WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 5, 795-802 Research Article ISSN 2278 – 4357

SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION OF S-AMLODIPINE AND NEBIVOLOL IN A PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

P. Praneetha Praveen, S. Nagunath, P. USha Sree and S. D. Shanmuga Kumar*

Department of Pharmaceutical Analysis. Smt. Sarojini Ramulamma College of Pharmacy. Mahabubnagar. Telangana. India.

Article Received on
23 Feb. 2018,
Revised on 15 March 2018,
Accepted on 05 April 2018,
DOI: 10.20959/wjpps20185-11461

***Corresponding Author**
S. D. Shanmuga Kumar
Department of Pharmaceutical Analysis. Smt. Sarojini Ramulamma College of Pharmacy. Mahabubnagar. Telangana. India.

ABSTRACT

A simple, rapid, selective, sensitive, accurate and precise HPLC method with UV-Detection method has been developed for S-Amlodipine and Nebivolol and validated in its pure form as well as tablet dosage form. Chromatogrpahic separation was achieved by Sunfire column C_{18} (4.6x250mm) with 5µm thickness with the mobile phase containing a mixture of acetonitrile: water (60:40v/v). The flow rate was 1mL/min and the detection was carried out 220nm. The RT values of the S- Amlodipine and Nebivolol was found to be 5-25µg/mL and 10-50µg/mL respectively with regression co-efficient (r^2) =0.9990. The recovery was found to be 98.0%.

KEYWORDS: Nebivolol, S-Amlodipine, RP-HPLC, Accuracy, Linearity, Specificity, Precision.

INTRODUCTION

S-Amlodipine also known as levo amlodipine. It is antihypertensive drug. Chemically, it is described as (S) -3- ethyl- 5- methyl -2 –(2- amino ethoxy) methyl] -4- (2- chloro phenyl) -6- methyl-1,4 – dihyro pyridine -3,5 dicarboxylate. Nebivolol is an adrenergic agent which is used as an antihypertensive perspective. Chemically, It is described as (1RS, 1'RS) -1-1' – (2RS, 2'RS) –bis –(6-Fluoro -3,4 dihydro -2H-1- benzopyran -2yl) 2,2' –iminodiethanol. Several studies related to stability indicated RP-HPLC methods related to simultaneous estimation of valsartan and amlodipine.[1,2] Spectrophotometric methods for simultaneous Estimation of valsartan and amlodipine besylate.[3] *In vitro* dissolution studies of amlodipine

and valsartan.[4] The objective of the present work was to develop a simple, accurate, reproducible and sensitive method for determination of rapid, convenient and simple RP-HPLC method.

MATERIALS AND METHODS

1. **Preparation of Mobile phase:** The mobile phase acetonitrile: water (60:40v/v) was filtered through 0.45µm membrane filter and sonicated on ultra sonic bath for 15 min.
2. **Preparation of standard stock solution[5]:** S-Amlodipine and Nebivolol standard stock solution was prepared by transferring 10mg of each drug in to a working standard 10mL dry volumetric flask. The drugs were dissolved using 7mL of methanol as a solvent and sonicated for 10 min to remove the air bubble contamination. The volume was make up to 10mL with the same solvent. From the stock solution, 15mL of S-Amlodipine & 3mL of Nebivolol were further diluted in to 10mL with methanol in a volumetric flask.
3. **Mobile Phase Optimization:** Intially, various proportions of methanol: water and acetonitrile: water were been tried out for optimized chromatogram.[6] It is achieved finally with acetonitrile: water in the proportion of 40:60 v/v respectively.
4. **Method development:** The optimized method [acetonitrile: water 40:60v/v] was performed with various columns like symmetery, hypersil and sunfire C_{18} [4.6x150mm, 5µm] which was found to ideal as been selected to produce an good peak shape optimized chromatogram which a flow rate of 1mL/min **[Fig.1 Table.1].**
5. **Instrument used:** Waters HPLC with autosampler equipped with PDA detector 996 model.

   Column Temperature: 35°C.

Type of Column: Sunfire C_{18} [4.6X150mm,5µm]

Mobile Phase: Acetonitrile: water [40:60v/v]

Deduction wavelength: 220nm

Injection volume: 10µL

Runtime: 6 min

6. **Validation[7]:** The developed method were been validated as per ICH guidelines. The analytical method validation includes determination of system suitability, specificity, linearity, precision, robustness, LOD and LOQ, Accuracy, Assay.

1. **System suitability:** Weigh accurately 10mg of S-Amlodipine and 10mg of Nebivolol in a 10mL of clean dry volumetric flask and volume is make up with methanol diluents.

Further 15mL of each stock solution has been diluted to 10mL with diluents and the corresponding standard solution was been injected for 5 times in HPLC to determine the % RSD [**Table.2; Fig 2].**

2. **Specificity:** Analysis of blank sample should be performed to rule out the presence of impurities, degradation products & matrix components **[Fig.3]**
3. **Linearity:** Linearity studies were been carried out in the working standard stock solution of S-Amlodipine & Nebivolol in the concentration range of 5ppm-25ppm for S-Amlodipine and 10ppm-50ppm for nebivolol. The results were shown in **Table.3-4 ; Fig.4-5.**
4. **Accuracy and Precision:** The standard stock solution of S-Amlodipine & Nebivolol were been prepared in individual concentrations [50%,100%,150%] made under optimized conditions. The corresponding chromatograms were been recorded and the peak responses were been measured.
5. **Precision:** Both intra –day and inter –day precision were been estimated within batch [S-Amlodipine and Nebivolol]. The % RSD for the area after 5 replicate injections were been found and calculated.
6. **Robustness:** Amlodipine and Nebivolol were been subjected to varying flow conditions and the chromatograms were been recorded.
7. **LOD & LOQ:** The deduction limits of S-Amlodipine and Nebivolol can be detected by using the following formula:

   LOD = 3.3xσ/s

   LOQ =10xσ/s

   Σ = standard deviation of the response.

   S = Slope of calibration.
8. **Assay:** The % purity of S-Amlodipine and Nebivolol both in standard and pharmaceutical dosage form were being estimated by using the formula:

Amount of drug present in the pharmaceutical dosage form

Sample area x weight of standard x dilution of sample x purity x weight of tablet/ standard area x dilution of standard weight of sample x100 x label claim.

% of assay = The amount of individual drug x100

RESULTS AND DISCUSSION

Method development: Different trials of S-Amlodipine and Nebivolol were been carried out with varying proportions of mobile phases and the mobile phase optimized chromatogram were been achieved with acetonitrile and water [40:60v/v] with retention time of 3.006 and 3.853 respectively.

Validation

1. **System suitability:** The optimized chromatogram of S-Amlodipine and Nebivolol were been achieved with peak area of 658995 and 3096188 with USP tailing factor of 1.1 and 1.2 with plate count of 7442 and 7331 respectively. From the above data, it was found that all the system suitability parameter for the develop methods are within the limits.
2. **Specificity:** As per the ICH guidelines, the obtained chromatogram of S-Amlodipine and Nebivolol prove to be free from impurities, degradation products and matrix compounds.
3. **Linearity:** The linearity studies of S- amlodipine and Nebivolol shows the straight line [y=43950x+8388] with correlation co-efficient [r^2] =0.999 and [y=6622x+10151] with correlation co-efficient [r^2] =0.998 respectively. These values meet the validation criteria.
4. **Precision:** The % RSD of S-Amlodipine and Nebivolol in the intra –day study were shown to be 0.554601 and 1.035429 respectively. The acceptance criteria resemblance the %RSD for the sample should be not more than 2. In similar lines, the inter-day precision % RSD were found to be 0.887613 and 0.679742 respectively. Hence the method is précised.
5. **Robustness:** The Robustness were performed for the flow rate variations from 0.8mL to 1.1 mL per minute. It was observed that there were no significant change the parameters like resolution, tailing factor, Rt value and Plate count.
6. **LOD & LOQ:** The LOD and LOQ value of S-Amlodipine and Nebivolol use found to be 0.7µg/mL & 2.7µg/mL and2.1µg/mL & 8.3µg/mL respectively.
7. **Accuracy:** Accuracy at different concentrations [50%,100% & 150%] were prepared and the percentage recovery was calculated. It was found to be 99.88,99.89 and 101% for S-Amlodipine and 99.7,99 & 99% for Nebivolol. Hence the method is accurate.

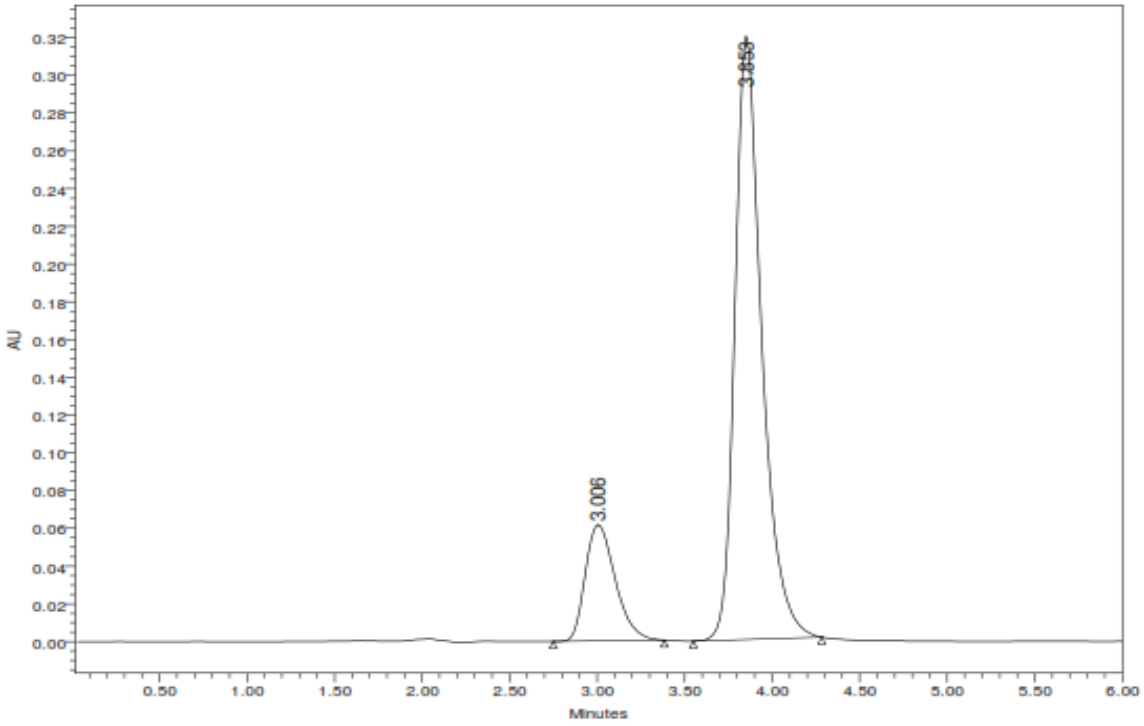

Fig. 1 Optimized chromatogram.

Table. 1: Optimized chromatogram.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	S-Amlodipine	3.006	731322	61677	1.2	8574
2	Nebivolol	3.853	3421257	319786	1.1	9664

Table 2: System suitability.

S.No	Name	RT	Area	Height	USP Tailing	USP PlateCount
1	S-Amlodipine	3.005	658995	61772	1.1	7442
2	Nebivolol	3.848	3096188	324054	1.2	7331

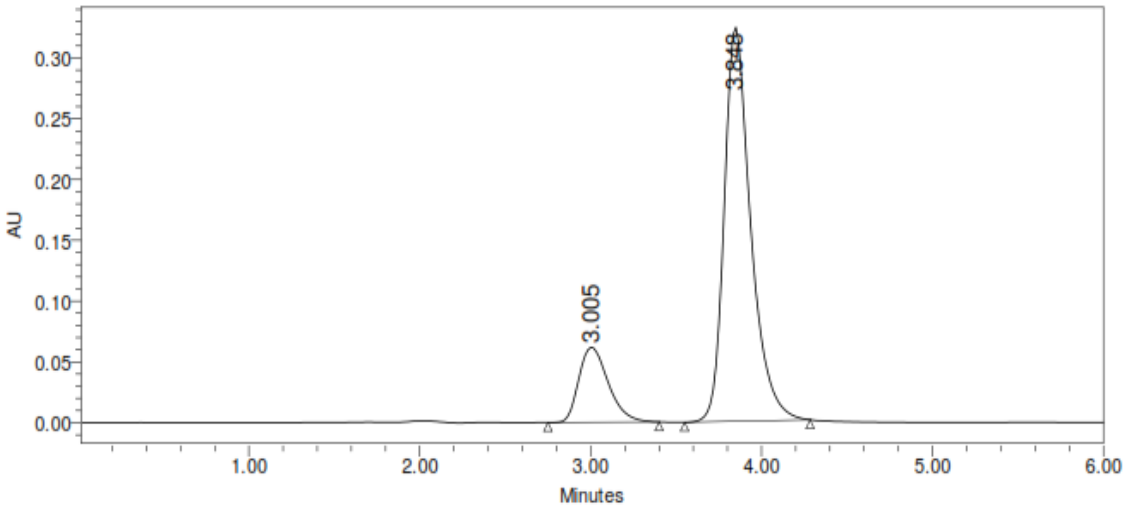

Fig. 2 System suitability.

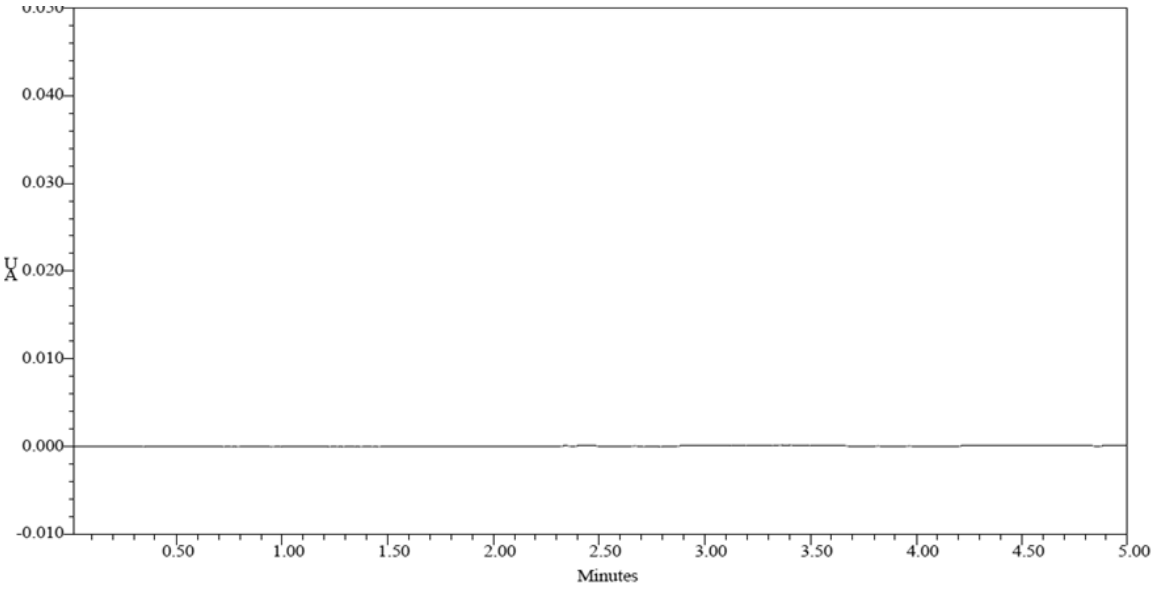

Fig 3: specificity.

Table 3: Linearity study for S-Amlodipine.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	230247
66.6	10	462332
100	15	659905
133.3	20	892989
166.6	25	1101075

Table 4: Linearity study of Nebivolol.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	15	1215225
66.6	30	2135937
100	45	3020839
133.3	60	4078841
166.6	75	5058145

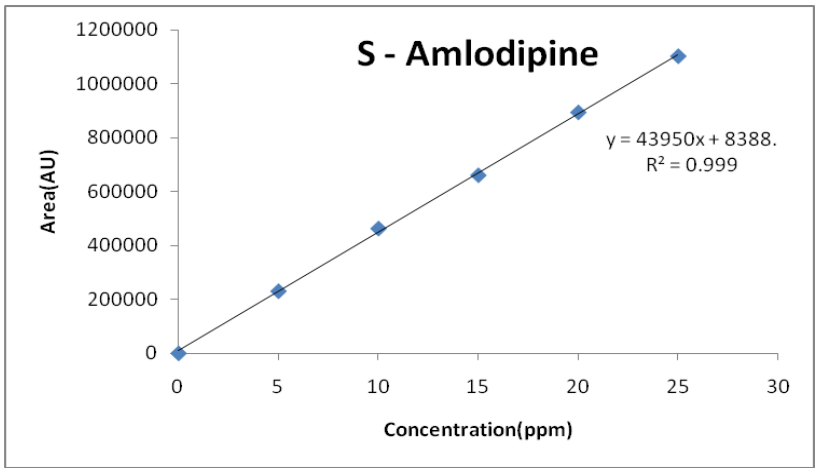

Fig 4: Linearity level of S-Amlodipine.

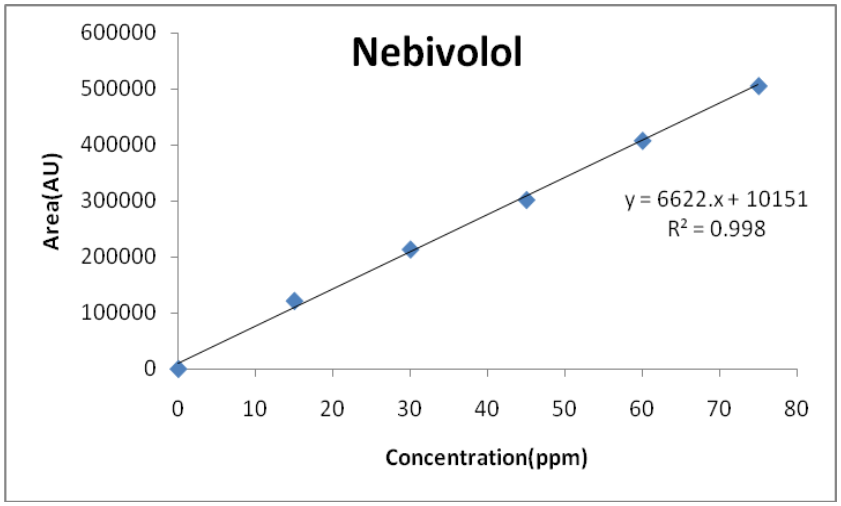

Fig 5: Linearity level of Nebivolol.

CONCLUSION

A simple simultaneous analytical method for S-Amlodipine and Nebivolol has been developed and method was validated as per US FDA guidelines. The proposed methods were found to be simple, accurate, precise and reproducible. It can be applied for the estimation of bioavailability, bioequivalence and pharmacokinetic studies.

ACKNOWLEDGEMENT

The authors are thankful to the chairman **Dr. P. Balraj** and director **P. Ushasree** for providing research facilities and sura labs Hyderabad for providing me the analytical samples.

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