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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND CHLORTHALIDONE IN TABLET DOSAGE FORM

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ABSTRACT: Simple, specific, accurate, precise and reproducible and robust method have been developed and validated for the Simultaneous Estimation of Azelnidipine and Chlorthalidone in tablet Dosage from. The Reverse Phase High Performance Liquid Chromatography, the chromatographic system was equipped with Kromasil C_{18} column (150 x 4.6 x 5 µm) and UV detector set at 235 nm, in conjunction with a mobile phase of 20 mM diammonium hydrogen phosphate and Methanol in the ratio of 65:35% v/v (Gradient) (pH 5.5, adjusted with 1% orthophosphoric acid) at a flow rate of 1.2 mL/min. The described method was linear over a concentration range of 40.39-121.16 µg/ml for Azelnidipine and 31.16-93.47 µg/ml for Chlorthalidone. Retention time of Azelnidipine and Chlorthalidone were found to be 10.414 and 4.616 min respectively. The %Recoveries of Azelnidipine and Chlorthalidone at the three different levels were found in the range of 98.9-101.6 % and 99.3-100.9 % respectively. Methods were statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines and can be used for analysis of combined tablet formulation. Azelnidipine and Chlorthalidone were subjected to stress conditions of acid degradation, base degradation, oxidative degradation and thermal degradation under same chromatographic condition. The stress samples were assayed on RP-HPLC system.

KEYWORDS:

Azelnidipine, Chlorthalidone, RP-HPLC method, Force Degradation Study, Validation of RP-HPLC method

I . INTRODUCTION:

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. It is most common disorder affecting the heart and blood vessels. The major cause of heart failure, kidney diseases and stroke **[1]**. Azelnidipine - 3-(1-Benzhydrylazetidin-3-yl) 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3, 5-dicarboxylate is a dihydropyridine calcium channel blocker **[2&4]** and , Chlorthalidone - 2-chloro-5-(1-hydroxy-3-oxo-2*H*-isoindol-1-yl) benzene sulfonamide is thiazide diuretics and it is sulphonamide derivative **[3&5]**. Structure of Azelnidipine and Chlorthalidone is shown in Figure **[6-7]**.Azelnidipine inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. Ca2+ channels are classified into various categories, including L-type,. The L-type Ca2+ channels. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure. Chlorthalidone inhibits sodium reabsorption at the level of the distal convoluted tubule and thus chloride via inhibition of the Na/Cl symporter. By removing sodium reabsorption at this location, the distal convoluted tubules of the nephron retain higher sodium content. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, etc. and separation of drug from degradation products **[17-18]**. Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of Azelnidipine and Chlorthalidone. Also, it is validated for market product named UNIAZ CH 8/6.25 containing Azelnidipine and Chlorthalidone in tablet dosage form. **[18]**

II . LITERATURE SURVEY

Azelnidipine and Chlorthalidone are given into combination. By the literature survey it was found that analytical methods are available for estimation of Azelnidipine and Chlorthalidone alone and with other combination. Only UV method are available for Combination **[8]**.No RP-HPLC method was found for estimation of Azelnidipine and Chlorthalidone in combined dosage form **[9-16].** So, there is thought to perform force degradation study of Azelnidipine and Chlorthalidone in their combined tablet dosage form.

III .MATERIALS AND METHODS

Standard Azelnidipine and Chlorthalidone were obtained as gift sample from TORRENT PHARMACEUTICALS LTD. Shimadzu HPLC, LC 2010 CHT model and LC Solution software was used. Acetonitrile, methanol, Diammonium hydrogen phosphate, MiliQ water and ortho phosphoric acid of AR grade from Merck Life Science Pvt. Ltd, was used. A commercial dosage form UNIAZ CH 8/6.25 was purchased from local market.

IR identification and wavelength selection

IR Spectra of Drug standards were obtained by IR Spectrophotometer. Small quantities of standards were kept directly in the sample compartment of IR and they were scanned in the range of 400-4000 cm^{-1}. An IR spectrum of Drug was interpreted. , UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

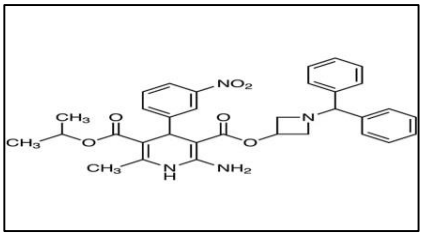

Figure 1. Structure of Azelnidipine

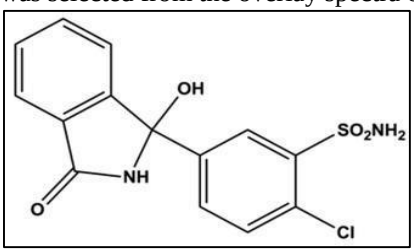

Figure 2. Structure of Chlorthalidone

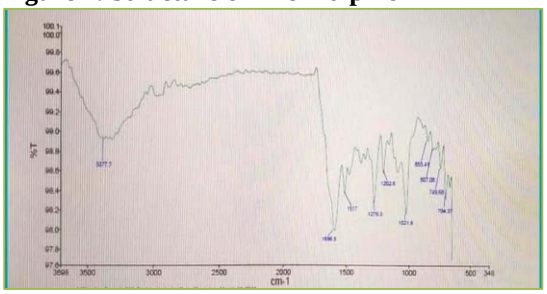

Figure 3: IR spectrum of Azelnidipine (Std.)

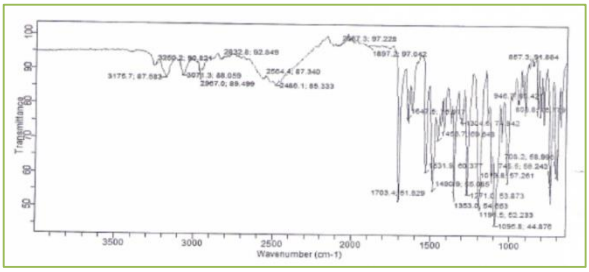

Figure 4: IR Spectrum of Azelnidipine

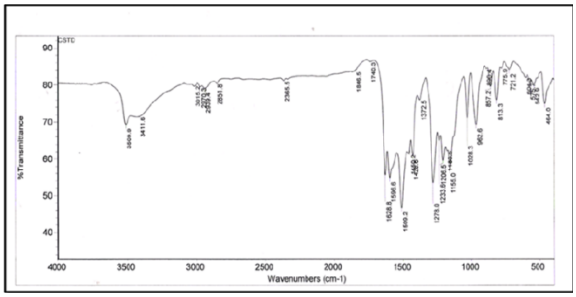

Figure 5: IR spectrum of Chlorthalidone (STD)

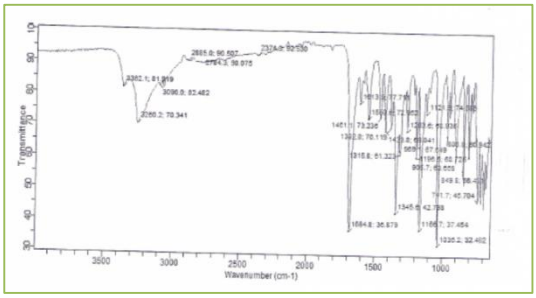

Figure 6: IR Spectrum of Chlorthalidone

Table 1: IR spectrum of Azelnidipine

Sr. No.	Functional group	Standard value	Observed value
1	N-H Stretching	3550-3060	3175.7
2	C=O Stretching	1730-1700	1703.4
3	N-H Bending	1640-1550	1531.9
4	N-O Stretching	1550-1350	1490.9
5	C-H Stretching	1465-1375	1458.7
6	C-O Stretching	1300-1000	1090.8

Table 2: IR spectrum of Chlorthalidone

sr. No.	Functional group	Standard value	Observed value
1	O-H Stretching	3500-3200	3362.1
2	N-H Stretching	3550-3060	3260.2
3	C-H Bending	1465-1375	1684.8
4	S=O Stretching	1335-1370	1345.6
5	C-O Stretching	1300-1000	1166.7
6	C-CL Stretching	800-600	849.8

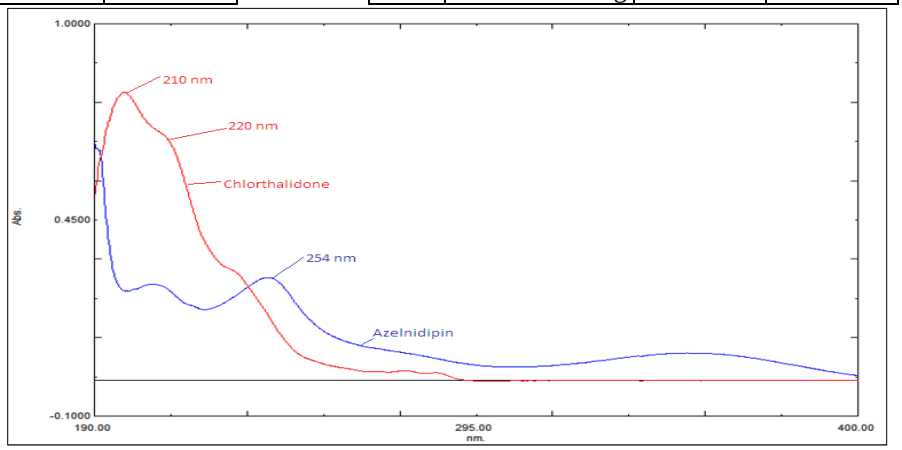

Figure 7: Determination of wavelength maximum(235 nm)

Selection of Mobile phase

Various mobile phases were tried. Trial contains various mobile phases which consisted of, methanol, Diammonium hydrogen phosphate, sodium hydroxide and hydrochloric acid, MiliQ water ortho phosphoric acid in different proportions with various pH and different volumes at flow rate 1.2 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

(A) Buffer preparation (20 mM diammonium hydrogen phosphate, pH 5.5): Accurately weigh and transfer 2.64 g of diammonium hydrogen phosphate in clean beaker. Add about 200 mL MiliQ water. Sonicate for 15 minute to dissolve. Then add 800 mL MiliQ water to make volume 1000 mL Adjust pH 5.5 with ortho-phosphoric acid. Buffer is always freshly prepared for analysis.

(B) Diluent: Buffer: Methanol (50:50)

(C) Chlorthalidone stock solution (625 mcg/ml): About 62.5 mg of Chlorthalidone working standard was accurately weighed and transferred into 100 ml volumetric flask. To this, 20 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

(D) Azelnidipine stock solution (800 mcg/ml): About 80 mg of Chlorthalidone working standard was accurately weighed and transferred into 100 ml volumetric flask. To this, 20 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

(E) Azelnidipine Standard Solution (80 mcg/ml): Pipette out 2 ml of azelnidipine stock solution into 20 ml volumetric flask. The solution was then diluted up to the mark with diluent.

(F) Chlorthalidone Standard Solution (62.5 mcg/ml): Pipette out 2 ml of chlorthalidone stock solution into 20 ml volumetric flask. The solution was then diluted up to the mark with diluent.

(G) Mixed Standard Solution (62.5 mcg/ml Chlorthalidone + 80 mcg/ml Azelnidipine): Pipette out 2 ml of chlorthalidone stock solution and 2 ml of azelnidipine stock solution into 20 ml volumetric flask. Make volume with diluent.

(H) Sample preparation (62.5 mcg/ml chlorthalidone & 80 mcg/ml azelnidipine):To determine the content of chlorthalidone and azelnidipine simultaneously in a pharmaceutical dosage form,5 tablets were accurately weighed and crushed into fine powder. An accurately weighed portion of the powder equivalent to 6.25 mg of chlorthalidone and 8 mg of azelnidipine was transferred to 100 ml volumetric flask containing 20 ml of methanol. The mixture was sonicated for 10 minute to dissolve the content. Then volume was made up to the mark with diluent with intermittent shaking. The resultant solution was filtered through what man 0.45 µm membrane filters to give a concentration of 62.5 mcg/ml of chlorthalidone and 80 mcg/ml of azelnidipine.

METHOD DEVELOPMENT

Trial-1

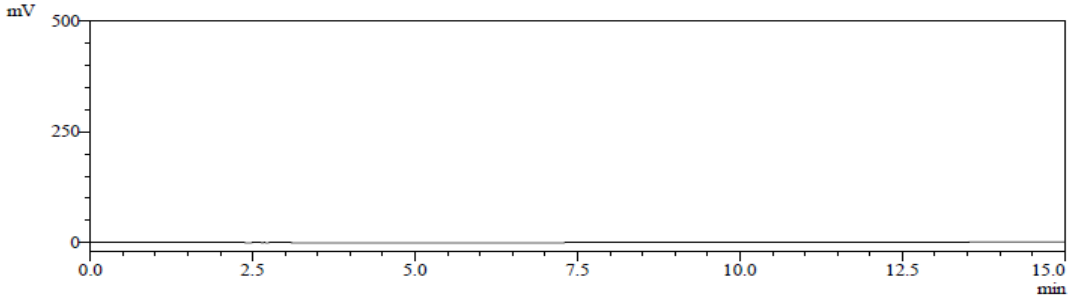

Figure 8: Chromatogram for Azelnidipine in Water: Methanol 50:50

Trial-2

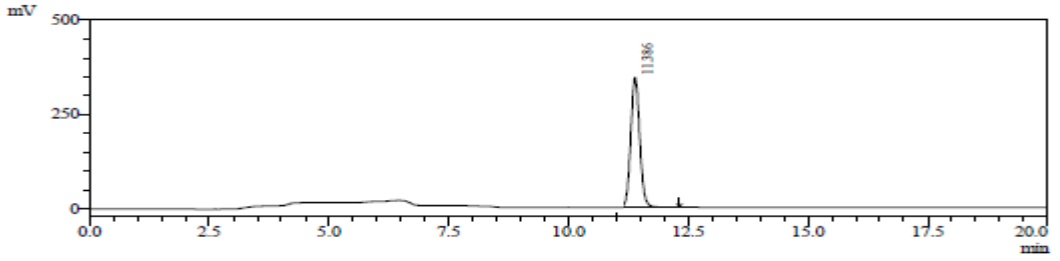

Figure 9: Identification peak of Azelnidipine in Water: Methanol 20:80

Trial-3

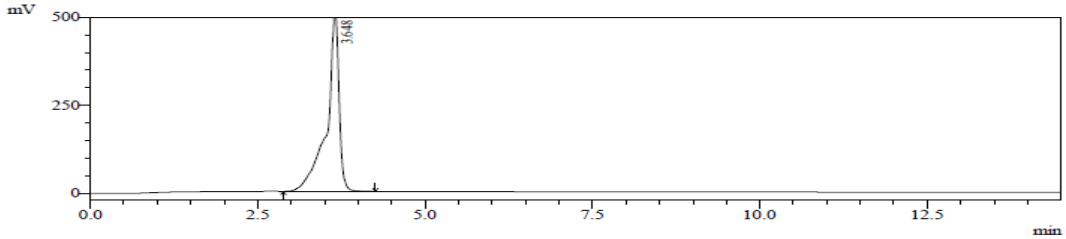

Figure 10: Identification peak of Chlorthalidone in Water: Methanol 50:50

Trial-4

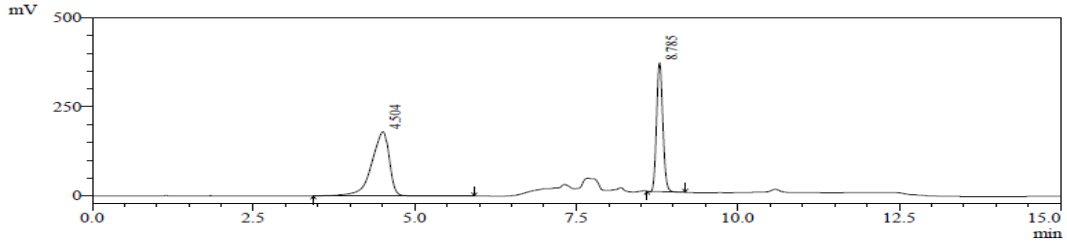

Figure 11: Identification peak of Chlorthalidone and azelnidipine in Water: Methanol Gradient (not proper peak)

Trial-5

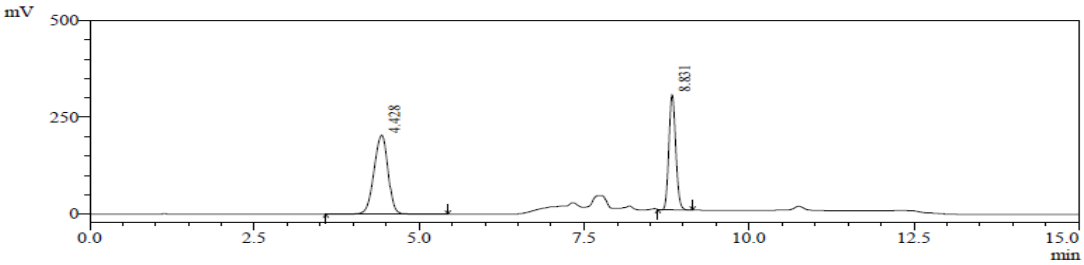

Figure 12: Identification peak of Chlorthalidone and Azelnidipine (20 mM diammonium hydrogen phosphate, pH 5.5: Methanol- Gradient, Azelnidipine peak merge with baseline)

Trial-6 (Final)

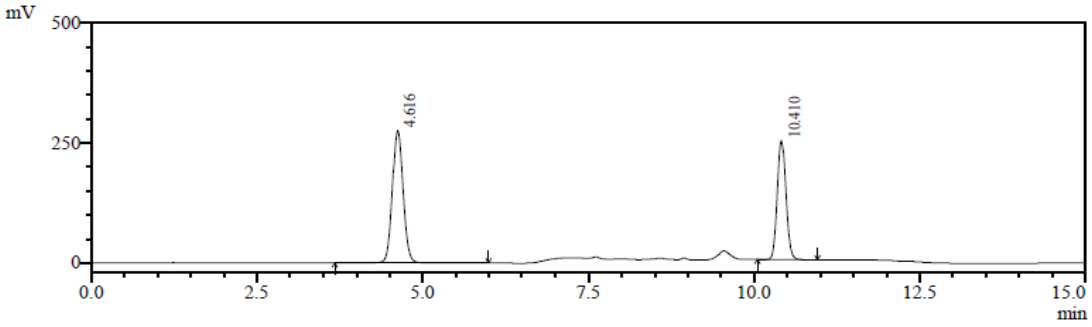

Figure 13: Identification peak of Chlorthalidone and Azelnidipine (20 mM diammonium hydrogen phosphate, pH 5.5: Methanol- Gradient)

Table: 3 Mobile phase selection

Sr. no	Mobile phase composition	Inference
1	Water: Methanol (50:50%v/v)	No peak
2	Water: Methanol (20:80%v/v)	azelnidipine was noted to be 11.38 with Water
3	Water: Methanol (50:50%v/v)	Only one peak (Chlorthalidone)
4	Water: Methanol (Gradient)	Chlorthalidone Peak shape is poor and theoretical plate is less than normal range so used buffer to control ph of mobile phase.
5	20 mM diammonium hydrogen phosphate (pH 5.5),: Methanol- Gradient (Gradient)	Azelnidipine peak merged with baseline noise Hence, further trials were taken to optimize method parameters.
6	20 mM diammonium hydrogen phosphate (pH 5.5),: Methanol- Gradient (Gradient)	Flow rate was increased to 1.2 mL/min to shorten run time. Organic ratio adjusted in gradient program to achieve optimum separation from baseline nose. Tailing factor and theoretical plates within normal limits. Hence, trials was finalized.

Table: 4 Optimized Chromatographic Conditions

Parameters	Chromatographic Condition
Mode of elution	Gradient
Mobile Phase	20 mM diammonium hydrogen phosphate, pH 5.5: Methanol- Gradient

Time(min)	Buffer	Methanol
0	65	35
4.5	65	35
5	19	81
10	19	81
10.5	65	35
15	65	35

Column	Kromasil C-18 column (150 mm × 4.6 mm, 5.0µm)
Flow rate	1.2 ml/min
Temp	25 °C
Runtime	15 min
Injection volume	20 µl
Detection wavelength	235 nm

IV. METHOD VALIDATION

Specificity

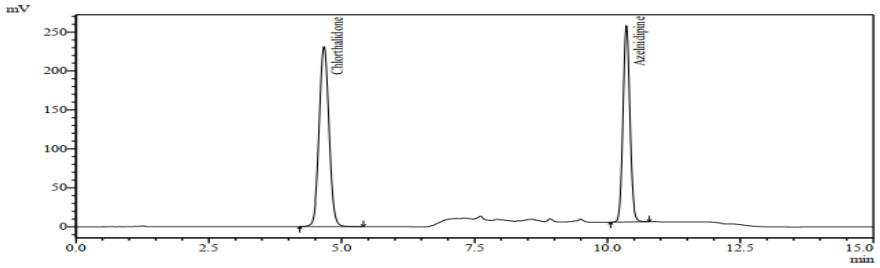

Figure 14: Chromatogram of Standard Azelnidipine and Chlorthalidone

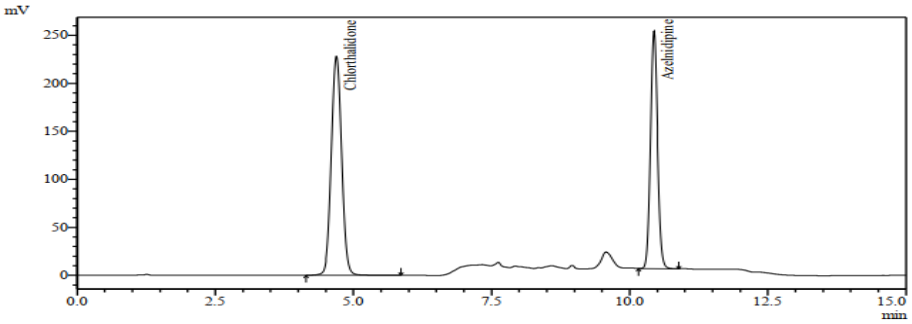

Figure 15: Chromatogram of Sample Azelnidipine and Chlorthalidone

Linearity

For the linearity study 40.39,60.58,80.78,100.97,121.16 ml of Azelnidipine, 31.16,46.73,62.31,77.89,93.47 ml of Chlorthalidone was mixed in six 10ml volumetric flask and volume was made up to mark by Methanol. Calibration curve for Azelnidipine and Chlorthalidone are shown in figure.

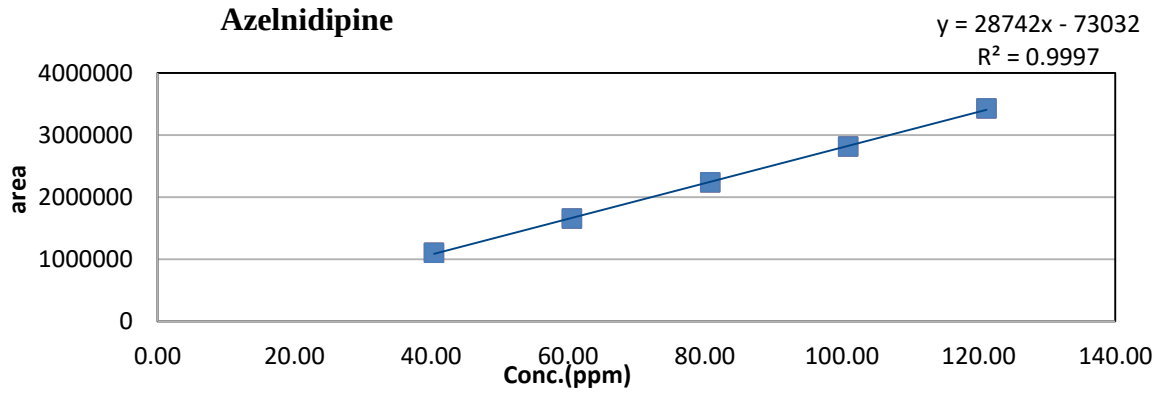

Figure 16: Calibration Curve of Azelnidipine

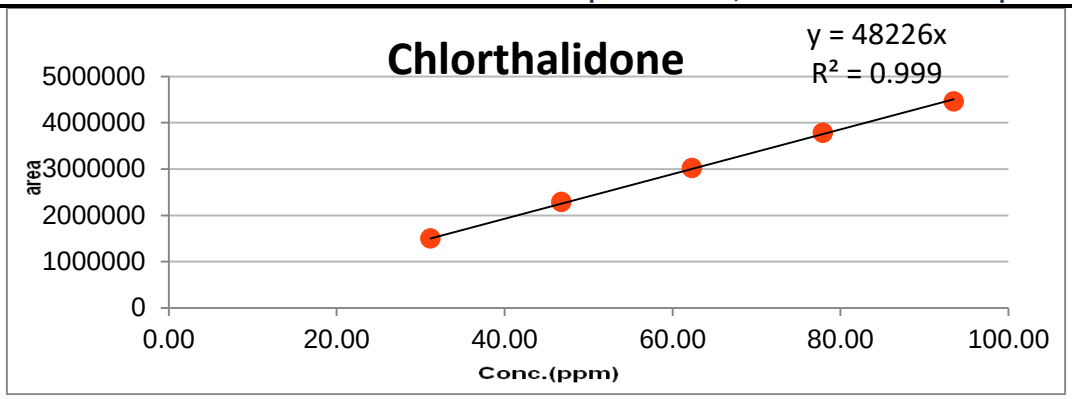

Figure 17: Calibration curve of Chlorthalidone

Table 5: Linearity study of Azelnidipine and Chlorthalidone

Azelnidipine		Chlorthalidone	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
40.39	1105255	31.16	1499295
60.58	1658263	46.73	2285544
80.78	2235707	62.31	3019612
100.97	2814783	77.89	3783360
121.16	3429049	93.47	4460579

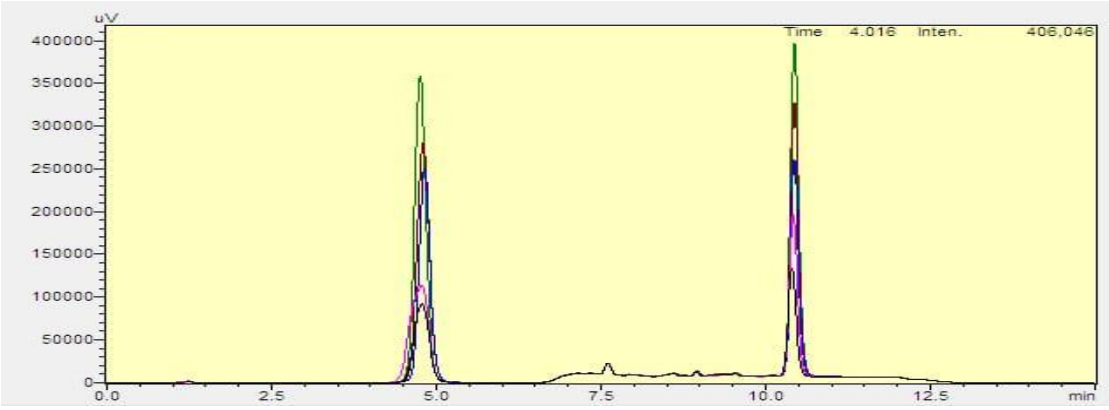

Figure 18: Overlain linearity chromatogram of Azelnidipine and Chlorthalidone

Repeatability

The data for repeatability of peak area measurement for Azelnidipine and Chlorthalidone based on six measurements of same solution. The % RSD for Azelnidipine and Chlorthalidone are shown in table.

Intra-day and inter-day Precision

In the intra-day studies, three repeated injections of standard solution were made and response factor of drug peaks and % RSD were calculated. In inter-day variation studies, three injections of standard solution were made for three consecutive days and response of drug peaks and % RSD were calculated.

Table 6: Repeatability study

Concentrationof Azelnidipine (µg/ml)	Azelnidipine		Concentration of Chlorthalidone(µg/ml)	Chlorthalidone	
	Mean ± SD (n=6)	**% RSD**		**Mean ± SD (n=6)**	**% RSD**
80	2270592±3867.428	0.2	62.5	2965032±3370.203	0.1

Table 7: Intraday & Interday precision study of Azelnidipine

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD(n=3)	**% RSD**	**Mean ± SD (n=3)**	**% RSD**
Azelnidipine	80	2279921±14319.71	0.6	2278933±10090.779	0.4

Table 8: Intraday & Interday precision study of Chlorthalidone

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD (n=3)	**% RSD**	**Mean ± SD (n=3)**	**% RSD**
Chlorthalidone	62.5	2965442±2231.317	0.1	2982447±9389.500	0.3

Accuracy:

Table 9: Recovery study for Azelnidipine

Sr. No	Conc. Levels%	Sample amount	Amount Added	Amount recovered	% Recovery	% Mean Recovery	% RSD
1	50%	1	3.99	3.95	99.0	98.9	0.2
2		1	3.99	3.94	98.7		
3		1	3.99	3.95	99.1		
4	100%	1	7.98	7.94	99.5	99.6	0.1
5		1	7.98	7.95	99.7		
6		1	7.98	7.95	99.7		
7	150%	1	11.97	12.16	101.6	101.6	0.1
8		1	11.97	12.15	101.5		
9		1	11.97	12.17	101.7		

Table 10: Recovery study for Chlorthalidone

Sr. No	Conc. Levels%	Sample amount	Amount Added	Amount recovered	% Recovery	% Mean Recovery	% RSD
1	50%	1	3.15	3.16	100.2	100.2	0.2
2		1	3.15	3.16	100.4		
3		1	3.15	3.15	100.1		
4	100%	1	6.30	6.35	100.8	100.9	0.1
5		1	6.30	6.36	100.9		
6		1	6.30	6.36	101.0		
7	150%	1	9.45	9.38	99.2	99.3	0.1
8		1	9.45	9.38	99.3		
9		1	9.45	9.39	99.3		

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 10 %).
2. Temp of Mobile phase was changed (± 5°C).
3. Ratio of Mobile phase was changed (± 2 %). The results were shown in table.

Table: 11 Robustness data for Azelnidipine

Sr. No.	Area at Temp. (-5°C)	Area at Temp. (+5°C)	Area at Flow (-10% ml/min)	Area at Flow (+10% ml/min)	Area at Mobile Phase (-2%)	Area at Mobile Phase (+2%)
1	2280953	2324560	2557868	2121430	2294362	2340770
2	2299201	2325417	2550065	2119277	2274963	2339193
3	2295427	2330932	2555103	2125405	2281994	2343959
Mean	2291860	2326970	2554345	2122037	2283773	2341307
% R.S.D	0.4	0.1	0.2	0.1	0.4	0.1

Table: 12 Robustness data for Chlorthalidone

Sr. No.	Area at Temp. (-5°C)	Area at Temp. (+5°C)	Area at Flow (-10% ml/min)	Area at Flow (+10% ml/min)	Area at Mobile Phase (-2%)	Area at Mobile Phase (+2%)
1	3045547	3050962	3389806	2783821	3061300	3071819
2	3049992	3053715	3384935	2872954	3069165	3067162
3	3053167	3050617	3392423	2793220	3069854	3073299
Mean	3049569	3051765	3389055	2816665	3066773	3070760
% R.S.D	0.1	0.1	0.1	1.7	0.2	0.1

LOD and LOQ

Calibration curve was repeated for three times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts. The results were shown in table.

Table 13: Limit of Detection and Limit of Quantitation Data of Azelnidipine and Chlorthalidone

Azelnidipine	Chlorthalidone
LOD = 3.3 x (SD / Slope) LOD =3.3 x (917867.7 / 28741.88740) = 105.38498 mg/ml = 0.1053 µg/ml	LOD = 3.3 x (SD / Slope) LOD =3.3 x (1173608 / 47633.35767) = 81.3066 mg/ml = 0.08130 µg/ml
LOQ = 10 x (SD / Slope) LOQ =10 x (917867.7 / 28741.88740) = 319.3484433 mg/ml = 0.3193 µg/ml	LOQ = 10 x (SD / Slope) LOQ =10 x (1173608 / 47633.35767) = 246.3836 mg/ml = 0.24638 µg/ml

Assay of marketed formulation

Applicability of proposed method was tested by analysing tablet formulation (UNIAZ CH 8/6.25). The result is shown in table.

Table 14: Assay of Azelnidipine

Sr. No.	Area of Sample	% Assay	Average % Assay	SD	%RSD
1	2271631	100.7	100.5	3720.514	0.2
2	2268313	100.5			
3	2264204	100.4			

Table 15: Assay of Chlorthalidone

Sr. No.	Area of Sample	% Assay	Average % Assay	SD	%RSD
1	2968460	99.8	99.7	3731.189	0.1
2	2963616	99.6			
3	2961122	99.6			

V. Forced Degradation Condition

1. Acid Degradation

- **Acid degradation Standard:** 2 ml azelnidipine standard stock + 2 ml chlorthalidone standard stock into 20 ml volumetric flask. Add 0.5 ml 1 N HCL. Set aside at room temperature for 4 hours. Neutralize with 0.5 ml 1 N NaOH. Make volume with diluents and inject into HPLC.
- **Acid degradation Sample:** 10 ml tablet stock solution in 20 ml volumetric flask+ 0.5 ml 1 N HCL. Set aside at room temperature for 4 hours. Neutralize with 0.5 ml 1 N NaOH. Make volume with diluents and inject into HPLC.

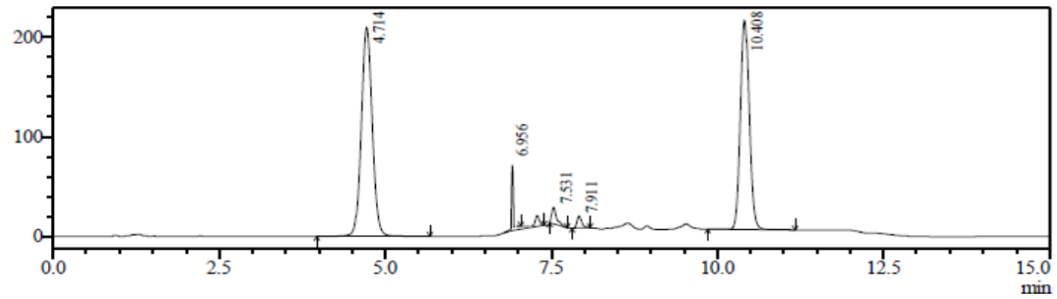

Figure 19: Acid Degradation Standard (0.5 ml 1 N HCL, Room Temp, 4 hours)

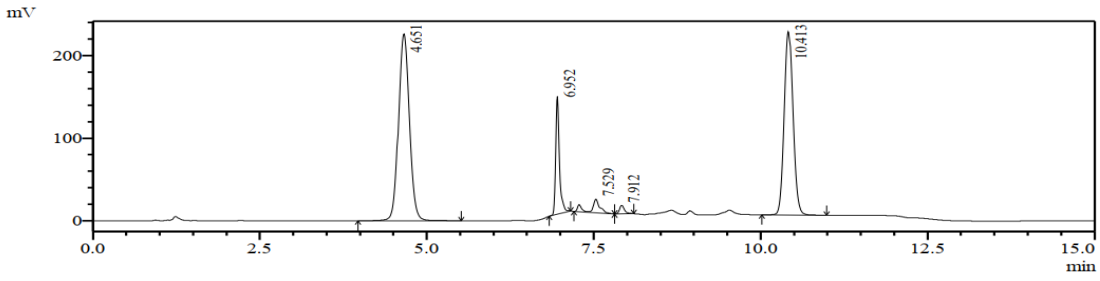

Figure 20: Acid Degradation Sample (0.5 ml 1 N HCL, Room Temp, 4 hours)

2 .Base Degradation

- **Base degradation Standard:** 2 ml azelnidipine standard stock + 2 ml chlorthalidone standard stock into 20 ml volumetric flask. Add 0.3 ml 1 N NaOH. Set aside at room temperature for 4 hours. Neutralize with 0.3 ml 1 N HCL. Make volume with diluent and inject into HPLC.
- **Base degradation Sample**:10 ml tablet stock solution into 20 ml volumetric flask+ 2 ml chlorthalidone standard stock into 20 ml volumetric flask. Add 0.3 ml 1 N NaOH. Set aside at room temperature for 4 hours. Neutralize with 0.3 ml 1 N HCL. Make volume with diluents and inject into HPLC.

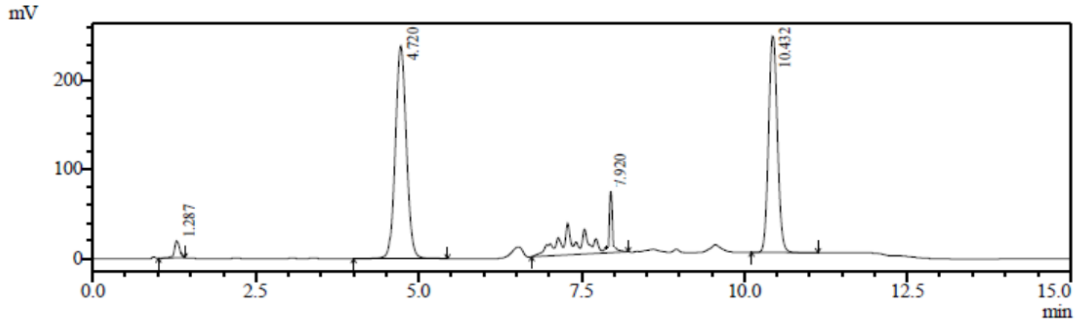

Figure 21: Base Degradation Standard (0.3 ml 1 N NAOH, Room Temp, 4 hours)

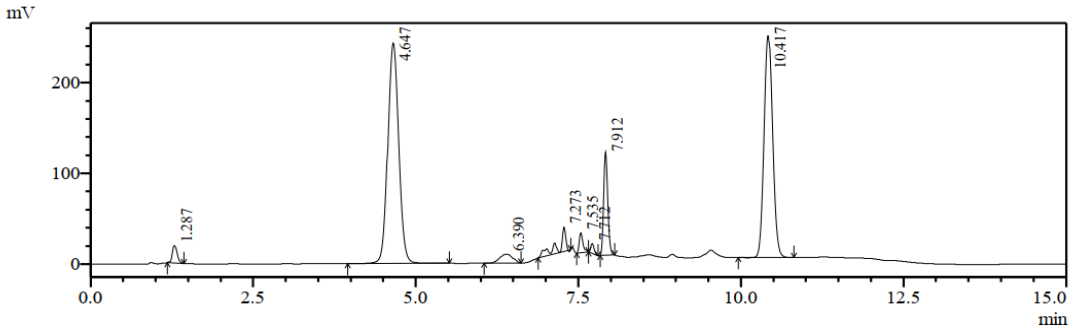

Figure 22: Base Degradation Sample (0.3 ml 1 N NAOH, Room Temp, 4 hours)

3. Oxidative Degradation

- **Peroxide degradation Standard:**2 ml azelnidipine standard stock + 2 ml chlorthalidone standard stock into 20 ml volumetric flask. 0.2 ml 3% H2O2. Set for 4 hours at room temperature. Volume made with diluent and injected into HPLC.
- **Peroxide degradation Sample:** 5 ml tablet stock solution into 20 ml volumetric flask + 0.2 ml 3% H2O2. Set for 4 hours at room temperature. Volume made with diluents and injected into HPLC.

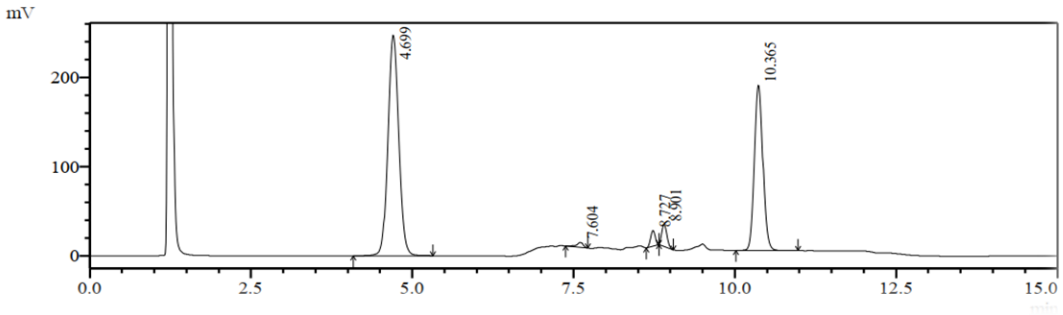

Figure 23: Oxidative Degradation Standard (0.2 ml 3% H2O2, Room Temp, 4 hours)

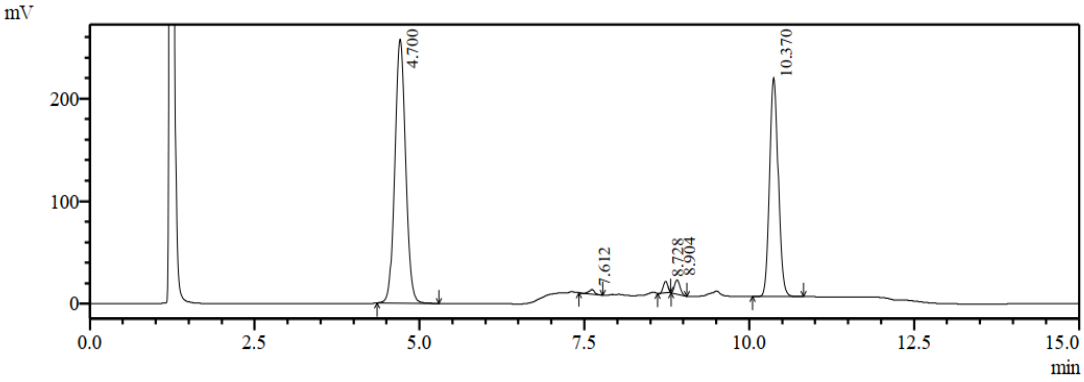

Figure 24: Oxidative Degradation Sample (0.2 ml 3% H2O2, Room Temp, 4 hours)

4. Photo Degradation

- **Photo degradation Standard:** API powders were kept in sunlight for 3 days. Solution was prepared according to test method and injected into HPLC.
- **Photo degradation Sample:** tablet powders were kept in sunlight for 3 days. Solution was prepared according to test method and injected into HPLC.

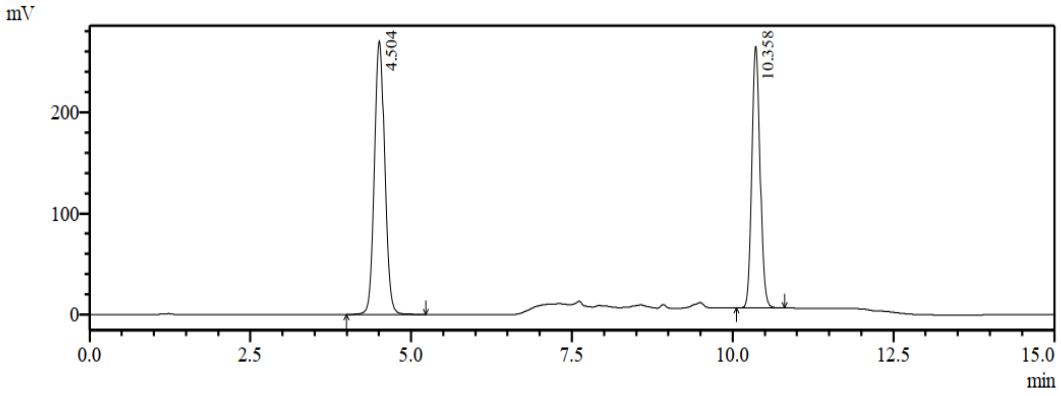

Figure 25: Photo Degradation Standard (Sunlight, 3 days)

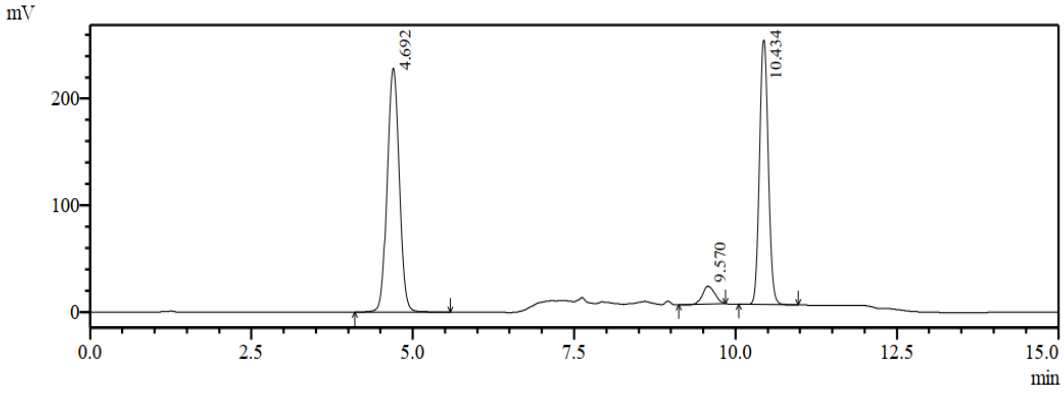

Figure 26: Photo Degradation Sample (Sunlight, 3 days)

5. Thermal Degradation

- **Thermal degradation Standard:** API powders were kept in hot air oven at 80 ℃ temperature for 12 hours. Solution was prepared according to test method and injected into HPLC.
- **Thermal degradation Sample:** tablet powders were kept in hot air oven at 80 ℃ temperature for 12 hours. Solution was prepared according to test method and injected into HPLC.

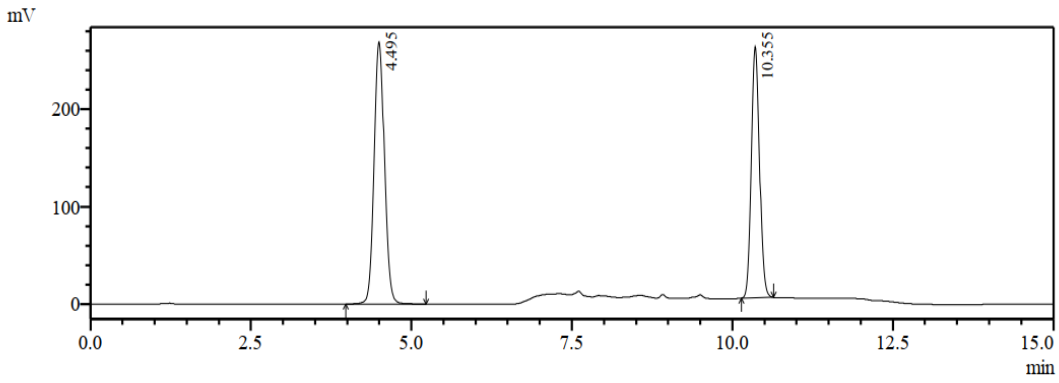

Figure 27: Thermal Degradation Standard (hot air oven at 80 ℃ temperature for 12 hours)

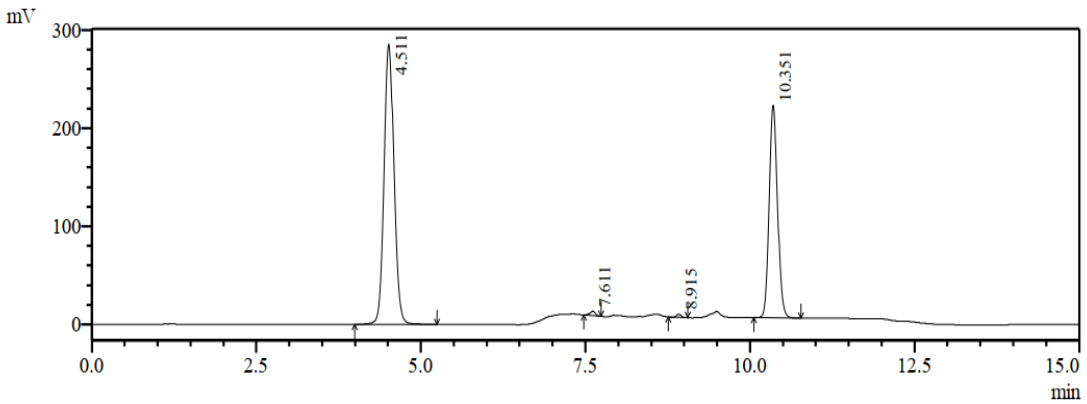

Figure 28: Thermal Degradation Sample (hot air oven at 80 ℃ temperature for 12 hours)

Table 16: Result of stability study of Azelnidipine and Chlorthalidone

Degradation method	Optimized condition	% degradation (standard)		% degradation (sample)	
		AZE	CHL	AZE	CHL
Acid	1 N HCl (37 °C for 4 hours)	15.78%	19.33%	9.73%	17.32%
Base	1 N NaOH (37 °C for 4 hours)	3.01%	5.63%	0.41%	8.70%
Oxidation	3% H_2O_2 (37 °C for 4 hours)	26.33%	3.35%	12.74%	5.30%
Thermal	80 °C for 12 hours)	0.39%	0.26%	0.15%	1.59%
Photolytic	Sunlight(3 days)	0.25%	0.27%	2.97%	3.87%

RESULT AND DISCUSSION

RP-HPLC method was developed for simultaneous estimation Azelnidipine and Chlorthalidone. In RP-HPLC method, good resolution and separation of two drugs was achieved. Buffer (pH 5.5): Methanol (65:35 v/v, Gradient) was used as mobile phase. Retention time of Azelnidipine and Chlorthalidone were found to be 4.616 and 10.414 min respectively with a flow rate of 1.2 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Azelnidipine and Chlorthalidone in tablets. Forced degradation study of Azelnidipine and Chlorthalidone was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit. The linearity regression analysis data for the calibration plots showed good linear relationship with concentration 40.39-121.16 µg/ml for Azelnidipine and 31.16-93.47 µg/ml for Chlorthalidone with the correlation co-efficient 0.99 and 0.9992 for Azelnidipine and Chlorthalidone respectively and the Precision data obtained with less than 2% RSD.The LOD and LOQ for Azelnidipine and Chlorthalidone were found to be 0.1053, 0.08130 µg/ml and 0.3193, 0.24638 µg/ml.The %Recoveries of Azelnidipine and Chlorthalidone at the three different levels were found in the range of 98.9-101.6 % and 99.3-100.9 % respectively. The method was validated for accuracy, precision, linearity, repeatability and robustness as per ICH guideline Q2 (R1) for method validation.

CONCLUSION

Azelnidipine inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. Ca2+ channels are classified into various categories, including L-type, The L-type Ca2+ channels. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure. Chlorthalidone inhibits sodium reabsorption at the level of the distal convoluted tubule and thus chloride via inhibition of the Na/Cl symporter. By removing sodium reabsorption at this location, the distal convoluted tubules of the nephron retain higher sodium content. So, Azelnidipine and Chlorthalidone are given into combination.

RP-HPLC method was developed for simultaneous estimation Azelnidipine and Chlorthalidone. In RP-HPLC method, good resolution and separation of two drugs was achieved. 20 mM diammonium hydrogen phosphate and Methanol in the ratio of 65:35% v/v (Gradient) (pH 5.5, adjusted with 1% orthophosphoric acid). Retention time of Azelnidipine and Chlorthalidone were found to be 4.616 and 10.414 min respectively with a flow rate of 1.2 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Azelnidipine and Chlorthalidone in tablets. Forced degradation study of Azelnidipine and Chlorthalidone was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit.

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