Stability-Indicating TLC-Densitometric Determination of Nebivolol Hydrochloride in Bulk and Pharmaceutical Dosage Form

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Abstract

A simple, selective, precise, and stability-indicating high-performance thin-layer chromatographic (HPTLC) method for densitometric determination of nebivolol hydrochloride both as a bulk drug and in formulation was developed and validated as per the international conference on harmonization guidelines (ICH). The method employed TLC aluminium plates precoated with silica gel 60F254 as the stationary phase. The solvent system consisted of toluene–methanol–triethylamine (3.8:1.2:0.2 v/v/v). Densitometry analysis of nebivolol hydrochloride was carried out in the absorbance mode at 281 nm. The system was found to give compact spot for nebivolol hydrochloride (R_f value of 0.33 ± 0.02). The linear regression analysis data for the calibration plots showed good relationship with r^2= 0.9994 ± 0.0002 in the concentration range 500–3000 ng/spot. The mean value ± SD of slope and intercept were 3.761 ± 0.017 and 127.39 ± 19.53 with respect to peak area. The limits of detection (LOD) and limit of quantitation (LOQ) were 63.10 ng/spot and 191.23 ng/ spot, respectively. Nebivolol hydrochloride was subjected to acid and alkali hydrolysis, oxidation, thermal degradation, and photodegradation. All the peaks of degradation products were well-resolved from the standard drug with significantly different R_f values. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of said drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of nebivolol hydrochloride in the bulk and pharmaceutical dosage form.

Introduction

Nebivolol hydrochloride, α,α'[iminobis (methylene)] bis [6-fluro-3, 4- dihydro-2H -1-benzopyran-2-methanol] hydrochloride is an antihypertensive (1,2). Nebivolol hydrochloride occurs in two isomeric forms (3). (+) Nebivolol acts as strong adrenergic β_1 blocker whereas (–) nebivolol as vasodilator (4,5).

In literature, various methods have been reported for the estimation of nebivolol hydrochloride from pharmaceutical dosage form and biological fluids. The first order UV-spectrophotometry (6), reverse-phase high-performance liquid chromatography (HPLC)–UV detection (7), enantiomeric resolution on normal and reversed amylase based-chiral phases using HPLC (8), enantioseparation using chiral stationary phase (9), high-speed determination in human urine by LC–tandem mass spectrometry (10), rapid quantification in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectroscopy (11), are some of those. High-performance thin-layer chromatography (HPTLC) densitometric quantitative analysis from tablet formulation (12, 13) has been reported. All of these methods have their advantages and limitations. However, to our knowledge, no article related to the stability-indicating HPTLC determination of nebivolol hydrochloride has been described in literature.

The aim of this work is to develop an accurate, specific, repeatable, and stability-indicating method for the determination of nebivolol hydrochloride in the presence of its degradation products as per ICH guidelines.

Experimental

Nebivolol hydrochloride was supplied as a gift sample from Cadila Pharmaceuticals, (Ahmedabad, India). All chemicals and reagents used were of analytical-grade and purchased from Qualigens Fine Chemicals (Mumbai, India).

HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with Camag microliter syringe on precoated silica gel aluminium Plate 60F254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Darmstadt, Germany) using Camag Linomat V (Muttenz, Switzerland). A constant application rate of 150 nL/s was employed, and the space between two bands was 15 mm. The slit dimension was kept 6 mm × 0.45 mm. The mobile phase consisted of toluene–methanol–triethylamine (3.8:1.2:0.2, v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 25 min at room temperature. The length of chromatogram run was approximately 80 mm. Subsequent to the development, TLC plates were dried in current of air with the help of an air dryer. Densitometric

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scanning was performed using Camag TLC scanner III in the absorbance mode at 281 nm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190– 400 nm.

Calibration curve of nebivolol hydrochloride

A stock solution of nebivolol hydrochloride (1000 µg/mL) was prepared in methanol. Different volume of stock solution 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 µL were spotted in six replicates on TLC plates to obtain concentration of 500, 1000, 1500, 2000, 2500, and 3000 ng/spot of nebivolol hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

Method validation (14–18)

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (2000 ng/spot of nebivolol hydrochloride). The intra-day and inter-day variation for the determination of nebivolol hydrochloride was carried out at three different concentration levels of 1000, 1500, and 2000 ng/spot.

Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phase having different composition like toluene–methanol–trimethylamine (3.6:1.4:0.2, v/v/v), (4.0:1.0:0.2, v/v/v) were tried, and chromatograms were run. The amount of mobile phase was varied as 5.02 and 10.02 mL. Development distance was varied as 7, 7.5, and 8 cm. Plate was developed at relative humidity 55% and 65%. The duration of saturation was also varied from 20, 25, and 30 min. The effects on results were examined.

Limit of detection and limit of quantification

In order to the determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. Stock solution of nebivolol hydrochloride (1000 µg/mL) was prepared and different volume of stock solution 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 µL were spotted in triplicate. The amount of nebivolol hydrochloride by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (SD) of responses were calculated. The average of standard deviations was calculated (ASD). Detection limit was calculated by (3.3 × ASD)/b and quantification limit was calculated by (10 × ASD)/b, where b corresponds to the slope obtained in the linearity study of method.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for nebivolol hydrochloride in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of nebivolol hydrochloride was accessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions of the spot.

Table I. Linear Regression Data for the Calibration Curve

Linearity range (ng /spot)	500–3000
Coefficient of Correlation r^2 ± SD	0.9994 ± 0.0002
Slope ± SD	3.761 ± 0.0172
Intercept ± SD	127.39 ± 19.534

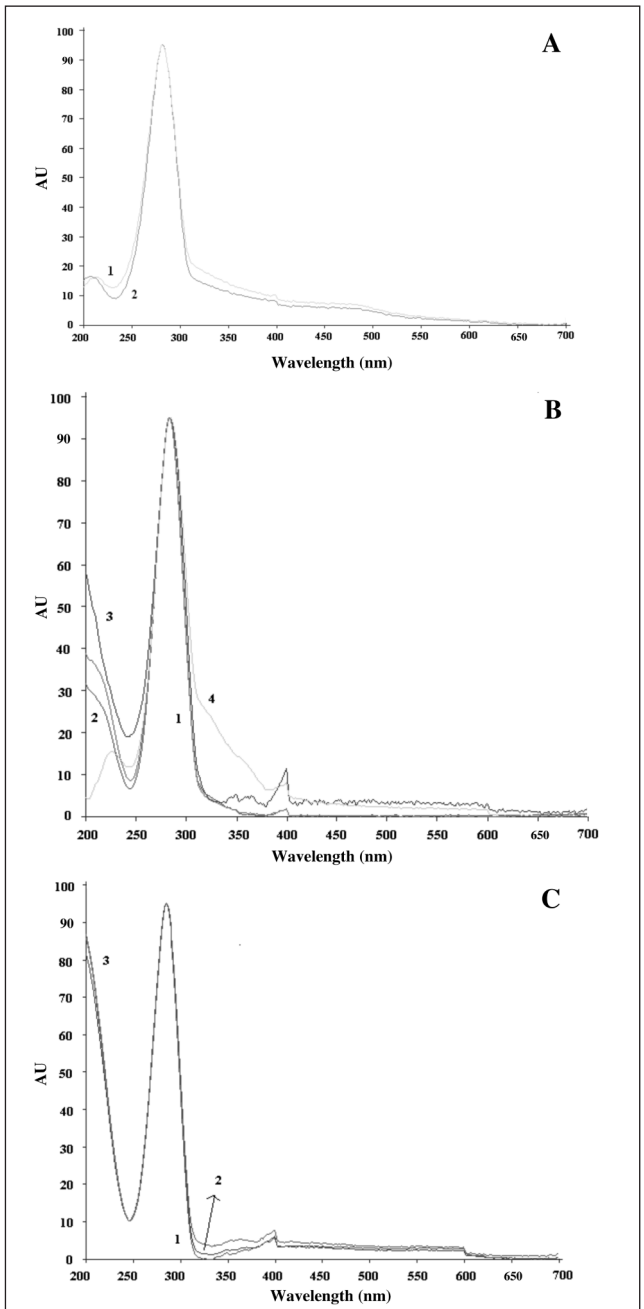

Figure 1. (A) Peak purity spectra of standard nebivolol hydrochloride, sample 2 extracted from Nebicard tablet, scanned at the peak-start-peak-apex r(s, m) and peak-apex- peak end r (m, e) positions of the spot (correlation > 0.99).

(B) Peak purity spectra of standard nebivolol hydrochloride (line 1) [r (s, m) = 0.99, r (m, e) = 0.99]; Acid degraded (line 2) [r (s, m) = 0.99, r (m, e) = 0.88]; Base degraded (line 3), [r (s, m) = 0.98, r(m, e) = 0.96]; and hydrogen peroxide degraded (line 4) [r (s, m) = 0.97,r (m, e) = 0.85] (B).

(C) Peak purity spectra of standard nebivolol hydrochloride (line 1), [r (s, m) = 0.99, r(m, e) = 0.99]; Dry heat degraded (line 2), [r (s, m) = 0.99, r (m, e) = 0.99] and Photo degraded (line 3) [r (s, m) = 0.99, r (m, e) = 0.99].

Table II. Intra-Day and Inter-Day Precision*

Amount ng /spot	Amount found	SD	%RSD
Intra-day precision			
1000	993.82	8.47	0.85
1500	1507.24	7.10	0.47
2000	1999.14	10.18	0.50
Inter-day precision			
1000	999.15	3.40	0.34
1500	1498.03	13.08	0.87
2000	1992.62	6.86	0.34

* Mean of three estimations at each level.

Table III. Recovery Studies*

Initial amount (ng)	Excess drug added to analyte (%)	Amount recovered (ng)	Recovery (%)	%RSD
1000	0	997.33	99.73	1.80
1000	80	798.11	99.76	1.59
1000	100	994.12	99.41	1.62
1000	120	1196.94	99.74	0.87

* Mean of three determinations at each level.

Recovery studies

The pre-analyzed sample was over spotted with extra 80, 100, and 120% of the standard nebivolol hydrochloride, and it was analyzed by the proposed method. At each level of the amount, three determinations were performed. This was done to check the recovery of drug at different levels in the formulation.

Analysis of nebivolol hydrochloride in prepared formulation

To determine the concentration of nebivolol hydrochloride in tablets (label claim: 5 mg per tablet), twenty Nebicard tablets were weighed, mean weight determined, and ground to a fine powder. The powder equivalent to 10 mg of Nebivolol was weighed. The drug from the powder was extracted by methanol. To ensure the complete extraction of the drug, it was sonicated for 20 min, and the volume was made up to the 50 mL. The 5 µL of the previously mentioned solution (1000 ng/spot) was applied on TLC plate followed by development and scanning as described previously. The analysis was repeated in six times. The possibility of excipients interferences in the analysis was studied.

Forced degradation of nebivolol hydrochloride (19–20)

In all degradation studies the average peak area of nebivolol hydrochloride after application (1000 ng/spot) of seven replicates was obtained. The plate was developed and scanned under the previously established chromatographic conditions. Peak area was recorded for each concentration of degraded drug.

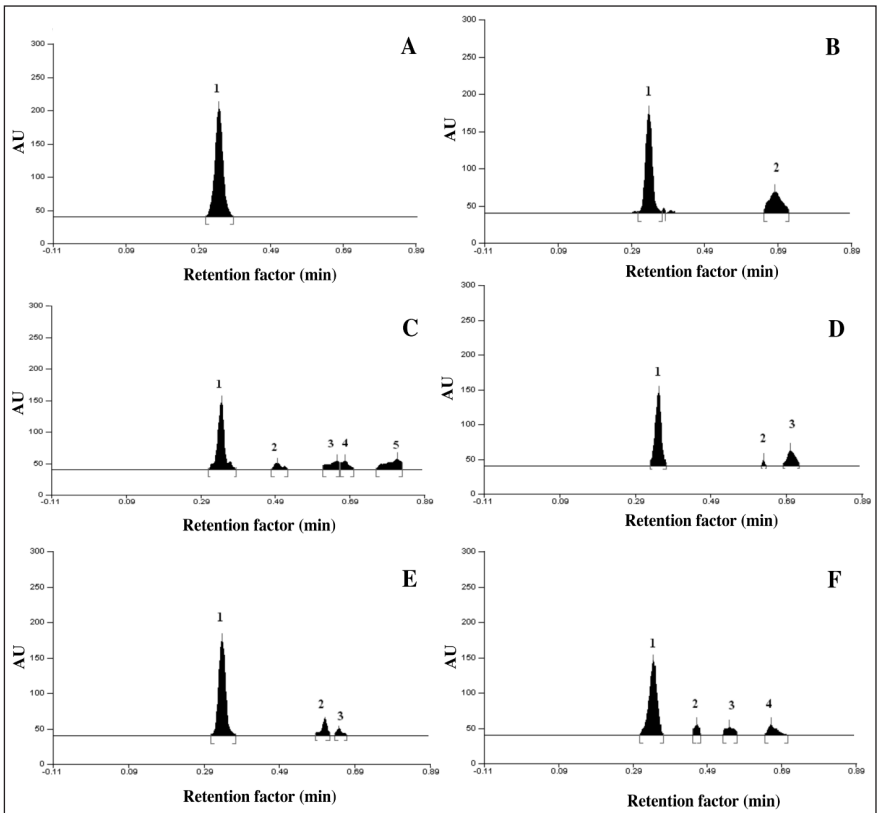

Figure 4. A typical HPTLC chromatogram of Nebivolol hydrochloride from pharmaceutical dosage form (R_f = 0.33) (A). HPTLC chromatogram of acid treated Nebivolol hydrochloride (B). HPTLC chromatogram of base treated Nebivolol hydrochloride (C). HPTLC chromatogram of dry heat degraded Nebivolol hydrochloride (D). HPTLC chromatogram of hydrogen peroxide (3% v/v) degraded Nebivolol hydrochloride (E). HPTLC chromatogram of Photo degraded Nebivolol hydrochloride (F).

Acid- and base-induced degradation

The 10 mg of nebivolol hydrochloride was separately dissolved in 10 ml methanolic solution of 0.1 M HCl and 0.1 M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. 1 mL of the described solution was taken, neutralized, and diluted up to 10 mL with methanol. The resultant solution were applied on TLC plates, (10 µL each, of 1000 ng/spot). The chromatograms were run as described earlier.

Hydrogen peroxide-induced degradation

The 10 mg of nebivolol hydrochloride was separately dissolved in 10 mL of methanolic solution of hydrogen peroxide (3.0%, v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. 1 mL of described solution was taken and diluted up to 10 mL with methanol. The resultant solution was applied on TLC plate, (10 µL each, of 1000 ng/spot). The chromatograms were run as described in section 2.1.

Dry heat degradation products

Nebivolol hydrochloride 10 mg was stored at 55°C for 3 h in oven. It was transferred to 10-mL volumetric flask containing methanol and volume was made up to the mark. 1 µL

(1000 ng/spot) was applied on TLC plate and chromatogram were run as described earlier.

Light heat degradation products

The 10 mg of nebivolol hydrochloride was dissolved in 10 mL of methanol. The solution was kept in the sun light for 8 h. 1 mL of the described solution was taken and diluted up to 10 mL with methanol. The resultant solution was applied on TLC plate (10 µL each of 1000 ng/spot). The chromatograms were run as described earlier.

Nebivolol hydrochloride in acid, base, hydrogen peroxide, photo, and dry heat degraded sample solutions and in standard drug solutions were spectral scanned to access the peak purity.

Results and Discussion

Development of optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay of method. Initially, mobile phase consisted of toluene–methanol (4:2, v/v) gave good resolution with R_f value of 0.33 for nebivolol hydrochloride, but a typical peak nature was missing. Finally, the mobile phase consisting of toluene–methanol–triethylamine (3.8:1.2:0.2 v/v/v) gave a sharp and well-defined peak at R_f value of 0.33. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 25 min at room temperature.

Table IV. Analysis of Prepared Formulation

Component	Labeled claim (mg)	Amount Found ± SD*	% Found ± SD
Nebivolol	5	5.00 ± 0.06	100.08 ± 1.36

* mean of six determinations

Table V. Summary of Validation Parameters

Parameters	Observation
Linearity range (ng/spot)	500 – 3000
Correlation coefficient	0.9994 ± 0.0002
Limit of detection (ng/spot)	63.10
Limit of quantitation (ng/spot)	191.23
Recovery (% RSD)	0.87–1.80
Precision (% RSD)	
Repeatability (*n* = 7)	1.26
Intra- day (*n* = 3)	0.47–0.85
Inter-day (*n* = 3)	0.34–0.87
Ruggedness (% RSD)	
Analyst –I (*n* = 6)	1.74
Analyst –I (*n* = 6)	1.89
Robustness	Robust
Specificity	Specific

Calibration curve

The linear regression data for the calibration curve (n = 6) as shown in Table I showed a good linear relationship over the concentration range 500–3000 ng/spot with respect to peak area.

No significant difference was observed in the slopes of standard curves.

Validation of the method

Precision

The repeatability of sample application and measurement of peak area were expressed in the terms of % RSD, and the results are depicted in Table II, which revealed intra-day and inter-day variation of nebivolol hydrochloride at three different concentration levels 1000, 1500, and 2000 ng/spot.

Robustness of the method

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low values of % RSD indicated robustness of the method.

LOD and LOQ

Detection limit and quantification limit was calculated by the method as described earlier and were found to be 63.10 and 191.23 ng, respectively. This indicates adequate sensitivity of the method.

Specificity

The peak purity of the nebivolol hydrochloride was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot as shown in Figure 1.

Recovery studies

The proposed method when used for extraction and subsequent estimation of nebivolol hydrochloride from pharmaceutical dosage form after over spotting with 80, 100, and 120% of additional drug afforded recovery of 98–102 % as listed in Table III.

Analysis of prepared formulation

A single spot of R_f 0.33 ± 0.02 was observed in chromatogram of the nebivolol hydrochloride samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. The nebivolol content was found to be as per the labeled claim. The results are shown in Table IV. It may therefore be inferred that degradation of nebivolol hydrochloride had not occurred in the formulation that were analyzed by this

Table VI. Forced Degradation of Nebivolol Hydrochloride

Sample exposure condition	No degradation product (R_f value)	Recovery (%)
0.1M HCl,8h,RT*	1 (0.68)	82.88
0.1M NaOH,8H,RT*	4 (0.49, 0.67, 0.69, 0.75)	76.07
3 % H2O2, 8h,RT*	2 (0.65, 0.68)	96.42
Dry heat, 3 h, 55°C	2 (0.63, 0.70)	91.90
Light heat,8 h	3 (0.47, 0.54, 0.68)	86.69

* RT = Room temperature.

method. The low % RSD value indicated the suitability of this method for routine analysis of nebivolol hydrochloride in pharmaceutical dosage form. The typical chromatogram of nebivolol is as shown in Figure 2.

The summery of validation parameters are listed in Table V.

Stability-indicating property

Acid and base induced degradation product

The chromatogram of the acid degraded sample for nebivolol hydrochloride showed one peak at R_f value 0.68 (Figure 2). The chromatogram of the base degraded sample showed four peaks at R_f value of 0.49, 0.67, 0.69, and 0.75, respectively (Figure 2). The areas of degraded peaks were found to be lesser than the area of standard drug concentration (1000 ng/spot), indicating that nebivolol hydrochloride undergoes degradation under acidic and basic conditions.

Dry heat degradation product

The samples degraded under dry heat conditions (Figure 2) showed additional peaks at R_f values of 0.63 and 0.70. The spots of degraded products were well resolved from the drug spot.

Hydrogen peroxide induced degradation product

The sample degraded with hydrogen peroxide (Figure 2) showed additional peaks at R_f values of 0.65 and 0.68. The spots of degraded products were well-resolved from the drug spot.

Photochemical degradation product

The photo degraded sample showed three additional peaks at R_f values of 0.47, 0.54, and 0.68 when drug solution was left in day light for 8 h (Figure 2). From the stability-indicating studies, it proves that the drug is susceptible to acid-base hydrolysis, oxidation, dry heat degradation, and photo degradation. The results are listed in Table VI.

Conclusion

The developed HPTLC technique is precise, specific, accurate, and stability-indicating. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of nebivolol hydrochloride as bulk drug and in pharmaceutical dosage form. The method can be used to determine purity of drug available from the various sources by detecting various impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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