METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC ASSAY METHOD FOR NIFEDIPINE

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RESEARCH ARTICLE

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ABSTRACT

A simple and rapid stability indicating, ultra performance liquid chromatographic (UPLC) method was developed and validated for the determination of Nifedipine. The quantitative determination of Nifedipine drug was performed on a Sunniest C-18-HT, 2m, (50 2.1nm) column with gradient elution. For UPLC method, UV detection was made at 335nm. During method validation, parameters such as precision, linearity, stability, robustness and specificity were evaluated, which remained within acceptable limits. Forced degradation studies of Nifedipine were studied under acidic and alkaline stress conditions. Mild degradation of the drug substance was observed during acidic hydrolysis and no degradation was observed during basic hydrolysis.

Keywords: Nifedipine, UPLC, Stability indicating assay, Validation.

INTRODUCTION

Nifedipine (3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) (Fig. 1) is a dihydropyridine calcium channel blocker that primarily blocks L-type calcium channels. (1) Nifedipine is used to treat high blood pressure and to control angina (chest pain). (2) It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. (3) It lowers blood pressure by relaxing the blood vessels so the heart does not have to pump as hard. It controls chest pain by increasing the supply of blood and oxygen to the heart. (4)

Literature survey for Nifedipine revealed several analytical methods based on different techniques, viz UV Spectrophotmetric (5,6), method for simultaneous determination, method Validation of Nifedipine Solid Dosage Form, HPLC (7,8) analysis of Nifedipine Residues on Stainless-Steel Surfaces in the Manufacture of Pharmaceuticals, method for the determination of Nifedipine in pharmaceutical preparation and RP-HPLC. (9-13)

1. UPLC system

Ultra performance liquid chromatography is a recent technique in liquid chromatography, which enables significant reduction in separation time and solvent consumption. Literature reports reveals that UPLC system allows about nine fold decrease in analysis time as compared to conventional HPLC system using 5 µm particle size analytical columns and about three fold decrease in analysis time in comparison to 3 µm particle size analytical column without compromise on overall separation. (14-16)

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity, and light and enables recommendation of storage conditions, retest periods and shelf life to be established. The present investigation was undertaken to establish the stability indicating UPLC assay method for the estimation of Nifedipine as recommended by the International Conference on Harmonization (ICH) guidelines (17) and USP. (18)

2. Experiments

2.1. Chemicals and reagents

Reference standard of Nifedipine was bought from commercial sources (Sigma Aldrich). Methanol of UPLC grade was obtained from Qualigens, Mumbai, India. Analytical reagent grade and Mili Q was used throughout the study.

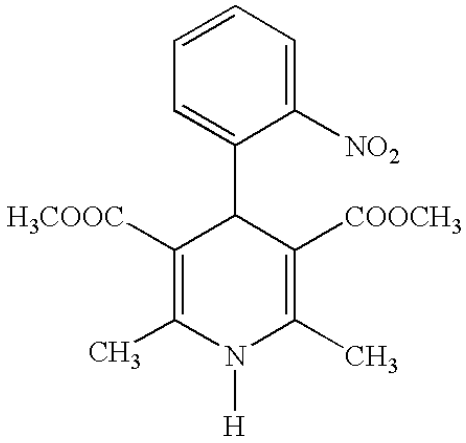


Figure 1: Chemical structure of Nifedipine

Table 2.1: UPLC conditions

S.No.	UPLC System condition	
1	Column	Sunniest C-18-HT, 2µm, (50×2.1)
2	Injection volume	3 µL
3	Column Temperature	40°C
4	Sample Temperature	5°C
5	Flow rate	0.7 mL/min
6	Run Time	5 min
7	Detection System	(PDA) wavelength 210-400 nm
8	Chromatogram Extracted at	335 nm
9	Mobile Phase A	Water: ACN (950:50 mL) and formic Acid (0.5 mL)
10	Mobile Phase B	ACN (1000 mL) and formic acid (0.5 mL)

Table 2.2: Gradient flow characteristics

S.No.	Time (min)	% Mobile Phase A	% Mobile Phase B
1	0	100	0
2	0.5	100	0
3	2.5	40	60
4	3.2	40	60
5	3.3	10	90
6	3.8	10	90
7	4.0	100	0
8	5.0	100	0

2.2. Chromatographic system

Analyses were performed on Acquity UPLC™ system (Waters, Milford, USA), consisting of binary solvent manager, sample manager and PDA detector. The data collection and data processing were accomplished using Waters EmpowerTM chromatography data software. The analytical column used was UPLC Sunniest C-18-HT, 2µm, (50 2.1 nm) column. The separation of Nifedipine was achieved by gradient elution using Mobile Phase A (ACN: Milli Q water 950: 50 mL with 0.5 mL Formic Acid) and Mobile Phase B (ACN and Formic acid). The optimized conditions were as follows:

2.3. Preparation of stock solution

The stock solution was prepared by weighing 10 mg of Nifedipine in 10 mL volumetric flask. The drug was dissolved in diluent, i.e. in Methanol: water (90:10) sonicated for one minute to give 1 mg/mL solution. The samples were prepared from this stock solution after suitable dilutions.

2.4. Preparation of standard solution

Standard Solution (50 µg/mL) was prepared from the stock solution, 5 mL solution of stock solution was transferred to 100 mL volumetric flask and volume was made up to 100 mL by using diluent Methanol: water (90:10) get the final sample concentration of 50 µg/mL.

2.5. Method validation

System suitability

System suitability parameters were measured so as to verify the system performance. System precision was determined on six replicate injections of standard preparations.

System Precision

System Precision was investigated using sample preparation for six replicate injections and analyzed by proposed method. The % RSD was calculated for the results obtained.

Method precision

Method precision was investigated using sample preparation procedure for six real samples and analyzed by proposed method. The % RSD was calculated for the results obtained.

Linearity

Linearity was demonstrated from 70% to 130% of standard concentration using minimum five calibration level (70%, 80%, 90%, 100%, 120%, and 130%) for the compound. The method of linear regression was used for data evaluation. Peak areas of sample compound were plotted against respective concentrations.

Robustness

The robustness is a measure of method capacity to remain unaffected by small but deliberate changes in chromatographic conditions such as change in wavelength of detection (± 5%) flow rate (± 10%) as well as ratio of mobile phase (± 2 units).

Recovery

The API recovery was analyzed by spiking the sample with a known concentration and analyzed by proposed method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the lowest amount of analyte that can be detected in a sample, but not necessarily quantified, under the stated experimental conditions. The LOQ was identified as the lowest concentration of the standard curve that could be quantified with acceptable accuracy, precision and variability. They were determined by signal-to-noise method.

Specificity

Specificity was analyzed by preparing a blank solution containing diluent without drug and analyzed by the proposed method.

Forced Degradation Studies

Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed method. The samples of Nifedipine were exposed to acidic and alkaline degradation conditions. All the exposed standards and samples were than analyzed by proposed method.

3. Results and discussion

3.1. Method development

For analysis of Nifedipine, different chromatographic conditions were tried on UPLC and results obtained were compared. Among various columns available for UPLC analysis Sunniest C-18-HT, 2µm, (50 2.1nm) column was preferred, because it provides appreciable peak shape, resolution and absorbance were good. Among different mobile phase employed the mobile phase consisted of Mobile Phase A : Water: ACN (950:50 mL) and formic Acid (0.5 mL) and Mobile Phase B: ACN (1000 mL) and formic acid (0.5 mL) was found to be suitable for analysis of Nifedipine. Further a flow rate of 0.7 ml/min, an injection volume of 5 µl and UV detection at 335 nm for drug was found to be suitable for analysis. Fig. 2 indicates the peak obtained for the sample by the selected method.

3.2. Analytical Parameters and Validation

After satisfactory development of method it was subjected to method validation as per ICH guidelines. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (precision,

linearity, robustness, stability indicating capability).

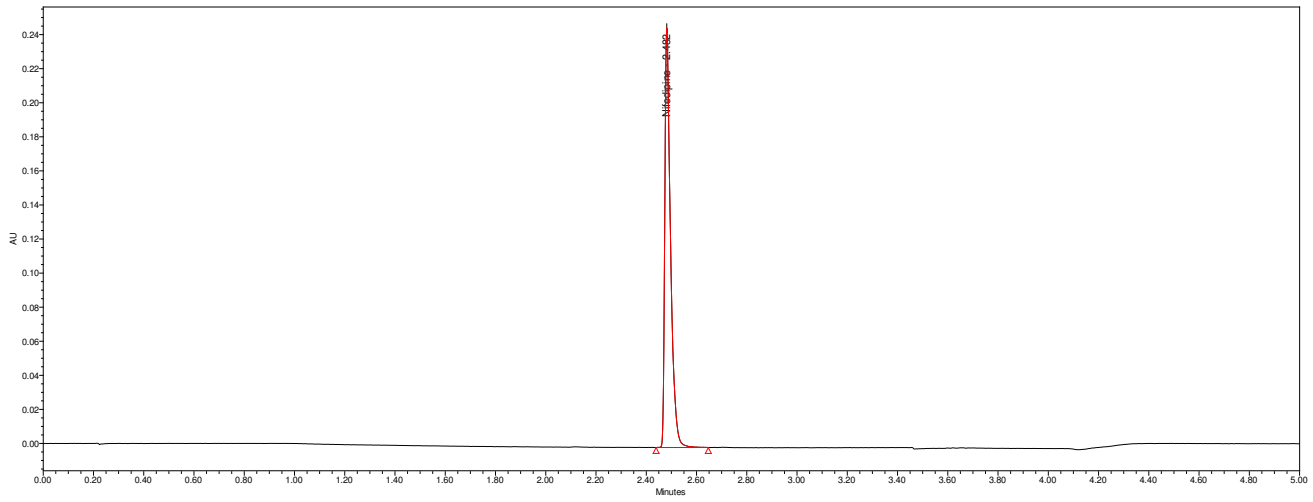


Figure 2: Nifedipine Chromatogram

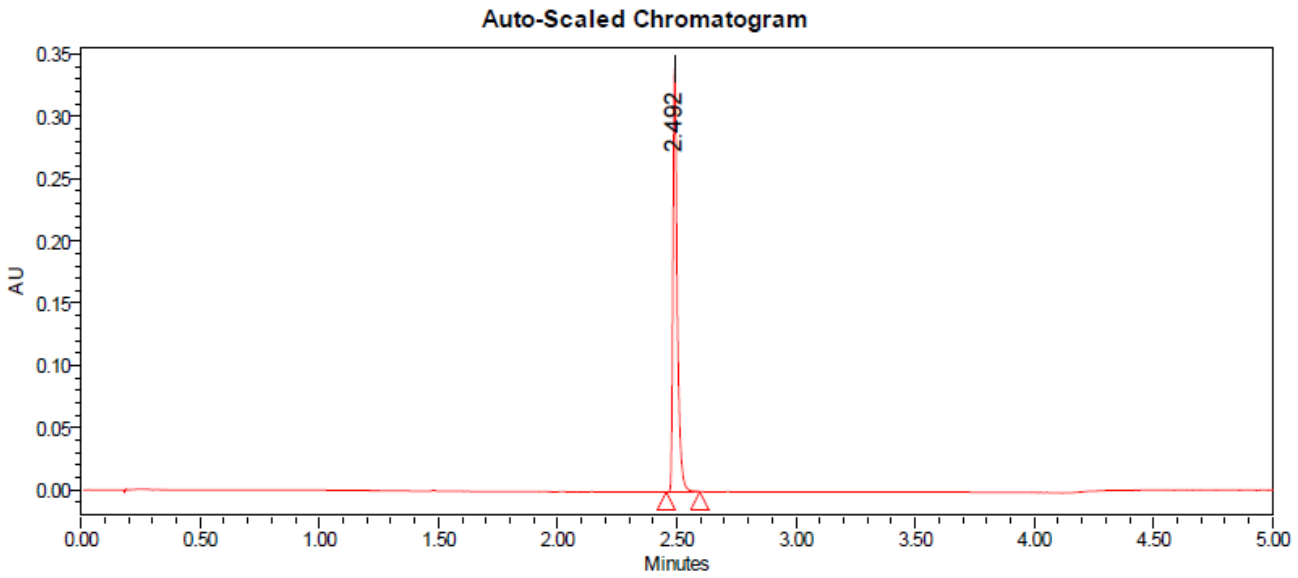


Figure 3: Chromatogram of Nifedipine showing system suitability

System suitability

Results of other system suitability parameters such as theoretical plates, purity angle, purity threshold and tailing are presented in Table 3.1. Figure 3 shows the chromatogram of Nifedipine for system suitability. The data presented in Table 3.1 indicated the acceptable system suitability parameters, as the tailing factor was not more than 2 and theoretical plates are more than 1000 and purity angle was less than purity threshold.

System precision

Six replicate of the standard solution were used to calculate system precision. Table 3.2 showed that %RSD was found to be <1%. The chromatogram for system precision of Nifedipine is shown in figure 4

Table 3.1: System Suitability Parameters for Nifedipine

Retention time	Purity Angle	Purity Threshold	USP Tailing	USP Plate Count	K Prime
2.492	0.313	1.023	1.53	104158	1.49

Table 3.2: System precision for Nifedipine

S.no.	Sample	RT	Area
1.	Standard Nifedipine Injection 1	2.49	430028
2.	Standard Nifedipine Injection 2	2.50	429848
3.	Standard Nifedipine Injection 3	2.49	428007
4.	Standard Nifedipine Injection 4	2.50	427538
5.	Standard Nifedipine Injection 5	2.49	427897
6.	Standard Nifedipine Injection 6	2.50	428490
%RSD			0.2

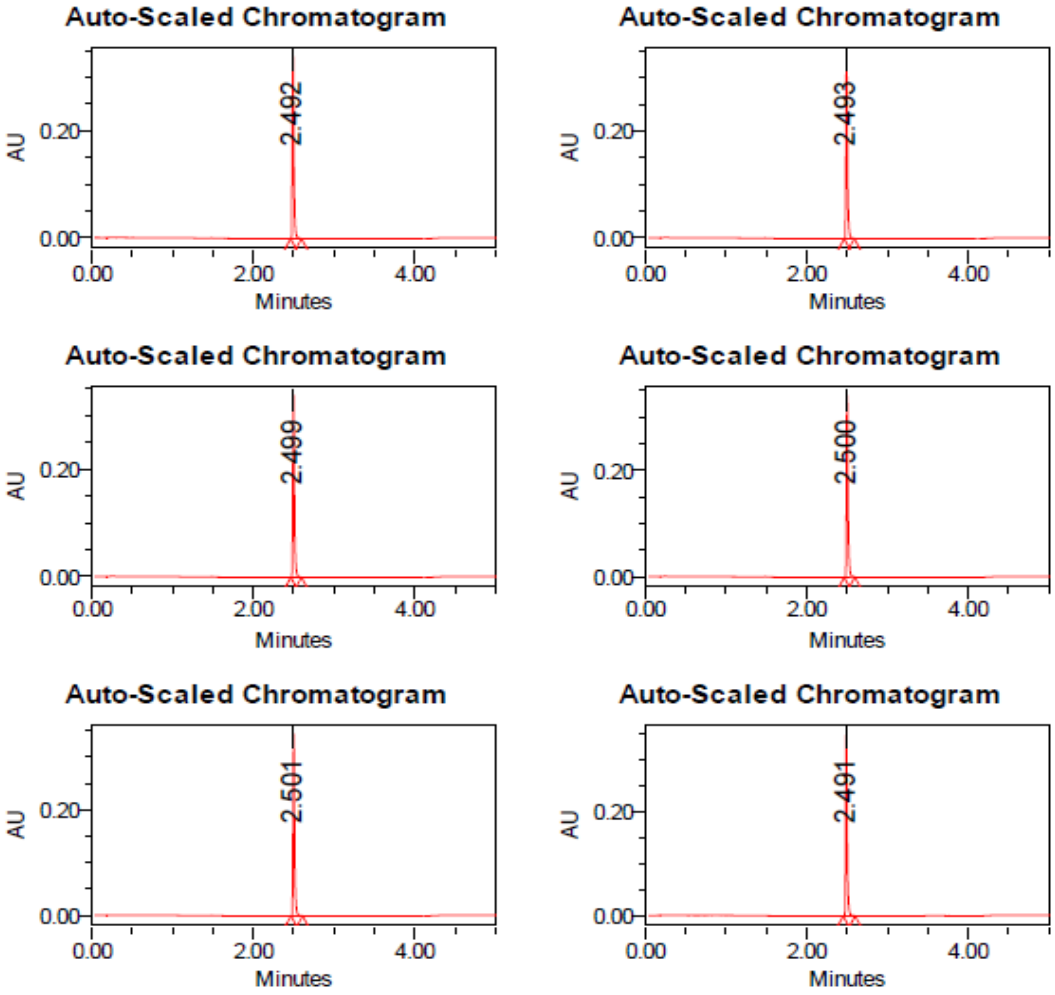


Figure 4: Chromatogram of Nifedipine showing System Precision

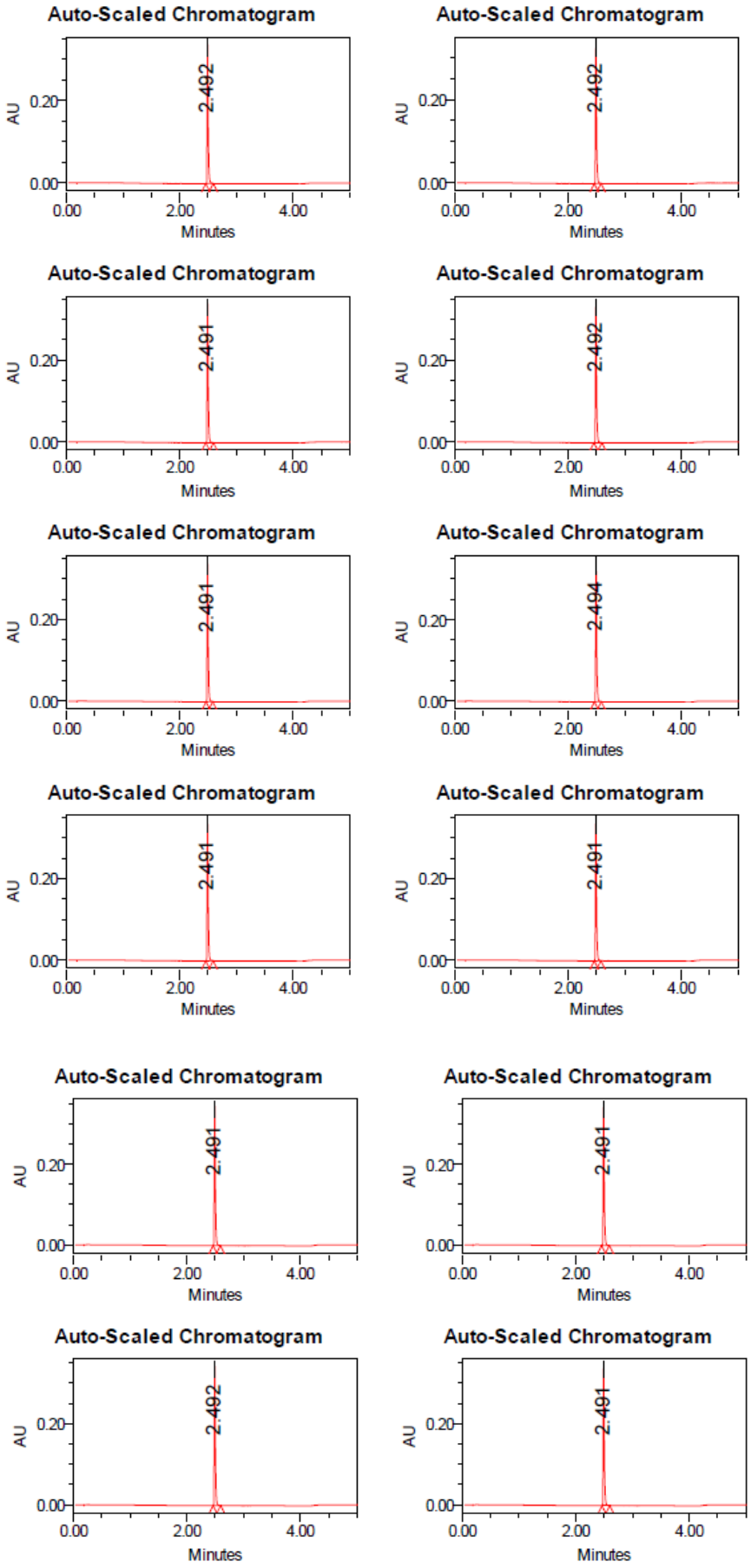


Figure 5: Chromatogram of Nifedipine for Method precision

Table 3.3: Method precision for Nifedipine

Sr. no.	Sample	RT	Area	Assay
1	Nifedipine Sample Injection 1	2.49	421047	99.43
	Nifedipine Sample Injection 2	2.49	420409	
2	Nifedipine Sample Injection 1	2.49	430288	96.72
	Nifedipine Sample Injection 2	2.49	429580	
3	Nifedipine Sample Injection 1	2.49	431768	98.81
	Nifedipine Sample Injection 2	2.49	432575	
4	Nifedipine Sample Injection 1	2.49	434282	100.75
	Nifedipine Sample Injection 2	2.49	432728	
5	Nifedipine Sample Injection 1	2.49	439292	98.85
	Nifedipine Sample Injection 2	2.49	439438	
6	Nifedipine Sample Injection 1	2.49	436078	98.94
	Nifedipine Sample Injection 2	2.49	436441	
%RSD				1.32

Method precision

Sample of one batch were prepared and analyzed separately six times in duplicate as per the method. The % assay was calculated using the formula:

$$\%\ Assay\ (w/w) = \frac{Area\ of\ sample\ \times Dilution\ of\ standard\ \times Purity\ \times 100}{Area\ of\ standard\ \times Dilution\ of\ sample}$$

The % RSD was calculated by the result obtained and was found to be <2% as shown in table 3.3. Figure 5 shows the chromatogram of Nifedipine for method precision.

Linearity

The graph of concentration of Nifedipine vs. Area was plotted. The response was found to be linear for 70% to 130% standard concentration. The correlation coefficient was found to be 0.9983. Table 3.4 showed the data for linearity of Nifedipine. Figure 6 and Figure 7 shows the linearity curve and chromatogram of Nifedipine for linearity respectively.

Table 3.4: Linearity for Nifedipine

Linearity Range	Concentration (in mcg/mL)	Area
70%	35	312206
80%	40	346332
90%	45	392649
100%	50	435092
110%	55	471672
120%	60	512339

130%	65	563024
Correlation Coefficient (r^2)	**0.9983**	

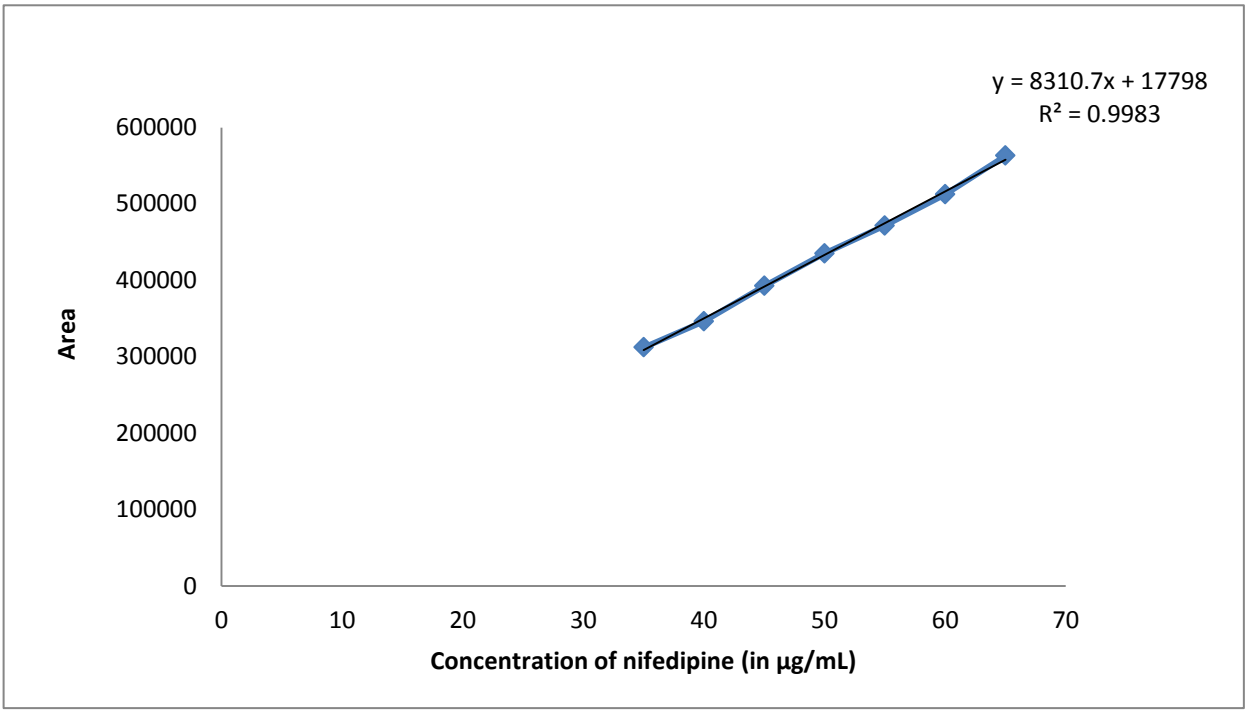


Figure 6: Linearity Curve for Nifedipine

Table 3.5: System suitability parameters and robustness

System suitability parameters	Robustness parameters	NIF
Column efficiency	No change (repeatability)	104158
	Wavelength of detection (+2 units)	100025
	Wavelength of detection (- 2 units)	100126
	Flow (+10%)	100183
	Flow (-10%)	102324
	Organic content (-2%)	92936
Purity angle	No change (repeatability)	0.313
	Wavelength of detection (+2 units)	0.305
	Wavelength of detection (- 2 units)	0.305
	Flow (+10%)	0.318
	Flow (-10%)	0.329
	Organic content (-2%)	0.325
Purity threshold	No change (repeatability)	1.024
	Wavelength of detection (+2 units)	1.022
	Wavelength of detection (- 2 units)	1.023
	Flow (+10%)	1.023
	Flow (-10%)	1.024
	Organic content (-2%)	1.022

Tailing	No change (repeatability)	1.53
	Wavelength of detection (+2 units)	1.527
	Wavelength of detection (- 2 units)	1.510
	Flow (+10%)	1.537
	Flow (-10%)	1.519
	Organic content (-2%)	1.519

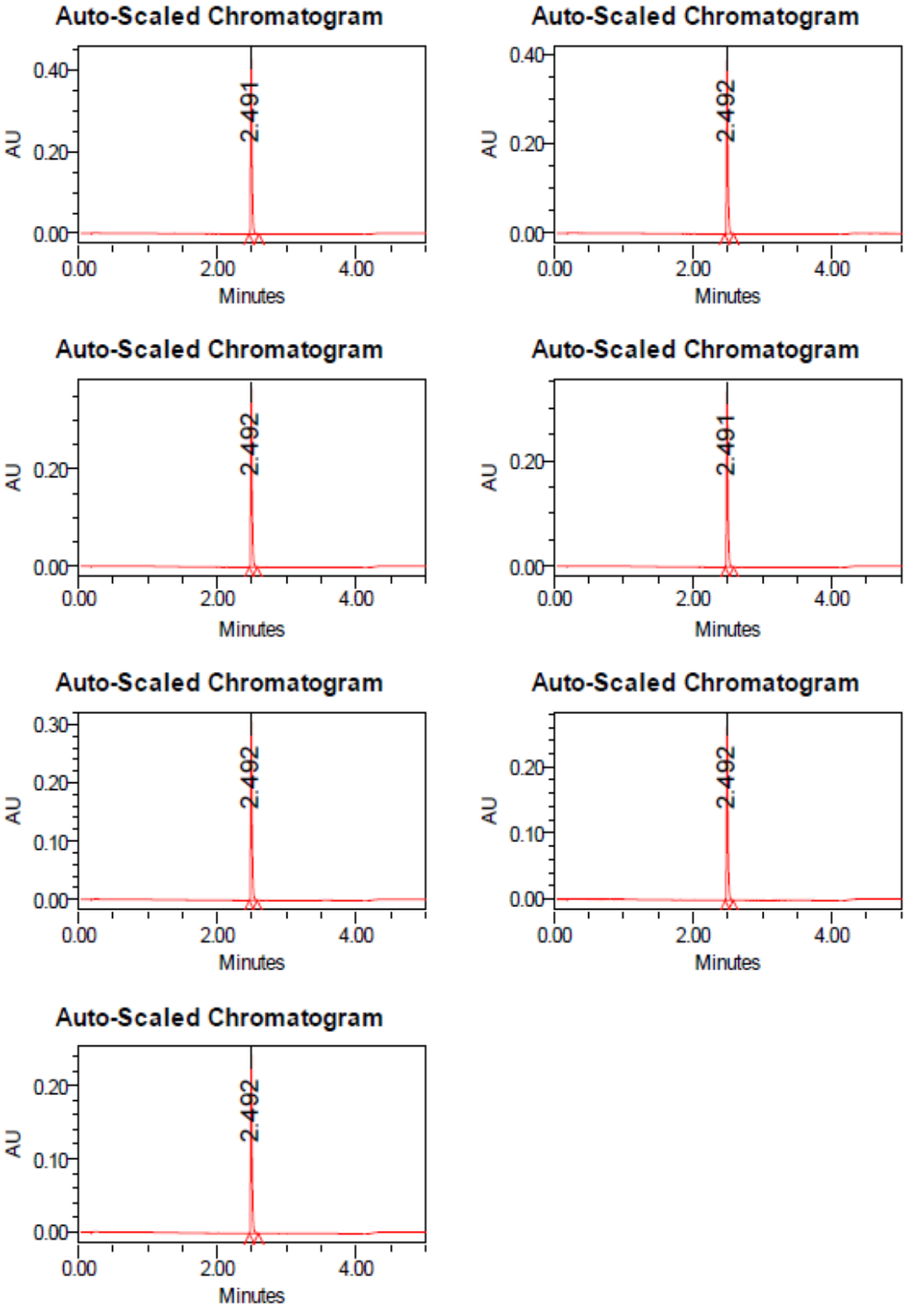


Figure 7: Chromatogram of Nifedipine for Linearity

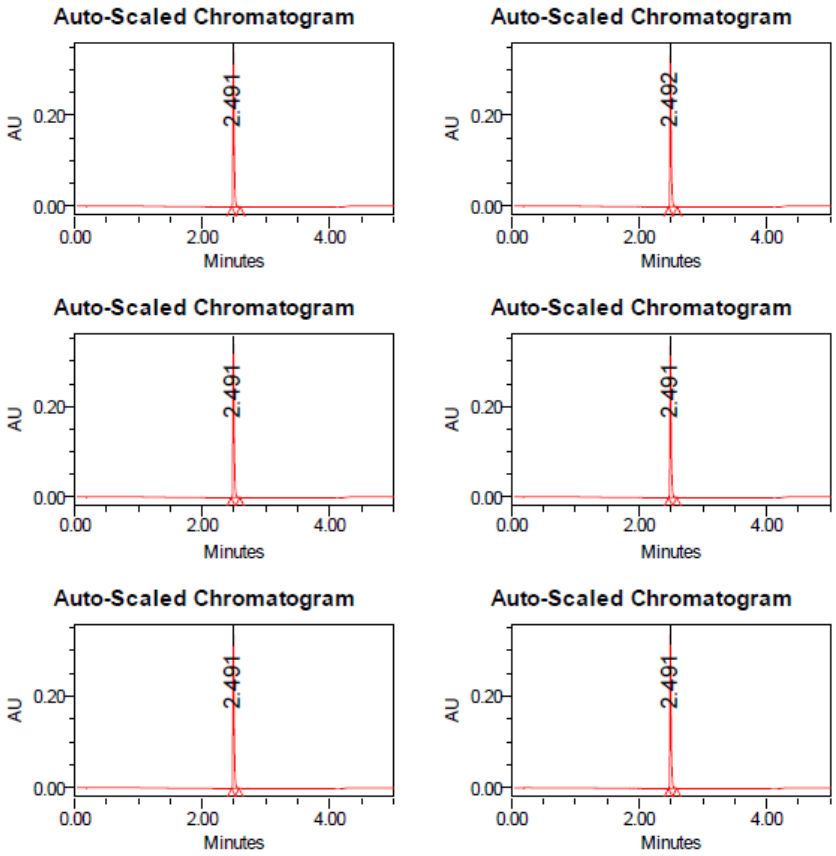
Robustness

No significant effect was observed on system suitability parameters such as capacity factor, purity angle, purity threshold and tailing, when small but deliberate changes were made to Three samples were prepared and analyzed under variable conditions i.e. change in flow rate (± 10%), wavelength of detection (± 2 unit), organic content (± 2%) in duplicate. Table 3.6 shows the %RSD was found to be <2% for every chromatographic conditions such as change in flow rate (± 10%), wavelength of detection (± 2 unit), organic content (± 2%). The results are presented in Table 3.5, along with system suitability parameters of normal methodology.

condition. Thus, the method was found to be robust with respect to variability in above condition. The Chromatogram for different conditions are shown in Figure 8.

Table 3.6: Robustness results for Nifedipine

Sample	RT	Area	Assay	%RSD
+10% flow rate				
1	2.48	420094	98.16	**1.58**
2	2.48	421548	96.88	
3	2.48	427245	96.7	
-10% flow rate				
1	2.51	445842	97.67	**1.69**
2	2.51	445544	99.1	
3	5.51	449904	101.72	
-2% organic				
1	2.43	434010	98.14	**1.89**
2	2.43	433909	99.73	
3	2.43	439989	102.8	
+2 unit wavelength of detection				
1	2.49	430239	96.51	**1.72**
2	2.49	430867	99.01	
3	2.49	436932	96.16	
-2 unit wavelength of detection				
1	2.49	433046	97.92	**1.62**
2	2.49	433724	100.5	
3	2.49	439753	101.05	



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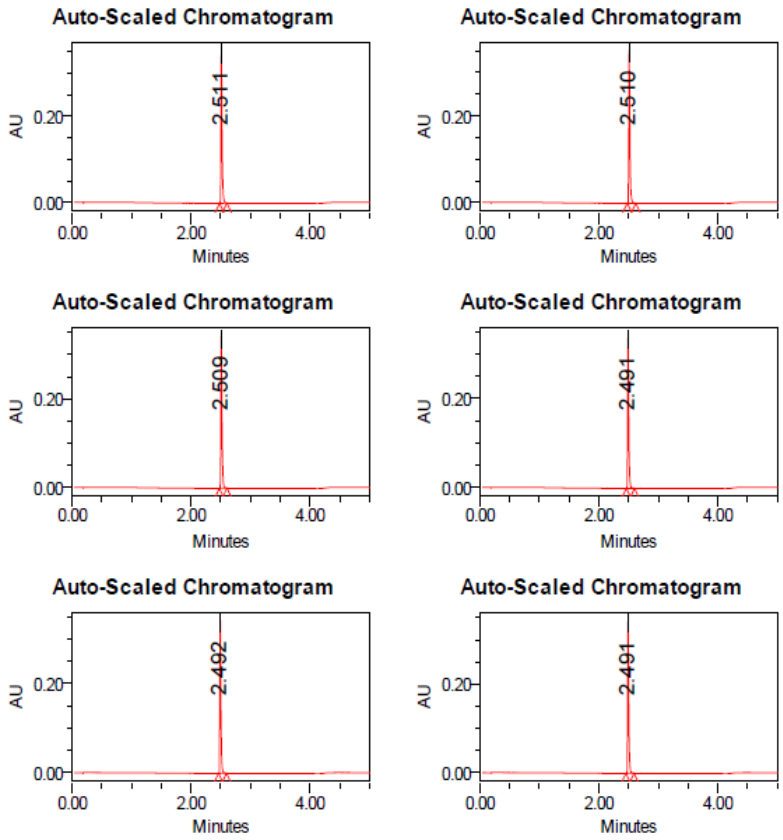

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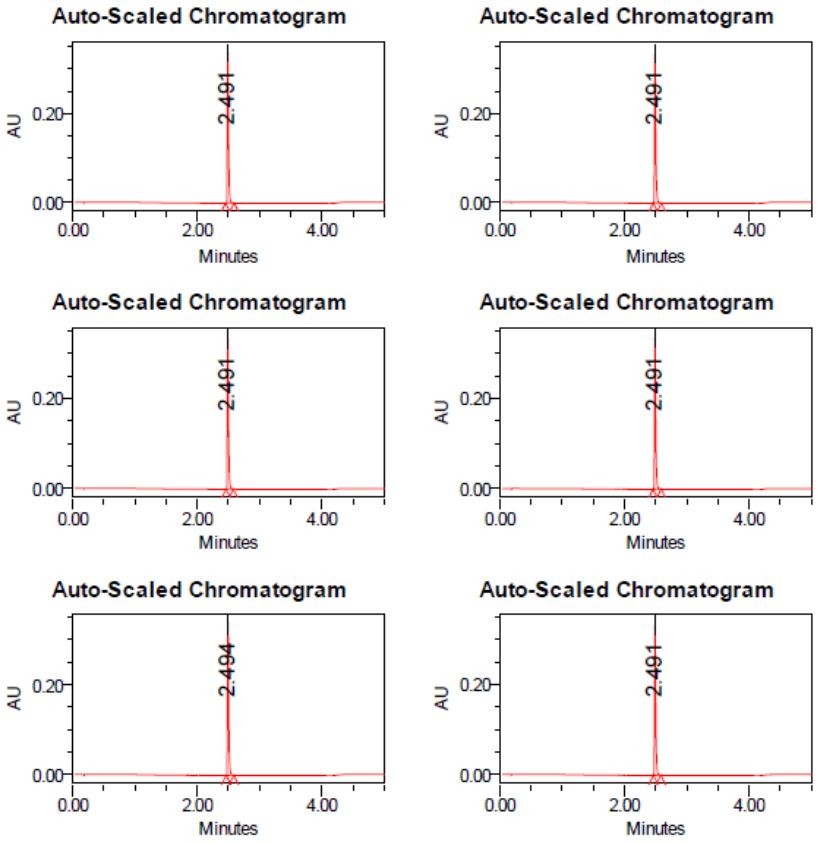

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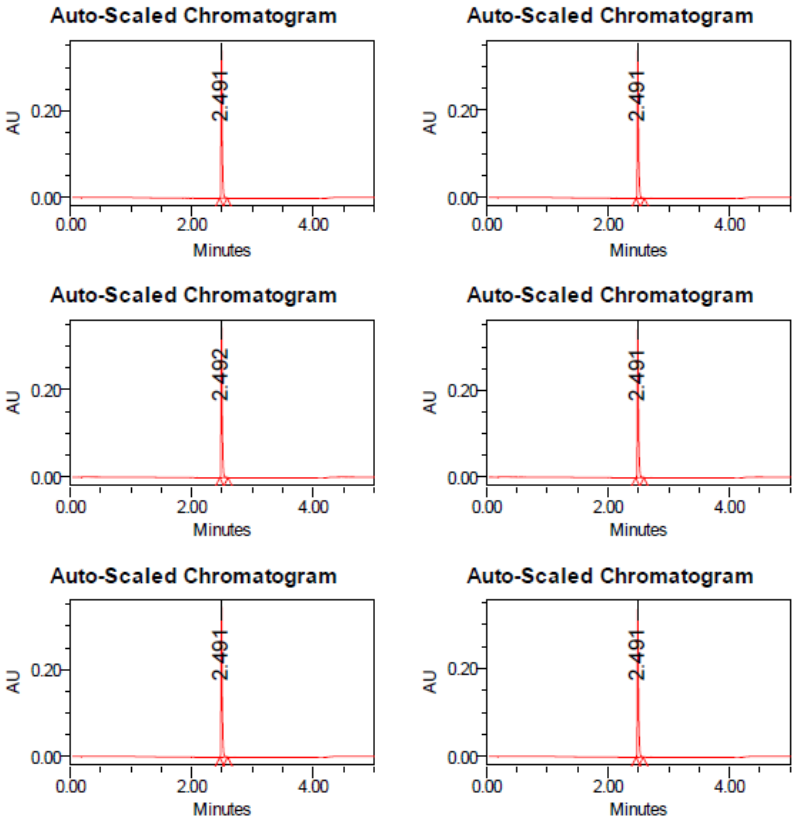

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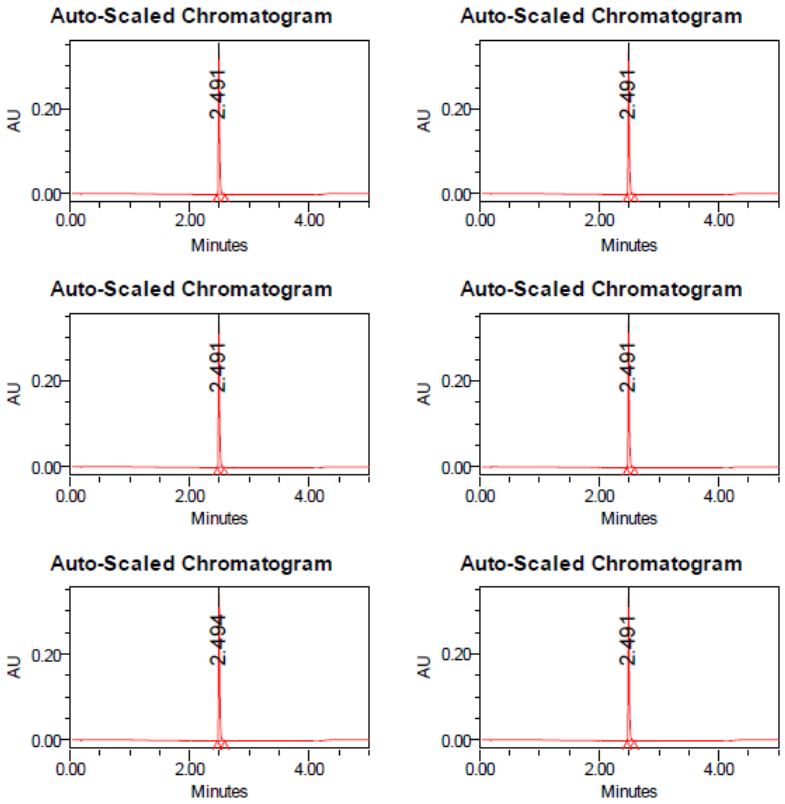


(e)

Figure 8: Chromatogram for Robudtness (a) +10% flow rate (b) -10% flow rate (c) +2 unit wavelength of detection (d) -2 uni0t wavelength of detection (e) -2% organic content

Recovery % recovery can be calculated using the formula:

The standard sample was spiked with 20% API (Nifedipine) and analyzed as per the method.

$$\%\, Recovery = \frac{Area\ of\ sample\ \times Dilution\ of\ standard\ \times Purity\ \times 100}{Area\ of\ standard\ \times Dilution\ of\ sample}$$

The % Recovery was found to be 120%

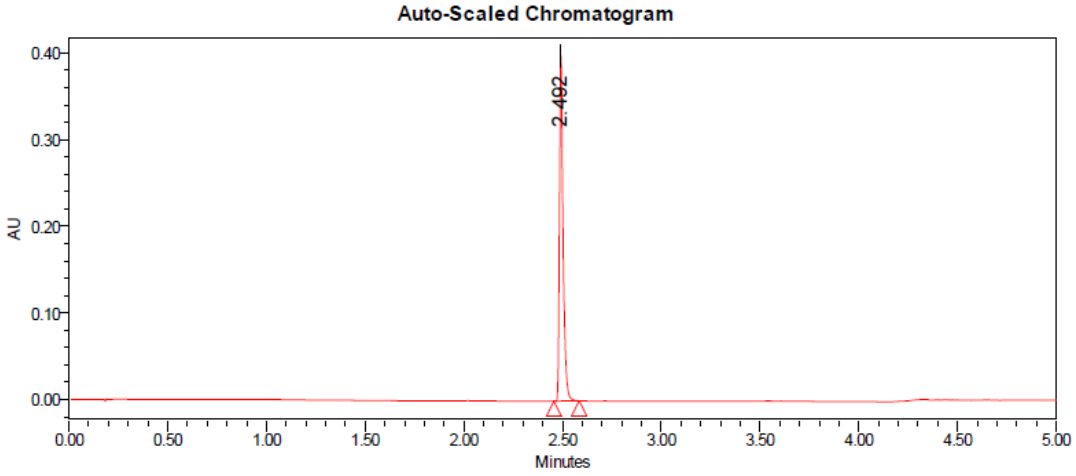


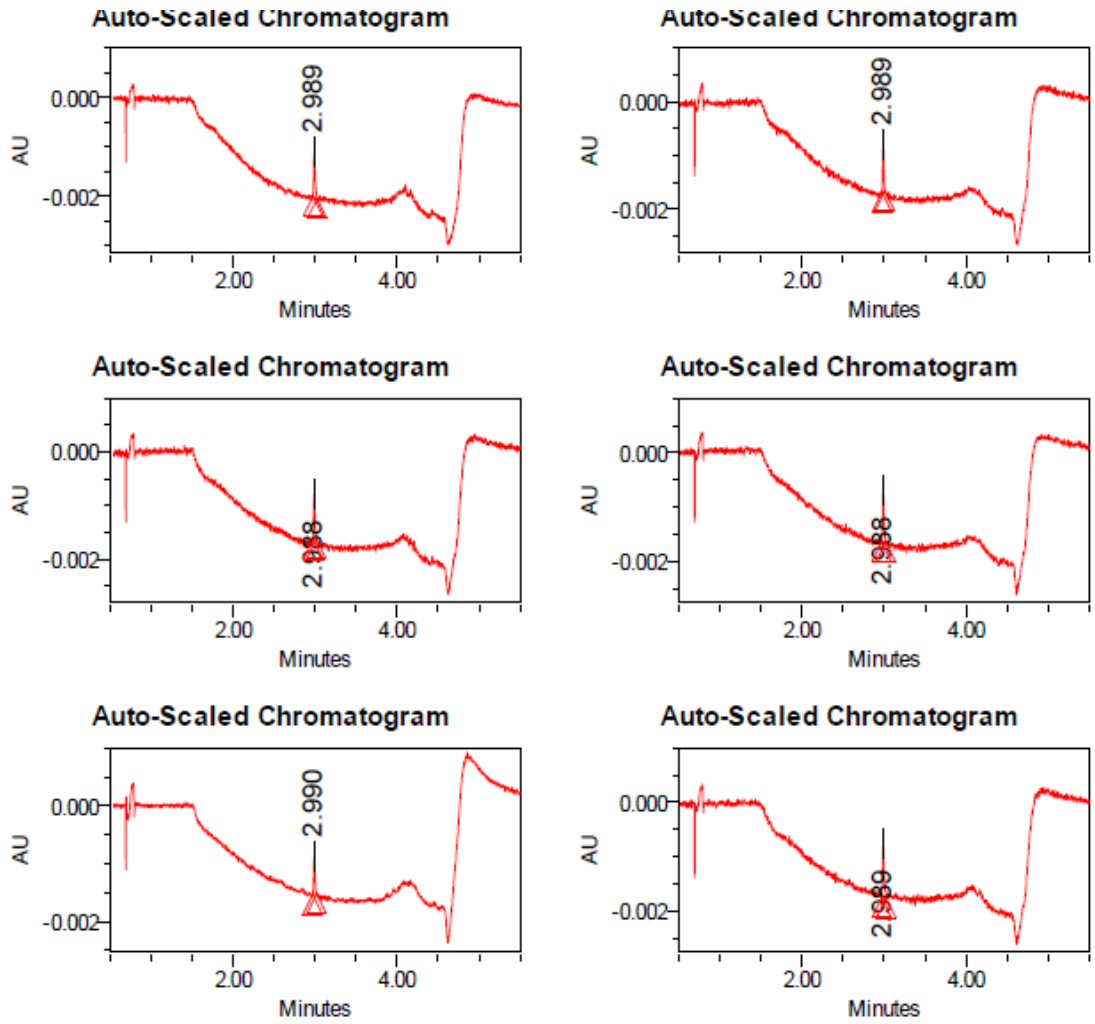
Figure 9: Chromatogram of Nifedipine showing recovery at 120%

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were determined using signal-to-noise (s/n) method by comparing result of test of samples with known concentration of analyte to blank samples. A signal-to-noise ratio of 3:1 is used for LOD whereas a signal-to-noise ratio of 10:1 is used for LOQ. The LOD and LOQ values of Nifedipine were found to be 180 ng/mL and 300 ng/mL respectively.

Table 3.7: s/n values for LOD and LOQ samples

LOD	s/n	LOQ	s/n
Sample 1	3.87	Sample 1	9.79
Sample 2	3.06	Sample 2	10.19
Sample 3	3.32	Sample 3	9.64
Sample 4	3.35	Sample 4	12.42
Sample 5	3.49	Sample 5	10.20
Sample 6	3.75	Sample 6	10.67
Mean s/n	3.47	**Mean s/n**	10.49



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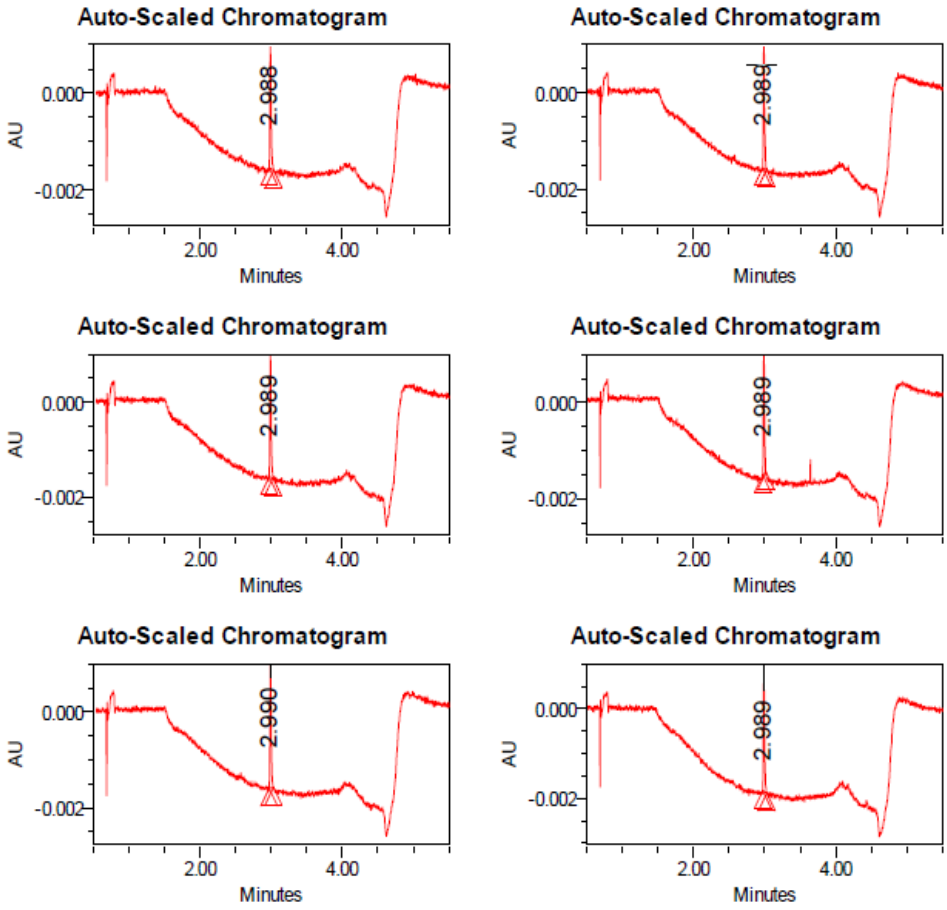
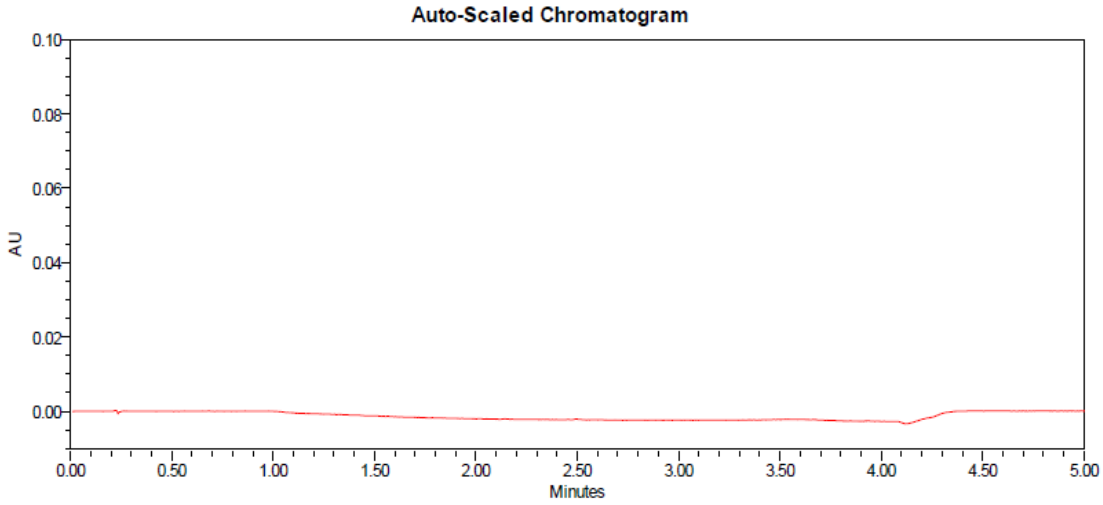


(b)

Figure 10: Chromatogram of Nifedipine for (a) LOD (b) LOQ

Specificity

Specificity was tested against the standard Nifedipine solution and a blank solution (i.e. diluent) under optimized test conditions. The comparison of the chromatograms of blank and standard solution revealed that there was no other peak co-eluting with the peaks of Nifedipine in sample solution.no interference was observed from diluent at retention time of Nifedipine. Therefore it can be concluded that the method is specific and can access unequivocally the analyte of interest in presence of possible interferences. The chromatogram for standard Nifedipine and diluent are shown in figure 11.



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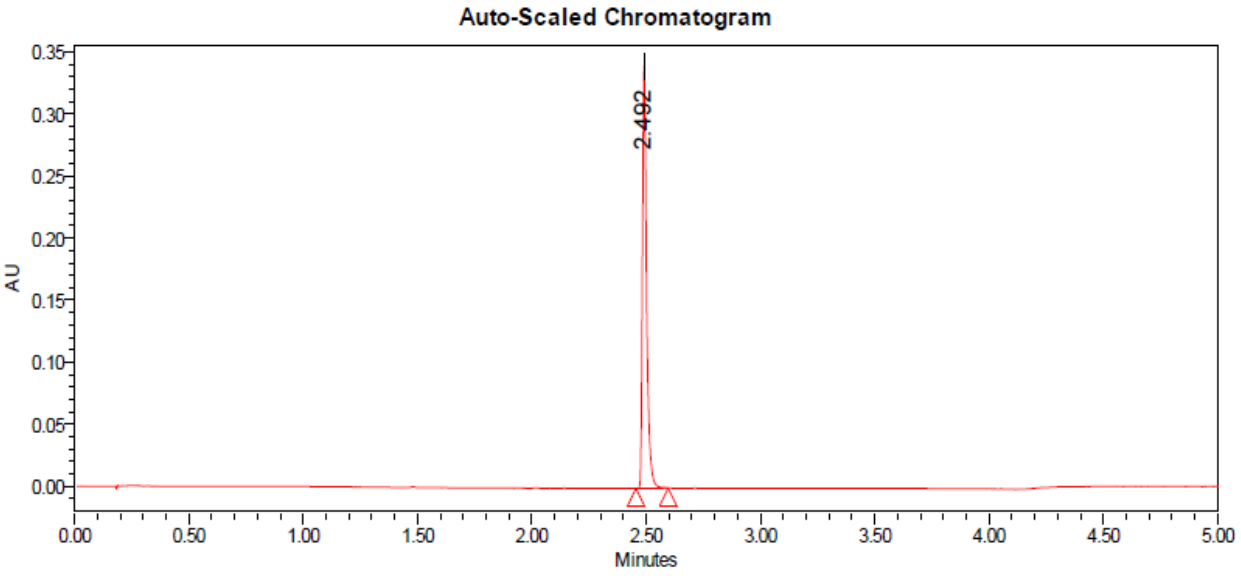
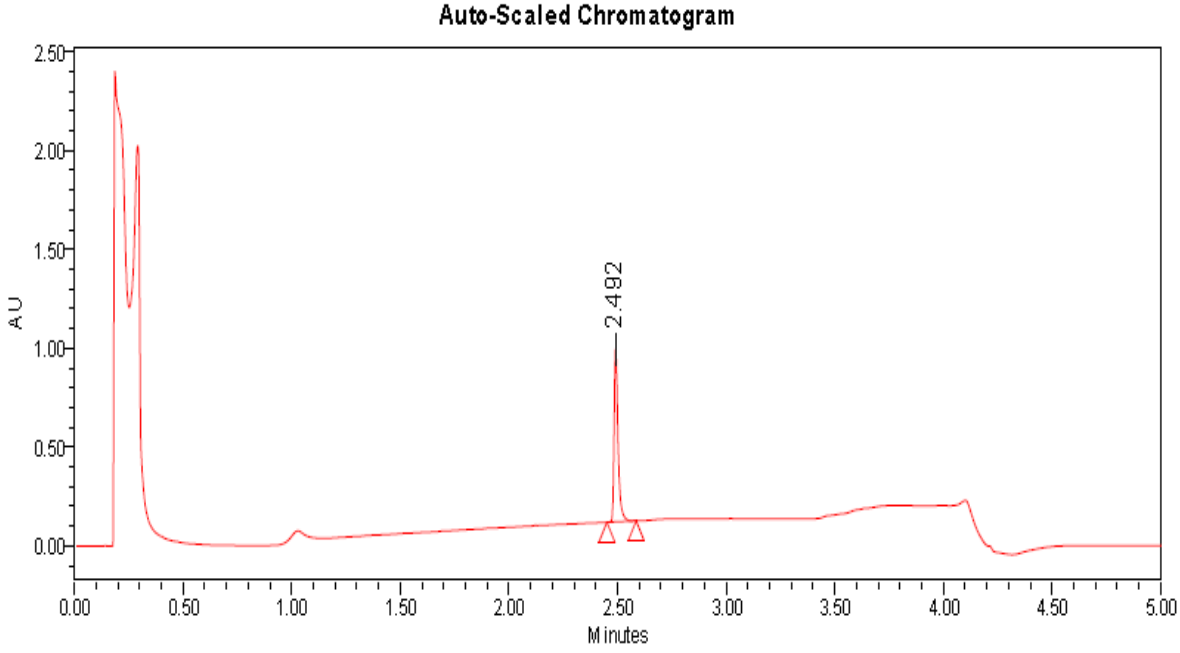


(b)

Figure 11: Chromatogram (a) Diluent (b) Nifedipine Standard solution

Forced Degradation Studies

Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed method. The samples of Nifedipine were exposed to acidic and alkaline degradation conditions. All the exposed standards and samples were than analyzed by proposed method. In acidic condition no degradation was observed when Nifedipine was treated with 0.1N Hydrochloric Acid (HCl). But when the concentration of HCl was increased to 1N 11% degradation was observed. The degradation product's peak were not visible at 335 nm, therefore the chromatogram was extracted at 220 nm. Figure 12 shows the chromatogram for the degradation products obtained after acidic degradation.



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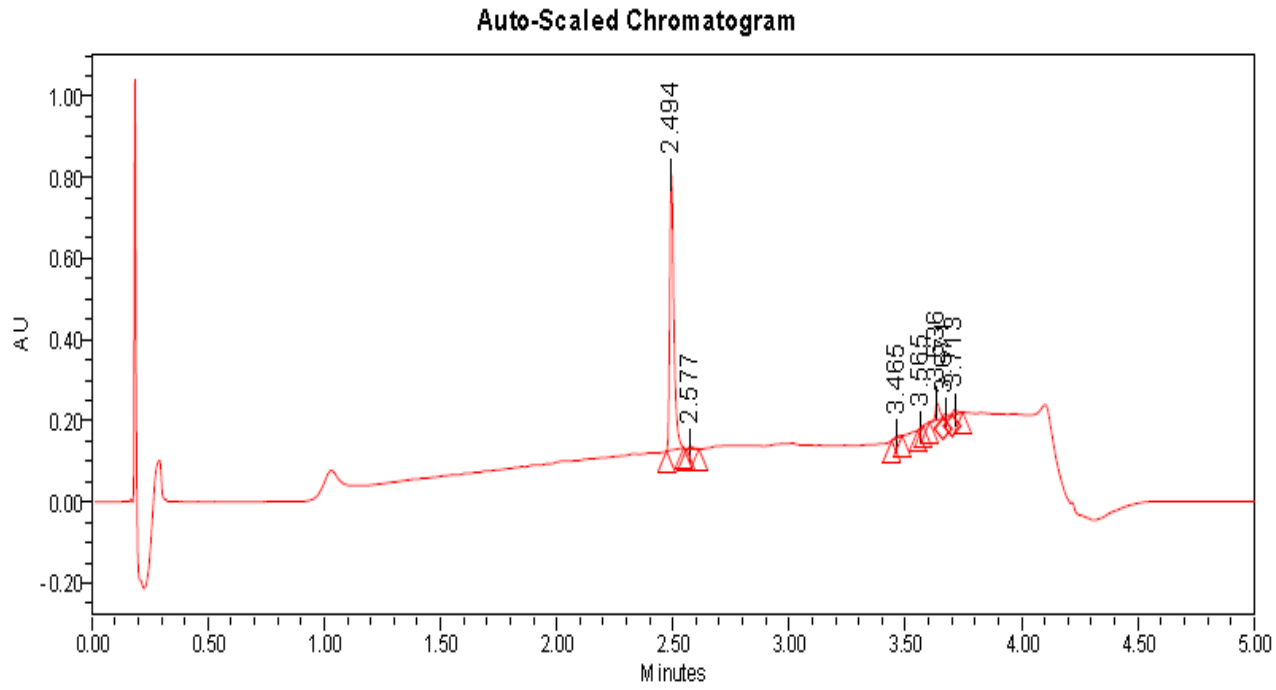
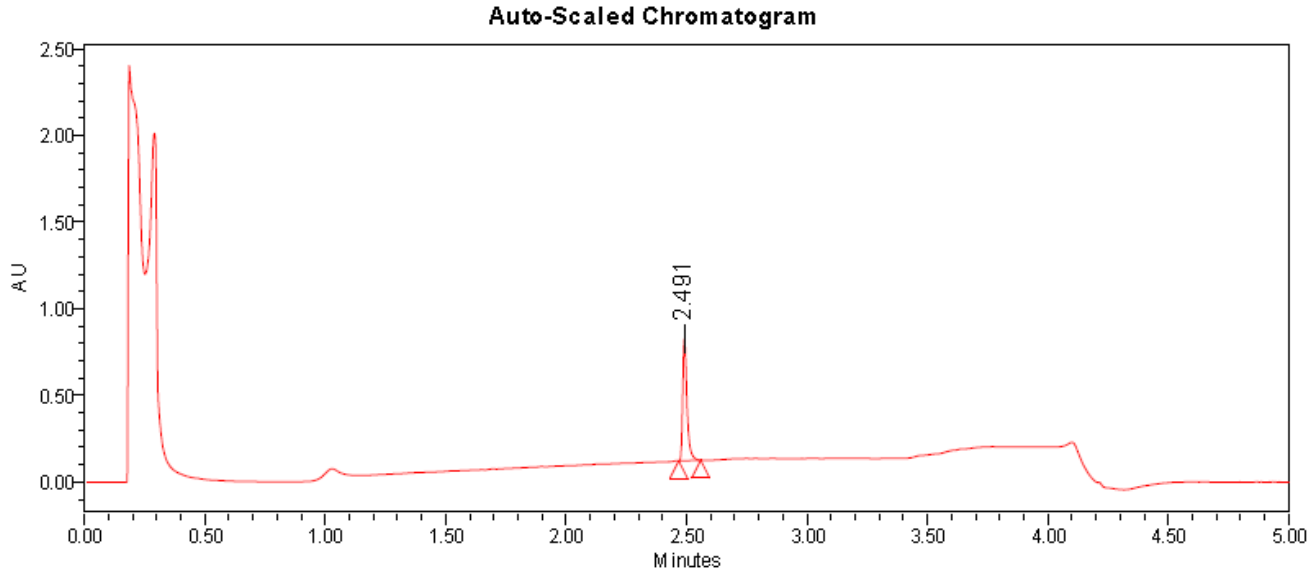


(b)

Figure 12: Chromatogram for Nifedipine in acidic conditions (a) 0.1 N HCl (b) 1 N HCl extracted at 220 nm

Nifedipine showed no degradation in alkaline conditions when treated with 0.1N and 1 N Sodium Hydroxide (NaOH). The chromatogram was extracted at 335 nm as well as 220 nm but no degradation product were observed. Figure 13 shows the chromatogram for this condition.



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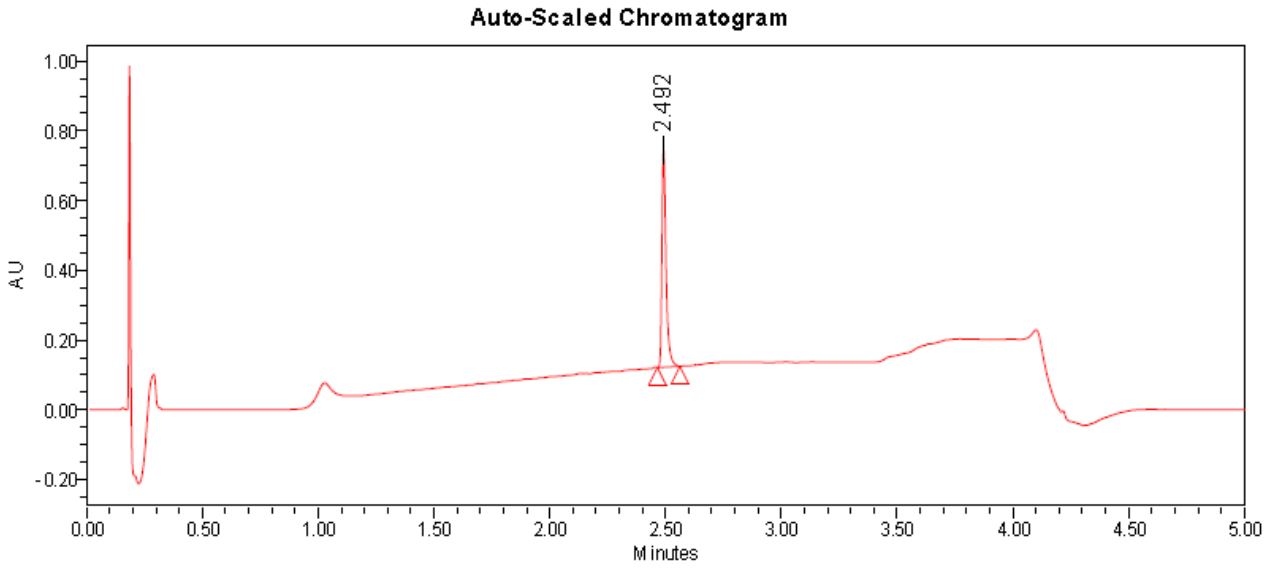

(b)

Figure 13: Chromatogram for Nifedipine in alkaline conditions (a) 0.1N NaOH (b) 1N NaOH extracted at 220 nm; No degradation observed

CONCLUSION

A novel UPLC method was successfully developed and validated for determination of Nifedipine. The total run time was 5 min, within which drug got eluted. Method validation results have proved the method to be selective, precise, accurate, and robust and stability indicating. Sample solution stability was established for determination of assay as well as impurities. This method can be successfully applied for the routine analysis as well as stability study. Also it can be utilized for determination of content uniformity and dissolution profiling of this product, where sample load is higher and high throughput is essential for faster delivery of results. Overall, the method provides high throughput solution for determination of Nifedipine with excellent selectivity, precision and accuracy.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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