# RP-HPLC and HPTLC Methods for the Estimation of Nebivolol hydrochloride in Tablet Dosage form

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Two simple, specific, accurate and precise methods, namely, reverse phase high performance liquid chromatography and high performance thin layer chromatography were developed for estimation of nebivolol hydrochloride in tablet dosage form. For the HPLC method, Lichrospher 100 C-18, 5 µm column consisting of 200×4.6 mm i.d. in isocratic mode, with mobile phase containing 50 mM $KH_2PO_4$ buffer (pH 3.0±0.1):acetonitrile: (45:55 v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 282 nm. The retention time was found to be 3.76±0.02 min. For the high performance thin layer chromatographic method a Camag system comprising of Linnomat V automatic sample applicator, Hamilton syringe, Camag TLC Scanner-3, Camag Win CAT software with stationary phase precoated silica gel $60F_{254}$ and mobile phase consisting of ethyl acetate:toluene:methanol: ammonium hydroxide (1:6:2:0.1 v/v/v/v) were used. The detection of spot was carried out at 282 nm. The $R_f$ value was found to be 0.33±0.02. The methods were validated in terms of linearity, accuracy and precision. The linearity curves were found to be linear over 10-150 µg/ml for high performance thin layer chromatography and 100-600 ng/spot for high performance thin layer chromatography. The limit of detection and limit of quantification for high performance thin layer chromatography were found to be 2.0 and 10 µg/ml, respectively, and for the high performance thin layer chromatography, 30 and 100 ng/spot, respectively. The proposed methods were successfully used for estimation of nebivolol hydrochloride in tablet dosage form.

Key words: Nebivolol, HPLC, HPTLC, validation, analysis

Nebivolol is chemically, α,α[1]-[imino bis (methylene)]bis[6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol][1], which is a selective $\beta_1$-receptor antagonist without partial agonist activity[2]. Liquid chromatography-mass spectroscopic (LC-MS) methods[3-5] for analysis of nebivolol in biological fluids, are reported in the literature. The present investigation describes two precise, accurate and specific, reverse phase high performance liquid chromatography (RP-HPLC) and high performance thin layer chromatography (HPTLC) methods for the estimation of nebivolol hydrochloride in tablet formulation.

All the reagent used were of HPLC and analytical

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grades. Reference standard of nebivolol hydrochloride (NBH) was obtained from Cadila Pharmaceuticals Limited, Ahmedabad. Tablets of two different brands, Nodon tablet (5.0 mg) of Cadila Pharmaceuticals Ltd. Ahmedabad (Brand I) and Nebicard tablet (2.5 mg) of Torrent Pharmaceuticals Ltd. Ahmedabad (Brand II) were purchased from local pharmacy. A standard stock solution of NBH (1 mg/ml) was prepared by dissolving 25 mg of the drug in 25 ml of methanol. For HPLC method, working standard solution (500 µg/ml) was obtained from stock solution by dilution with mobile phase and for HPTLC method working standard solution (100 µg/ml) was obtained from stock solution by dilution with methanol.

HPLC including a Hitachi pump L-7110 equipped with universal injector 77251 (Rheodyne) with injection volume 20 µl, Hitachi L- 7420 UV/Vis detector, Merck-Hitachi HSM software, Lichrospher 100 C-18, 5 µm column having 200 mm length and 4.6 mm i.d. was used. Mobile phase was prepared by mixing 50 mM $KH_2PO_4$ (pH was adjusted to 3.0±0.1 with 10% v/v o-phosphoric acid) and acetonitrile in proportion of 45:55 v/v, respectively. The mobile phase was filtered through 0.45 µm cellulose nitrate filter paper and degassed by ultrasonication for 15 min.

Linearity of the method was investigated by serially diluting the stock solution to give a concentration range of 10 to 150 µg/ml and injected 20 µl with universal injector 77251(Rheodyne). The flow rate was maintained at 1.0 ml/min. Temperature of the column was kept ambient and the effluent was monitored at 282 nm. Calibration curve was constructed by plotting concentration against peak area.

A Camag HPTLC system comprising of Linnomat V automatic sample applicator, Hamilton syringe, Camag TLC scanner-3, Camag Win CAT software, Camag twin trough chamber and as stationary phase, precoated silica gel $60F_{254}$ were used. TLC plates were prewashed with methanol. Activation of plates was done in an oven at $50^0$ for 5 min. The chromatographic conditions maintained were precoated silica gel $60F_{254}$ aluminum sheets as stationary phase, ethyl acetate: toluene: methanol: ammonium hydroxide (1:6:2:0.1 v/v/v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance allowed was 80 mm, scanning was done at 282 nm keeping the slit dimension at 6 x 0.45 mm. A deuterium lamp provided the source of radiation.

Aliquots (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 µl) of standard solution (100 µg/ml) of NBH were applied on the precoated silica gel $60F_{254}$ TLC plate. The TLC plate was dried, developed and analyzed photometrically as described earlier. Calibration curve was constructed by plotting peak area against concentration.

Assay of two different marketed tablets with brand names Nodon (5.0 mg, Brand I) and Nebicard (2.5 mg, Brand II) were performed. Twenty tablets of the above brands were separately weighed and powdered. The powder equivalent to 25 mg of NBH was dissolved in methanol to obtain 1 mg/ml, and was ultrasonicated and filtered through 0.45 micron membrane filter. The solution was further diluted with the mobile phase for HPLC and with methanol for HPTLC, and subjected for HPLC and HPTLC analysis as described earlier. From the peak area of NBH, the amount of drug in sample was computed.

To optimize the HPLC parameters, several mobile phase compositions were tried. Satisfactory peak symmetry was obtained with mobile phase consisting of 50 mM $KH_2PO_4$ (pH was adjusted to 3.0 ± 0.1 with 10% v/v o-phosphoric acid):acetonitrile (45:55 v/v). Quantification was achieved with UV detection

**TABLE: 1 VALIDATION AND SYSTEM SUITABILITY PARAMETERS**

| Parameter | RP-HPLC | HPTLC |
|---|---|---|
| Retention time (min) | 3.76 ± 0.02 | - |
| Rf value | - | 0.33±0.02 |
| Linearity range | 10-150 µg/ml | 100-600 ng/spot |
| Correlation coefficient (r2) | 0.9997 | 0.9950 |
| Regression equation (y=mx+c) | | |
| Slope (m) | 11371 | 4.4182 |
| Intercept (c) | 21143 | 203.15 |
| Tailing factor | 1.34 | - |
| Theoretical plates | 1360 | - |
| % RSD (n = 5) | 0.002 | 1.860, 1.649 |
| Limit of detection (LOD) | 2 µg/ml | 30 ng/spot |
| Limit of quantification (LOQ) | 10 µg/ml | 100 ng/spot |

y = peak area, x = Concentration in µg/ml

**TABLE 2: INTRA-DAY AND INTER-DAY PRECISION STUDY**

| Concentration | | Intra - day (%RSD) | | Inter - day (%RSD) | |
|---|---|---|---|---|---|
| HPLC µg/ml | HPTLC ng/spot | HPLC | HPTLC | HPLC | HPTLC |
| 20 | 150 | 1.253 | 0.841 | 1.634 | 1.343 |
| 40 | 250 | 1.025 | 0.805 | 1.138 | 2.095 |
| 60 | 350 | 0.128 | 1.656 | 0.696 | 1.717 |
| 80 | 450 | 0.189 | 0.828 | 1.385 | 1.006 |
| 100 | 650 | 0.780 | 0.824 | 1.212 | 1.059 |

RSD = Relative standard deviation (n=6)

**TABLE 3: ASSAY RESULTS AND % RECOVERIES FOR NEBIVOLOL HYDROCHLORIDE TABLETS**

| Formulation | Label Claim mg | RP-HPLC method | | | HPTLC method | | |
|---|---|---|---|---|---|---|---|
| | | Amount found* mg±SD | % Assay | % Recovery* ±SD | Amount found* mg±SD | % Assay | % Recovery* ±SD |
| Brand I | 5.0 | 5.087±0.020 | 101.75±0.40 | 102.08±0.76 | 5.25±0.009 | 101.04±1.14 | 99.72±1.04 |
| Brand II | 2.5 | 2.549±0.002 | 101.95±0.08 | 100.48±0.19 | 2.54±0.048 | 101.52±1.93 | 101.18±0.59 |

*Average of three determinations, SD = Standard deviation

at 282 nm based on peak area. The retention time was 3.76±0.02.

As per the USP XXIII[7], system suitability tests for HPLC were carried out on freshly prepared standard stock solution of NBH and the parameters studied and results obtained with 20 µl injection volumes are summarized in Table 1.

In HPTLC method several combinations of solvents were tried to accomplish separation. Using solvent system ethyl acetate: toluene: methanol: ammonium hydroxide (1:6:2:0.1 v/v/v/v) and precoated silica gel $60F_{254}$ aluminum plate as stationary phase, good separation was attained, where $R_f$ was found to be at 0.33±0.02. The quantification of the drug was carried at 282 nm wavelength.

The linear regression data showed a good linear relationship over a concentration range of 10 to 150 µg/ml for HPLC and 100 to 600 ng/spot for HPTLC. The limit of detection and the limit of quantification for HPLC was found to be 2.0 and 10.0 µg/ml, respectively, and for HPTLC as 30 and 100 ng/spot, respectively. The intra-day and inter-day precision were determined by analyzing standard solutions in the concentration range of 20 to 100 µg/ml for HPLC and 150 to 550 ng/spot for HPTLC. The intra-day and inter-day results indicate that both methods are precise (Table 2). Repeatability of HPLC method was assayed by injecting 40 µg/ml for five times and peak area was measured. The % RSD was found to be 0.002. In HPTLC, 350 ng/spot was applied five times on a TLC plate followed by development of plate and recording the peak area. The % RSD was found to be 1.860. The spot was scanned five times without changing the position of the plate and % RSD was found to be 1.649 (Table 1).

Assay results of both the brands are very close to the label claim. To study accuracy of the developed methods, recovery studies were carried out using standard addition method at four different levels for both the brands and the % recoveries were calculated (Table 3). The results revealed no interference of excipients. The proposed RP-HPLC and HPTLC methods are accurate, precise, sensitive, selective and rapid. Both the methods can be used for routine analysis of nebivolol hydrochloride in the tablet dosage form.



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