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Analysis of Nebivolol hydrochloride and Valsartan in Pharmaceutical Dosage Form by High Performance Thin Layer Chromatographic Method

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ABSTRACT

A simple, accurate and precise high performance thin layer chromatographic method has been developed for the estimation of Valsartan and Nebivolol hydrochloride simultaneously from a tablet dosage form. The method employed silica gel 60 F_{254} precoated plates as stationary phase and a mixture of Ethyl acetate: Methanol: Ammonia (6.5:2.5:0.5 %v/v/v) as mobile phase. Densitometric scanning was performed at 280 nm using Camag TLC scanner 3. Beer's law was obeyed in the concentration range of 800ng/spot-2400ng/spot for Nebivolol hydrochloride and 200ng/spot-1000ng/spot for Valsartan. The Retention factors for Nebivolol hydrochloride is 0.75 ± 0.04 and for Valsartan is 0.27 ± 0.01. The method was validated as per ICH Guidelines, proving its utility in estimation of Valsartan and Nebivolol hydrochloride in combined dosage form.

Keywords:

Valsartan, Nebivolol hydrochloride, simultaneously, ICH guidelines.

INTRODUCTION

Nebivolol hydrochloride is chemically known as{α, α'- [iminobis (methylene)] bis [6 fluoro- 3, 4 – dihydro– 2H -1- benzopyran– methanol]} (1). Valsartan is chemically known as L-valine, N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl.

Valsartan is angiostensin II blocker and is used as an antihypertensive drug. Nebivolol hydrochloride is selective β_1 adrenoreceptor antagonist, has vasodilating properties unrelated to β_2 stimulation or alpha blockade (2). Literature survey reveals that assay of Valsartan in bulk and dosage form is official in USP 2007 (3). Several analytical methods that have been reported for estimation of Valsartan and Nebivolol hydrochloride are HPLC (4-8), Spectrophotometric (9), thin layer chromatography (10-11) .The present paper describes a simple, accurate and precise method for simultaneous estimation of Valsartan and Nebivolol hydrochloride in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

EXPERIMENTAL

Instruments:

HPTLC system (Camag, Muttenz, Switzerland) comprising of, Linomat 5 sample applicator, twin-trough developing chamber and TLC Scanner 3 with WinCATS evaluation, ATS software (version 1.2.6) was used in the studies.

Reagents and Chemicals:

Methanol (AR grade) was procured from Ashonuj specialties Pvt. Ltd., Mumbai. AR Grade Ethyl acetate, Ammonia was procured from Loba chemie, Mumbai.

Working Standards:

Working standards of Nebivolol hydrochloride was procured from Burgeon pharmaceuticals Pvt.Ltd, Pondicherry, India and Valsartan was procured from Lupin Research Park, Pune, India as a gift samples. Marketed formulation Nebicard-V (Nebivolol hydrochloride- 5 mg, Valsartan 80 mg/tablet) was procured from local market.

PROCEDURE

Preparation of standard stock solution

25 mg of Valsartan and 25 mg of Nebivolol hydrochloride were weighed and transferred into 25 ml volumetric flasks separately. To it 15ml methanol was added and shaken to dissolve the drug and volume was then made upto 25 ml so as to get the concentration 1mg/ml for both the drugs. Required amount of standard stock solution of both the drugs was diluted appropriately so as to get the mixed standard solution containing 0.2mg/ml Nebivolol hydrochloride and 0.1mg/ml Valsartan. Each standard was applied as bands on TLC plates in five replicates. Plates were developed by linear ascending development using neat solvents like toluene, methanol, chloroform, ethyl acetate, acetone, acetonitrile, etc. with chamber saturation. Based on the results of these initial chromatograms binary and ternary mixtures of solvents were tried to achieve optimum resolution between Valsartan and Nebivolol hydrochloride respectively. After several trials, mixture of Ethyl acetate: Methanol: Ammonia (6.5: 2.5: 0.5 v/v/v) was chosen as the mobile phase for analysis. The linearity of the method was determined at five concentration levels ranging from 200 to 1000 ng/spot for Valsartan and 800 to 2400 ng/spot for Nebivolol hydrochloride.

Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately and powdered. Powder quantities equivalent to 5 mg of Nebivolol hydrochloride and 5 mg of Valsartan were weighed separately and transferred in two 10 ml volumetric flasks. To each 5 ml of methanol was added and sonicated for 10 min. Volume

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was then made upto 10 ml with methanol. Each solution was then filtered through whatman filter paper No.41. From the filtrate of both, appropriate volumes were spotted to obtain final concentration of 400 ng/spot for Valsartan and 1200 ng/spot for Nebivolol hydrochloride. The peak areas of the spots were measured at 280 nm and concentrations in the samples were determined from the respective calibration curves. The amount of each drug present per tablet was calculated. The densitogram is shown in Fig 1.

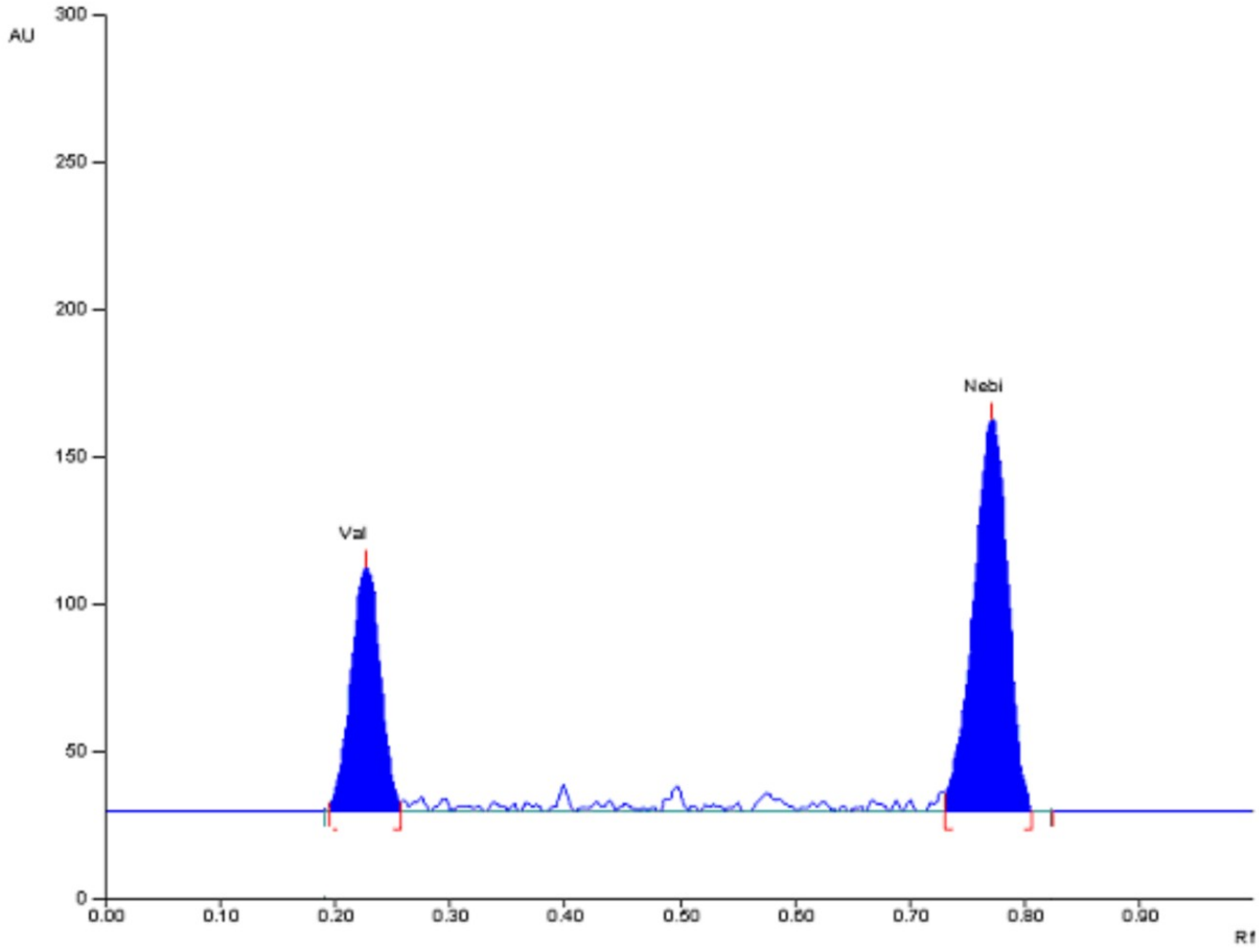

Figure 1: Densitogram of Nebivolol hydrochloride (1600ng/spot) and Valsartan(600ng/spot)

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METHOD VALIDATION

As per the ICH guidelines, the method validation parameters checked were linearity & range, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity and Range

Linearity of the method was checked using five different concentrations in the range of 200 to 1000 ng/spot for Valsartan and 800 to 2400 ng/spot for Nebivolol hydrochloride. The linearity is indicated by regression equation. The Linear regression equations obtained are:

For Nebivolol hydrochloride $\mathbf{y = 4.261x - 1552.8\ (r = 0.998)}$

For Valsartan $\mathbf{y = 2.537x + 162.27 (r = 0.997)}$

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated and presented in Table 1. Recovery was within the range of 100 ± 2% which indicates accuracy of the method.

Table-1 Results from recovery Studies

% Level	Labelled (mg/tab)	Amount added (mg)	Amount recovered (mg)	% Recovery ± S.D*
	Valsartan			
80	80	64	144.12	98.80 ± 0.168
100	80	80	159.83	99.98 ± 0.159
120	80	96	177.06	100.88 ± 0.233
	Nebivolol hydrochloride			
80	5	4	8.89	99.48 ± 1.112
100	5	5	10.09	100.12 ± 0.857
120	5	6	10.87	100.10 ± 0.170

*Standard deviation of three determinations

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, three different concentrations of the mixed standard were analyzed in a day and percentage RSD were calculated and was found to be less than 1.5%. In the inter day variation studies, three different concentrations of the mixed standard were analyzed on 3 consecutive days and percentage RSD were calculated and was found to be less than 1.5%. The results of

interday and intraday studies are shown in Table 2. The data obtained indicates that the developed HPTLC method is precise.

Table 2: Validation Parameters for Valsartan and Nebivolol hydrochloride

PARAMETERS	**VALSARTAN**	**NEBIVOLOL HYDROCHLORIDE**
Beer's law range (ng/spot)	200-1000	800-2400
Regression equation y= mx + c		
Slope (m)*	2.537	4.261
Intercept (c)*	162.27	-1552.80
% RSD (n=3)		
Intraday precision	0.759	0.629
Interday precision	0.747	0.645
LOD_a	4.77	63.67
LOQ_b	14.46	192.94

LOD_a – Limit of detection

LOQ_b – Limit of Quantitation

* Average of five determination

Sensitivity

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD was calculated using the following formula

$$\mathbf{LOD} = \frac{\mathbf{3.3 \times Standard\ deviation\ of\ response}}{\mathbf{Slope\ of\ caliberation\ curve}}$$

The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ was calculated using the following formula

$$\mathbf{LOQ} = \frac{\mathbf{10 \times Standard\ deviation\ of\ response}}{\mathbf{Slope\ of\ caliberation\ curve}}$$

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Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in procedure. Slight and deliberate changes were made to the following parameters like changing the mobile phase ratio and chamber size, and the effect on the Rf values and peak areas were noted. In case of mobile phase ratio the % change in Rf Value was not more than 0.08% & the % change in area was not more than 0.189 %. In case of Chamber change % change in Rf value was not more than 0.22% & the % change in area was not more than 0.117%. The method was found to be robust since the monitored parameters were not significantly affected.

Specificity

Specificity is the ability to access unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products.

It is done by spotting common excipients during analysis and it was found that there is no interference by excipients at the Rf value of the drug.

Spectra of standard drug also match with the sample spectra and hence it is confirmed that the method is specific. The spectrum is shown in Fig 2.

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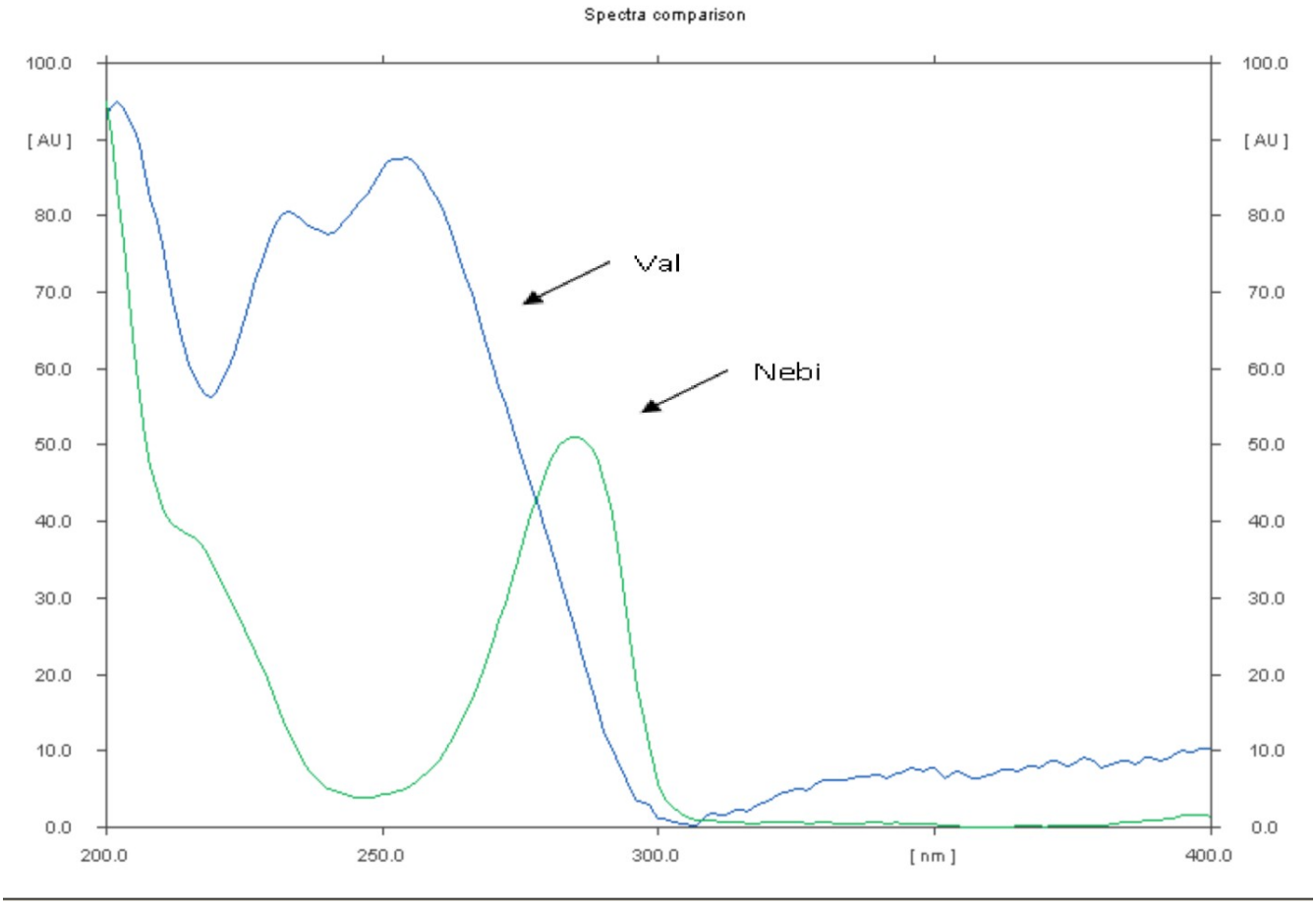

Figure 2: Spectra for Valsartan and Nebivolol hydrochloride

RESULTS AND DISCUSSIONS

As per the validation parameters checked, precision was indicated by % RSD value that is less than 1.5. Recovery of Valsartan on an average was 99.89% and for Nebivolol hydrochloride the value was 99.90%, indicating accuracy of the method. The details are shown in Table 2. The % assay values for Valsartan were found to be 100.13 ± 1.15% and for Nebivolol hydrochloride were found to be 100.49 ± 1.35%.

CONCLUSION

The proposed HPTLC method for the simultaneous estimation of Valsartan and Nebivolol hydrochloride in combined dosage forms was found to be sensitive, accurate, precise, simple and rapid. Hence the present HPTLC method may be used for routine analysis of the raw materials and formulations.

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