An accurate, sensitive, rapid and precise HPTLC method for analysis of Nebivolol Hydrochloride and Valsartan in the combination has been developed and validated. Literature survey reveals few stability indicating methods for Nebivolol Hydrochloride and Valsartan as single drugs. There is no stability indicating method reported for these two drugs in combination. By use of HPTLC technique, an optimum separation was achieved on plates precoated with silica gel 60 F254 with mobile phase consisting of Ethyl Acetate: Methanol: Acetic acid. Detection and quantization was performed at 280nm and 240nm for Nebivolol Hydrochloride and Valsartan respectively. Method was validated according to the ICH guidelines. The validation parameters checked were specificity, linearity, accuracy, precision, LOD, LOQ. The drugs were subjected to stress conditions like hydrolysis under acidic, basic and neutral conditions, oxidation, heat and photolysis. The degradation products resulting from stress conditions did not interfere with the detection of drug peak. This method can be used for stability monitoring of these drugs.

Keywords: Valsartan; Nebivolol Hydrochloride; HPTLC; stability indicating method.

INTRODUCTION
Nebivolol hydrochloride (NEBI) is chemically known as 2-[2-(1H-tetrazol-5-yl)[1,12-biphenyl]-4-yl]methyl]-L-valine. Valsartan (VAL) is chemically known as \(N\)-(1-Oxopentyl)-\(N\)-[[22 -(1H-tetrazol-5-yl)[1,12-biphenyl]-4-yl]methyl]-L-valine. VAL is angiotensin II receptor antagonist and is used as an antihypertensive drug. NEBI is selective \(\beta\), adrenoceptor antagonist, has vasodilating properties unrelated to \(\beta\) stimulation or alpha blockade. Literature survey reveals that assay of VAL as bulk drug is official in USP 2007. Few stability indicating methods are reported for VAL and NEBI. To the best of our knowledge, there is no published stability indicating HPTLC method for these drugs in combination. The present paper describes a simple, accurate and precise method for simultaneous estimation of VAL and NEBI in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.

EXPERIMENTAL
Materials
Nebivolol hydrochloride and VAL as bulk drugs were kindly provided by Burgeon Pharma Pvt. Ltd. Pondicherry, India and Lupin Research Park, Pune, India respectively. All chemicals and reagents were of analytical grade. Marketed formulations, Nebicard-V manufactured by Torrent Pharmaceuticals (Nebivolol hydrochloride 5 mg, Valsartan 80 mg/tablet) was procured from local market.

Apparatus
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 1.4.3) was used in the studies.

Procedure
Standard and sample preparation
NEBI and VAL (25mg each) were weighed and transferred to separate 25ml volumetric flasks. 10ml of methanol was added and sonicated, final volume was made with methanol to get concentration of \(1000 \text{ g/ml}\) of stock solutions. Both the solutions were further diluted with methanol to get final concentration of \(200 \text{ g/ml}\) of NEBI and \(100 \text{ g/ml}\) of VAL. For analysis of tablet dosage form, twenty tablets were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 4mg of NEBI and 64mg of VAL were transferred to 10ml volumetric flask. Methanol was added, sonicated and final volume was made up with same solvent. These solutions were then centrifuged for 30 min and then decanted and filtered through Whatman filter paper No. 41. Appropriate amounts from this solution were diluted with methanol to get two separate solutions with
concentrations of 20: g/ml of NEBI and 320: g/ml of VAL separately.

Chromatography was performed on 10 cm x 10 cm aluminum plates precoated with silica gel 60 F_254. Before use, the plates were prewashed with methanol and activated at 110°C for 5 min. Drug solutions were applied to the plates as bands 6 mm wide and 6.8 mm apart by means of a Camag Linomat 5 sample applicator equipped with a 100 µL syringe. Linear ascending development was performed in a 20 cm x 10 cm twin trough glass chamber using ethyl acetate, methanol and acetic acid (6:1:0.5, v/v/v) as mobile phase, after saturation of the chamber with mobile phase vapors for 15 min. The development distance was 9 cm. After development, the plates were dried in a current of air by use of a dryer. Densitometric scanning was performed with a Camag TLC Scanner 3 at 240nm for VAL and 280nm for NEBI. The scanner was operated by Wincats software Version 1.4.3.

Stress Degradation Studies

Drug concentration of 100µg/ml of VAL and 200µg/ml of NEBI was used in all degradation studies. Appropriate volume of standard stock solution was taken in 50ml volumetric flask, to it either 5ml of 10M HCl or 10M NaOH, or 30% H_2O_2 (1ml) was added; volume was made with methanol and the solution was refluxed at 60°C for 1hr. Neutral hydrolysis was also carried out by refluxing both the drug solutions with water. After refluxing, the solutions were cooled and volume made up with methanol. The bulk drugs in dry form were subjected to effect of temperature (80°C, 4hr) and photolysis. After exposure, appropriately weighed quantity was dissolved in methanol and diluted suitably. Appropriate amount was spotted in form of band to get concentration of 600ng/band for Valsartan and 1200ng/band for Nebivolol Hydrochloride having band width 6mm. Chamber was saturated for 15min with mobile phase and development was done up to 90mm at ambient temperature. After development, the plates were dried in a current of air by using a dryer. Densitometric scanning was performed with a Camag TLC Scanner 3.

RESULTS AND DISCUSSION

The representative densitogram of NEB and VAL by the developed method is shown in Figure 1.

Forced degradation studies

HPTLC studies performed on the stressed samples of VAL and NEBI resulted in the following observations. NEBI degraded in acid stressed conditions showing well resolved peak of degradation product at Rf 0.14 and VAL also degraded to some extent. But VAL showed degradation product at Rf slightly less than Rf for VAL. This was observed after keeping the refluxed solution for two days and spotting 10 times higher concentration. In this case, the drug peak had shifted to Rf 0.89 and the degradation peak appeared at Rf 0.84, probably since the method is not as robust as it should have been (Figure 2 and 3). But the spectral comparison confirmed the spot at Rf 0.89 to be Valsartan in this case as shown in Figure 4. The percent degradation for VAL was found to be 69% and for NEBI 82.85%. The results are reported in Table 1.

NEBI and VAL degraded to the extent of approximate 11% upon base stressed studies. Stability studies show that both the drugs are stable to action of heat and photolysis while 98.98% of NEBI and 91.95% of VAL...
was recovered after oxidative stress. The drugs were also found to be stable to neutral hydrolysis.

Table 1: Stress Degradation Studies

<table>
<thead>
<tr>
<th>Stress Conditions</th>
<th>Exposure Time</th>
<th>% degradation NEBI</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis (10% reflux)</td>
<td>1</td>
<td>52.68%</td>
<td>34.90%</td>
</tr>
<tr>
<td>Base Hydrolysis (5% reflux)</td>
<td>1</td>
<td>11.12%</td>
<td>11.18%</td>
</tr>
<tr>
<td>Neutral Hydrolysis (reflux)</td>
<td>1</td>
<td>0.59%</td>
<td>2.24%</td>
</tr>
<tr>
<td>Oxidation 30% (reflux)</td>
<td>1</td>
<td>1.02%</td>
<td>0.55%</td>
</tr>
<tr>
<td>Heat 80°C</td>
<td>4</td>
<td>0.51%</td>
<td>0.97%</td>
</tr>
<tr>
<td>Photolysis UV fluorescence temp (320 nm to 400 nm)</td>
<td>200 hour</td>
<td>0.78%</td>
<td>2.33%</td>
</tr>
<tr>
<td>Cool white fluorescent temp</td>
<td>1.2 million hour</td>
<td>0.21%</td>
<td>0.66%</td>
</tr>
</tbody>
</table>

Validation Parameters

Specificity
Wincats software afforded automatic calculation of the peak purity by comparing the UV spectra, at the start, max and tail of the peak of each response. The correlation value $r > 0.99$ confirmed peak purity indicating that this HPTLC method is perfectly able to resolve and accurately measure two drugs in the presence of the degradation products. The peak purity values were found to be more than 0.9950 which show that the method is specific for the two drugs.

Linearity
Five-point calibration curves were obtained in a concentration range from 600-1400ng/band and 1200-2800ng/band for VAL and NEBI respectively. Five independent determinations were performed at each concentration. The response for the drug was linear and the calibration equation was $y = 3.5478x + 1585.5$ for NEBI and $y = 4.1234x + 3195.1$ for VAL with $r^2 = 0.9974$ and 0.9995 respectively.

Accuracy and Precision
The accuracy of the proposed method was ascertained with analysis of the marketed formulation by standard addition method at 80, 100 and 120% levels. Percent Recovery was found to be in the range of 98.40 to 100.13 % for Valsartan and from 98.39 to 101.49 % for Nebivolol Hydrochloride.

The precision study was performed during the same day and consecutive days. For the intermediate precision, a study was carried out on three different days. The intraday precision was determined at three levels on the same day (six replicates) giving good R.S.D. values for peak areas. In all these cases, the R.S.D. values obtained were far below the percentage limit set for the precision study, thus showing that the method used was highly repeatable.

Limit of Detection and Limit of Quantification
The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated by the method based on the standard deviation ($\sigma$) of responses and the slope of the calibration plot, using the formula $LOD = 3.3\sigma / S$ and $LOQ = 10\sigma / S$. The LOD and LOQ values are shown in Table 2.

Table 2: Validation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NEBI</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (ng/band)</td>
<td>1200-2600</td>
<td>200-1400</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 3.5478x + 1585.5$</td>
<td>$y = 4.1234x + 3195.1$</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.9974</td>
<td>0.9995</td>
</tr>
<tr>
<td>Accuracy (% recovery)</td>
<td>Within 98 – 102%</td>
<td>Within 98 – 102%</td>
</tr>
<tr>
<td>Precision (% R.S.D.)</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>LOD</td>
<td>180.86 ng/band</td>
<td>295.87 ng/band</td>
</tr>
<tr>
<td>LOQ</td>
<td>271.45 ng/band</td>
<td>105.28 ng/band</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
</tr>
</tbody>
</table>

Fig. 4: Spectral comparison of drug peak at Rf 0.89 and degraded product at Rf 0.84

Robustness
This method is very sensitive to ratio of mobile phase and saturation time so it is necessary to be controlled meticulously. Slight variations in Rf values can be observed if these two parameters are not controlled.

ACKNOWLEDGEMENTS
The authors express their gratitude to University of Pune for sponsoring this research, to the management of A.I.S.S.M.S. College of Pharmacy for providing the research facilities, to M/s. Lupin Research Park & M/s. Torrent Pharmaceuticals for providing the drug samples.

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