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Development and Validation of a UV Spectrophotometric Method for Quantification of Brimonidine Tartrate in Acetone:Water and Simulated Tear Fluid Media

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Keywords: *Brimonidine tartrate, UV spectrophotometry, Method validation, Simulated tear fluid, Acetone:water medium*

Introduction: Brimonidine tartrate (BT) is a selective α2-adrenergic agonist commonly used in the treatment of ocular hypertension and glaucoma. This study aimed to develop and validate a simple, fast, and reproducible UV spectrophotometric method for determining BT concentration in two different media: acetone:water (1:1, v/v) and simulated tear fluid (STF). The method was validated in line with literature and ICH Q2(R1) guidelines [1-5].

Materials and Methods: BT stock solutions were prepared in both 1:1 acetone:water and Simulated Tear Fluid (STF) media. λmax was determined as 322 nm in both environments. Calibration standards were prepared at seven concentration levels (5–60 µg/mL). Method validation included the evaluation of linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Accuracy and intra-day precision were assessed at 10, 30, and 50 µg/mL. Absorbance values were measured using a UV-visible spectrophotometer with appropriate blank correction.

Results: In the acetone:water medium, the method showed excellent linearity with the regression equation y = 0.0147x – 0.0068 and a correlation coefficient (R^2) of 0.9990. The LOD and LOQ were calculated as 0.98 µg/mL and 2.96 µg/mL, respectively. The accuracy results at 10, 30, and 50 µg/mL were 99.11% ± 1.04, 99.74% ± 0.35, and 99.87% ± 0.21, respectively. Precision values were within acceptable limits, with %RSD < 2%.
For the STF medium, the method demonstrated even stronger linearity, with a regression equation y = 0.0108x + 0.0042 and R^2 = 0.9999. The calculated LOD and LOQ were 1.33 µg/mL and 4.04 µg/mL, respectively. Recovery at the same concentration levels was found to be 99.66% ± 1.07 (10 µg/mL), 99.58% ± 0.47 (30 µg/mL), and 99.57% ± 1.30 (50 µg/mL), indicating high accuracy. Precision results were also consistent with ICH criteria, with %RSD below 2% at all levels.

Conclusion: The developed UV spectrophotometric method is sensitive, accurate, and reproducible for the quantification of brimonidine tartrate in both acetone:water and simulated tear fluid media. The method demonstrates excellent linearity and precision, with recovery rates near 100% and low %RSD values. These findings confirm the suitability of this validated method for routine analysis and *in vitro* release studies of BT.

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