ejpmr, 2018,5(4), 300-307

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL AND S – AMLODIPINE BY RP-HPLC METHOD

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Article Received on 23/01/2018 **Article Revised on 13/02/2018** **Article Accepted on 06/03/2018**

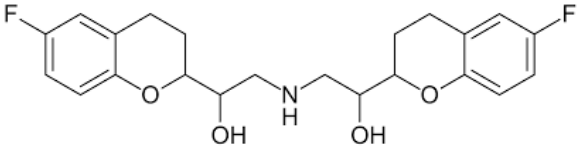
ABSTRACT

The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. A rapid, simple and precise HPLC method was developed for simultaneous estimation of two drugs Nebivolol and S – Amlodipine from pharmaceutical dosage forms. The estimation was carried out using Sunfire C18 (4.6×250mm, 5µ) column; mobile phase consisting of Acetonitrile: Water (40:60 v/v); the flow rate of 0.9mL/min and ultraviolet detection at 220nm. Both the drugs were properly resolved having runtime of 6 min. the method was validated as a final verification of method development with respect to Precision, Linearity, Accuracy, Ruggedness and Robustness. The validated method was successfully applied to the commercially available pharmaceutical dosage forms, yielding very good and reproducible results.

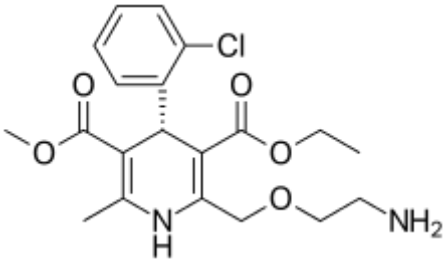
KEYWORDS: Nebivolol, S – Amlodipine, HPLC, Method development and Validation.

INTRODUCTION

Analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy.[1] A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. It should be simple as possible, yet it should allow the use of sophisticated tools such as computer modeling.[2] Reversed-Phase HPLC offers multiple parameters for optimizing a separation. To plan separation by RP-HPLC, the analyst must select both a stationary phase and a mobile phase appropriate to the analyte under investigation.[3] Review of literature for Nebivolol and S - Amlodipine gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.[4,5] The primary objective of proposed work is to develop new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Nebivolol and S - Amlodipine. To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Nebivolol and S - Amlodipine in dosage form.



Nebivolol



S – Amlodipine

Experimental Work

Hplc Method Development[6,7,8]

Preparation of standard solution

Accurately weighed and transferred 10 mg of S - Amlodipine and Nebivolol working standard into a 10ml of clean dry volumetric flask added about 7mL of Methanol, sonicated to dissolve and remove air completely and made volume up to the mark with the same Methanol. Further pipetted 0.15mL of the S - Amlodipine and 0.3mL of the Nebivolol stock solutions into a 10mL volumetric flask and diluted up to the mark with Methanol.

Procedure

Injected the samples by changing the chromatographic conditions and recorded the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 40:60 v/v respectively.

Optimization of Column

The method was performed with various columns like Symmetry, Hypersil and Sunfire C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1mL/min flow.

Optimized Chromatographic Conditions

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Sunfire C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (40:60v/v)
Flow rate : 0.9mL/min
Wavelength : 220nm
Injection volume : 10 µL
Run time : 6min

Validation[9,10,11,12]

Preparation of Mobile Phase

Preparation of mobile phase

Accurately measured 600mL (60%) of Water, 400mL of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio :Acetonitrile:Water 40:60v/v)
Column : Sunfire C18 (4.6×250mm) 5µ
Column temperature : 35ºC
Wavelength : 220nm
Flow rate : 0.9mL/min
Injection volume : 10µL
Run time : 6minutes

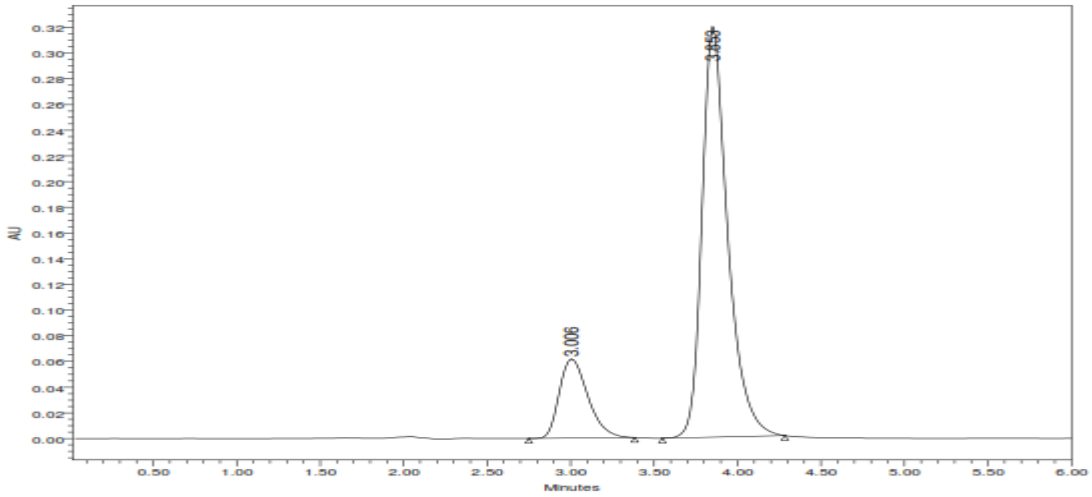


Fig. No. 1: Optimized Chromatogram (Standard).

Table No.1: Optimized Chromatogram (Standard).

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

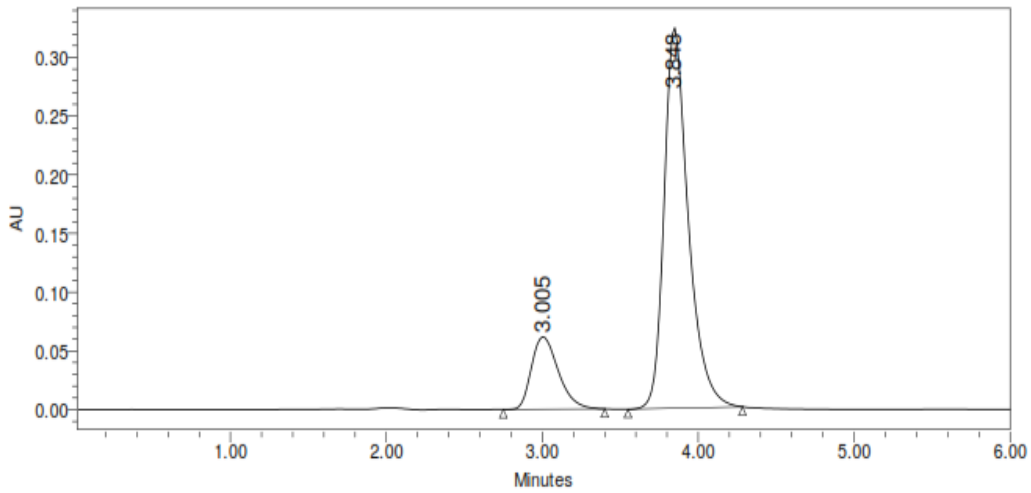


Fig. No. 2: Optimized Chromatogram (Sample).

Table No. 2: Optimized Chromatogram (Sample).

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

Validation

Blank

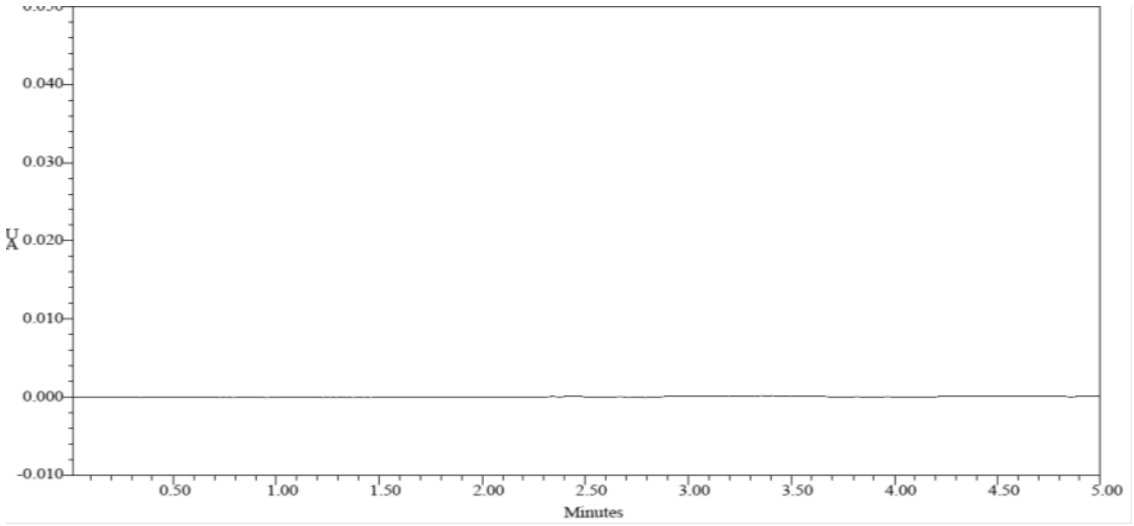


Fig. No. 3: Chromatogram showing blank (mobile phase preparation).

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantities S-Amlodipine and Nebivolol in drug product.

Table No. 3: Peak results for assay standard of S-Amlodipine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	S-Amlodipine	3.008	658263	61335	7462	1.2
2	S-Amlodipine	3.009	658264	61947	8264	1.1
3	S-Amlodipine	3.008	653426	61049	6627	1.2
4	S-Amlodipine	3.010	653058	61141	7264	1.1
5	S-Amlodipine	3.006	657393	61735	6645	1.1
Mean			656080.8			
Std. Dev.			2618.946			
% RSD			0.39918			

Table No. 4: Peak results for assay standard of Nebivolol.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nebivolol	3.857	3028176	381011	9583	1.1
2	Nebivolol	3.859	3018373	381645	8927	1.2
3	Nebivolol	3.857	3018462	381663	8465	1.1
4	Nebivolol	3.861	3081711	381746	9222	1.2
5	Nebivolol	3.853	3075143	381193	8462	1.1
Mean			3044373			
Std. Dev.			31427.07			
% RSD			1.0323			

Table No. 5: Peak results for Assay sample of S-Amlodipine.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	S-Amlodipine	3.008	651712	61173	1.2	8563
2	S-Amlodipine	3.005	657635	61936	1.1	7462
3	S-Amlodipine	3.007	658917	61196	1.1	9264

Table No. 6: Peak results for Assay sample of Nebivolol.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nebivolol	3.854	3029472	361938	1.1	6476
2	Nebivolol	3.853	3017462	361746	1.1	7264
3	Nebivolol	3.855	3028171	371864	1.2	6545

Linearity

Table No. 7: Chromatographic data for Linearity study for S-Amlodipine.

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

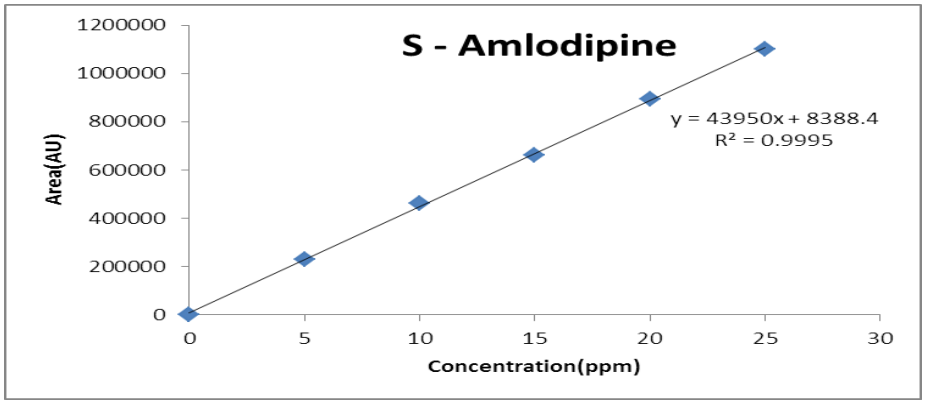


Fig. No. 4: Graph showing linearity level.

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of S-Amlodipine is a straight line. Results are given in Table 7 and 8.

Y = mx + c
Slope (m) =43950
Intercept (c) = 8388
Correlation Coefficient (r) = 0.999

Table No. 8: Chromatographic Data for Linearity study for Nebivolol.

Concentration Level (%)	Concentration µg/mL	Average Peak Area
33.3	10	1215225
66.6	20	2135937
100	30	3020839
133.3	40	4078841
166.6	50	5058145

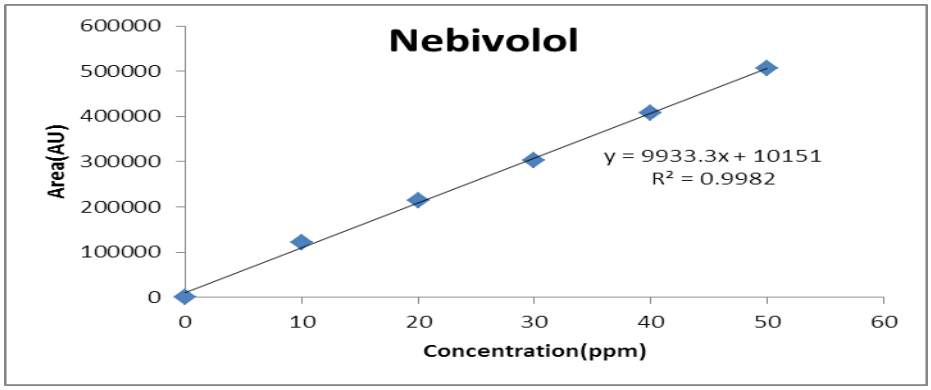


Fig. No. 5: Graph showing linearity level.

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Nebivolol is a straight line.

Y = mx + c

Slope (m) =9933

Intercept (c) = 10151

Correlation Coefficient (r) = 0.999

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Results are given in Table 11 to 14.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD. Results are given in Table 9 and 10.

Table 9: Results of repeatability for S-Amlodipine.

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	S-Amlodipine	3.003	654426	61521	8474	1.1
2	S-Amlodipine	3.005	659862	61937	8262	1.2
3	S-Amlodipine	3.007	650837	62018	8117	1.1
4	S-Amlodipine	3.008	651433	61893	7917	1.2
5	S-Amlodipine	3.005	652752	61867	8011	1.1
Mean			653862			
Std.dev			3626.323			
%RSD			0.554601			

Table No. 10: Results of repeatability for Nebivolol.

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nebivolol	3.851	3028371	381736	6881	1.1
2	Nebivolol	3.852	3009188	380138	9363	1.2
3	Nebivolol	3.854	3067464	386615	7844	1.1
4	Nebivolol	3.853	3076611	380183	9746	1.2
5	Nebivolol	3.851	3011912	379471	7883	1.2
Mean			3038709			
Std.dev			31463.69			
%RSD			1.035429			

Intermediate Precision

Table No. 11: Results of Intermediate precision Day-1 for S-Amlodipine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	S-Amlodipine	3.007	658911	60173	9141	1.1
2	S-Amlodipine	3.005	650383	61936	9662	1.2
3	S-Amlodipine	3.005	658813	60383	9746	1.1
4	S-Amlodipine	3.005	651138	60774	7746	1.1
5	S-Amlodipine	3.005	659937	61947	8264	1.2
6	S- Amlodipine	3.010	653715	61893	7836	1.1
Mean			655482.8			
Std. Dev.			4258.945			
% RSD			0.649742			

Table No. 12: Results of Intermediate precision Day-1 for Nebivolol.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Nebivolol	3.851	3021731	369771	8564	1.1
2	Nebivolol	3.848	3019183	372746	9227	1.1
3	Nebivolol	3.848	3029847	371866	7565	1.2
4	Nebivolol	3.850	3028471	369017	7726	1.1
5	Nebivolol	3.849	3088641	376453	6746	1.2
6	Nebivolol	3.860	3056633	386621	5977	1.1
Mean			3040751			
Std. Dev.			26990.09			
% RSD			0.887613			

Day 2

Table No. 13: Results of Intermediate precision Day- 2 for S-Amlodipine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	S-Amlodipine	3.006	648822	61847	6983	1.1
2	S-Amlodipine	3.008	640863	59882	7728	1.2
3	S-Amlodipine	3.008	643382	60774	9576	1.1
4	S-Amlodipine	3.007	641884	58928	8275	1.2
5	S-Amlodipine	3.007	647822	61483	9837	1.1
6	S-Amlodipine	3.005	649181	60928	8744	1.2
Mean			645325.7			
Std. Dev.			3711.009			
% RSD			0.57506			

Table No. 14: Results of Intermediate precision Day- 2 for Nebivolol.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Nebivolol	3.853	3075833	389911	7039	1.1
2	Nebivolol	3.857	3029583	379019	9857	1.2
3	Nebivolol	3.854	3021991	381875	7881	1.1
4	Nebivolol	3.855	3022485	391099	7902	1.2
5	Nebivolol	3.854	3085833	389222	9285	1.1
6	Nebivolol	3.853	3019482	391184	8955	1.2
Mean			3042535			
Std. Dev.			30022.42			
% RSD			0.986757			

Accuracy

Accuracy at different concentrations (50%, 100% and 150%) were prepared and the % recovery was calculated. Results are given in Table 15 to 19.

Table No. 15: Results of Accuracy for concentration-50%.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	S-Amlodipine	3.006	335352	31861	1.1	8573
2	S-Amlodipine	3.022	336153	39371	1.1	5891
3	S-Amlodipine	3.006	330183	37857	1.2	6573
4	Nebivolol	3.855	1593716	179472	1.1	9164
5	Nebivolol	3.877	1583631	178947	1.2	8264
6	Nebivolol	3.854	1579482	176534	1.1	7248

Accuracy100%

Table No. 16: Results of Accuracy for concentration-100%.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	S-Amlodipine	3.007	657351	61655	1.1	7842
2	S-Amlodipine	3.006	657874	61948	1.1	6018
3	S-Amlodipine	3.005	658292	61183	1.1	7544
4	Nebivolol	3.855	3078171	386641	1.2	8922
5	Nebivolol	3.853	3076144	378656	1.1	9355
6	Nebivolol	3.850	3097262	386521	1.2	8456

Accuracy150%

Table No. 17: Results of Accuracy for concentration-150%.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	S-Amlodipine	3.004	974626	89388	1.1	8462
2	S-Amlodipine	3.006	975411	89749	1.2	9771
3	S-Amlodipine	3.008	970815	88937	1.2	8947
4	Nebivolol	3.847	4598264	436613	1.1	7917
5	Nebivolol	3.851	4589462	439282	1.1	9364
6	Nebivolol	3.853	4501948	437167	1.2	8462

Table No. 18: Accuracy results for S-Amlodipine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	331938	7.5	7.3	99.88	100.166
100%	658274	15	14.7	98.89	
150%	970963	22.5	22.2	101	

Table No. 19: The accuracy results for Nebivolol.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	7.5	7.49	99.7%	99%
100%	420697.7	15	14.9	99%	
150%	631550.7	22.5	22.48	99%	

Limit of Detection for S-Amlodipine and Nebivolol

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Robustness

The robustness was performed for the flow rate variations from 0.8mL/min to 1.0mL/min and mobile phase ratio variation from more organic phase to less organic phase ratio for S-Amlodipine and Nebivolol. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard samples of S-Amlodipine and Nebivolol were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Results are given in Table 20 to 21.

Table No. 20: Results for Robustness -S-Amlodipine.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	658211	3.006	8793	1.2
Less Flow rate of 0.8mL/min	621077	3.441	7269	1.3
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	642190	2.663	9446	1.2
Less organic phase	542402	3.185	8126	1.1
More organic phase	642112	2.867	5854	1.3

Table No. 21: Results for Robustness-Nebivolol.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	3.853	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.426	6328	1.58
More Flow rate of 1.0mL/min	392497	3.415	6217	1.54
Less organic phase	391379	4.291	6996	1.61
More organic phase	391703	3.583	6120	1.50

SUMMARY AND CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Nebivolol and S-Amlodipine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Nebivolol and S-Amlodipine was freely soluble in ethanol, methanol and sparingly soluble in water. Water and Acetonitrile (60:40% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Nebivolol and S-Amlodipine in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

The authors are thankful to the management of St. Mary's College of Pharmacy, Secunderabad and Chaitanya Institute of Pharmaceutical Sciences, Rampur Warangal, for providing laboratory facilities and financial support.

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