EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 5
270-275
Year: 2023

DEVELOPMENT OF NEW ANALYTICAL METHOD FOR ESTIMATION OF CALCIUM CHANNEL BLOCKER AZELNIDIPINE

Prakash S. Sarsambi*, Ramesh D. Avute and Preeti J. Jadhav

Department of Pharmaceutical Analysis, HKES'S Mathoshree Taradevi Rampure Institute of Pharmaceutical Science, Sedam Road, Kalaburagi, Karnataka-585105, India.

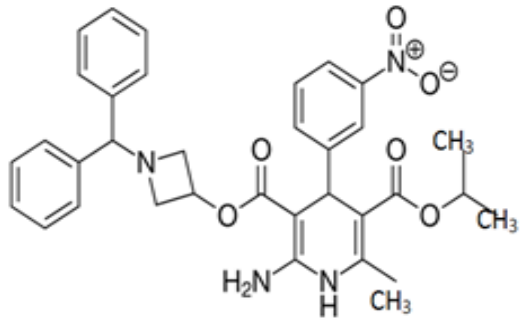
***Corresponding Author: Prakash S. Sarsambi**
Department of Pharmaceutical Analysis, HKES'S Mathoshree Taradevi Rampure Institute of Pharmaceutical Science, Sedam Road, Kalaburagi, Karnataka-585105, India.

Article Received on 13/03/2023 **Article Revised on 03/04/2023** **Article Accepted on 24/04/2023**

ABSTRACT
Azelnidipine is a third generation and long acting dihydropyridine calcium channel antagonist used for effective antihypertensive agent. Three simple, sensitive, accurate and economical spectophotometric methods have been developed for the estimation of Azelnidipine in bulk drug and pharmaceutical formulation. Method A is based on UV-spectophotometric measurements in which Azelnidipine was dissolved in 0.1N HCl and exhibited absorption maxima at 220nm and obeyed Beer's law in the concentration range of 5-30µg/ml. In Method B and C the amine group of reduced Azelnidipine forms colouredchromogens. In method B Azelnidipine forms colouredschiff's base with PDAC(Para- Dimethyl amino cinnamaldehyde) reagent to yield orange red colouredchromogen exhibitingabsorption maxima at 530nm and obeyed Beer's law in the concentration range of 20-100µg/ml. In Method C the reduced Azelnidipine is diazotized with sodium nitrite and HCl to form a diazonium salt and coupled with BMR(Bratton Marshal reagent) i.e.N-1-[Napthylethylenediaminedihydrochloride] reagent and exhibited absorption maxima at 557nm and obeyed Beer's law in the concentration range of 20-100µg/ml. Results of the analysis were validated for accuracy, precision, limit of detection (LOD), Limit of quantification(LOQ) were found to be satisfactory. The proposed methods are simple, sensitive, rapid economical and suitable for the routine quality control application in pharmaceutical formulations.

KEYWORDS: Azelnidipine, 0.1N HCl, PDAC, BMR.

1. **INTRODUCTION**: Azelnidipine[1] is chemically (±) -3-(1-diphenylmethylazethidin 3-yl) 5-isoprophyl12-amino-1,4dihydro-6-methyl-4-(3nitro-phenyl) 3,5-pyridinedicarboxylate. Clinically, it is used as treatment of hypertension.[2] Literature survey reveals that few analytical methods were reported for the estimation of Azelnidipine like, RP-HPLC method development and validation of Azelnidipine[3], Method development and validation of azelnidipine by RP-HPLC[4], A novel method for the simultaneous determination of Azelnidipine and Olmesartan in Human plasma by using liquid chromatography- electro spray Ionization tandem mass spectrometry and application to a pharmacokinetic study[5], UV- spectrophotometric method development and validation of Azelnidipine and in pharmaceutical dosage form[6], Simple validation of Azelnidipine by RP- HPLCmethods[7], Method Development and validation for the simultaneous determination of azelnidipine and telmisartan in tablet dosage form by RP- HPLC[8], Analytical method development and validation of azelnidipine by uv-visible spectroscopy[9], Mathematically Processed UV Spectroscopic Method for Quantification of Chlorthalidone and Azelnidipine in Bulk and Formulation: Evaluation of Greenness and Whiteness[10], UV Spectrophotometric method for estimation of Azelnidipine from bulk drug and pharmaceutical formulation[11], A Stability Indicating RP-HPLC Method Validation for Simultaneous Estimation of Azelnidipine and Telmisartan in a Fixed-dose Combination.[12] As per literature survey there is only few analytical methods reported for estimation of Azelnidipine using visible spectroscopy. The analytical important functional group of Azelnidipine have not been exploited completely for designing sensitive, precise, accurate and flexible spectrophotometric methods for the determination of Azelnidipine bulk drugs and pharmaceutical formulations. Based on different chemical reactions two visible spectrophotometric methods and one uv method have been developed.



Azelnidipine

2 EXPERIMENTAL METHODS

2.1Equipment: All spectral measurement where made on Shimadzu 1800 UV/Visible spectrophotometer with 1cm matched quartz cell used.

2.2chemical and reagents: All the chemical used were of analytical grade . Azelnidipine bulk drug was obtained from moleculochempvt ltd, Ahmedabad, India as a gift sample. The tablets are purchased from the market under tha trade name of Azovas16, zeblong 16. The reagent like PDAB(P-dimethyl amino benzaldehyde), PDAC(0.1% w/v in alcohol). Sodium nitrite [$NaNO_2$ 0.1% w/v indistiled water], o.5NHCl, ammonium sulfamate(0.5%w/v in water) and BMR(O.1%w/v in distilled water).

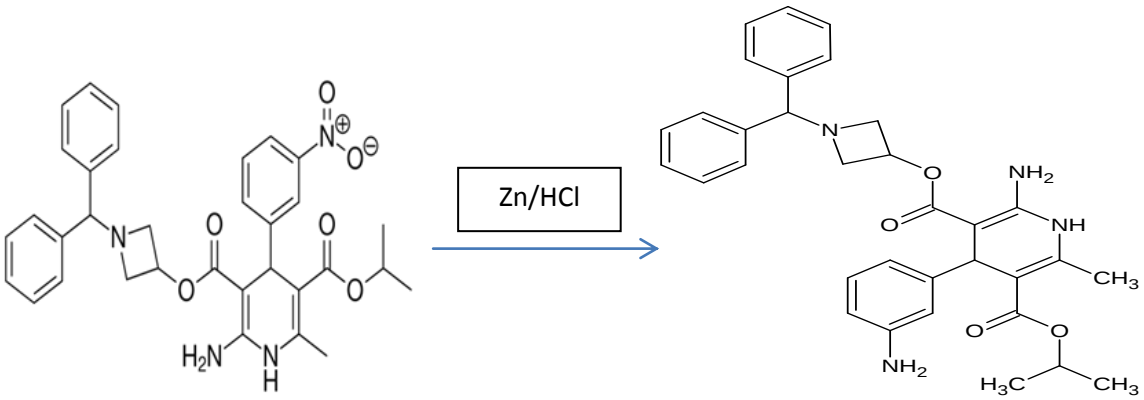
2.3 Preparation of standard and sample solutions

Soluiiton-I : accurately weighed 100mg of Azelnidipine or tablet powder equivalent to 100mg of Azelnidipine was dissolved in 30ml of ethanol in 100ml volumetric flask and then add 10ml of 4N HCl and 500mg of Zn granules.After keeping it for one hour with occasional shaking at room temperature. The solution was filtered through cotton wool, the residue was brought 100ml with distilled ethanol(I.e 1000µg/ml). The concentration of reduced Azelnidipine was brought to(100µg/ml) solution-I by further dilution with ethanol.

2.4 Procedure

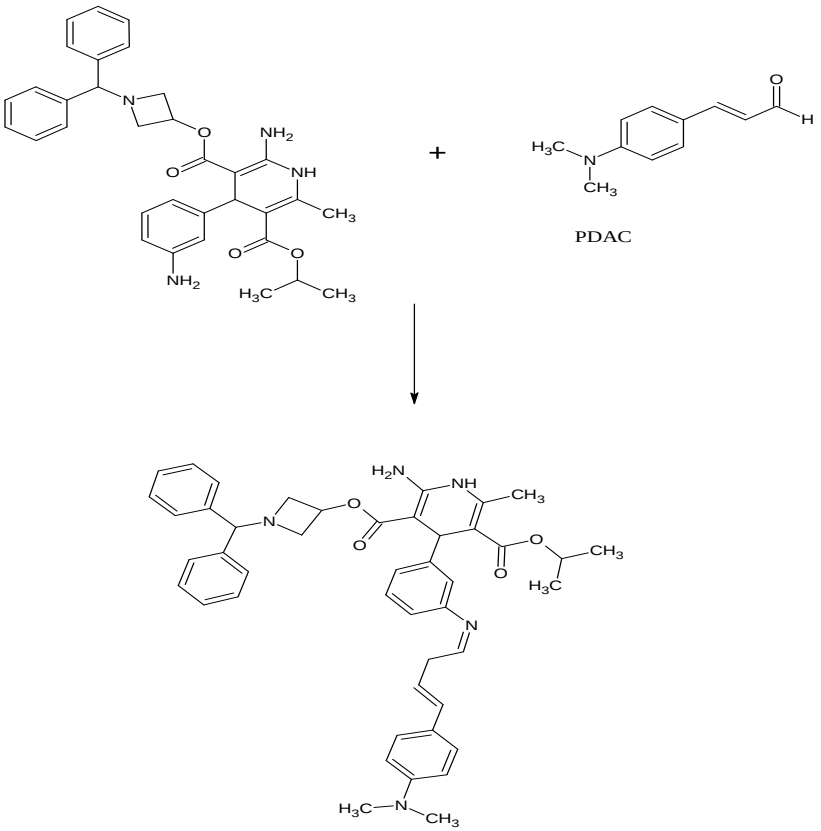
Method A: UV spectroscopy

For the selection of analytical wavelength 15µg/ml solution(unreduced) was prepared by subsequent dilutions and the solution was scanned in the spectrum made from 200-400nm.From then spectra of drug (Fig 1)$\lambda_{max}$of Azelnidipine 220nm was selected for the analysis. The calibration curve (Fig 2) was prepared in the concentration range of 5-30µg/ml. The amount of drug present in the sample solution was computed from its calibration curve.



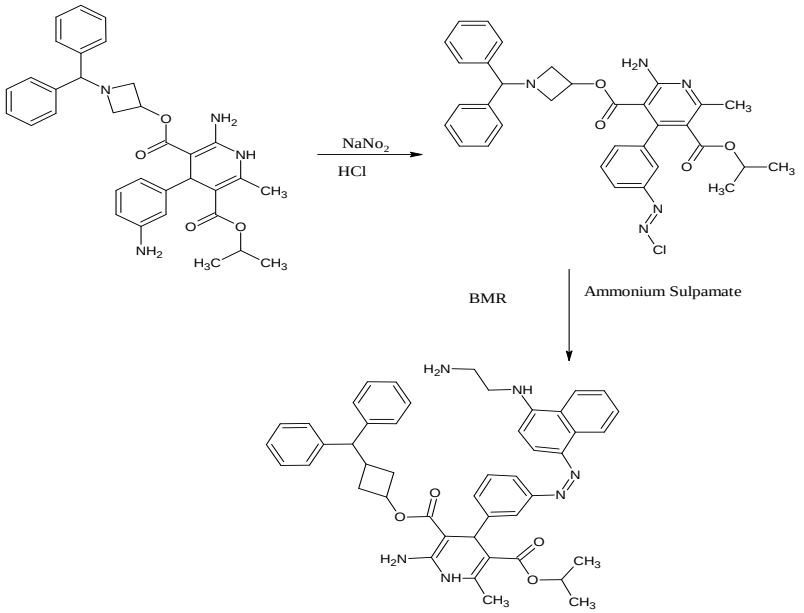
Method B: Condensation reaction by using PDAC Reagent

In this method reduced Azelnidine60µg/ml solution was prepared by appropriate dilution of standard stock solution-I and scanned in the spectrum mode from 400-800nm. From the spectra of drug(Fig 3)λ_{max} of Azelnidipine 530nm was selected for the analysis. The calibration curve was prepared by taking fresh Aliquots from solution-I ranging from 2 to 10ml(1ml=100µg/ml) were transferred into a series of 10ml graduated volumetric flask to get concentration range of 20-100µg/ml. To each flask add 1.0ml of alcoholic PDAC (0.1%w/v) solution and two drops of concentrated HCl were added. The solution were kept at normal room temperature and made upto mark with ethanol. The absorbance of orange red colourchromogen was measured at 530nm against the blank. The amount of Azelnidipine present in the sample solution was computed from its calibration curve(Fig 4)



Method C: Diazotization followed by coupling with BMR

In this method, the reduced Azelnidipine 60µg/ml solution was prepared by appropriate dilution of standard stock solution-I and scanned in the spectrum mode of 400-800nm. From the spectra of drug(Fig 5) λ_{max} of Azelnidipine 539nm was selected for the analysis. The calibration curve was prepared by taking fresh aliquots of reduced Azelnidipine ranges from 0.2-1.0ml (1ml=100µg/ml) were transferred into a series of 10ml graduated volumetric flask to get concentration range of 20-100µg/ml. To each flask 1ml of 0.1%w/v of $NaNO_2$, 1ml of o.5N HCl and wait for 3 minutes and add 1ml of 0.5%w/v ammonium sulfamate and wait for 3 minutes and then add 0.1% BMR reagent. The solvent were kept at normal room temperature and make up the volume upto 10ml mark with ethanol. The absorbance of the pink colourchromogen was measured at 557nm against the reagent blank (Fig 5). The amount of Azelnidipine present in the sample solution was computed from its calibration curve(Fig 6).



2.5 Assay

The sample of the powered tablet claimed to have 16 mg of Azd taken, the quantity equivalent to 100 mg of AZD was transferred to a 100 ml vol flask and dissolved in 30 ml of 0.1 N HCl, the solution was sonicated for about 10min and filtered through Waltman filter paper. The filter paper was washed with an adequate volume of 0.1 N HCl and then the volume was made up to 100 ml with 0.1 N HCl. Subsequent dilutions of this solution were made with 0.1 N HCl to obtain 10mcg/ml and obtained concentration range of 5-30 mcg /ml and analysed at the 220 nm and their results were analytically validated.

The sample of the powder tablet claimed to contain 16 mg, accurately weighed to an equivalent quantity of 100mg of AZD and the weighted sample was dissolved in 10ml of 4N HCl followed by adding Zn granules and shaken for hours and filtered through cotton swab placed in funnel and added ethanol upto 100ml to get stock solution of 100 mcg/ml, from the above stock solution 10ml was taken and diluted to 100 ml with ethanol to make final dilution of concentration of 0.1mg/ml and the final solution were prepared as per the above calibration curve procedure and analyzed at 530 nm and 557 nm respectively for both Colerimetric method B & C and results were statistically validated.

3 RESULTS AND DISCUSSION

Table 1: Optical Characteristics and Precision.

Parameter	UV	PDAC	BMR
λ max (nm)	220nm	530nm	557
Beer's law limits (µg/mL) (c)	5-30	20-100	20-100
Molar absorptivity(lit.mol-1cm-1)	12.46 X 103	1.36 X 103	1.46X103
Limit of Detection (LOD/mcgmL-1)	0.551	5.1	2.89
Limit of Quantification (LOQ/mcgmL-1)	1.682	15.4	8.73
Sandell'ssensitivity (µg/mL0.001absunit)	0.020	0.028	0.025
Regression equation (Y*) Slope (b)	0.02152	2.341X10-3	2.59X10-3
Intercept(a)	9.39X10-4	1.2X10-3	2.9X10-3
Correlationcoefficient(r)	0.999	0.999	0.9998
%RSD	1.13	1.97	2.207
Range of Errors** Confidencelimitswith0.05 levelConfidencelimitswith0.01level	0.0030 0.0042	0.00235 0.00447	0.0023 0.0034
Standard Error	0.00127	0.000997	0.000997

***** average of eight determinations

** average of three determinations

Table 2: Assay results of Azelnidipine in pharmaceutical formulations.

Sample	Labelled amount (mg)	Amount found* (mg)			% recovery by the proposed method	
		Proposed method		Reference method		
		PDAC	BMR	(UV)	PDAC	BMR
T1	16	15.53	15.71	15.92	97.55	**98.68**
T2	**16**	**15.55**	**15.73**	**15.95**	**97.49**	**98.60**

T_1 and T_2 are tablets from different manufactures,

*Average of five determinations.

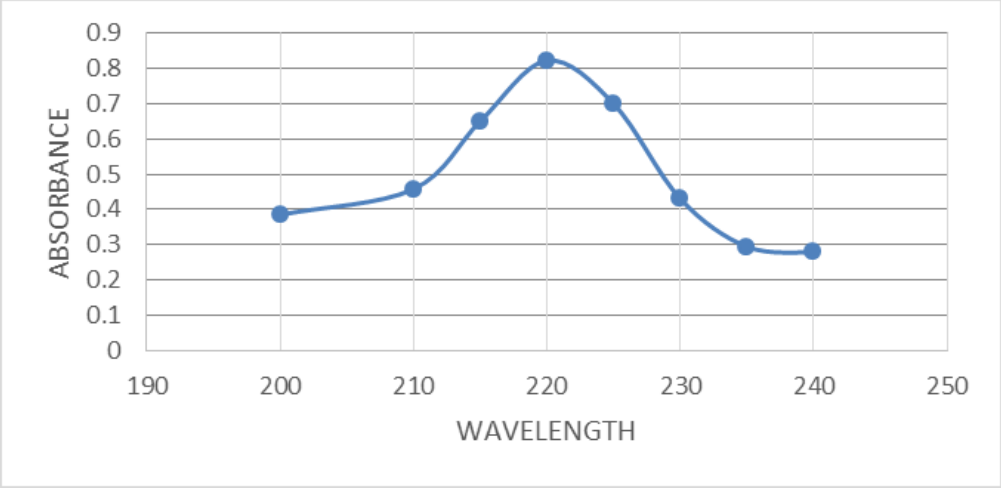


Fig. 1: Absorption spectra of Azelnidipine using UV method.

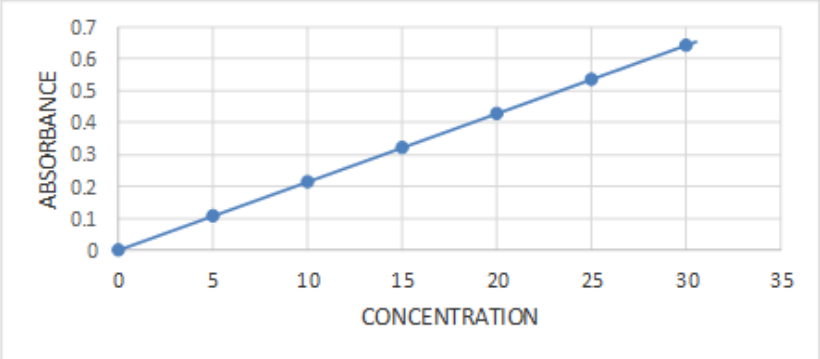


Fig. 2: Calibration curve of Azelnidipine using UV method.

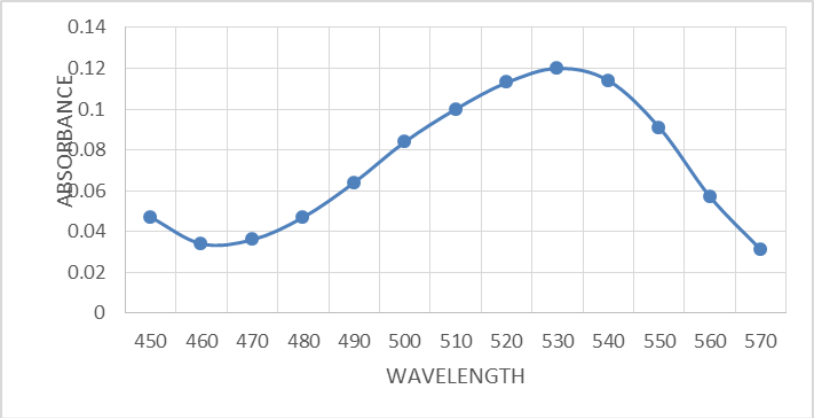


Fig. 4: Calibration curve for AZELNIDIPINE with PDAC (0.1%w/v) at 530nm.

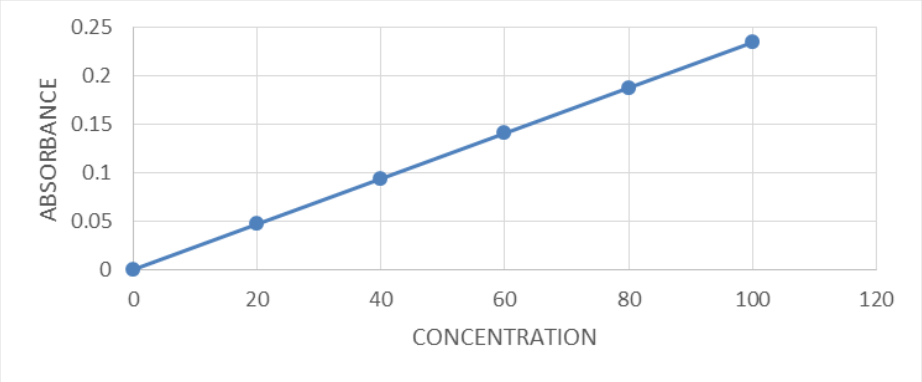


Fig. 3: Absorption spectra for AZELNIDIPINE by using PDAC (0.5%w/v) (10mcg/ml).

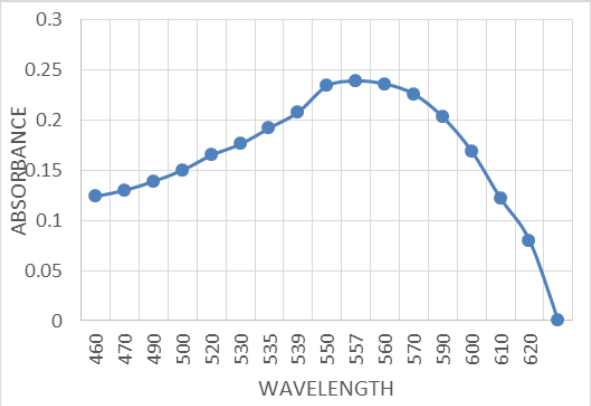


Fig. 5: Absorption spectra for AZELNIDIPINE by 0.1% BMR (100mcg/ml).

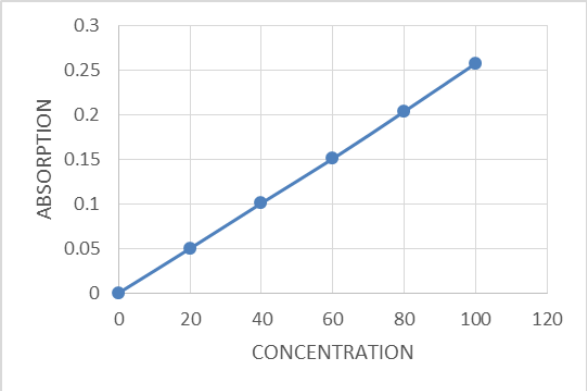


Fig. 6: Calibration curve for AZELNIDIPINE with BMR (0.1%w/v) at 557nm.

4. CONCLUSION

The optical characteristics such as beer's law limits, sandell's sensitivity, molar absorptivity, percent relative standard deviation was calculated and the results are summarized in table no. 01. Regression characteristics like slope, intercept, correlation coefficient and percentage of error (0.005 and 0.01) confidence limit were also calculated and represented in table no 1. Commercial formulations of Azelnidipine were successfully analyzed by the proposed methods as well as UV method and the results are represented in the table no: 02. To evaluate validity and reproducibility of the methods, fixed amount of the drug was added to the preanalyzed formulations. These results of percentage recovery are summarized in table no. 02. The methods are validated for various parameters like accuracy standard deviation and % RSD and the result is presented in table 3. There is no interference of additives and excipients in the proposed analytical methods. The proposed spectrophotometric method for estimation of Azelnidipine are simple, sensitive, accurate, and precise and can be used for routine estimation of the drug in bulk as well as in pharmaceutical formulation.

5 AKNOWLEDGEMENT

The authors are thankful to moleculochempvt ltd, Ahmedabad, India for providing the drug sample, the principal HKES'S Mathoshree Taradevi Rampure Institute of Pharmaceutical science, kalaburagi for providing the facilities to carryout the present work.

6 REFERENCES

1. Wikipedia(en.m.wikipedia.org)
2. Liu LS, Writing Group of 2010 Chinese Guidelines for the Management of Hypertension 2010 Chinese guidelines for the management of hypertension. ZhonghuaXinXue Guan Bing ZaZhi, 2011; 39(7): 579–615. Chinese. [PubMed] [Google Scholar]
3. Megha G Gore and Dabhade Ps, RP-HPLC method development and validation of Azelnidipine. IJPSR, 2016; 5111-5114.
4. Prabhakar D, Sreekanath J. Jayaveera K. Method development and validation of Azelnidipine by 'RP-HPLC. International Journal of Chem Tech Research, 2018; 11(01): 07-12.
5. Adepu R., et al. "A novel method for the simultaneous determination of Azelnidipine and Olmesartan in Human plasma by using liquid chromatography- electro spray Ionization tandem mass spectrometry and application to a pharmacokinetic study". International Journal of Pharmaceutics, 2017; 7.3: 111-124.
6. RD Raskapur. MM Patel et al. UV-spectrophotometric method development andvalidation of Azelnidipine and in pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(1): 238-240.
7. SelvaduraiMuralidharan, S. Parasurraman V, Venugopal. Simple validation of Azelnidipine by RP- HPLCmethods. Rapports De Pharmacie, 2015; 1(1): 43-45.
8. Silky Agrawal, TahirNizami; Method Development and validation for the simultaneous determination of azelnidipine and telmisartan in tablet dosage form by RP- HPLC; International Journal of Pharmaceutical Sciences and Medicine, 2021; 6(10): 26-36.
9. Imdad Husen Mukeri, Amit Kumar Kushwaha, Netra Prasad Neupane, Ashutosh Kumar, Allen Sushant, Anant Nag and P. Malairajan* Analytical method development and validation of azelnidipine by uv-visible spectroscopy world journal of pharmaceutical research, 10(10): 858-872.
10. Mahesh Attimarad, Muhammad ShahzadChohan, VenugopalaKatharigattaNarayanaswamy, AnroopBalachandran Nair, N. Sreeharsha, SheebaShafi, Marysheela David, Abdulmalek Ahmed Balgoname,1 Abdulrahman Ibrahim Altaysan,1 Efren II Plaza Molina, and Pran Kishore Deb "Mathematically Processed UV Spectroscopic Method for Quantification of Chlorthalidone and Azelnidipine in Bulk and Formulation: Evaluation of Greenness and Whiteness" Hindawi Journal of Spectroscopy, 2022; Article ID 4965138, 13.
11. Rele R. V, Patil S.P. UV Spectrophotometric method for estimation of Azelnidipine from bulk drug and pharmaceutical formulation. Asian J. Research Chem., 2010; 3(4): 1077-1079.
12. Manish Kumar*, Umesh Chandra, ArunGarg, Pankaj Gupta A Stability Indicating RP-HPLC Method Validation for Simultaneous Estimation of Azelnidipine and Telmisartan in a Fixed-dose Combination International Journal of Pharmaceutical Sciences and Drug Research, 2021; 13(3): 288-294.