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Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RELATED SUBSTANCES OF ATENOLOL AND NITRENDIPINE BY RP-HPLC

THULASEEDHAR ALUMURI[a], NAMBURI L. A. AMARABABU[b], ARAVİND KURNOOL[c], PHANI RAJA KANUPARTHY[d], KARUNASREE MERUGU[a*]

[a*]Department of Chemistry, GITAM (Deemed to be University), Bengaluru 560034, Karnataka, India, [b]New Generation Materials Lab (NGML), Department of Science and Humanities, Vignan's Foundation for Science Technology and Research University (VFSTR) (Deemed to be University), Vadlamudi, Guntur 522213, Andhra Pradesh, India, [c]Department of Chemistry, Osmania University, Hyderabad 500007, Telangana, India, [d]Department of Chemistry, GITAM (Deemed to be University), Hyderabad 502329, Telangana, India
[*]Email: kmerugu@gitam.edu

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ABSTRACT

Objective: A validated stability-indicating RP-HPLC method for Atenolol and Nitrendipine was developed by separating its related impurities.

Methods: By using Waters HPLC e-2695 quaternary pump with a PDA detector of 2998 instrument, the chromatographic separation of Atenolol, Nitrendipine and its related impurities was achieved on the column of Agilent eclipse C_{18} (150x4.6 mm, 3.5 µ) using gradient elution with a buffer containing 0.1percent formic acid and acetonitrile as a mobile phase with a flow rate of 1 ml/min at ambient temperature. A detector wavelength of 218 nm utilizing the PDA detector was given in the instrumental settings. The linearity was studied between the concentration range of 6.25-37.5 µg/ml of Atenolol, 0.75-4.5 µg/ml each of Atenolol imp-A, imp-B and 5-30 µg/ml of Nitrendipine, 0.5-3 µg/ml each of Nitrendipine imp-1, imp-2 were injected with a run time of 40 min. Validation of the proposed method was carried out according to an International Conference on Harmonization (ICH) guidelines.

Results: LOD and LOQ for the Atenolol and its impurities were established with respect to test concentration. The plotted calibration curves were linear with a regression coefficient of R^2>0.999, indicating that the linearity was with in the limit. As a part of method validation the parameters like specificity, linearity, accuracy, ruggedness, robustness were determined and the results were found to be within the allowable limit.

Conclusion: The method developed was found to be applicable to routine analysis and to be used for the measurement of active pharmaceutical ingredients (i. e, Atenolol, Nitrendipine and their related impurities). Since there is no HPLC method reported in the literature for the estimation of Atenolol, Nitrendipine and their related impurities, there is a need to develop quantitative methods under different conditions to achieve improvement in specificity selectivity etc.

Keywords: Atenolol, Nitrendipine, Related impurities, HPLC, Validation

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INTRODUCTION

Atenolol is a beta-blocker [1, 2] medication primarily used to treat high blood pressure [3] and heart-associated chest pain [4]. Atenolol, however, does not seem to improve mortality in those with high blood pressure [5, 6]. Other uses include the prevention of migraines and treatment of certain irregular heartbeats. It is taken by mouth or by injection into a vein. It can also be used with other blood pressure medications. Common side effects include feeling tired, heart failure [7, 8], dizziness [9], depression, and shortness of breath [10, 11]. Other serious side effects include bronchospasm. Use is not recommended during pregnancy and alternative drugs are preferred when breastfeeding. It works by blocking β1-adrenergic receptors [12] in the heart, thus decreasing the heart rate and workload. Atenolol is used for a number of conditions, including hyperthyroidism [13], hypertension, angina [14, 15], long QT syndrome [16], acute myocardial infarction [17, 18], supraventricular tachycardia [19], ventricular tachycardia [20], and the symptoms of alcohol withdrawal. Hypertension treated with a β-blocker such as atenolol, alone or in conjunction with a thiazide diuretic, is associated with a higher incidence of new-onset type 2 diabetes [21] mellitus compared to those treated with an ACE inhibitor or angiotensin receptor blocker [22, 23]. β-blockers, of which atenolol is mainly studied, provides weaker protection against stroke and mortality in patients over 60 y old compared to other antihypertensive medications [24, 25]. Diuretics may be associated with better cardiovascular and cerebrovascular outcomes than β-blockers in the elderly [26].

Nitrendipine is a dihydropyridine calcium channel blocker. It is used in the treatment of primary (essential) hypertension to decrease blood pressure and can reduce the cardiotoxicity of cocaine [27]. Nitrendipine is given to hypertensive individuals in 20 mg oral tablets every day [28]. This amount is effective in reducing blood pressure by 15–20% within 1–2 h of administration. With long-term treatments, the dosage may rise to as much as 40 mg/day; in elderly individuals, a lower dosage of up to 5 mg/day may be equally effective (this reduction in drug amount is attributed to decreased liver function [29] or "first pass" metabolism). Once digested, nitrendipine is absorbed into the blood and binds to plasma proteins [30]. The majority (98%) is bound to plasma proteins and 70-80% of its inactive polar metabolites are also bound to plasma proteins. Following hepatic metabolism, 80% of the 20 mg dose can be recovered in the first 96 h as inactive polar metabolites [31]. In terms of drug half-life, nitrendipine has a half-life of 12–24 h. The reported side effects include headache, flushing, edema and palpitations [32]. These side effects can all be attributed to the vasodilation effect of this drug. So, we developed a method for the estimation of Atenolol by using RP-HPLC.

Till today there are no HPLC methods reported in the literature, So, it has more interested to develop a novel and reliable HPLC strategy for the establishment of Atenolol, Nitrendipine and their related impurities.

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade orthophosphoric acid, and water were purchased from Merck India Ltd, Mumbai, India. Candila health care ltd, Ahmedabad, India provided the reference criteria for Atenolol, Nitrendipine, and their related impurities.

The ınstrumentation

Waters alliance liquid chromatography (model e-2695) was monitored with empower 2.0 data handling system and a detector of photodiode array (model 2998) [33] was used for this study.

Preparation of mobile phase-A: 1 ml of formic acid was dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

Mobile phase-B: Acetonitrile

Optimization of mobile phase

Different trails have done, and different buffers and different mobile phases were used to develop the method. In all trails peaks are not separated properly. Finally, for the proposed method all the peaks are separated and the entire suitability conditions are within the limit.

Chromatographic conditions

The HPLC analysis was performed on a reverse-phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% formic acid and Agilent eclipse C_{18} (150x4.6 mm, 3.5 µ) column with a flow rate of 1 ml/min.

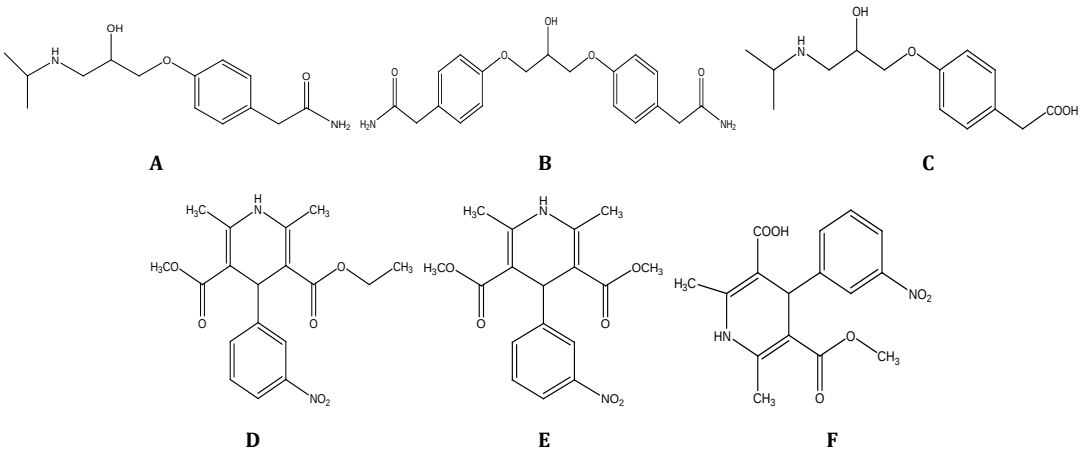


Fig. 1: Chemical structures of (A) Atenolol (B) Impurity-A (C) Impurity-B (D) Nitrendipine (E) Impurity-1 (F) Impurity-2

Table 1: Gradient program

Time (min)	Mobile phase-A	Mobile phase-B
0.00	80	20
5	80	20
10	30	70
15	30	70
20	80	20
40	80	20

Diluent

Mobile phase was used as a diluent.

Validation procedure

The analytical parameters [34-38] such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines.

Standard stock solution

Weighed accurately 25 mg of Atenolol and 20 mg of Nitrendipine, transferred into a 100 ml volumetric flask, added 70 ml of diluent and sonicated for 10 min to completely dissolved the contents and made up to the mark with diluent.

Preparation of Impurity stock solution-A

Weighed accurately 5 mg each of Atenolol imp-A, imp-B into a 10 ml volumetric flask. Added 7 ml of diluent, sonicated until dissolved the contents and made up to the mark with diluent.

Preparation of Impurity stock solution-B

Weighed accurately 5 mg each of Nitrendipine imp-1, imp-2 into a 10 ml volumetric flask. Add 7 ml of diluent, sonicated until dissolved the contents and made up to the mark with diluent.

Preparation of ımpurity stock solution

Takeen 6 ml of impurity stock solution-A and 4 ml impurity stock solution-B into another 100 ml volumetric flask and made up to the mark with diluent.

Spiked standard solution

Transferred 5 ml of standard stock into a 50 ml volumetric flask, added 40 ml of diluent, and also add 5 ml of impurity standard stock solution and made up to the mark with diluent and filtered through 0.45µ syringe filter.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients. In order to provide a good performance, the chromatographic conditions were optimized.

Method validation

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

System suitability

Device suitability was performed by injecting a spiked standard solution containing 25 µg/ml of Atenolol, 3 µg/ml each of Atenolol imp-A, imp-B and 20 µg/ml of Nitrendipine, 2 µg/ml each of Nitrendipine imp-1, imp-2 in six replicates. The results show that the machine fitness parameter is within the limit provided by ICH [39]. The results were shown below in table 2 and 3.

Specificity

In this test method, standard solution was analyzed individually to examine the interference. The below fig. shows that the active ingredients and their related substances were well separated from the blank. Hence the method is specific.

Table 2: Suitability results of atenolol

System suitability parameter	Atenolol		Imp-A		Imp-B	
	Mean	Std dev	Mean	Std dev	Mean	Std dev
USP Plate count	5422	4.326	8324	6.257	44401	4.214
USP Tailing	1.08	0.025	1.05	0.016	1.06	0.014
USP Resolution	-	-	3.76	1.06	29.77	2.41
% RSD	0.04	459.069	0.01	49.521	0.15	300.142
Retention Time	3.570	0.376	4.294	0.547	9.955	0.968

mean±SD (n=6)

Table 3: Suitability results of nitrendipine

System suitability parameter	Nitrendipine		Imp-1		Imp-2	
	Mean	Std dev	Mean	Std dev	Mean	Std dev
USP Plate count	60090	4.025	64799	4.518	62559	4.414
USP Tailing	0.95	0.059	0.98	0.011	0.95	0.067
USP Resolution	4.37	0.147	18.74	0.452	5.80	1.037
% RSD	0.03	878.633	0.39	441.433	0.16	445.249
Retention Time	16.273	0.329	13.786	0.329	15.137	0.752

mean±SD (n=6)

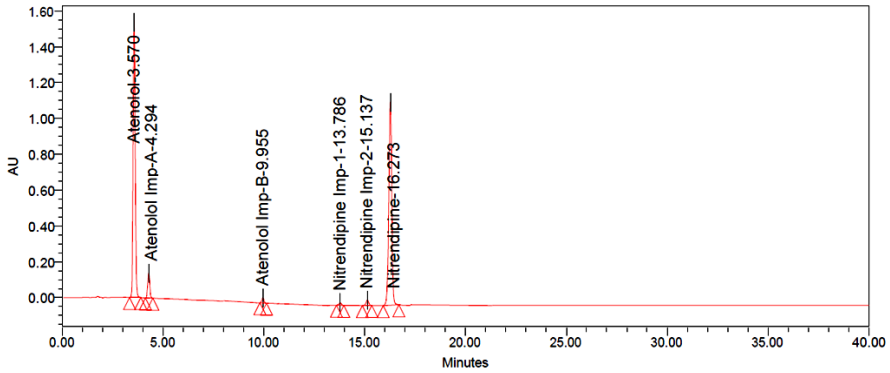


Fig. 2: Chromatogram of standard

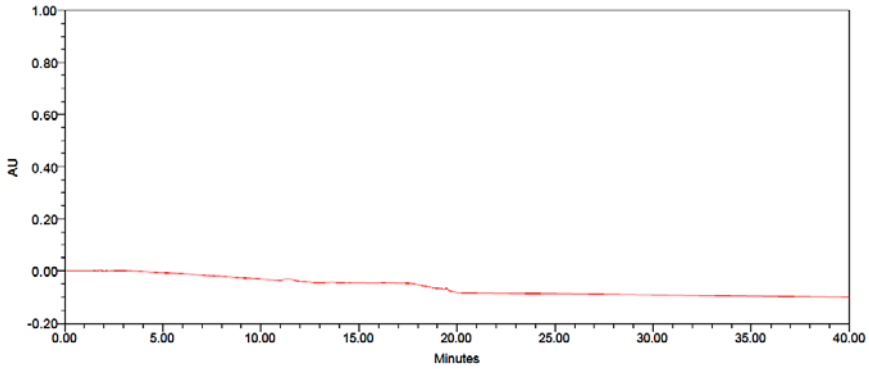


Fig. 3: Chromatogram of blank

Linearity

Linearity was calculated by plotting a calibration curve of the peak area against its respective concentration, and linearity was determined. From this calibration curve, it was noticed that the curve was linear between the concentration range of 6.25-37.5 µg/ml of Atenolol, 0.75-4.5 µg/ml each of Atenolol imp-A, imp-B and 5-30 µg/ml of Nitrendipine, 0.5-3 µg/ml each of Nitrendipine imp-1, imp-2. Linearity results were shown in table 4.

Accuracy

The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs with concentrations of 12.5, 25 and 37.5 µg/ml of Atenolol and 10, 20 and 30 µg/ml of Nitrendipine were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery results were similar to 100% and also, the RSD values were less than±2%. The percentage recovery, mean and relative standard deviations were determined. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in table 5.

Intraday precision

Six replicates of a standard solution containing Atenolol, Nitrendipine and their related substances were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and % RSD values.

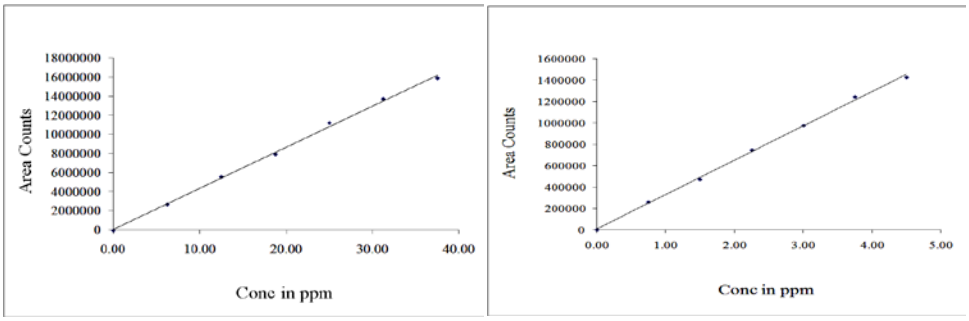
Table 4: Linearity results of atenolol and its impurities

A

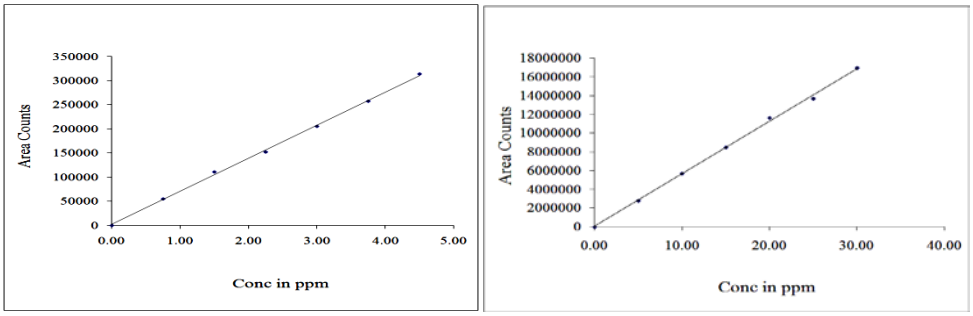
Linearity	Atenolol		Imp-A		Imp-B	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	6.25	2706763	0.75	258491	0.75	55024
Linearity-2	12.50	5599555	1.50	471148	1.50	111015
Linearity-3	18.75	7956421	2.25	745210	2.25	152304
Linearity-4	25.00	11231327	3.00	975283	3.00	205612
Linearity-5	31.25	13727814	3.75	1244049	3.75	257621
Linearity-6	37.50	15916933	4.50	1426751	4.50	314182
CC	0.99914		0.99926		0.99947	
Slope	430998.13		321690.67		68682.71	
Intercept	81472.61		7757.71		2000.75	

B

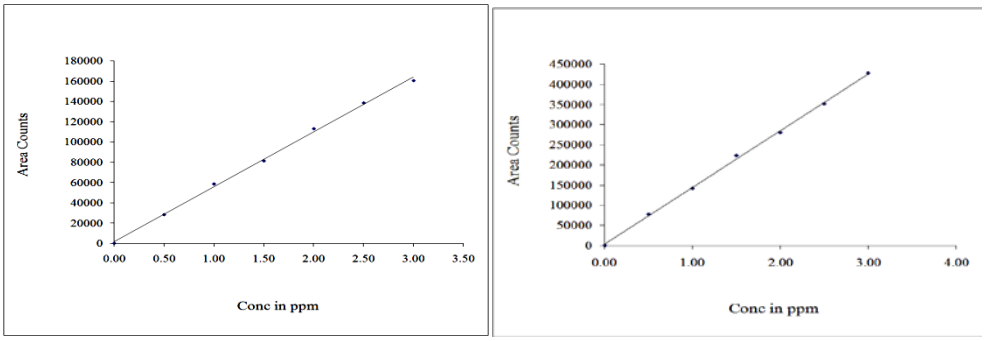
Linearity	Nitrendipine		Imp-1		Imp-2	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	5.00	2803365	0.50	28296	0.50	77707
Linearity-2	10.00	5714212	1.00	58639	1.00	141916
Linearity-3	15.00	8463259	1.50	81367	1.50	223056
Linearity-4	20.00	11624812	2.00	113184	2.00	280081
Linearity-5	25.00	13683081	2.50	138649	2.50	351568
Linearity-6	30.00	16948625	3.00	160666	3.00	427459
CC	0.99934		0.99907		0.99948	
Slope	560827.91		54089.21		140590.29	
Intercept	50060.54		1837.75		3655.57	



Atenolol **Atenolol Imp-A**



Atenolol **Imp-B Nitrendipine**



Nitrendipine **Imp-1 Nitrendipine Imp-2**

Fig. 4: Calibration plots of atenolol, nitrendipine and their related impurities

Table 5: A and B gives the results of accuracy

A

S. No.	% Level	Atenolol % recovery		Imp-A % recovery		Imp-B % recovery	
		Mean	Std dev	Mean	Std dev	Mean	Std dev
1	50	100.6	0.557	99.3	0.666	100.5	1.012
2	100	100.8	0.802	100.1	0.153	99.3	0.586
3	150	99.9	0.603	100.7	1.193	100.7	1.332

mean±SD (n=3)

B

S. No.	% Level	Nitrendipine % recovery		Imp-1 % recovery		Imp-2 % recovery	
		Mean	Std dev	Mean	Std dev	Mean	Std dev
1	50	99.5	1.159	100.7	0.643	100.1	0.611
2	100	99.4	0.493	99.7	1.002	100.5	0.737
3	150	100.1	1.429	99.4	0.551	99.0	0.458

mean±SD (n=3)

Table 6: Intraday precision results of allantoin and permethrin

S. No.	% of related substances		
	Spiked impurities	Total impurities	% Purity (100-Total impurities)
1	1.12	0.52	99.48
2	1.14	0.61	99.39
3	1.11	0.62	99.38
4	1.25	0.65	99.35
5	1.23	0.67	99.33
6	1.25	0.65	99.35
Average	1.18	0.62	99.38
Std dev	0.067	0.054	0.054

mean±SD (n=6)

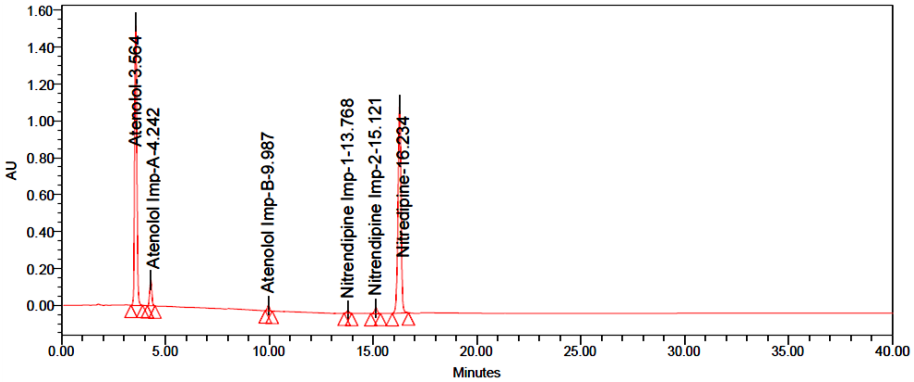


Fig. 5: Chromatogram of sample

Inter-day precision

Six replicates of a standard solution containing Atenolol, Nitrendipine and their related substances were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values [40]. The present method was found to be precise as the RSD values were less than 2% and also, the percentage assay values were close to be 100%. The results are given in table 6.

Table 7: Inter-day precision results

Sample No.	% of related substances		
	Spiked impurities	Total impurities	% Purity (100-total impurities)
1	1.20	0.65	99.35
2	1.22	0.62	99.38
3	1.21	0.56	99.44
4	1.19	0.71	99.29
5	1.15	0.57	99.43
6	1.24	0.63	99.37
Average	1.20	0.62	99.38
Std dev	0.031	0.055	0.055

mean±SD (n=6)

LOD and LOQ

LOD and LOQ were determined separately using the calibration curve technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard solution. The LOD and LOQ concentrations and their S/N values of Atenolol, Nitrendipine and their related standards were represented in the following table. This method is validated as per the ICH guidelines [41, 42].

Robustness

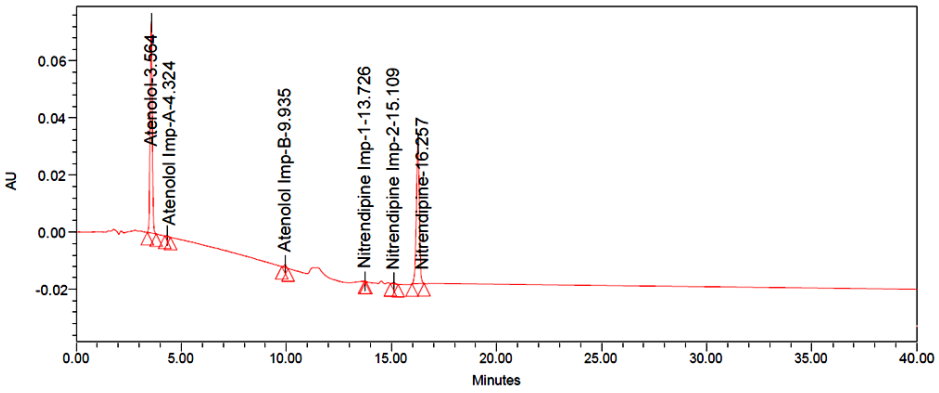
The conditions of the experiment was designed to measure the robustness of the intentionally changed conditions such as flow rate and mobile phase in organic percentage in all these varied conditions [43]. Robustness results for Atenolol, Nitrendipine and their impurities were found to be within the limit and results were tabulated in table 8.

Stability

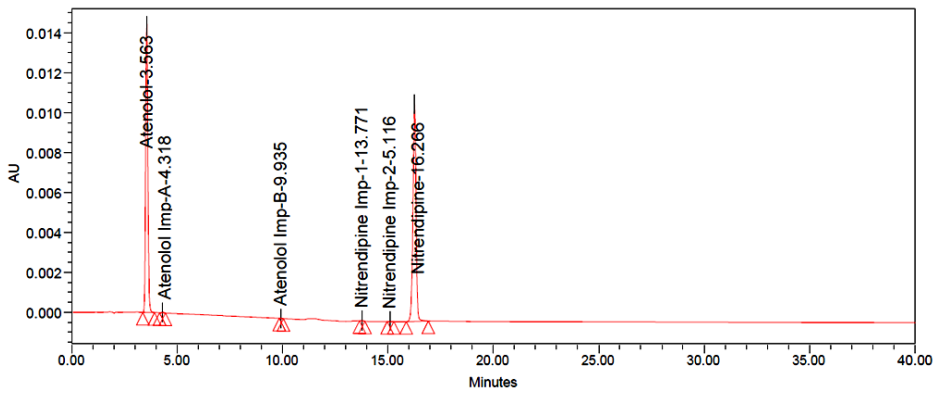
Normal solution was kept at room temperature and 2-8 °C for up to 24 h. These solutions were then pumped into the system and the percent deviation from the initial to 24 h was measured [44]. No major variations were found and verified that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Atenolol, Nitrendipine and their related impurities. Stability results were tabulated in table 9.

Table 8: Results of LOD and LOQ

Name	LOD Conc. (µg/ml)	S/N	LOQ Conc. (µg/ml)	S/N
Atenolol	0.248	8	2.5	27
Imp-A	0.09	5	0.3	23
Imp-B	0.09	4	0.3	22
Nitrendipine	0.6	7	2	25
Imp-1	0.06	4	0.2	22
Imp-2	0.06	4	0.2	22



A



B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Table 9: Robustness results

Parameter name	Atenolol % assay		Nitrendipine % assay	
	Mean	Std dev	Mean	Std dev
Flow rate (0.8 ml/min)	99.4	0.049	99.9	0.042
Flow rate (1.2 ml/min)	99.2	0.043	100.3	0.035
Org Plus (+10%)	99.7	0.015	99.6	0.027
Org Minus (-10%)	99.5	0.026	101.4	0.062

mean±SD (n=3)

Table 10: Stability results at RT

Stability	Atenolol		Nitrendipine	
	% Purity	% deviation	% Purity	% deviation
Initial	99.99	0.01	99.99	0.01
6 h	99.76	0.24	99.64	0.36
12 h	98.56	1.14	99.37	0.63
18 h	98.23	1.47	99.01	0.99
24 h	98.01	1.79	98.86	1.14

Table 11: Stability results at 2-8 °C

Stability	Atenolol		Nitrendipine	
	% Purity	% deviation	% Purity	% deviation
Initial	99.99	0.01	99.99	0.01
6 h	99.54	0.45	99.66	0.33
12 h	99.22	0.78	99.14	0.86
18 h	98.91	0.19	98.83	0.17
24 h	98.64	0.36	98.55	0.45

Degradation studies

Atenolol, Nitrendipine and their related substances were subjected to various conditions of forced degradation [45, 46] in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials [47, 48]. In addition, the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities [49].

Acid degradation

For 15 min, add 1 ml of 1N HCl to 5 ml of standard solution in a volumetric flask of 50 ml. A solution of 1N NaOH should be added to the solution after 15 min.

Alkali degradation

1 ml of 1N NaOH is added to a 50 ml volumetric flask containing 5 ml of standard stock solution and left for 15 min. Adding 1 ml of 1N HCl and diluting it with diluents was done after 15 min.

Peroxide degradation

Five ml of stock solution were put into a 50 ml volumetric flask, and 0.3 ml of 30 percent hydrogen peroxide was added.

Reduction degradation

Five ml of the standard stock solution were transferred to a 50 ml volumetric flask, and one ml of a 30 percent sodium bi sulphate solution was added and the diluents level was reached.

Thermal degradation

The standard solution was set at 105° in the oven for 6 h. The resultant solution was injected into HPLC.

Hydrolysis degradation

Five ml of the standard stock solution were transferred to a 50 ml volumetric flask, and one ml of HPLC water was added and the diluents level was reached.

Table 12: Forced degradation results

Degradation condition	Atenolol % deg		Nitrendipine % deg	
	Mean	Std dev	Mean	Std dev
Acid deg	12.4	0.264	11.9	0.229
Alkali deg	12.1	0.341	11.4	0.335
Peroxide deg	14.3	0.259	13.1	0.249
Reduction deg	9.5	0.163	10.6	0.161
Thermal deg	1.2	0.047	2.5	0.048
Hydrolysis deg	0.9	0.059	1.1	0.066

mean±SD (n=3)

CONCLUSION

The developed method gave good results between Atenolol, Nitrendipine and their related impurities with run time of 40 min, high efficiency and complies with modified SST specifications of USP. The utilization of Agilent eclipse C_{18} column within the present work has shown better elution of analytes with good resolution, improved plate count and tailing. Therefore the C_{18} columns are often wont to achieve high specificity in shorter time of study of Atenolol, Nitrendipine as per ICH Q 3A (R_2) guidelines. The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination and quantification of Atenolol, Nitrendipine and their impurities. The sample recovery was in good agreement with their respective label claims suggested non-interference within the estimation. Hence, the technique is often easily and conveniently adopted for routine analysis of Atenolol, Nitrendipine in the combined dosage form.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Author declares that there have been no conflicts of interest.

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