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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL AND VALSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination nebivolol and valsartan in pharmaceutical dosage form. The using Altima C_{18} (4.6 x 150mm, 5µ) column in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (52:48 v/v) adjusted to pH 4.8 with dilute ortho phosphoric acid solution. The flow rate was 1.0 ml/ min and effluents were monitored at 282 nm. The retention times of nebivolol and valsartan were found to be 2.325 min and 5.172 min, respectively. The linearity for nebivolol and valsartan were in the range of 2.5-7.5µg/ml and 40-120µg/ml respectively. The recoveries of nebivolol and valsartan were found to be 100.04% to 101.36% and 99.86% to 101.62%, respectively. The proposed method was validated and successfully applied for the estimation of nebivolol and valsartan in combined tablet dosage forms.

Key words: Nebivolol, Valsartan, HPLC

INTRODUCTION

Nebivolol is chemically known as 1-(6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-yl)-2-{[2-(6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2-hydroxyethyl] amino}ethan-1-ol. It is a highly cardioselective vasodilatory beta1 receptor blocker used in treatment of hypertension. Nebivolol is a selective β1-receptor antagonist. Activation of β1-receptors by epinephrine increases the heart rate, blood pressure and the heart consumes more oxygen. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. In addition, beta blockers prevent the release of renin, which is a hormone produced by the kidneys which leads to constriction of blood vessels. At high enough concentrations, this drug may also bind beta 2 receptors[1].

Valsartan is chemically known as (2S)-3-methyl-2-[N-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl) pentanamido] butanoic acid. Valsartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure[2].

Different analytical methods have been reported in the literature for the assay of nebivolol in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC, LC-MS[3–19]. The present investigation reports a simple UV spectrophotometric

method for the analysis of nebivolol in bulk as well as in tablet dosage form. The developed method was validated as per ICH guidelines [20].

EXPERIMENTAL

Reagents

Nebivolol and Valsartan were kindly supplied by Torrent Pharmaceutical Limited (Gujarat, India). Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A capsule of NEBICARD-V(Torrent) containing 5mg of nebivolol and 80mg of valsartan.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic with mobile phase consisting a mixture of buffer (accurately weighed and transferred 2.72gm of Potassium dihydrogen orthophosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and sonicate. finally make up the volume with water, then pH adjusted to 4.8 with dil. Ortho phosphoric acid solution) and acetonitrile (52 : 48 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. An Altima C_{18} column (150 x 4.6mm x 5 µ) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~25 ^{o}C). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 282nm. A typical chromatogram of nebivolol and valsartan is shown in (Fig. 1).

Diluent: Methanol and Water (50:50) v/v

Standard Preparation

Accurately weighed and transferred 5mg of nebivolol and 80mg of valsartan working standards into a 100ml clean dry volumetric flask, add 70ml of diluents, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1ml was pipette out in a 10ml volumetric flask and then make up to the final volume with diluent to get concentration of 5µg/ml and 80µg/ml.

Sample Preparation

About20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 5mg and 80mg were transferred to a 100 ml volumetric flask, dissolved in diluent. Transfer 1ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 5µg/ml and 80µg/ml.

METHOD VALIDATION

The developed method was validated as per ICH guidelines [13-14] for its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table 9.

System suitability

System suitability and chromatographic parameters w ere validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of tinidazole at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2 & 3). The responses were found to be linear in the range 2.5-7.5µg/ml and 40-120µg/ml for nebivolol and valsartan. The data was given in table 1.

Accuracy

Accuracy was performed in triplicate for various concentrations of tinidazole equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table 2.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 3.

B) Intermediate Precision (Day to Day variability)

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of nebivolol were found to be 0.043µg/ml and 0.129µg/ml respectively. The LOD and LOQ of valsartan were found to be 1.534µg/ml and 4.648µg/ml.

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of tinidazole was noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table 4 and 5.

Ruggedness of the method was checked by using different days and instruments. The relative standard deviation of the results obtained from different days and instruments was <2.0%. The results were given in table 6 and 7.

Assay

The assay and percentage were calculated for NEBICARD-V with label claim 5mg and 80mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table 8.

RESULTS

A reverse-phase column procedure was proposed as a suitable method for the determination of nebivolol and valsartan dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 52:48v/v was used as mobile phase, which showed good resolution of nebivolol and valsartan peak. The wavelength of detection selected was 282nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of nebivolol and valsartan were about 2.325mins and 5.172mins and none of the impurities were interfering in its assay.

DISCUSSIONS

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of nebivolol and valsartan in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of nebivolol and valsartan in bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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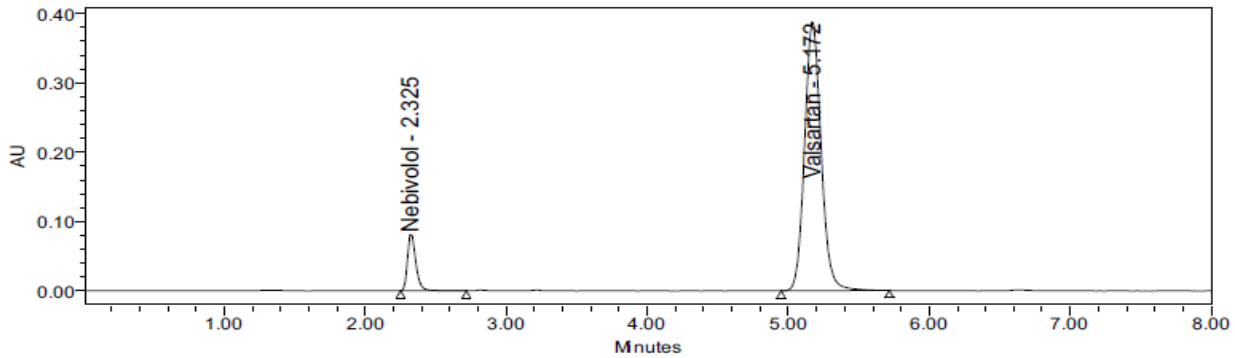

Fig - 1: HPLC chromatogram of Nebivolol and Valsartan in optimized chromatographic conditions

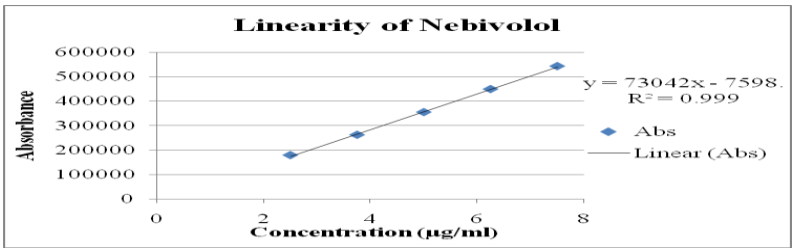

Fig – 2: Linearity of Nebivolol in the range 2.5 to 7.5 µg/ml.

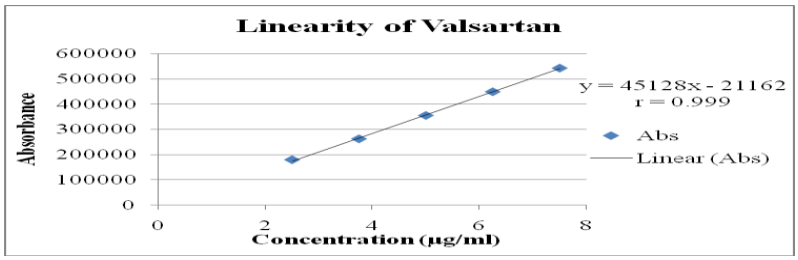

Fig – 3: Linearity of Valsartan in the range 40 to 120 µg/ml.

Table 1: Linearity data of Nebivolol and Valsartan

	Nebivolol			Valsartan		
S.No	**Conc(µg/ml)**	**Rt(mins)**	**Area**	**Conc(µg/ml)**	**Rt(mins)**	**Area**
1	2.5	2.202	179382	40	4.702	1814217
2	3.75	2.247	262312	60	4.899	2665061
3	5.0	2.270	354840	80	5.015	3572162
4	6.25	2.281	448931	100	5.028	4468773
5	7.5	2.285	542582	120	5.050	5425156
		r = 0.9997			r = 0.9998	
		y = 73042x - 7598			y = 45128x - 21162	

Table 2: Accuracy data

		Nebivolol			Valsartan		
S.No	**Spiked level**	**Amount added (µg/ml)**	**Amount present (µg/ml)**	**Average %Recovery***	**Amount added (µg/ml)**	**Amount present (µg/ml)**	**Average %Recovery***
1(n=6)	50%	2.49	2.51	100.63 ± 0.18	39.92	40.44	101.29 ± 0.35
2(n=6)	100%	4.98	4.99	100.14 ± 0.15	79.71	79.68	99.96 ± 0.15
3(n=6)	150%	7.47	7.55	101.09 ± 0.24	119.59	120.86	101.06 ± 0.24

*n=3 (Average of 3 determinations)

Table 3: Precision data of Nebivolol and Valsartan

	Nebivolol			Valsartan		
S.No	**Conc(µg/ml)**	**Rt(mins)**	**Area**	**Conc(µg/ml)**	**Rt(mins)**	**Area**
1	5	2.321	355342	80	5.15	3557561
2	5	2.325	357559	80	5.156	3528405
3	5	2.33	357025	80	5.17	3547014
4	5	2.33	355515	80	5.17	3557433
5	5	2.331	355906	80	5.172	3554056
6	5	2.337	357142	80	5.173	3505046
Mean			356415			3541586
Std.dev			941			20977
%RSD			0.26			0.59

Table 4: Robustness data relating to change in flow rate (1.0ml/min)

		Nebivolol			Valsartan		
S.No	**Flow rate (ml/min)**	**Average Peak Area***	**Std.dev**	**%RSD**	**Average Peak Area***	**Std.dev**	**%RSD**
1	0.9ml/min	353514	1427	0.40	3556637	10618	0.30
2	1.0ml/min	354283	925	0.26	3544909	6480	0.18
3	1.1ml/min	352768	1182	0.33	3557419	10154	0.29

*n=3 (Average of 3 determinations)

Table 5: Robustness data relating to change in mobile phase composition

		Nebivolol			Valsartan		
S.No	**Mobile phase variation (%)**	**Average peak area***	**Std.dev**	**%RSD**	**Average peak area***	**Std.dev**	**%RSD**
1	M.P-1 (Buffer:ACN:: 50:50)	353557	1001	0.28	3540204	6242	0.18
2	M.P-2 (Buffer:ACN:: 52:48)	354477	778	0.22	3545576	5919	0.17
3	M.P-3 (Buffer:ACN:: 54:46)	352589	1038	0.29	3554085	6820	0.19

*n=3 (Average of 3 determinations)

Table 6: Ruggedness data relating to change of day

		Inter-day precision				
		Day – 1 Peak area			**Day – 2 Peak area**	
S.No	**Conc (µg/ml)**	**Nebivolol**	**Valsartan**	**Conc (µg/ml)**	**Nebivolol**	**Valsartan**
1	5	355765	3559823	80	355366	3551209
2	5	357354	3568405	80	357432	3523405
3	5	357955	3541098	80	357025	3547632
4	5	355515	3557433	80	355642	3567918
5	5	355906	3554047	80	355906	3534056
6	5	357142	3505046	80	357238	3532018
Mean		356606	3547642		356435	3542706
SD		1005	22687		899	16086
%RSD		0.28	0.64		0.25	0.45

Table 7: Ruggedness data relating to change of instrument

		Instrument to Instrument				
		Inst – 1 Peak area			**Inst – 2 Peak area**	
S.No	**Conc (µg/ml)**	**Nebivolol**	**Valsartan**	**Conc (µg/ml)**	**Nebivolol**	**Valsartan**
1	5	355343	3562817	80	354787	3557843
2	5	357354	3587366	80	357432	3528753
3	5	357955	3572893	80	357025	3547632
4	5	355515	3557433	80	355323	3567918
5	5	355906	3554047	80	354906	3534056
6	5	357323	3508732	80	355135	3535612
Mean		356566	3557215		355768	3545302
Std.dev		1110	26634		1154	15287
%RSD		0.31	0.75		0.32	0.43

Table-8: Results of analysis of laboratory samples (Assay)

S.No	Sample	Label	Nebivolol		Valsartan	
			Amount found	%Purity ± RSD*	Amount found	%Purity ± RSD*
1	Brand-1 (NEBICARD-V)	5mg/80mg	4.99	99.62 ± 0.10	79.87	99.64 ± 0.10

***n=3 (Average of 3 determinations)**

Table 9: System suitability parameters

Validation parameter	Results	
	Nebivolol	Valsartan
Linearity range (µg/ml)	2.5	7.5
Regression equation	y = 73042x - 7598	y = 45128x – 21162
Correlation Coefficient(r)	0.9997	0.9998
Accuracy	100.04% to 101.36%	99.86% to 101.62%
Precision (%RSD)	0.26	0.59
Robustness (%RSD)		
Flow rate: (0.9ml/min & 1.1ml/min)	NMT 0.40	NMT 0.30
Mobile phase: Buffer : ACN(50:50 & 54:46)	NMT 0.29	NMT 0.19
Ruggedness (%RSD)		
Interday – (Day 1 & Day 2)	NMT 0.28	NMT 0.64
Instrument to Instrument (Inst-1 & Inst-2)	NMT 0.32	NMT 0.75

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