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Method Development and Validation of Azelnidipine in Bulk and Dosage Form by Visible Spectroscopy in NED Reagent

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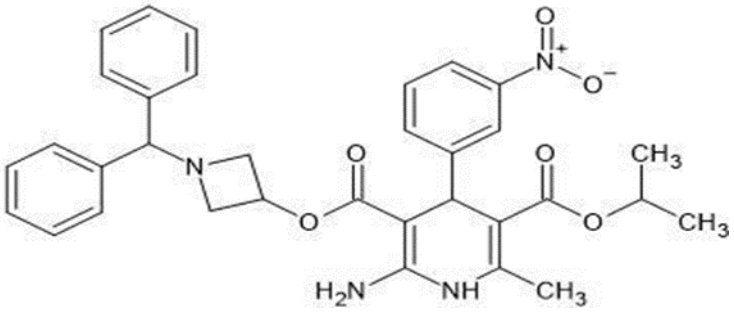
ABSTRACT
A simple, rapid, accurate, precise and specific Visible spectroscopic method has been developed using ethanol, distilled water as a solvent for the determination of AZEL in bulk and pharmaceutical dosage form. A colorimetric for the analysis of AZEL has been developed based on the formation of pink color complex, when the drug is treated with NED reagent after diazotization of aromatic amino group. The absorption maxima at 554nm, the linear in the range of 0.04-0.21 µg/ml and exhibit a good correlation coefficient (0.997).

Key words: visible spectroscopy, Azelnidipine, NED Reagent.

INTRODUCTION

Azelnidipine, chemically 3-(1-Benzhydrylazetidin-3-yl) 5-isopropyl 2-amino-6 methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (C33H34N4O6) is yellow powder, slightly soluble in methanol and ethanol sparingly soluble in water[1]. Azelnidipine is a new and long acting dihydropyridine derivative with antagonistic activity. Azelnidipine under the class of calcium channel blockers in third generation. It is used to treatment of high blood pressure (hypertension) and to prevent angina. Azelnidipine inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. Ca2+ channels are classified into various categories, including L-type, T-type, N-type, P/Q-type, and R-type Ca2+ channels. The L-type Ca2+ channels 6. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure. Structure have two methyl group located at the 2-and 6-positioin of the di-hydropyridine ring, one methyl group at the 2-position is structured by an amino group in the azelnidipine molecule. Azelnidipine is used in the treatment of patient with hypertension and recommended dosage form is 8-16 mg orally once daily.



azelnidipine

Review of the literature show that few UV[2,5,6,8,10], NMR[7], UPLC[9],RP HPLC[3,4] Mass spectroscopy[11] have been reported for the determination of AZEL. There is an increase in number of publications describing AZEL with other combination. The present study aim to develop accurate, precise, simple, rapid method for the estimation of AZEL by colorimetric method using NED reagent. however, no visible work done yet.

MATERIAL AND METHOD'S

List of instruments:

- A SHIMADZU model PHARMASPEC -180 UV -Visible double beam spectrometer with 1cm quartz cell was for recording spectra an absorbance measurement.
- Shimadzu electronic model AY 220 Ultra sonicator - Enertech
- Cuvettes -quartz cells.

Drug sample:

Pure sample - azelnidipine was gifted by Rubicon pharma Ltd in Mumbai and alembic pharma Ltd in Gujarat

Formulation - azelnidipine were produced from market uniaz 8

Chemical reagents:

- NED
- $NaNO_2$(Sodium Nitrite)
- HCl (hydrochloric acid)
- $[NH_4]SO_3NH_2$ (Ammonium sulphamate)
- Ethanol
- Methanol

Methods

Selection of wavelength

Azelnidipine standard solution prepared and note the maximum absorbance 554nm. Then azelnidipine solution where suitably prepared and diazotized and coupled with NED. The solution scanned in the range of 400-800nm.The overlay UV-spectra of azelnidipine were shown in fig.no 1. For the estimation of wavelength where selected ,554nm for azelnidipine.

Preparation of stock solution

20 mg azelnidipine was weighed and transfer to 50ml standard flask, dissolved in ethanol and finally made up to 50ml ethanol to give standard solution of 400 (µg/ml) of azelnidipine.

Optimized method

Aliquot of the standard drug solution was pipetted out in to separate 10ml standard flask containing mixture of 1ml of 5N HCL and 0.3% sodium nitrate for preparing five calibration standard solutions containing 0.04, 0.08, 0.12, 0.16 ,0.21 µg/ml of azelnidipine. Mix well and 1.5ml of 0.1% ammonium sulphamate was added. 1ml of 0.1% NED reagent was added and made up to volume with distilled water, stand for 3minute. Absorbance was measured against blank at 554nm for azelnidipine. Overlay spectra of azelnidipine are shown in fig no.2. Calibration graph were constructed by plotting the absorbance against the concentration of the drug as shown in fig no 3.

METHOD VALIDATION

Linearity and range

Five concentrations of the standard azelnidipine 0.04,0.08,0.12,0.16,0.21µg/ml were prepared and regression coefficients were found out.

Accuracy

The accuracy of the method was determined using recovery analysis. A known quantity of the pure drug was added to the pre- analysed sample formulation at 80%, 100%, and 120% levels. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation was calculated.

Precision

From Formulation, pure drug solutions (azelnidipine) at a particular concentration level 4 µg/ml of azelnidipine were prepared and analysed in three replicates during the same day (intra- day) and on three consecutive days (inter-day). And the percentage relative standard deviation (%RSD) was also calculated.

Robustness

Robustness of the method was estimated by introducing change in the solvent system from ethanol to methanol for dilution.

Ruggedness

Ruggedness was determined by performing analysis of the formulation following the recommended procedures by three different analysts.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the intercept standard deviation and the curve slope.

LOD = 3.3 x S.D/slope

LOQ = 10 x S.D/ slope

Where,

SD is standard deviation

S is slope

RESULT AND DISCUSSIONS:

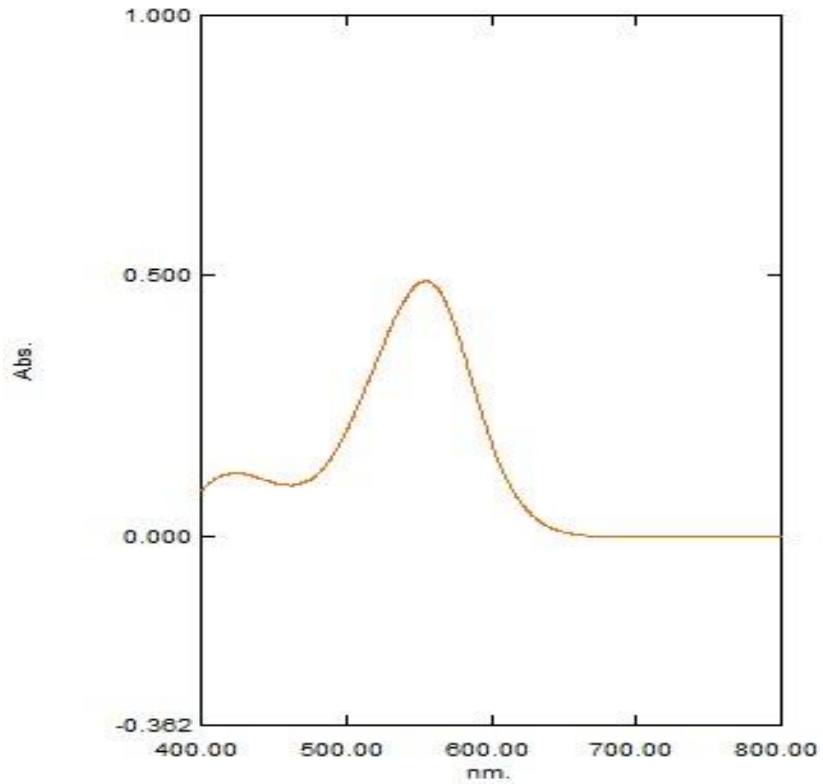


Fig no:1visible spectrum of azelnidipine showing at λ_{max} 554 nm

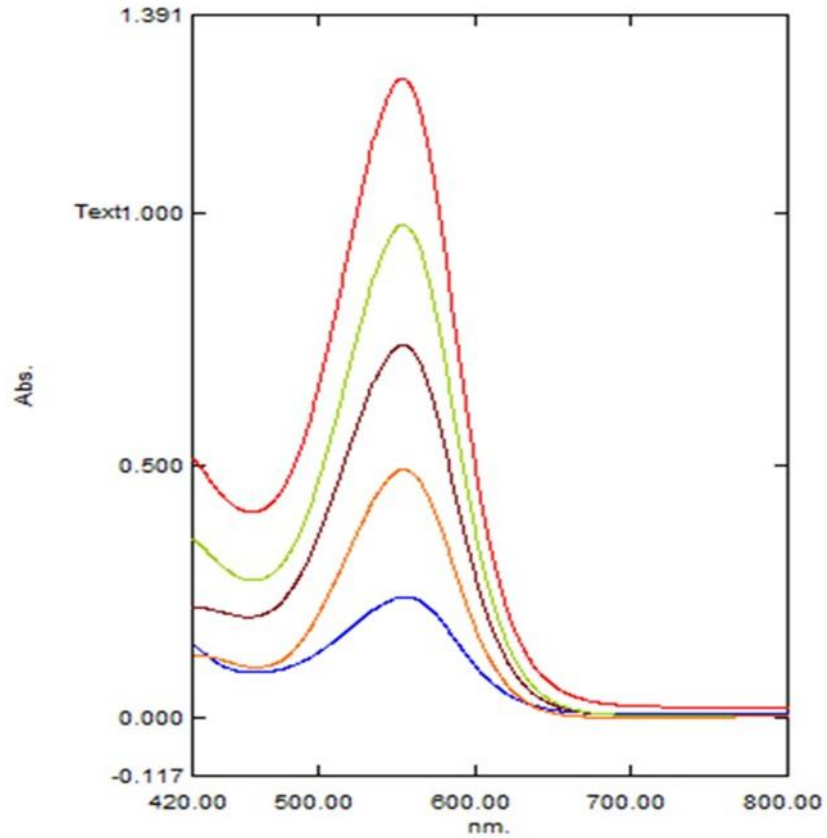


Fig no:2 overlay spectrum of azelnidipine

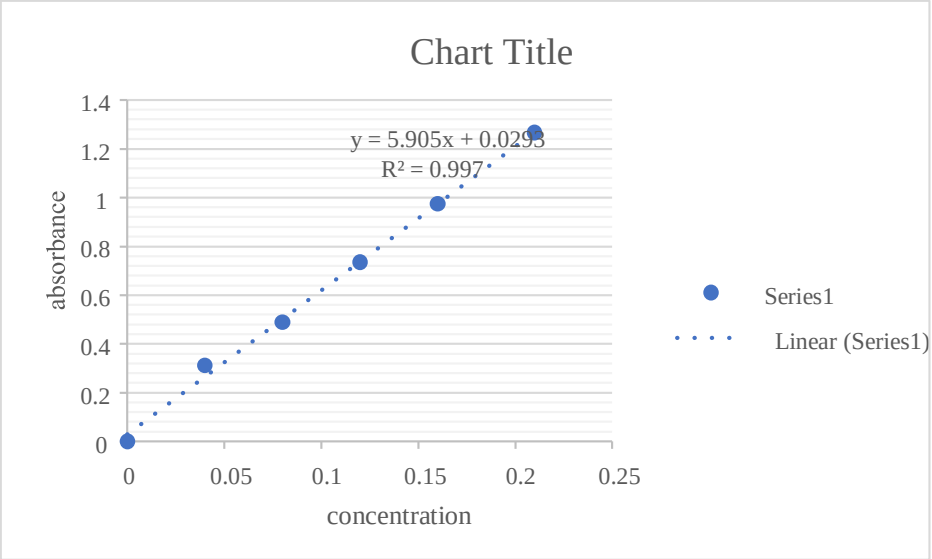


Fig no:3 calibration curve of azelnidipine at 554nm

Drug	Theoretical% target level	*Amount recovered (g)	%recovery	%RSD
Azelnidipine (Label claim 8mg)	80 100 120	0.0075 0.0079 0.0083	95.17% 98.89% 103.14%	0.54

Table:1Result of marketed formulation of azelnidipine

Sample	Drug	Test Conc. (µg/ml)	*Estimated Amount (g)	%Purity	%RSD
UNAIAZ 8	AZELNIDIPINE (Label claim 8mg)	0.12	0.0078 0.0077 0.0078	98.21% 97.00% 98.53%	0.42

Table :2 Result of accuracy of Azelnidipine

Table: 4 Result of robustness of azelnidipine

Drug	Day/Hour	Amount Taken(µg/ml)	Intra Day		Inter Day	
			*%content	%RSD	*%content	%RSD
Azelnidipine	1	0.12	99.25	0.819	98.12	1.099
	2		99.70		100.2	
	3		100.84		99.78	

Table:5 Result of robustness of azelnidipine

Drug	Parameter altered	Amount taken (µg/ml)	*Amount recovered (g)	%content	%RSD
azelnidipine	Solvent system(methanol)	0.12	0.0085	107.07	0.86

Table: 6 Result of ruggedness of azelnidipine

Conc. (µg/ml)	Absorbance
0.04	0.312
0.08	0.490
0.12	0.735
0.16	0.975
0.21	1.266

Drug	Analyst	Amount taken (µg/ml)	Amount found (g)	%content	%RSD
Azelnidipine	ANALYST 1	0.12	0.0079	99.37	0.79
	ANALYST 2		0.0080	100.66	
	ANALYST 3		0.0081	102.11	

Table: 7 Result of LOD AND LOQ

Drug	LOD(µg/ml)	LOQ(µg/ml)
Azelnidipine	0.015	0.047

ANALYTICAL DATA

Parameter	Azelnidipine
Detection wavelength	554nm
Beer's law limit	0.04-0.21(µg/ml)
Regression equation	Y=5.905x+0.028
Correlation co-efficient (r^2)	0.997
Slope	5.905
intercept	0.028
LOD(µg/ml)	0.015
LOQ(µg/ml)	0.047

SUMMARY AND CONCLUSIONS

Conclusively, the visible spectroscopic method described in this paper is simple, rapid, accurate, precise, specific and straight forward to perform. the sample recovery from all formulation were good agreement with their respective label claim moreover, this method economical and time-consuming method. It could be precisely quantified and calibration curve show linear coefficient was 0.997. The low standard deviation and good percentage recovery indicate the reproducibility and accuracy of the method.

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REFERRNCE

1.Imdad Husen Mukeri, Amit Kumar Kushwaha; Analytical Method Development and Validation Of Azelnidipine By UV-Visible Spectroscopy; World Journal Of Pharmaceutical Research, Vol.10 No.10 (2021) 858-872.

2.Dinesh P. kawade, Mayur A. Ikhankar; UV spectroscopic method for the estimation of azelnidipine; World Journal of Pharmaceutical Research, Vol.11 No.2(2022) 974-982.

3.Vangallu spandana ,Siddartha; Analytical Method Development and Validation of Azelnidipine and telmisartan by RP HPLC;Journal of Pharmaceutics and Nanotechnology, Vol 10 No.2(2022) 2347-7849

4.D. Prabhakar, J. Sreekanth; Method Development and Validation of Azelnidipine By Rp-Hplc; International Journal Of Chem Tech Research, Vol.10 No.10 (2017) 418-423.

5.Sneha Ubale, M.S Kalshetti; UV Spectrophotometric Method Development and Validation of Azelnidipine in Bulk and Dosage Forms; International Journal of Creative Research Thoughts (Ijcrt) Vol.9. No.7 (2021) 608-611.

6.Paul Richardsm, Madankumar; Method Development and Validation of Azelnidipine Drug by Ultraviolet Visible Double Beam Spectrophotometer; Journal of Pharmaceutical Sciences, Vol. 13 No.1(2022) 18-22.

7.Nirma Chavda, Suresh Kumar; A Review Article on Analytical Method Development for The Combination of Azelnidipine and Telmisartan; Asian Journal of Pharmaceutical Analysis, Vol 11. No 3 (2021) 227-234.

8.Kunti D Raskapur, Mrunali M Patel; UV Spectrophotometric method Development and Validation for The Determination of Azelnidipine in Pharmaceutical Dosage Forms; International Journal of Pharmacy and Pharmaceutical Sciences, Vol.4 No.1 (2012) 238-240.

9.Kunala Anush, G. Sowjanya; A New Rp-Uplc Method Developed for The Simultaneous Determination of Telmisartan and Azelnidipine in Dosage Forms, International Journal of Pharmaceutical Sciences and Research, Vol 14. No 5(2022) 4963-4972.

10.Rele R.V, Patil S.P;Ultra Violet Spectrophotometric Method for Estimation of Azelnidipine From Bulk Drug And Pharmaceutical Formulations; Asian Journal Of Research In Chemistry, Vol.3 No.4 (2010) 1077-1079.

11. kiyosi Kawabata yoko urasaka; simultaneous determination of azelnidipine and two metabolite in human plasma using liquid chromatography-tandem mass spectrometry; journal of chromatography B, Vol.844 No.1(2006)45-52