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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AZELNIDIPINE AND CHLORTHALIDONE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise and accurate stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Azelnidipine and Chlorthalidone in combined pharmaceutical formulation. Chromatographic separation was achieved on Inertsil ODS C18 (250 mm x 4.6 mm, 5 µm) with UV detection at 253 nm. The mobile phase consists of acetonitrile and ammonium formate buffer pH 3.2 in the ratio of 30:70 v/v and at a flow rate of 1.0 mL/min. The method was linear over the concentration range of 2-12 µg/mL for Azelnidipine and 3.25-19.50 µg/mL for Chlorthalidone. The retention times for Azelnidipine and Chlorthalidone were found to be 7.740 min and 4.356 min respectively. The mean percentage recoveries of Azelnidipine and Chlorthalidone were found to be 99.83% and 100.00% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Azelnidipine and Chlorthalidone in combined pharmaceutical formulation.

Keywords: Azelnidipine, Chlorthalidone, HPLC and Validation.

INTRODUCTION

Azelnidipine (Fig. 1) is a dihydropyridine calcium channel blocker and used in the treatment of hypertension[1]. Chemically it is 3-(1-Benzhydrylazetidin-3-yl)5-isopropyl-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydro pyridine -3,5-dicarboxylate. Azelnidipine inhibits trans-membrane Ca^{2+} influx through the voltage-dependent channels of smooth muscles in vascular walls[2].

Chlorthalidone (Fig. 2) is a thaizide-like diuretic and used in the treatment of hypertension[3]. Chemically it is 2-Chloro-5-(1-hydroxy-3-oxo-2*H*-isoindol-1-yl) benzenesulfonamide. Chlorthalidone prevents reabsorption of sodium and chloride through inhibition of the Na+/Cl-symporter in the cortical diluting segment of the ascending limb of the loop of henle[4].

For improvement activity of hypertension, Azelnidipine and Chlorthalidone newer combination is available in the market. This combination was developed to improve medication for stage II hypertension.

Literature survey revealed that no HPLC method was reported for simultaneous estimation of Azelnidipine and Chlorthalidone in combined pharmaceutical dosage form. Hence the objective of this method is to develop and validate a novel, simple, specific, precise and accurate stability indicating RP-HPLC method for the simultaneous estimation of Azelnidipine and Chlorthalidone in combined pharmaceutical dosage form in accordance with ICH guidelines[5,6].

MATERIALS AND METHODS

MATERIALS

Azelnidipine and Chlorthalidone pure drugs were obtained as gift samples from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. Commercial formulations of Azelnidipine and Chlorthalidone (UNIAZ CH: Azelnidipine-8 mg, Chlorthalidone-12.5 mg) tablets were procured from local pharmacy store. Acetonitrile, ammonium formate, formic acid and distilled water were

obtained from Rankem Chemicals Ltd., Mumbai, India.

Instrumentation
The analysis of drugs was carried out on Waters Alliance e 2695 seperation module HPLC system with PDA Detector at 253 nm on Inertsil ODS C18 (250 mm x 4.6 mm, 5 µm). The instrument is equipped with auto injector with 10 µL sample loop. A 10 µL Hamilton syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. A double-beam Schimadzu UV-1800 UV-Visible spectrophotometer was used for measuring absorbance for Azelnidipine and Chlorthalidone solutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Sartorius balance was used for weighing the materials.

Mobile phase
A mobile phase consisting of mixture of acetonitrile and ammonium formate buffer pH 3.2 in the ratio of 30:70 v/v was prepared.

Preparation of standard solution
Weighed accurately and transferred about 8 mg of Azelnidipine and 13 mg of Chlorthalidone working standards taken separately in 100 mL volumetric flasks added about 70 mL of mobile phase. Sonicated the solution for few minutes to dissolved the drugs completely and make upto the final volume with diluent. The solutions were filtered through 0.45 µm filter. Further transfer 5 mL of the above solutions into 50 mL volumetric flask with diluent and mixed well.

Preparation of sample preparation
Twenty commercial tablets were weighed and powdered. Weighed accurately the tablet powder equivalent to 8 mg of Azelnidipine and 13 mg of Chlorthalidone, transferred into 100 mL volumetric flask and was dissolved in 70 mL of the diluent. Sonicated the solution for few minutes and dissolved the drugs completely and make upto the final volume with diluent. The solution was filtered through 0.45 µm filter. Further transfer 5 mL of the above solution into 50 mL volumetric flask with diluent and mix well.

METHOD DEVELOPMENT
Various trails were performed by using different mobile phases and based on peak parameters the chromatographic conditions (Table 1) were optimized and optimize chromatogram was shown in Fig. 3.

METHOD VALIDATION
System suitability parameters
The system suitability parameters were determined by preparing standard solutions of Azelnidipine and Chlorthalidone and the solutions were injected six times and the parameters like USP plate count, peak tailing and resolution were determined. All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The results were furnished in Table 2.

Specificity
Specificity is the parameter used to check the interference in the optimized method. We should not find interfering peaks in blank, placebo, standard and sample at retention times of these drugs in this method. So this method was said to be specific.

Linearity
Six linear concentrations of Azelnidipine (2-12 µg/mL) and for Chlorthalidone (3.25-19.50 µg/mL) are prepared and injected. The results were furnished in Table 3 and linearity curves were shown in Fig. 4 & 5.

Precision
Precision of method was studied by performing system precision and method precision. System precision (Table 4) and method precision (Table 5) was studied by injecting the 6 replicates of standard solution in a single day and six days. Calculate the %RSD and it should not be more than 2.0.

Accuracy
The accuracy of the method was established by calculating percentage recovery of Azelnidipine and Chlorthalidone by the method of addition. Known amount of Azelnidipine and Chlorthalidone at 50%, 100% and 150% was added to a prequantified sample solution. The recovery studies (Table 6 & 7) were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery at each level was not less than 98% and not more than 102%.

Sensitivity
Limit of detection (LOD) was calculated by standard deviation method. Limit of quantitation (LOQ) was calculated by standard deviation method.

DEGRADATION STUDIES
Acid degradation studies
To 5 mL of stock solution of Azelnidipine and Chlorthalidone into 50 mL volumetric flask, 3 mL of 1N HCl was added and refluxed at 60^0C for 6 hours and then neutralized with 1 N NaOH and make up to 50 mL with diluent. 10 µL solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Alkali degradation studies

To 5 mL of stock solution of Azelnidipine and Chlorthalidone into 50 mL volumetric flask, 3 mL of 1N NaOH was added and refluxed at 60^{0}C for 6 hours and then neutralized with 1 N HCl and make up to 50 mL with diluent. 10 µL solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Oxidative degradation studies

To 5 mL of stock solution of Azelnidipine and Chlorthalidone into 50 mL volumetric flask, 1 mL of 3% v/v of hydrogen peroxide was added and refluxed at 60^{0}C for 6 hours and make up to 50 mL with diluent. 10 µL solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Reductive degradation studies

To 5 mL of stock solution of Azelnidipine and Chlorthalidone into 50 mL volumetric flask, 1 mL of 10% sodium bisulphate and kept on bench top for 10 min. 10 µL solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Neutral degradation studies

To 5 mL of stock solution of Azelnidipine and Chlorthalidone into 50 mL volumetric flask, 1 mL of water was added and kept at room temperature for 15 min. 10 µL solution was injected into the system and the chromatogram was recorded to assess the stability of sample.

Degradation studies results of Azelnidipine & Chlorthalidone were tabulated in Table 8 & 9.

RESULTS AND DISCUSSION

A stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Azelnidipine and Chlorthalidone by using mobile phase consisting of acetonitrile and ammonium formate buffer pH 3.2 in the ratio of 30:70 v/v. The retention times for Azelnidipine and Chlorthalidone were found to be 7.740 min and 4.356 min respectively. The proposed method was validated as per ICH guidelines. The theoretical plates for Azelnidipine and Chlorthalidone were found to be 6842 and 3641 respectively, which indicates the efficient performance of the column. Linearity range was found to be 2-12 µg/mL for Azelnidipine and 3.25-19.50 µg/mL for Chlorthalidone. The %RSD values for system precision values of Azelnidipine and Chlorthalidone were found to be 0.34 and 0.56 respectively. The %RSD values for method precision values of Azelnidipine and Chlorthalidone were found to be 0.37 and 0.81 respectively and hence the proposed method is precise. The mean percentage recoveries of Azelnidipine and Chlorthalidone were found to be 99.83% and 100.00% respectively and the method is found to be accurate. Degradation studies were carried out in acid, alkali, oxidative, reductive and neutral stressed conditions. The results revealed that both the drugs are stable in described conditions. Thus it is evident that the described method can be adopted for routine estimation of Azelnidipine and Chlorthalidone in combined pharmaceutical dosage form.

CONCLUSION

The present method was proposed for the simultaneous estimation of Azelnidipine and Chlorthalidone by using RP-HPLC in tablet dosage form is found to be novel, simple, specific, precise and accurate. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be applied in regular quality control tests in pharmaceutical industries.

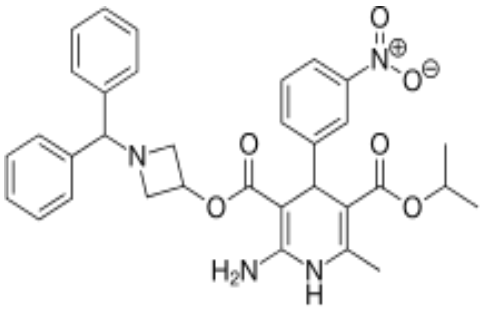

Fig. 1: Chemical structure of Azelnidipine

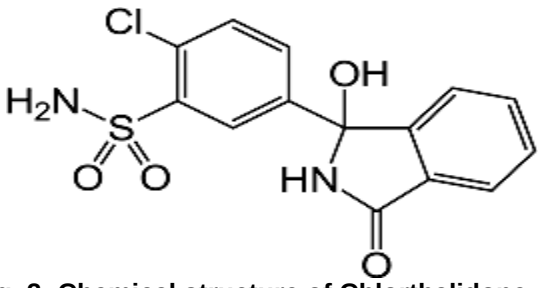

Fig. 2: Chemical structure of Chlorthalidone

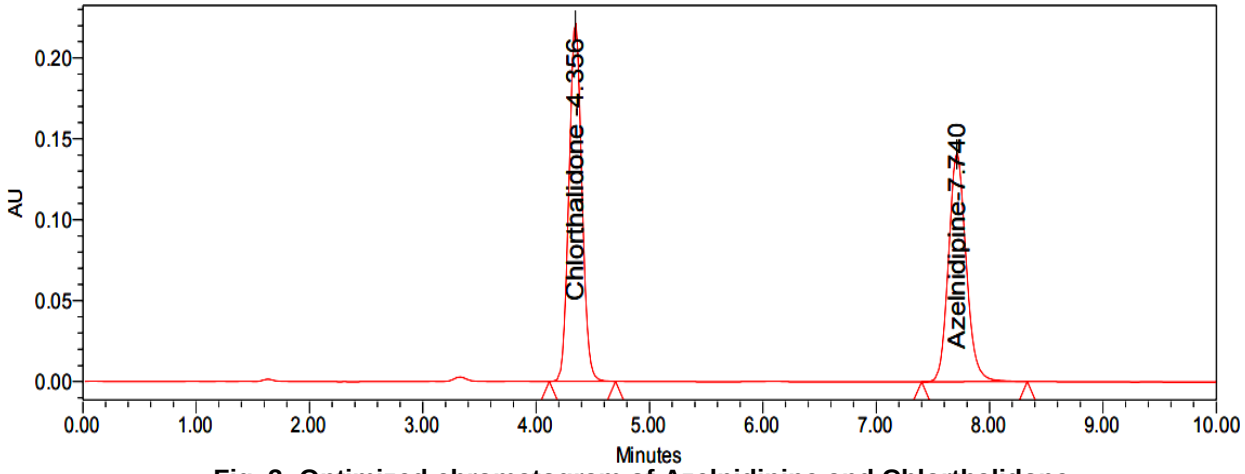

Fig. 3: Optimized chromatogram of Azelnidipine and Chlorthalidone

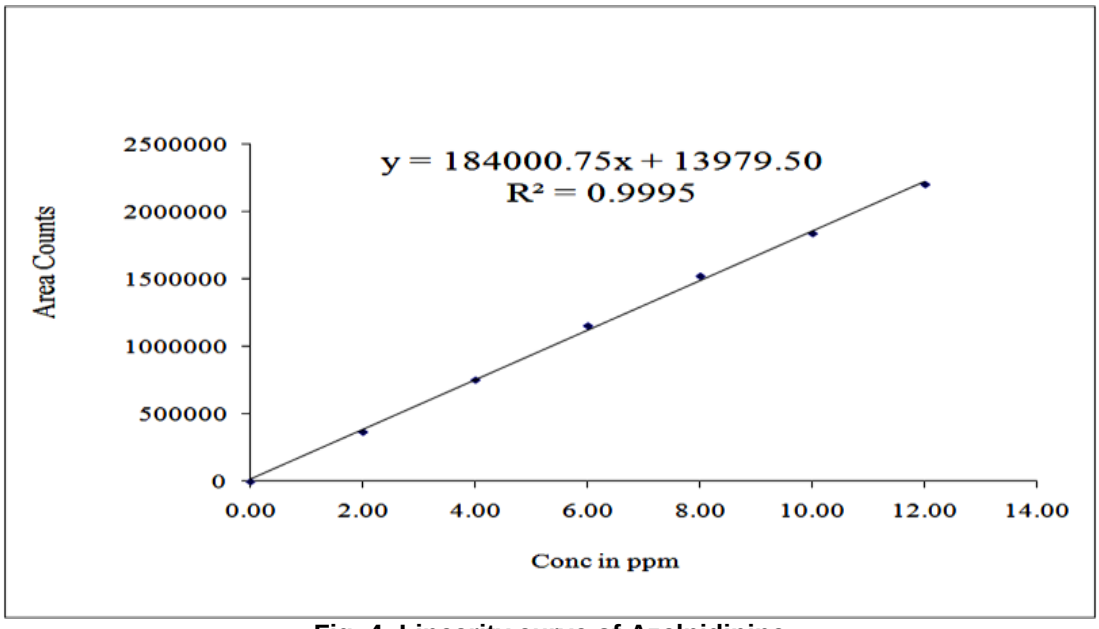

Fig. 4: Linearity curve of Azelnidipine

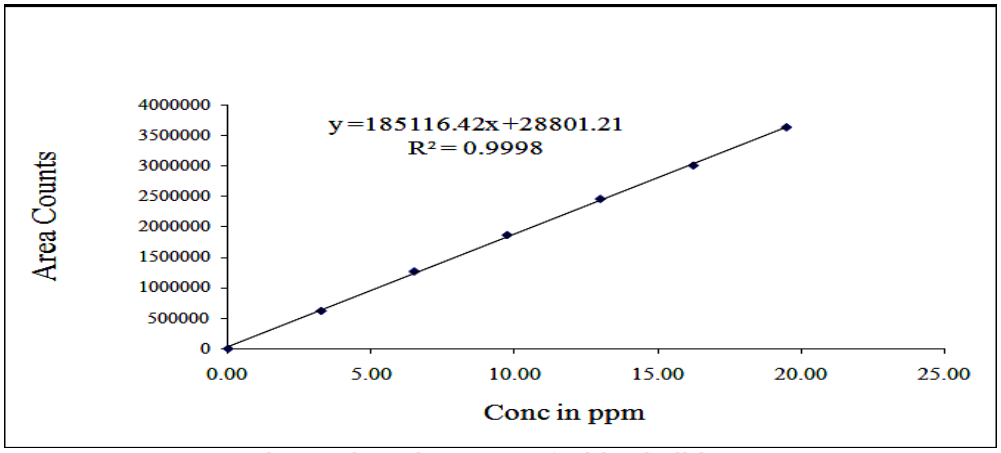

Fig. 5: Linearity curve of Chlorthalidone

Table 1: Optimized chromatographic conditions

Mobile phase	Acetonitrile and ammonium formate buffer pH 3.2, 30:70 v/v
Flow rate	1 mL/min
Column	Inertsil ODS C18 (250 mm × 4.6 mm, 5 µm)
Detector wave length	253 nm
Column temperature	Ambient
Injection volume	10 µL
Run time	10 min
Diluent	Mobile phase

Table 2: System suitability parameters of proposed method

S. No.	Parameters	Azelnidipine	Chlorthalidone
1	Linearity (µg/mL)	2-12	3.25-19.50
2	Correlation coefficient	0.999	0.999
3	Retention time (min.)	7.740	4.356
4	Resolution	8.60	--
5	Tailing factor	1.09	1.17
6	Theoretical plates (N)	6842	3641
7	LOD (µg/mL)	0.24	0.39
8	LOQ (µg/mL)	0.72	1.17

Table 3: Linearity results for Azelnidipine and Chlorthalidone

S. No.	Concentration of Azelnidipine (µg/mL)	Peak area	Concentration of Chlorthalidone (µg/mL)	Peak area
1	2	366501	3.25	612212
2	4	751257	6.50	1265675
3	6	1152746	9.75	1863729
4	8	1521068	13.00	2455532
5	10	1835715	16.25	3003211
6	12	2198601	19.50	3629046

Table 4: System precision results for Azelnidipine and Chlorthalidone

S. No.	Azelnidipine Peak area	Chlorthalidone Peak area
1	1559347	2458674
2	1575478	2471122
3	1575784	2453149
4	1556781	2464386
5	1574571	2450793
6	1562457	2469014
Mean	2461190	1567403
Std. Dev.	8356.55	8820.61
%RSD	0.34	0.56

Table 5: Method precision results for Azelnidipine and Chlorthalidone

S. No.	Azelnidipine Peak area	Chlorthalidone Peak area
1	2400189	1571991
2	2406873	1551387
3	2410703	1538647
4	2401657	1558475
5	2390107	1571305
6	2388102	1561250
Mean	2399605	1558843
Std. Dev.	8978.554	12629.891
%RSD	0.37	0.81

Table 6: Accuracy results of Azelnidipine

% Concentration	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery	Mean Recovery
50%	4	3.97	99.3	99.83
100%	8	8.02	100.3	
150%	12	11.99	99.9	

Table 7: Accuracy results of Chlorthalidone

% Concentration	Amount added (µg/mL)	Amount found (µg/mL)	% Recovery	Mean Recovery
50%	6.5	6.53	100.5	100.00
100%	13.0	13.0	100.0	
150%	19.5	19.41	99.5	

Table 8: Degradation data of Azelnidipine

S. No.	Degradation condition	Peak area	% Degradation
1	Acid	1363424	13.1
2	Alkali	1376110	12.3
3	Oxidative	1329341	15.2
4	Reduction	1398585	10.8
5	Neutral	1551046	1.1

Table 9: Degradation data of Chlorthalidone

S. No.	Degradation condition	Peak area	% Degradation
1	Acid	2034478	14
2	Alkali	2012034	14.9
3	Oxidative	1981103	16.2
4	Reduction	2074625	12.3
5	Neutral	2349935	0.6

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