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Research Article

New Smartphone Based Colorimetric Method Development and Validation of Drugs Containing Nitrogen, Phosphorus and Sulphur

Tejasbharthi Pravinbharthi Goswami*, Apeksha Kadam, Rajashree Mashru

The Maharaja Sayajirao University of Baroda, G.H Patel Pharmacy building, Donor's Plaza, Fatehgunj, Vadodara 390001, Gujarat, India

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*Address for Correspondence:

Tejasbharthi Pravinbharthi Goswami, The Maharaja Sayajirao University of Baroda, G.H Patel Pharmacy building, Donor's Plaza, Fatehgunj, Vadodara 390001, Gujarat, India

Abstract

Colorimetry is a method for determining the concentration of coloured substances in a solution. The intensity of colour is directly proportional to the concentration of compound being measured. Smartphone based colorimetry has 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. The colorimetric method based on a smartphone and the UV method both are based on the detection of colour intensity. The ammonium metavanadate is having different oxidation states and it produce different colours in its oxidation state. The +5-oxidation state shows yellow colour, +4 oxidation state shows blue colour, +3 oxidation state shows green colour and +2 oxidation state shows purple colour. The ammonium metavanadate reagent is orange red in colour; however, it transforms into green colour complex when it reacts with drugs containing nitrogen, phosphorus and sulphur in its structure. In this article the developed method for all the drugs exhibits good linearity having Correlation coefficient about 0.998(Amoxicillin trihydrate), 0.998 (Silodosin) and for 0.999(Sofosbuvir). With increasing the concentrations of API, the colour intensity increases. All photographs were taken with smartphone and analysed using the photometrix PRO software. This application converts an image to an RGB histogram and regression models are included into the photometrix PRO application. By using Photometrix PRO and UV technique, the percent RSD of for all the three drugs was < 2. Using statistical tool i.e two paired test on both procedures for all the three different drugs, the results show that both are equally significant.

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

INTRODUCTION:

Introduction of Amoxicillin (Sulphur atom containing drug):

Amoxicillin is a semisynthetic, acid stable antibiotic that belongs to the Penicillin (beta lactam antibiotics) class of medicines.

In both humans and animals, it has been found to be effective against a wide range of illnesses caused by a wide spectrum of Gram positive and Gram-negative bacteria.

It is a congener of ampicillin semisynthetic amino penicillin) that differs only in the hydroxylation of the phenyl side chain from the parent medication.

After oral administration, it has found a niche in the treatment of ampicillin-resistant infections.

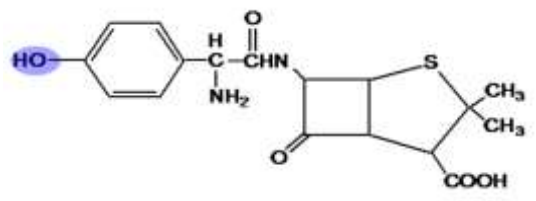

Figure 1: Chemical structure of amoxicillin [1]

Amoxicillin is a chemical compound that is used to treat infections (2S,5R,6R)-6-[[(2R)-2-amino-2 -(4-hydroxyphenyl) acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid is a kind of heptane-2-carboxylic acid.[1,2]Amoxicillin trihydrate have various HPLC methods[3–6], UV spectroscopic methods[7,8], colorimetric methods[9–11] and HPTLC methods[12–14] have been reported for estimation alone or in combination of other drugs.

Introduction of silodosin (Nitrogen atom containing drug):

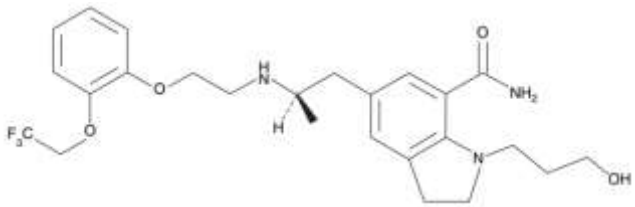

Figure 2: Structure of silodosin[41]

Silodosin is a 1A-adrenoceptor antagonist that has been licenced to treat the signs and symptoms of benign prostatic hyperplasia.[15]

The three a1-adrenoceptor subtypes have quite different functions: Prostate contraction is mediated mostly by a1A receptors, whereas the human urethra has exclusively a1A receptors, whereas a1A, a1B, and a1D all mediate blood vessel dilation.

As a result, compared to less selective α-adrenoceptor blockers, silodosin's strong selectivity for the a1A subtype should result in greater cardiovascular tolerance without a loss of efficacy on urinary tract symptoms. Indeed, both in the animal model and in investigations with healthy individuals, insignificant effects on the cardiovascular system have been found. In dogs given oral dosages of silodosin up to 200 times the normal therapeutic dose indicated for humans, there were moderate effects on blood pressure but no effects on cardiac repolarisation. In healthy individuals given silodosin at a dose three times larger than the normal therapeutic dose, no significant changes in heart rate, pulse rate, or QRS interval duration were found (24 mg).

Silodosin have various HPLC methods[16–18] UV spectroscopic methods[19,20], colorimetric methods and HPTLC methods[21] have been reported for estimation alone or in combination of other drugs.

Introduction of sofosbuvir (Phosphorus atom containing drug):

Sofosbuvir is a phosphoramidate prodrug that is converted into the potent antiviral agent 2'-deoxy-2'-a-fluoro-b-C-methyluridine-5'- monophosphate in the liver.

Although phosphoramidate prodrugs have been researched as a technique of boosting nucleoside potency in cell culture by raising the concentration of active nucleotide, at the time of sofosbuvir's discovery, no phosphoramidate prodrug technology had been applied in the treatment of HCV. The triphosphate derivative of b-D2'-deoxy-2'-R-F-2'-b-C-methyluridine, a uridine nucleoside, was discovered to be a particularly effective inhibitor of HCV protease NS5B in previous research.

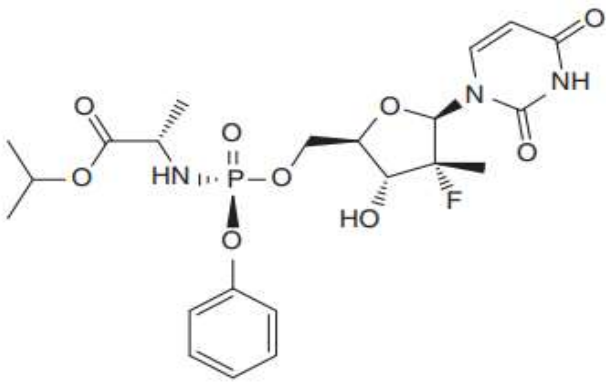

Figure 3: Structure of Sofosbuvir[22]

The monophosphate derivative of b-D-2'-deoxy-2'-R-F-2'-b-C-methyluridine can be converted to the triphosphate derivative enzymatically in HCV replicon cells.

Intracellular monophosphorylation of b-D-2'-deoxy-2'-R-F-2'-b-C-methyluridine, on the other hand, does not happen. As a result, the monophosphate derivative of b-D-2'-deoxy-2'-R-F-2'-b-C-methyluridine was chosen as a candidate for therapeutic development.

The development of the phosphoramidate prodrug sofosbuvir was based on the hypothesis that first-pass metabolism would produce the required triphosphate antiviral nucleotide at the targeted site of action, the liver[22].

Sofosbuvir have various HPLC methods[23–25] UV spectroscopic methods[26,27], colorimetric methods and HPTLC methods[28–30] have been reported for estimation alone or in combination of other drugs.

For work in the visual region the type of instrument largely used consists of a light source, a monochromator, a photometer, an eyepiece for observing the photometric field, and a holder for the sample. The holder is a cell for transmission measurements of a liquid, or a device for supporting ran opaque object on which reflection measurements are to be made. Spectrophotometric measurements are not limited to coloured systems, unlike chemists' "colorimetric" results. For many years, photographic methods were used to determine absorption spectra in the ultraviolet and infrared parts of the spectrum.

A spectrophotometer's fundamental data shows the proportion of light incident on a sample that is reflected or transmitted by it. For a particular wave length, 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 wave length as the abscissas. When creating a curve that covers part or all of the visible range, the question of what wave-length interval to employ to define individual points and what spectral band width to use for the light source arises. If somehow the curve is steep and has small sharp absorption bands, the points may need to be taken at every millimicron with the narrowest feasible spectral band.[31]

Colorimetric analysis is a handy method for determining the concentration of coloured substance in a solution. Light in the visible spectrum is absorbed by coloured substances, and the amount of light absorbed is proportional to the concentration of the substance in solution.[32]

Due to their ease of use and adaptability to portable equipment, colour shifts recorded using Smartphone-based sensors are attracting significant interest in chemical investigation. Smartphones have gained popularity as analytical instruments because they are widely available at a low cost and allow data gathering, storage, and processing all in one device. The mobile camera is used as a detector in smartphone colorimetry.[33]

A variety of smartphone-based colorimetric applications are available. One of them is Photo Metrix-PRO. Photo Metrix PRO was free to download from the Windows Phone Store and the Google Play Store. For univariate analysis, this programme uses basic linear correlation, and for multivariate exploratory analysis, it uses principal components analysis (PCA). The image data is taken by the smartphone camera and transformed into RGB histograms (red, green, and blue).[34]

The RBG colour model is based on a colour perception hypothesis in which the human eye has various sensitivity peaks located around red, green, and blue. Multivariate analysis (e.g., partial least squares, PLS) could be used in this software to improve Colorimetry's RGB colour system applicability.[35]

The colour intensity is proportional to the concentration of the substance that is being measured. The visible band of light in the electromagnetic spectrum has a wavelength of 400 nm to 800 nm. A colorimeter/visible spectrophotometer is a device that measures the absorbance of a given wavelength of light to determine the concentration of a solution. When choosing a reagent for colorimetric analysis, consider its specificity and sensitivity.[36]

This procedure necessitated the use of sophisticated tools. The goal of this study is to establish a simple, cost-effective method for estimating amoxicillin trihydrate.

The method uses ammonium metavanadate as a colouring ingredient, which reacts with sulphur containing amoxicillin trihydrate[37]Nitrogen containing Silodosin and Phosphorus containing Sofosbuvir[38–40] to produce a green colour. Photo Metrix-PRO application captured and analysed the data image.

MATERIAL AND METHOD:

Chemicals and reagents:

5% Ammonium Metavanadate solution, amoxicillin trihydrate, silodosin, Sofosbuvir, double distilled water, 0.1M H_2SO_4, 40% H_2SO_4.

Apparatus and Applications:

The API samples were weighed on an electronic balance (A×120) (Shimadzu). Smartphone camera and uploaded to the mobile (photometrix Pro) Application.

Preparation of 0.1M H_2SO_4:

To make 0.1M solution, slowly add 0.136ml of 98% H_2SO_4 to around 6.25ml of double distilled water. Adjust the final volume of solution to 25ml with double distilled water.

Preparation of 5% ammonium metavanadate reagent:

Weigh about 5gm of ammonium metavanadate reagent in 100ml of 40% H_2SO_4 and heat on water bath until solid residue dissolve.

Preparation of standard stock solution:

Weigh about 10mg of Amoxicillin trihydrate, silodosin and sofosbuvir then transferred all of them into previously calibrated 10ml volumetric flasks respectively. The final volume was made up to the mark using 0.1M H_2SO_4 to obtain the standard stock solution of 1000µg/ml concentration each.

Method development:

UV-Vis. spectroscopy:

Selection of wavelength:

Using ammonium metavanadate as a blank, the drug solution was scanned across the range 400-800nm. Amoxicillin trihydrate, silodosin and sofosbuvir was found to have an absorbance of 762nm. Prepare a calibration curve using the working solution, ranging from 30-150µg/ml for amoxicillin trihydrate, 20-100 µg/ml for silodosin and sofosbuvir.

Reaction mechanism:

Ammonium metavanadate is inorganic oxidizing agent. 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 Amoxicillin trihydrate, silodosin and sofosbuvir were done in acidic medium. Heat is given during chemical reaction to prevent reoxidation. Ammonium metavanadate is orange red color complex but when it reacts with Amoxicillin trihydrate, silodosin and sofosbuvir individually it forms green color complex.

Oxidation state: From +5 it comes to +3 of vanadium.

Method optimization:

Optimization of regent concentration for the drugs:

Ammonium metavanadate was allowed to react with Amoxicillin trihydrate, silodosin, sofosbuvir to form a green colour with absorption maxima at 762nm, by keeping another parameter constant. The optimization of the experiment was established by varying the concentration of reagent in the range of 1.25%–10%, where, maximum absorbance of reagent was found at 5%; as shown in Table 1.

Table 1: Optimization of reagent concentration

Sr. No.	Concentration of reagent	Observation
1	1.25%	No colour change
2	2.5%	No stable colour change
3	**5%**	**Stable colour change**
4	7.5%	No stable colour
5	10%	Ammonium metavanadate powder didn't dissolved

Optimization of reagent volume for Amoxicillin trihydrate:

The effect of reagent volume was carried out in range from 0.25-6ml. From green colour complex and absorbance maxima optimized volume was selected. A linear increment in absorbance was observed with the increase in volume of reagent up to 4ml. However, above its linearity was disturbed and hence 4ml was selected as optimum reagent volume.

Table 2: Optimization of reagent volume for Amoxicillin trihydrate

ml of reagent	**Absorbance**
0.25	0.027
0.5	0.041
1	0.068
2	0.141
3	0.238
4	**0.279**
5	0.492
6	0.578

Optimization of reagent volume for Silodosin:

The effect of reagent volume was carried out in range from 2-6ml.Optimized volume was selected taking into consideration green colour complex & absorbance maxima. 4ml of reagent volume was selected for method.

Table 3: Optimization Of volume reagent for silodosin

ml of reagent	**Absorbance**
2	0.172
3	0.249
4	**0.341**
5	0.325
6	0.314

Optimization of reagent volume for sofosbuvir:

The effect of reagent volume was carried out in range from 3-7ml. Optimized volume was selected taking into consideration green colour complex & absorbance maxima. 5ml of reagent volume was found to be optimum for method.

Table 4: Optimization Of volume reagent for Sofosbuvir

ml of reagent	Absorbance
3	0.068
4	0.115
5	**0.197**
6	0.162
7	0.161

Optimization of reaction time for amoxicillin trihydrate:

The effect of reaction time was carried out in from 10-50 min. From colour complex reaction observed between 10- 50 min, slight increase in colour intensity was observed at 30 minutes (Figure 4).

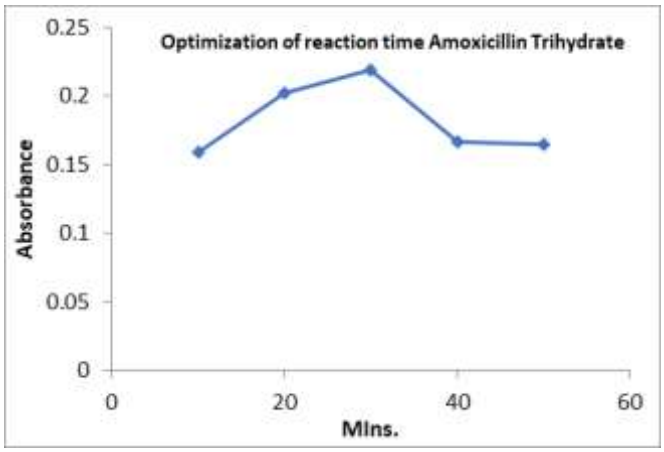

Figure 4: Optimization graph of reaction time for amoxicillin trihydrate

Optimization of reaction time for silodosin:

The effect of reaction time carried out in from 10-50 min. From colour complex reaction observed between 10- 50 min, slight increase in colour intensity was observed at 30 minutes (Figure 5).

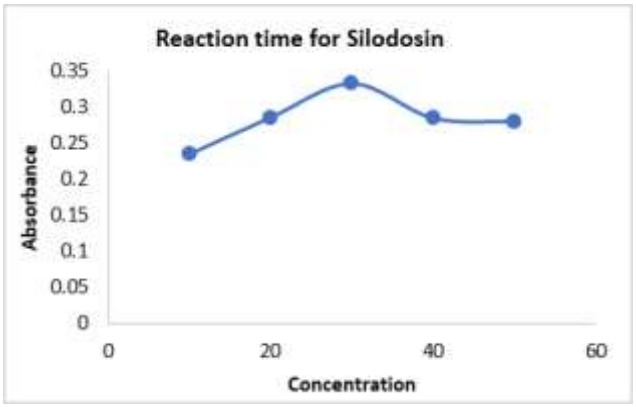

Figure 5: Optimization graph of reaction time for silodosin

Optimization of reaction time for sofosbuvir:

The effect of reaction time carried out in from 20-60 min. From colour complex reaction observed between 10- 50 min, slight increase in colour intensity was observed at 50 minutes (Figure 6).

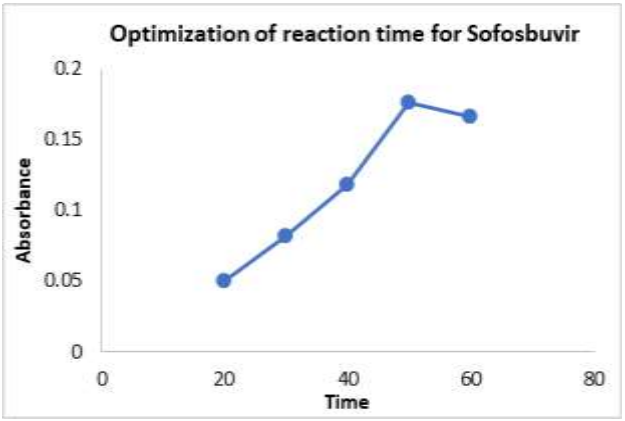

Figure 6: Optimization graph of reaction time for sofosbuvir

Preparation of calibration graph:

Aliquots of standard solution of Amoxicillin trihydrate corresponding to 30-150 µg/ml, Silodosin and Sofosbuvir corresponding to 20-100 µg/ml were taken into 10ml volumetric flask. To each flask 4 ml of 5% Ammonium metavanadate reagent was added and solutions were heated on water bath for respective time for each as mentioned above. 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 Amoxicillin Trihydrate Using Smartphone Application:

Experimental Setup:

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 shown in Figure 7.

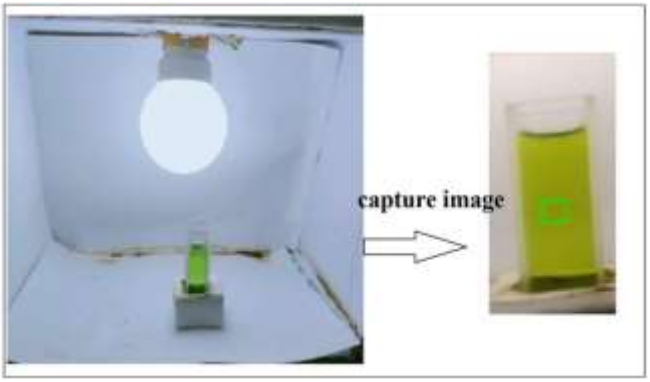

Figure 7: Experimental set up

The image of a colour complex solution was taken with a smart phone and analysed 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.

Figure 8: Steps for run the photometrix pro application

Using univariate and multivariate analysis, this programme processes and displays the results. For the best results, many smartphone types were used. Figure 8 depicts the steps for utilising the PhotoMetrixPRO 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 of Amoxicillin trihydrate was reported in the range of 30-150 µg/ml, Silodosin and sofosbuvir was reported in the range of 20-100 µg/ml. In the case of UV-vis 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:

Amoxicillin trihydrate was linear with the concentration range of 30-150 µg/ml at 762 nm, Silodosin and sofosbuvir were linear with the concentration range of 20-100 µg/ml at 762 nm, by obeying Beer's law (Figure 9). A calibration curve was plotted between concentration Vs absorbance. The plot was found to be linear (Figure 10).

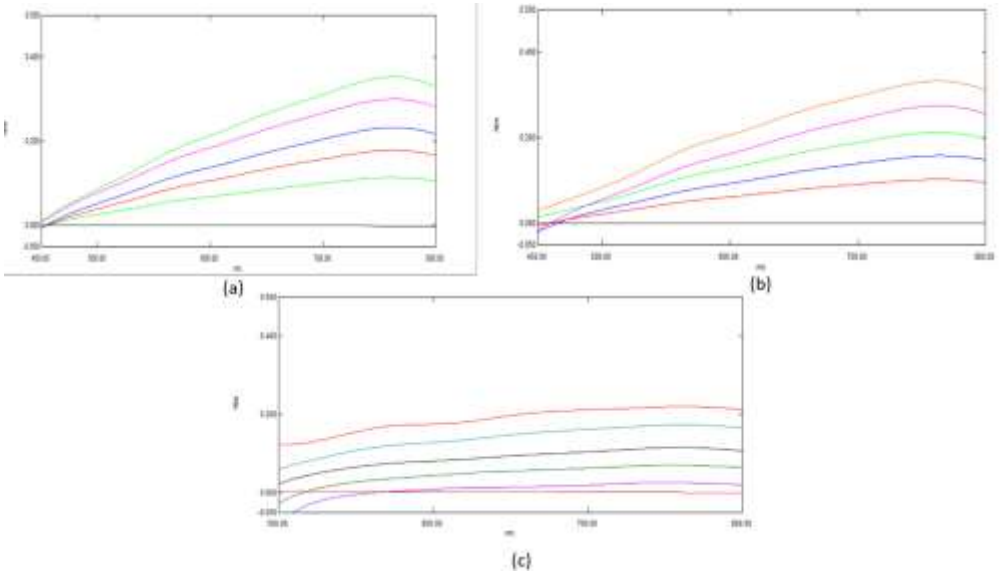

Figure 9: linearity of Amoxicillin trihydrate(a), Silodosin(b), Sofosbuvir(c)

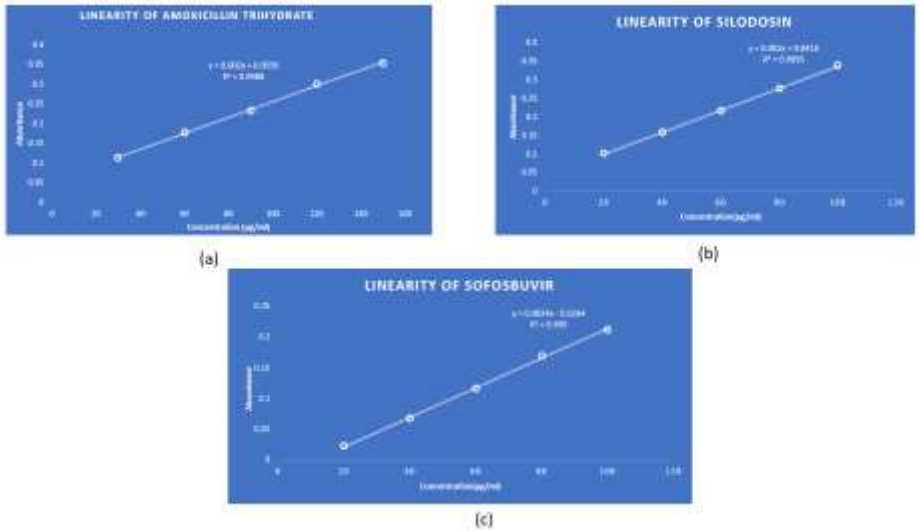

Figure 10: Calibration graph for Amoxicillin trihydrate(a), Silodosin(b), Sofosbuvir(c)

2. Precision:

The precision of an analytical method refers to the degree of agreement between a set of measurements acquired by sampling the same homogeneous sample many times under the method's specified circumstances. The intraday (Repeatability) and interday precision were calculated here. Three-concentration samples with lowest, upper, and middle limits of both medicines were taken and analysed three times at the same concentration level on the same day for intra-day precision and three times on three different days for inter-day precision for three drugs. The percent RSD of Amoxicillin trihydrate(Table 5), Silodosin(Table 6) and Sofosbuvir(Table 7) was calculated and determined to be less than 2.

Table 5: Intraday and Interday precision of Amoxicillin trihydrate

	Concentration(µg/ml)	Mean ± SD (n = 3)	% RSD
Intraday	30	0.112 ± 0.002	1.79
	60	0.178 ± 0.00057	0.32
	90	0.236 ± 0.0037	1.6
	120	0.288 ± 0.0045	1.59
	150	0.348 ± 0.0047	1.36
Interday	30	0.113 ± 0.0015	1.34
	60	0.176 ± 0.0032	1.82
	90	0.223 ± 0.0026	1.19
	120	0.285 ± 0.047	1.66
	150	0.355 ± 0.0023	0.62

Table 6: Intraday and Interday precision of Silodosin

	Concentration(µg/ml)	Mean ± SD (n = 3)	% RSD
Intraday	20	0.104 ± 0.0008	0.96
	40	0.158 ± 0.0012	0.96
	60	0.218 ± 0.0008	0.46
	80	0.282 ± 0.0041	1.78
	100	0.342 ± 0.0014	0.60
Interday	20	0.105 ± 0.0012	1.45
	40	0.163 ± 0.0032	0.96
	60	0.219 ± 0.0012	0.70
	80	0.282 ± 0.0012	0.54
	100	0.353 ± 0.0035	1.23

Table 7: Intraday and Interday precision of Sofosbuvir

	Concentration(µg/ml)	Mean ± SD (n = 3)	% RSD
Intraday	20	0.022 ± 0.0004	1.23
	40	0.070 ± 0.0016	1.45
	60	0.116 ± 0.0032	1.79
	80	0.172 ± 0.0020	1.46
	100	0.215 ± 0.0040	1.17
Interday	20	0.022 ± 0.0032	1.23
	40	0.071 ± 0.0012	1.79
	60	0.118 ± 0.0008	0.85
	80	0.172 ± 0.0024	1.74
	100	0.216 ± 0.0029	1.67

3. Accuracy:

Recovery tests were used to determine the method's accuracy. At 80 percent, 100 percent, and 120 percent, a known quantity of the pure drug was added to the pre-analysed sample formulation. The percentage recovery and percentage relative standard deviation of the percentage recovery of different formulations were determined and are shown in Table 8 (Amoxicillin trihydrate), Table 9 (Silodosin) and Table 10 (Sofosbuvir).

Table 8: Accuracy data for marketed formulations of Amoxicillin trihydrate

	Conc. From formulation (µg/ml)	% Spiked	Standard conc. Added (µg/ml)	Concentration recovered (µg/ml)	% Recovery	%RSD
Formulation 1 (DT)	60	80	48	47.37	98.70	0.37
	60	100	60	61.05	101.75	0.51
	60	120	72	71.77	99.69	0.64
Formulation 2 (Capsule 500mg)	60	80	48	47.92	99.84	0.21
	60	100	60	59.99	99.99	0.34
	60	120	72	73.26	101.76	0.18
Formulation 3 (Capsule 250mg)	60	80	48	47.99	99.99	0.37
	60	100	60	59.99	99.99	0.34
	60	120	72	73.17	101.63	0.22

Table 9: Accuracy data for marketed formulations of Silodosin

	Conc. From formulation (µg/ml)	% Spiked	Standard conc. Added (µg/ml)	Concentration recovered (µg/ml)	% Recovery	%RSD
Formulation 1	40	80	32	32.30	100.95	0.80
	40	100	40	39.67	99.19	0.21
	40	120	48	48.78	101.64	0.32
Formulation 2	40	80	32	32.45	101.41	0.22
	40	100	40	39.95	99.88	0.21
	40	120	48	48.19	100.4	1.23
Formulation 3	40	80	32	32.30	100.95	0.80
	40	100	40	40.23	100.58	1.06
	40	120	48	48.49	101.03	0.68

Table 10: Accuracy data for marketed formulation of Sofosbuvir

	Conc. From formulation (µg/ml)	% Spiked	Standard conc. Added (µg/ml)	Concentration recovered (µg/ml)	% Recovery	%RSD
Formulation 1	40	80	32	31.98	99.94	0.66
	40	100	40	40.31	100.78	0.33
	40	120	48	48.26	100.56	1.08

4. Analysis of the marketed formulation:

*The assay of **different formulations** was determined which falls within the acceptance criteria (98-102%) for Amoxicillin trihydrate (Table 11), Silodosin (*Table 12*) and Sofosbuvir (Table 13).*

Table 13

Table 11: Assay results for different marketed formulations of Amoxicillin trihydrate

Marketed Formulations	Concentration taken (µg/ml)	Concentration found (µg/ml)	% Recovery	% RSD
Formulation 1 (Dispersible tablets)	100	100.6	100.6	0.81
Formulation 2 (Capsule 500mg)	100	99.1	99.1	0.83
Formulation 3 (Capsule 250mg)	100	100.2	100.2	0.96

Table 12: Assay results for different marketed formulations of Silodosin

Marketed Formulations	Concentration taken (µg/ml)	Concentration found (µg/ml)	% Recovery	% RSD
Formulation 1	50	49.345	98.69	0.59
Formulation 2	50	49.95	99.9	1.70
Formulation 3	50	49.73	99.46	0.89

Table 13: Assay results for marketed formulation of Sofosbuvir

Marketed Formulations	Concentration taken (µg/ml)	Concentration found (µg/ml)	% Recovery	% RSD
Formulation 1	50	50.49	100.98	0.59

5. Specificity:

The specificity was done by using the blank and marketed formulation which having excipients and 100µg/ml solution was prepared from the marketed formulation. The specificity of the method is demonstrated in following Figure 11 for all the three drugs in which graphs shows the specific absorbance at 762 nm for each. Hence, it can be concluded that this method is specific.

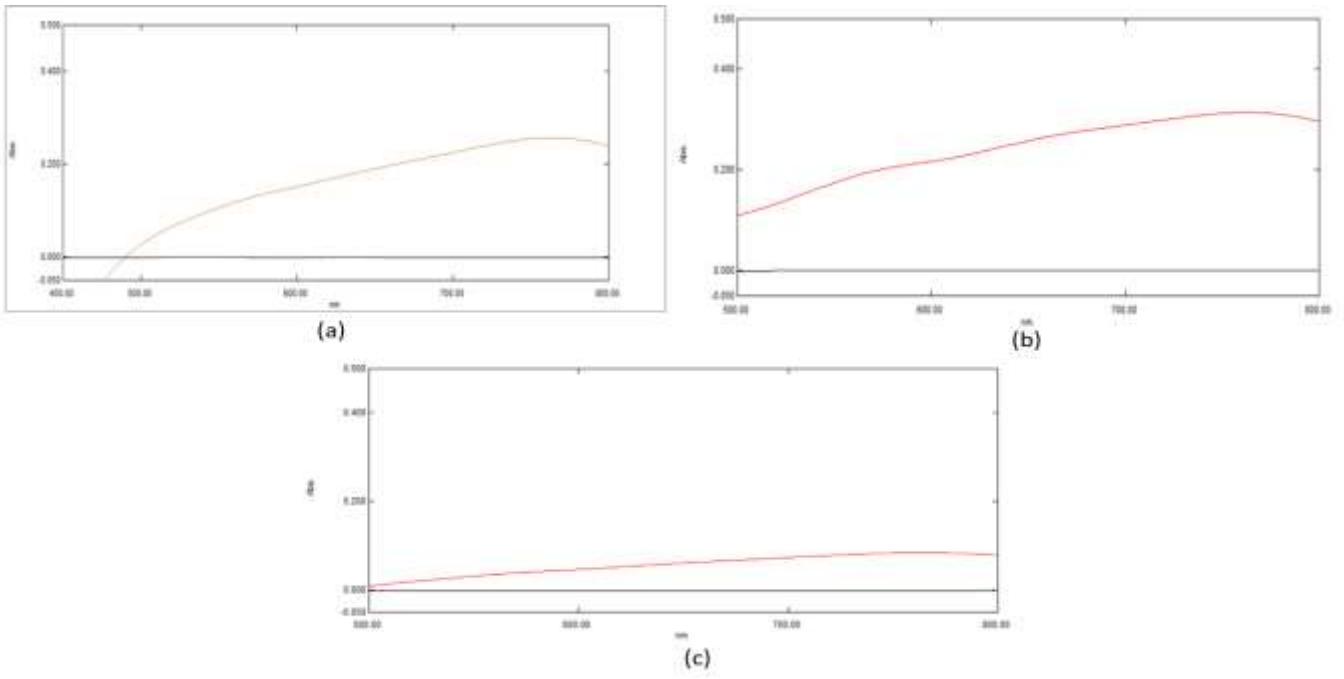

Figure 11: Specificity indicating graph of Amoxicillin trihydrate(a), Silodosin(b), Sofosbuvir

6. 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 14(Amoxicillin trihydrate),Table 15(Silodosin) and Table 16(Sofosbuvir).

Table 14: Method Ruggedness Result for Amoxicillin trihydrate

Parameter	Mean assay %	SD	%RSD
Lab 1	100.13	0.62	0.76
Lab 2	100.06		
Smartphone 1	99.75	0.46	0.57
Smartphone 2	100.32		

Table 15: Method Ruggedness Result for Silodosin

Parameter	Mean assay %	SD	%RSD
Lab 1	100.39	0.02	0.14
Lab 2	100.46		
Smartphone 1	100.46	0.04	0.07
Smartphone 2	100.55		

Table 16: Method Ruggedness Result for Sofosbuvir

Parameter	Mean assay %	SD	%RSD
Lab 1	100.49	0.18	0.46
Lab 2	100.25		
Smartphone 1	100.49	0.02	0.06
Smartphone 2	100.18		

Estimation of Amoxicillin trihydrate using Smartphone application:

By using PhotoMetrix PRO application the image was captured and according to concentration which shows the gradients of colours for all the three drugs (Figure 12). The linear regression equation was observed (Figure 13). Regression equation data of both methods for all the three drugs shown in Table 17.

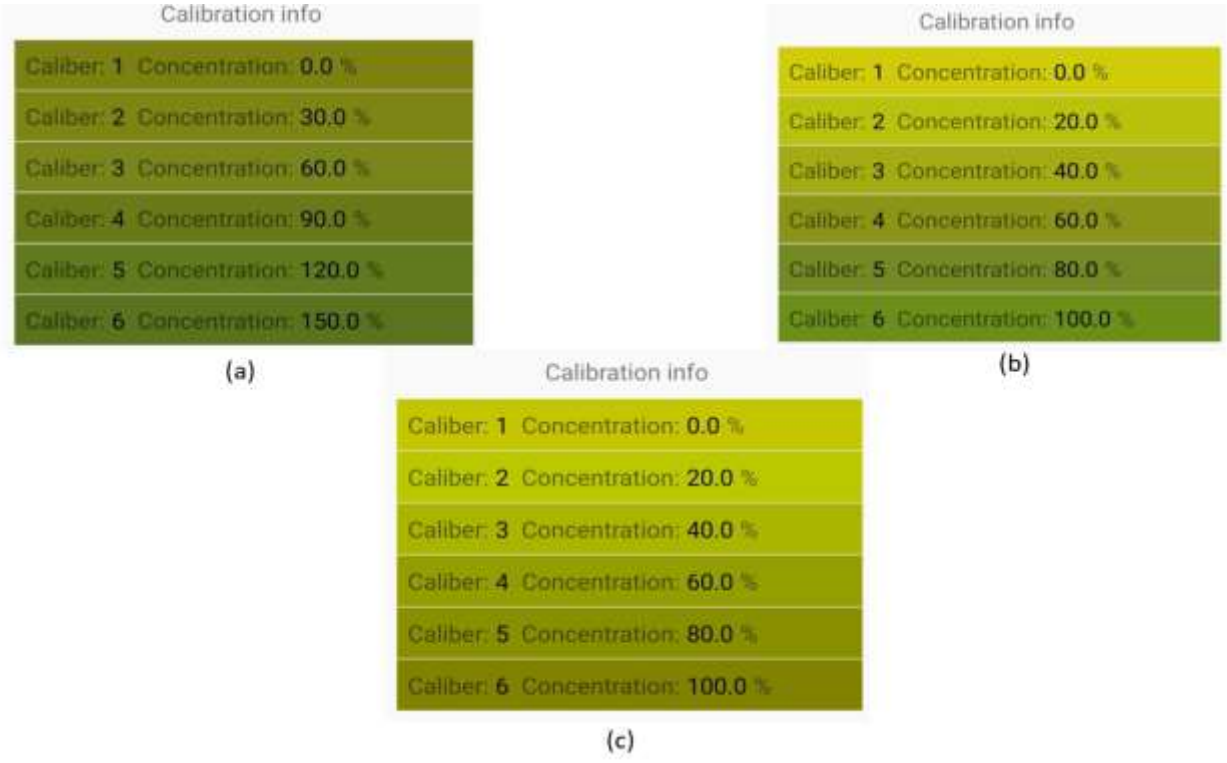

Figure 12: Chart of colour intensity corresponding to the concentration of amoxicillin trihydrate(a), Silodosin(b), Sofosbuvir(c)

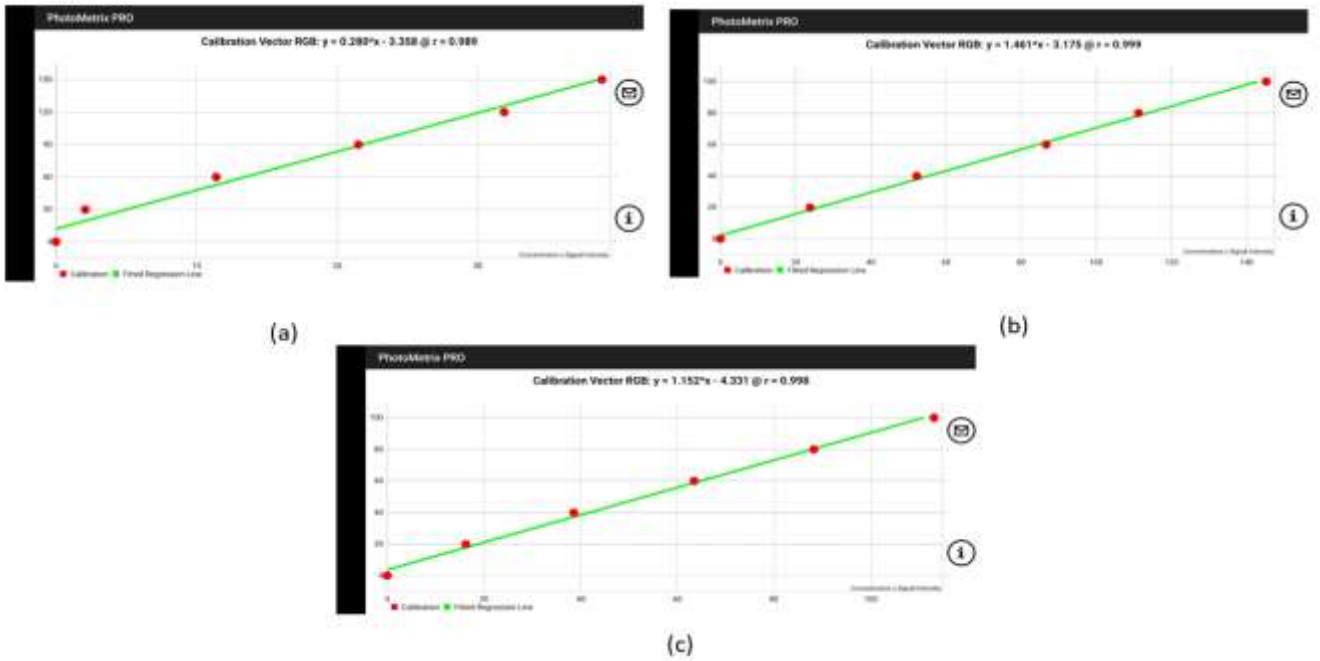

Figure 13: Calibration curve of the amoxicillin trihydrate(a), Silodosin(b), Sofosbuvir(c) by photometrix pro application

Table 17: Regression data for both UV and photometrix application

Parameter	Drugs	UV Method	Photometric application
Linearity(µg/ml)	Amoxicillin trihydrate	30-150	30-150
	Silodosin	20-100	20-100
	Sofosbuvir	20-100	20-100
Regression equation	Amoxicillin trihydrate	y=0.002x + 0.055	y=0.280x - 3.358
	Silodosin	y=0.003x + 0.0418	y=1.146x - 3.175
	Sofosbuvir	y=0.0024x + 0.0264	y=1.108x – 2.258
Slope	Amoxicillin trihydrate	0.002	0.280
	Silodosin	0.003	1.146
	Sofosbuvir	0.0024	1.108
Intercept	Amoxicillin trihydrate	0.055	3.358
	Silodosin	0.0418	3.175
	Sofosbuvir	0.0264	2.258
Correlation coefficient (R^2)	Amoxicillin trihydrate	0.998	0.989
	Silodosin	0.998	0.999
	Sofosbuvir	0.999	0.999
LOD(µg/ml)	Amoxicillin trihydrate	8.59	3.25
	Silodosin	0.57	0.69
	Sofosbuvir	4.52	2.38
LOQ(µg/ml)	Amoxicillin trihydrate	26.04	9.87
	Silodosin	1.73	2.09
	Sofosbuvir	13.72	7.22

The linearity of the standard amoxicillin trihydrate was taken in the range of 30-150 µg/ml, Silodosin and Sofosbuvir 20-100 µg/ml. The calibration curve and regression equation generated by the application was shown in the Figure 13. The % assay was found to be within the acceptance criteria.

Assay of formulation:

The assay was performed on the different marketed formulations 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 18.

Table 18: Assay results of different formulation for both the methods

		UV				Photometrix			
Drugs	**Formulations**	**Amount taken (µg/ml)**	**Amount recovered (µg/ml)**	**%Recovery**	**% RSD**	**Amount taken (µg/ml)**	**Amount recovered (µg/ml)**	**%Recovery**	**% RSD**
Amoxicillin trihydrate	1	100	100.6	100.6	0.81	100	99.33	99.33	1.52
	2	100	99.1	99.1	0.83	100	99.66	99.66	1.16
	3	100	100.1	100.1	0.60	100	99.33	99.33	1.52
Silodosin	1	50	49.345	98.69	0.59	50	49.34	98.69	0.59
	2	50	49.95	99.9	1.70	50	49.95	99.9	1.70
	3	50	49.73	99.46	0.89	50	49.73	99.46	0.89
Sofosbuvir	1	50	50.49	100.98	0.59	50	50.93	101.87	1.01

Statistical Comparison of two methods:

The obtained assay results from the PhotoMetrix 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 PhotoMetrix application can be used to identify Amoxicillin trihydrate, Silodosin as well as Sofosbuvir using colorimetry. Table 19 displays the information.

Table 19: Applied Pair t-Test Result

Parameters	**Drugs**	**UV method**	**Photometrix PRO**
Mean (X)	Amoxicillin trihydrate	99.92	99.9184
	Silodosin	50.398	49.5384
	Sofosbuvir	50.44	50.2726
Variance (s^2)	Amoxicillin trihydrate	0.337	0.501533
	Silodosin	0.63467	0.132223
	Sofosbuvir	0.112	0.044958
Observations (n)	Amoxicillin trihydrate	5	5
	Silodosin	5	5
	Sofosbuvir	5	5
Pearson Correlation	Amoxicillin trihydrate	-0.87532	
	Silodosin	0.153739	
	Sofosbuvir	-0.81117	
Hypothesized mean difference	Amoxicillin trihydrate	0	
	Silodosin	0	
	Sofosbuvir	0	
df	Amoxicillin trihydrate	4	
	Silodosin	4	
	Sofosbuvir	4	
t stat	Amoxicillin trihydrate	0.003907	
	Silodosin	2.194896	
	Sofosbuvir	0.944	
P (T<=t) one-tail	Amoxicillin trihydrate	0.498489	
	Silodosin	0.035298	
	Sofosbuvir	0.188106	
t Critical one-tail	Amoxicillin trihydrate	1.859548	
	Silodosin	1.94318	
	Sofosbuvir	1.894579	
P (T<=t) two-tail	Amoxicillin trihydrate	0.996978	
	Silodosin	0.070597	
	Sofosbuvir	0.376212	
t Critical two ail	Amoxicillin trihydrate	2.306004	
	Silodosin	2.446912	
	Sofosbuvir	2.364624	

CONCLUSION:

Ammonium metavanadate is reagent used as an oxidizing agent and it converts +5 to +3 oxidation state, here we utilizing its oxidation power as a colorimetric reagent which shows the significance colour change in presence of acidic medium and heat it gives the colour when reacting with compound containing sulphur, nitrogen and phosphorus atom in its structure. The smartphone-based PhotoMetrix PRO application is used to develop a novel and cost-effective colorimetric detection method for Amoxicillin trihydrate, Silodosin and Sofosbuvir. The approach relied on a basic colouring ingredient and a quick operation. The main aim of this work was to make the colorimetric measurement of drug content easier with the use of such smartphone-based applications. The approach was also compared to a UV method created using the same reagent and protocol, and no significant differences in assay results were identified. In quantitative drug estimate in pharmaceutical dosage forms, this unique method can be utilised as an alternative to analytical science.

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