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Research Article

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE AND NEBIVOLOL IN RAW AND TABLET FORMULATION

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ABSTRACT

The main objective of this study is to determine Amlodipine and Nebivolol in tablet formulations using reverse phase high pressure liquid chromatography method. The HPLC separation was carried out on Thermo hypersil – keystone C18 (250 x 4.6mm, 0.5µ) column in isocratic mode, using a mobile phase mixture comprising a 67: 33(% v/v) of Acetonitrile: phosphate buffer and UV detection at 280 nm. The percentage recoveries 99.70 % and 99.62% for Amlodipine and Nebivolol respectively. The proposed method of RP-HPLC shows good separation of Amlodipine and Nebivolol, were retention time was found to 2.1min for Amlodipine and 5.3 min for Nebivolol, which show very less time consuming analysis. No previous method of analysis shows good separation at less retention time and less cost. Linearity range for Amlodipine and Nebivolol were 10-50µg/ml, 10–50µg/ml. The developed method of analysis can be applied for the Amlodipine and Nebivolol pharmaceutical dosage form.

Keywords: *Amlodipine Besylate, Nebivolol HCL, RP-HPLC, UV spectroscopy, Mobile phase and simultaneous estimation..*

INTRODUCTION

Amlodipine is widely used in the treatment of high blood pressure. Amlodipine comes under the class of calcium channel blockers; it acts on blood vessels relaxation, by means heart does not have to pump hard and lowers the blood pressure [1-2]. Amlodipine increases the flow of blood to the heart [3]. The chemical name of Amlodipine is 3-Ethyl 5-Methyl (4RS)-2-[(2aminoethony) Methyl]-4-(2-Chlorophenyl)-6-Methyl-14–dihydro pyridine-3,5-dicarbonylate benzenesulphate, the structure is given in figure-1[4]. Nebivolol is used treat high blood pressure. Nebivolol comes under the class of beta blockers [5]. Nebivolol relaxes the blood vessels and improves in lowering the blood pressure and blood flow [6-7]. The chemical name of Nebivolol is 1-(6-Fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol, the structure is given in figure-2 [8]. Analytical methods such as HPLC and UV spectrophotometer have been reported for these drugs [8-12]. There are no many HPLC analytical methods reported for the estimation and determination of these two drugs in pharmaceutical formulations

[13-14]. In this paper we present a specific, simple and rapid analytical method of determination for Amlodipine and Nebivolol in tablet formulations.

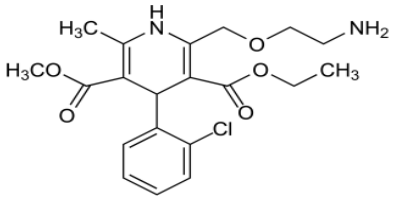

Figure 1: Structure of Amlodipine

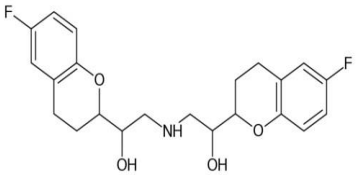

Figure 2: Structure of Nebivolol

Materials and methods

2.1Reagents

Working standards of Amlodipine and Nebivolol were purchased from Sigma, UK. Combined Amlodipine with Nebivolol tablet brand name Nobi-AM strength Amlodipine 5 mg and Nebivolol 5 mg, manufactured by Aristo, marketed by Aristo Pharmaceuticals was purchased from local pharmacy in Jazan, Saudi Arabia. Acetonitrile, water, Potassium dihydrogenortho-phosphate and Phosphoric acid was purchased from E. Merck, Germany.

2.2 Chromatographic conditions

A Shimadzu class LC-10AHPLC system equipped with LC – 10ATvp pump, SPD – 10A UV detector, and Rheodyne injector was used. Compounds were separated on a C18 hypersil (250 x 4.5mm, 0.5µ) column, pore size of the column 95Å and flow rate 1.3 ml/min. The detector was set at wavelength of 280nm. The peak area responses were recorded and integrated using Shimadzu chromatographic software.

2.3 Preparation of Mobile phase

The Phosphate buffer was prepared using 1.35gm of KH_2PO_4 dissolved in 1000 ml of analytical grade water, the pH adjusted to 6 using triethylamine and filtered by 0.45µm membrane filtration. The ratio of the mobile phase is Acetonitrile: phosphate buffer (67:33) (% v/v).

2.4 Standard stock solution

5mg of Amlodipine and Nebivolol reference standard was taken separately in 100ml flask and diluted with mobile phase to the mark and filtered with 0.45µ disc filter.

2.5 Standard solution

From standard stock solution 1 ml was taken and made up to 5 ml by mobile phase to obtain a mixed concentration of 10 µg/ml of Amlodipine and Nebivolol.

2.6 Sample solution

20 tablets were weighed and grinded to fine powder. 25 mg of Nebivolol powder equivalent was weighed and dissolved in 50 ml mobile phase made and up to 100 ml mark with mobile phase and membrane filtered. 6 ml of the

filtrate made up to 50 ml of mobile phase to achieve effective concentration of 30 µg/ml of Amlodipine and 30 µg/ml of Nebivolol.

2.7 Assay

20µl of the prepared standard and sample solutions was injected individually at different time point, into an HPLC injector, from the obtained HPLC peak area of liquid chromatography, from Amlodipine and Nebivolol peak area the amount of drug in sample was calculated.

2.8 Method validation

The proposed analysis method was conducted to obtain, sensitive, easy and rapid estimation and determination of Amlodipine and Nebivolol by HPLC method for tablet form**.** Based on the ICH guidelines recommendations and USP-30 for parameters the method was validated.

2.9 System suitability

System parameters like resolution, retention time, column theoretical plates and tailing factor and were performed by five and three replicates of standard and sample drugs.

2.10 Linearity

For the linearity studies specific range was determined at 10 - 50 µg/ml for Amlodipine and Nebivolol was injected into the HPLC. For 30 minutes the column was equilibrated with the mobile phase before injection of the solutions.

2.11Accuracy

The recovery experiments show the proposed method of accuracy. The recovery was performed by adding Amlodipine and Nebivolol working standards in the range of test concentration (40%, 80% and 100 %,) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery results are well within the range (S.D < 2) value for Amlodipine and Nebivolol.

2.12Precision

The intraday and interday precision of the method was analyzed separately from the peak area ratios obtained by five replicates from a fixed amount of drug on the same day and 3 different days of a week for a period of a week for 4 different concentrations 10 - 50 µg/ml respectively. The results are represented in terms of % RSD.

2.13 Specificity

Specificity is to analyze the analyte accurately in presence of excipients, matrix components and degradants. The RP-HPLC of standard mixture and formulation shows specificity of method. The proposed RP-HPLC method is able to access the analyte in presence of excipients.

2.14 Statistical Parameters

The assay results obtained were subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

2.15 Recovery studies

The recovery experiments were carried out for accuracy and reproducibility and the precision study for the proposed method. A 50% of pre-analyzed sample was taken and 50% of standard drug was added together. Three repeats were carried over for each level. The lower RSD values of assay show the method is precise and accurate. The mean recoveries of Amlodipine and Nebivolol were in the range of 99.70% and 99.62 %, which shows there is no interference from excipients.

RESULT AND DISCUSSION

3.1 Chromatography

The optimum mixture of mobile phase comprising a 67:33(% v/v) Acetonitrile: phosphate buffer was selected because it was found to ideally resolve peaks of Amlodipine (2.1) and Nebivolol (5.3) as the retention shown in "Figure 3" and "Figure 4". By scanning all standard drugs over a wide range of wavelength 200-400nm wavelength was selected. Amlodipine and Nebivolol show good response at 280 nm. Linearity was evaluated by plotting peak area as a functional of analyte concentration for both Amlodipine and Nebivolol. The graphical representation was given in "Figure 5" and data is presented in "Table 1". According to USP-30 system suitability the analysis were carried out on freshly prepared standard stock solution of Amlodipine and Nebivolol. The parameters obtained with Amlodipine and Nebivolol are shown in "Table 2". From the linearity studies, the specific range was determined for both drugs are 10-50 µg/ml for Amlodipine and Nebivolol. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression equation were also determined and the data are presented in "Table 2".

The parameters like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method is presented in "Figure 6" the data are presented in "Table 3". The retention time is well within the specific limits of 10 minutes. The high-resolution value for Amlodipine and Nebivolol indicates complete separation of the drugs. The tailing factor for Amlodipine and Nebivolol was found to be 0.808 and 0.860 . The peaks are symmetrical and theoretical plates for Amlodipine and Nebivolol were 8948 and 8308 respectively, this shows column efficient performance. The limit of detection and limit of quantification for Amlodipine and Nebivolol are presented in "Table 4". The quantitative estimation of Amlodipine and Nebivolol tablet formulation was carried out by RP-HPLC method using 67:33 (% v/v) Acetonitrile: phosphate buffer using C18 column as the stationary phase. The quantitative estimation and statistical data are presented in "Table 5", and graphically presented in "Figure 6". The chromatogram of Amlodipine and Nebivolol in raw drug is shown in "Figure 7". The Recovery studies for spiked concentration of drugs to the pre analyze form is show in "Table 6".

Conclusions

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

Acknowledgements

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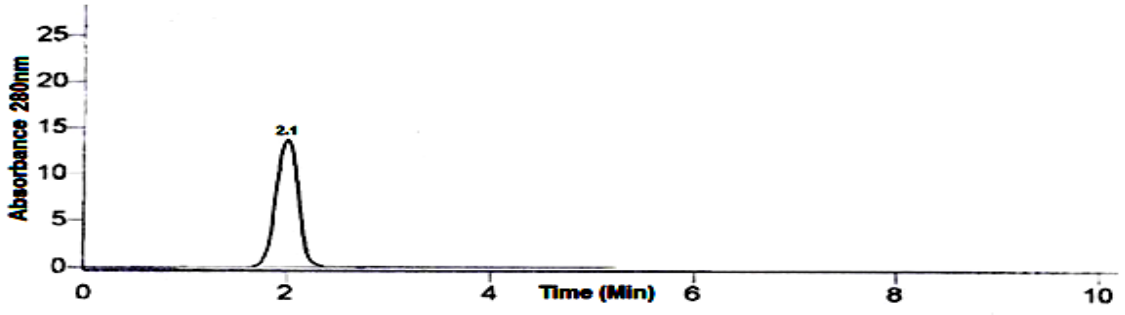

Figure 3. Chromatogram of Amlodipine in single injection

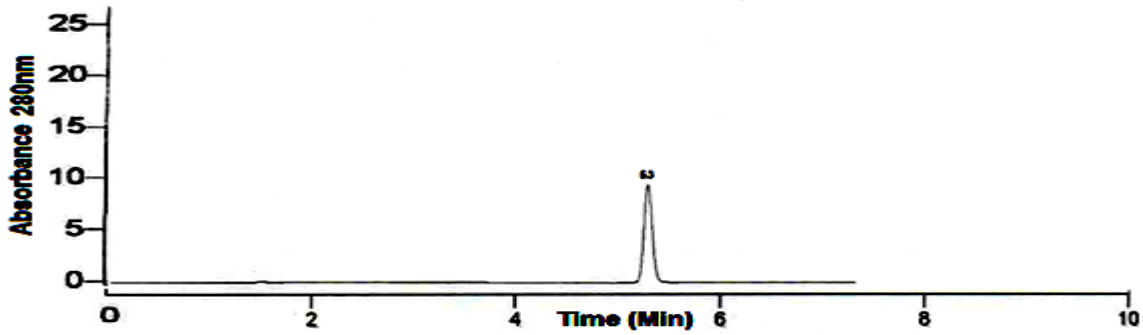

Figure 4. Chromatogram of Nebivolol in single injection

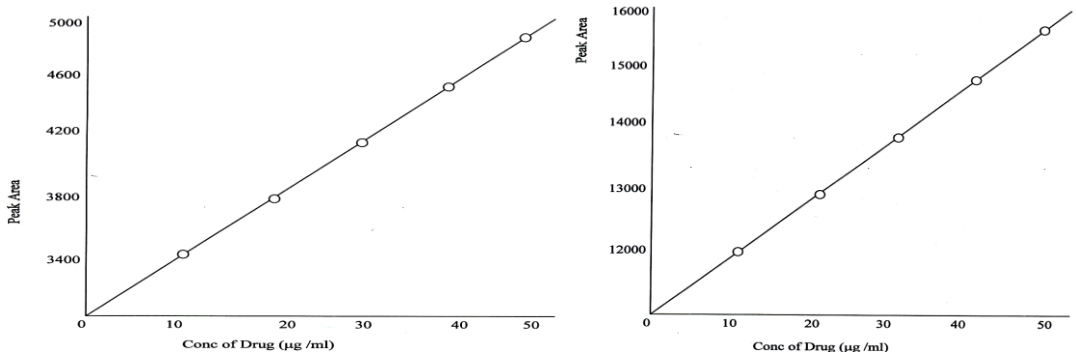

Figure 5. Calibration curve of Amlodipine and Nebivolol

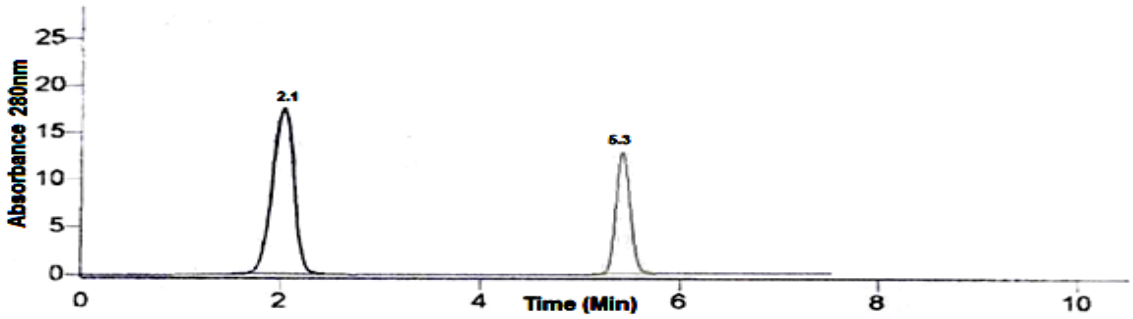

Figure 6. Quantitative (Assay) of Amlodipine and Nebivolol in tablet

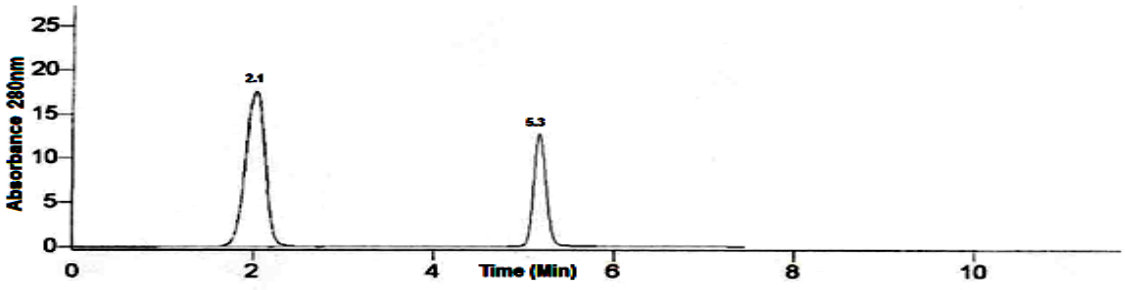

Figure 7. Chromatogram of Amlodipine and Nebivolol in raw drug

SNo	Concentration (µg/ml)	Peak area
1	10	3436.94
2	20	3760.64
3	30	4090.95
4	40	4427.75
5	50	4759.29

SNo	Concentration (µg/ml)	Peak area
1	10	11903.78
2	20	12763.24
3	30	13628.45
4	40	14493.95
5	50	15358.19

Table 1. RP-HPLC linearity data for Amlodipine and Nebivolol

SNo	Parameters	Amlodipine	Nebivolol
1	Standard deviation (SD)	4.353	13.933
2	Relative standard deviation (RSD)	0.00574	0.0101
3	% RSD	0.574	1.011
4	Standard error (SE)	0.02347	0.0836
5	Correlation Coefficient (r)	0.9983	0.9996
6	Slope (a)	33.1181	86.395
7	Intercept (b)	1503.26	11037.67
8	Regression equation Y = (a X + b)	Y=33.1181 X + 1503.26	Y = 86.395 X + 11037.67

Table 2. Results of statistical parameters

Parametrs	Amlodipine	Nebivolol
R T	2.1	5.3
Theoretical plates	8948	8308
Tailing factor	0.808	0.860
Resolution factor	8.33	8.33
Calibration range (or) Linear dynamic range	10-50	10-50

Table 3. Results of system suitability parameters

Parameters	Amlodipine	Nebivolol
LOD (ng/ml)	430	530
LOQ (ng/ml)	1310	1610

Table 4. Results of Limit of detection (LOD) & limit of quantification LOQ

Drug	Label claim (mg/ml)	Amount found	Mean amount	Percentage purity	Mean percentage	% Deviation
Amlodipine	5	4.94 4.96 5.05 5.02 4.98	4.95	98.90 99.20 101.01 100.40 99.62	99.82	-1.1 -0.8 +1.1 +0.4 -0.4
Nebivolol	5	4.97 4.91 5.02 5.04 5.01	4.99	99.40 98.22 100.44 100.80 100.20	99.80	-0.6 -1.8 +0.4 +0.8 +0.2

Table 5. Quantitative (Assay) of data of in tablet

Drug	Amount of Drug present in preanalyzed Sample	Amount of Standard drug (RS) added (µg/ml	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
Amlodipine	30	30 40 50	60.81 70.97 80.92	99.36 99.92 100.48	99.70
Nebivolol	30	30 40 50	60.92 70.77 80.87	99.70 99.42 99.74	99.62

Table 6. Recovery studies for spiked concentration of drugs to the pre analyze form.

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