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Development and Validation of Stability Indicating RP-HPLC Method for Azelnidipine for bulk drug.

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Abstract:

A Stability Indicating RP HPLC method was develop and validated for the determination of Azelnidipine using Phenomenex C_{18} column (25 cm × 4.6 mm,5µm) with mobile phase consisting of Methanol: Water (75:25% v/v). The flow rate was kept constant 1.0 mL/min and eluent was detected at 256 nm. In calibration curve experiments, Linearity was found to be in concentration range 2-14 µg/ml (R^2=0.9985) with regression equation ***y = 160134x + 3313.7*** Azelnidipine was subject to stress condition including alkaline, acidic, oxidation, wet heat, thermal degradation and photolysis. Azelnidipine is more sensitive towards acid degradation. Also there was no interference of excipient and degradation product at retention time of Azelnidipine, indicating specificity of the method.

Keywords: HPLC, Azelnidipine, Method Development and Validation, ICH Guideline.

INTRODUCTION:

Forced degradation/stress testing in which drug substance and drug product under conditions more than those used for accelerated stability test.Stability indicating methods included trial and error approach is usually cost, labor, along with time intensive with systematic approach [1-4].

Azelnidipine chemical name is (±)-3-[1-(diphenylmethyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate Fig.1. Azelnidipine(AZP) is a new and long acting dihydropyridine calcium channel blocker. This drug has been shown to decrease blood pressure [5].As per literature review, several methods were there for the determination of its pharmacologic action. Azelnidipine was estimated by only few method UV spectroscopy, HPLC, UPLC & one stability indicating RP-HPLC method. [5-16]. The aim of present work was to develop and validate a accurate, cost effective and precise stability indicating RP-HPLC method for determination Azelnidipine.

MATERIALS AND METHODS:

Apparatus and Equipments:

HPLC instrumentation consisting of pump PU-2080 plus (JASCO, Tokyo, Japan), with Rheodyne manual loop injector 7725*i* (injection loop capacity 20 μL) was used. Detection was carried out using UV-2075 detector (JASCO, Tokyo, Japan).

Reagents and Chemicals:

Pharmaceutical grade Azelnidipine was supplied as a gift sample from Macleod Pharmaceutical Pvt. Ltd. Gujarat, India. All chemicals and reagents were of analytical grade and were purchased from SD Fine Chemicals, Mumbai, India. Azelnidipine tablets prepare in lab (label claim25 mg/tablet).

Chromatographic Conditions.

All chromatographic separations were carried out on Phenomenex HyperClone C 18 column (250 × 4.6 mm, 5 μ), using mobile phase comprising methanol: water 75:25% v/v. The flow rate was kept constant throughout analysis at 1.0 mL/min and eluent was detected at 256 nm by UV- detector.

Standard Preparation: (Azelnidipine 1000µg/ml)

The chromatogram of standard Azelnidipine solution was shown in Fig.2. And the average retention time was found to be 5.625 min.

METHOD VALIDATION:

System suitability:

A Standard solution of Azelnidipine working standard was prepared as per procedure and was injected six times into the HPLC system.Six replicate injections are within range and results were shown in Table

Linearity:

Linearity is the ability of the method to elicit test results that are proportional to concentration of the analyte in the sample.[4]

It was found to be in the range of 2-14 µg/ml. The calibration graph was plotted, equation was obtained ***y = 160134x + 3313.7*** and the drug was found to be linear with a correlation coefficient (r2) of 0.9985 .

Accuracy and Precision:

It was determined by studying repeatability, intra-day and inter-day precision of method.[4] The average recovery of the analyte of 80%, 100% and 120% solution.

Specificity:

Dextrose was used as excipient. Three solution of 10 µg/mL was injected and one excipient solution as a blank injected and compare the chromatogram with the standard solution of Azelnidipine.

Robustness:

The analysis was performed by slightly changing the wavelength (254 nm and 258 nm), mobile phase composition (60:40 ,80:20 and75:25%v/v) and flow rate (0.8 and 1.2 mL/min). Th

Limit of Detection (LOD):.

It is calculated to be 0.12µg/mL by using the formula, LOD= 3.3σ/S Where, σ = Standard deviation of the response,S = Slope of calibration curve.

Limit of Quantitation (LOQ):

It was calculated to be 0.38µg/mL by using the formula,

LOQ=10σ/S Where,σ = Standard deviation of the response,S = Slope of calibration curve.

Degradation studies:

Acid degradation:

To 0.1 ml of stock solution of Azelnidipine, 10 ml of 0.1N Hydrochloric acid was added and refluxed for 15 mins at 60ºC

Alkali degradation studies:

To 0.1 ml of stock solution of Azelnidipinee, 10 ml of 0.1 N Sodium hydroxide was added and refluxed for 15mins at 60ºC

Thermal degradation studies:

In a Petri plate drug powder form was placed in oven at 80ºC for 30mins to study thermal degradation.

Photo Stability studies:

In a petri plate drug powder form was directly expose to sunlight for 48 and 72 hours for photo stability study.

Neutral Degradation Studies:

To 0.1 ml of stock solution of Azelnidipinee, 10 ml of water was added and refluxed for 30mins at 60ºC .

Oxidation studies:

To 0.1 ml of stock solution of Azelnidipinee, 1 ml of 30% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 24 hours at room temperature (R.T).

Chromatographic Analysis of Forced Degraded Samples:

. The % degradation was calculated by using below formula and was result shown in Table 5.

$$\%\ Degradation = \frac{Peak\ area\ of\ stressed\ sample}{Peak\ area\ of\ unstressed\ sample} \times 100$$

RESULTS AND DISCUSSION:

Table 1: System suitability for Azelnidipine

Sr.No.	R.T	Peak area	Theoretical plates
1	5.62	1895645.23	10836
2	5.61	1895874	10848
3	5.63	1894644.12	10830
4	5.60	1893243	10822
5	5.624	1896421.31	10840
6	5.626	1896646	10841
Mean	-	1895412	10836.17
SD	-	1162.146	8.335
%RSD	-	0.061314	0.076918

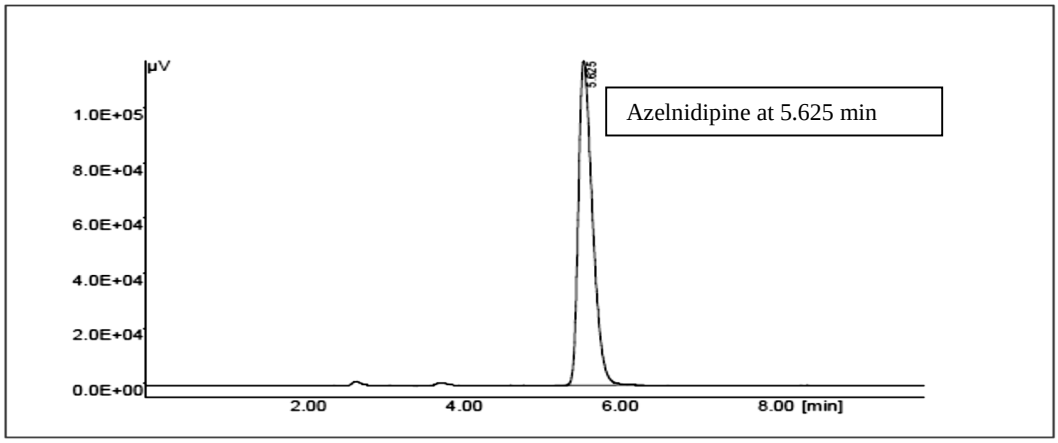

Fig 1: System Suitability Chromatogram of Azelnidipine

Table 2: Linearity for Azelnidipine

Sr. No.	Conc. (µg/ml)	Mean Peak area
1	2	332408
2	4	630367.7
3	6	954788
4	8	1276774
5	10	1618765
6	12	1971297
7	14	2206296

Table 3: Accuracy and Precision studies

Amount added(mg)	Amount found(mg)		
	Day 1	Day 2	Day 3
80%(12.8mg)	12.608	12.693	12.854
	12.796	12.757	12.721
	12.842	12.714	12.875

Mean	12.74867	12.72133	12.81667
Mean %Recovery	99.53	99.38	100.13
SD	0.101224	0.026637	0.068188
%RSD	0.793994	0.209392	0.532023
100%(16mg)	16.014	15.993	15.948
	15.972	15.941	15.916
	16.183	16.163	16.012
Mean	16.05633	16.03233	15.95867
Mean %Recovery	100.31	100.18	99.68
SD	0.091193	0.094803	0.039911
%RSD	0.567959	0.591322	0.25009
120%(19.2mg)	19.174	18.9199	18.894
	18.990	19.260	19.190
	19.184	18.987	19.223
Mean	19.116	19.05563	19.10233
Mean %Recovery	99.56	99.24	99.49
SD	0.089189	0.147083	0.147929
%RSD	0.466567	0.771858	0.774401

Table 4: Degradation Data for Azelnidipine

Sr. No.	Degradation Condition	% Degradation	Degradation RT
1	Acid	18.14	8.007
2	Alkali	24.50	3.083
3	Wet heat	23.26	8.19
4	Oxidation	stable	2.99(H_2O_2)
5	Thermal	Stable 80 °-150 °C	-
6	Photolytic	Photo stable	-

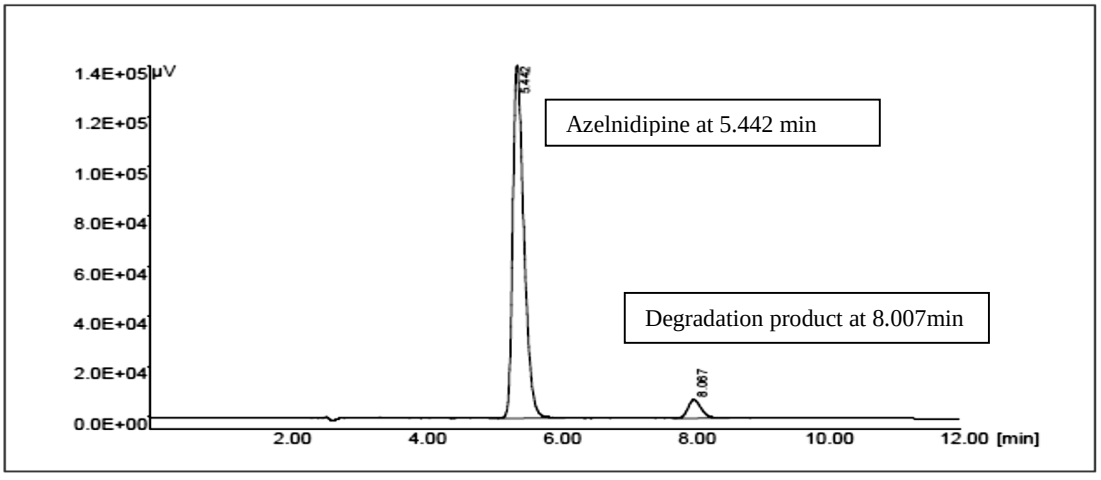

Fig 2: Representative Chromatogram of Acid Degradation of Azelnidipine

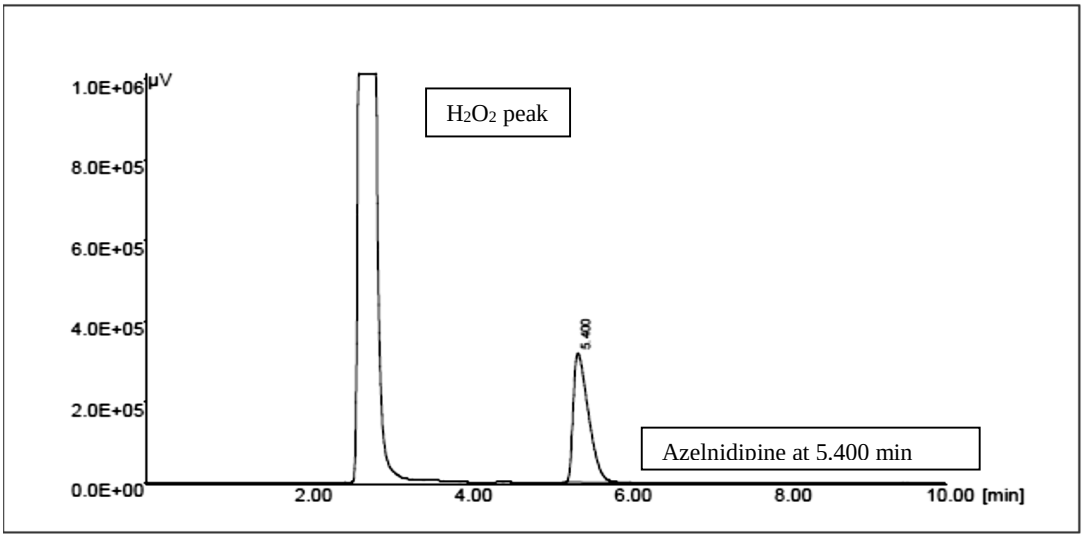

Fig 3: Representative Chromatogram of Peroxide Degradation of Azelnidipine

CONCLUSION:

In conclusion, a simple, selective, sensitive and accurate stability indicating RP-HPLC method was developed and validated for the analysis of Azelnidipine. Further the method was found to be linear, precise, accurate and robust. The degradation studies reveal the stability of the drug. Hence the proposed method can be used for the estimation of Azelnidipine in routine analysis.

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