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Original Article

VALIDATION OF STABILITY INDICATING ULTRA-FAST LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL and NIFEDIPINE IN BOTH BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: The study depicts improvement of ensuing validation of a stability indicating technique for the simultaneous estimation of Atenolol and Nifedipine using Ultra-fast liquid chromatographic method (UFLC).

Methods: The analysis is performed on Phenomenex Kinetex C_{18}, (150 × 4.6 mm, 5µm) column using methanol and 0.1%ortho-phosphoric acids (75:25 v/v) as mobile phase with a flow rate of 1.3 ml/min. The eluents were checked with PDA detector at 237 nm.

Results: In this optimized conditions Atenolol and Nifedipine elutes at a retention time of 2.79 and 4.50 min respectively individually the considered optimized condition is having linearity in the range from 10 to 50µg/ml of Atenolol and 4-20µg/ml of Nifedipine. The method was validated by following the ICH guidelines and their combination drug yield was exposed to acid and base stress, thermal stress, photolytic stress, hydrolytic stress, and oxidative stress conditions. All samples were studied by the given optimized method. In this Calibration curves were linear over studies ranges with correlation coefficient found between the ranges of 0.99 to 1.00.

Conclusion: The proposed method was found to be accurate, precise, and specific and suitable for determination of both the drugs.

Keywords: Atenolol, ICH guidelines, Nifedipine, Stability indicating studies, UFLC

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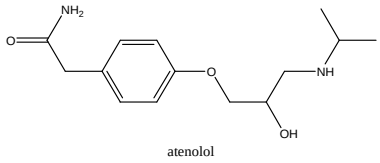
INTRODUCTION

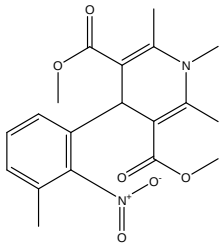
Atenolol, 4-[2-hydroxy-3-[(1-methyl ethyl) amino] propoxy]-benzeneacetamide] [1] (fig. 1) is a cardio-selective β_1-adrenergic receptor blocking agent recommended for the treatment of hypertension, angina pectoris, and cardiac arrhythmias. It is a Beta blocker that intrudes with binding to the receptor of epinephrine and different stress hormones and decreases the impacts of these hormones. Beta blockers are especially utilized for the management of cardiovascular arrhythmias, shielding the heart from second attack (myocardial infarction) after a first heart attack and hypertension [2].



atenolol

Fig. 1: Chemical structure of atenolol

Nifedipine is dimethyl-,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine 3,Sdicarboxylate [3]. (fig. 2) Nifedipine is a calcium channel blocking agent. The principal activity of calcium channel blockers incorporate dilatation of coronary and fringe coronary and peripheral arteries and arterioles, negative in tropic activity, decrease the heart rate, and decelerate the atrioventricular (AV) conduction. It restrains the Trans layer influx of calcium ions into vascular smooth muscle and cardiovascular muscle. Nifedipine restrains calcium ions influx across cell membranes specifically, with a more impact on vascular smooth muscle compared to cardiac muscle cells[4] Combined use of Atenolol with Nifedipine decreases the properties of cardiac muscles especially in patients with ventricular or conduction abnormalities [5-7].



Nifedipine hydrochloride

Fig. 2: Chemical structure of nifedipine

The proposed technique was optimized and validated as per International Conference on Harmonization (ICH) guidelines. [8-10]. The aim of the present work is to develop a simple, fast, precise and accurate reversed-phase chromatographic method together with stability indicating studies for the both mix drugs Atenolol and Nifedipine in bulk and its pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

The HPLC grade methanol is acquired from Merck Pvt Ltd, Mumbai. The chemicals utilized are of analytical grade (AR grade) like ortho-phosphoric acid obtained from Loba Chemie, Mumbai.

Instrumentation

The SHIMADZU, UFLC with PDA detector and LC solution software was utilized in the current research work. The separation was accomplished using C18 column. The mobile phase contains of 0.1% orthophosphoric acid in water and methanol (75:25 v/v). The mobile phase was filtered before use through membrane filters (0.45µ). The upgraded chromatographic conditions were mentioned in given table 1.

Table 1: Optimized chromatographic conditions

Column	C_{18} (150 × 4.6 mm, 5µm) Phenomenex Kinetex
Flow rate	1.3 ml/min
Run time	10 min
Wavelength	238 nm
Injection Volume	20µL
Detector	PDA Detector
Elution	Isocratic
Mobile Phase	Methanol and 0.1 % ortho-phosphoric acid (75:25 v/v)
Column oven temperature	25±5 °C

Preparation of mobile phase

The mobile phase is prepared by adding 1 ml of orthophosphoric acid in 1000 ml water (ie; 0.1% orthophosphoric acid in 1000 ml water) and methanol this mobile phase is ultra-sonicated used for 20 min were used in the ratio of 75:25(v/v).

Preparation of standard solutions

A standard stock solution of Atenolol and Nifedipine was prepared by dissolving 50 mg Atenolol and 20 mg of Nifedipine drugs in 50 ml of methanol made up to the volume by dissolving completely using the methanol to get the standard stock solutions of concentration 1000µg/ml for Atenolol and 400µg/ml Nifedipine.

Preparation of calibration curve

From the standard stock solutions, different aliquots of Atenolol and Nifedipine were pipetted into series of 10 ml volumetric flask from the above stock preparation (1000 µg/ml). HPLC grade methanol was used for making up the volume. 20µl solution was injected to the column and peak areas are measured. The calibration curve was established linear correlations were found between peak scales. Atenolol and Nifedipine concentration are defined my means of regression equation (fig. 3 and fig. 4 respectively). The Beer's law is observed in the concentration scale of 10-50µg of Atenolol and Nifedipine 4-20µg/ml Estimation of two drugs was done through PDA detector at 238 nm.

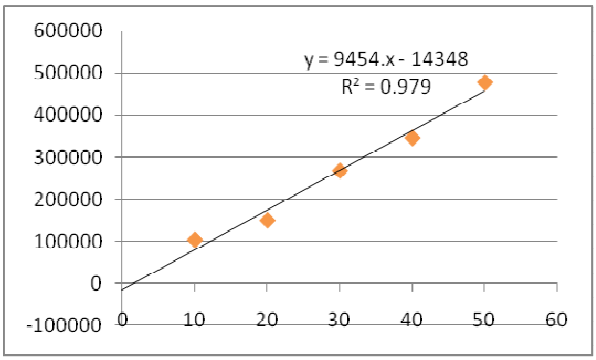


Fig. 3: Linearity graph of atenolol

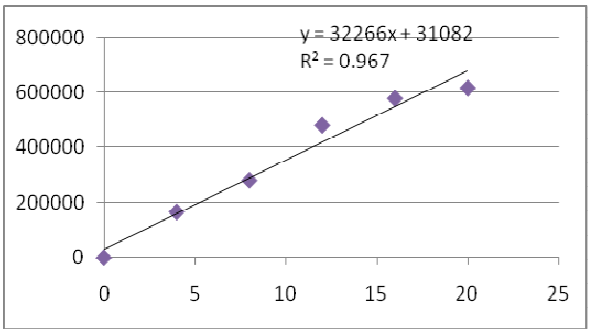


Fig. 4: Linearity graph of nifedipine

Preparation of sample solution of formulation

Into a dry 50 ml volumetric flask finely grounded and mixed contents of 20 capsules with equivalent weights of 50 mg Atenolol and 20 mg of Nifedipine were taken and ultra-sonicated until the drug dissolved in methanol then made up to the volume. At 238 nm area of each peak was measured. From the peak area, we determine the amount of each drug, atenolol and Nifedipine respectively present in the pure mixture. Upon further quantitative dilution of this solution with mobile phase, a final concentration of 50 mg/ml of Atenolol and Nifedipine was obtained.

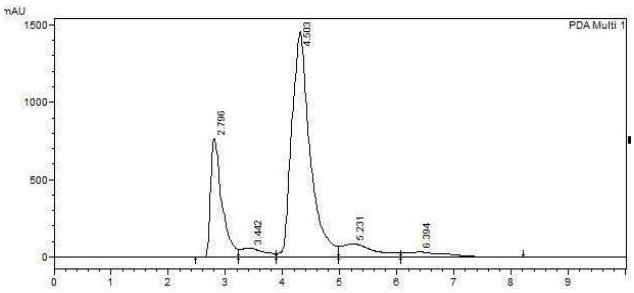


Fig. 5: Standard chromatogram of atenolol and nifedipine

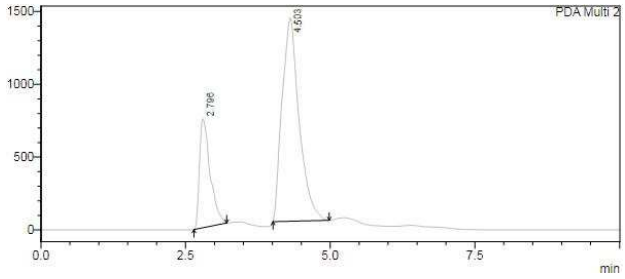


Fig. 6: Sample chromatogram of atenolol and nifedipine

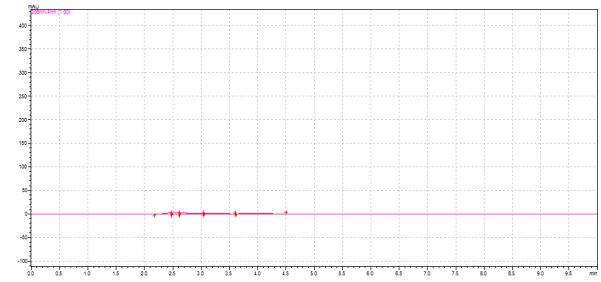


Fig. 7: Chromatogram of blank

RESULTS AND DISCUSSION

Linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ), robustness is the parameters to be validated for all samples according to the ICH guidelines using above chromatography conditions.

Linearity

Linear calibration curves of both Atenolol and Nifedipine were obtained based on the above chromatographic conditions. The r^2 for Atenolol and Nifedipine were found to be 0.979and 0.967 respectively. Between the peaks area of Atenolol and Nifedipinelinear correlations were found and are described by using regression equation. Table 2 specifies the results. For system suitability, Atenolol and Nifedipine and the linearity range were found to be10-50µg/ml and 4-20 µg/ml respectively.

Precision

Repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility) are the terms to determine the method precision mentioned below in table 3 and 4.

Accuracy

According to the test procedure triplicates of samples solutions by spiking with the test solutions of Atenolol and Nifedipine 50%, 100% and 150%. Prepared separately and injected into UFLC system to establish the accuracy of the test method. The results were summarized in below table 5 by calculating the spike levels of the amount of drug added, amount of drug found and average % recovery for atenolol and Nifedipine 50%, 100% and 150%.

Robustness

A measure of capacity to stay unaffected by small, but deliberate variations in the final method optimized conditions', called robustness for an analytical procedure as per ICH guidelines. The method development with predictable variations in the optimized method parameters is the most significant feature here. In the development phase of a method, robustness should be considered earlier-stated by ICH guideline.

Mobile phase composition, pH, flow rate, temperature, wavelength are the parameters to find characteristic variations and the results are shown in table 6 and 7 for Atenolol and Nifedipine respectively.

Table 2: System suitability parameters for atenolol and nifedipine

Parameters	Atenolol	Nifedipine
Linearity range (µg/ml)	10-50µg/ml	4-20 µg/ml
Regression equation	y = 9454.2x-14348	y = 32266x+31082
Slope	9454.2	32266
Intercept	14348	31082
Correlation coefficient	0.9797	0.9679
Retention Time (ret.) min	2.79 min	4.50 min
LOD (µg/ml)	1.698	0.786
LOQ (µg/ml)	5.147	2.382
Tailing factor	1.073	1.323
Theoretical plates	4141.47	3967.10

Table 3: Results for method precision intraday studies

Precision-intraday							
Injection no	Atenolol			Nefidipine			
	10µg	30 µg	50 µg	Injection no	4 µg	12 µg	20 µg
1	103594	268106	478153	1	165874	481173	615869
2	103545	267402	458241	2	165258	481654	614258
3	103492	267105	475142	3	165159	484429	616369
4	103684	264254	474157	4	165753	482554	617859
5	103798	264456	468122	5	165456	482844	615741
6	103882	263501	488153	6	165729	481433	615789
AVG	103665.8	265343.6	473661.3	AVG	165538.2	482582.8	615980.8
STD DEV	138.1454	1594.351	9138.676	STD DEV	265.947	1064.163	1060.909
%RSD	0.13326	0.600863	1.929369	%RSD	0.160656	0.220514	0.172231

Table 4: Results for method precision interday studies

Precision-interday							
Injection no	Atenolol			Nifedipine			
	10 µg	30 µg	50 µg	Injection no	4 µg	12 µg	20 µg
1	103594	268104	478153	1	165874	481173	615869
2	102597	264146	488248	2	165788	481754	614278
3	104562	267408	464854	3	167159	485229	616372
4	105414	268809	475149	4	168153	483554	617259
5	107453	258456	478654	5	167456	483864	615741
6	103475	258714	488153	6	168929	481473	618789
AVG	104515	264272.8	478868.5	AVG	167226.5	483174.8	616384.7
STD DEV	1583.815	4278.55	8010.15	STD DEV	1133.775	1396.66	1394.25
%RSD	1.515383	1.61899	1.67272	%RSD	0.677988	0.28905	0.22619

Forced degradation studies

The stress studies were performed on Atenolol and Nifedipinedrug at 50µg/ml concentration. unstressed sample(fig. 8) Here the bulk drug is subjected to acidic stress by adding 1.0 ml of 0.1M HCl (fig. 9) to drug solution and neutralized with 1.0 ml of 0.1M NaoH, at 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, 6 h and 32 h respectively. Similarly, the basic stress studies were performed by adding 1.0 ml of 0.1 M NaOH (fig. 10) and neutralized with 1 ml of 0.1M HCl. Thermal studies were performed by heating the sample at 60 °C (fig. 11) Oxidation studies were performed on the bulk drug by adding 2 ml of 3% H_2O_2,(fig. 12) and UV studies were also carried out by the sample at UV-Lamp 450C

(fig. 13)respectively. All samples were placed in a different volumetric flask (10 ml) and dissolved in HPLC grade methanol. Chromatographic system injected with final drug concentration for assay made with methanol. For all these stability study, the formation of degradable product was confirmed by comparing with the chromatogram of the solution kept under normal unstressed condition. All stressed samples were analysed by optimized UFLC method. The degradation data for Atenolol and Nifedipine was shown in below table 8.

Table 5: Recovery results for atenolol and nifedipine

Level of % recovery	Amount of std drug added (µg/ml)	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Difference	% Recovery	Mean
50	20	10	30	150652	100.36	99.19
				148151	98.69	
				147884	98.51	
100	20	20	40	148341	98.82	99.62
				150541	100.28	
				149780	99.77	
150	20	30	50	150047	99.95	99.00
				148442	98.88	
				147391	98.18	

Table 6: Results of robustness for atenolol

Condition		Tailing	% RSD	Theoretical plates	%RSD
As such condition (optimized method)		1.073	--	4158.49	--
Mobile phase ratio	70:30	1.059	0.66	4037.3	1.48
As such (75:25)	85:15	1.179	0.28	4048.3	1.34
% of Ortho-phosphoric acid	Decreased (-0.2 units)	1.847	1.29	4284.9	1.50
	Increased (+0.2 units)	0.98	1.87	4255.1	1.15
Flow rate	Decreased (-0.2 ml/min)	1.020	0.89	4267.39	1.29
	Increased (+0.2 ml/min)	1.099	1.20	4250.43	1.09
Column temperature	Decreased (-5 °C)	1.267	1.32	4048.29	1.34
	Increased (+5 °C)	1.183	0.83	4302.93	1.71
Wave length	Decreased (1 nm)	0.545	1.37	4249.39	1.08
	Decreased (2 nm)	1.288	1.60	4312.2	1.81
	Increased (1 nm)	1.373	1.74	4313.22	1.83
	Increased (2 nm)	1.218	1.47	4292.08	1.58

Table 7: Results of robustness for nifedipine

Condition		Tailing	%RSD	Theoretical plates	%RSD
As such condition (optimized method)		1.323		4358.49	
Mobile phase ratio	70:20	0.959	0.141	3732.39	1.47
As such (75:25)	85:15	1.865	1.19	3348.30	1.28
% of Ortho-phosphoric acid	Decreased (-0.2 units)	1.846	0.84	4946.9	1.51
	Increased (+0.2 units)	1.298	1.16	3456.74	1.42
Flow rate	Decreased (-0.2 ml/min)	1.170	1.93	4861.39	1.84
	Increased (+0.2 ml/min)	1.249	0.97	4285.43	0.81
Column temperature	Decreased (-5 °C)	1.167	0.82	4948.29	1.59
	Increased (+5 °C)	1.583	1.22	4202.93	1.82
Wave length	Decreased (1 nm)	0.835	1.22	4839.39	1.39
	Decreased (2 nm)	1.18	1.34	3893.92	1.92
	Increased (1 nm)	1.448	0.46	4839.22	1.58
	Increased (2 nm)	1.78	1.93	3772.08	1.59

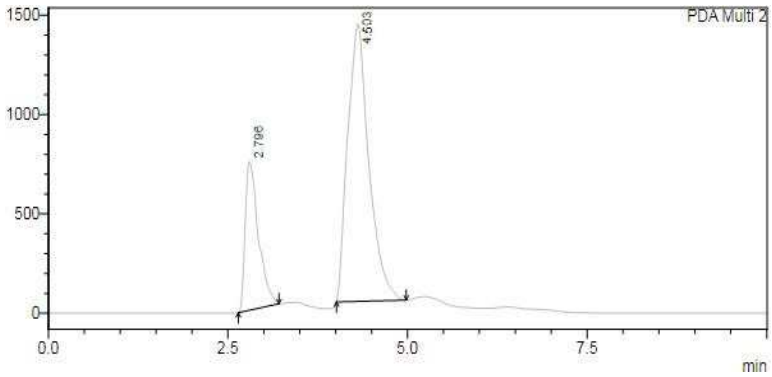


Fig. 8: Chromatogram of unstressed sample

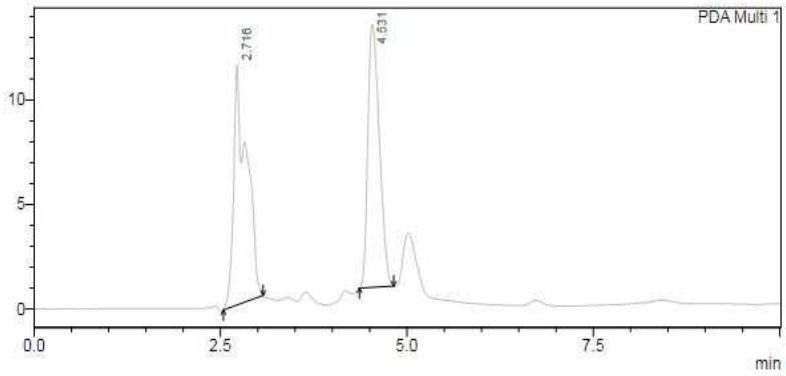


Fig. 9: Chromatogram of acid hydrolysis

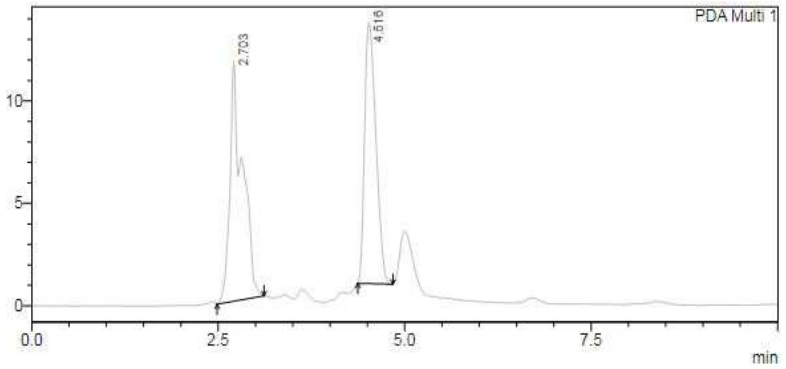


Fig. 10: Chromatogram of base hydrolysis

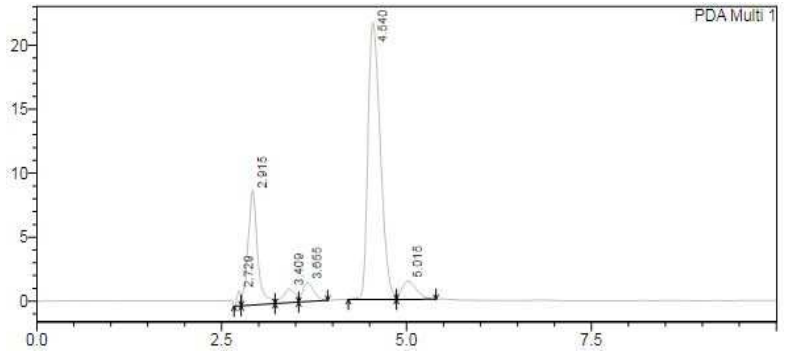


Fig. 11: Chromatogram of thermal stress

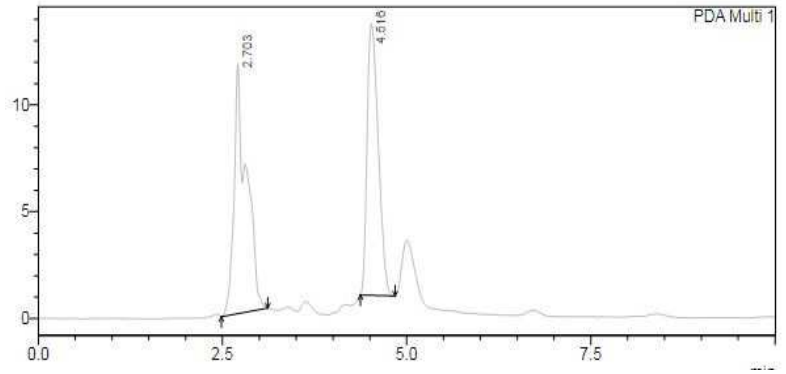


Fig. 12: Chromatogram of peroxide stress

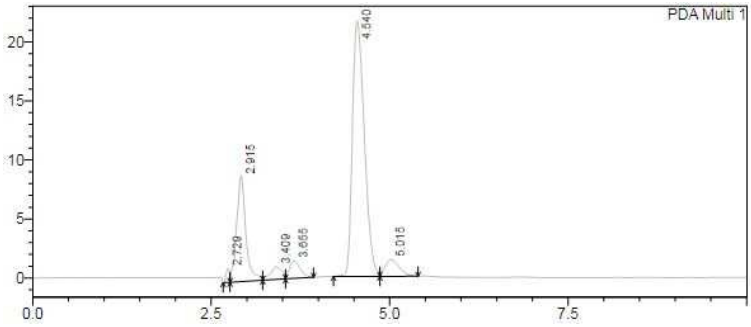


Fig. 13: Chromatogram of UV stress

Table 8: **Results for recovery studies of atenolol and nifedipine after the stress conditions (% recovery of drug)**

Time	Drug	UV	Thermal	0.1N HCL	0.1N NaoH	3%H_2O_2
0 Min	Atenolol	82.24%	73.11%	71.65%	72.34%	56.47%
	Nifedipine	84.23%	76.76%	87.79%	89.35%	81.34%
30 Min	Atenolol	77.34%	60.76%	57.29%	61.34%	44.19%
	Nifedipine	80.34%	67.31%	84.14%	87.34%	74.34%
1 h	Atenolol	69.32%	47.86%	52.3%	54.34%	32.47%
	Nifedipine	72.43%	50.16%	78.86%	80.34%	68.23%
2 h	Atenolol	61.73%	28.66%	37.47%	42.34%	25.19%
	Nifedipine	67.34%	37.14%	74.78%	78.38%	60.87%
4h	Atenolol	54.22%	19.81%	28.07%	30.87%	15.47%
	Nifedipine	59.34%	21.69%	67.27%	70.34%	44.34%
8h	Atenolol	47.82%	8.89%	14.64%	13.32%	4.43%
	Nifedipine	52.23%	30.15%	59.65%	57.23%	32.62%
16h	Atenolol	39.22%	---	6.34%	---	---
	Nifedipine	43.87%	---	44.64	43.24%	22.23%
32h	Atenolol	22.43%	---%	---	---	---
	Nifedipine	---44.24%	---	--	---	--

CONCLUSION

A simple, quick, sensitive, reliable, and precise stability indicating UFLC method was developed and validated for the estimation of Atenolol and Nifedipine. The method was observed to be linear, accurate, precise, and turned out to be sensitive, convenient and successful with good resolution for the estimation of Atenolol and Nifedipine in both bulk and pharmaceutical dosage forms in industries and research labs for routine sample analysis.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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