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Research Article

# New Smartphone Based Colorimetric Method Development and Validation of Emtricitabine in Bulk and Tablet Dosage Form

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### Abstract

The novel smartphone based colorimetric application called PhotoMetrix-PRO has been used for quantitative analysis of Emtricitabine in bulk and tablet dosage form and it compared with conventional UV –visible spectrophotometry. Smartphone based colorimetric method and UV method was based on detection of intensity of color with increasing concentration Ammonium metavanadate (inorganic oxidizing agent) which was used for preparation Mandeline reagent they used as coloring agent for colour development. The reagent is orange red colour but when it react with Emtricitabine it convert into green color complex. The developed methods have good linearity 50-250 µg/ml. The color intensity of API increase with increasing concentration. All images were captured through mobile phone and analysed in PhotoMetrix PRO application. This application convert image into Red, Green and Blue (RGB) histogram and regression models were built into PhotoMetrix-PRO Application both the method have correlation coefficient  $R^2 > 0.995$ . The LOD and LOQ for PhotoMetrix PRO 4.77 µg/ml and 14.92 µg/ml and UV-visible spectrophotometry method 4.99 µg/ml and 14.96 µg/ml respectively. The % RSD of Emtricitabine by PhotoMetrix PRO and UV method was 99% (%RSD < 2). Applying statistical tool i.e paired two test on both the methods result indicate that both methods are equally significant.

**Keywords:** Smartphone, PhotoMetrix PRO, UV Spectrophotometry, RGB

## INTRODUCTION:

Color changes recorded with Smartphone based are gaining increasing interest in chemical analysis due to their simplicity of use and easy adaption to portable devices. 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. The smartphone based Colorimetry uses mobile camera as a detector. <sup>1</sup> There is numbers of smartphone based colorimetric application. PhotoMetrix-PRO is one of them. PhotoMetrix PRO 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 <sup>2</sup>. The RGB color model is based on a theory of color perception by the human eye, which has 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. <sup>3</sup>

Colorimetry is a technique which involves the quantitative estimation of Colors frequently used in biochemical investigation. Color can be produced by any substance when it binds with color forming chromogens. The difference in color intensity results in 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 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. Specificity and sensitivity are to be considered in the selection of a reagent for colorimetric analysis. <sup>4</sup>

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults and children. This drug is most widely used in antiviral therapy. It is indicated in combination with other antiretroviral agents to treat HIV infection <sup>5</sup>. Chemically, Emtricitabine is 4-amino-5-fluoro-1- [(2R, 5S)-2-(hydroxymethyl) -1, 3-oxathiolan-5-yl] - 1, 2-dihydropyrimidin-2-one Figure 1

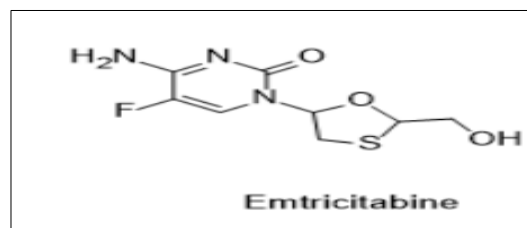


Figure 1: Structure of Emtricitabine

Emtricitabine have various HPLC methods <sup>6,7,8</sup>. UV spectroscopic method <sup>9,10,11</sup>. HPTLC method <sup>12</sup> and colorimetric method <sup>5</sup> have been reported for estimation alone or in combination of other drugs. All this method was done using sophisticated instruments. Thus the aim of present work is develop simple, economical method for estimation of emtricitabine. The method involves ammonium metavanadate as coloring agent which gives green color on reaction with emtricitabine. The data image captured and analysed by PhotoMetrix-PRO application.

## MATERIALS AND METHOD:

**Apparatus and Applications:-**The Emtricitabine API samples were weighed on an electronic balance (A×120) by Shimadzu. A picture of the sample was taken using the camera of a smartphone and uploaded to the mobile (photometrix Pro) Application.

### Chemicals and reagents

5%Ammonium Metavanadate solution was prepared 5gm of Ammonium Metavanadate was dissolved in concentrated H<sub>2</sub>SO<sub>4</sub> and Distilled Water.

### Preparation of standard stock solution

For the preparation of the standard stock solution 50mg of Emtricitabine was accurately weighed and transferred into a previously calibrated 50ml volumetric flask. The final volume was made up to the mark using 1M H<sub>2</sub>SO<sub>4</sub> to obtain the standard stock solution of 1000µg/ml concentration.

### Preparation of Calibration Curve by application of Smartphone:-

From the standard stock solution prepared above, aliquots of 0.5ml, 1ml 1.5ml, 2.0ml and 2.5ml are taken and transferred to a series of previously calibrated 10ml volumetric flasks. 2ml of Ammonium Metavanadate is added, and then heat on water bath at 40 °C for 40 min. The volume is then made up to the mark using cold distilled water to produce concentrations of 50µg/ml to 250µg/ml. Against a white background a clear picture of these solutions was taken using the camera of a smartphone and the picture is uploaded to the RGB application. This application will provide us with the various colour intensities. The colour intensity is then plotted against the concentration to get the calibration curve.

### Assay of tablet formulation:-

20 tablet of tablet formulation (Tafero EM10) containing 200mg of Emtricitabine were weight accurately. The average weight of tablet was calculated and tablet was powdered. The powered equivalent to 200mg of Emtricitabine was weighed and transfer into 50 ml of volumetric flask and volume made up to mark using 1M H<sub>2</sub>SO<sub>4</sub> to get 1000µg/ml of solution. The solution was filtered through 0.45 micro filtered papers to get clear solution. From above filtered solution withdraw 2ml and transferred to 10 ml Volumetric flask to get 200 µg/ml concentrations. Added 2 ml of prepared reagent solution and heated the solution on water bath for 40°C for 40 mins.final volume made up to mark using cold distilled water.

## METHOD DEVELOPMENT:-

### UV Vis –spectroscopy:-

#### Selection of detection wavelength:-

The solution of drug were scanned over the range 400-800nm by using ammonium metavanadate as blank. It was observed that Emtricitabine showed absorbance at

761nm.Using working solution prepared calibration curve, from range 50-250µg/ml and generate linear regression equation.

### Experimental optimization:-

#### Optimization of concentration of reagent:-

Ammonium metavanadate was allowed to react with Emtricitabine to form a green colour with absorption maxima at 761nm, by keeping other parameter constant. The optimization of the research was first established by varying the reagent range from (1%–9%), where we found maximum absorbance at 5%; hence, it was coined as the optimized volume of reagent, as shown in Figure 2

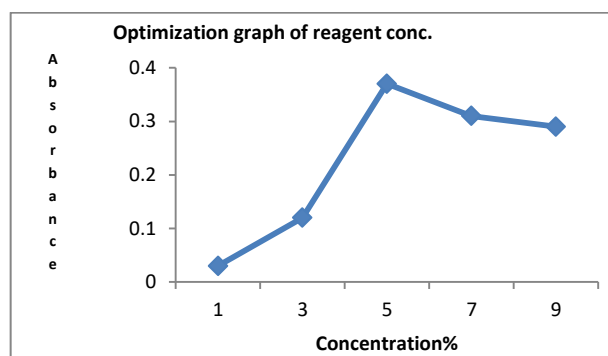


Figure 2: Optimization Graph of Reagent Conc.

#### Optimization of reagent volume:-

The effect of reagent volume was carried out in range from 1-4ml. From green color complex and absorbance maxima optimized volume was selected. 2ml of reagent volume was selected for method Figure.3

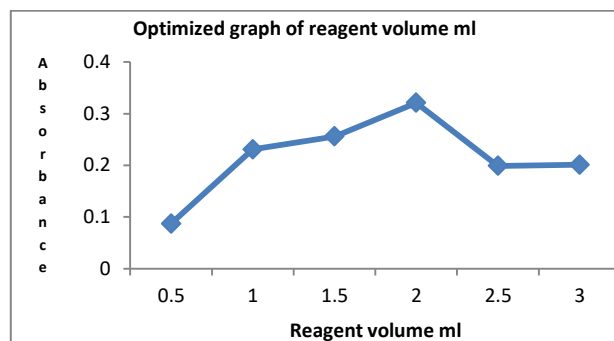


Figure 3: Optimized Of Reagent Volume (ml)

#### Optimization of heating time:-

The effect of heating time carried out in from 20-50 min. From color complex reaction observed between 20- 45 min. Slightly increasing color intensity was observe from 20 mins. Figure 4

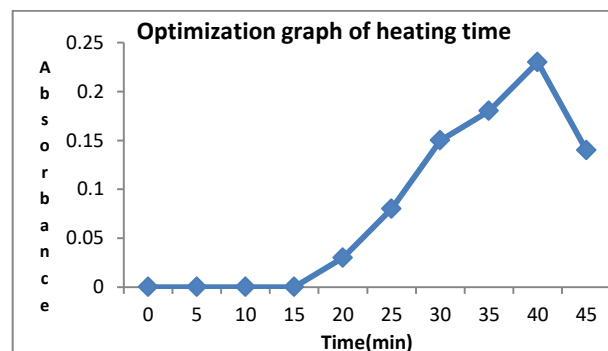
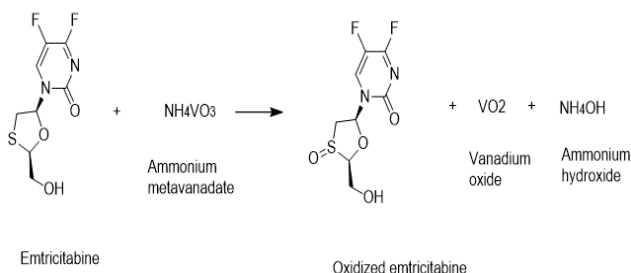


Figure 4: Optimization Graph of Heating Time

### Reaction mechanism:-

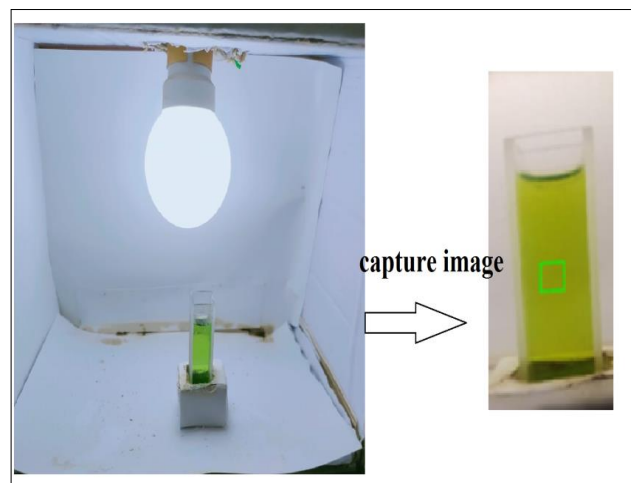
Ammonium metavanadate is inorganic oxidizing agent, which is used to prepare Mandeline reagent. The vanadate has oxidation states in its compound of +5, +4, +3 and +2. The usual source of vanadium in the +5 oxidation state is ammonium metavanadate. The reaction for oxidation of Emtricitabine was done in acidic medium. Heat is given during chemical reaction to prevent reoxidation. Ammonium metavanadate is orange red color complex but when it reacting with Emtricitabine it forms green color complex. Probable reaction mechanism Figure 5



**Figure 5: Probable Reaction Mechanism**

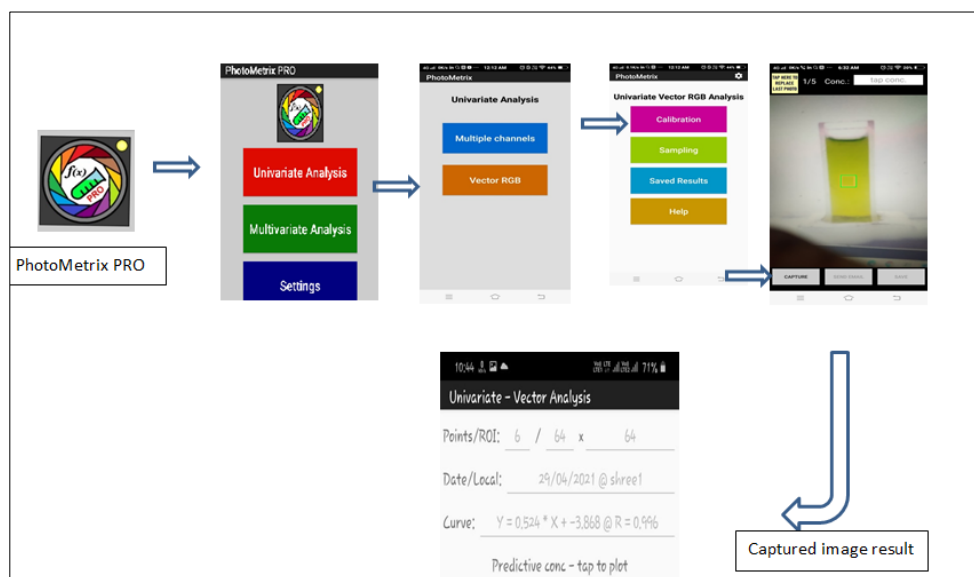
### Analysis using smartphone based photometric application:

The coloured solution was transfer into slandered glass cuvette which was placed in 18cm×18cm of white box and 6W LED (Light Emitting Diode) bulb was connected to control the intensity throughout the experiment Figure 6.



**Figure 6: Experimental set up**

The image of color complex solution was captured using smart phone with photometric application to evaluate the red-green-blue intensities (RGB scale) of recorded image. The image captured from PhotoMetrix PRO was converted to concentration, which was calculated from linear regression equation. The application of PhotoMetrix builds and analyzed color histogram on RGB scales and convert into calibration curve. This application performs the processing and presentation of result using univariate and multivariate analysis. Different models of smartphone were used for best result. Stapes for using PhotoMetrixPRO application Figure7.



**Figure 7: Stapes for Photometrix PRO**

### Method validation:-

The UV -visible spectrophotometry and PhotoMetrix application both method was validate in terms of linearity and ruggedness according to validation guidelines. Assay of the formulation was carried out for both the methods. The good linearity was found in range from 50-250 µg/ml under optimized condition .concentration of tablet formulation was carried out using regression equation for in case of UV-vis spectrophotometry and for photometrix it was estimated within the application.

### RESULT AND DISCUSSION:-

Using uv-vis spectrophotometry measured absorbance the calibration cure was plotted against concentration (figure 8) overly spectrum of Emtricitabine color complex at 761nm (figure 9). By using PhotoMetrix PRO application the image was captured and according to concentration (figure 10). The linear regression equation was observed (figure 11). Correlation coefficient (r<sup>2</sup>) for PhotoMetrix PRO 0.996 and 0.9957 for UV vis spectrophotometry respectively. Regression equation data for both methods Table:-1

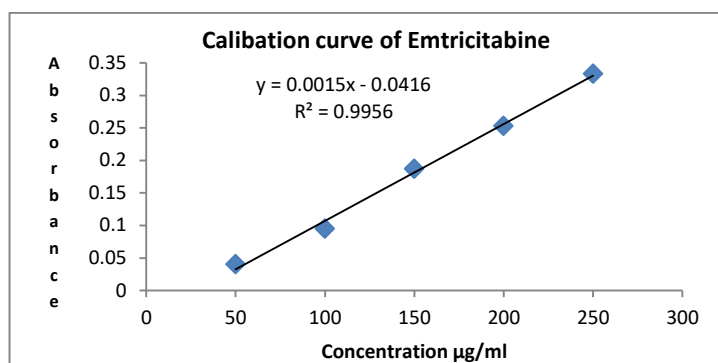


Figure 8: Calibration Curve of Emtricitabine

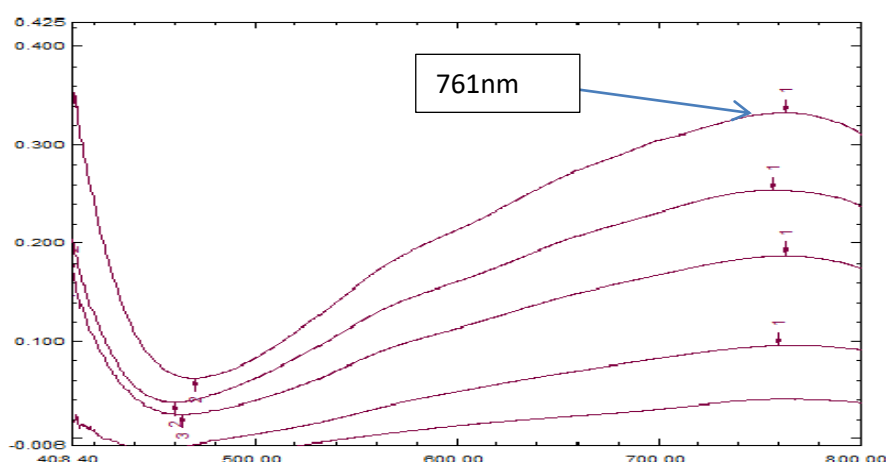


Figure 9: Overly Spectrum of UV Method Showing Linearity at 761nm

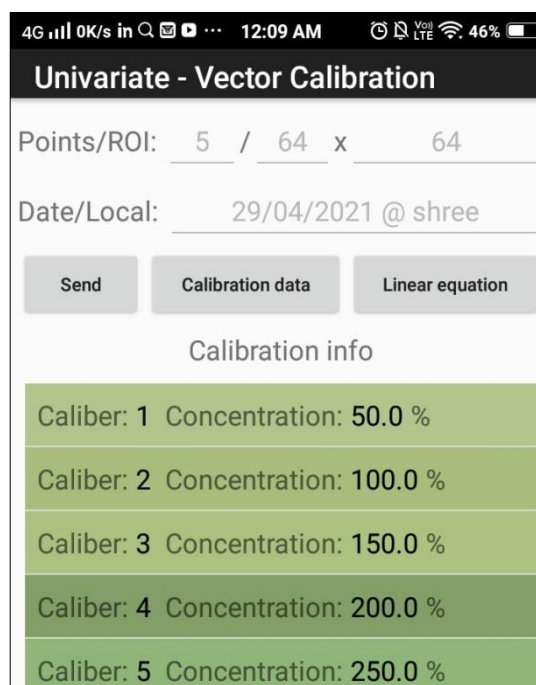
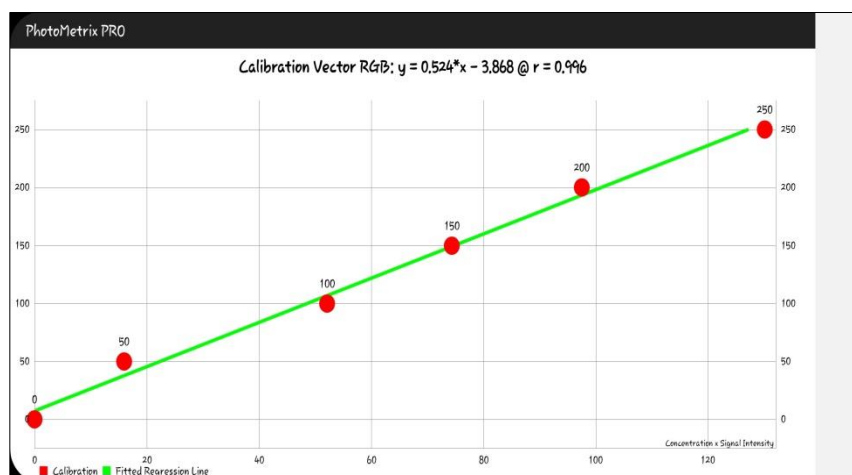


Figure 10: Colour Intensity of Captured Image in Photometrix PRO



**Figure 11: Calibration graph obtain from PhotoMetrix PRO**

**Table 1: Regression Equation Data of Both the Drug**

Parameter	UV Method	Photometric Application
Linearity ( µg/ml)	50-250	50-250
Regression Equation	$Y=0.0015x - 0.0416$	$Y=0.524x - 3.868$
Slop	0.0015	0.524
Intercept	0.0416	3.868
Correlation Coefficient	0.9956	0.996
LOD (µg/ml)	4.99	4.97
LOQ(µg/ml)	14.96	14.92

#### Ruggedness of 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 Result**

Parameter	Mean Assay%	SD	%RSD
Lab 1	99.64	0.73	0.733
Lab2	99.91		
Smartphone1	99.99	1.74	1.67
Smartphone2	99.68		

#### Assay of formulation:-

The assay was performed on the marketed formulation (Tafero EM10) with label claim of Emtricitabine 200mg 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 Result Obtain From Both Method**

Method	Amount taken µg/ml	Amount recovered	%Recovery ±SD (n=5)	%RSD
Uv	200	199.08	1.061	0.533
Photometrix application	200	199.25	0.759	0.381



### Comparison of Methods:-

Obtained assay results from PhotoMetrix application and UV method were compared by applying paired t-test (two tails) to the assay results. By applying a t-test to both the methods it was found that  $t_{\text{stat}}$  values were less than the  $t_{\text{critical}}$  values and P values were greater than applied alpha value (\* $P > 0.05$ ). It denotes that there is 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 Pair t-Test Result**

	UV method	PhotoMetrix PRO
Mean (X)	199.084	199.254
Variance ( $s^2$ )	1.12568	0.57673
Observations (n)	5	5
Pearson Correlation	0.990030485	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.162096076	
P(T<=t) one-tail	0.154904403	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.309808806	
t Critical two-tail	2.776445105	

### CONCLUSION:-

The novel and rapid, economical colorimetric detection method of Emtricitabine using smartphone based PhotoMetrix PRO application is developed. The method used simple coloring agent with 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.

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