CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/11401295

https://www.iajps.com/volumes/volume11-may-2024/28-issue-05-may-24/

Available online at: http://www.iajps.com *Research Article*

VALIDATED SPECTROPHOTOMETRIC ESTIMATION OF LABETALOL HYDROCHLORIDE USING DYE DRUG REACTION

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Abstract:
The validated spectrophotometric method for the estimation of labetalol hydrochloride (LBH) using a dye-drug reaction demonstrates a robust and reliable analytical technique for routine pharmaceutical analysis. The method involves the formation of a colored complex between LBH and methyl orange, which is measured spectrophotometrically. LBH showed varying solubility in different solvents: sparingly soluble in water, freely soluble in 0.1 N HCl, slightly soluble in 0.1 N NaOH, and soluble in both methanol and ethanol. Among various dyes tested, methyl orange was selected due to its positive reaction with LBH, forming a stable colored complex. The maximum wavelength (λmax) for the methyl orange-LBH complex was determined to be 433 nm. The method exhibited excellent linearity over a concentration range of 2-10 µg/ml with a correlation coefficient (r^2) of 0.99984, indicating a strong linear relationship between absorbance and concentration. The method showed high repeatability with low %RSD values across different concentrations. Analyst-to-analyst variation and day-to-day variation studies confirmed the method's precision and robustness, with %RSD values within acceptable limits. Recovery studies at 80%, 100%, and 120% levels demonstrated the method's accuracy, with mean recovery percentages close to 100% and low %RSD values. The LOD and LOQ were determined to be 0.25 mg/ml and 1.75 mg/ml, respectively, indicating the method's sensitivity. This validated spectrophotometric method for estimating labetalol hydrochloride using the methyl orange dye-drug reaction is precise, accurate, and reliable. The method's high sensitivity, excellent linearity, and robust recovery studies make it suitable for routine quality control and pharmaceutical analysis of LBH. The low variability in repeatability and inter-analyst tests further confirm the method's applicability in ensuring consistent and reproducible results in various laboratory settings. Overall, this method provides a valuable analytical tool for the effective quantification of LBH in different formulations and samples.

Keywords: *Labetalol hydrochloride, Spectrophotometric method, Dye-drug reaction, Methyl orange, Analytical technique, Pharmaceutical analysis*

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Please cite this article in press Sunil Gupta et al., ***Validated Spectrophotometric Estimation Of Labetalol Hydrochloride Using Dye Drug Reaction****., Indo Am. J. P. Sci, 2024; 11 (05).*

INTRODUCTION:

Labetalol hydrochloride, a unique antihypertensive agent, functions as both an alpha and beta-adrenergic receptor blocker, offering a dual mechanism of action. It is widely prescribed for the management of hypertension and hypertensive emergencies. Accurate and reliable analytical methods are essential for the quality control and therapeutic monitoring of labetalol hydrochloride in pharmaceutical formulations. Spectrophotometric methods are favored for their simplicity, cost-effectiveness, and sensitivity.

Spectrophotometry, which measures the absorbance of light by a sample at specific wavelengths, is a fundamental technique in pharmaceutical analysis. It is particularly useful for quantifying drugs in bulk and dosage forms due to its straightforwardness and high precision. Various spectrophotometric methods have been developed to estimate labetalol hydrochloride, including those involving complex formation with dyes, which enhance sensitivity and selectivity (Skoog et al., 2017).

The use of dye-drug reactions in spectrophotometric analysis involves the formation of a colored complex between the drug and a dye, which can be measured at a specific wavelength. This method not only increases the sensitivity of detection but also allows for the selective estimation of the drug in the presence of excipients and other potential interferences (Beckett & Stenlake, 2004). Common dyes used in these reactions include bromothymol blue, methyl orange, and bromocresol green, each offering unique advantages depending on the nature of the drug and the matrix.

Several studies have reported the use of spectrophotometric methods for the determination of labetalol hydrochloride. For instance, Mahmoud and Abdine (2000) described a method using bromothymol blue, achieving good sensitivity and accuracy. However, limitations such as lengthy reaction times and the requirement for stringent control of pH conditions have been noted. More recently, Taha et al. (2013) improved upon these methods by optimizing the reaction conditions and employing more stable dye complexes, yet challenges remain in terms of reproducibility and interference from excipients.

The primary objective of this study is to develop and validate a simple, rapid, and sensitive spectrophotometric method for the estimation of labetalol hydrochloride using a dye-drug reaction. The method aims to overcome the limitations of previous techniques by optimizing reaction conditions and ensuring robustness and accuracy in various pharmaceutical formulations.

MATERIAL AND METHODS:

Selection of particular dye

Different dyes used for confirmation of reaction with dye by following procedure:-

Solutions of 100µg/ml of Labetalol hydrochloride was prepared in distilled water, in 3 ml of drug solutions add 1 ml dye and extracted with 3ml chloroform and same manner control also prepared shake both the solution and stand aside for 10 min and the colour change compared to control for dye drug reaction (Venkatesh et al., 2020).

Linearity range and calibration graph

Selection of wavelength for linearity

Solutions of 100µg/ml of Labetalol hydrochloride was prepared in 0.1 N HCl, in 3 ml of drug solutions add 1 ml dye and extracted with 3ml chloroform and same manner control also prepared shake both the solution and stand aside for 10 min and compare the colour change compared to control for dye drug reaction. Pipette out the coloured layer of solution and scan between 400 to 800nm as control as blank.

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving 10mg of drug in 8mL 0.1 N HCl, the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with 0.1 N HCl to get a concentration of 1000µg/ml (Stock-A) for drug (Krishnamoorthy et al., 2012).

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 1.0ml withdrawn with help of pipette from standard stock solution A of Labetalol hydrochloride and transferred into 10ml volumetric flask separately and diluted up to 10ml with 0.1 N HCl that gave concentration of 100µg/ml (Stock-B).

Preparation of Working Standard Solution (For reaction with Methyl orange)

Aliquots of 0.2ml, 0.4 ml, 0.6ml, 0.8ml and 1.0 ml withdrawn with help of pipette from standard stock solution (Stock-B) in 10 ml volumetric flask and volume was made up to 10 ml with 0.1 N HCl. This

gave the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml respectively for Labetalol hydrochloride. Take 3ml of each standard and react with 1 ml (2%) methyl orange add chloroform (3ml) was added to each volumetric flask; the flask was shaken well for thorough mixing of two phases and was allowed to stand for clear separation of the layers. The absorbance of the separated chloroform layers were measured against the reagent blank at 506nm and a calibration curve was drawn for the standard dilutions.

Analysis of Tablets sample

Take 20 tablets and determine the average weight, weight equivalent to 10mg of Labetalol hydrochloride was taken in 10ml volumetric flask, and volume make up to 10ml. Dilute suitably to 10µg/ml. Take 3 ml of this solution in three different volumetric flasks, Then 1 ml of dye solution (2%, Methyl orange) was added and 3 ml chloroform, the flask was kept aside for about 10 min. Pipette out the colored layer and the absorbances of were observed at selected wavelengths and the concentrations were obtained from calibration curve method.

Validation of developed method

The validation of analytical methods is done as per ICH guidelines (ICH; 2005).

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to absorbance of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 2 to 10µg/ml for LBH) and absorbance for each concentration were recorded three times and mean absorbance was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. The response ratio (response factor) was found by dividing the mean absorbance with respective concentration (Sheetal; 2013).

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Patel et al., 2016).

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 3, 6, 9, 12 and 15µg/ml for LBH indicates the precision under the same operating condition over short interval time (Sri et al., 2015).

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for LBH reported in table.

Analyst to Analyst variation

Analyst-to-analyst variation in methods development is a common challenge in analytical laboratories, influenced by factors like experience, interpretation of method requirements, instrument calibration, and data interpretation. To mitigate this, labs use standardized protocols, training, calibration, peer review, and quality control measures to ensure consistency and reliability in analytical results (Suraj et al., 2018).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

RESULTS AND DISCUSSION:

The linearity of the method was evaluated by analyzing labetalol hydrochloride at different concentrations ranging from 2 to 10µg/ml. The absorbance values at 433.0 nm were recorded for each concentration, and the data is presented in Table 1.

The mean absorbance values were plotted against the respective concentrations to generate a calibration curve, which showed excellent linearity with a correlation coefficient (r^2) of 0.99984. The slope of the calibration curve was found to be 0.1457, and the intercept was 0.0327, indicating a strong linear relationship between concentration and absorbance.

The maximum absorption wavelength (λmax) of labetalol hydrochloride in the presence of methyl orange was determined to be 433.0 nm. This wavelength was used for the spectrophotometric analysis as it provided the highest sensitivity for detection. The developed method was applied to the analysis of labetalol hydrochloride in tablet formulations.

As shown in Table 2, the concentration found was 98.96 mg, corresponding to 98.96% of the labeled claim. This result demonstrates the method's accuracy and applicability for routine quality control of labetalol hydrochloride tablets.The response ratio, calculated as the mean absorbance divided by the concentration, was assessed for its consistency across different concentrations of labetalol hydrochloride (Table 3).

The mean response ratio was 0.157 with a standard deviation (SD) of 0.012 and a relative standard deviation (%RSD) of 7.722%. This indicates acceptable variability and confirms the method's reliability over the studied concentration range.

The method's validation parameters are summarized in Table 4. Recovery studies at 80%, 100%, and 120% levels yielded recovery rates of 99.12%, 97.56%, and 98.70%, respectively, with low standard deviations, indicating the method's accuracy. Repeatability tests showed a mean recovery of 96.163% with minimal variability. Day-to-day and analyst-to-analyst variations were also low, with mean recoveries of 98.540% and 98.565%, respectively, demonstrating the method's precision and reproducibility.

The LOD and LOQ were determined to be 0.25 mg/ml and 1.75 mg/ml, respectively (Table 5). These values indicate the method's sensitivity, allowing for the detection and quantification of labetalol hydrochloride at low concentrations.

Table 1: Linearity of Labetalol hydrochloride at λ_{max} = 433.0nm

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
2	0.355	0.356	0.354	0.353	0.355	0.355
4	0.625	0.624	0.625	0.623	0.624	0.624
6	0.912	0.915	0.916	0.914	0.914	0.914
8	1.198	1.197	1.196	1.197	1.196	1.197
10	1.476	1.475	1.476	1.478	1.475	1.476
Correlation Coefficient (r^2)						0.99984
Slope (m)						0.1457
Intercept (c)						0.0327

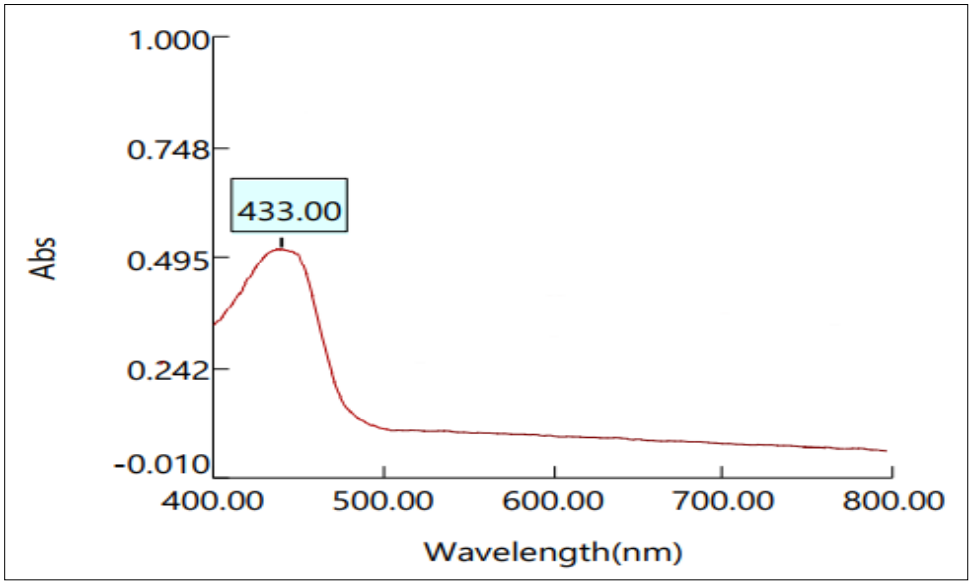

Figure 1: Determination of λ_{max} in methyl orange

Table 2: Analysis of tablets formulation of Labetalol hydrochloride

Conc. Present	Methyl orange	
	Conc. Found (mg)	% Conc. Found
100	98.96	98.96

Table 3: Response ratio data for linearity of LBH

Concentration (µg/ml)	Mean AUC	Response Ratio
2	0.355	0.178
4	0.624	0.156
6	0.914	0.152
8	1.197	0.150
10	1.476	0.148
Mean		0.157
SD		0.012
%RSD		7.722

Table 4: Results of validation Parameters

Parameters		Results
Recovery study	**80%**	99.12±0.350
	100%	97.56±0.822
	120%	98.70±0.588
Repeatability		96.163±0.033
Day-to-Day variation		98.540±0.067
Analyst to analyst		98.565±0.038

Table 5: Results of LOD and LOQ of LBH

Name	LOD (µg/ml)	LOQ (µg/ml)
LBH	0.25	1.75

CONCLUSION:

The developed and validated spectrophotometric method for the estimation of labetalol hydrochloride using a dye-drug reaction with methyl orange is simple, sensitive, and reliable. The method demonstrates excellent linearity, accuracy, and precision, making it suitable for routine analysis of labetalol hydrochloride in pharmaceutical formulations. The low LOD and LOQ further enhance its applicability for detecting and quantifying the drug at low concentrations. This method provides a valuable tool for quality control in pharmaceutical industries, ensuring the efficacy and safety of labetalol hydrochloride-containing products.

REFERENCES:

1. Skoog, D.A., Holler, F.J., & Crouch, S.R. (2017). *Principles of Instrumental Analysis*. Belmont, CA: Cengage Learning.
2. Beckett, A.H., & Stenlake, J.B. (2004). *Practical Pharmaceutical Chemistry*. New Delhi, India: CBS Publishers and Distributors.
3. Mahmoud, A.M., & Abdine, H. (2000). Spectrophotometric determination of labetalol hydrochloride in pharmaceutical preparations. *Journal of Pharmaceutical and Biomedical Analysis*, 23(5), 849-856.
4. Taha, E.A., Salama, N.N., & Omara, S. (2013). Stability-indicating spectrophotometric methods

for the determination of labetalol hydrochloride. *Journal of Applied Pharmaceutical Science*, 3(7), 86-92.
5. Sama Venkatesh, Sirimalla Jyothi, Rajeswari Chiluka, Yenumula Padmavathi. Development of UV- Spectrophotometric Method for the Simultaneous Estimation of Nateglinide and Piperine in Combined Tablet dosage form. Asian J. Pharm. Ana. 2020; 10(3): 134-140.
6. G. Krishnamoorthy, C. Diana Priyadarshini, R. Senthamarai. Spectrophotometric method of Choline Bitartrate in bulk and its tablet formulation. Asian J. Pharm. Ana. 2012; 2(4): 114-115.
7. Validation of analytical procedures: Text and methodology. International Conference on Harmonization (ICH) Q2 (R1) guideline. Geneva, Switzerland; 2005.
8. Mahawar Sheetal. A Simple Ultraviolet Spectrophotometric Method for the Estimation of Docetaxel in Bulk Drug and Formulation. Asian J. Pharm. Ana. 2013; 3(2): 48-52.
9. Jetal Patel, Dhara Patel, Sharav Desai. Simultaneous Spectrophotometric Estimation of Naphazoline Nitrate and Hydrocortisone in Nasal drops. Asian J. Pharm. Res. 2016; 6(2): 61-66.
10. K. Vijaya Sri, S. Sravani, M. Shiva Kumar. Development and Validation of UV Spectrophotometric Method for Estimation of Lurasidone in Bulk and Pharmaceutical Formulations. Asian J. Pharm. Res. 2015; 5(2): 102-107.
11. Suraj R. Chaudhari, Amod S. Patil, Atul A. Shirkhedkar. Studies on Derivative Spectroscopy and Area Under Curve UV-Spectrophotometric Methods for Estimation of Apremilast in Bulk and In-house Tablets. Asian J. Pharm. Res. 2018; 8(1): 11-16.