Journal of Comprehensive Pharmacy

Research Article

Available Online at: www.jcponline.in

ISSN NO: 2349-5669

Analytical Method Development and validation for Simultaneous Determination of Atenolol and Nitrendipine in Pharmaceutical Dosage Form by RP-HPLC Method

Prathap Boobalan ^{*,a}, Srinivasa Rao Gorantla ^a, Gobalakrishnan Pandurangan ^b, Venkata Swathikrishna Kavuluru ^a, Priyanka Vadthey ^a

^aDepartment of Pharmaceutical analysis, Saastra College of Pharmaceutical Education & Research, Nellore-524311, Andhra Pradesh, India.

^bDepartment of Pharmaceutical Chemistry, Vagdevi College of Pharmacy and Research Centre, Nellore-524346, Andhra Pradesh, India..

ARTICLE INFO

Article history:

Received 12 December 2015

Accepted 22 January 2016

Available online 27 February 2016

*Corresponding author:

Prathap Boobalan

Email:

prathapnila@gmail.com

Tel.:+91-8125974910.

ABSTRACT

A simple, specific, precise, accurate, rapid and reproducible efficient reversed phase HPLC method has been developed and validation for simultaneous determination of atenolol and nitrendipine in pharmaceutical dosage form. Chromatography was performed on a 250mm X 4.6mm, 5µm particle size, Phenomenox Luna C18 column with a 40:40:20 v/v/v mixture of methanol: acetonitrile: water and pH was adjusted to 3.0 with ortho-phosphoric acid as a mobile phase. The detection of the combined dosage form was carried out at 235nm and flow rate employed was 1.5ml/min. The retention times were 2.621 and 5.169 min for atenolol and nitrendipine respectively. Linear was established in the concentration range of 30.0 to 70.0µg/ml for atenolol and 6.0 to 14.0µg/ml for nitrendipine with a correlation coefficient for 0.9989 for atenolol and 0.9992 for nitrendipine respectively. The percentage recoveries obtained were 99.52 -100.01% for atenolol and 99.75 - 100.71% for nitrendipine. Similarly the %RSD value for precision was also found to be within the acceptable limit. The method was validated according to international conference of harmonization guidelines in terms of accuracy, precision, specificity, robustness, linearity and other aspects of analytical validation. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method. Developed method was rapid and convenient which could be successfully applied for the routine control of both the component.

KEY WORDS: RP-HPLC, Atenolol, Nitrendipine, ICH

INTRODUCTION

Atenolol is a competitive β_1 -selective adrenergic receptor blocking agent without membrane stabilizing or intrinsic sympathomimetic activities. Atenolol inhibits β_2 -adrenoreceptors, chiefly located in the bronchial and vascular musculature [1]. Chemically it is known as 2-[4-2(RS)-2-hydroxy 3[(1-methylethyl) amino] propoxyl] phenyl] acetamide. It is a white powder and molecular weight is 266.3 and freely soluble in methanol and soluble in acetic acid and dimethyl sulfoxide Figure [1]. Atenolol is also used to

treat myocardial infarction and arrhythmias, angina and disorders arising from decreased circulation and vascular constriction, including migraine. Atenolol may be used alone or with other antihypertensive agents including thiazide-type diuretics, hydralazine, prazosin, and α -methyldopa [2].

Nitrendipine is a dihydropyridine calcium channel blocker, is used alone or with an angiotension converting enzyme inhibitor, to treat hypertension, chronic stable angio pectoris and Prinzmetal's variant angina. Chemically it is Ethyl methyl (4RS) -2,6-

dimethyl-4-(3-nitrophenyl)- 1, 4-dihydropyridine-3,5-dicarboxylate. It is a yellow crystalline powder and molecular weight is 360.3 and freely soluble in ethyl acetate, soluble in ethanol and methanol [3] Figure [2].

Figure 1: Chemical Structure of Atenolol

Figure 2: Chemical Structure of Nitrendipine

Literature survey revealed that several methods have been reported estimation of Atenolol and Nitrendipine individually or in combination with other drugs in pharmaceutical dosage forms and/or in biological fluids [4-20]. However, no HPLC method has been reported so far for the estimation of these two drugs simultaneously in combined dosage forms. Hence, in the present study, a new reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Atenolol and Nitrendipine in tablets. The method was validated for accuracy, precision, specificity, detection limit, quantitation limit, robustness and solution stability as per ICH guidelines [21]. Present work describes rapid, accurate, reproducible, and economical methods for simultaneous determination of these drugs in pharmaceutical dosage form.

MATERIALS AND METHODS

Reagents and Chemicals Used:

HPLC grade acetonitrile and methanol was procured from Rankem (Mumbai, India) and Millipore water obtained from (Milli Q) was used in all experiments. Ortho phosphoric acid was procured from Qualigens fine chemicals, (Mumbai, India). Pharmaceutical dosage form containing labeled amount of Atenolol (50mg) and Nitrendipine (10 mg) (Cardif beta-10, Concept

Pharmaceuticals Pvt. Ltd.) was purchased from the local pharmacy.

Instrumentation and Conditions:

The chromatographic separation was performed by using Shimadzu LC 2010 AHT HPLC system with UV-Visible detector and Rheodyne injector. Chromatographic analysis was performed on a Phenomenex Luna C-18 with 250×4.6 mm and 5 μm particle size. The mobile phase was composed of mixture of acetonitrile, methanol and water in the ratio of 40:40:20 v/v v (pH adjusted to 3.0 with orthophosphoric acid). It was filtered through a 0.45 μm membrane filter and degassed for 10 minutes. Before analysis the mobile phase was filtered through a 0.2 μm filter and degassed using sonicator at the flow rate of 1.0 ml per minutes. Sample solutions were also filtered through a 0.2 μm filter and aliquots of 10 μL were injected into the chromatographic system. The flow rate of the mobile phase was maintained at 1.5 ml/min. Detection was carried out at 235 nm [Figure 3]. The HPLC system was used in air-conditioned laboratory atmosphere temperature (25±2°C).

Figure 3: UV overlay spectra of Atenolol (a) and Nitrendipine (b) in methanol

Preparation of mobile phase:

The mobile phase was prepared by mixing of methanol: Acetonitrile: water in the ratio of 40:40:20 v/v/v and it is filtered and degassed. The Chromatogram was obtained is shown in [figure-4]

Preparation of Standard Solution:

50mg of Atenolol and 10mg of Nitrendipine working standard was weighed accurately into a 100ml volumetric flask, dissolved with small quantity of methanol (10ml) and was made up to volume with mobile phase. Solution was filtered through 0.45 μm membrane filter. First few ml of the filtrate was discarded. 5ml of stock solution was pipette out and transferred into a 50ml volumetric flask and was made up to the volume with mobile phase.

Preparation of Sample Solution:

Twenty tablets were weighed accurately and powdered. Powder equivalent to 50mg of atenolol was weighed

Figure 4: Chromatogram for Mobile Phase (Blank)

Figure 5: Chromatogram for Placebo

Figure 6: Chromatogram for Atenolol and Nitrendipine

and transferred to 100ml volumetric flask and dissolved in small quantity of methanol (10ml) by sonicating the flask for 15 mins and was made up to volume with mobile phase. The solution was filtered through 0.45 μ m membrane filter. Take 5ml of the above filtrate and diluted to 50ml with mobile phase.

Optimization of Chromatographic Conditions:

The initial literature search indicated that many UV, HPLC and HPTLC methods are available for individual drugs and their combination with different drugs or formulations. Based on literature search, attempts were made to develop a simple method which has less retention time and high selectivity, top priority was given for complete separation of Atenolol and Nitrendipine. Several mobile phase were tested until good resolution, theoretical plate and tailing factors obtained between two drugs.

In preliminary experiments all the two drugs i.e., Atenolol and Nitrendipine were subjected to separation by reverse phase HPLC equipped with the Phenomenex Luna C-18 (250mm X 4.6 mm X 5 μ m) column and with flow rate 1.5mL/min and detection wavelength of 235nm. Column temperature was maintained at ambient. Injection volume is 20 μ L.

The mobile phase consists of buffer pH 4, acetonitrile and methanol (35:30:35% v/v/v). These drugs were able to be separated on the chromatogram but peak shape was not good and broad, retention time was more and tailing was more than specified limit. The effect of mobile phase composition was checked with different ratio and different composition and different solvents. It improved peak purity and peak shape and its pass all the system suitability parameters. Finally a method developed with methanol: acetonitrile: water (40:40:20% v/v/v). The chromatogram obtained was better than the previous one in all aspects with good peak shape, tailing factor, resolution and theoretical plate as per ICH and USP requirement. The retention times of Atenolol and Nitrendipine peaks are about 2.621 and 5.169 minutes respectively. The chromatograms were shown in the [Figure 6].

RESULTS:

Validation:

The method was successfully validated as per ICH guideline Q2B (R1): validation of analytical procedures: text and methodology, international conference on harmonization, Food and Drug Administration, USA, November 2005. The method was validated and parameters were linearity, range, accuracy, precision, LOQ, LOD, robustness, system suitability and solution stability.

Specificity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. The comparison of the chromatograms of the synthetic placebo mixture and the spiked drug solution revealed that there was no interference of placebo with the peaks of Atenolol and Nitrendipine in sample solution. No interference from placebo was observed at the retention time of the drugs. Therefore, it was concluded that the method is specific. The Chromatogram was shown in [Figure 5].

Linearity:

Appropriate aliquots of standard stock solutions of Atenolol and Nitrendipine were diluted mobile phase to obtain final concentrations in the range of 30-70 μ g/ml of Atenolol and 6-14 μ g/ml of Nitrendipine. The solutions were injected in triplicates for each concentration using a 20 μ L fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average content of the drug versus respective concentrations and regression equations were computed for Atenolol and Nitrendipine. The plots of average content Vs respective concentration of Atenolol and Nitrendipine were found to be linear in the range of 30-70 μ g/ml and 6-14 μ g/ml with coefficient of correlation 0.9989 and 0.9992 for Atenolol and Nitrendipine respectively. The data of linearity curve was summarized in the Tables 1, 2 and [Figures 7 and 8].

Table 1: Linearity data for Atenolol

S. No.	Concentration	Peak Area
1	30 μ g/ml	1218292
2	40 μ g/ml	1723148
3	50 μ g/ml	2157782
4	60 μ g/ml	2589426
5	70 μ g/ml	3021724
Correlation Coefficient (R^2)		0.9989

Table 2: Linearity Date for Nitrendipine

S. No.	Concentration	Peak Area
1	6 μ g/ml	519916
2	8 μ g/ml	667884
3	10 μ g/ml	847280
4	12 μ g/ml	1017826
5	14 μ g/ml	1188728
Correlation Coefficient (R^2)		0.9992

Figure 7: Linearity graph for Atenolol

Figure 8: Linearity graph for Nitrendipine

Precision:

To check the system precision (repeatability) for peak response obtained with five replicates of standard at specified concentration. The %RSD found to be within 2.0%. To check repeatability (method precision) of the method six individual sample preparations from same batch were prepared and injected the % RSD with six samples found to be within 2.0%. The results obtained were presented in Table 6 and 7.

Table 3: Data for Limit of Detection and Quantitation

Parameters	Atenolol	Nitrendipine
L.O.D. ($3.3 \times \sigma/m$)	1.964 µg/ml	0.342 µg/ml
L.O.Q. ($10 \times \sigma/m$)	5.952 µg/ml	1.036 µg/ml

Accuracy:

The accuracy of an analytical method is established across its range. Accuracy is performed in three different levels for Atenolol and Nitrendipine. The known quantity of Atenolol and Nitrendipine at 80%, 100% and 120% level is analysed for each level. The % recovery values for these drugs were found to be in between 99.52% to 100.71% and %RSD values were found to be less than 2.0%. The accuracy results were tabulated in the Table 4 and 5.

LOD and LOQ:

These methods were evaluated on the basis of signal-to-noise ratio between 3 or 2:1 is generally considered

acceptable for estimating the detection limit. A typical signal-to-noise ratio required for LOQ is 10:1. According to a formula given by Miller, the limit of detection (LOD) and limit of quantification (LOQ) were calculated. The results are given in Table 3.

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness was done by changing the flow rate (± 0.2 ml/min) and the Wave length (± 20 nm). The results obtained were presented in Table 8 and 9.

System Suitability:

According to USP system suitability tests are an integral part of chromatographic method validation. The tests were used to verify that the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness system suitability tests were carried out on freshly prepared standard solution. 20 µL of solution was injected into the optimized chromatographic system. For system suitability six replicates of working standard samples were injected and the parameters like retention time (RT), theoretical plate (N), peak area, tailing factor and resolution of sample were calculated. These results are presented in Table 11.

Solution Stability:

Solution stability study of Atenolol and Nitrendipine was done by preparing sample and standard solution (Initial, 24Hr and 48Hr). It was done to check whether it was stable up to given hours. The results are shown in Table 10.

DISCUSSION:

To optimize the mobile phase various proportions of buffers with methanol and acetonitrile were tested. Mobile phase composition was changed and the method development was started by Phenomenex Luna C-18 (250mm X 4.6 mm X 5 µm) column and with flow rate 1.5 mL/min and detection wavelength of 235 nm. Column temperature was maintained at ambient. Injection volume is 20 µL. The mobile phase consists of methanol: acetonitrile: water (40:40:20% v/v/v) pH 3.0 was adjusted with orthophosphoric acid was used. The retention times of Atenolol and Nitrendipine peaks are about 2.621 and 5.169 minutes respectively.

Quantitative linearity was observed over the concentration range of 30-70 µg/ml of Atenolol and 6-14 µg/ml of Nitrendipine. The regression equations of concentration of Atenolol and Nitrendipine are found to be $y = 44731x - 94497$ and $y = 84378x + 4543.8$ of

Table 4: Recovery Data for Atenolol

Level	Peak Area	% Recovery	Mean Recovery	S.D.	% RSD
80%	1701948	99.136	99.865	0.691	0.692
	1715893	99.948			
	1725574	100.512			
100%	2135756	99.057	99.525	0.691	0.573
	2159557	100.161			
	2142193	99.356			
120%	2558129	99.251	100.014	0.665	0.665
	2589522	100.469			
	2585762	100.323			

Table 5: Recovery Data for Nirendipine

Level	Peak Area	% Recovery	Mean Recovery	S.D.	% RSD
80%	654681	99.350	100.460	0.995	0.990
	663958	100.758			
	667352	101.273			
100%	858422	101.603	100.714	0.770	0.764
	846931	100.242			
	847384	100.296			
120%	1029824	100.667	99.750	0.807	0.809
	1014218	99.141			
	1017312	99.444			

Table 6: Repeatability Data for Atenolol and Nitrendipine

S. No	Atenolol		Nitrendipine	
	Peak Area	Amount (%)	Peak Area	Amount (%)
1	2156778	100.42	844122	100.97
2	2154088	100.29	839816	100.45
3	2157194	101.44	832488	99.57
4	158254	101.49	831982	99.51
5	2154682	100.32	838819	100.33
6	2118996	98.65	824978	98.67
R.S.D	0.710		0.822	

Table 7: Data for Intermediate precision (Day to Day analysis)

S. No	Atenolol		Nitrendipine	
	Day 1	Day 2	Day 1	Day 2
1	100.51	101.38	101.08	101.26
2	100.33	100.59	100.46	100.88
3	99.63	101.10	99.34	100.56
4	100.53	101.38	99.62	100.68
5	100.42	101.27	100.25	99.35
6	100.02	100.07	99.58	100.03
R.S.D	0.351	0.539	0.661	0.675

Table 8: Data for robustness - Changes in flow rate

Conditions		Atenolol			Nitrendipine		
Parameters	AUC	USP Tailing	R.S.D	AUC	USP Tailing	R.S.D	
1.3 ml/min	2456250	1.215	0.285	954340	1.154	0.195	
1.7 ml/min	1894820	1.218	0.226	748793	1.157	0.373	

Table 9: Data for robustness Changes in Wavelength

Conditions		Atenolol			Nitrendipine		
Parameters	AUC	USP Tailing	R.S.D	AUC	USP Tailing	R.S.D	
230 nm	2024016.667	1.219	0.263	854352.666	1.157	0.449	
240 nm	2215540	1.222	0.874	792688	1.157	0.191	

Table no 10: Data for Sample solution stability for atenolol and nitrendipine

Time	Atenolol		Nitrendipine	
	AUC	%Assay	AUC	%Assay
Initial	2141458	100.528	822782	100.480
24hrs	2140682	100.491	811536	99.107
48hrs	2117894	99.421	804888	98.295
SD	13386.29	0.628	9044.92	1.104
%RSD	0.6274		1.112	

Table 11: Data for System suitability for atenolol and nitrendipine

Drugs	RT (min)	AUC	No. of Theoretical plates (n)	Tailing factor
Atenolol	2.622	2158821.33	5489.67	1.222
Nitrendipine	5.162	846381.66	4069.35	1.160

Atenolol and Nitrendipine respectively, where y is the peak area and x is the concentration of drugs ($\mu\text{g/mL}$). The correlation coefficient of Atenolol and Nitrendipine was found to be 0.9989 and 0.9992 respectively.

The numbers of theoretical plates obtained were 5489.67 and 4069.35 for Atenolol and Nitrendipine respectively which indicates the efficiency of the column. The high percentage recovery indicates that the proposed method is highly accurate. There is no interference of filters with standard and sample solutions as the difference in responses is within the limit. The %RSD was found to be less than 2.0%.

CONCLUSION:

A simple, specific, accurate, precise, reproducible and efficient reverse phase high performance liquid chromatography method has been developed which can be used accurately for quantitative estimation of Atenolol and Nitrendipine for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R2) so it can be used by analytical department.

REFERENCE:

1. Physicians Desk Reference-PDR, 51st ed, Montvale, NJ: Medical Economics Company, Inc; 1999:1548.
2. Wadworth AN, Murdoch D, Brogden RN. Atenolol. A reappraisal of its pharmacological properties and therapeutic use in cardiovascular disorders. *Drugs*. 1991;42(3):468-510.
3. Goodman and Gillman; The Pharmacological Basis of Therapeutics. New York, MacMillan, 1996:767-774.
4. Kumar N, Verma N, Singh O, Singh KG. Estimation of atenolol by reverse phase high Performance liquid chromatography. *E-Journal of Chem*. 2010;7(3):962-966.
5. Sridharan D, Thenmozhi A, Sundaranandavalli S. Bioanalytical method development and validation of atenolol in human plasma by LC-MS. *Asian J Pharm Clin Res*. 2010;3(2):93-94.
6. de Abreu LR, de Castro SA, Pedrazzoli J Jr. Atenolol quantification in human plasma by high-performance liquid chromatography: application to bioequivalence study. *AAPS Pharm Sci*. 2003; 5(2):E21.
7. Barman RK, Islam MA, Ahmed M, Ibne Wahed MI, Islam R, Khan A et al. Simultaneous high-performance liquid chromatographic determination of atenolol and amlodipine in pharmaceutical-dosage form. *Pak J Pharma Sci*. 2007;20(4):274-279.
8. Argekar AP, Powar SG. Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography. *J Pharm Biomed Anal*. 2000;21(6):1137-1142.
9. Choudhari VP, Suryawanshi VM, Mahabal RH, Deshchougule SG, Bhalerao KP, Kuchekar BS. Simultaneous spectrophotometric estimation of atenolol and lercandipine hydrochloride in combined dosage form by ratio derivative and dual wavelength method. *Int J of Pharm Sci Rev Res*. 2010;3(1):73-76.
10. Kavitha J, Muralidharan S. Determination and validation of new method for atenolol, hydrochlorothiazide and losartan potassium by RP-HPLC: Its application to routine quality control analysis. *Inter J Chem Tech Res*. 2010;2(2):880-884.
11. Santhana Lakshmi K, Lakshmi S. Simultaneous Analysis of Losartan potassium, Amlodipine Besylate, and Hydrochlorothiazide in Bulk and in Tablets by High-Performance Thin Layer Chromatography with UV-Absorption Densitometry. *J Anal Methods Chem*. 2012;2012:108281.
12. Sivakumar T, Venkatesan P, Manavalan R, Valliappan K. Development of a HPLC method for the simultaneous determination of losartan potassium and atenolol in tablets. *Indian J Pharm Sci*. 2007;69(1):154-157.
13. Gupta P, Issa C, Bansal AK. Simultaneous determination of atenolol and furosemide in intestinal perfusion samples by validated reversed-phase high-performance liquid chromatography. *Indian J Pharm Sci*. 2005;67(6):672-676.
14. Marinkovic V, Agbaba D, Vladimirov S, Stankovic S. Simultaneous HPLC determination of nitrendipine and impurities of the process of synthesis. *J Pharm Biomed Anal*. 2001;24(5-6):993-998.
15. Oh SY, Kim KY, Kim YG, Kim HG. High-performance liquid chromatographic analysis of nitrendipine in human plasma using ultraviolet detection and single-step solid-phase sample preparation. *J Pharm Biomed Anal*. 2003;32(2):387-392.
16. Kowalczyk D. Determination of nitrendipine in tablets by HPTLC-densitometry. *J Planar Chromatogr Mod TLC*. 2006;19(108):135-138.
17. Baranda AB, Jimenez RM, Alonso RM. Simultaneous determination of five 1,4-dihydro pyridines in pharmaceutical formulations by high-performance liquid

- chromatography-amperometric detection. J Chromatogr A. 2004;**103(1-2)**:275-280.
- 18.** Pastera J, Mejstrikova L, Zoulova J, Macek K, Kvetina J. Simultaneous determination of nitrendipine and one of its metabolites in plasma samples by gas chromatography with electron-capture detection. J Pharm Biomed Anal. 2007;**44(3)**:674-679.
- 19.** Baranda AB, Etxebarria N, Jimenez RM, Alonso RM. Development of a liquid-liquid extraction procedure for five 1,4-dihydropyridines calcium channel antagonists from human plasma using experimental design. Talanta. 2005;**67(5)**:933-941.
- 20.** Argekar AP, Sawant JG. Simultaneous determination of atenolol and nitrendipine in pharmaceutical dosage form by HPTLC. J Liquid Chrom Related Tech. 1999;**22(10)**:1571-1578.
- 21.** ICH, Q2B- Validation of Analytical Procedure, International Conference on Harmonization, November, 1996.

Cite this article as: Boobalan P, Gorantla SR, Pandurangan G, Kavuluru VS, Vadthey P. Analytical Method Development and validation for Simultaneous Determination of Atenolol and Nitrendipine in Pharmaceutical Dosage Form by RP-HPLC Method. J Compr Phar 2016;3(1):14-22.