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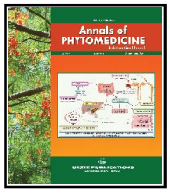

Review Article : Open Access

A review of analytical method development and validation of labetalol hydrochloride

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Abstract

Combined alpha- and beta-adrenoceptor blockers, such as labetalol hydrochloride, are administered orally and intravenously to treat hypertension. It is a competitive antagonist of postsynaptic nascent 1-adrenoceptors and an unselective antagonist of beta-adrenoceptors. It is a medication used to treat hypertension. A significant amount of research has been conducted on the synthesis, pharmacology, mechanism of action, and other aspects of labetalol hydrochloride. However, the authors of this paper have only addressed the analytical methodologies that have been published, thus far for the estimation of labetalol hydrochloride in pharmaceutical dosage forms. Reviewing various analytical techniques and validating the related substance of labetalol hydrochloride is the main goal of this article.

1. Introduction

In (RS)-2-hydroxy-5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl] benzamide monohydrochloride is the chemical name for labetalol hydrochloride. Labetalol often appears as a powdered white or off-white crystal. Both ethanol and water will dissolve beta-lactamol hydrochloride (Ashok Chakravarthy *et al*., 2016). The boiling point of labetalol hydrochloride is 552.7°C, and a melting point of labetalol hydrochloride is reported to be around 136-139°C. Labetalol is a weak base, and its pKa (acid dissociation constant) is around 10.5. The molecular weight of labetalol hydrochloride is approximately 364.91 g/mol (Ciciliati *et al*., 2018). Labetalol hydrochloride has a half-life of approximately 5 to 8 h in the body, meaning that it takes this long for half of the medication to be removed from circulation. Labetalol hydrochloride needs to be kept out of the light and moisture in a cold, dry location (Pradeep Singh *et al*., 2022). Labetalol contains an amide functional group, specifically in the benzamide moiety. The compound's ability to inhibit beta-adrenergic receptors is partly attributed to its hydroxyl groups. Labetalol is a racemic mixture, meaning it has both R and S enantiomers (MacCarthy and Bloomfield,1983). The presence of chiral centers contributes to the different stereoisomers in the compound. Labetalol has multiple chiral centers, leading to different stereoisomers. In its hydrochloride salt form (Labetalol hydrochloride), the compound is ionized. The hydrochloride salt helps improve the solubility of labetalol in aqueous solutions (Sivakumar *et al*., 2022).

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2. Drug profile

Table 1: Drug profile of labetalol hydrochloride

Properties	Details
IUPAC name	2-hydroxy-5-{1-hydroxy-2-[(4-phenylbutazone-2-yl)amino]ethyl} benzamide
Appearance	White or off-white crystalline powder
Molecular weight	$C_{19}H_{24}N_2O_3$
Melting point	188 ºC
Boiling point	552.7 ºC
Solubility	Soluble in water and alcohol, insoluble in ether and chloroform
pH	3.0 to 4.5
Therapeutic activity	Labetalol hydrochloride is used to treat high blood pressure (hypertension) and heart-related chest pain (Angina).
Dosage form	Tablet, coated, film-coated, solution, injection.
Route of administration	Oral, parenteral

2.1 Mechanism of action

The action of beta receptors in the heart is inhibited by labetalol hydrochloride. Usually, adrenaline (epinephrine) and norepinephrine stimulate beta receptors, leading to increased heart rate and force of contraction (Ganesan *et al*., 2010; Indumathy *et al*., 2023). By blocking these beta receptors, labetalol reduces the heart rate and the

force with which the heart pumps blood. This results in a decreased cardiac output, helping to lower blood pressure. Labetalol also blocks alpha1 receptors. These receptors are found in the walls of blood vessels, and their stimulation by norepinephrine causes blood vessels to constrict (Nahata, 1991). By inhibiting alpha1 receptors, labetalol causes vasodilation (relaxation of blood vessels). The peripheral vascular resistance is decreased as a result of this dilatation, facilitating the heart's ability to pump blood against less resistance. The combined beta and alpha-blocking actions of labetalol result in a balanced antihypertensive effect (Natchiappan Senthilkumar *et al*., 2022). The beta-blockade decreases cardiac output, while the alpha-blockade reduces peripheral vascular resistance. The net effect is a reduction in blood pressure, making labetalol effective in the management of hypertension (Nivetha *et al*., 2023).

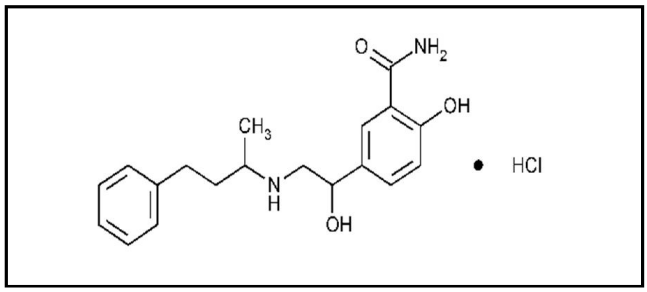

Figure 1: Structure of labetalol hydrochloride.

3. Method development

Ashok Chakravarthy *et al*. (2016) established a stability-indicating RP-HPLC method for measuring labetalol and its breakdown products in tablet form simultaneously. The chromatographic separation of labetalol and its breakdown product in tablets was performed using a Zorbax Eclipse plus C-8 (100*4.6 mm, 3.5 µm) column. Using a linear gradient method, the sample was composed of 0.1% trifluoro acetic acid (TFA) (v/v) in 1000 milliliters of water and 0.1% TFA in 1000 milliliters of acetonitrile: methanol (1:1). The flow rate was 1.0 ml/min using a detection wavelength of 230 nm and a column temperature of 35°C. During the forced degradation studies, labetalol tablets were exposed to acidic, basic, heat, humidity, and photolytic environments. The method robustness, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and precision were all validated by ICH criteria. Using this method, it was observed that all pollutants, known and unknown, were within the linear range of LOQ to 120%. The impurity known LOQ and LOD values were determined to be between 0.3593 and 0.7187 µg/ml, and at several concentration levels, the percentage recovery values ranged from 95.5 to 105.2%. Comparative results for the division for perfection and moderate perfection were set to be less than five percent. For every compound, the correlation coefficient was not less than 0.99. The established method's ability to indicate stability is demonstrated by the validation trials' findings.

Kulkarni *et al.* (2015) described a straightforward, quick, and precise stability-indicating RP-HPLC method for measuring labetalol hydrochloride in pharmaceutical formulations. An ultraviolet sensor from the Younglin (S.K) grade system was used to analyze the medication. Outfitted with an Autochro3000 software-powered reverse phase C18 column (4.6 mm x 250 mm; 5 µm), a UV 730D absorbance detector, a 20 µl injection loop, and an SP930D pump. The mobile phase is a combination of H_2O and MeOH in a (70:30% v/v) ratio; the pH was adjusted using 0.05% OPA. At a flow rate of 1 milliliter per minute from the solvent reservoir to the column, the mobile phase was pumped. The temperature of the column was kept at ambient. At 246 nm, UV detection was carried out. Using the mobile phase running through the system, the ultrasonic water bath degassed it for at least thirty minutes. When preparing the standard and test samples, the mobile phase is utilized as diluents. Using MeOH and H_2O solution in the ratio (70:30) as the mobile phase, the method demonstrated a linear response for concentrations in the range of 10-50 µg/ml with detection at 246 nm, a flow rate of 1 ml/min, and a retention time of 6.3 min. The intercept, slope, and correlation coefficient values were, in order, 7.019, 15.30, and 0.999. Under situations of acidity, basicity, peroxide, and heat deterioration, the medication degrades. Analysing samples from accelerated stability tests and isolating the analyte from related chemicals, degradation products, and excipients included in tablet dosage forms can be accomplished with the help of the suggested technique.

Attia *et al*. (2015) created a spectrophotometric method for determining the existence of the oxidative degradation product in the presence of labetalol hydrochloride at two wavelengths that are dependent on stability. Method B: Bivariate at 302 and 246 nm; Method A: Dual wavelength at 300 and 280 nm. The suggested styles' ranges of refinement, linearity, and delicacy were identified. Lab set fusions were used to test the suggested styles' selectivity. Standard addition fashion is used to evaluate the results' validity. The F and t-test were used to statistically compare the developed styles and the specified technique; the findings showed no discernible difference in accuracy or precision. The advanced styles have been effectively employed for the study of LBT in pharmaceutical lozenge form. The dual wavelength approach was found to have superior selectivity and sensitivity when compared to the other two methods. This particular spectrophotometric analysis feature is highly interesting to analytical chemists because it provides a unique opportunity to assay LBT in its pharmaceutical formulation free from interference from degradation products or excipients.

Nafisur Rahman *et al.* (2011) developed a simple and accurate kinetic spectrophotometric technique to measure labetalol hydrochloride. The protocol was based on a kinetic examination of the drug's oxidation with alkaline potassium permanganate at room temperature (25 ± 1°C). At 605 nm, the absorbance of colored manganite ions increased. Every experimental factor influencing the color's development was examined and optimized. The medicine attention was determined using the original rate and fixed time styles, which were set at 6 nanoseconds, with the initial rate and fixed time approaches. In the concentration ranges of 2-14 µg/ml and 1-10 µg/ml, respectively, the calibration graphs were linear. The technique worked well for figuring out how much labetalol was in both store-bought and homemade tablets. The results were statistically validated by recovery studies.

Nahata *et al*. (1991) established the stability of three fruit juices, distilled water, and simple syrup for labetalol hydrochloride**.** both in glass and plastic prescription bottles, both at room temperature and refrigerated for four weeks, labetalol HCL tablets (Trandate, Glaxo) were triturated and suspended in distilled water, simple syrup, apple juice, grape juice, and orange juice to approach a concentration of 7-10 mg/ml. Following filtration, the liquids were kept at 4 and 23 in

five prescription bottles made of plastic and five made of glass. Samples were collected at 1.2 and 4 weeks, as well as at 0, 24, and 72 h after the trial's commencement. The HCL of labetalol was measured with an HPLC system. Since there was no discernible difference in the amount of attention that labetalol HCL received over the study time, the tablet lozenge form can be reformulated, stored, and administered as a liquid lozenge to elderly or pediatric patients. The stability statistics of labetalol HCL in the five liquid vehicles were seen to be consistent over four weeks when kept in plastic bottles at 4 and 23, and in glass bottles at 23 (Raghavi *et al.,* 2023). Based on the findings of this study, patients who would rather take liquid than tablets can have labetalol HCL tablets reformulated and given to them in a liquid dosage form. This may be significant, especially when treating young or elderly patients until a commercially available liquid dose form becomes available. These investigations are necessary for several drugs that may be given to elderly or pediatric patients in the absence of a liquid dosage form. It should be mentioned, nonetheless, that we did not carry out any research to rule out the possibility of microbial growth, especially during prolonged room-temperature storage. For this reason, the suspension that has been reformulated and kept for a long time should be kept refrigerated.

Yuen *et al.* (1983) determined a labetalol hydrochloride's stability and compatibility with commonly used large-volume parenterals (LVPs) were assessed. Following the acquisition of the initial samples, the admixtures were made, divided evenly, and kept between 4 and 25^0C for 72 h. We examined the medication concentrations, pH, osmolarity, and visible alterations in the first and 72 h samples. High-performance liquid chromatography (HPLC) was used in the experiment, and the American Public Health Association's color-testing technique was used to evaluate the color. Within 6 h, a white precipitate developed in the mixture with the injection of 5% sodium bicarbonate. At either of the storage temperatures, there was little change in the initial drug concentration in any of the LVP solutions. No detectable variations in pH or osmolarity were observed, nor were there any other HPLC peaks identified.

Table 2: Summary of various analytical techniques for the estimation of labetalol

Analytical techniques	Parameters	Reference
Stability indicating HPLC	Reverse phase Thermo C18 column, UV absorbance detector, Mobile phase-MEOH: H2O (70:30% v/v).	Kulkarani *et al.* (2015)
Stability indicating RP-HPLC degradation products	Zorbax eclipse plus-8column, Dual wavelength detector, 0.1% TFA in 1000 ml of ACN: MEOH (1:1) - gradient program.	Ashok Chakravarthy *et al.* (2016)
Spectrofluorimetric determination in pharmaceutical preparations and urine samples	A-Native fluorescence was measured at 432 nm after excitation at 312 nm. B-Formation of a ternary complex between zinc (II), eosin, and LBT.	Nafisur Rahman, *et al.* (2011)
Spectrofluorimetric and spectro-photometric determination in human plasma	Drug with 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) in an alkaline medium (pH 7.5). 1. The reaction product was measured spectrofluorimetrically at 540 nm after excitation at 476 nm. 2. Spectro-photometrically at 480 nm.	Omar *et al.* (2018)
Simple TLC-densitometric method for the estimation of labetalol hydrochloride in tablets	Stationary phase: Aluminium-packed silica gelMobile phase-Ethyl acetate: Methanol: Ammonia, (8:2:0.2, v/v/v/).Wavelength: 309 nm.	Wankhede *et al.* (2012)
A simple and sensitive liquid chroma-tography-tandem mass spectrometry method was developed and validated for the estimation of labetalol in human plasma	Column: Phenomenex Luna C18Mobile phase: Amm-onium formate: Methanol, (20:80% v/v),The mass transition m/z 329.01–>161.95 and 267.99–>115.86 were used to measure labetalol.	Ganesan *et al.* (2010)
HPTLC method development and validation for the estimation of labetalol hydrochloride in tablet dosage form	Stationary phase: Al plate (60F254) Mobile phase: Chloroform: Methanol: Ammonia, (8:2:0.2%v/v/v), Wavelength: 254 nm.	Monali Bhalerao (2014)

The thoroughness of this review extends beyond a mere enumeration of techniques, delving into the intricate details of each method's principles, advantages, and limitations (Alka Rani and Wamik Azmi, 2021). Special emphasis is placed on the rigorous validation processes undertaken to ensure the reliability and accuracy of these analytical approaches. In essence, this comprehensive review serves as a compendium of knowledge, offering a deep and nuanced understanding of the analytical methodologies sanctioned for labetalol evaluation it provides a valuable resource for researchers (Akella Anuradha *et al.*, 2023).

4. Conclusion

Numerous techniques, including stability indicating, spectro-fluorimetry, HPLC, HPTLC method improvements, *etc.*, have been described for labetalol hydrochloride. However, this medication review will be helpful for future investigations. The creation of a novel RP-HPLC technology is a praiseworthy initiative in the plan for the labetalol and related compounds separated and quantified chemicals in their medicinal dose form. The literature review, method development, method validation, quantification of related substances, documentation and reporting, method transferability, publication,

and knowledge sharing are some of the important phases and factors involved in this endeavor. By following these procedures, a unique RP-HPLC method for labetalol and related chemicals may be developed, which can greatly improve analytical capabilities for pharmaceutical quality control and guarantee the medication's safety and efficacy.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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