

STABILITY INDICATING REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF LABETALOL AND ITS DEGRADATION PRODUCTS IN TABLET DOSAGE FORMS

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ABSTRACT

Objective: The objective of the present work is to develop a simple, efficient, and reproducible stability indicating reverse phase high-performance liquid chromatographic method for simultaneous determination of labetalol and its degradation products in tablet dosage forms.

Methods: The chromatographic separation of labetalol and its degradation products in tablets was carried out on Zorbax Eclipse Plus C-18 (100 × 4.6 mm, 3.5 μm) column using 0.1% trifluoroacetic acid (TFA) (v/v) in 1000 ml of water and 0.1% TFA (v/v) in 1000 ml of acetonitrile: Methanol (1:1) by linear gradient program. Flow rate was 1.0 mL min⁻¹ with a column temperature of 35°C, and detection wavelength was carried out at 230 nm. Known impurity is well resolved from the main active drug within 14 minutes run time.

Results: The forced degradation studies were performed on labetalol tablets under acidic, basic, oxidation, thermal, humidity, and photolytic conditions. No degradation products were observed from the forced degradation studies, and the known impurity is well resolved from the main active drug. The method was validated in terms of specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, and robustness as per the ICH guidelines. The method was found to be linear in the range of LOQ to 120% for all the known and unknown impurities. The LOD and LOQ values of known impurity were found between 0.3593 and 0.7187 μg mL⁻¹, and the percentage recovery values were in the range of 95.5-105.2% at different concentration levels. Relative standard deviation for precision and intermediate precision results were found to be <5%. The correlation coefficient found for all compounds was not <0.99. The results obtained from the validation experiments prove that the developed method is a stability indicating method.

Conclusion: The developed method can be successfully applied for routine analysis, quality control analysis and also suitable for stability analysis of the simultaneous determination of labetalol and its degradation products in tablet dosage forms as per the regulatory requirements.

Keywords: Labetalol, Development, Validation, Reverse phase high-performance liquid chromatography.

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INTRODUCTION

Labetalol hydrochloride (labetalol) 2-hydroxy-5-[(1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl) benzamide hydrochloride is a mixed α/β-adrenergic antagonist, which is used to treat high blood pressure [1,2]. The minimum requirement for adrenergic agents is a primary or secondary amine separated from a substituted benzene ring by one or two carbons. This configuration results in strong agonist activity. As the size of the substituent attached to the amine becomes greater, particularly with respect to a t-butyl group, then the molecule typically is found to have receptor affinity without intrinsic activity and is, therefore, an antagonist. Labetalol, with its 1-methyl-3-phenylpropyl substituted amine, is greater in size relative to a t-butyl group, and therefore, acts predominantly as an antagonist. The overall structure of labetalol is very polar. This was created by substituting the isopropyl group in the standard β-blocker structure with an aralkyl group, including a carboxamide group on the meta position, and by adding a hydroxyl group on the para position [3,1]. Structure of labetalol is shown in Fig. 1.

Labetalol has two chiral carbons and consequently exists as four stereoisomers [4]. Two of these isomers, the (S,S)- and (R,S)-forms are inactive. The third, the (S,R)-isomer, is a powerful α1 blocker. The fourth isomer, the (R,R)-isomer which is also known as dilevalol, is a mixed nonselective β-blocker and selective α1 blocker [3]. Labetalol is typically given as a racemic mixture to achieve both α- and β-receptor blocking activity, which lowers the blood pressure [5,1]. It can be given intravenously in severe hypertensive situations or by mouth

for long-term hypertension management [6]. Labetalol's dual α- and β-blockade has different physiological effects in short- and long-term situations. In short-term, acute situations, labetalol decreases blood pressure by decreasing systemic vascular resistance with little effect on stroke volume, heart rate, and cardiac output [7]. During long-term use, labetalol can reduce heart rate during exercise while maintaining cardiac output by an increase in stroke volume [8].

Labetalol is a dual α1- and β1/β2-adrenergic receptor blocker and competes with other catecholamines for binding to these sites [5]. Its action on these receptors is potent and reversible. Labetalol is highly selective for postsynaptic α1-adrenergic and non-selective for β-adrenergic receptors. It is about equipotent in blocking both β1- and β2-receptors [3]. Labetalol acts by blocking α- and β-adrenergic receptors, resulting in decreased peripheral vascular resistance without significant alteration of heart rate or cardiac output. The β:α antagonism of labetalol is approximately 3:1 [9,10]. Labetalol hydrochloride is having a pKa value of 9.3 [11,12]. Labetalol is a white to off-white powder. Melts at about 180°C, with decomposition. Soluble in water and alcohol; insoluble in ether and chloroform [13].

From the literature survey, it is evident that very few methods are available for the determination of labetalol and its impurities using high-performance liquid chromatography (HPLC). Jinqi *et al.* developed a method for the determination of content and related substances of labetalol hydrochloride by HPLC [14]. Meredith *et al.* developed an HPLC method for determination of labetalol in plasma by HPLC using fluorescence detection [15]. Vaishali *et al.*

developed stability indicating reverse phase HPLC (RP-HPLC) method for determination of labetalol hydrochloride in pharmaceutical formulation [16]. Ganesan *et al.* developed a method of rapid analysis of labetalol in human plasma using liquid chromatography-tandem mass chromatography [17].

Methods are available in the United States Pharmacopeia [13] for the determination of labetalol and its related impurities in drug substance by thin layer chromatography (TLC) and determination of assay content in tablet dosage form by HPLC. Similarly, analytical method for related substances of labetalol hydrochloride drug substance is available in European pharmacopoeia [18] for related substances of labetalol hydrochloride drug substance.

As per the literature review, no method was reported for the estimation of labetalol and its degradation products in finished dosage forms using HPLC. The present work describes the simultaneous estimation of labetalol and its degradation products in tablet dosage forms using HPLC and also developed method gives a sensitive, specific, and stability indicating method for the determination of impurities of labetalol in a short run time by HPLC. Time required for the analysis, manpower, and solvent consumption for performing TLC analysis can be saved using the current HPLC method and finally supporting toward the green environment by following health safety and environment guidelines. Developed LC method was validated with respect to specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, precision, accuracy, and robustness. Forced degradation studies were carried out to verify the stability indicating nature of the LC method.

EXPERIMENTAL

Chemicals and reagents

Qualified standards (labetalol purity ~99.3%, 2-hydroxy impurity ~99.4) are obtained from Spectrum Pharma research solutions and were used without any further purification. Samples of LABEBET 100 mg tablets are purchased from local pharmacy. HPLC grade methanol (MeOH purity ~99.8%) and acetonitrile (ACN purity ~99.9%) were obtained from Rankem (Mumbai, India). Trifluoroacetic acid (TFA) (purity ~99%) was received from Acros Organics (India).

Instrumentation

The waters LC system (Milford, MA, USA) equipped with a diode array detector was used for method development and forced degradation studies. The output signal was monitored and processed using Empower software. Waters LC consists of 2695 separation module and 2996 photodiode array detector used for the validation study. Intermediate precision was carried out using waters 2695 separation module with 2487 dual wavelength detector. Photolytic chamber was used for photolytic degradation, and thermal degradation samples were kept at 50°C for 2 days in an oven.

Chromatographic conditions

The chromatographic separation was achieved on a Zorbax Eclipse Plus C-18 (100 × 4.6 mm, 3.5 μm) column using mobile phase-A composed of 0.1% TFA (v/v) in 1000 ml of water and mobile phase-B composed of 0.1% TFA (v/v) in 1000 ml of ACN: Methanol (1:1) by linear gradient program. The mobile phase-A was filtered with 0.45 μm nylon filter. Gradient program used for chromatographic separation was shown in Table 1. Flow rate was set to 1.0 mL min⁻¹ with a column temperature of 35°C. Detection wavelength was carried out at 230 nm. Injection volume was 10 μL. Water and MeOH in the ratio of 75:25 was used as diluent for the preparation of standards and samples.

Preparation of standard and sample solutions

Standard stock solution of labetalol

Accurately weighed and transferred 50 mg of labetalol working standard into a 50 mL volumetric flask. Added about 30 mL of diluent and sonicated to dissolve with intermittent shaking. The resulting solution is diluted up to the mark with diluent and mixed well.

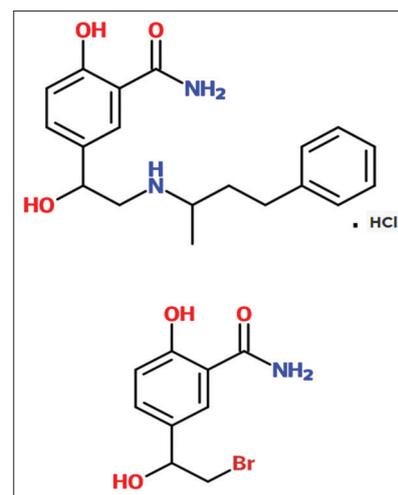


Fig. 1: Structures of labetalol hydrochloride and its related impurity 2-hydroxy impurity

Table 1: Gradient program

Time in minutes	Mobile phase-A	Mobile phase-B
0	75	25
1	75	25
10	20	80
11	75	25
14	75	25

Preparation of standard solution

Transferred 1 mL of labetalol standard stock solution into a 100 mL volumetric flask and diluted up to the mark with the diluent and mixed well.

Preparation of sample solution

Determined the average weight of 10 tablets and transferred 10 tablets equivalent to 1000 mg of labetalol directly into a 1000 mL volumetric flask. Added about 200 mL of diluent and sonicated for 10 minutes with intermittent shaking until tablets were completely disintegrated. Added 600 mL of diluent and sonicated further for 30 minutes with intermittent shaking. Made up the volume to 1000 mL volumetric flask with diluent and centrifuged the sample solution for 15 minutes at 3000 rpm. Transferred the resultant sample solution into HPLC vials for analysis.

METHOD VALIDATION

Specificity/stress studies

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, and matrix [19]. The specificity of the developed method was established to prove the absence of interference from placebo peaks (excipients) which is part of required pharmaceutical preparation. Degradation study was performed by subjecting the tablets to accelerated degradations such as acid, alkaline, oxidation, thermal, humidity, and photolytic conditions to evaluate the interference of degradation impurities. Thermal degradation was performed by keeping the tablets in a Petri dish and then placed them in an oven at 50°C for 2 days. Humidity degradation was performed by placing the tablets in a Petri dish and kept in a humidity chamber at 75% relative humidity, at 25°C for 2 days. Photolytic study was carried out by placing the tablets in a Petri dish in a photolytic chamber at 1.2 million lux hour's illumination and 200 W hrs/m² ultraviolet energy. Acid, base, and oxidation degradations were performed by adding 3 mL of 1 N HCl, 3 mL of 1 N NaOH, and 3 mL

of 30% peroxide solution (H₂O₂), respectively, to the sample solutions, and these samples are kept on bench top for 3 hrs.

LOD and LOQ

The LOD and LOQ were important for the estimation of impurities at trace levels in an analytical method. The LOD is generally quoted as the concentration yielding a signal-to-noise ratio of 2:1 or 3:1, and LOQ is quoted as the concentration yielding a signal-to-noise ratio of 10:1. The signal-to-noise ratio is determined by the following equation:

$$s = H/h$$

Where, H = height of the peak corresponding to the component.

h = Absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.

LOD and LOQ are also determined based on the standard deviation of the response and the slope. The detection limit expressed as "3.3σ/S" and quantification limit expressed as "10σ/S" where, σ is the standard deviation of the response and S is the slope of the calibration curve.

Linearity

Linearity is the ability of the method to obtain results which are either directly or after mathematical transformation proportional to the concentration of the analyte within a given range. The linearity of response for labetalol and their related impurities were determined in the range from LOQ to 120%. The five concentrations of each component were subjected to regression analysis by least-squares method to calculate correlation coefficient and calibration equation. The method of linear regression was used for the data evaluation.

Precision

Precision is a measure of the reproducibility of the whole analytical method under normal operating conditions. The precision was expressed as the relative standard deviation (RSD).

$$\% \text{ RSD} = (\text{Standard deviation/average}) \times 100$$

The precision of the developed method was carried out by 6 determinations (preparations) of the test solution by injecting the impurity spiked solution and calculated the % RSD for impurity.

Accuracy

Accuracy or trueness was determined by applying the method to samples in which known amounts of analyte have been added. These should be analyzed against the standard and blank solutions to ensure that no interference exists. The accuracy was calculated from the test results as a percentage of the analyte recovered by the assay.

The accuracy of the present method was carried out by injecting the impurities spiked solution at three different concentration levels of LOQ, 100% and 120% to their specification limit, in triplicate determinations. Percent recovery and the mean percentage recovery were calculated for known impurity.

Robustness

Robustness of the method indicates the reliability of analysis to assess the system suitability parameters under the influence of small but deliberate variations in method parameters. It was performed by injecting the impurity spiked solution by changing several parameters including different batches of the same column, flow rate, column temperature, and minor change in organic composition.

Solution stability

The impurity spiked sample solution and the standard solutions containing labetalol were prepared as per the test procedure. All these solutions were divided into two portions. One portion was stored at room temperature, and another portion was stored in the refrigerator at 2-8°C. Freshly prepared solutions and the solutions, which were

stored at room temperature and in the refrigerator (2-8°C) up to 24 hrs, were injected at different time intervals. The % impurity obtained at initial was compared with the % impurity obtained at different time intervals.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main purpose of the current chromatographic method is to develop an LC method for the separation and quantification of known and unknown degradation products of labetalol in labetalol tablets at trace level. Labetalol and its known impurity structures were shown in Fig. 1. From the structure of labetalol, it was observed that labetalol has pKa value of 9.3. In spite of the fact that in the reversed-phase separations, pH of selected buffer should have the pH ± 1.5 units from the pKa values of the analytes [20], the selection of buffer with proper pH leads to ionization of analytes which consequences the sharp and symmetric peak shapes and reproducible retention times (RTs). The pH of the mobile phase was selected at lower side as the pH increases silica dissolves slowly and results in inconsistent RTs and results. KH₂PO₄ has a wide range of pKa values; hence, initially selected a buffer of 10 mM KH₂PO₄ and set the pH of this solution to 2.00 ± 0.05 using diluted orthophosphoric acid. ACN was used in the mobile phase along with Inertsil ODS 3V, C-18, 150 × 4.6 mm, 5 μm column at a column temperature of 30°C. Flow rate was 1.0 mL/minute. ACN was selected as solvent for initial method development trials as it produces sharp, symmetrical peaks with less column back pressure. Gradient program used for chromatographic separation was shown in Table 2. Broad peak shape observed for labetalol peak, and also baseline humps are observed.

Next trials were performed on Hypersil BDS, C-18, 150 × 4.6 mm, 5 μm column at a column temperature of 30°C using the same above chromatographic conditions with the gradient program as shown in Table 2; however, peak shapes for both labetalol and 2-hydroxyimpurity are not symmetrical, and also baseline humps are observed. Further trials were performed on Hypersil BDS, C-8, 150 × 4.6 mm, 5 μm column at a column temperature of 30°C using the same above chromatographic conditions except gradient program. Gradient program used for chromatographic separation was shown in Table 3. Peak fronting is observed for 2-hydroxy impurity, and baseline peaks are observed.

Hence, mobile phase changed to 0.1% of TFA (v/v) in water and ACN was used as solvent with Zorbax Eclipse Plus C-18 (100 × 4.6 mm, 3.5 μm) column at a column temperature of 25°C. Gradient program used for chromatographic separation was shown in Table 4. Both labetalol and 2-hydroxy impurity peaks eluted early at 1.6 minutes, 2.9 minutes and the tailing factor is more than 2 for labetalol peak. To reduce the tailing factor, next trial was performed with 0.1% of TFA (v/v) in

Table 2: Gradient program

Time in minutes	Mobile phase-A	Mobile phase-B
0	85	15
3	85	15
20	30	70
20.5	85	15
25	85	15

Table 3: Gradient program

Time in minutes	Mobile phase-A	Mobile phase-B
0	90	10
3	90	10
10	50	50
15	40	60
15.5	90	10
20	90	10

water as mobile phase-A and 0.2% TFA (v/v) in ACN: MeOH as mobile phase-B with linear gradient as mentioned in Table 5 with the same HPLC column. Although labetalol tailing is reduced in this run when compared to the previous trial, still tailing factor value is more than 2. Further trial was taken with same chromatographic conditions except change in mobile phase-B to 0.1% TFA in ACN: MeOH with column temperature 35°C and the gradient program is as shown in Table 6. Tailing factor for labetalol peak reduced below 2 with RTs of 2-hydroxy impurity at 2.7 minutes and labetalol at 7.1 minutes. Final trial was taken to reduce the RTs of both labetalol and 2-hydroxy impurity peaks by increasing the % organic composition. Gradient program used is same as in Table 1. RTs of 2-hydroxy impurity, labetalol were observed at 2.5 minutes and 6.0 minutes with tailing factor values of 1.0 and 1.3.

The screening studies were performed on a variety of columns to cover a wide range of stationary phase properties including carbon chain length, carbon loading, and surface area. Each of the selected columns was screened with different mobile phase ratios, different column temperatures, and different types of organic solvents including MeOH and ACN. Zorbax Eclipse Plus C-18 (100 × 4.6 mm, 3.5 μm) column was selected for the final method due to reproducible results and better peak shapes. In most of the trials, 2-hydroxy impurity of labetalol was well separated from the labetalol peak; however, baseline humps and more tailing factor for labetalol were observed. The chromatograms of blank run, 1% standard solution (concentration -0.01 mg mL⁻¹), control sample (concentration -1.0 mg mL⁻¹), and 0.15% impurity spiked samples are shown in Figs. 2-5.

The elution order of the impurity in different chromatographic conditions was 2-hydroxy impurity followed by labetalol peak in all the trials performed with different ratios of ACN, MeOH with different buffer solutions. Elution order did not change with respect to change in chromatographic conditions and also with different brands of HPLC columns used for the trials.

Selection of wavelength for labetalol and its impurity

Spectra for known impurity and labetalol were measured from 200 to 640 nm for wavelength maxima. The corresponding spectrum of labetalol is shown in Fig. 6. Based on the spectra maxima, 230 nm was selected for identification and quantification of 2-hydroxy impurity and labetalol.

Optimization of column temperature

To study the temperature effect on resolution between the impurity peak and of labetalol peak, injected the impurity spiked solution at different column temperatures. It was observed that at a column temperature of 35°C, known degradation impurity was well separated and also better baseline observed when compared to the other column

temperatures. Although resolution between 2-hydroxy impurity peak and labetalol peak was more than 3.5 from the initial trials, better baseline was observed at a column temperature of 35°C.

METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended use. The described HPLC method has been extensively validated for its known impurity and unknown impurities as per the ICH guidelines [19]. After successful completion of method development [20-22], method validation [23-31] was performed to ensure that the developed method was capable of giving reproducible and reliable results when used by different operators employed on the same equipment of the same labor of different laboratories. Stress testing needs to be performed to elucidate the inherent stability characteristics of the active drug substance and also to prove the stability indicating the capability of the method. The developed HPLC method was validated to quantify the degradation impurities of labetalol in its tablet dosage form by determining the parameters including specificity, LOD, LOQ,

Table 4: Gradient program

Time in minutes	Mobile phase-A	Mobile phase-B
0	75	25
1	75	25
10	20	80
12	75	25
15	75	25

Table 5: Gradient program

Time in minutes	Mobile phase-A	Mobile phase-B
0	85	15
1	85	15
10	35	65
12	85	15
15	85	15

Table 6: Gradient program

Time in minutes	Mobile phase-A	Mobile phase-B
0	83	17
1	83	17
10	20	80
12	83	17
15	83	17

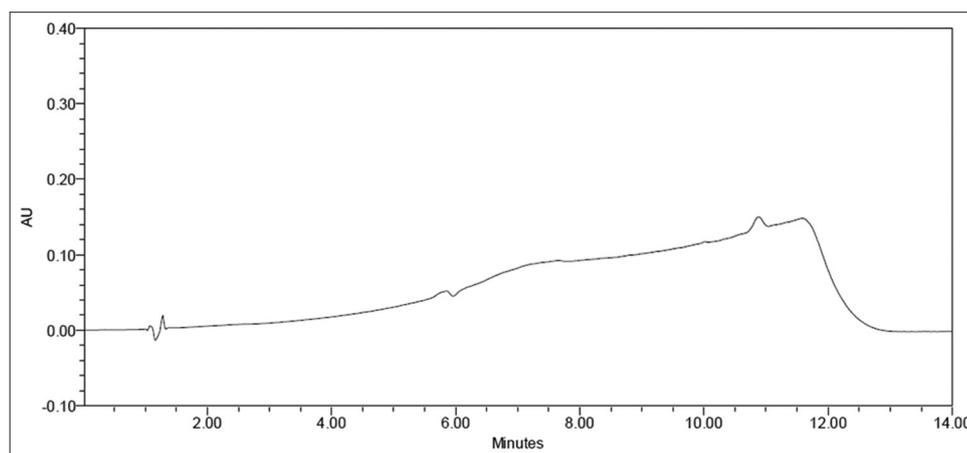


Fig. 2: Blank chromatogram

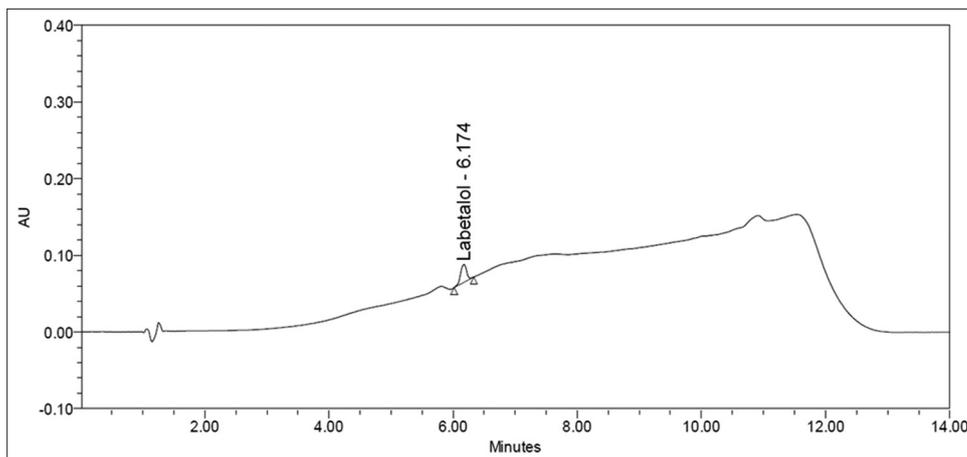


Fig. 3: Diluted standard chromatogram

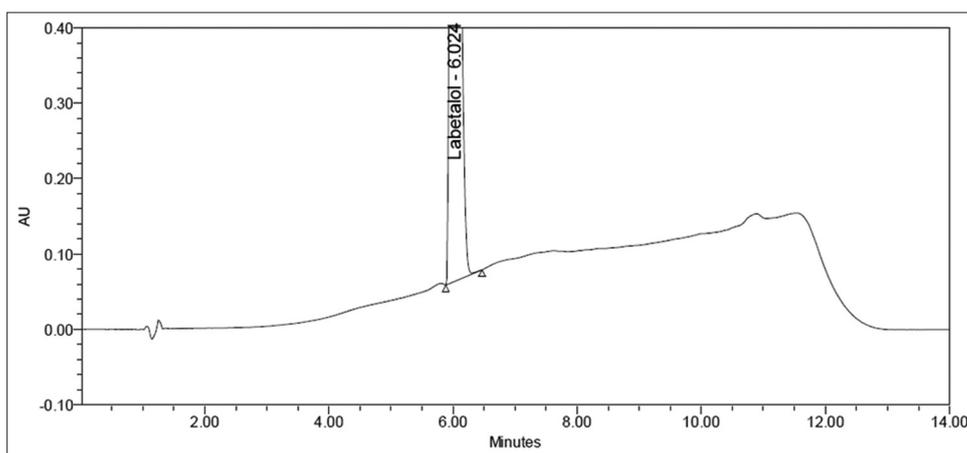


Fig. 4: Control sample chromatogram

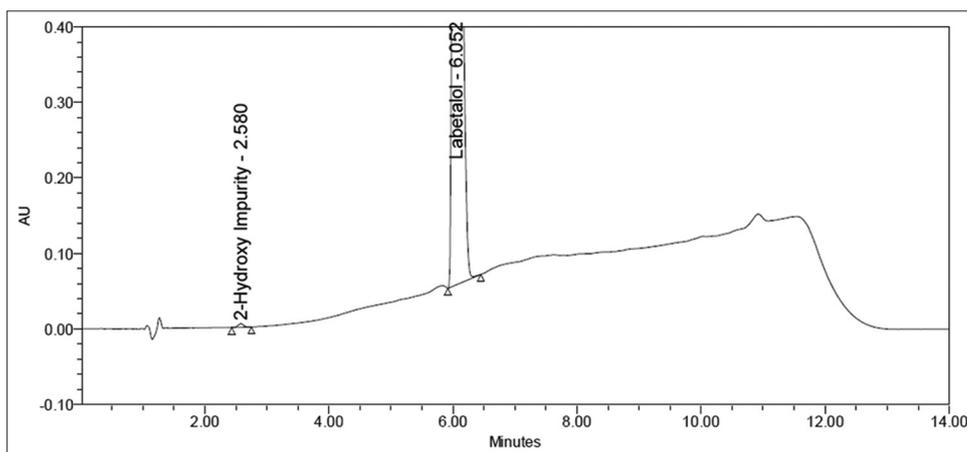


Fig. 5: Impurity spiked sample chromatogram

linearity, accuracy, precision, and robustness according to the ICH guidelines.

Specificity

Specificity of the developed method was performed by injecting the stressed degradation samples and the impurity spiked solutions. The degradation study was carried out using the samples which include tablets containing labetalol and blank solutions.

Labetalol was found to be best able in all the degradation conditions. Spectral homogeneity of labetalol and its known impurity were verified. Peak purity passed for both the main active and the known impurity. Purity angle value was less than the purity threshold for all peaks indicating all peaks are spectrally homogeneous. Furthermore, spectral homogeneity of labetalol peak in degradation samples found to be similar with those obtained for the control sample, suggests that no peak was being coeluted at the RT of labetalol peak. Degradation

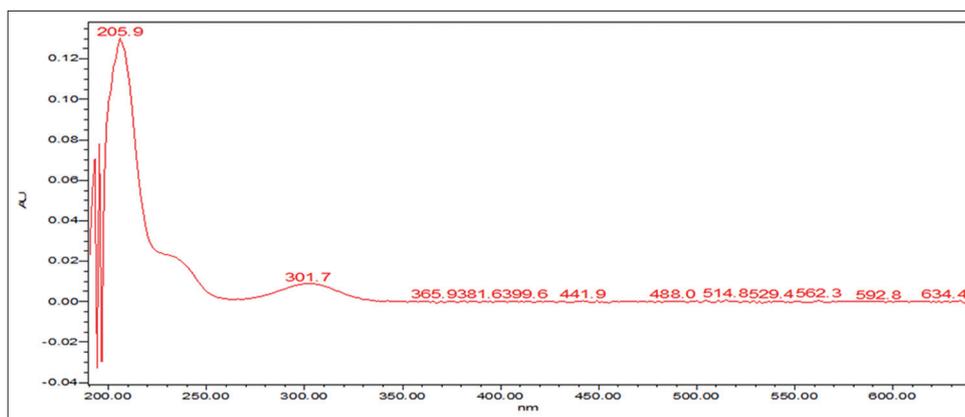


Fig. 6: Spectra of labetalol

results of labetalol in various stress conditions indicate that labetalol undergoes no degradation in the presence of respective stress conditions to for many unknown impurities.

LOD and LOQ

The LOD and LOQ were determined for labetalol and its known impurity by injecting a series of solutions with known concentrations. S/N ratio was calculated for these solutions and selected the concentration at which level S/N was about 3 for LOD, and the S/N ratio was about 10 for LOQ. S/N values of LOD and LOQ for labetalol and its known impurity were shown in Table 7.

Linearity

The linearity graphs were plotted between the peak areas versus concentration to obtain the calibration curve. Linearity graphs for labetalol and its known impurity were shown in Figs. 7 and 8. The response obtained for labetalol and its known impurity was found to be linear from LOQ to 120% of standard concentration. The correlation coefficient found for all compounds was not <0.99. The relative response factor for 2-hydroxyimpurity was determined against its respective standard and presented in Table 7 and also statistical values of all compounds were shown in Table 7. The results demonstrate that an excellent correlation between the peak area and concentration of known impurity and labetalol.

Precision

Method precision was determined by injecting the known impurity spiked solution of six determinations and the observed values of % RSD were shown in Table 7. % RSD for known compound in impurity spiked solution for six determinations was not more than 2.5%. The intermediate precision of the method was studied by injecting the impurity spiked solution of six determinations and the values were shown in Table 7. The % RSD difference between the two analysts is 1.7%. Less difference between the two analysts shows that the developed method is precise and has good intermediate precision.

Accuracy

The percentage recovery results for known impurity of labetalol were varied from 95.5% to 105.2% at three different concentration levels, and the results were shown in Table 8. Based on the % recovery data, it was concluded that the developed method is capable for the estimation of its related substances and is adequate for routine analysis.

Robustness

In all the robust conditions (flow rate, column temperature, organic composition change in mobile phase and columns), the resolution between 2-hydroxy impurity and labetalol was not <11. Relative RT and resolution values for different robustness parameters were shown in Table 9. Furthermore, elution pattern of the known impurity and labetalol peaks remained unchanged. The peak shapes for 2-hydroxy

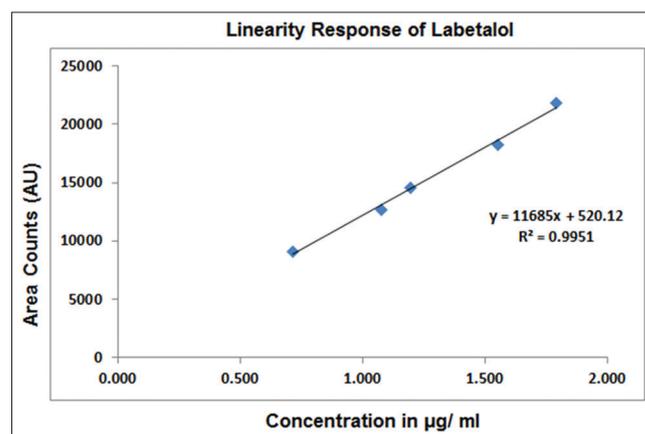


Fig. 7: Linearity graph of labetalol

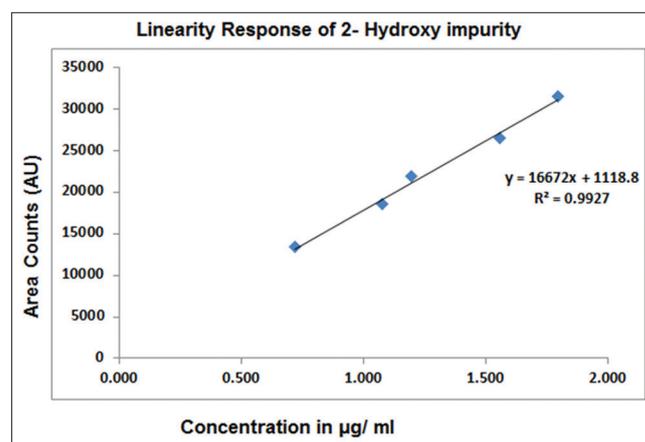


Fig. 8: Linearity graph of 2-hydroxy impurity

impurity and labetalol were found to be symmetrical. Peak purity for 2-hydroxy impurity and labetalol peaks were also tested to observe no interference in all the robust conditions.

Solution stability

The impurity percent difference was determined for solutions stored at room temperature and at refrigerated condition (2-8°C) in different time intervals up to 24 hrs. 2-hydroxy impurity and labetalol peaks were found to be stable up to 24 hrs at room temperature and also at refrigerator condition. Solution stability results at room temperature and refrigerated condition were shown in Table 10.

Table 7: LOD, LOQ, linearity and precision data

Parameter	Labetalol	2-hydroxy impurity
LOD ($\mu\text{g mL}^{-1}$)	0.3583	0.3593
S/N ratio	12	16
LOQ ($\mu\text{g mL}^{-1}$)	0.7166	0.7187
S/N ratio	23	27
Relative response factor	-	0.701
Method precision (% RSD) ^a	-	2.5
Intermediate precision (% RSD) ^b	-	4.2
Regression statistics		
Slope	11,685.1	16,671.9
Intercept	520.1	1118.8
Coefficient of determination (R^2)	0.9951	0.9927
Intercept at 95% confidence interval (lower value-upper value)	1478.5-2518.7	2356.3-4593.8
Slope at 95% confidence interval (lower value-upper value)	10,171.2-13,198.9	14,047.7-19,296.2

^aMethod precision calculated from six preparations of impurity spiked solutions, ^bIntermediate precision calculated from six preparations of impurity spiked solutions. LOQ: Limit of quantitation, LOD: Limit of detection, RSD: Relative standard deviation

Table 8: Accuracy results

Percentage impurity level	Percentage recovery range for triplicate preparations
	Percentage 2-hydroxy impurity
LOQ	98.8-105.2
100	98.4-102.3
120	95.5-102.3

LOQ: Limit of quantitation

Table 9: Robustness results

Robustness parameters	2-hydroxy impurity	
	RRT	Resolution
Control sample	0.43	16.5
Using other batch column	0.43	15.0
Flow rate (1.1 mL/min)	0.41	12.8
Flow rate (0.9 mL/min)	0.45	12.7
Low column temperature 30°C	0.43	19.2
High column temperature 40°C	0.41	15.7
Minor component change lower acetonitrile concentration at 10 min to 75%	0.41	12.7
Major component change higher acetonitrile concentration at 10 min to 85%	0.44	11.8

RRT: Relative retention time

CONCLUSIONS

A novel RP-HPLC method was developed for the separation and quantification of labetalol and its related impurities in its pharmaceutical dosage forms. Degradation behavior of labetalol was studied under various degradation conditions. No degradation peaks were observed in the respective stress conditions. 2-hydroxy impurity is well separated from the labetalol peak, and no unknown degradation impurities were observed which shows the stability indicating capability of the method. The developed method can be used for the quantification of related substances of labetalol in routine analysis.

Table 10: Solution stability results of standard and control sample at room temperature and at refrigerated condition

Solution stability	Time interval		
	Initial	After 24 hrs	Percentage difference
At room temperature			
Percentage assay of standard solution	100.6	99.5	1.1
Percentage assay of sample solution	100.2	99.3	0.9
Percentage 2-hydroxy impurity	0.154	0.148	0.01
At refrigerated condition			
Percentage assay of standard solution	100.6	100.1	0.5
Percentage assay of sample solution	100.2	99.5	0.7
Percentage 2-hydroxy impurity	0.154	0.142	0.01

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REFERENCES

- Available from: <https://www.en.wikipedia.org/wiki/Labetalol>.
- Fahed S, Grum DF, Papadimos TJ. Labetalol infusion for refractory hypertension causing severe hypotension and bradycardia: An issue of patient safety. Patient Saf Surg 2008;2:13.
- Louis WJ, McNeill JJ, Drummer OH. Labetalol and other vasodilator/Beta-blocking drugs. In: Handbook of Hypertension. Amsterdam, Netherlands: Elsevier Sciences Publishing Co.; 1988. p. 246-73.
- Riva E, Mennini T, Latini R. The alpha- and beta-adrenoceptor blocking activities of labetalol and its RR-SR (50:50) stereoisomers. Br J Pharmacol 1991;104(4):823-8.
- Robertson D, Biaggioni I. Adrenoceptor antagonist drugs. In: Katzung BG, Masters SB, Trevor AJ, editors. Basic & Clinical Pharmacology. 12th ed. San Francisco: McGraw-Hill Lange Medical; 2012. p. 151-68.
- Koda Kimble MA, Alldredge BK. Koda Kimble and Young's Applied Therapeutic: The Clinical Use of Drugs. Philadelphia, PA: Lippincott Williams & Wilkins; 2013.
- MacCarthy EP, Bloomfield SS. Labetalol: A review of its pharmacology, pharmacokinetics, clinical uses and adverse effects. Pharmacotherapy 1983;3(4):193-219.
- Louis WJ, McNeill JJ, Drummer OH. Pharmacology of combined alpha-beta-blockade. I. Drugs 1984;28 Suppl 2:16-34.
- Bertram KG. Basic and Clinical Pharmacology. New York: McGraw-Hill Medical; 2006. p. 170.
- Richards DA, Tuckman J, Prichard BN. Assessment of alpha- and beta-adrenoceptor blocking actions of labetalol. Br J Clin Pharmacol 1976;3(5):849-55.
- Xu QA, Timothy LM. Analytical Methods for Therapeutic Drug Monitoring and Toxicology. Hoboken, NJ: John Wiley and Sons; 2011.
- Available from: <https://www.pubchem.ncbi.nlm.nih.gov/compound/labetalol#section=Caco2-Permeability>.
- The United States Pharmacopoeia. USP-37, NF-32. Rockville, MD, USA: United States Pharmacopoeial Convention; 2014.
- Jinqi Z, Lina FU, Chao L, Guogang Z. Determination of content and related substances of labetalol hydrochloride by HPLC. Chin J Modern Appl Pharm 2013;1.
- Meredith PA, McSharry D, Elliott HL, Reid JL. The determination of labetalol in plasma by highperformance liquid chromatography using fluorescence detection. J Pharmacol Methods 1981;6(4):309-14.
- Vaishali CK, Bhavika RC, Bavaskar SR, Barhate SD. Stability indicating RP-HPLC method for determination of labetalol hydrochloride in pharmaceutical formulation. World J Pharm Res 2015;4(4):1149-61.
- Ganesan M, Nanjundan S, Rauthan KS, Eswaran K, Tripathi P.

- Rapid analysis of labetalol in human plasma using liquid chromatography- tandem mass chromatography. *Int J Pharm Sci Res* 2010;1(12):209-18.
18. European Pharmacopoeia 8.0. Labetalol Hydrochloride Monograph in European Pharmacopoeia. Reference No. 04/2013:0923; 8.0:2577-78.
 19. ICH. Q2 (R1), Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology. In: Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2005.
 20. Snyder LR, Kirkland JJ, Glajch JL. Handbook of Practical HPLC Method Development. 2nd ed. Hoboken, NJ, USA: Wiley-Blackwell; 1997.
 21. Basak AK, Raw AS, Al Hakim AH, Furness S, Samaan NI, Gill DS, et al. Pharmaceutical impurities: Regulatory perspective for abbreviated new drug applications. *Adv Drug Deliv Rev* 2007;59(1):64-72.
 22. Bakshi M, Singh S. Development of validated stability-indicating assay methods – critical review. *J Pharm Biomed Anal* 2002;28(6):1011-40.
 23. United States Food and Drug Administration. Guidance for Industry: Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation. Draft Guidance. Rockville, MD: USFDA; 2001.
 24. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A* 2003;987(1-2):57-66.
 25. Center for Drug Evaluation and Research (CDER). Reviewer Guidance: Validation of Chromatographic Methods, CMC 3. USFDA; 1994.
 26. Guidance for Industry, Analytical Procedures and Methods Validation for Drugs and Biologics. Draft Guidance, U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), CMC; 2014.
 27. Ravichandran V, Shalini S, Sundramand KM, Rajak H. Validation of analytical methods-strategies & importance. *Int J Pharm Pharm Sci* 2010;2(3):18-22.
 28. Pranshu T, Singh RP, Vikash J. Validation: A critical parameter for quality control of pharmaceuticals. *J Drug Deliv Ther* 2012;2(3):34-40.
 29. Validation of Compendial Methods. United States Pharmacopeia 37, National Formulary 32. Ch. 1225. Rockville, MD, USA: The United States Pharmacopeial Convention; 2014.
 30. Ashok CV, Sailaja BB, Praveen KA. Development and validation of a dissolution method for Frovatriptan tablets by reverse phase UPLC. *Int J Pharm Pharm Sci* 2015;7(4):125-30.
 31. Venkata RG, Pavani G, Supriya P, Madhavalatha N. Reverse phase high performance liquid chromatography method development and validation of atorvastatin in bulk drug and formulation. *Asian J Pharm Clin Res* 2015;8(6):84-7.