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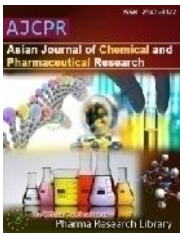

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Atenolol and Nitrendipine by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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A B S T R A C T

A new method was established for simultaneous estimation of Atenolol and Nitrendipine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Atenolol and Nitrendipine by using Phenomenox Luna C_{18}Column, 5µ (250 × 4.6mm), flow rate was 1.5ml/min, mobile phase ratio was Methanol : Acetonitrile : Water (40 : 40 : 20) pH was adjusted to 3.0 with Orthophosphoric acid, detection wave length was 235nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.621 mins and 5.169 mins. From the Linearity study, it was found that, the drug obeys beer's law and the specified range for estimation of Atenolol and Nitrendipine was found to be 60% to 140%. Correlation coefficient was found to be R^2=0. 0.9989 and R^2=0.9992 respectively for Atenolol and Nitrendipine. Limit of Detection and Quantitation for Atenolol was found to be 1.964 µg/ml and 5.952 µg/ml respectively. Limit of Detection and Quantitation for Nitrendipine was found to be 0.342 µg/ml and 1.036 µg/ml respectively. Precision RSD was found to be 0.710 for Atenolol and 0.822 for Nitrendipine. Whereas Intermediate Precision RSD was found to be 0.445 for Atenolol and 0.668 for Nitrendipine. The precision was found to be within the specified limit which indicates that the method is precise. From the accuracy study, it was found that recovery value of pure drug from the solution were between 98.0 % to 102% which indicates that the method is accurate. RSD with decreased flow rate of mobile phase for Atenolol was 0.285% and for Nitrendipine it was 0.195%. While with increased flow rate of mobile phase RSD for Atenolol was 0.226% and for Nitrendipine it was 0.373 %. RSD with decreased Wavelength of Detector for Atenolol was 0.263% and for Nitrendipine it was 0.449%. While with increased Wavelength of Detector RSD for Atenolol it was 0.874 % and for Nitrendipine it was 0.191%. In both conditions the R.S.D. was less than 2%.

Keywords: Atenolol, Nitrendipine, HPLC

A R T I C L E I N F O

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1. Introduction

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

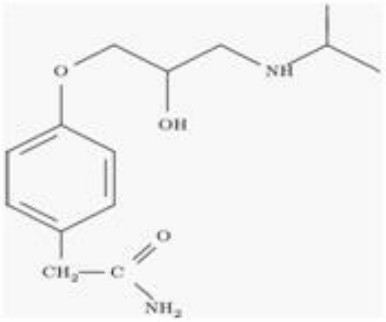

Figure 1: Atenolol

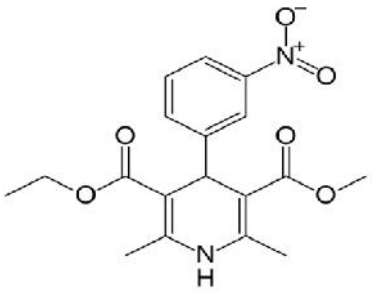

Figure 2: Nitrendipine

2. Materials and Methods

Apparatus:

The instrument used for the study was WATERS, software: Empower, 2695 separation module, PDA detector.

Materials: The solvents used were Ammonium acetate, Orthophosphoric acid, Methanol, Acetonitril & Water [10].

Selection of detection wavelength:

Absorption maxima (λmax) for atenolol was observed at 224 nm where as for nitrendipine it was observed at 238 nm. Here both spectra were coincided at 235 nm (Isobestic Point), hence suitable wavelength for detection of atenolol and nitrendipine was 235 nm [11].

Selection of mobile phase

Methanol: Acetonitrile: Water (50: 30: 20) pH was adjusted to 3.0 with Orthophosphoric acid. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation [12]. Good Response, Area, Tailing factor, Resolution will be achieved.

Optimization Chromatographic trials

Optimization chromatographic conditions

Column : Phenomenox Luna C18Column, 5µ(250× 4.6mm)
Mobile phase: Methanol : Acetonitrile : Water (40:40:20)
Detection wavelength : 235nm
Flow rate : 1.5ml/min
Injection volume : 20µl Column
Temperature : Ambient

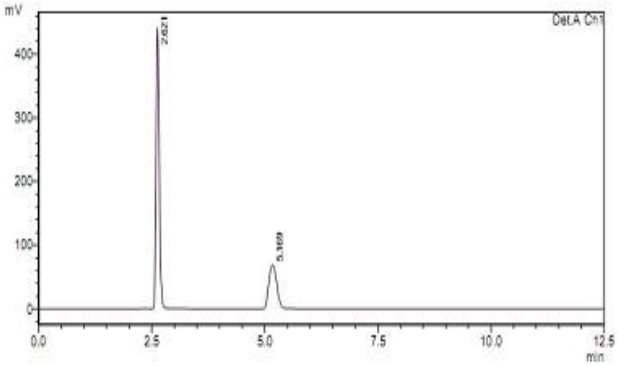

Figure 3: Optimization Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Procedure

Standard Preparation:

50mg of Atenolol and 10mg of Nitrendipine working standard was weighed accurately into a 100ml volumetric flask, dissolved with small quantity of methanol and was made up to volume with mobile phase. Solution was filtered through 0.45 µ membrane filter. First few ml of the filtrate was discarded. 5ml of stock solution was pipetted out and transferred into a 50ml volumetric flask and was made up to the volume with mobile phase [13].

Preparation of sample solution:

Twenty tablets were weighed accurately and powdered. Powder equivalent to 50mg of Atenolol was weighed and transferred to 100 ml volumetric flask and dissolved in small quantity of methanol by sonicating the flask for 15mins and was made up to volume with mobile phase. The solution was filtered through 0.45µ membrane filter [14]. Take 5ml of above filtrate and diluted to 50ml with mobile phase.

3. Results and discussion

Method Validation Parameters

1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak [15]. The specificity was performed by injecting blank [16].

2. Linearity

The linearity study was performed for the concentration of 30 µg/ml to 70 µg/ml of Atenolol and 6µg/ml to 14µg/ml of Nitrendipine was prepared. Each level was injected into chromatographic system [17]. The area of each level was used for calculation of correlation coefficient.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

The linearity study was performed for concentration range of 30µg - 70µg and 6µg-14µg of Atenolol and Nitrendipine and the correlation coefficient was found to be 0.999[18].

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there [19].

a) Standard addition method [20]**:**

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration

Percentage method:

For these assay method samples are prepared in three concentrations of 80%, 100%, and 120% respectively.

Acceptance criteria: The mean % recovery of the Ezetimibe and Simvastatin at each level should be not less than 95.0% and not more than 105.0%.

5.Precision: The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day [21]. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported

Repeatability:

Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration

Intermediate Precision

Intermediate Precision is demonstrated by carrying out the complete experiment by the different analyst on different days, on different instruments in the same laboratory. The concentrations of 100% Test solution prepared as given in following procedure

Recovery studies

Sample solutions at different concentrations (80%, 100%, and 120%) were prepared and the % recovery was calculated [25].

Detection limit: The LOD was performed for Atenolo and Nitrendipine was found to be 1.964and 0.342 respectively.

Quantitation limit

The LOQ was performed for Atenolol and Nitrendipine was found to be 5.952 and 1.036 respectively.

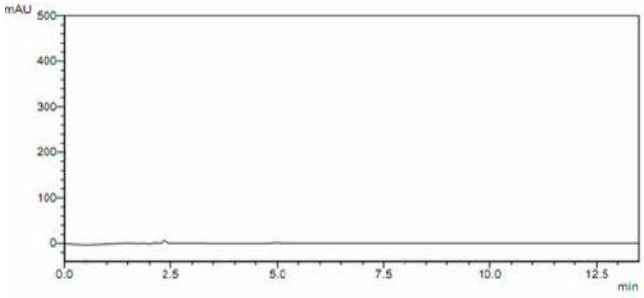

Figure 4: Chromatogram of Blank

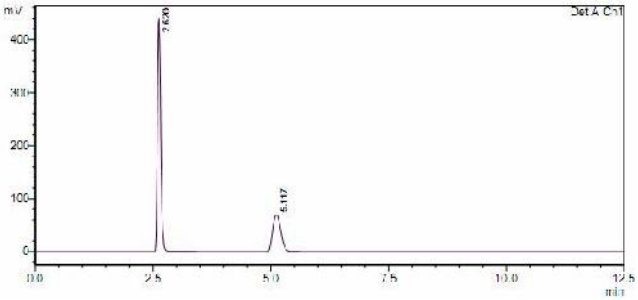

Figure 5: Chromatogram of Sample

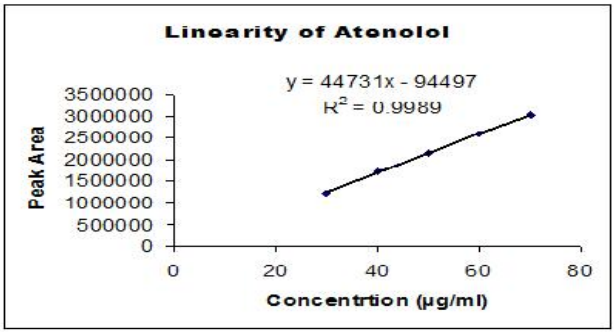

Figure 6: Calibration graph of Atenolol

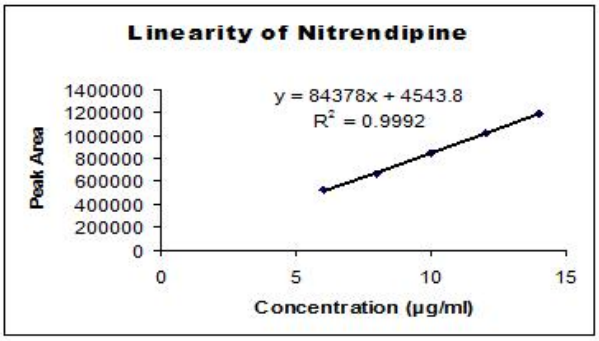

Figure 7: Calibration graph of Nitrendipine

4. Conclusion

The chromatographic conditions were successfully developed for the separation of Atenolol and Nitrendipine by using Phenomenox Luna C_{18}Column, 5µ (250 × 4.6mm), flow rate was 1.5ml/min, mobile phase ratio was Methanol : Acetonitrile : Water (40 : 40 : 20) pH was adjusted to 3.0 with Orthophosphoric acid, detection wave length was 235nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.621 mins and 5.169 mins. From the Linearity study, it was found that, the drug obeys beer's law and the specified range for estimation of Atenolol and Nitrendipine was found to be 60% to 140%. Correlation coefficient was found to be R^2=0. 0.9989 and R^2=0.9992 respectively for Atenolol and Nitrendipine. Limit of Detection and Quantitation for Atenolol was found to be 1.964µg/ml and 5.952µg/ml respectively. Limit of Detection and Quantitation for Nitrendipine was found to be 0.342µg/ml and 1.036µg/ml respectively. Precision RSD was found to be 0.710 for Atenolol and 0.822 for Nitrendipine. Whereas Intermediate Precision RSD was found to be 0.445 for Atenolol and 0.668 for Nitrendipine. The precision was found to be within the specified limit which indicates that the method is precise. From the accuracy study, it was found that recovery value of pure drug from the solution were between 98.0% to 102% which indicates that the method is accurate. RSD with decreased flow rate of mobile phase for Atenolol was 0.285% and for Nitrendipine it was 0.195 %. While with increased flow rate of mobile phase RSD for Atenolol was 0.226% and for Nitrendipine it was 0.373 %. RSD with decreased Wavelength of Detector for Atenolol was 0.263% and for Nitrendipine it was 0.449%. While with increased Wavelength of Detector RSD for Atenolol it was 0.874 % and for Nitrendipine it was 0.191%. In both conditions the R.S.D. was less than 2%.

Table 1: Accuracy results for Atenolol

Level	S. No.	Peak Area	% Recovery	Mean	S.D.	% RSD
80%	1	1701948	99.136	**99.865**	**0.691**	**0.692**
	2	1715893	99.948			
	3	1725574	100.512			
100%	1	2135756	99.057	**99.525**	**0.691**	**0.573**
	2	2159557	100.161			
	3	2142193	99.356			
120%	1	2558129	99.251	**100.014**	**0.665**	**0.665**
	2	2589522	100.469			
	3	2585762	100.323			

Table 2: Accuracy (recovery) data for Nitrendipine

Level	S. No.	Peak Area	% Recovery	Mean	S.D.	% RSD
80%	1	654681	99.350	**100.460**	**0.995**	**0.990**
	2	663958	100.758			
	3	667352	101.273			
100%	1	858422	101.603	**100.714**	**0.770**	**0.764**
	2	846931	100.242			

	3	847384	100.296			
120%	1	1029824	100.667	**99.750**	**0.807**	**0.809**
	2	1014218	99.141			
	3	1017312	99.444			

Table 3: Data for robustness at flow rate 1.3 ml/min

S.NO	**Atenolol**			**Nitrendipine**		
	AUC	**USP Tailing**	**R.T.**	**AUC**	**USP Tailing**	**R.T.**
1	2462076	1.214	3.569	952120	1.157	5.971
2	2448472	1.217	3.564	954208	1.151	5.973
3	2458202	1.214	3.561	956694	1.156	5.982
Mean Area	**2456250**	**1.215**	**3.564**	**954340**	**1.154**	**5.975**
S.D.	**7008.918**			**1869.682**		
R.S.D.	**0.285**			**0.195**		

Table 4: Data for robustness at flow rate 1.7 ml/min

S.NO	**Atenolol**			**Nitrendipine**		
	AUC	**USP Tailing**	**R.T.**	**AUC**	**USP Tailing**	**R.T.**
1	1891056	1.216	1.841	748820	1.159	3.918
2	1893904	1.215	1.843	752209	1.161	3.915
3	1899500	1.224	1.846	745350	1.151	3.919
Mean Area	**1894820**	**1.218**	**1.843**	**748793**	**1.157**	**3.91733**
S.D.	**4295.879**			**2800.240**		
R.S.D.	**0.226**			**0.373**		

Table 5: Repeatability results of Atenolol and Nitrendipine

S. No	**Atenolol**		**Nitrendipine**	
	AUC	**Amount (%)**	**AUC**	**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
Avg.	**2149998.66**	**100.10**	**835367.5**	**99.92**

S.D	**15268.95**	**0.710**	**6870.780**	**0.821**
R.S.D	**0.710**	**0.710**	**0.822**	**0.822**

Table 6: Ruggedness results of Atenolol and Nitrendipine

S. No	**Atenolol**			**Nitrendipine**		
	Day 1	**Day 2**	**Avg.**	**Day 1**	**Day 2**	**Avg.**
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	
Avg.	**100.24**	**101.03**	**100.63**	**100.06**	**100.46**	**100.26**
S.D	**0.351**	**0.546**	**0.448**	**0.661**	**0.678**	**0.669**
R.S.D	**0.351**	**0.539**	**0.445**	**0.661**	**0.675**	**0.668**

Table 7: Data for Limit of Detection and Quantitation

S.No	**Atenolol**		**Nitrendipine**	
	Conce (µg/ml)	**AUC**	**Conce (µg/ml)**	**AUC**
1	30 µg/ml	1218292	6 µg/ml	519916
2	40 µg/ml	1723148	8 µg/ml	667884
3	50 µg/ml	2157782	10 µg/ml	847280
4	60 µg/ml	2589426	12 µg/ml	1017826
5	70 µg/ml	3021724	14 µg/ml	1188728
S.D. (σ)		**26626.53**		**8744.68**
Slope (m)		**44731**		**84378**
L.O.D. (3.3 × σ/m)		**1.964 µg/ml**		**0.342 µg/ml**
L.O.Q. (10 × σ/m)		**5.952 µg/ml**		**1.036 µg/ml**

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