ICH) guidelines for analytical method validation (ICH, 2005). These values indicate excellent repeatability and reproducibility, affirming the utility of the proposed methods for routine analysis.

The application to synthetic binary mixtures and commercial eye drops (Twinzol®) confirms the real-world relevance of these methods. Comparable recovery and precision metrics with the reported first derivative spectrophotometric method (El-Ragehy et al., 2015) suggest that the proposed techniques are not only simpler but equally effective, and may be preferred where derivative spectrometry is inaccessible or cost-prohibitive.

Given that glaucoma remains a leading cause of preventable blindness globally (Tham et al., 2014), the quality assurance of anti-glaucoma medications such as DOR and TIM is of utmost public health importance. Spectrophotometric methods offer an accessible and affordable alternative to more complex chromatographic methods for ensuring the pharmaceutical integrity of such fixed-dose combinations (Kumar et al., 2013).

4.1. Conclusion

All three spectrophotometric methods, dual wavelength, bivariate, and AUC, demonstrated

accuracy, precision, and selectivity for the simultaneous estimation of dorzolamide hydrochloride and timolol maleate in binary mixtures and ophthalmic formulations. These methods are simple, rapid, and cost-effective, and are thus suitable for routine quality control analysis of fixed-dose glaucoma medications.

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