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Research Article

Development and Validation of New Smartphone Based Colorimetric Method for Metoprolol succinate in Bulk and Tablet Dosage Form

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Abstract

A method for determining the concentration of coloured compounds in a solution is colorimetry. The intensity of the colour is related to the chemical concentration being measured. Because of its low cost and ability to collect, store, and interpret data all in one device, smartphone-based colorimetry has increased in appeal as an analytical tool. The camera on the phone is used as a detector in smartphone colorimetry. Both the smartphone colorimetric method and the UV method relied on the detection of colour intensity as concentration rose. The ammonium metavanadate reagent is orange red in colour, but when it reacts with Metoprolol succinate, it changes to a green colour complex. The developed method has good linearity in the 20-40 µg/ml range. The colour intensity increases as the concentration of API increases. All of the photos were captured on a smartphone and analyzed with photometrix PRO software. The photometrix PRO application turns an image to an RGB histogram, and it also includes regression models. LOD and LOQ value for UV visible spectrophotometry technique is 1.05 µg/ml and 3.189 µg/ml, respectively. Photometrix PRO LOD and LOQ are 0.0338 µg/ml and 0.102 µg/ml, respectively. The percent RSD of Metoprolol succinate was <2% utilizing Photometrix PRO and UV method. The results of a statistical tool called a two-paired test on both procedures show that they are both equally significant.

Keywords: UV spectrophotometry, Metoprolol succinate, Smartphone based colorimetry, Photometrix PRO, RGB Histogram

INTRODUCTION:

Metoprolol is a propanol amine that is 1-(propan-2-ylamino) propan-2-ol substituted by a 4-(2-methoxyethyl) phenoxy group at position 1. Iupac name of metoprolol succinate is butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol. It has a role as a beta-adrenergic antagonist, an antihypertensive agent, a xenobiotic, an environmental contaminant, and a geroprotector. It is a propanol amine, aromatic ether, secondary alcohol, and a secondary amino compound.

Metoprolol is a selective beta-1 blocker commonly employed as the succinate and tartrate derivatives depending if the formulation is designed to be of immediate-release or extended-release. The possibility of the generation of these formulations comes from the lower systemic bioavailability of the succinate derivative. To this date, it is one of the preferred beta-blockers in general clinical guidelines and it is widely prescribed in the Netherlands, New Zealand, and the US. Metoprolol was developed in 1969 by US Pharmaceutical Holdings I and FDA approved in 1978.

Metoprolol is a cardioselective beta-blocker that is widely used in the treatment of hypertension and angina pectoris. Metoprolol has been linked to rare cases of drug-induced liver injury. Metoprolol is indicated for the treatment of angina, heart failure, myocardial infarction, atrial fibrillation, atrial flutter, and hypertension. Some off-label uses of metoprolol

include supraventricular tachycardia and thyroid storm. All the indications of metoprolol are part of cardiovascular diseases. These conditions correspond to a number of diseases that involve the function of the heart and blood vessels. The underlying causes of these conditions are variable and can be due to genetic disposition, lifestyle decisions such as smoking, obesity, diet, and lack of exercise, and comorbidity with other conditions such as diabetes. Cardiovascular diseases are the leading cause of death on a global scale¹.

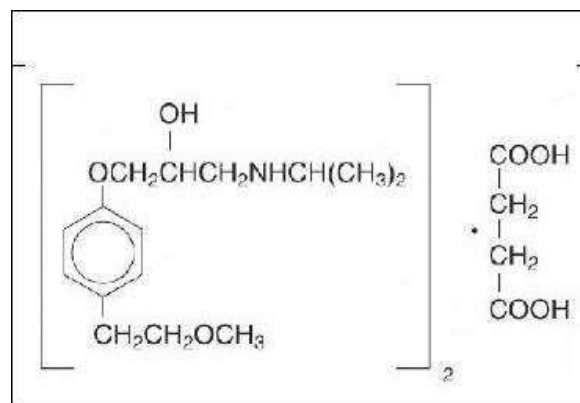


Figure 1: Metoprolol succinate (2)

Various analytical methods are developed for Metoprolol succinate. Different HPLC methods ³⁻⁷, UV spectroscopic

methods ^{2, 8-10}, and HPTLC ¹¹⁻¹⁵ methods have been reported for estimation alone or in combination of other drugs.

A light source, a monochromator, a photometer, an eyepiece for monitoring the photometric field, and a holder for the sample are commonly used for work in the visual region. The holder is either a cell for liquid transmission experiments or a device for supporting a large opaque object for reflection measurements. Unlike chemists' "colorimetric" results, spectrophotometric measurements are not limited to colored systems. Photographic methods have been used to determine absorption spectra in the ultraviolet and infrared regions of the spectrum for many years. A spectrophotometer's fundamental data shows the proportion of light incident on a sample that is reflected or transmitted by it. For a particular wavelength, a single value may be obtained, or values may be determined to cover the entire visible range. The results in the latter situation are usually displayed as a curve, with transmission or reflection as the ordinates and wavelength as the abscissas. Despite the fact that spectrophotometry's analytical applications are only discussed infrequently in books on general analytical methods or even in works dealing with colorimetric methods of analysis, a review of the literature reveals a large number of studies. These spans a wide range of specific applications and can be found in a variety of industries ¹⁶.

A colorimetric analysis is a simple way to figure out how much of a colored component is in a solution. Colored compounds absorb light in the visible spectrum, and the amount of light absorbed is proportional to the concentration of the material in the solution. A tungsten halogen lamp is used as the light source in colorimetric biochemistry analyzers. The lamp must be modified with filters or a monochromator to get the desired wavelength ¹⁷.

Color shifts recorded using Smartphone-based sensors are generating a lot of interest in chemical research because of their ease of use and flexibility to portable equipment. Smartphones have grown in popularity as analytical instruments due to their low cost and ability to collect, store, and process data all in one device. In smartphone colorimetry, the camera on the phone serves as a detector ¹⁸.

There are a number of smartphone-based colorimetric apps available. PhotoMetrix-PRO is one of them. PhotoMetrix PRO was available for free in the Windows Phone Store and Google Play Store. This programme employs simple linear correlation for univariate analysis and principal components analysis for multivariate exploratory analysis (PCA). The smartphone camera captures visual data, which is then converted into RGB histograms (red, green, and blue) ¹⁹.

The RGB colour model is based on the colour perception hypothesis, which states that the human eye has various sensitivity peaks around red, green, and blue. To improve Colorimetry's RGB colour system applicability, multivariate analysis (e.g., partial least squares, PLS) could be applied ²⁰.

Colorimetry is a method of determining the quantitative value of colours that is frequently used in biological research. When chromogens connect to a substance, colour is created. Light absorption varies according to the strength of the colours.

The intensity of the colour corresponds to the concentration of the material being tested. The visible band of light has a wavelength of 400 nm to 800 nm in the electromagnetic spectrum. A colorimeter/visible spectrophotometer is a device that determines the concentration of a solution by measuring the absorbance of a specific wavelength of light. Consider the specificity and sensitivity of a reagent when choosing one for colorimetric analysis ²¹.

The usage of advanced tools was required for this technique. The purpose of this research is to develop a simple, low-cost method for calculating Metoprolol succinate. The method uses ammonium metavanadate as a colouring agent, which produces a green colour when combined with Metoprolol succinate. The data image was acquired and analyzed using the PhotoMetrix-PRO application.

MATERIAL AND METHOD:

Chemicals and reagents:

Double distilled water, 40% H₂SO₄, 5% Ammonium Metavanadate solution was freshly prepared.

Apparatus and Applications:

The metoprolol succinate API samples were weighed on an electronic balance (A×120) by Shimadzu. Ultraviolet and visible spectrophotometry was carried out through Shimadzu UV-1700 double beam spectrophotometer. A picture of the sample was taken using the camera of a smartphone and uploaded to the mobile (photometrix Pro) Application.

Preparation of 5% ammonium metavanadate reagent:

Dissolve 5gm of ammonium metavanadate reagent in 100ml of 40% H₂SO₄ and heat it on water bath until solid residue dissolve.

Preparation of standard stock solution:

For the preparation of the standard stock solution 10mg of Metoprolol succinate was accurately weighed and transferred into a previously calibrated 10ml volumetric flask. The final volume was made up to the mark using double distilled water to obtain the standard stock solution of 1000µg/ml concentration.

Method development:

UV-Visible spectroscopy:

Selection of wavelength for metoprolol succinate:

Using ammonium metavanadate as a blank, the drug solution was scanned across the range 400-800nm. Metoprolol succinate was found to have a maximum absorbance at 762nm. Prepare a calibration curve using the working solution, ranging from 20-40µg/ml, and construct a linear regression equation.

Reaction mechanism:-

Ammonium metavanadate, an inorganic oxidizing agent. Vanadate has oxidation states of +5, +4, +3, and +2 in its compound. Ammonium metavanadate is the most common source of vanadium in the +5 oxidation state. The oxidation of Metoprolol succinate was carried out. To prevent reoxidation, heat is applied during the chemical reaction. Ammonium metavanadate is an orange red colour complex that turns green when it reacts with Metoprolol succinate. Vanadium's oxidation state comes from +5 to +3.

Experimental optimization:

Optimization of concentration of reagent:

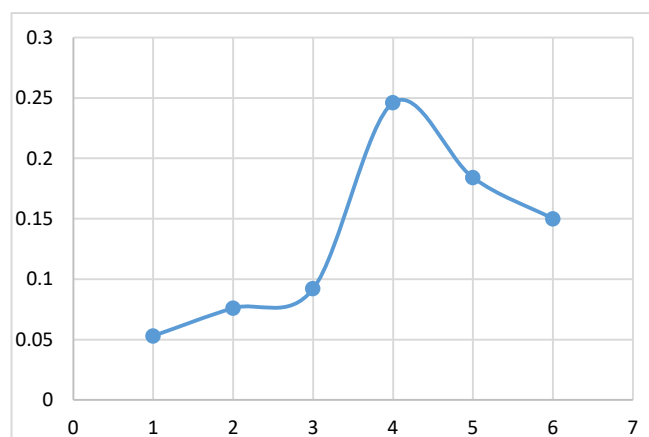
Ammonium metavanadate was allowed to react with metoprolol succinate to form a green colour with absorption maxima at 762nm, by keeping other parameter constant. The optimization of the research was first established by varying the reagent range from (1.25%–10%), where we found maximum absorbance at 5%; hence, it was coined as the optimized volume of reagent, as shown in Table 1.

Table 1: Optimization of reagent concentration

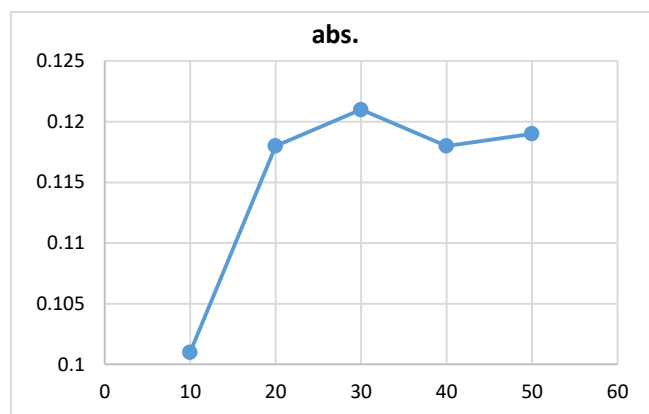
S. N.	Concentration of reagent	Observation
1	1.25%	No color change
2	2.5%	Color changed but not stable
3	5%	Stable color
4	7.5%	Color is too dark
5	10%	Ammonium metavanadate powder is undissolved even after put it on water bath for long time

Optimization of reagent volume:

The effect of reagent volume was carried out in range from 1-6ml. From green color complex and absorbance maxima optimized volume was selected. 4ml of reagent volume was selected for method.

**Figure 2: Optimization of reagent volume (ml)****Optimization of heating time:**

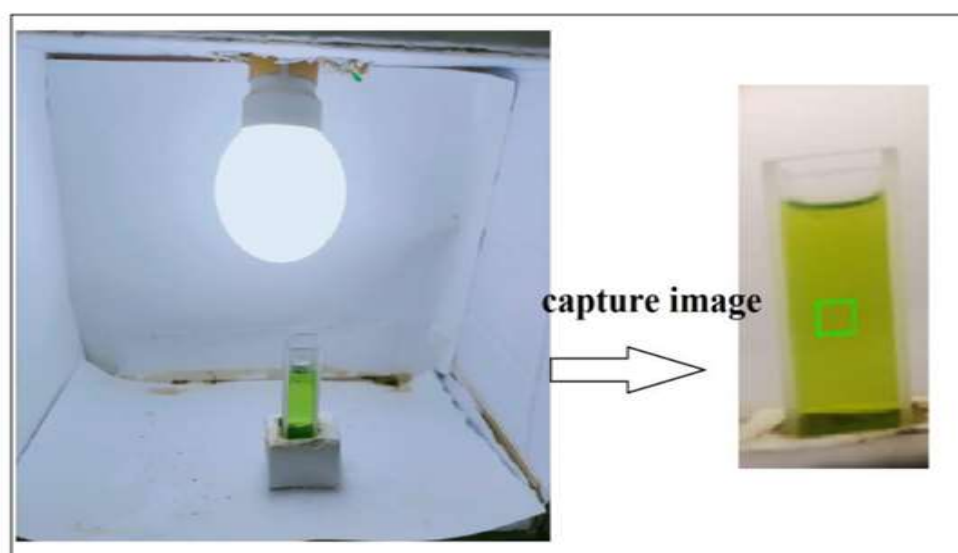
The effect of heating time carried out in from 10-50 min. From color complex reaction observed between 10- 50 min. Maximum color intensity was observed at 30 mins.

**Figure 3: Optimization of heating time (min.)****Preparation of calibration graph for Metoprolol succinate:**

Aliquots of standard solution of Metoprolol succinate corresponding to 20-40 µg/ml was taken into 10 ml of volumetric flask. To each flask 4 ml of 5% Ammonium metavanadate reagent was added and solution was heated for 30 min at 50°C on water bath. The solution was allowed to cool at room temperature and then volume was made up to 10 ml with distilled water. The absorbance of the solution was measured at 762 nm against blank.

Estimation of Metoprolol Succinate using Smartphone Application:**Experimental Setup:**

The colored 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 shown in Figure 4.

**Figure 4: Experimental setup**

The image of a colour complex solution was taken with a smart phone and analyzed using a photometric application to determine the red-green-blue intensities (RGB scale) of the image. The concentration of the image taken by Photometrix PRO was estimated using a linear regression equation. Photometrix creates and analyses colour histograms on RGB

scales, which it then converts into a calibration curve. Using univariate and multivariate analysis, this programme processes and displays the results. For the best results, many smartphone types were used. Figure 5 depicts the steps for utilizing the PhotoMetrixPRO application.



Figure 5: Steps for run the photometrix pro application

Method validation:

According to validation requirements, the UV-visible spectrophotometry and PhotoMetrix applications were

separately validated in terms of linearity and robustness. For both approaches, a formulation assay was carried out. Under optimal conditions, excellent linearity was reported in the range of 20-40 µg/ml. In the case of UV-visible spectrophotometry, the concentration of tablet formulation was calculated using a regression equation, while photometrix was calculated within the programme.

RESULT AND DISCUSSION:

Method Validation:

1. Linearity:

Metoprolol succinate was linear with the concentration range of 20-40 µg/ml at 762 nm, by obeying Beer's law (Figure 6). A calibration curve was plotted between concentration Vs absorbance (Figure 7). The plot was found to be linear.

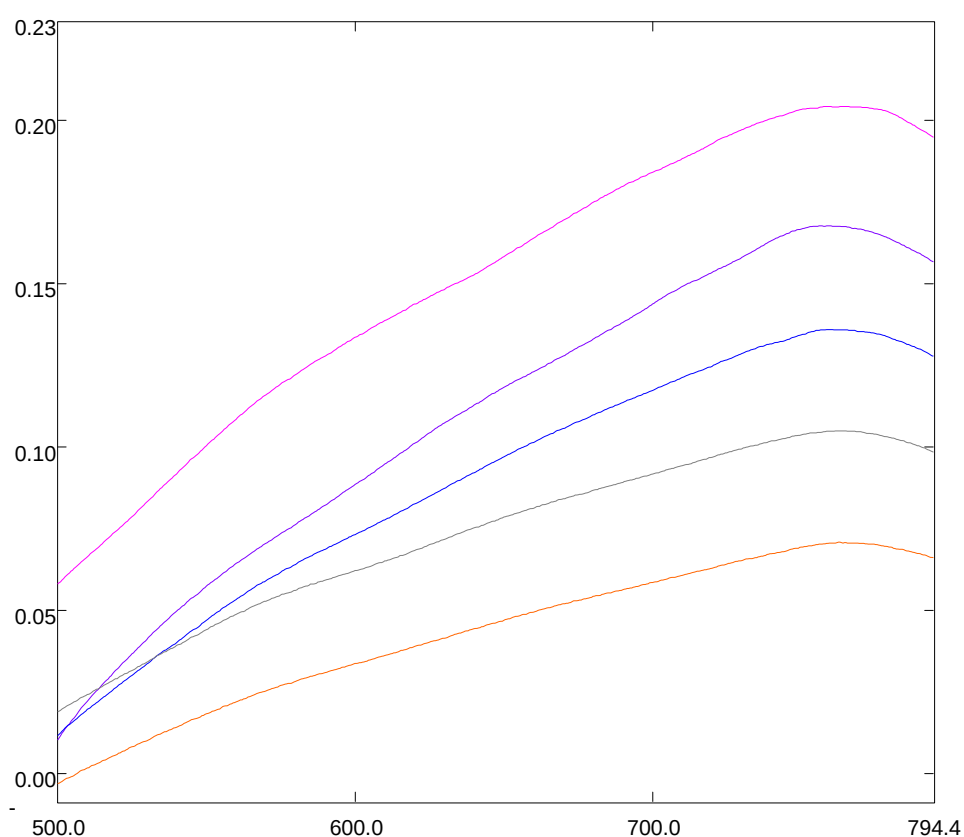


Figure 6: Overlay UV spectra of Metoprolol succinate

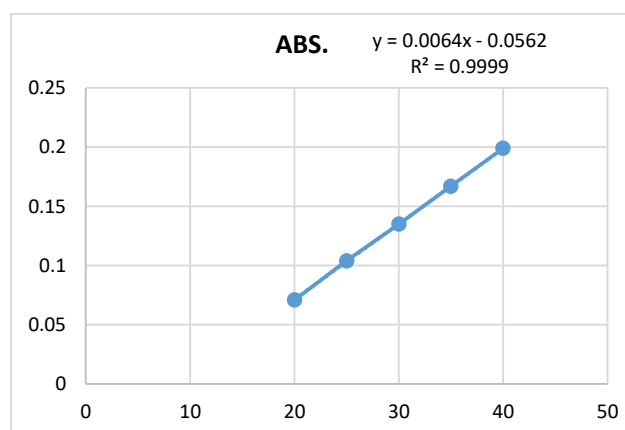


Figure 7: Calibration graph of Metoprolol succinate

2 Precision:

The degree of agreement between a series of measurements obtained by sampling the same homogenous sample numerous times under the method's defined circumstances is referred to as the precision of an analytical method. Here, we calculated the intraday (Repeatability) and interday precision.

Three-concentration samples of each drugs' lowest, upper, and middle limits were taken and analysed three times on the same day at the same concentration level for intra-day precision and three times on three different days for inter-day precision. The % RSD was calculated (Tables 2) and found to be less than 2.

Table 2: Intra-day and Inter-day precision of Metoprolol succinate

	Conc.(µg/ml)	Mean ± SD	%RSD
Intra-day	20	0.070±0.00080	1.43
	25	0.105±0.00047	0.55
	30	0.135±0.00169	1.54
	35	0.166±0.00047	0.35
	40	0.202±0.00124	0.75
Inter-day	20	0.071±0.0023	1.43
	25	0.105±0.00081	0.95
	30	0.135±0.00047	0.43
	35	0.168±0.00094	0.69
	40	0.201±0.00216	1.32

3 Accuracy:

The method's accuracy was determined by recovery experiments. A known quantity of the pure medication was

added to the pre-analyzed sample formulation at 80 percent, 100 percent, and 120 percent. Table 3 shows the percentage recovery as well as the percentage relative standard deviation of the percentage recovery.

Table 3: Accuracy data of Metoprolol succinate

Drug	Standard concentration (µg/ml)	%spiked	Concentration added from formulation (µg/ml)	Concentration recovered (µg/ml) (mean)	%recovery ± SD	%RSD
Metoprolol succinate (Extended release Tablets)	20	80	16	16.20	100.55±0.2189	0.95
	20	100	20	20.45	101.12±0.21	0.53
	20	120	24	24.53	101.21±0.38	1.73

Analysis of the marketed formulation:

The assay of Marketed Formulation (Extended release Tablets) was found to be 101.125 % which is within the acceptance criteria (98-102%) (Table 4).

Table 4: Assay results of the Marketed Formulation

Drug	Concentration (µg/ml) (Mean)	Concentration Found (µg/ml) (Mean)	%recovery ± SD	%RSD
Formulation 1	32	32.36	101.125±0.00125	1.11
Formulation 2	32	32.14	100.441±0.00152	1.12
Formulation 3	32	31.97	99.916±0.00251	1.85

4 Specificity:

The specificity was determined by preparing a 100 µg/ml solution from the marketed formulation and a blank. The method's specificity is shown in the following figure 8. It indicates that the excipient does not interfere and only the drug shows absorbance.

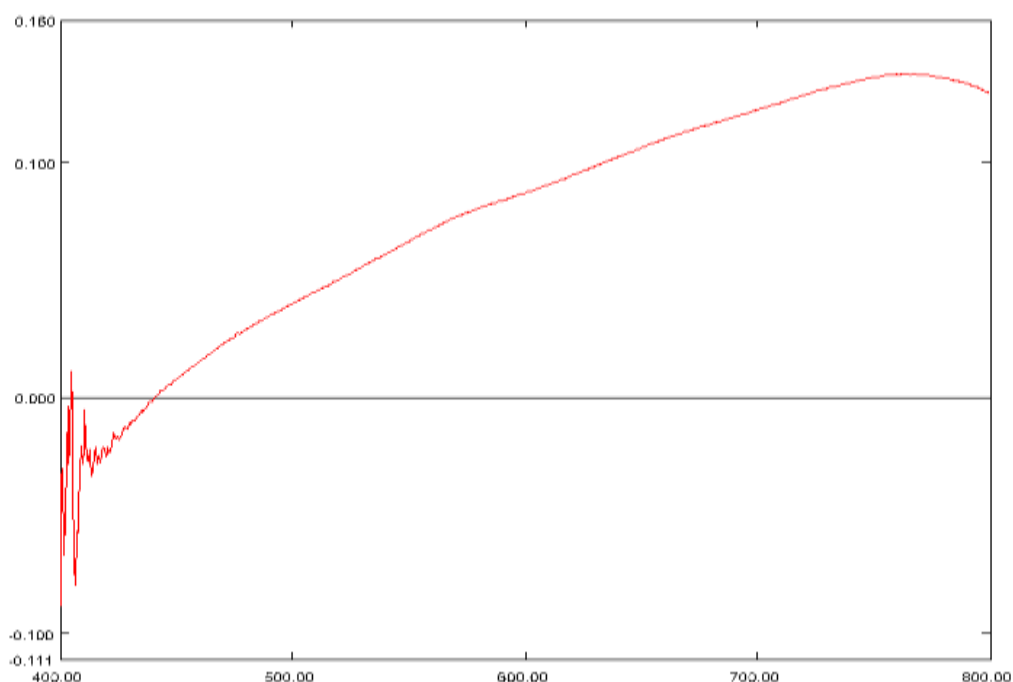


Figure 8: Specificity indicating graph

5 Method's ruggedness:

The created method's ruggedness was studied in two labs and with the use of 2 separate smartphones. As indicated in Table 5, the percent RSD of both of these parameters was less than 2.

Table 5: Method Ruggedness Result

Parameter	Mean assay %	SD	%RSD
Lab 1	99.28	0.40	0.40
Lab 2	100.13		
Smartphone 1	101.32	0.61	0.61
Smartphone 2	100.78		

Metoprolol succinate estimation using a smartphone application:

The image was captured according to concentration using the PhotoMetrix PRO programme (figure 10). It was discovered that the linear regression equation exists (figure 9). PhotoMetrix PRO and UV-Vis spectrophotometry have correlation coefficients of 0.989 and 0.999, respectively. Table 6 shows the regression equation data for both techniques.

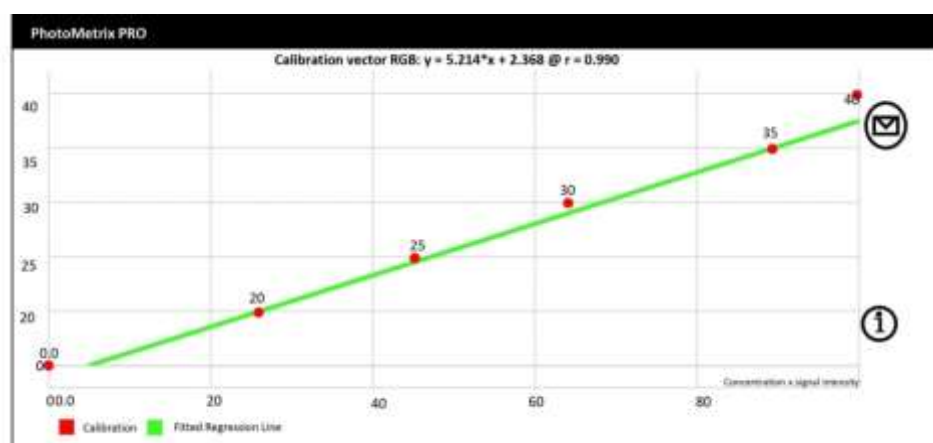


Figure 9: Calibration curve of the Metoprolol succinate by photometrix pro application

Caliber: 1 concentration: 0.0 %
Caliber: 2 concentration: 20.0 %
Caliber: 3 concentration: 25.0 %
Caliber: 4 concentration: 30.0 %
Caliber: 5 concentration: 35.0 %
Caliber: 6 concentration: 40.0 %

Figure 10: Chart of colour intensity corresponding to the concentration of Metoprolol succinate

Metoprolol succinate estimation using a smartphone app:

The linearity of standard Metoprolol succinate was measured between 20 - 40 µg/ml. Figure 9 depicts the calibration curve and regression equation obtained by the application. The sample solution had a concentration of 31.73 µg/ml (nominal 32 µg/ml). The percent assay resulted in a result of 99.15%, which is within the acceptable range.

Table 6: Regression data for both UV and photometric application

Parameter	UV Method	Photometric application
Linearity (µg/ml)	20-40	20-40
Regression equation	$y = 0.0064x - 0.0562$	$Y = 5.214x + 2.368$
Slope	0.00646	5.214
Intercept	0.0562	2.368
Correlation coefficient	0.999	0.990
LOD (µg/ml)	1.05	0.0338
LOQ (µg/ml)	3.189	0.102

Assay of formulation:

Both methods were used to test the marketed formulations with the label claim of Metoprolol succinate 50mg. The concentration of sample solutions was calculated using a linear regression method and expressed as a percent recovery. For both approaches, the assay findings were found to be within acceptable limits and significant. Table 7 shows the results of the assays.

Table 7: Assay results of ABMETOP 50XL obtain from both method

Method	UV				Photometric			
	Amount taken (µg/ml)	Amount recovered (µg/ml)	%Recovery	% RSD	Amount taken (µg/ml)	Amount recovered (µg/ml)	%Recovery	% RSD
Formulation 1	32	32.36	101.25	1.11	32	31.73	99.15	1.52
Formulation 2	32	32.14	100.441	1.12	32	31.48	98.37	1.76
Formulation 3	32	31.97	99.916	1.85	32	31.63	98.84	1.09

Comparison of methods:

The obtained assay results from the Photometric application and the UV technique were compared using a paired t-test (two tails). Using a t-test, it was discovered that tstat values

were lower than tcritical values and P values were higher than the applied alpha value (*P>0.05). It signifies that there is no discernible difference between the approaches' means. As a result, the Photometric application can be used to identify drugs using colorimetry. Table 8 displays the information.

Table 8: Applied pair t-Test Result

	UV	Photometric
Mean (X)	32.134	31.916
Variance	0.08253	0.10638
Observations (n)	5	5
Pearson Correlation	0.396944	
Hypothesized mean difference	0	
Df	4	
t stat	1.121538	
P (T<=t) one-tail	0.147296	
t Critical one-tail	1.859548	
P (T<=t) two-tail	0.294592	
t Critical two tail	2.306004	

CONCLUSION:

The Photometrix PRO application for smartphones is being utilized to build a new and cost-effective colorimetric detection method for Metoprolol succinate. The method depended on a simple coloring additive and a short procedure. The major goal of this study was to use smartphone-based applications to make colorimetric drug content measurements easier. The method was also compared to a UV method created using the identical reagent and methodology, and there were no significant variations in assay findings. This innovative method can be used as an alternative to analytical science for quantitative drug estimation in pharmaceutical dosage forms.

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