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Utilization of Photometrix Mobile Application for Colorimetric Detection of Pantoprazole Sodium by Ammonium Metavanadate in Bulk and Dosage Form

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ABSTRACT
The novel mobile application called PhotoMetrix has been used as a colorimetric analysis tool for quantitative estimation of Pantoprazole in bulk and dosage form and compared with conventional UV-Spectrophotometry. The method was based on the detection of color intensities and the evaluation of relationship between measured color and concentration of sample. Ammonium Metavanadate was used as a colouring agent for the color development. It is orange-red in color but forms green color complex when reacts with Pantoprazole. Different concentrations of drug API were prepared in series with increasing color intensity. All samples were captured through mobile camera and analysed in PhotoMetrix application. This application converts image data into Red, Green and Blue (RGB) histograms and regression models were built within the app. Calibration curve was plotted by RGB scale and concentration of drug standard was found from linear equation. The Method was also developed by UV-Spectrophotometer with the same reagent and procedure. The developed method shows satisfactory results with good linearity in the range of 20-100 µg/ml.

KEYWORDS: Pantoprazole, PhotoMetrix, RGB Scale, Colorimetric method

I. INTRODUCTION

Colorimetry is a technique which involves the quantitative estimation of the colours commonly used in biochemical investigation. Color can be produced by any substance when it reacted with color forming chromogens. The difference in color intensity results in a difference in the absorption of light. The intensity of color is directly proportional to the concentration of the compound being measured. Wavelength between 400 nm to 800 nm forms the visible band of light in the electromagnetic spectrum. A colorimeter/visible spectrophotometer are a device used to test the concentration of a solution by measuring the absorbance of a specific wavelength of light **[1, 2].**

Smartphones have gained interest as analytical devices because they are fully available at a reasonable cost and they allow data acquisition, storage, and processing in the same device. Smartphone based colorimetry uses a mobile camera as a detector [**3**]. There are a number of systems that are demonstrated based on this principle. PhotoMetrix application is one of them. PhotoMetrix was available free in Windows Phone Store and Google Play Store.

This application employs the techniques of simple linear correlation for univariate analysis and principal components analysis (PCA) for multivariate exploratory analysis. The image data are captured by the camera of smartphone and converted into red, green and blue (RGB) histograms **[4**]. The RBG color model is based on a theory of color perception by the human eye, which have different sensitivity peaks situated around red, green and blue. In this app, multivariate analysis (e.g., partial least squares, PLS) could be performed to improve the applicability of colorimetry through RGB color system **[5].**

Pantoprazole is a first-generation proton pump inhibitor (PPI) used for the management of gastroesophageal reflux disease (GERD), for gastric protection to prevent recurrence of stomach ulcers or gastric damage from chronic use of NSAIDs, and for the treatment of pathological hypersecretory conditions including Zollinger-Ellison (ZE) Syndrome **[6].** It can also be found in quadruple regimens for the treatment of H. pylori infections. Chemically it is 6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methanesulfinyl]-1H-1,3-benzodiazole.(**Figure 1**)

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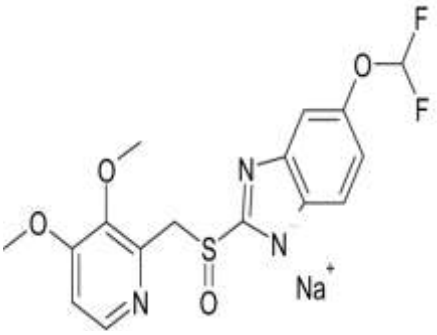

Figure 1 structure of pantoprazole sodium

Various titrimetric methods **[7,8]** , colorimetric methods [**9,10]** ,HPTLC methods **[11**] and HPLC methods **[12,13**] have been reported for estimation of pantoprazole either alone or in combination with other drugs **[14,15,16**]. All these methods have been involved with sophisticated and expensive instruments. Thus the aim of the present work is to develop simple and economical method for quantitative estimation of pantoprazole. This method involves green color reaction between Ammonium Metavanadate and pantoprazole. The image data was captured by smartphone and concentration of drug was analysed through PhotoMetrix smartphone application.

II. MATERIALS AND METHODS

Instruments/ Application

PhotoMetrix smartphone based application was used for smartphone based colorimetric analysis. 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 samples were weighed on an electronic balance (A×120) by Shimadzu.

Chemicals and reagents

5% Ammonium metavanadate solution was prepared by dissolving 5 gm of Ammonium metavanadate in concentrated H_2SO_4.

Preparation of standard stock solution

Accurately weigh and transffer 10 mg of Pantoprazole sodium in 10 ml volumetric flask. Volume was made upto the mark using doubly distilled water to get final concentration of 1000 µg/ml.

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 20-100 µg/ml. Add 2 ml of freshly prepared Ammonium Metavanadate reagent to all the flask. All solutions were heated on waterbath for 40 mins. After heating time solutions were cooled down and volume was made upto the mark using distilled water.

Assay of the formulation

20 tablets of formulation (PANTOSEC) containing 50 mg of Pantoprazole were weighed accurately. The average weight of tablets was founded and tablets were powdered. The tablet powder equivalents to 50 mg of Pantoprazole was weighed and transferred into 50 ml volumetric flask and volume is made upto the mark using distilled water to get 1000 µg/ml solution. The content was filtered through the whatman filter paper to get clear solution.

From the above solution 5 ml was withdrawn to the 10 ml volumetric flask to get 50 µg/ml concentration. Add 2 ml of reagent and heat the solution in waterbath for 40 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 20-100 µg/ml were scanned between 400-800 nm in the UV-Spectrophotometer by using Ammonium Metavanadate reagent as a blank. At the 760 nm maximum absorbance was observed and it was selected as the detection wavelength. Calibration graph was plotted for the concentration range of 20-100 µg/ml. Linear regression equation was generated from calibration graph.

Experimental optimization

Optimization of concentration of Ammonium Metavanadate reagent

Effect of change in concentration of reagent in the range of 1% to 7% were performed by keeping other parameters constant. Maximum absorbance was seen with 5% Ammonium Metavanadate reagent. Then the concentration of reagent was chosen to be 5 %.(**Figure 2)**

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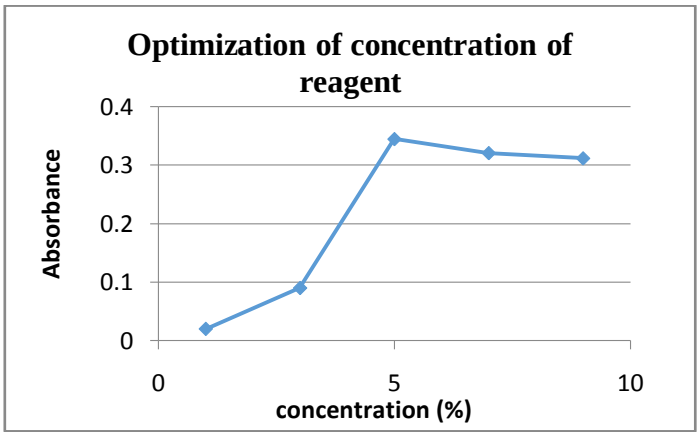

Figure 2 optimization graph for reagent concentration

Optimization of volume of Reagent

The effect of reagent volume on the drug-reagent complex was carried out in the range of 1-3 ml. from the color intensity and the absorbance in the UV spectrophotometer optimum concentration was chosen. 2 ml of reagent was found to be optimum for the method**.(figure 3)**

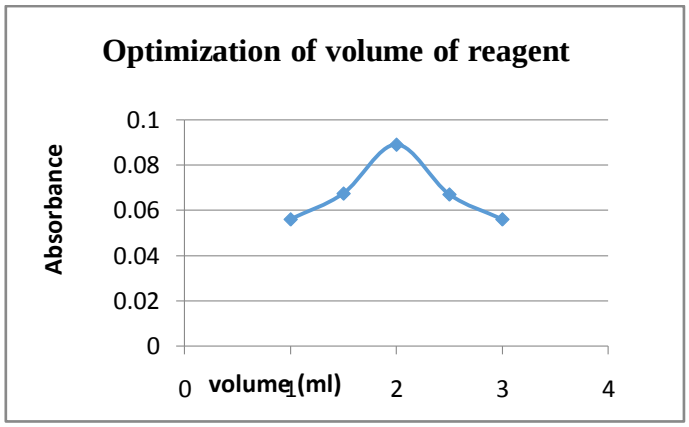

Figure 3 optimization graph for volume of reagent

Optimization of heating time

The effect of heating time on complex formation was examined. The reaction between the drug and reagent was occurred between 25-40 mins heating time. Slight increase in color intensity was observed after heating for 30 mins(**figure 4)**

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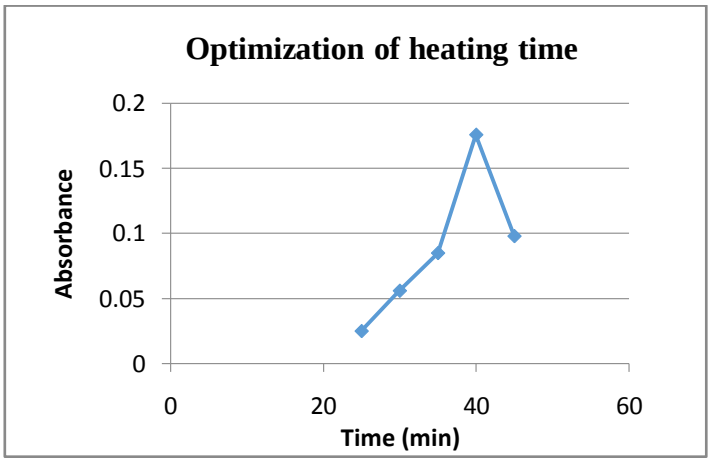

Figure 4 optimization graph for heating time

Possible drug-reagent reation mechanism

Ammonium Metavanadate is the inorganic oxidizing compound. The usual source of vanadium in the +5 oxidation state is ammonium metavanadate, Reaction for oxidation of pantoprazole was done in acidic medium. Heat is given during chemical reaction to prevent re-oxidation of vanadium. Ammonium metavanadate is orange-red in color but it forms green colored complex after reacting with pantoprazole. Probable mechanism is shown in **figure 5**.

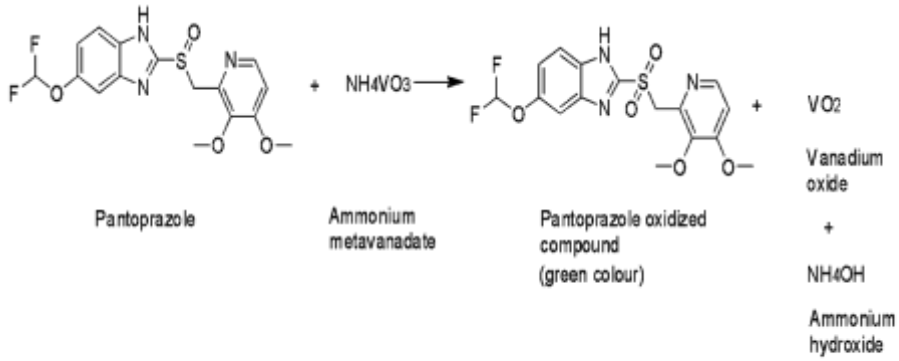

Figure 5 Possible reaction mechanism for color complex

Quantitative analysis using Smartphone based PhotoMetrix Application

The complex solutions were transferred into standard glass cuvettes placed against the white background and 6W LED bulb was connected to control the intrnsity of light emitted **(figure 6)**. The images of colored solutions were captured through android smartphones and PhotoMetrix application was applied to evaluate the red-green-blue intensities (RGB scale) of the recorded images.

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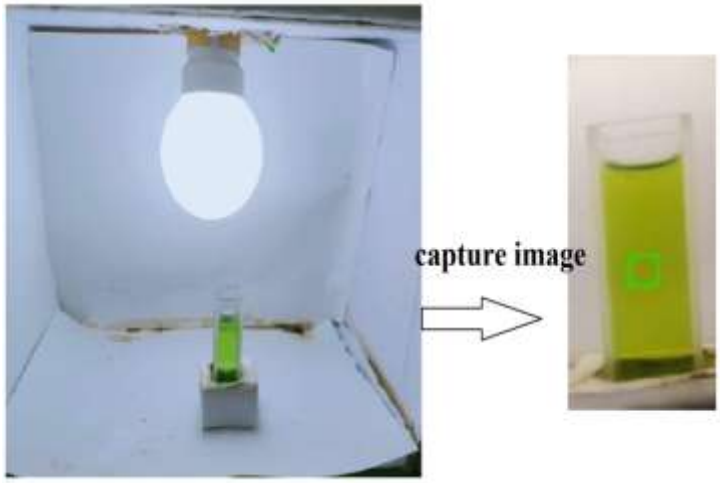

Figure 6 Solutions kept in cuvett placed against white background

Data images were converted to the concentration which was calculated from the relationship of linear equation. The application PhotoMetrix builds and analyzes the color histograms on the RGB scales and converts into calibration graph. This application performs the processing and presentation of results using univariate or multivariate analysis methods. Different models of smartphones were used for analysis of sample solutions for better results. Steps for using this application is depicted in **figure 7.**

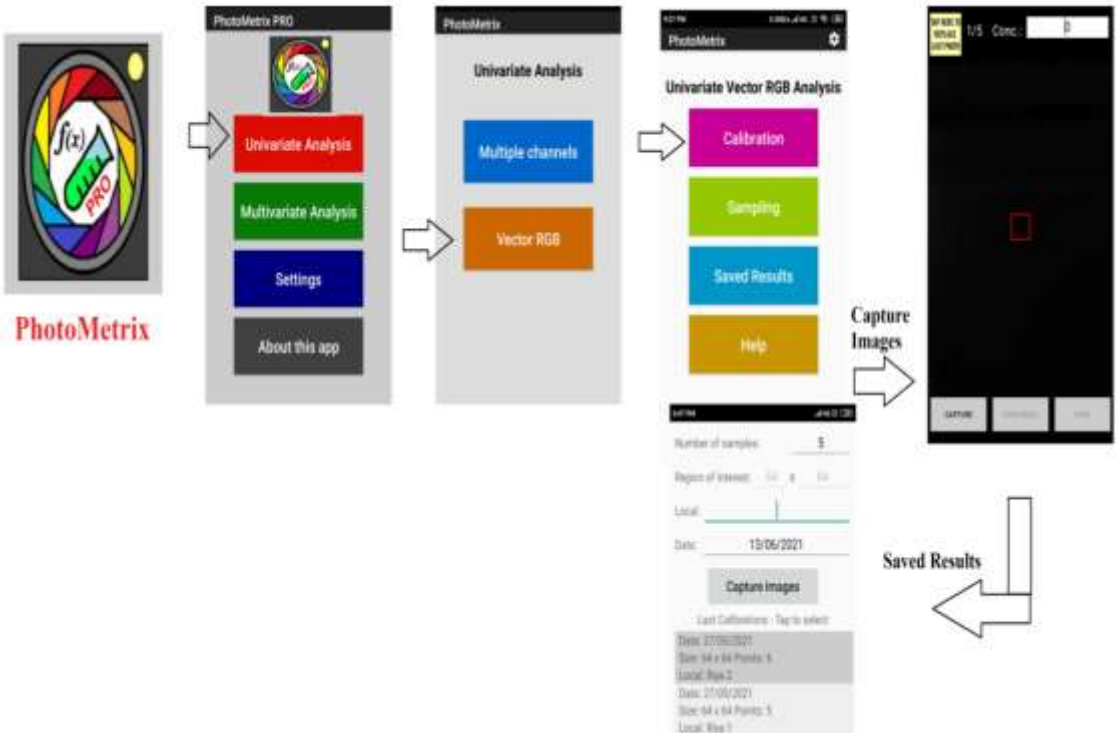

Figure 7 steps for using PhotoMetrix Application

Method Validation

Both the methods were validated in terms of linearity and method ruggedness corresponding to the validation guidelines. Assay of the formulation was also carried out using both methods. A good linearity was founded in the range

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of 20-100 µg/ml under optimized conditions for both methods. Concentration of drug in formuation was estimated by linear regression equation in case of UV spectrophotometry. In photoMetrix application drug concentration was estimated within the application.

III. RESULTS AND DISCUSSION

Linearity and calibration graph

After capturing images through smartphone camera, calibration graph and linear regression equation (**Figure 8**) was generated according to color intensity (**figure 9**) and concentration of the samples in PhotoMetrix app itself. In UV spectroscopy, after measuring the absorbance calibration graph was plotted against the concentration (**figure 10).** The overlay spectra of UV is shown in (**figure 11**)The correlation coefficient (r^2) were found to be 0.996 and 0.998 in PhotoMetrix application and UV-Spectrophotometry respectively. The regression data are shown in **Table 1**.

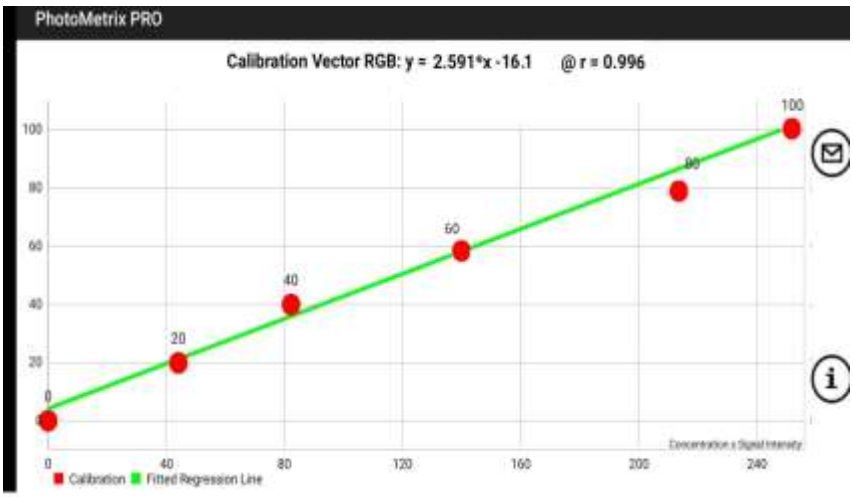

Figure 8 Calibration graph obtained from PhotoMetrix Application

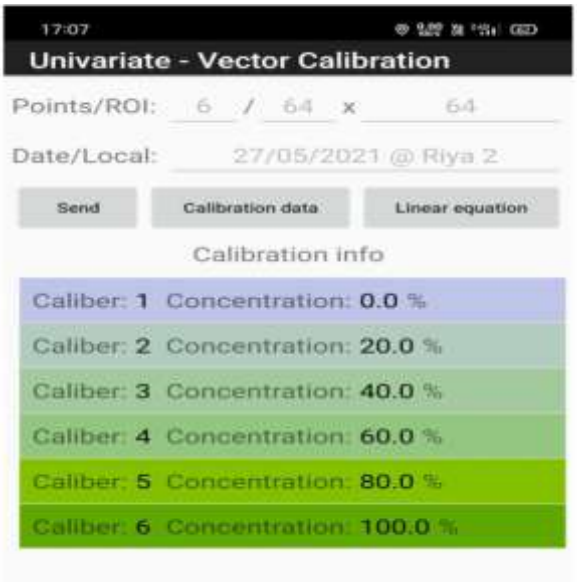

Figure 9 Color intensities of captured images in PhotoMetrix

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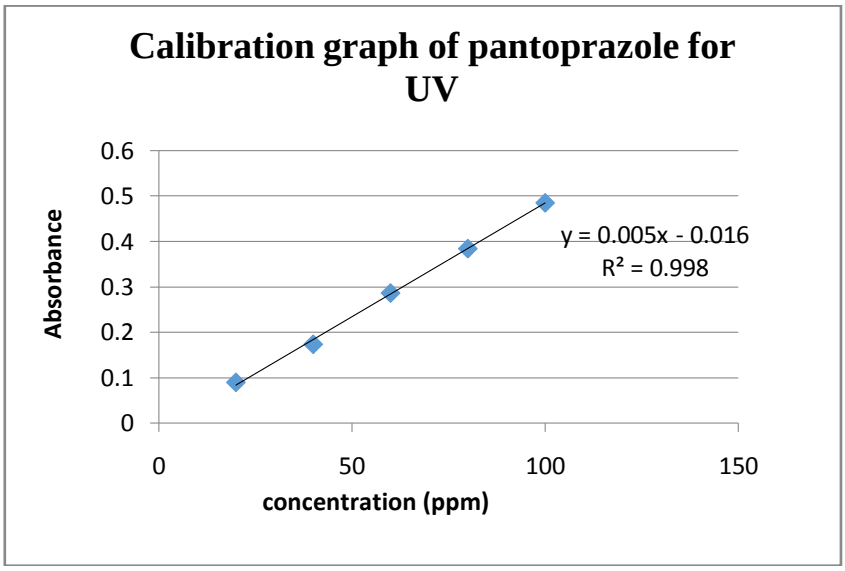

Figure 10 Calibration graph for UV method

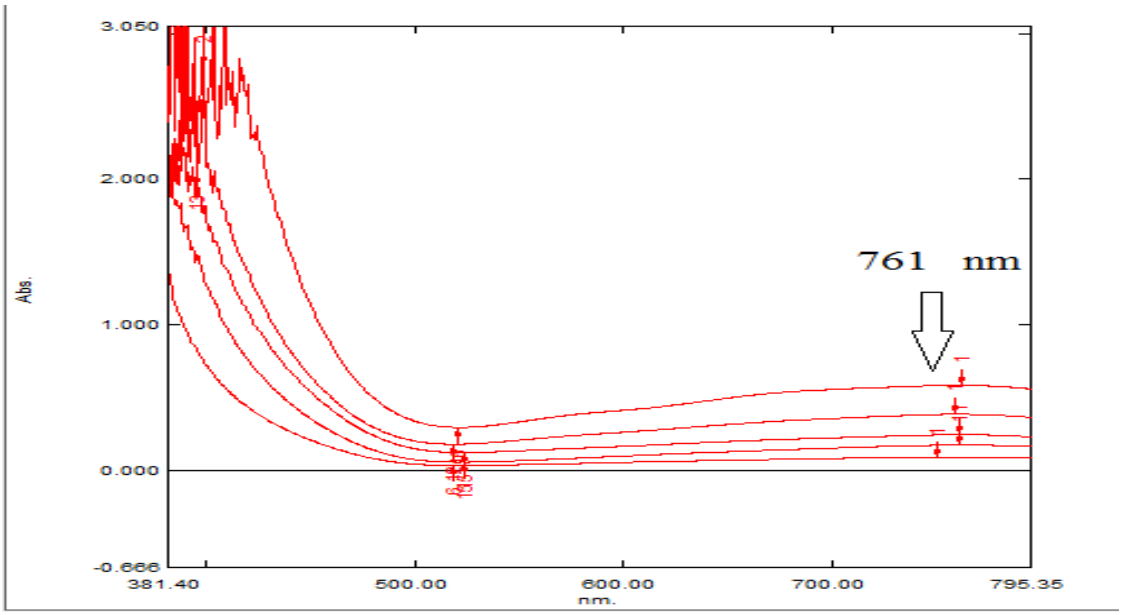

Figure 11 overlay spectra of UV method showing linearity at 761nm

Table 1 Regression equation data for both methods

Parameters	**Photometrix Application**	**UV-Method**
Linearity	20-100 Mg/Ml	20-100 Mg/Ml
Regression Equation	Y = 2.591x – 16.1	Y = 0.005x - 0.016
Slope	2.591	0.005
Intercept	16.1	0.016
Correlation Coefficient (R^2)	0.996	0.998

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Ruggedness of the method

The ruggedness of the developed method was studied in two labs as well as with the use of two different smartphones. The %RSD of both these parameters was found to be less than 2 as shown in the **Table 2**.

Table 2 Method ruggedness results

Parameter	Mean Assay%	SD	%RSD
Lab 1	97.96	0.494	0.503
Lab 2	98.66		
Smartphone 1	98.12	0.565	0.574
Smaetphone 2	98.92		

Assay of formulation

The assay was performed on the marketed formulation PANTOSEC with label claim of pantoprazole 50mg by both the methods. Sample solutions were analysed 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 3.**

TABLE 3 Assay results obtained from both methods

Method	Amount taken (µg/ml)	Amount recoverd	%Recovery ± SD (n=6)	%RSD
PhotoMetrix Application	50	49.19	98.28 ± 0.726	1.47
UV method	50	49.31	98.62 ± 0.799	1.62

Comparison Of Methods

Obtained assay results from PhotoMetrix application and UV method were compared by applying paired t-test (two tail) to the assay results. By applying a t-test to both the methods it was found that t_{stat} values were less than the $t_{critical}$ values and P values were greater than applied alpha value (P>0.05). It denotes that there are no significant difference between the means of the methods. So, PhotoMetrix application can be used as a colorimetric detection of the drugs. The data are shown in **Table 4**.

TABLE 4 Applied paired t-test results

Parameters	PhotoMetrix Application	UV method
Mean	98.28	98.62
Variance	0.527	0.639
Observations	6	6
Hypothesized mean difference	0	
d_f	5	
t_{stat}	-0.72	
P(T<=t) two tail	0.5	
$t_{critical}$ two tail	2.57	

IV. CONCLUSION

The novel and rapid colorimetric detection method of pantoprazole 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 were 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.

REFERENCRS

[1]. Gummadi S, Kommoju M. Colorimetric Approaches To Drug Analysis And Applications–A.
[2]. Beckett A.H, Stenlake J.B, UV-visible Spectrophotometry: Practical

[3]. Konnaiyan KR. Smartphone Based 3D Printed Colorimeter for Biomedical Applications.
[4]. 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 Feb;28(2):328-35.
[5]. 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 Mar 1;32:675-83.
[6]. Pantoprazole sodium – Drug Bank [Available From : https://go.drugbank.com/salts/DBSALT000386]
[7]. Basavaiah K, Rajendraprasad N, Tharpa K, Anilkumar UR, Hiriyanna SG, Vinay KB. Titrimetric and spectrophotometric assay of pantoprazole in pharmaceuticals using permanganate. Journal of the Mexican Chemical Society. 2009 Mar;53(1):34-40.
[8]. Devi OZ, Basavaiah K, Revanasiddappa HD, Vinay KB. Titrimetric and spectrophotometric assay of pantoprazole in pharmaceuticals using cerium (IV) sulphate as oxidimetric agent. Journal of Analytical Chemistry. 2011 May;66(5):490-5.
[9]. KALAICHELV R, Rose MF, Vadivel K, Jayachandran E. Simple extractive colorimetric determination of pantoprazole sodium by aciddye complexation method in solid dosage form. International Journal of Chemistry Research. 2010 Jan 1:6-8.
[10]. MAHAJAN N, JADHAV R. NEW SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PANTOPRAZOLE IN BULK AND PHARMACEUTICAL FORMULATION.
[11]. Patel GH, Prajapati ST, Patel CN. HPTLC Method development and validation for simultaneous determination of cinitapride and Pantoprazole in capsule dosage form. Research Journal of Pharmacy and Technology. 2011 Sep 28;4(9):1428-31.
[12]. Hemalatha PV. Validated Method Development for the Quantification of Cinitapride and Pantoprazole by Spectrophotometry and RP-HPLC in Bulk and Oral Dosge form (Doctoral dissertation, College of Pharmacy, Madras Medical College, Chennai).
[13]. Prasanna Kumar Reddy B, Ramanjaneya Reddy Y, Ramachandran D. Determination of pantoprazole sodium and lansoprazole in individual tablet dosage forms by RP-HPLC using single mobile phase. E-Journal of Chemistry. 2009;6(2):489-94.
[14]. Wahbi AA, Abdel-Razak O, Gazy AA, Mahgoub H, Moneeb MS. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis. 2002 Nov 7;30(4):1133-42.
[15]. Hemalatha PV, Suresh AJ, Niraimathi V. Visible spectrophotometric methods for the estimation of cinitapride and pantoprazole in bulk and oral dosage form. Research Journal of Pharmacy and Technology. 2012 Mar 1;5(3):441.
[16]. Dighade NR, Shende MD, Kasture AV. Simultaneous Estimation of Cinitapride Hydrogen Tartrate (CNT) and Pantoprazole Sodium Sesquihydrate (PNP) in Capsule by Q-analysis UV-Spectrophotometric Method. Asian Journal of Research in Chemistry. 2013 Oct 28;6(10):911-5.