Research Article

A Validated HPTLC Method for Determination of Nebivolal from Tablets

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ABSTRACT
A simple, specific and precise high performance thin layer chromatographic method of analysis of Nebivolal, both as a bulk drug and in formulation was developed and validated. The method employed TLC (Thin Layer Chromatography) aluminum plates pre-coated with silica gel 60 F254 as the stationary phase. The solvent system consisted of ethyl acetate: methanol: 25%ammonia (8:2:0.1v/v). This system was found to give compact bands for nebivolal (Rf 0.63±0.05). Densitometric analysis of nebivolal was carried out in the absorbance mode at 285nm. Linear regression analysis data for the calibration spots showed good relationship with regression coefficient r^2 = 0.9917 in the range of 20-140 ng/ band. The limits of detection and quantitation were 6 ng/ band and 20 ng/ band respectively. The proposed method was found to be simple, precise, accurate, reproducible for the estimation of nebivolal in pure drugs and its formulations.

Keywords: Nebivolal, HPTLC method validation, Densitometric

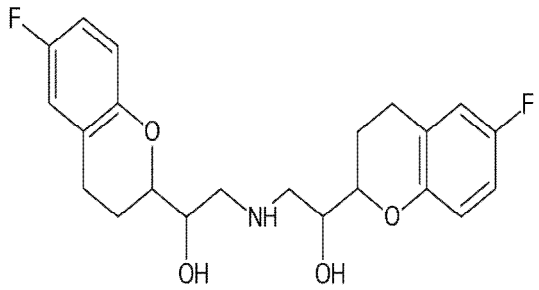
1. INTRODUCTION

Nebivolal is chemically1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} having antihypertensive action given orally[1-3]. Nebivolol is a β1 receptor blocker with nitric oxide-potentiating vasodilatory effect used in treatment of hypertension and, in Europe, also for left ventricular failure. It is highly cardioselective under certain circumstances. Literature assessment showed that Various analytical methods have been reported for determination of nebivolol including UV spectrophotometry[4], high performance liquid chromatography (HPLC)[5-11], liquid chromatography-mass spectroscopy (LC-MS)[12] , High Performance thin layer Chromatography (HPTLC)[13-15] for estimation of NEB in dosage formulations and in biological fluids. Valsartan (VAL,), 3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid, is an angiotensin II receptor antagonist[16]. This HPTLC method was developed which is simple, accurate, and precise for the determination of bulk drug and its formulation**.**

Medical uses

Nebivolal is a beta-blocker, prescribed for hypertension and also for left ventricular failure either alone or combined with other medications. It decreases the amount of blood pumped out from heart. This helps to decrease blood pressure, helps the heart pump more efficiently, and reduces the workload on the heart[1].

Molecular structure



2. MATERIALS AND METHODS

Nebivolal (purity 99.78%) was provided as a gift sample by USV Ltd. Mumbai, India and was used without further purification. All the other reagents used were of analytical grade. Toluene (AR grade), Methanol (AR grade), Acetone (AR grade) were purchased from Merck (chemicals) Pvt Limited,Germany.

2.1 Instrumentation

Chromatographic separation of drug was performed on Merck TLC plate pre-coated with silica gel 60 F254 (10 cm ×10 cm with 250 mm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a band with 8 mm width using CAMAG 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using CAMAG TLC scanner 3 in the range of 20-140 ng per band and operated by winCATS software (V 1.4.6, CAMAG).

2.2 Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that the drug showed considerable absorbance at 285 (Figure I). So, 285nm were selected as the wavelength for detection.

2.3. Method validation

2.3.1 Linearity

A stock solution of Nebivolal (100 µg /µl) was prepared in methanol and diluted suitably to obtain concentration of 0.01µg /µl. Different volumes of the dilution, 2, 4,6,8,10,12, 14µl were spotted on TLC plate to obtain concentration of 20 to 140 ng/ band of Nebivolal, respectively. The data of peak area v/s drug amount were treated by linear least-square regression analysis.

2.3.2. Precision

The intra and inter-day variation for the determination of Nebivolal was carried out at three different concentration levels of 40, 80 and 120 ng per spot. The % RSD values were determined for intra-day and inter-day variation.

2.3.3. Accuracy

The analysed samples were spiked with 80, 100 and 120 % of the standard Nebivolal and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug by standard addition method.

2.3.4. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the standard formula as per the ICH guidelines.

2.3.5. Specificity

The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peaks was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on winCATS software.

2.4. Analysis of the marketed formulation

To determine the content of Nebivolal in tablet (label claim: 5mg per tablet). Methanol was added gradually upto 10 ml. then from this stock solution 1ml diluted to 10 ml, again from this diluted stock solution we took 1 ml in volumetric flask. This volumetric flask was kept covered with aluminum foil. Finally volume was made upto 10ml with methanol to get stock solution of (0.01 µg/µl). The solution was suitably diluted. Appropriate volume of solution was applied on TLC plate followed by development and scanning.

3. RESULTS AND DISCUSSION

3.1. Development of the optimum mobile phase

TLC procedure was optimized with a view to develop an accurate assay method. The drug reference standard was spotted on the TLC plate and developed in different solvent systems. The mobile phase ethyl acetate: methanol: 25%ammonia (8:2:0.1v/v) gave sharp and symmetrical peak with Rf 0.65. Well-

defined bands were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature. The representative densitogram is given in (Figure III).

3.2 Validation of the method

3.2.1 Linearity

The response for the drugs was found to be linear in the concentration range 20-140 ng / band with correlation co-efficient of 0.9994. The representative linearity graph is given in (Figure II).

3.2.2 Precision

The % RSD value for intra-day and inter-day variation study was found to be not more than 0.725 % and 1.21 % respectively, thus confirming precision of the method.

3.2.3 Recovery

Acceptable recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80 %, 100 %, 120 % level) indicated the mean recovery 94.28%.

3.2.4 Limit of Detection and limit of Quantitation

The limit of detection and limit of quantitation as calculated by standard formula as given in ICH guidelines was found to be 6 ng / band and 20 ng/ band respectively.

3.2.5 Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s, m) 0.9978 and r (m, e) 0.9959 for Nebivolal, indicating the non interference of any other peak of degradation product, impurity or matrix. The validation results are listed in Table I.

3.3 Analysis of marketed formulation

There was no interference from the excipients present in the suspension. The drug content was found to be 103.08 %.

4. CONCLUSION

The developed method was found to be simple, precise, and sensitive. High throughput ability of HPTLC makes it a very useful method for routine analysis of bulk drug as well as formulation.

Table 1: Validation Parameters

S.No.	Validation Parameter	Nebivolal
1	Linearity Equation (r^2) Range	Y = -209.5 + 46.08 * X (0.9994) 20 – 120 ng per band
2	Precision (% RSD) Intraday Interday	 NMT 0.725 % NMT 1.21 %
3	Accuracy (% mean recovery)	94.28 %
4	LOD	6ng
5	LOQ	20ng
6	Specificity Peak Purity	Specific r (s,m) = 0.9978 r (m,e) = 0.9959

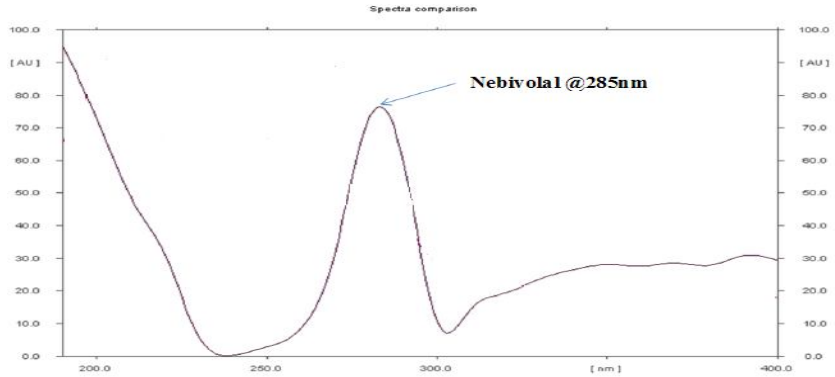


Fig. I: Spectrum of Nebivolal

Regression via area: Linear Y = -209.5 + 46.08 * X r = 0.99974 sdv = 1.46

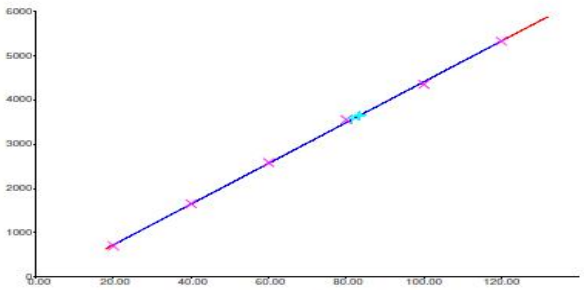


Fig. 2: Linearity of Nebivolal

Nebivolal @285nm

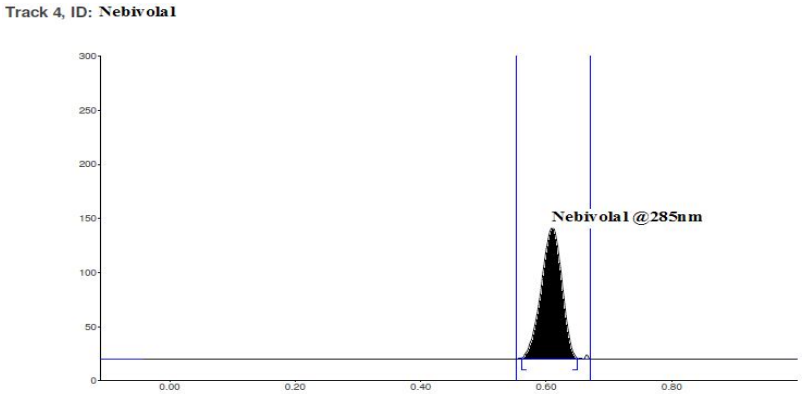


Fig. 3: Representative Densitogram of Nebivolal

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