Original Research Article **Analitical Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD FOR THE ASSAY OF AZELNIDIPINE IN BULK AND PHARMACEUTICAL FORMULATIONS

RAJAN V RELE.*AND SWAPNIL A. SAWANT

Central research laboratory, D.G. Ruparel College, Matunga, Mumbai 400016

ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of azelnidipine from active pharmaceutical ingredients and its pharmaceutical dosage form. The separation of drug was achieved on Waters Symmetry C8 shield (15 x 4.6 mm i.d.) with 5 µ particle size column. It showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of 0.1% orthophosphoric acid in water and acetonitrile (60:40 % v/v). The detection was carried out at wavelength 256 nm. The mixture of 0.1% (v/v) orthophosphoric acid in water and acetonitrile (60:40% (v/v)) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. All the peaks of degradation products were resolved from the active pharmaceutical ingredient with significantly differ retention time. As the method could effectively separate the drug from the its degraded products. Hence above method can be also used for the study of stability indicating parameters.

KEYWORDS: **Azelnidipine, acetonitrile, ortho-phophoric acid**

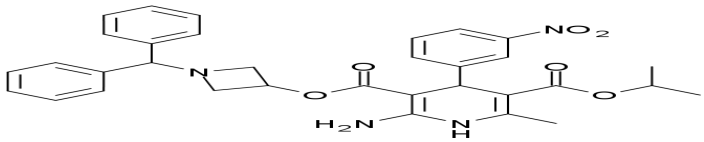
RAJAN V RELE
Central research laboratory, D.G. Ruparel College, Matunga, Mumbai 400016

INTRODUCTION

In this communication the present work proposes reverse phase high pressure liquid chromatographic method for assay and stability study of azelnidipine from active pharmaceutical ingredients. Azelnidipine is a lipophilic calcium channel antagonists. Its chemical name is O-3-[1-[di (phenyl) methyl] azetidin-3-yl] O-5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Azelnidipine can restrain Ca^{+} ions outside the cardiac muscle and vascular smooth muscle. They enter the cells through cell membrane; it expands blood vessel, lower peripheral vascular resistance and arterial pressure. In clinic, it is used for treatment of essential hypertension and angina pectoris[1]. This drug is not official in any pharmacopoeia. In literature survey only HPLC[2, 3], spectrophotometric[4-7] and titrimetric[8] and methods have been reported for its validation of drugs. Simple, sensitive and reproducible RP-HPLC method has been developed here for the estimation of azelnidipine from bulk drug and pharmaceutical formulation. The developed method will be useful for routine analysis in pharmaceutical industries and research organizations. The structure of azelnidipine is as shown.

Chemical structure of Azelnidipine



MATERIALS AND METHODS

Chemical and reagents

Reference standard of azelnidipine was obtained from reputed firm with certificate of analysis. Acetonitrile and ortho-phosphoric acid were used of analytical grade and HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent of 0.1% orthophosphoric acid in water and acetonitrile (60:40% (v/v)).

Instrumentation

The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software. A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation

Standard solution

A 10 mg of standard azelnidipine was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 2 minutes. The volume was adjusted up to the mark with diluent to give a concentration as 1000 µg /ml. The working standard solution was prepared by diluting 1 ml of 1000 µg /ml solution to 10 ml with diluent to get concentration 100 µg /ml.

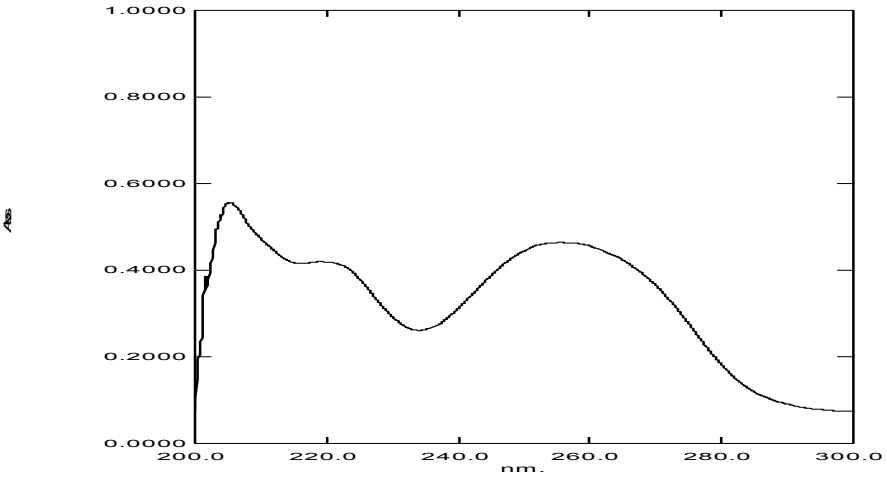
Sample preparation

Twenty tablets were weighed accurately and finely powdered. Powder equivalent to 10 mg of azelnidipine sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The sample solution was prepared by diluting 1 ml of 1000 µg/ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Chromatographic condition

Chromatographic separation was performed on a reverse phase Waters Symmetry C8 shield (15 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of 0.1% (v/v) orthophosphoric acid in water and acetonitrile (60:40 % (v/v)). The flow rate of the mobile phase was adjusted to 1.5 ml /min. The detection was carried out at wavelength 256 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 10.0 µl.

Figure 1
UV spectra of Azelnidipine



Method validation

System suitability

System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, area and % area were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1

System suitability parameters evaluated on standard solution of azelnidipine

Retention Time	Area	%Area	USP Plate Count	Symmetry
2.75	8825181	100	6082	1.65

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard azelnidipine was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2

Typical chromatogram of azelnidipine (standard)

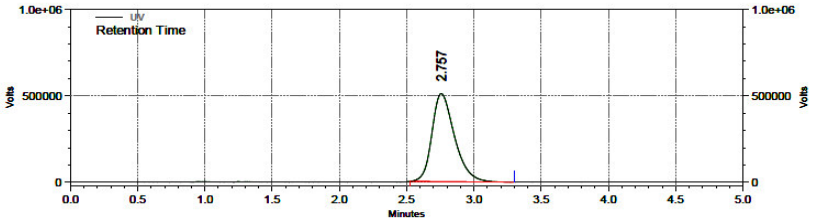
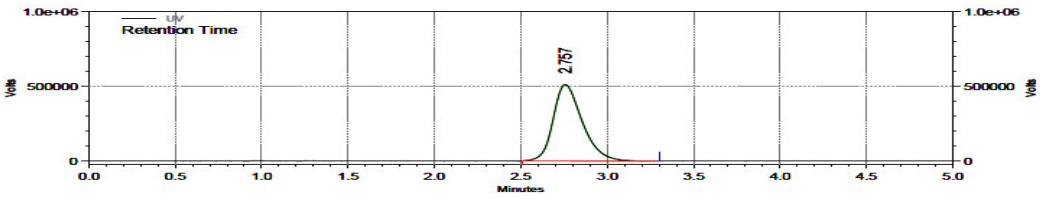


Figure 3

Typical chromatogram of Azelnidipine (sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2

Statistical evaluation of the data subjected to regression analysis

Parameters	Azelnidipine
Correlation Coefficient (r)	0.999
% Intercept (y)	190.7
Slope (m)	60422

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 120 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Table 3

Statistical evaluation of the data subjected to accuracy of azelnidipine

level	test	area	quantity added in µg/ ml	quantity recovered in µg/ ml	% recovery	mean recovery
80%	1	4800700	82.50	83.54	100.90	**101.77**
	2	4842063	82.50	84.26	101.77	
	3	4798638	82.50	83.51	100.85	
100%	1	5999378	103.50	104.37	100.89	**100.86**
	2	5999336	103.50	104.40	100.87	
	3	5999184	103.50	104.40	100.87	
120%	1	7292125	124.20	126.90	102.17	**101.85**
	2	725202	124.20	125.73	101.23	
	3	7290615	124.20	126.87	102.50	
				Mean		**101.49**

Precision

The method precision was established by carrying out the analysis of azelnidipine. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table no. 4.

Table 4

Statistical evaluation of the data subjected to method precision of azelnidipine

Test	wt of test sample	Area	% assay
Test solution -1	10.16	5960890	98.38
Test solution -2	10.42	5935553	100.47
Test solution -3	10.35	5959767	100.20
Test solution -4	1036	5936052	99.90
Test solution -5	10.33	5913344	99.23
Test solution -6	10.25	6046656	100.68
	Mean Assay		**99.81**
	SD		**0.864**
	RSD		**0.866**

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in the flow rate by ± 0.2 ml /min

Variation in mobile phase composition by ± 2 %

Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application

Sample equivalent to 10 mg of azelnidipine sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 minutes to dissolve it. Further volume was made up to the mark with the diluent to give 1000 µg /ml. Further the 1 ml of this solution was diluted to 10 ml with diluent to give 100 µg /ml of azelnidipine. From this solution 10.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of azelnidipine in the product meets the requirement.

Degradation studies

Acid degradation Studies

To 1ml of stock solution of azelnidipine, 1ml of 1N hydrochloric acid was and added and refluxed for 2hrs at 60°C. The resultant solution was diluted with diluent to obtain 100 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Base degradation Studies

To 1ml of stock solution of azelnidipine 1ml, of 1N sodium hydroxide was and added and refluxed for 2hrs at 60°C. The resultant solution was diluted with diluent to obtain 100 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation Studies

To 1ml of stock solution of azelnidipine 1ml 10% hydrogen peroxide was and added and refluxed for 2hrs at 60°C. The resultant solution was diluted with diluent to obtain 100 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation Studies

The Standard drug solution was placed in oven at 105°C for 6 hours. The resultant solution was diluted with diluent to obtain 100 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photostability degradation Studies

The Standard drug solution was exposed to UV light by keeping the beaker in UV chamber for 24hrs. The resultant solution was diluted with diluent to obtain 100 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation Studies

The Standard drug solution was refluxed in water bath for 6hrs at 60°C. The resultant solution was diluted with diluent to obtain 100 µg /ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The percent of drug degraded in the presence of acidic, alkali, oxidative, dry heat, photostability and neutral conditions were studied. The amount of drug recovered or degraded is calculated by compared the area of the standard with that of the area of the degraded sample. The results are furnished in Table 5.

Table no.5

Results of degradation studies

No.	Test	Weight of test sample in mg	Peak area of standard	Peak area of sample	% Assay	Mean Assay
1	**Acid hydrolysis (0.1 N HCl)**	10.16	6443736	3972084	60.51	60.88
		10.42	6443736	3920268	61.25	
2	**Base Hydrolysis (0.1 N NaOH)**	10.35	6443736	6241299	96.86	96.69
		10.36	6443736	6214022	96.53	
3	**Oxidation 0.1% H_2O_2**	10.33	6443736	3278730	50.78	50.00
		10.25	6443736	3201724	49.21	
4	**Neutral hydrolysis**	10.33	6443736	6256270	96.90	97.67
		10.25	6443736	6404774	98.44	
5	**UV Radiation**	10.33	6443736	6492720	100.57	100.16
		10.25	6443736	6489981	99.74	

RESULTS

In the proposed method, the retention time of azelnidipine was 2.757 min. The linearity was in the range of 8-12 µg/ml. The regression equation of the linearity was given as Y=

60422X+190.7 where X is concentration of azelnidipine in µg /ml. and Y is corresponding peak area. The coefficient of co-

relation was 0.9999. The result shows that an excellent co-relation between peak area and concentration of azelnidipine in the range indicated. The relative standard deviation for method precision was 0.866(limit %RSD < 2.0%). The mean recovery of the azelnidipine was 99.81. The high percentage recovery indicates that the proposed method is highly accurate. The use of 0.1% orthophosphoric acid in water and acetonitrile (60:40% (v/v) gave peak with good resolution. The robustness studies indicated that there was no effect on the drug study. No interfering peaks were found in the chromatogram of the formulation within the run time indicted that excipents used in the formulation did not interfere the estimation of drug. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation.(Table no.4) The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. (table no.3) The stability- indicating nature of the proposed method was established by performing force degradation, which provided degradation behavior of azelnidipine under various conditions. The results of force degradation were given in table no.5.

DISCUSSION

The proposed stability indicating reverse phase HPLC method is useful to separate various degradants in alkaline, acidic, oxidative, neutral and thermal conditions (table no.5). The drug undergoes degradation in acidic medium with 60.88% and 50% in oxidation in recovery respectively. It gave recovery of 96.69 % and 97.67% in base and neutral hydrlosis. While good stability in UV radation. This can be successfully used for validation of drug as well as for determining stability of drug in various conditions as per ICH guidelines.

CONCLUSION

Thus the proposed RP-HPLC method is used for estimation of azelnidipine from active pharmaceutical ingredient. It is more economical, precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation and degradation of drug in various conditions as per ICH guidliness.

ACKNOWLEDGMENT

Authors express sincere thanks to the principal, Dr. Tushar M. Desai, of D.G. Ruparel College, Mumbai for encouragement and providing laboratory facilities.

REFERENCES

1. Chemical Abstract Service Register Number.1949; 20-8.
2. Hua-min, WANG Ju-cai**.** Determination of content and related substance of azelnidipine by HPLC West China Journal of Pharmaceutical Science,2006; 21(06):581-582.
3. Pan Y, Zhang J, Ding J and Wang T. Determination of azelnidipine tablets by HPLC. Qilu Pharmaceutical Affairs,2008; 07: 398-399.
4. Rele, R.V.,Patil, S.P. Ultraviolet spectrophotometric method for estimation of azelnidipine from the Bulk drug and pharmaceutical formulation, Asian journal Research Chem. 2010; 3(4):1-3.
5. Kunti R, Patel M, Anandkumari D. Captain. UV-spectrophotometric method development and validation for determination of azelnidipine in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2012:4(1); 238-240.
6. Rajan Rele. Spectrophotometric estimation of azelnidipine in bulk drug and pharmaceutical dosage form by first order derivative and area under curve methods, American journal of pharmtech research ,2014;4(2):126-135.
7. Nilam Patela, Jayvadan Patel. Simultaneous Determination of azelnidipine and olmesartan medoxomil by first derivative spectrophotometric method. Der Pharmacia Lettre, 2012; 4 (4):1080-1084.
8. Rele RV, Terse R H. A validated non aqueous potentiometric titration method for the quantitative determination of azelnidipine from pharmaceutical preparation. Journal of chemical and pharmaceutical research.2011; 3(3): 1-5.
9. ICH, Q2 (R1). Validation of Analytical Procedures: text and methodology; 2005**;**1-13.