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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF BETA BLOCKERS AGENT NEBIVOLOL AND S- AMLODIPINE IN BULK ANDPHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid reversed phase high performance liquid chromatographic (RP-HPLC) method with subsequent validation using ICH suggested approach for the simultaneous determination of Nebivolol and S- Amlodipine in bulk and pharmaceutical dosage form. The chromatographic separation was achieved on Symmetry C18 (4.6mm×150mm, 5.0 µm) particle size analytical column. A mixture of Acetonitrile and Triethyl amine (pH 4.2 adjusted with orthophosphoric acid) and acetonitrile in ratio (40:60 v/v) at flow rate of 1.0ml/min and detector wavelength 275 nm. The retention time of Nebivolol and S-Amlodipine Acid was found to be 2.781 and 4.048 minutes respectively. The validation of the proposed method was carried out for its specificity, linearity, accuracy, precision, limit of detection and quantification for both Nebivolol and S-Amlodipine. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation. The method is useful in the quality control of bulk and pharmaceutical formulations.

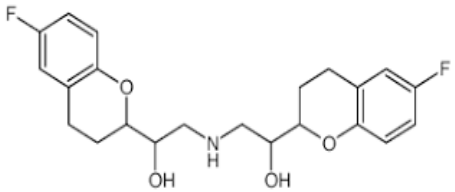
KEYWORDS: Nebivolol and S-Amlodipine, RP-HPLC, Accuracy, Validation.

INTRODUCTION

Nebivolol is a racemic mixture of 2 enantiomers where one is a beta-adrenergic antagonist and the other acts as a cardiac stimulant without beta adrenergic activity.[1] Treatment with nebivolol leads to a greater decrease in systolic and diastolic blood pressure than atenolol, propranolol, or pindolol. Nebivolol and other beta blockers are generally not first line therapies as many patients are first treated with thiazide diuretics.

Nebivolol is a highly selective beta-1 adrenergic receptor antagonist with weak beta-2 adrenergic receptor antagonist activity. Blocking beta-1 adrenergic receptors by d-nebivolol leads to decreased resting heart rate, exercise heart rate, myocardial contracility, systolic blood pressure, and diastolic blood pressure. The selectivity of d-nebivolol limits the magnitude of beta blocker adverse effects in the airways or relating to insulin sensitivity. Nebivolol also inhibits aldosterone, and beta-1 antagonism in the juxtaglomerular apparatus also inhibits the release of rennin.[2] Decreased aldosterone leads to decreased blood volume, and decreased renin leads to reduced vasoconstriction. l-nebivolol is responsible for beta-3 adrenergic receptor agonist activity that stimulates endothelial nitric oxide synthase, increasing nitric oxide levels; leading to vasodilation, decreased peripheral vascular resistance, increased stroke volume, ejection fraction, and cardiac output.[3] IUPAC name is 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. Chemical formula $C_{22}H_{25}F_2NO_4$.Molecular weight 405.

Amlodipine is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers.[4] Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure.[5] The option for single daily dosing of amlodipine is an attractive feature of this drug. IUPAC Name is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5 dicarboxylate; benzenesulfonic acid. Molecular formula is C26H31ClN2O8S. Molecular weight is 567 g/mol. It is slightly soluble in water and sparingly soluble in ethanol.



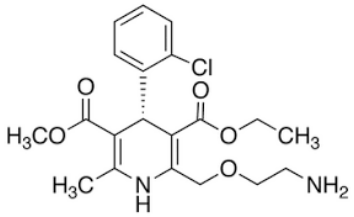


Figure 1: Structure of Nebivolol. Figure 2: Structure of S-Amlodipine.

The literature survey revealed that There are very few methods reported in the literature for analysis of Nebivolol and S-Amlodipine alone or in combination with other drugs in the pure form and pharmaceuticals formulations.[6-19] In view of the need for a suitable, cost-effective HPLC method for routine analysis of Nebivolol and S-Amlodipine Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Nebivolol and S-Amlodipine. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Nebivolol and S-Amlodipine in pharmaceutical dosage form by using HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: Nebivolol and S-Amlodipine were from Mylan laboratories, India. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector**.** Analysis was carried out at 275 nm with column Symmetry C18 (4.6mm×150mm, 5.0 µm), dimensions at 25 ^{0}C temperature. The optimized mobile phase consists of Acetonitrile and Triethyl amine (pH 4.2 adjusted with orthophosphoric acid) and acetonitrile in ratio (40:60 v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions

Preparation of Triethylamine (TEA) buffer (pH-3.8)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.2. Fliter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase

Accurately measured 400 ml (40%) of Acetonitrile and 600 ml of buffer (60%) a 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.

Validation ParametersSystem Suitability

Accurately weigh and transfer 10 mg of Nebivololand 10mg of S-Amlodipineworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.8ml of Nebivololand 2.5ml of S-Amlodipinefrom the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specifiedlimits.

Specificity Study of Drug

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Nebivolol and 10mg of S-Amlodipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.8ml of Nebivololand 2.5ml of S-Amlodipinefrom the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Nebivolol and S-Amlodipine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.8 ml of Nebivolol and 2.5ml S-Amlodipine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient

temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Symmetry C18 (4.6mm×150mm, 5.0 µm), the mobile phase of composition Acetonitrile and Triethyl amine (pH 4.2 adjusted with orthophosphoric acid) and acetonitrile in ratio (40:60 v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1,2.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Nebivolol and S-Amlodipine in their pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-3.

Validation of Analytical method

Linearity: The linearity study was performed for the concentration of 60-100µg/ml of Nebivolol and 187.5-312.5 µg/ml of S-Amlodipine. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 4,5 and figure 6,7.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Nebivolol and S-Amlodipine and calculate the individual recovery and mean recovery values. The results are shown in table 6 & 7.

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 8 & 9.

Intermediate precision: Intermediate precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 10 & 11.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The results are shown in table 12 to 13.

LOD and LOQ: The sensitivity of UPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 14.

LOD = 3.3 (SD/S) and

LOQ = 10 (SD/S), where

SD= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

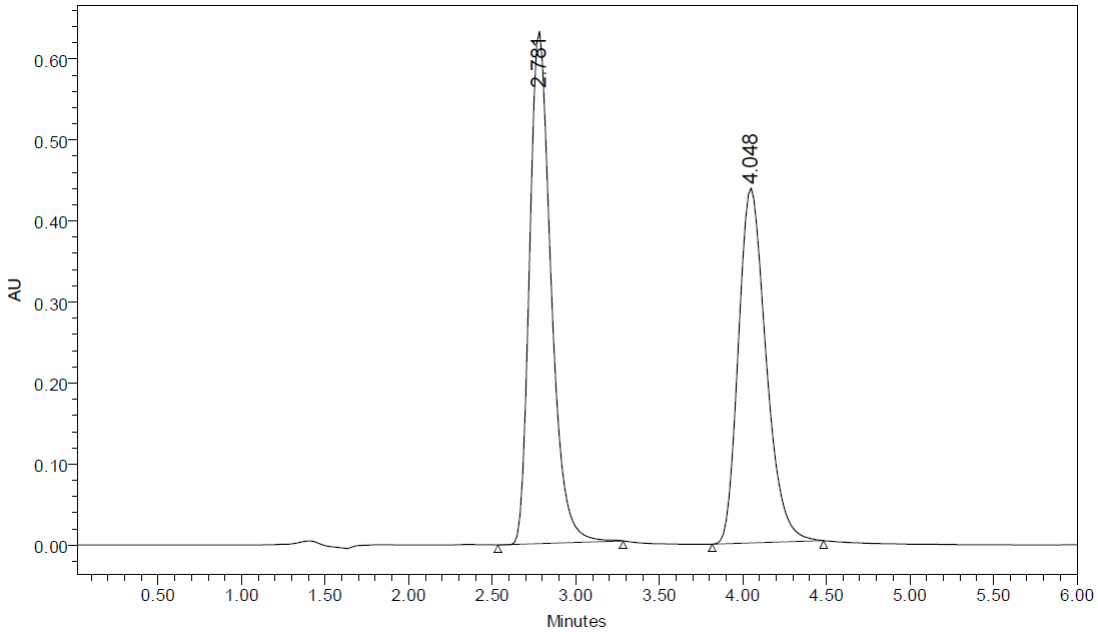


Figure 3: Standard chromatogram.

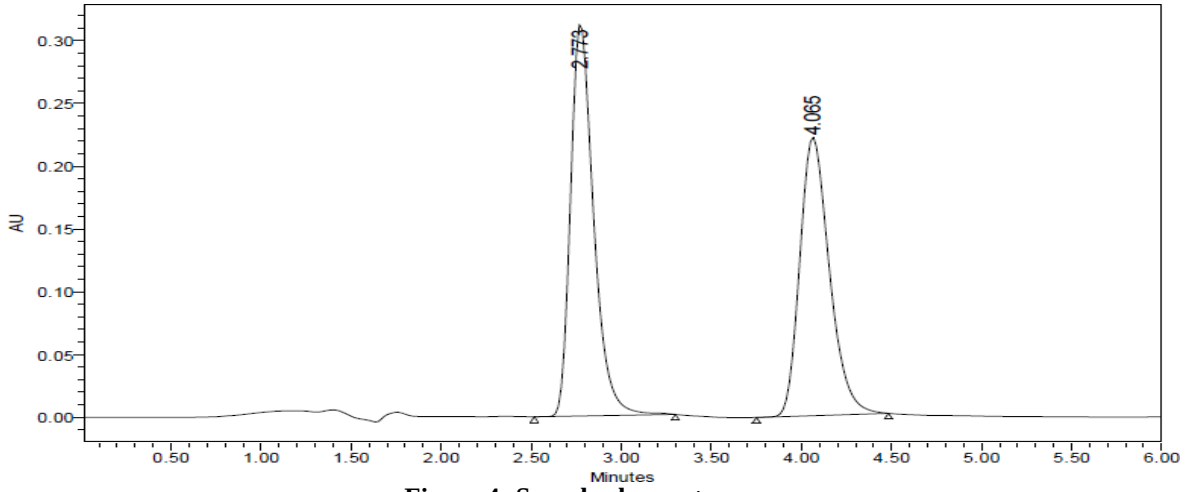


Figure 4: Sample chromatogram.

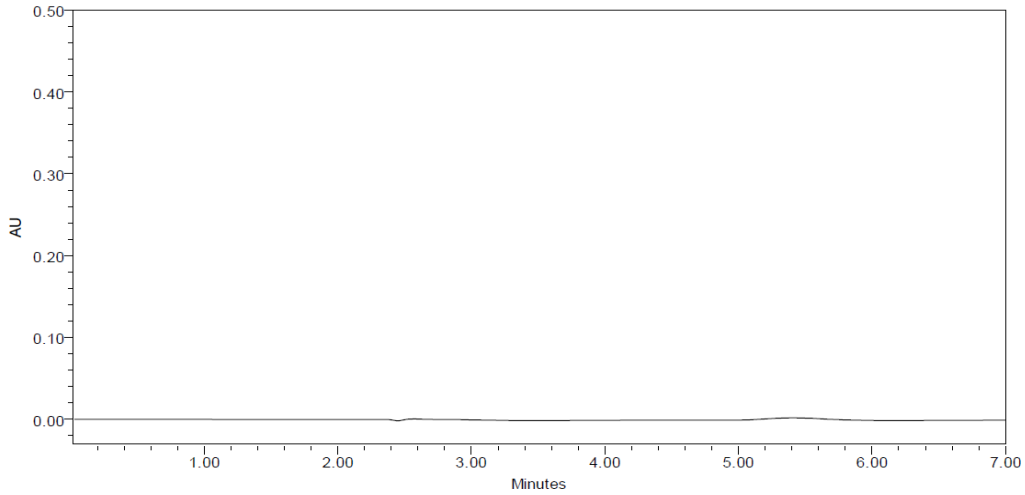


Figure 5: Blank chromatogram.

Table 1: Results of system suitability for Nebivolol.

S.No.	Peak Name	RT	Area (µV*sec)	Height(µV)	USP Tailing	USP PlateCount
1	Nebivolol	2.782	2762937	356859	1.3	6344.7
2	Nebivolol	2.766	2774613	387847	1.3	6368.2
3	Nebivolol	2.767	2762937	399481	1.3	6354.1
4	Nebivolol	2.795	2774613	386985	1.3	6341.7
5	Nebivolol	2.768	2776429	365478	1.3	6347.2
Mean			2770306			
Std. Dev.			6767.495			
V						
% RSD			0.2			

Table 2: Results of system suitability for S-Amlodipine.

S.No	Peak Name	RT	Area (µV*sec)	Height(µV)	USP Resolution	USP Tailing	USP PlateCount
1	S-Amlodipine	4.049	2540214	237854	4.6	1.3	5948.7
2	S-Amlodipine	4.025	2541284	225688	4.7	1.3	5254.8
3	S-Amlodipine	4.029	2534375	215324	4.6	1.3	5948.7
4	S-Amlodipine	4.067	2526189	224859	4.7	1.3	5265.8
5	S-Amlodipine	4.030	2546248	232547	4.7	1.3	5994.7
Mean			2537662				
Std.			7677.647				
% RSD			0.3				

Table 3: Assay results for Nebivolol and S-Amlodipine.

	Label Claim (mg)	% Assay
Nebivolol	5	100.9
S-Amlodipine	2.5	99.5

Table 4: Linearity results of Nebivolol and S-Amlodipine.

Concentration g/ml	Average Peak Area
60	1992464
70	2316364
80	2677423
90	3019213
100	3361317

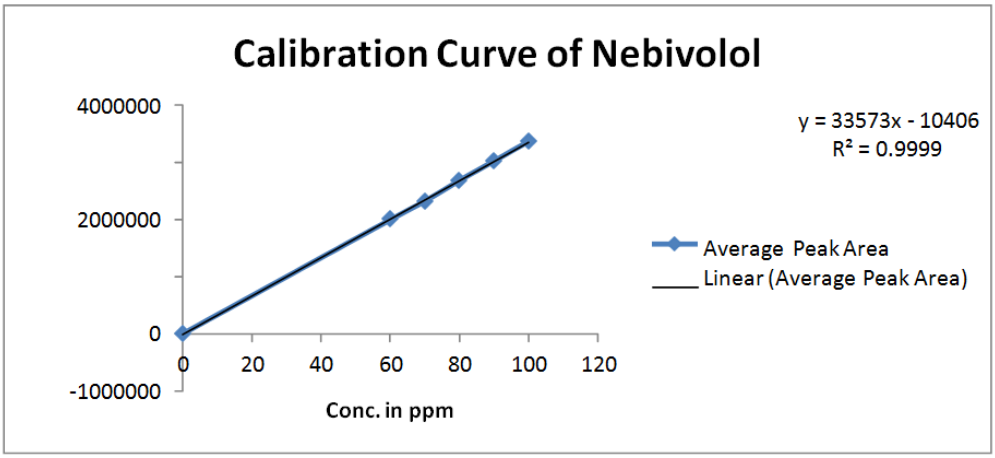


Figure 6: Linearity graph for Nebivolol.

Table 5: Linearity results of S-Amlodipine.

Concentration g/ml	Average Peak Area
187.5	2080032
218.75	2452782
250	2821426
281.25	3226009
312.5	3587393

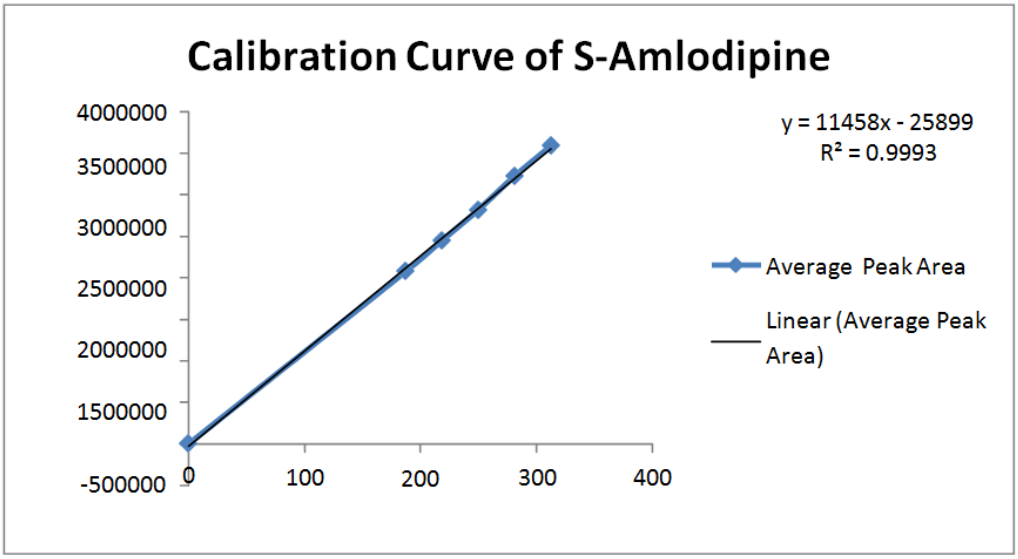


Figure 7: Linearity graph for S-Amlodipine.

Table 6: The accuracy results for Nebivolol.

%Concentration (at specificationLevel)	Area	AmountAdded (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	1361022	40	40.228	100.57	**100.387%**
100%	2698948	80	80.079	100.098	
150%	4059065	120	120.592	100.493	

Table 7: The accuracy results for S-Amlodipine.

%Concentration (at specificationLevel)	Area	AmountAdded (ppm)	AmountFound (ppm)	% Recovery	Mean Recovery
50%	1459598	125	125.126	100.100	100.098%
100%	2894368	250	250.346	100.138	
150%	4325099	375	375.213	100.056	

Precision results for Nebivolol and S-Amlodipine

Table 8: Results of repeatability for Nebivolol.

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Nebivolol	2.108	602223	128898	2586	1.6
2	Nebivolol	2.105	607748	129233	2947	1.4
3	Nebivolol	2.113	607302	127409	2468	1.6
4	Nebivolol	2.109	608674	127047	2146	1.9
5	Nebivolol	2.109	607376	129859	2307	1.7
Mean			606665			
Std. Dev			2542.3			
% RSD			0.42			
5 hr	5	5	5.17	5.24	103.4	104.8
	5	5	5.20	5.18	104	103.6
Mean					104.01	104.31
SD					0.94	1.001
%RSD					0.91	0.96

Table 9: Results of method precession for S-Amlodipine.

Sno	Name	Rt	Area	Height	USP platecount	USP Tailing	USP Resolution
1	S-Amlodipine	4.025	2534539	193240	5761	1.3	4.7
2	S-Amlodipine	4.040	2539247	201647	5489	1.3	4.6
3	S-Amlodipine	4.032	2544661	193472	5367	1.3	4.6
4	S-Amlodipine	4.041	2548839	196475	5845	1.3	4.6
5	S-Amlodipine	4.036	2558822	201394	5347	1.3	4.7
Mean			2545222				
Std. Dev			9329.852				
% RSD			0.3				

Intermediate precision

Table 10: Results of Intermediate precision for Nebivolol.

S no	Name	Rt	Area	Height	USP platecount	USP Tailing
1	Nebivolol	2.781	2715421	296585	6785	1.3
2	Nebivolol	2.780	2778540	284584	6856	1.3
3	Nebivolol	2.782	2754247	275698	6934	1.3
4	Nebivolol	2.780	2780545	282451	6484	1.3
5	Nebivolol	2.782	2777021	283654	6669	1.3
6	Nebivolol	2.774	2780254	296587	6584	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

Table 11: Results of Intermediate precision for S-Amlodipine

S no	Name	Rt	Area	Height	USP platecount	USP Tailing	USP Resolution
1	S-Amlodipine	4.048	2506927	212541	5486	1.4	4.6
2	S-Amlodipine	4.050	2504522	203658	5659	1.4	4.6
3	S-Amlodipine	4.049	2541270	198458	5857	1.4	4.7
4	S-Amlodipine	4.050	2507885	207554	5968	1.4	4.6
5	S-Amlodipine	4.049	2504587	206455	5784	1.4	4.6
6	S-Amlodipine	4.040	2504780	214521	5969	1.4	4.6
Mean			2511662				
Std. Dev			14572.01				
% RSD			0.5				

Robustness results Nebivolol and S-Amlodipine

Table 12: Robustness results of Nebivolol.

Parameter used for sampleanalysis	Peak Area	Retention Time	Theoreticalplates	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

Table 13: Robustness results of S-Amlodipine.

Parameter used for sampleanalysis	Peak Area	RetentionTime	Theoreticalplates	Tailingfactor
Actual Flow rate of 1.0mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5
Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

Table 14: LOD, LOQ of Nebivolol and S-Amlodipine.

S.NO	Drug	LOD (µg/ml)	LOQ (µg/ml)
1	Nebivolol	0.8	2.4
2	S-Amlodipine	0.7	2.19

CONCLUSION

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Nebivolol and S-Amlodipine in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Nebivolol and S-Amlodipine in pure and its pharmaceutical dosage forms.

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