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Method Development and Validation of Azelnidipine by RP-HPLC

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Abstract : The objective of this work is to develop a rapid, precise, accurate and sensitive reverse phase liquid chromatographic method for the estimation of azelnidipine in the plasma of rat animal model studies for transdermal drug delivery. The chromatographic method was standardized for azelnidipine using Shimadzu HPLC model reverse phase analytical inspire C18 column (250 mm x 4.5 mm, 5 µm particle size) with LC10AD pump and SPD-10A UV-Detector, The mobile phase consists of75:25 methanol: waterand 0.1%glacial acetic acid, wave length at 254nm, with flow rate of 1ml/min. The retention time of azelnidipine found to be 6.130 min. The method was statistically validated and %RSD was found to be less than 2 indicating high degree of accuracy and precision. Hence this is proposed method can be successfully applied for the estimation of azelnidipine in various dosage forms and animal model *in –vivo*studies.
Keywords : Azelnidipine, Chromatogram, Validation, Linearity.

Introduction

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-dicarboxylateFig.1. Azelnidipine(AZP) is a new and long-acting dihydropyridine calcium channel blocker. This drug has been shown to decreaseblood pressure with a similar potency as other dihydropyridines, such as amlodipine, but without increasing pulse rate[1].

Sankyo Co., Ltd. (President: TetsuoTakato) and Ube Industries, Ltd. (President KazumasaTsunemi) announced that AZP under the registered trademark of CALBLOCK®.CALBLOCK® is offered an oral tablet such as 8mg and 16mg per day for the treatment of hypertension, available from May 20, 2003 [2].

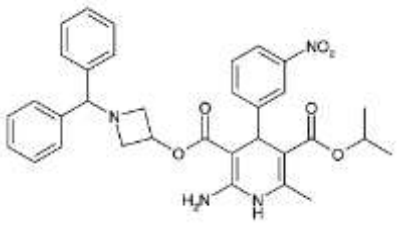

Fig.1:Chemical structure of Azelnidipine.

Calcium channel blockers (CCBs) have been shown to retard atherogenesis in animal models and to prevent the development of early lesions in human coronary arteries. They are used in the treatment of angina pectoris and hypertension [3].

A literature survey revealed that AZP is not yet official in any pharmacopoeia. Very few analytical methods have been reported for the determination of AZP, which include Uv-Spectroscopy [4], HPLC [4-6], LC-MS [7, 8], LC-ESI-MS [9, 10], and HPLC-MS-MS methods [11].

Materials and Methods :

Chemical and reagents

Azelnidipine was obtained as gift sample from Themis Medicare (India) Ltd. Methanol, Glacial acetic acid (Gaa) and water were HPLC grade obtained from sigma Aldrich chemicals Pvt.Ltd.

HPLC Method Development

Instruments and Chromatographic Conditions

The Shimadzu UV-1800 model was used to determine the absorption maximum (λ_{max}) of azelnidipine. Shimadzu HPLC model with LC10AD Pump and SPD-10A UV-Detector. The HPLC and column was maintained at room temperature. The reverse phase analytical inspire C18 column (250 mm x 4.5 mm, 5 µm), using of75:25 methanol: water (0.1%Gaa)was used as the mobile phase. The flow rate was set at 1ml/min and the injection volume was 20 µL. The HPLC detector was set to the wavelength of 254 nm.

Preparation of Standard solution

A primary stock solution was prepared by 50 mg of AZP dissolved in 50 ml of mobile phase to give a concentration of 1 mg/ml and stored at – 80^{o}C until use. The primary stock solution was diluted with mobile phase to give working solutions with concentrations of 10, 20, 30, 40, and 50µg /ml.

Method validation

The proposed method was validatedby parameters viz. linearity range, limit of quantification (LOQ), and limit of detection (LOD) precision, accuracy, specificity, robustness, selectivity [12-13].

Specificity and Selectivity

The chromatographic interference from endogenous compounds was assessed by comparing chromatograms with that of the AZP samples.

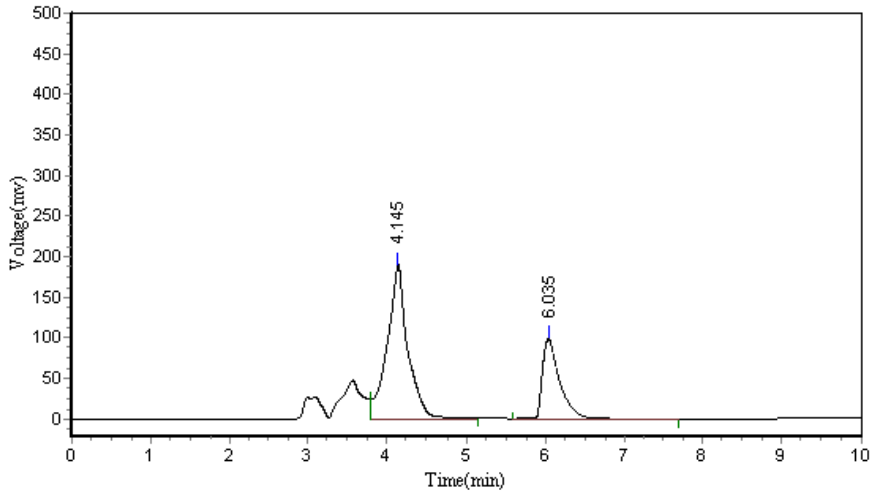

Fig.2:Chromatogram of Azelnidipine.

Sensitivity

The lowest limit of quantification (LLOQ) was determined as the minimum concentration that could be accurately and precisely quantified with the relative standard deviation of < ± 10%. The lowest limit of detection (LLOD) was defined as the amount that could be detected with a signal to-noise ratio of 4.

Table 1:Limit of Detection and Quantification.

Parameter(µg/ml)	Azelnidipine
Limit of Detection	0.193591
Limit of Quantification	0.586639

Linearity

Calibration curve was plotted by taking six concentrations of AZP ranging from 1.0 to 50 µg/ml. Blank samples were analyzed to confirm the absence of interferences. Calibration curve of azelnidipine was plotted by taking peak areas on Y-axis and concentrations on X-axis.

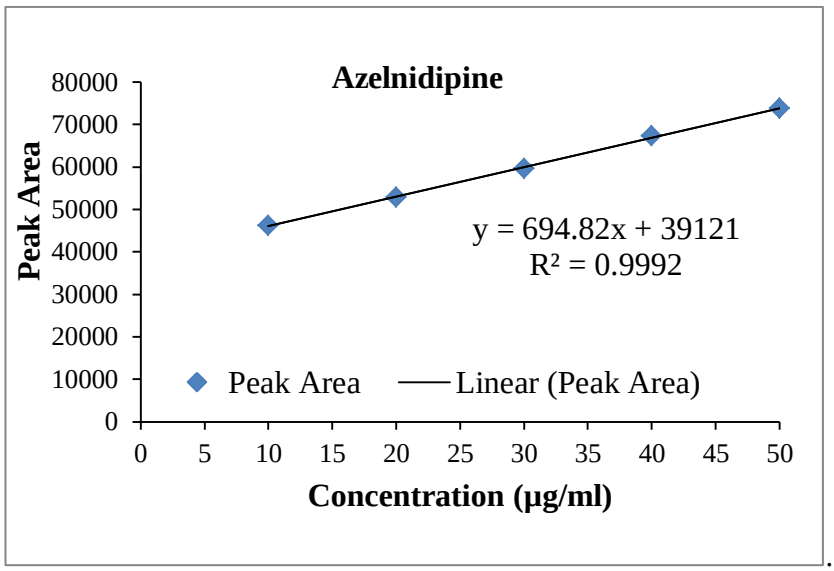

Fig.4: Linearity of Azelnidipine

Precision and Accuracy

In order to assess the intra and inter-day precision and accuracy for the method, AZP samples at low, medium and high concentrations were prepared as described above. The intra-day precision of the method was assessed by calculating the coefficient of variation (CV) for the analysis of samples in three replicates. Inter-day precision was determined by the analysis of samples on three consecutive days. Accuracy was calculated by comparing the measured values to the true values and was expressed in percentage or amount. The precision was accepted when the coefficient of variance for each concentration doesn't exceed ± 10%, and accuracy was accepted when the average values are > 95% of the true concentration except for the LLOQ where the limit was > 92%.

Table2:Precision Inter-Day and Intra-Day Variation of Azelnidipine Samples.

Drug	Theoretical Concentration (µg/ml)	Intra day		Inter day	
		Mean (µg/ml)	% RSD	Mean (µg/ml)	% RSD
Azelnidipine	10	9.98	0.49	9.68	0.65
	30	29.66	0.86	29.59	0.94
	50	49.52	0.47	49.26	0.78

*Mean of Six determinations

Table3:Accuracy (%recovery) data of Azelnidipine.

Drug	Theoretical Concentration(µg/ml)	Obtained concentration(µg/ml)	% Recovery
Azelnidipine	10	9.93	99.3
	30	29.61	98.7
	50	49.04	98.08

*Mean of Three Determinations

Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing the amount of analyte within or at the extremes of the specified range of the analytical procedure.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain, unaffected by small, but premeditated variations in method parameters and provides an indication of its consistency during normal usage. Robustness study was carried out by changing the flow rate and wavelength.

Table4:Robustness changing Flow rate and Wavelength.

Flow rate	Drug	TheoreticalConcentration (µg/ml)	Mean (Peak area)	Rt value	%RSD
0.9ml/min	**Azelnidipine**	10	46593.652	5.91	0.97
		30	59765.781	5.90	1.12
1.1ml/min	**Azelnidipine**	10	46581.622	6.00	1.24
		30	59662.521	6.02	0.91
Wavelength	**Drug**	**Theoretical Concentration(µg/ml)**	**Mean (Peak area)**	**Rt value**	**%RSD**
246 nm	**Azelnidipine**	10	46562.036	5.91	0.95
		30	59656.698	5.90	0.97
236 nm	**Azelnidipine**	10	46532.581	5.97	1.06
		30	59593.174	5.89	1.31

Ruggedness

In this a standard solution of the drug substance with in a matrix should be analyzed while systematically varying operating condition. The condition examined should include different operator in the same lab, different instrument in same lab, different laboratory, changing source of reagent and solvent, changing a new column. Ruggedness studies were carried out by changing the operator and instrument in same lab with same condition containing same concentration (1µg/mL) of azelnidipine in rat plasma.

Solution Stability Study

The stability of AZP in plasma was tested at the concentrations of the low and high QC samples. Short-term storage stability of sample up to 24 hours (6, 12, and 24 hrs) at room temperature in plasma was analyzed by the extraction from plasma and was assessed in triplicate. Results were expressed as the percent recovery relative to the initial (nominal) concentration at time zero. Stability was defined as less than 10% loss of the initial concentration.

System Suitability Test

The relative standard deviation (RSD) values for the peak areas, tailing factors, theoretical plates and retention times were the chromatographic parameters selected for the system suitability test [14].

Table 5:System Suitability data for Azelnidipine.

Parameters	Azelnidipine	Acceptance Limits
Theoretical plates	3964	> 2000
Tailing factor	1.24	< 2.0
Rt	6.1	-
Assymetry factor	1.32	< 2.0

Results and Discussion

Method development

The UV- Vis absorbance of AZP was scanned from wavelength of 200-400nm on a Shimadzu UV-Spectrophotometer (UV 1800) and maximum absorbance was found at wavelength of 254 nm in phosphate buffer. Therefore the wavelength of 254 nm was chosen for HPLC-UV detection in this method. The mobile phase used for the method was very simple and achieved optimal separation of AZP without interference from the other components. The flow rate was selected as 1 ml/min.

HPLC method validation

Specificity and Selectivity Fig. 2 represents chromatogram of AZP, No interference of endogenous peaks with AZP was observed with a retention time (Rt) of 6.1min. The sensitivity of AZP shown in table.1 the LLOD and LLOQ found to be 0.193591µg/ml and 0.586639µg/ml. The calibration curve of AZP was found to be linear over the different concentration range in mobile phase, the correlation coefficient was found to 0.999 Fig. 3. Thesummary of intra- and inter-day precision and accuracy. Intra- day accuracy of 10, 30 and 50 µg/ml was found to be 99.8, 98.8 and 99.0 respectively and inter- day accuracy was found to be 98.8, 98.6and 98.5 respectively. Therefore, the intra- and inter- day accuracies (% deviation) were within < ± 10% for the LLOQ. These results are indicated that the present method has very good precision and accuracy shown in table.2 and 3. The robustness results were shown in table 4,the relative standard deviation (RSD) was found to be less than 2%, and the low % of RSD value confirms that robustness of method. The table 5 reported values confirm that this method is suitable for estimation of AZP in formulations and in plasma samples. The stability of azelnidipine in plasma was found to be satisfactory results that confirmed that this method is suitable for animal model studies.

Conclusion

The proposed method was found to be rapid, precise, accurate and sensitive. It makes use of fewer amounts of solvents and shorter retention times than existing methods. Many samples can be suitably analyzed by this method. Hence developed method can be used for routine analysis of AZP in various formulations and animal model *in-vivo* studies.

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