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REVERSED-PHASE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF ATENOLOL AND NIFEDIPINE IN A CAPSULE FORMULATION

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ABSTRACT

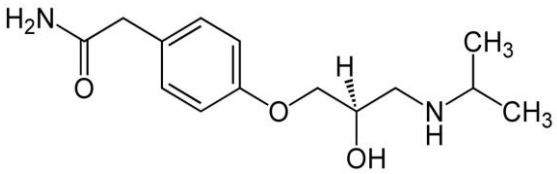
A simple, rapid, precise and accurate reversed phase high performance liquid chromatographic method has been developed for simultaneous determination of Atenolol in combination with Nifedipine. This method uses a mobile phase of 0.01M phosphate buffer solution: methanol (50:50 v/v, pH 4.0). The retention times for Atenolol and Nifedipine are 1.8 min and 7.7 min, respectively. The method is validated and shown to be linear. The linearity range for both Atenolol and Nifedipine was found to be 10-100 µg/ml. The Percentage recovery for Atenolol and Nifedipine are ranged between 99.32–100.02 and 99.10–100.4 respectively. The correlation coefficients of Atenolol and Nifedipine are 0.999 and 0.999 respectively**.** The relative standard deviation for six replicates is always less than 2%. The Statistical analysis proves that the method is suitable for analysis of Atenolol and Nifedipine as a bulk drug and in pharmaceutical formulation without any interference from the excipients. The propose method was validated as per the ICH guidelines parameters like Linearity, precision, accuracy, robustness limit of detection and limit of quantitation. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

KEYWORDS: Atenolol, Nifedipine, validation, ICH guidelines.

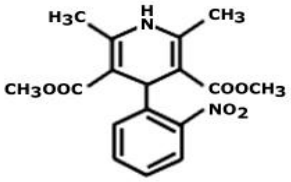
INTRODUCTION

Atenolol is a competitive, ß-1 selective adrenergic antagonist, similar to metoprolol. ß-adrenergic antagonists counter the effect of sympathomimetic neurotransmitters (i.e., catecholamines) by competing for receptor sites [1, 2]. It antagonizes ß1 receptors at doses 50 to 100 times less than those required to block ß2 receptors [3,4,5]. This cardioselectivty is more

pronounced at low doses and is lost at high doses. It lowers the blood pressure in hypertension and slows the heart rate. Nifedipine is the prototype of the dihydropyridine family of calcium channel blocker [6,7]. Chemically it 1, 4-dihydro-2, 6-dimethyl-4-(2-nirophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester [8,9,10]. In general, the dihydropyridine-type calcium-channel antagonists have more prominent effects on vasodilation and coronary flow than do diltiazem and verapamil [11, 12, 13]. Combined use of atenolol with nifedipine decrease the rate, conduction and contractility of heart particularly in patients of ventricular or conduction abnormalities by decreasing peripheral vascular resistance [14, 15, 16]. The chemical structures of the assayed compounds are given below.



Atenolol



Nifedipine

Fig: 1. Chemical structures of atenolol and nifedipine

The proposed method was optimized and validated in accordance with International Conference on Hormonization (ICH) guidelines [17, 18, 19]. The aim of present work is to develop a simple, rapid, precise, accurate and selective reversed phase chromatographic method and to estimate the Atenolol and Nifedipine in bulk and its solid dosage forms.

MATERIALS AND METHODS

The reference sample of Nifedipine and Atenolol standard was kindly supplied as gift sample by Cipla Ltd, Vikroli West, Mumbai, India and Ajanta Pharmaceutical, Mumbai, India, respectively. All the chemicals were of analytical grade. Methanol (HPLC grade) was used of Merck Pharmaceuticals Private Ltd., Mumbai, India. Potassium dihydrogen phosphate (monobasic) used was of HPLC grade and purchased from Loba Chemicals. Commercial capsules of Nifedipine and Atenolol in combination was procured from local market. Tenofed Capsule 40 mg are manufactured by Hetero drugs Pvt. Ltd. Hyderabad, A.P. The liquid

chromatographic system was of Perkin Elmer (USA), series 200, which consisted of following components: a gradient pump, variable wavelength programmable UV/Vis detector, a manual injection facility with 20 µl fixed loop. The chromatographic analysis was performed using Total Chrom Navigator version 6.3 software on a ODS metaphase C18-250×4.6 mm, particle size 5µm.

Preparation of Mobile Phase and Stock Solutions

Phosphate buffer 0.01 M solution was prepared by dissolving accurately about 1.369 gm of potassium dihydrogen phosphate in a 1000 ml of glass double distilled water. Mobile phase was prepared by mixing 250 ml of 0.01M potassium dihydrogen phosphate solution with 250 ml of methanol and its pH is adjusted to 4.0 by ortho phosphoric acid. This mobile phase was ultrasonicated for 20 min, and then it was filtered through 0.45 µm Nylon, 47 mm membrane filter paper. Stock solutions were prepared by weighing accurately about 50 mg of each of reference standard of atenolol and 20mg of nifedipine was weighed and transferred to 50ml volumetric flask. Both drugs were dissolved in 50 ml of methanol with shaking and then volume was made up to the mark with methanol to get 1000 µg/ml & 400 µg/ml of standard stock solution of each drug. These stock solutions were filtered through 0.2µm Nylon 6, 13 mm membrane filter paper. From the above stock solution 5 ml was then pipette out in 50 ml volumetric flask and diluted up to the mark with methanol to get 100 µg/ml & 40 µg/ml atenolol & nifedipine respectively.

Selection of Analytical Wavelength

By appropriate dilution of each standard stock solution with methanol, various concentrations of atenolol and nifedipine were prepared separately. Each solution was scanned using double beam UV visible spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra was overlaid. The wavelength selected was 237nm.

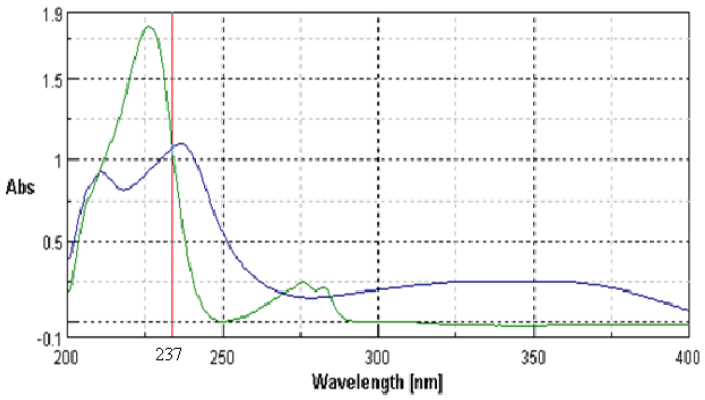


Fig: 2. Isoabsorptive point of atenolol and nifedipine.

Calibration Curves for Atenolol and Nifedipine

For each drug appropriate aliquots were pipetted out from each standard stock solution into a series of 10ml volumetric flasks. The volume was made up to the mark with methanol to get a set of solutions for atenolol having concentration range 20, 40, 60, 80 and 100 µg/ml and for nifedipine 20, 40, 60, 80 and 100 µg/ml. Triplicate dilutions of each concentration of each drug were prepared separately. From these triplicate solutions, 20µl injections of each concentration of each drug were injected into the HPLC system separately and chromatographed under the conditions as described above. Carbamazepine is used as internal standard. Evaluation of both drugs was performed with UV detector at 237 nm.

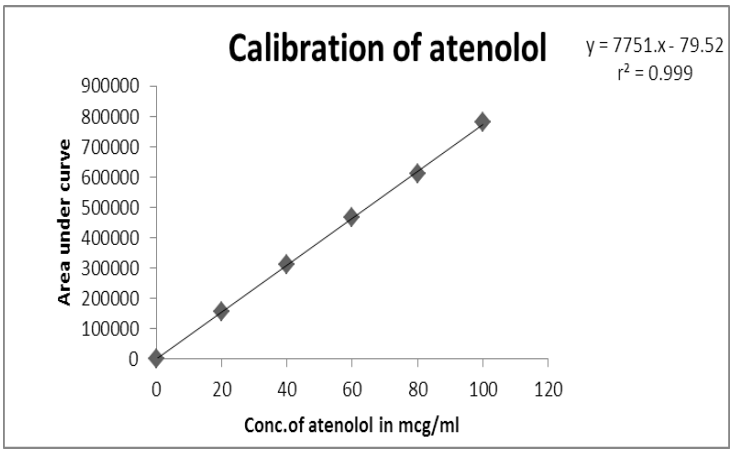


Fig: 3. Calibration curve for atenolol.

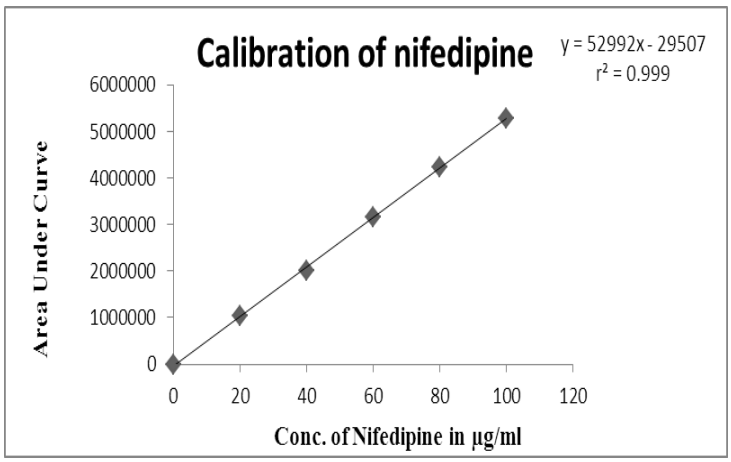


Fig: 4. Calibration curve for nifedipine.

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. It was found that methanol and 0.01M potassium dihydrogen phosphate buffer (pH- 4.0) gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of

the mobile phase was determined to be 0.01M phosphate buffer solution: methanol (50:50 v/v, pH 4.0). This mobile phase produced good resolution, reasonable retention times and acceptable peak symmetry for both the drugs. Using the optimized mobile phase, the flow rate was set to 1.5 ml/min and UV detection was carried out at 237 nm. The mobile phase and samples were degassed by ultrasonic vibrations for 20 min and filtered through 0.45µm Nylon, 47 mm membrane filter paper. The table 1 gives the R_t and peak area found in the estimation. Complete resolution of the peaks with clear baseline was obtained (fig.5). System suitability test parameters for atenolol and nifedipine for the proposed method are reported in table 2.

Table: 1 Results of simultaneous estimation of atenolol and nifedipine.

Parameter	Atenolol	Nifedipine	Carbamazepine
R_t	1.8	7.7	5.9
Peak area	779859	2012923	3969578

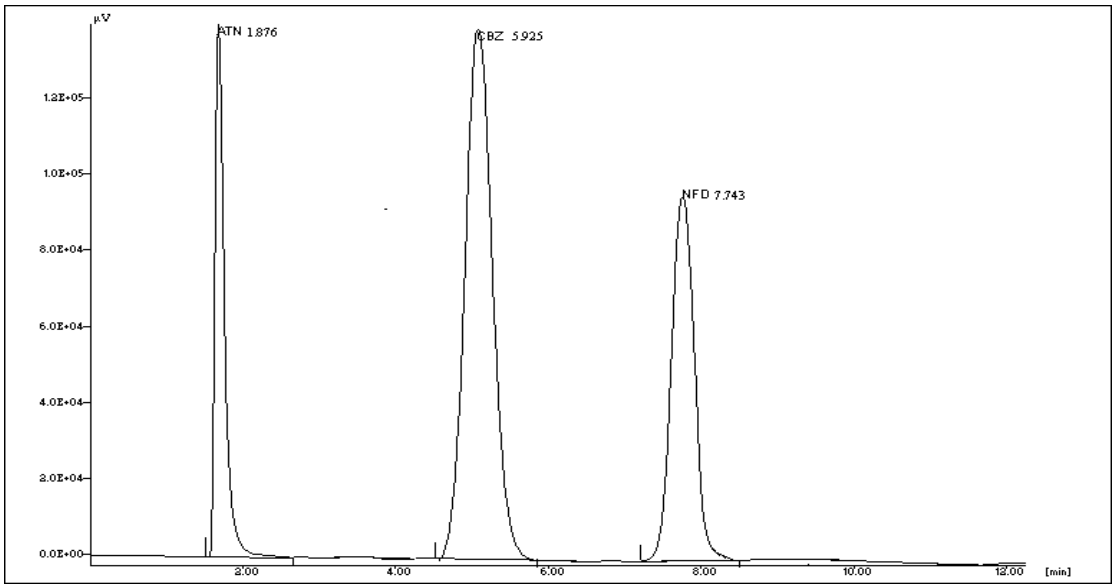


Fig: 5. Graph for simultaneous estimation of atenolol and nifedipine with internal standard.

Table 2: System Suitability Parameters.

Parameter	Atenolol	Nifedipine
Tailing factor	1.25	1.5
Resolution (Rs)	3.8	
Separation factor	1.857	
Capacity factor	2.07	4.15
Theoretical plates (N)	2752	3370
Retention time	1.8	7.7

Analysis of Capsule Formulation

Twenty capsules of atenolol and nifedipine in combination were weighed. The fine powder equivalent to 50 mg of atenolol and 20mg of nifedipine was weighed and transferred to 50 ml volumetric flask and dissolved in methanol and the content was kept in ultrasonicator for 30 min. The volume was made up to the mark with diluent. This capsule solution was further diluted with mobile phase to obtain mixed sample solutions in Lambert's- Beer's range for each drug containing 50µg/ml of atenolol and 20µg/ml of nifedipine respectively. A 20µl volume of each sample solution was injected into sample injector of HPLC six times under chromatographic condition as described above. Area of each peak was measured at 237 nm .The amount of each drug present in the sample (n=6) was determined from peak area of atenolol and nifedipine present in the pure mixture respectively. Typical chromatogram of atenolol and nifedipine present in capsule formulation is given in Fig: 6. and the results of analysis of capsule formulation and its statistical evaluation are given in the Table: 3 and 4 respectively.

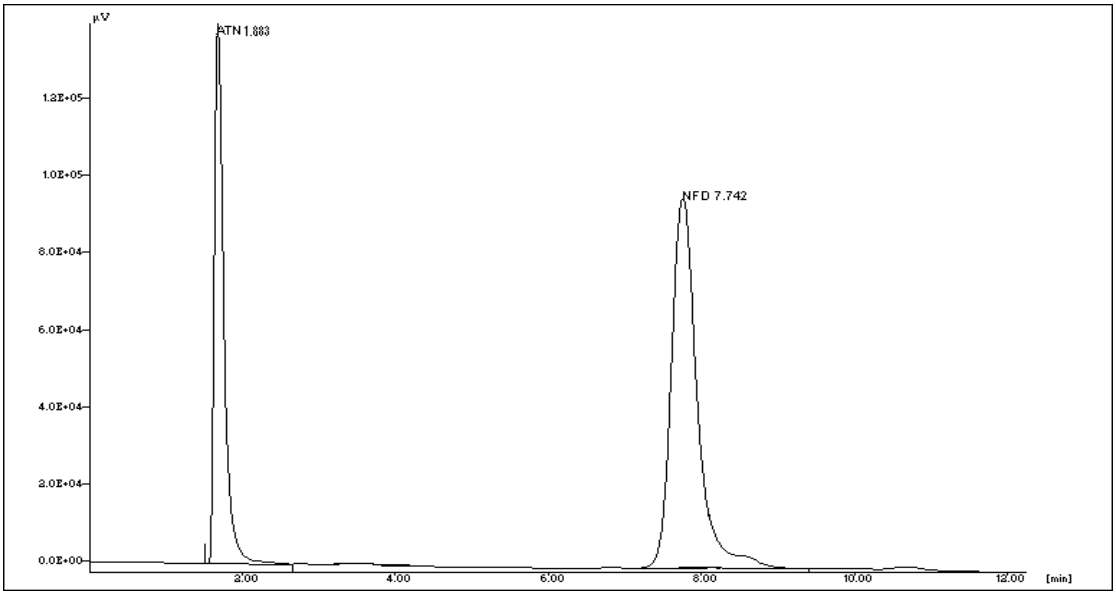


Fig: 6. Simultaneous estimation of atenolol and nifedipine in capsule of Tenofed.

Table 3: Analysis of Capsule Formulation.

S. No.	Label claim mg/capsule		Amount found mg/capsule		% amount drug found	
	ATN	NFD	ATN	NFD	ATN	NFD
1.	50	20	49.87	19.82	99.74	99.10
2.	50	20	49.99	20.08	99.98	100.4
3.	50	20	50.01	19.89	100.02	99.45
4.	50	20	49.66	19.55	99.32	99.25
5.	50	20	49.91	19.94	99.82	99.70
6.	50	20	49.89	19.91	99.78	99.55

Table 4: Statistical validation.

Capsule Sample	Type of recovery %	(%) Mean		SD		Std. error of mean	
		ATN	NFD	ATN	NFD	ATN	NFD
Tenofed	80	99.36	99.36	0.304	0.304	0.306	0.3061
	100	99.42	99.42	0.156	0.15	0.1571	0.157
	120	99.75	99.75	0.208	0.208	0.208	0.208

Validation Developed Method

The proposed method has been validated for the simultaneous determination of ATN and NFD in capsule dosage form. Calibration curves were constructed by plotting peak areas versus concentrations of ATN and NFD, and the regression equations were calculated. The calibration curves were plotted over the concentration range 20-100 µg/ml for ATN and 20-100 µg/ml for NFD. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described as above. Regression parameters are mentioned in Table 5.

Table 5: Summary for Validation Parameters.

Parameters	Atenolol	Nifedipine
Linearity range	10-100 µg/ml	10-100 µg/ml
Correlation Coefficient	0.999	0.999
Slope (m)	14128.76	7966
Intercept	2000	-628.072
Specificity	No Interference at R_t of the analyte peak	No Interference at R_t of the analyte peak
Method Precision (%Rsd)	0.1	0.0
Accuracy (%Rsd)	1.424	2.466
Robustness (%Rsd)	16.5	15.8
LOD	0.05 µg/ml	0.075 µg/ml
LOQ	0.15 µg/ml	0.22 µg/ml

Accuracy

To check the accuracy of proposed method, level of recovery carried out at 80, 100 and 120 % of the concentration as per standard addition method.To perform recovery studies of the test concentration, a powder of preanalysed capsule sample containing 50 mg of atenolol and 20 mg of nifedipine was weighed such that it should contain 50 mg of atenolol and 20 mg of nifedipine then transferred into 100 ml volumetric flask, add about 50 ml of methanol and sonicated for 20 minutes with intermediate shaking and volume make up up to the mark. 100 µg/ml and 40 µg/ml of a of atenolol & nifedipine pure drugs were used as standard

concentrations, finally % recovery was calculated and results and statistical validation are shown in Table 6 and 7 respectively.

Table 6. Recovery studies.

Capsule sample	Level of recovery (%)	Amount present (µg/ml)		Amt of std. added (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
		ATN	NFD	ATN	NFD	ATN	NFD	ATN	NFD
TENOFED	80	100	40	80	32	178.49	71.87	99.16	99.81
	80	100	40	80	32	179.38	71.53	99.71	99.34
	80	100	40	80	32	178.59	71.67	99.21	99.54
	100	100	40	100	40	198.64	79.88	99.32	99.85
	100	100	40	100	40	199.21	79.66	99.60	99.57
	100	100	40	100	40	198.68	79.91	99.34	99.88
	120	100	40	120	48	219.87	87.90	99.94	99.88
	120	100	40	120	48	218.98	87.81	99.53	99.78
	120	100	40	120	48	219.57	87.86	99.80	99.84

Table 7. Statistical validation.

Capsule Sample	Type of recovery %	(%) Mean		SD		Std. error of mean	
		ATN	NFD	ATN	NFD	ATN	NFD
TENOFED	80	99.36	99.36	0.304	0.304	0.306	0.3061
	100	99.42	99.42	0.156	0.15	0.1571	0.157
	120	99.75	99.75	0.208	0.208	0.208	0.208

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of ATN and NFD in capsule dosage forms on a column ODS metaphase C18- 250×4.6 mm, particle size 5µm with variable wavelength detection at 237 nm. The retention times for Atenolol and Nifedipine are 1.8 min and 7.7 min, respectively. The LOD and the LOQ for Atenolol and Nifedipine were found to be 0.05 and 0.15 µg/ml and 0.075 and 0.22 µg/ml, respectively. These data show that method is sensitive for the determination of Atenolol and Nifedipine The recovery experiment was performed by the standard addition method The Percentage recovery for Atenolol and Nifedipine are ranged between 99.32–100.02 and 99.10–100.4 respectively. The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine Atenolol and Nifedipine in their capsule dosage form. The results obtained for Atenolol and Nifedipine were comparable with the corresponding labelled amounts. No interference of the excipients with the absorbance of interest appeared; hence, the proposed method is applicable for the routine

simultaneous estimation of Atenolol and Nifedipine in pharmaceutical dosage forms. A simple, linear, accurate, specific and selective RP-HPLC method was developed and validated for estimation of Atenolol and Nifedipine in their combined dosage form. In this proposed method the linearity range for both Atenolol and Nifedipine was found to be 10-100 µg/ml with coefficient of correlation, (r^2)=0.999 and (r^2)=0.999 for Atenolol and Nifedipine, respectively at 237 nm. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the Atenolol and Nifedipine in combined dosage form without any interference of excipients.

CONCLUSION

In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Atenolol and Nifedipine in bulk drug and pharmaceutical formulations and a simple, sensitive, precise and accurate RP-HPLC method for the simultaneous estimation of Atenolol and Nifedipine in bulk drug and pharmaceutical formulations. These methods can be used for the routine determination of Atenolol and Nifedipine bulk drug and in pharmaceutical formulations.

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