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ADVANCED COLOURIMETRIC METHOD DEVELOPMENT FOR ESTIMATION OF AZELNIDIPINE USING A SMARTPHONE APPLICATION

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ABSTRACT

In the proposed research paper a simple and accurate colorimetric method has been developed for the estimation of AZELNIDIPINE in bulk. The proposed method utilizes a smartphone camera and an application instead of the conventional UV spectrophotometer for the measurement of color intensity of a solution under consideration. This colorimetric method was found to be linear in the range of 2µg/ml to 10µg/ml concentration with an r2 value of 0.9993. The limit of detection (LOD) and the limit of quantification (LOQ) was found to be 0.024 µg/ml and 0.068 µg/ml respectively.

KEYWORDS: AZL, Method development, Colorimetric method, LOD, LOQ.

INTRODUCTION

Azelnidipine (AZEL) is chemically (±)-3-(1-diphenylmethylazetidin-3-yl) 5-isopropy12-amino-1,4-dihydro-6 methyl-4-(3-nitrophenyl) -3,5- pyridine dicarboxylate. It is a dihydropyridine (DHP) type of calcium channel blocker (CCB) used for the treatment of hypertension. AZEL has two enantiomers due to an asymmetric carbon at the 4-position of the DHP ring. The pharmacological action of AZEL resides in the (R)-enantiomer. This is in marked contrast to other CCBs in which the (S)-enantiomer is responsible for the biological activity 1. The peculiar three-dimensional structure of the active enantiomer of AZEL may be related to its unique pharmacological features that are not shared by other DHPs such as long-lasting reduction in blood pressure, decreased heart rate and anti-atherosclerosis effect. AZEL also shows diuretic effect by increasing urine volume and thus reduction in retention of ions.

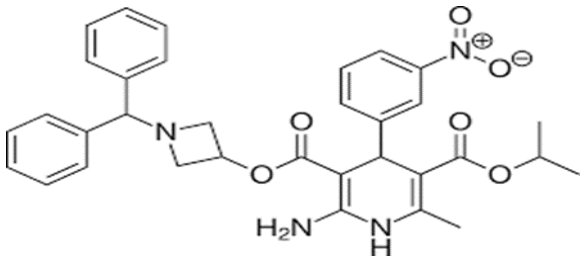

Figure 1: Chemical Structure of Azelnidipine.

Smartphone-based colorimetry has been gaining relevance because of the widespread advancement of devices with increasing computational power, their relatively low cost and portable designs with user-friendly interfaces, and their compatibility with data acquisition. Various methods has been reported for the estimation of drugs by using smart phone application in which mostly the RGB (Red, Blue and Green) principle has been used. In this study, the mobile phone application, called PhotoMetrix, which employs the techniques of simple linear correlation for univariate analysis and principal components analysis (PCA) for multivariate exploratory analysis was used. This PhotoMetrix application is available free in Google Play Store. The method was based on the detection of color intensities and the evaluation of relationship between measured color and concentration of sample.

MATERIALS AND METHODS

Apparatus and Software

Shimadzu UV-1700 double beam spectrophotometer connected to a computer with Shimadzu UV-Probe 2.10 software installed was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 400-800 nm. The samples were weighed on an electronic balance (A×120) by Shimadzu. Smart phone having application Photo Metrix was used to take images.

Chemicals and Reagents

Azelnidipine (API) was purchased from purechem LTD. Ankleshwar.

Preparation of Ethanolic Ninhydrin solution (3% W/V)

2gm of Ninhydrin was dissolved in 95 volume of methanol and 5 volumes of Glacial acetic acid.

Ammonium acetate buffer (pH 4.9)

10mM of ammonium acetate buffer was used and for pH adjustment acetic acid is used.(All chemicals used in the present study were of analytical grade).

Preparation of standard stock solution

10 mg of Azelnidipine was weighed accurately and transferred into separate 100 ml volumetric flask. This will give the conc. of 100 µg/ml of Azelnidipine.

Preparation of working solutions for calibration graph

From standard stock solution 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1 ml was taken out to the 10 ml volumetric flask to get the concentration range between 2-10 µg/ml. Add 2 ml of freshly prepared NINHYDRIN reagent And 1ml of buffer solution to all the flask. All solutions were heated on water bath for 25 mins. After heating time solutions were cooled down and volume was made up to the mark using methanol.

Assay of the formulation 20 tablets of formulation (Azedax-8) containing 8 mg of Azelnidipine were weighed accurately. The average weight of tablets was founded and tablets were powdered. The tablet powder equivalents to 8 mg of Azelnidipine was weighed and transferred into 100 ml volumetric flask and volume is made Upto the mark using distilled water to get 800 µg/ml solution. The content was filtered through the Whatman filter paper to get clear solution. From the above solution 0.5 ml was withdrawn to the 10 ml volumetric flask to get 8 µg/ml concentration. Add 2 ml of reagent, 1 ml of buffer and heat the solution in water bath for 25 mins. Final volume was made up to the mark using distilled water.

Method development

UV-Vis Spectroscopy Prepared working standard solutions in the range of 2-10 µg/ml were scanned between 400- 800 nm in the UV-Spectrophotometer by using Ninhydrin reagent as a blank. At the 574 nm maximum absorbance was observed and it was selected as the detection wavelength. Calibration graph was plotted for the concentration range of 2-10 µg/ml and overlay spectra are mention below.

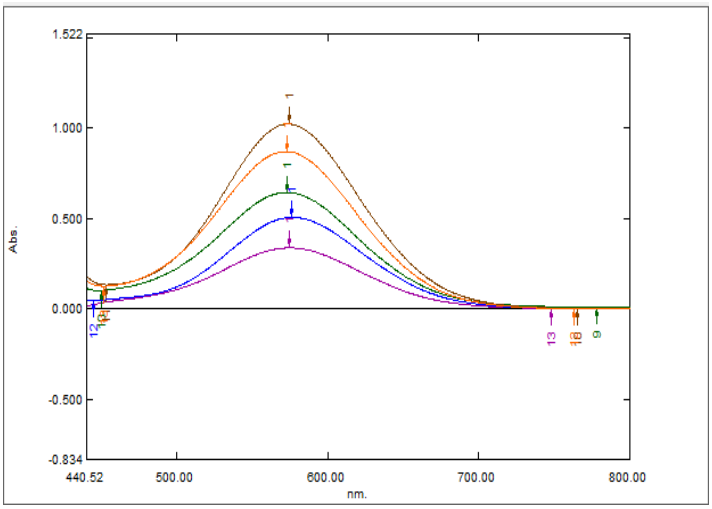

Figure 2: Overlay Spectra of AZL at 574 nm.

Experimental optimization

1. Optimization for concentration of ninhydrin reagent

Effect of change in concentration of reagent in the range of 1% to 5% was performed by keeping other parameters constant. Maximum absorbance was seen when the Concentration is 3 % for Ninhydrin reagent so the concentration of reagent was chosen to be 3 %.

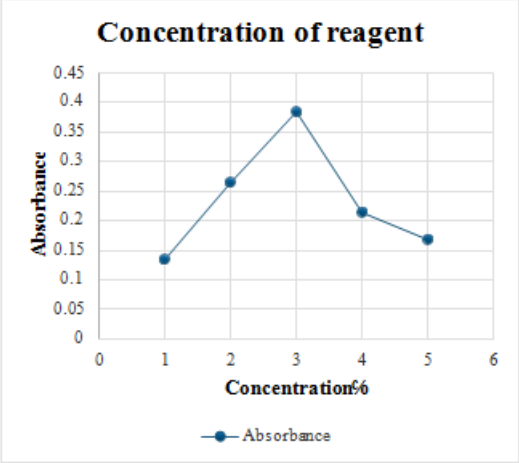

Figure 3: Optimization of concentration.

2. Optimization of reagent volume

The Effect of reagent volume on the drug reagent complex was carried out in the range of 1-3 ml. For the color intensity and absorbance in the UV spectrophotometer optimum concentration was found to be 2 ml of reagent for the method.

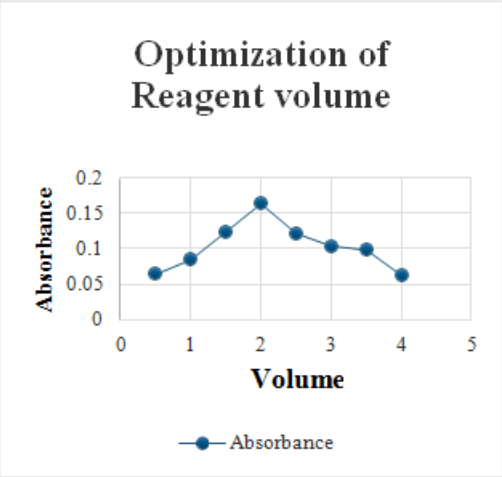

Figure 4: Optimization of reagent volume.

3. Optimization of heating time

The Effect of heating time on complex formation was examined. The reaction between the drug and reagent was occurred between 5-30 mins heating time. Slight increase in colour intensity was observed after heating for 25 mins.

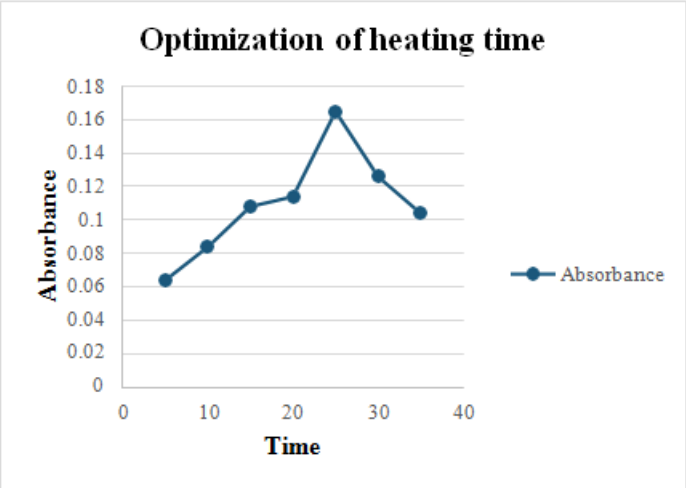

Figure 4: Optimization of heating time.

4. Optimization of temperature

To 1 ml stock solution of Azelnidipine, 2 ml of ninhydrin solution (3% w/v) was added. The reaction mixtures were heated for 10 min at 50-100°C. The colored product was diluted up to 10 ml with methanol and the absorbance was measured against a reagent blank at 574 nm. The results showed that the highest absorbance was obtained at 70±2°C. The developed color was stable for 48 h.

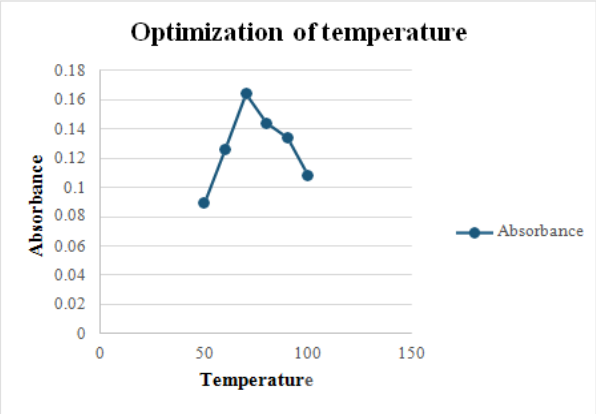

Figure 5: Optimization of temperature.

- **Optimized conditions for the colorimetric estimation of Azelnidipine**

Parameters	Optimized value
%Ninhydrin	3%
Volume of Ninhydrin	2ml
Heating time	25min
Temperature	70^0C

Reaction mechanism

Reaction of Ninhydrin with amines, alpha amino acids, peptides, and proteins yields an aldehyde with one carbon atom less than the alpha-amino acid; and carbon dioxide in stoichiometric amounts and varying amounts of ammonia, hydronation and a chromophoric compound known as Ruhlmann's Purple (2-(3-hydroxy-1-oxo- 1H-inden-2-ylimino)-2Hindene-1,3-dione). This pigment serves as the basis of detection and quantitative estimation of alpha-amino acids. Mechanism proposed (Figure-6) for the reaction involves removal of a water molecule from ninhydrin hydrate 1 to generate 1,2,3-indantrione 2 in the first step, which then, forms a Schiff's base with the amino group of pregabalin resulting in the ketamine 3. Removal of the aldehyde RCHO generates an intermediate amine 4 (2-aminol,3-indandione). Condensation of this intermediate amine with another molecule of ninhydrin follows to form the expected chromophore 5 (Ruhlmann's Purple).

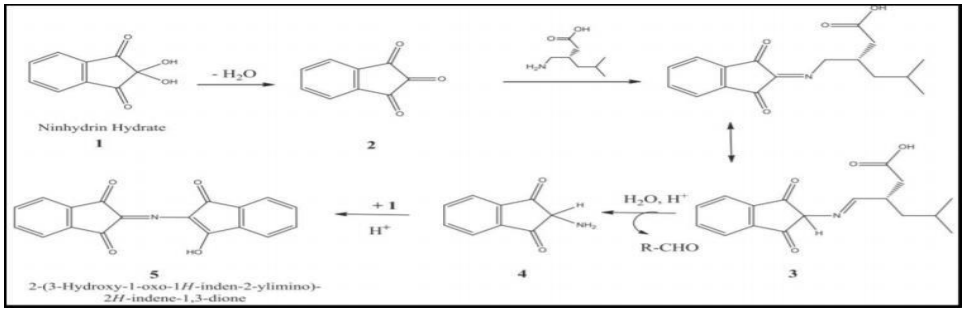

Figure 6: Mechanism of generation of chromophore (Ruhlmann's Purple) by reaction of Azelnidipine with Ninhydrin.

Estimation of azelnidipine using smartphone application

Experimental Setup: A self-designed box was built in the lab to improve accuracy and precision of the measurements. On the upper side of the box, an LED bulb was fitted to provide consistent incident light source. All the inner side wall of the box were covered with white paper to provide full reflection of the light. The front side is made in a manner that it can be open to insert a cuvette inside the box (Figure 9A). In the front side of the box, a small square shaped hole was made exactly in the middle to allow the camera to take photo of the object placed inside the box. Also, A cuvette holder was made from thermocol and was fixed in the middle of the box. The entire experimental setup is illustrated in the figure – 7.

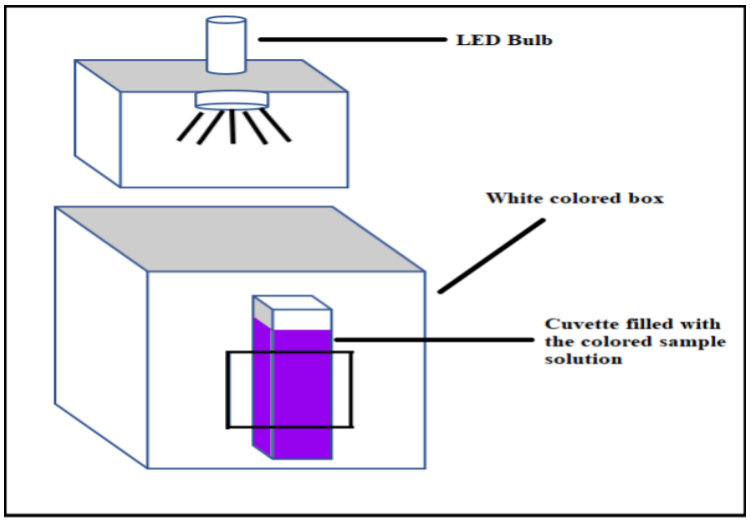

Figure 6: Illustration of experimental setup for image acquisition.

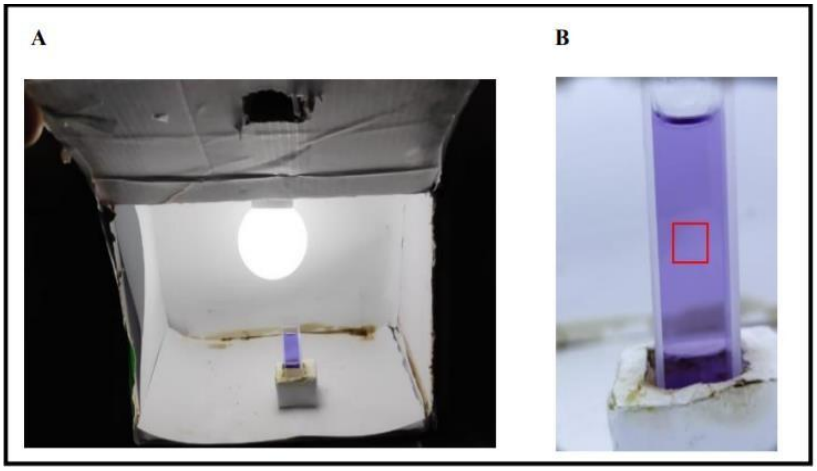

Figure 7: A. Cuvette placed in the arranged setup B. Image captured by mobile phone camera in the arranged setup.

Preparation of calibration graph by smart phone application

Aliquot of standard solution of AZELNIDIPINE corresponding to 2-10 µg/ml was taken into 10 ml volumetric flask. To each flask 2 ml of 3% Ninhydrin solution and 1 ml of buffer solution was added and solution was heated for 25 min at 70^0C. The solution was allowed to cool at room temperature and then volume was made up to 10 ml with methanol. Once the standard solutions were prepared, the images were captured one by one in the Photo Metrix Pro application. The interface of the application as well as the options to be choose in stepwise manner was shown in the figure -8. In application first Univariate Analysis, then in Univariate Analysis Vector RGB was selected. Then once you click on calibration, the app will ask about the number of samples. Here, in number of samples 6 was written (1 blank and 5 standards). Then first the blank solution was filled in the cuvette and was inserted in the box and after writing 0 in the concentration section, image was captured by putting the camera at the middle hole of the box. In same manner one by one the image was captured of all standard solution in an increasing order of concentration. Then the save button was clicked and the calibration graph as well as regression equation was shown by the application itself.

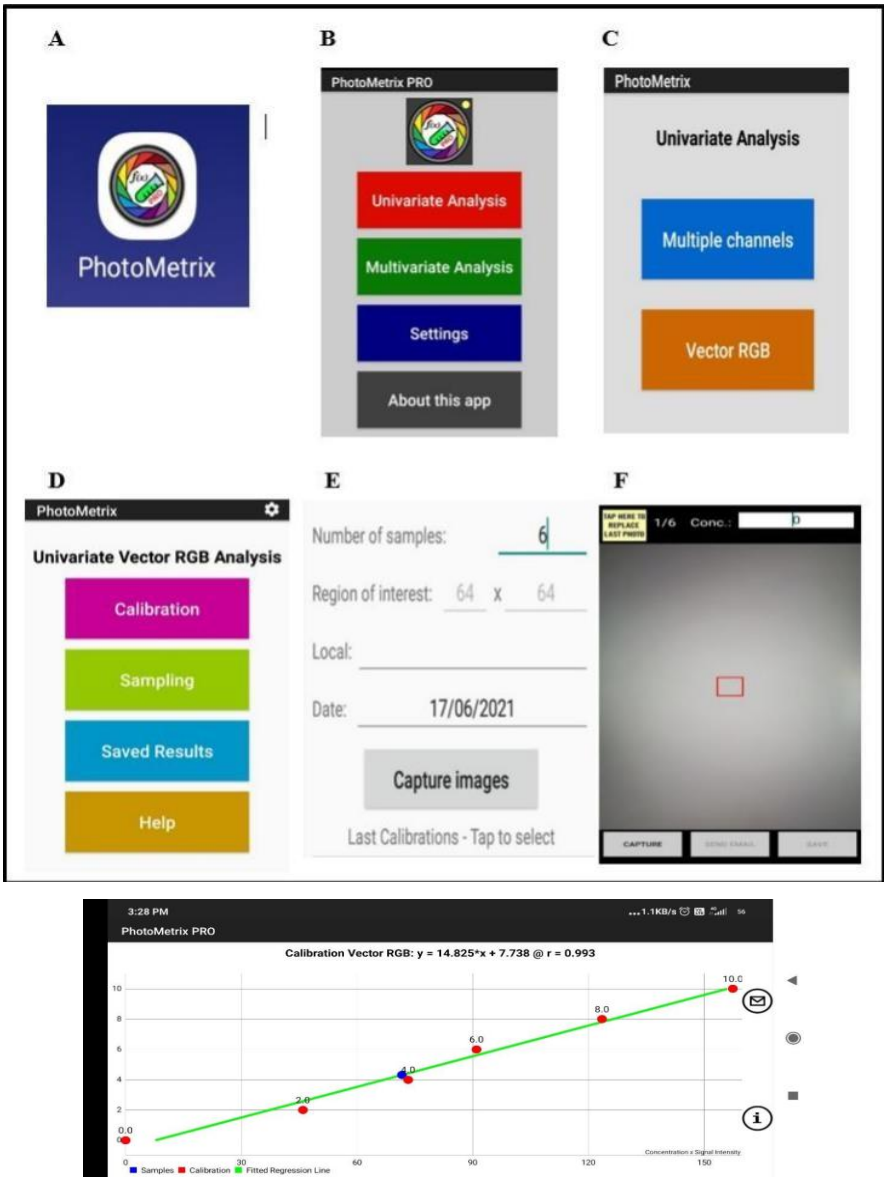

Figure 8: Graphic interface of the Photo Metrix Application and Steps to generate calibration graph in application.

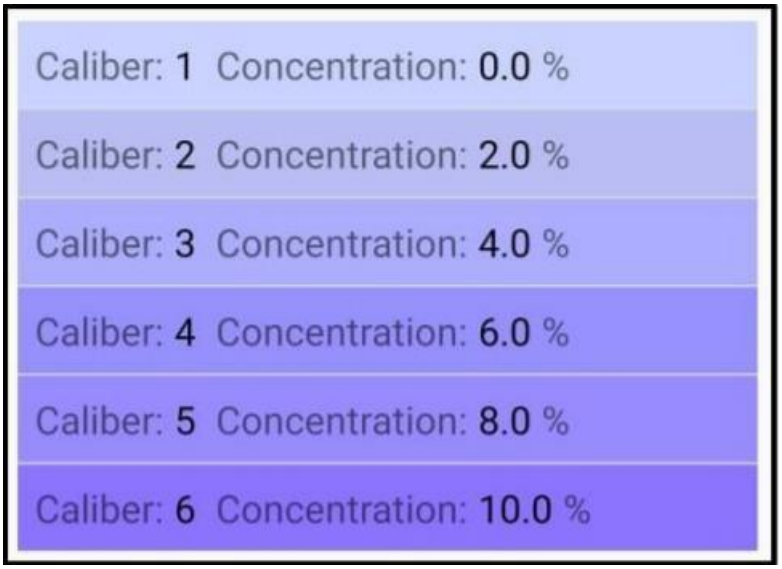

Figure 9: Colour intensities of captured images in Photo Metrix.

Once the regression equation was obtained, the concentration of the sample solution from formulation was estimated. Here, instead of calibration, sampling button was clicked and the image of sample solution prepared for assay was captured in the manner similar to standard solution. Then save button was clicked and the concentration of sample was given by application from the generated calibration graph.

Method validation

Linearity

Azelnidipine was linear with the concentration range of 2-10 µg/ml at 574 nm, by obeying Beer's law (Figure-10). A calibration curve was plotted between concentration Vs absorbance. The plot was found to be linear and shown in the figure below.

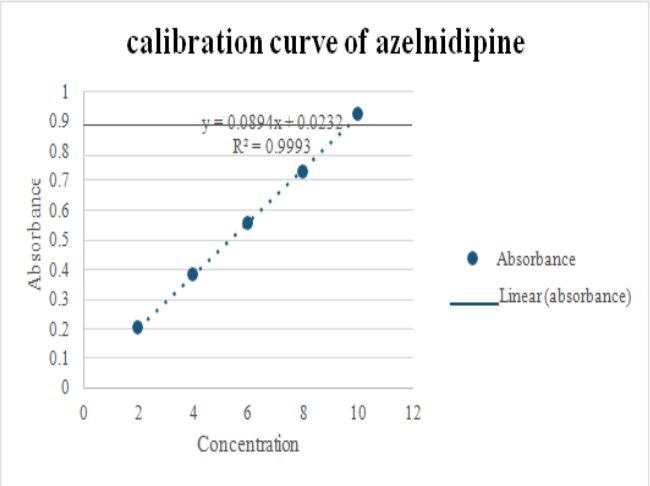

Figure 10: Calibration curve of Azelnidipine (2-10ug/ml).

Assay of formulation

The assay was performed on the marketed formulation azedex-8 with label claim of AZELNIDIPINE 8mg by both the methods. Sample solutions were analyzed and concentration was estimated as a % Recovery from linear regression equation. Assay results were found to be in acceptable range and significant for both the methods. Results of assays are shown in table below.

Table 1: Assay results obtained from both methods.

Method	Amount Lebeled2 (mg)	Amount Estimated (mg)	%Recovery ± SD (n=6)	%RSD
UV method	8	7.94	99.65 ± 0.321	0.80
Photometric	8	7.89	99.2 ± 0.131	0.83

- **Result table**: Statistical data for the regression equation of the proposed method

Parameters	Azelnidipine(uv)	photo matrix
Analytical wavelength	574nm	-
Linearity	2-10ug/ml	2-10ug/ml
Regression equation	0.0894x+0.0232	14.825*x+7.738
Slope	0.0894	14.825
Intercept	0.0232	7.738
Correlation coefficient	0.9993	0.993
Assay	99.65	99.2

CONCLUSION

The Novel and rapid colorimetric detection method of Azelnidipine using smartphone based PhotoMetrix application is developed. The method used simple coloring agent in simple and less time-consuming procedure. The main aim of this study was to make the colorimetric estimation of drug content easier with the help of such smartphone-based applications. Method was also compared with UV method developed with same reagent and procedure and it was found that there was no significant difference in assay results. This novel method can be used as an alternative for analytical science in quantitative drug estimation in pharmaceutical dosage forms.

REFERENCES

1. Kılıç V, Horzum N, Ertugrul Solmaz M. From Sophisticated Analysis to Colorimetric Determination: Smartphone Spectrometers and Colorimetry. Color Detect, 2020; 1–19.
2. Firdaus ML, Aprian A, Meileza N, Hitsmi M, Elvia R, Rahmidar L, et al. Smartphone coupled with a paper-based colorimetric device for sensitive and portable mercury ion sensing. Chemosensors, 2019; 7(2).

3. Coutinho MS, Morais CLM, Neves ACO, Menezes FG, Lima KMG. Colorimetric determination of ascorbic acid based on its interfering effect in the enzymatic analysis of glucose: An approach using smartphone image analysis. J Braz Chem Soc, 2017; 28(12): 2500–5.
4. Moonrungsee N, Pencharee S, Jakmunee J. Colorimetric analyzer based on mobile phone camera for determination of available phosphorus in soil. Talanta [Internet], 2015; 136: 204–9. Available from: http://dx.doi.org/10.1016/j.talanta.2015.01.024
5. Helfer GA, Magnus VS, Böck FC, Teichmann A, Ferrão MF, Da Costa AB. PhotoMetrix: An application for univariate calibration and principal components analysis using colorimetry on mobile devices. J Braz Chem Soc, 2017; 28(2): 328–35.
6. Konnaiyan KR. Smartphone Based 3D Printed Colorimeter for Biomedical Applications.
7. Helfer GA, Magnus VS, Böck FC, Teichmann A, Ferrão MF, Costa AB. PhotoMetrix: an application for univariate calibration and principal components analysis using colorimetry on mobile devices. Journal of the Brazilian Chemical Society, 2017; 28(2): 328.
8. Costa AB, Helfer GA, Barbosa JL, Teixeira ID, Santos RO, Santos RB, Voss M, Schlessner SK, Barin JS. PhotoMetrix UVC: A New Smartphone-Based Device for Digital Image Colorimetric Analysis Using PLS Regression. Journal of the Brazilian Chemical Society, 2021; 1, 32: 675-83.
9. Raskapur KD, PATEL MM, CAPTAIN AD. UV-Spectrophotometric method development and validation for determination of Azelnidipine in pharmaceutical dosage form. Toxicology, 2010; 106: 135-43.
10. Mandale D, Mistry R, Chauhan N. AN ANALYTICAL APPROACH OF AZELNIDIPINE: A REVIEW.
11. K Oizumi, H Nishino, H Koike, T Sada, M Miyamoto, T Kimura. Antihypertensive effects of CS-905, a novel dihydropyridine Ca2+ channel blocker. Jpn J Pharmacol, 1989; 51: 57–64.