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Research Article

New Smartphone based Colorimetric Method Development and validation for the Drugs containing Nitrogen, Sulphur and Phosphorus 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 their low cost and ability to collect, store, and interpret data all in one device, smartphone-based colorimetry has increased in popularity 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. Distinct oxidation states of ammonium metavanadate generate different colours depending on the oxidation state. The +5-oxidation state appears yellow, the +4-oxidation state appears blue, the +3-oxidation state appears green, and the +2-oxidation state appears purple. The ammonium metavanadate reagent is orange red in colour, but when it combines with pharmaceuticals that contain nitrogen, phosphorus, or sulphur in their structure, it turns green. The developed approach for all of the drugs in this article is linear. The colour intensity increases as the concentration of API increases. All of the photos were captured on a smartphone and analysed with photometrix PRO software. The photometrix PRO application turns an image to an RGB histogram, and it also includes regression models. The percent RSD for all three drugs was less than 2 employing Photometrix PRO and UV method. Using a statistical method called a two-paired test, the results reveal that both procedures are equally significant for all three drugs.

Keywords: UV spectrophotometry, Photometrix PRO, RGB Histogram, Sumatriptan Succinate, Gemifloxacin, Tenofovir disoproxil fumarate

INTRODUCTION

Sumatriptan Succinate (Sulphur Atom):

Triptans are a class of tryptamine-based drugs intended to relieve migraine headaches in the short term. One of them, sumatriptan succinate, is structurally similar to the neurotransmitter serotonin. Sumatriptan succinate (STS) is a 5-hydroxytryptamine (5-HT) receptor subtype with a low affinity for 5-HT1A, 5-HT5A, and 5-HT7 receptors. Sumatriptan Succinate is chemically designed as [3-[2-(Dimethylamino) ethyl]-1H-indol-5-yl]-N-methyl methane-sulfonamide hydrogen butanedioate. [1–3]

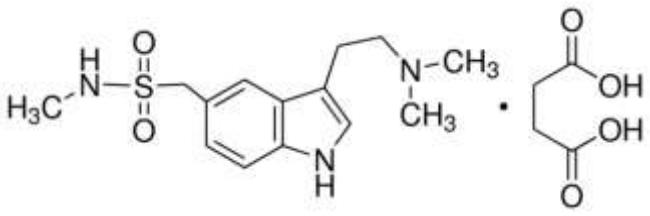


Figure 1: Structure of Sumatriptan Succinate[4]

Molecular weight: 413.5 g/mol

Molecular Formula: $C_{14}H_{21}N_3O_2S.C_4H_6O_4$

Solubility: Freely Soluble in Water (0.127 mg/ml)

STS works by selectively binding to serotonin type-1D receptors (serotonin agonists) to end a migraine episode while also removing accompanying symptoms like nausea, vomiting, and light and sound sensitivity. Sumatriptan operates as a vasoconstrictor on a serotonin (5-HT)1B/1D receptor, which is also found in peripheral arteries to a lesser level. [6]Sumatriptan also has a central inhibitory impact on the trigeminovascular system, which is engaged during migraine attacks. Triptans' potential mechanisms of action in migraine include cerebral vasoconstriction, decrease of neuropeptide and protein extravasation across dural arteries, and central inhibition of impulse transmission within the trigeminovascular system. [7,8]Triptans' major function in migraine, in our opinion, is to constrict dilated cranial extracerebral blood arteries, which is a 5-HT1B impact. When pure 5-HT1D receptor agonists have been produced and tested for efficacy in migraine, the potential contribution of triptans' neuronal impact can be assessed. [4,8]

Introduction to Gemifloxacin (Nitrogen Atom):

Gemifloxacin is a fourth generation, oral fluoroquinolones antibiotics used in the therapy of mild-to-moderate respiratory tract infections caused by susceptible organisms.

Gemifloxacin is a 1,4-dihydro-1,8-napthyridine with a carboxy group at the 3-position, an oxo substituent at the 4-position, a fluoro substituent at the 5-position and substituted pyrrolin-1-yl group at the 7-position. It is monocarboxylic acid, a 1,8-napthyridine derivative, a quinolone antibiotic and a fluoroquinolone antibiotic. [9]Like other fluoroquinolones, Gemifloxacin is active against a wide range of aerobic gram-positive and gram-negative organisms and is believed to act by inhibition of bacterial DNA gyrase and topoisomerase IV that are required for synthesis of bacterial mRNAs (transcription) and DNA replication. In contrast, DNA gyrase are not present in human cells and the equivalent topoisomerases are not sensitive to fluoroquinolone inhibition. Gemifloxacin was approved for use in the United States in 2003 and has not been as commonly used as other fluoroquinolones such as ciprofloxacin and levofloxacin.[10,11]

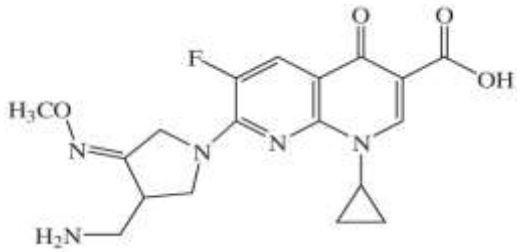


Figure 2: Structure of Gemifloxacin[10]

Molecular weight: 398.381 g/mol

Molecular Formula: $C^{18}H_2OFN^5O^4$

Solubility: Freely Soluble in neutral pH (350 mg/ml at 37°C, pH 7.0)

Current indications are limited to acute exacerbations of chronic bronchitis and community acquired pneumonia. Gemifloxacin is available under the commercial name Factive in 320 mg tablets. The recommended dose is 320 mg once daily for 5 to 7 days. Common side effects include diarrhoea, nausea, abdominal pain, headaches, skin rash and allergic reactions.[10,11,13]

Introduction to Tenofovir disoproxil fumarate (Phosphorus Atom):

Tenofovir disoproxil fumarate {9-[(R)-2-[[bis [[isopropoxycarbonyl] oxy] methoxy] phosphonyl] popyl] adenine fumarate} is a nucleotide analog reverse transcriptase inhibitor (NRTI) and is used for treating HIV infection in adults, in combination with other anti-retroviral agents. Due to the presence of a phosphonate group, tenofovir is negatively charged at neutral pH, which limits its oral bioavailability. During drug development, attention switched to the phosphonate ester derivative. Tenofovir disoproxil, which was the subject of extensive process chemistry to provide a viable manufacturing route. Tenofovir disoproxil, sold under the trade name Viread among others, is a medication used to treat chronic hepatitis B and to prevent and treat HIV/AIDS.[14,15]

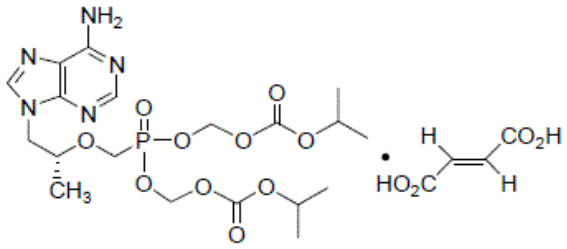


Figure 3: Structure of Tenofovir Disoproxil Fumarate[16]

Molecular weight: 287.213 g/mol

Molecular Formula: $C^{23}H^{34}N^5O^{14}P$

Solubility: Freely Soluble in Dimethyl Sulfoxide and Water, sparingly soluble in methanol.

Tenofovir disoproxil is a nucleotide analog reverse-transcriptase inhibitor (NtRTI). It selectively inhibits viral reverse transcriptase, a crucial enzyme in retroviruses such as human immunodeficiency virus (HIV), while showing limited inhibition of human enzymes, such as DNA polymerases α, β, and mitochondrial DNA polymerase Y. In vivo tenofovir disoproxil fumarate is converted to tenofovir, an acyclic analog of deoxyadenosine 5' – monophosphate (dAMP). Tenofovir lacks a hydroxyl group in the corresponding to the 3' carbon of the dAMP, preventing the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation. Common side effects include nausea, rash, diarrhoea, headache, pain, depression, and weakness. Severe side effects include high blood lactate and an enlarged liver.[17]

Introduction to Colorimetric Analysis based on Smartphone Application

A light source, a monochromator, a photometer, an eyepiece for monitoring the photometric field, and a sample holder are the most common instruments used for work in the visual region. The holder is either a cell for measuring liquid transmission or a device for supporting a ran opaque object on which reflection measurements are to be taken. Unlike chemists' "colorimetric" conclusions, spectrophotometric measurements are not limited to coloured systems. Photographic methods were utilised for many years to determine absorption spectra in the ultraviolet and infrared regions of the spectrum.[18,19]

The fundamental data of a spectrophotometer indicates the proportion of incident light on a sample that is reflected or transmitted by it. A single value for a certain wavelength can be produced, or values for the complete visible range can be computed. In the latter cases, the findings are typically shown as a curve, with transmission or reflection as the ordinates and wavelength as the abscissas. [18]The question of what wavelength interval to use to identify individual points and what spectral band width to utilise for the light source arises when building a curve that encompasses a portion or all of the visible range. If the slope is steep and features small sharp millimicrons with the narrowest spectral band possible.[18]

Colorimetric analysis is a useful technique for determining the concentration of a coloured material in a solution. Coloured compounds absorb visible light, and the amount of light absorbed is proportional to the concentration of the substance in solution.[20] The light source in colorimetric chemistry analysers is a tungsten halogen bulb. The lamp must be modified with filters or a monochromator to get the desired wavelength.[21]

Color changes recorded with Smartphone-based sensors are gaining popularity in chemical research due to their ease of use and flexibility to portable equipment[9]. 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 mobile camera serves as the detector.[22]

There are numerous smartphone-based colorimetric applications available. Photo Metric-PRO is one among them. Photo Metrix PRO could be downloaded for free from the Windows Phone Store and the Google Play Store. This programme uses simple linear correlation for univariate analysis and principal components analysis for multivariate exploratory analysis (PCA). The smartphone camera captures

visual data, which is then translated into RGB histograms (red, green and blue).[23–25]

The RGB colour model is based on the colour perception theory, which states that the human eye has different sensitivity peaks located around red, green, and blue. Multivariate analysis could be employed in this software to increase the RGB colour system applicability of colorimetry.[24]

Colorimetry is a technique used in biological research to calculate the quantitative value of colours. Color is produced when a substance binds with color-forming chromogens. Differences in colour intensity resulted in variations in light absorption.[20,26,27]

The intensity of the colour is related to the concentration of the material being tested. The wavelength of visible light in the electromagnetic spectrum ranges from 400 nm to 800 nm. [27]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 selecting one for colorimetric analysis.[25]

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 Sumatriptan Succinate, Gemifloxacin and Tenofovir Disoproxil Fumarate. As a colouring component, ammonium metavanadate[28–31] reacts with sumatriptan succinate, Gemifloxacin and Tenofovir Disoproxil Fumarate to generate green colour. The data image was acquired and analysed by the Photo Metrix-PRO application.

STS (Sumatriptan Succinate) has official monographs in BP (British Pharmacopoeia, 2009) and EP (European Pharmacopoeia, 2005), which describe liquid chromatographic methods for STS assay, as well as USP (The United States Pharmacopoeia, 2004), which describes a high-performance liquid chromatographic (HPLC) method for its determination.[32,33] A review of the literature finds that few analytical methods, such as high-performance liquid chromatography (HPLC)[24] and liquid chromatographic-mass spectrometry, have been reported for the analysis of STS in biological fluids.

EXPERIMENTAL:

- **Chemicals and reagents:**

5% Ammonium Metavanadate, Sumatriptan Succinate, Gemifloxacin, Tenofovir disoproxil fumarate, Double Distilled Water, 40% H_2SO_4.

- **Apparatus and Applications:**

The Sumatriptan Succinate, Gemifloxacin, and Tenofovir disoproxil fumarate API samples were weighed on an electronic balance (Ax120) (Shimadzu). Smartphone camera and uploaded to the mobile (Photometrix PRO) Application.

- **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 10 mg of Sumatriptan Succinate, Gemifloxacin and Tenofovir disoproxil fumarate and transferred into a previously calibrated 10ml volumetric flask. The final volume was made up to the mark using double distilled to obtain the standard stock solution of 1000µg/ml concentration.

Method development:

Uv-Vis Spectroscopy:

Selection of wavelength for Sumatriptan Succinate, Gemifloxacin and Tenofovir Disoproxil Fumarate:

Using Ammonium Metavanadate as a blank, the drug solution was scanned across the range 400-800 nm. Sumatriptan Succinate, Gemifloxacin and Tenofovir disoproxil fumarate was found to have an absorbance of 762 nm. Prepare a calibration curve using the working solution, ranging from 50-250 µg/ml for Sumatriptan Succinate and Gemifloxacin while 30-120 µg/ml and construct a linear regression equation.

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 in ammonium metavanadate. The reaction for oxidation of sumatriptan 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 sumatriptan succinate, Gemifloxacin and Tenofovir disoproxil fumarate it forms green colour complex

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

Method Optimization:

Optimization of reagent concentration:

Ammonium metavanadate was allowed to react with sumatriptan succinate to form a green colour with absorption maxima at 762 nm, by keeping another parameter constant. The optimization of the experiment was established by varying the concentration of reagent in the range of 2.5% - 20%, where, maximum absorbance of reagent was found at 5% as shown in Table 1.

Table 1: Optimization of reagent concentration

Sr.No.	Concentration	Observation
1	2.5%	No stable colour changes.
2	**5%**	**Stable colour changes.**
3	10%	No Stable colour changes.
4	15%	Reagent got Saturated (Dark red colour)
5	20%	Reagent doesn't dissolve.

Optimization of reagent volume for Sumatriptan Succinate:

The effect of reagent volume was studied in a range of 1 to 6 millilitres. The volume was tuned based on the green colour complex and the absorbance maxima. The absorbance increases with increasing reagent volume until 4ml, after which it decreases, hence 4ml was chosen for the procedure as shown in Table 2.

Table2: Optimization of reagent volume for Sumatriptan Succinate

Percent of Reagent (%)	Absorbance (nm)
1%	0.090 nm
2%	0.117 nm
3%	0.153nm
4%	**0.341nm**
5%	0.207nm
6%	0. 132nm

Optimization of reagent volume for Gemifloxacin:

The influence of reagent volume was investigated in a range of 1 to 6 mL. The optimal volume was chosen observing the green colour complex and absorbance maxima. As the volume of reagent was increased up to 4ml, the absorbance fell, so 4ml of reagent was chosen for the procedure as indicated in Table 3.

Table 3: Optimization of reagent volume for Gemifloxacin

Percent of Reagent (%)	Absorbance (nm)
1%	0.113
2%	0.153
3%	0.201
4%	**0.240**
5%	0.234
6%	0.229

Optimization of regent volume for Tenofovir Disoproxil Fumarate:

In a range of 1 to 6 mL, the effect of reagent volume was examined. The green colour complex and absorbance maxima were used to determine the appropriate volume. The absorbance decreased when the reagent volume was raised up to 4ml, hence 4ml of reagent was chosen for the experiment as shown in Table 4.

Table 4: Optimization of reagent volume for Tenofovir Disoproxil Fumarate

Percent of Reagent (%)	Absorbance (nm)
1%	0.112
2%	0.135
3%	0.152
4%	**0.179**
5%	0.169
6%	0.161

Optimization of reaction time for Sumatriptan Succinate:

The influence of reaction time was studied for 10 to 50 minutes. Between 10 and 50 minutes was recorded for the colour complex reaction. At 30 minutes, there was a little increase in colour intensity.

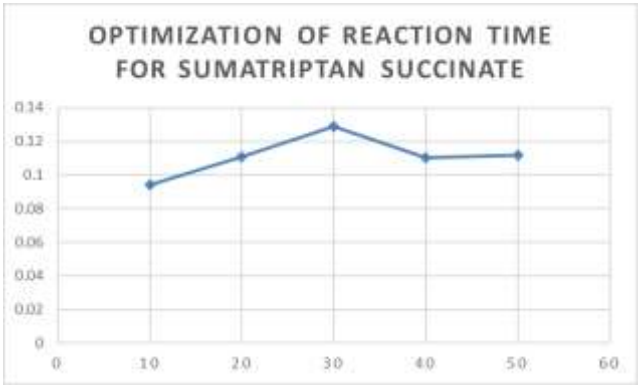


Figure 4: Optimization of reaction time for Sumatriptan Succinate

Optimization of reaction time for Gemifloxacin:

The influence of reaction time was studied for 10 to 50 minutes. Between 10 and 50 minutes was recorded for the colour complex reaction. At 30 minutes, there was a little increase in colour intensity.

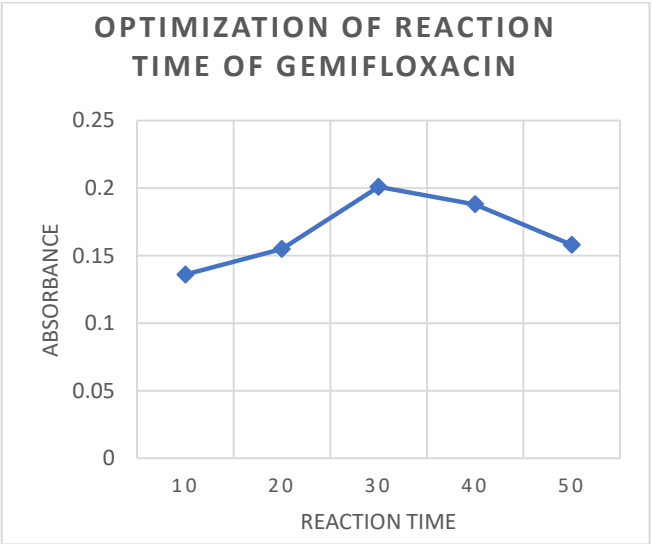


Figure 5: Optimization of reaction time for Gemifloxacin

Optimization of reaction time for Tenofovir Disoproxil Fumarate:

The influence of reaction time was studied for 10 to 50 minutes. Between 10 and 50 minutes was recorded for the colour complex reaction. At 30 minutes, there was a little increase in colour intensity.

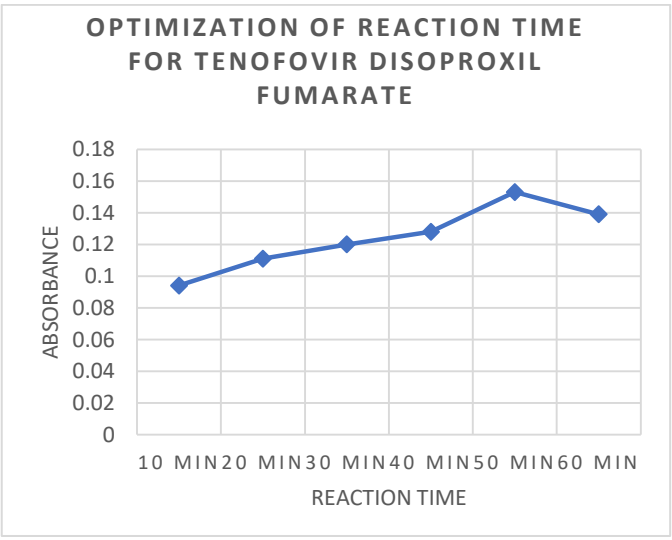


Figure 6: Optimization of reaction time for Tenofovir Disoproxil Fumarate

Preparation of Calibration graph for Sumatriptan Succinate, Gemifloxacin and Tenofovir Disoproxil Fumarate:

- Take a 10 mg of Sumatriptan Succinate, Gemifloxacin and Tenofovir disoproxil fumarate. Transfer and dissolve it with Double distilled water in 10 ml Volumetric flask to prepare 3 stock solution of 1000 PPM of drugs mentioned above.

- Prepare different aliquots from Stock solutions in 10ml Volumetric flask to obtain solutions from 50 to 250 PPM range for Sumatriptan Succinate and Gemifloxacin, While for Tenofovir disoproxil fumarate 30-150 PPM range was selected.
- Then add 4ml of freshly prepared 5 % Ammonium Metavanadate reagent and Heat on the Water bath for 30 minutes.
- Take an absorbance. Here, we got λmax was found at 762 nm.
- Calibration curve was plotted using working standard solutions of all three drugs by plotting absorbance at 762 nm vs. Concentration.

Estimation of Sumatriptan Succinate Using Smartphone Application:

Experimental Setup:

As indicated in Figure 8, the coloured solution was transferred into a slandered glass cuvette that was put in an 18cm x 18cm white box with a 6W LED (Light Emitting Diode) bulb to adjust the intensity during the experiment.

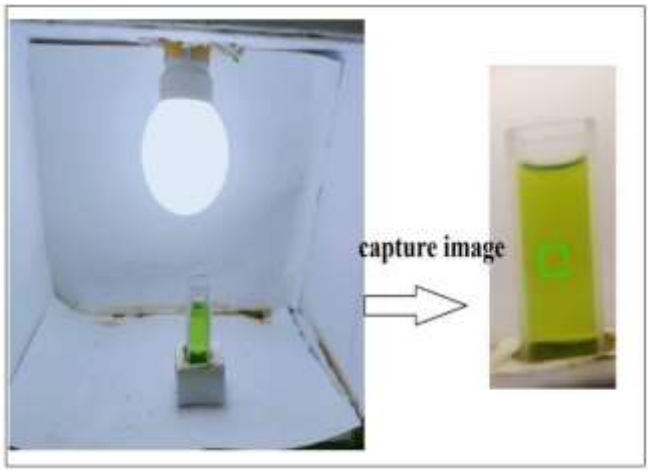


Figure 7: Experimental Set up

A smartphone image of a colour complex solution was obtained and analysed using a photometric tool to calculate the image's red-green-blue intensities (RGB scale). A linear regression equation was used to estimate the concentration of the image captured by Photometrix PRO. Photometrix generates and analyses RGB colour histograms before converting them to a calibration curve. This programme processes and shows the findings using univariate and multivariate analysis. Many different smartphone kinds were employed to achieve the best results. The methods for using the Photometrix application are shown in Figure 9.

Figure 8: 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 50-250 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.

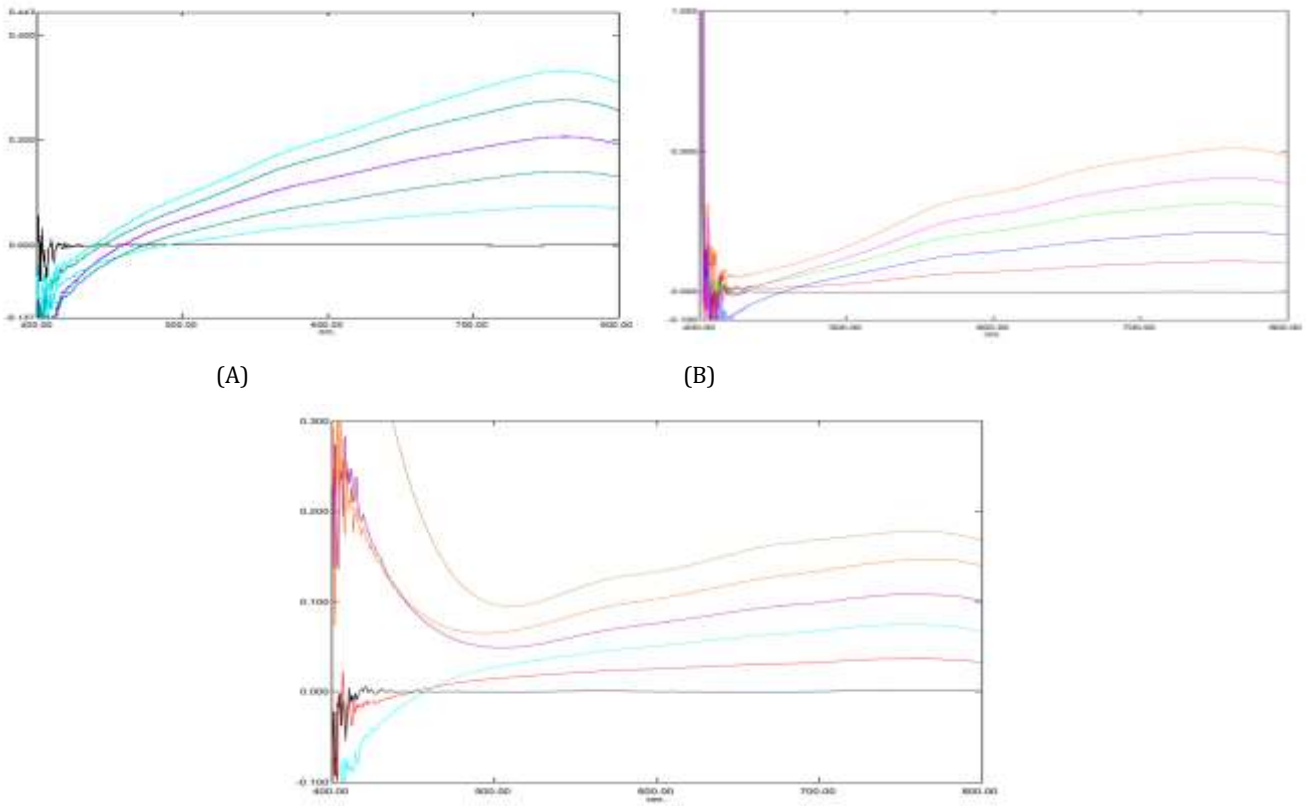
RESULT AND DISCUSSION:

Method Validation[34]:

The UV-visible spectrophotometry and PhotoMetrix applications were validated individually in terms of linearity and robustness, according to validation requirements. A formulation assay was performed for both techniques. Excellent linearity Sumatriptan Succinate and Gemifloxacin was recorded in the range of 50-250 µg/ml under ideal conditions, while Tenofovir Disoproxil Fumarate was reported in the range of 30-150 µg/ml. The concentration of tablet formulation was determined using a regression equation in UV-vis spectrophotometry, whereas photometrix was calculated within the programme.

1. Linearity:

By following Beer's law, Sumatriptan Succinate and Gemifloxacin were linear with concentrations ranging from 50 to 250 µg/ml at 762 nm, while Tenofovir disoproxil fumarate was linear with concentrations ranging from 30-150 µg/ml at 762 nm (Figure 10). Between concentration and absorbance, a calibration curve was produced. It was discovered that the plot was linear (Figure 11).



(A) (B)

(C)

Figure 9: Linearity of Sumatriptan Succinate (A), Gemifloxacin (B), Tenofovir Disoproxil Fumarate (C)

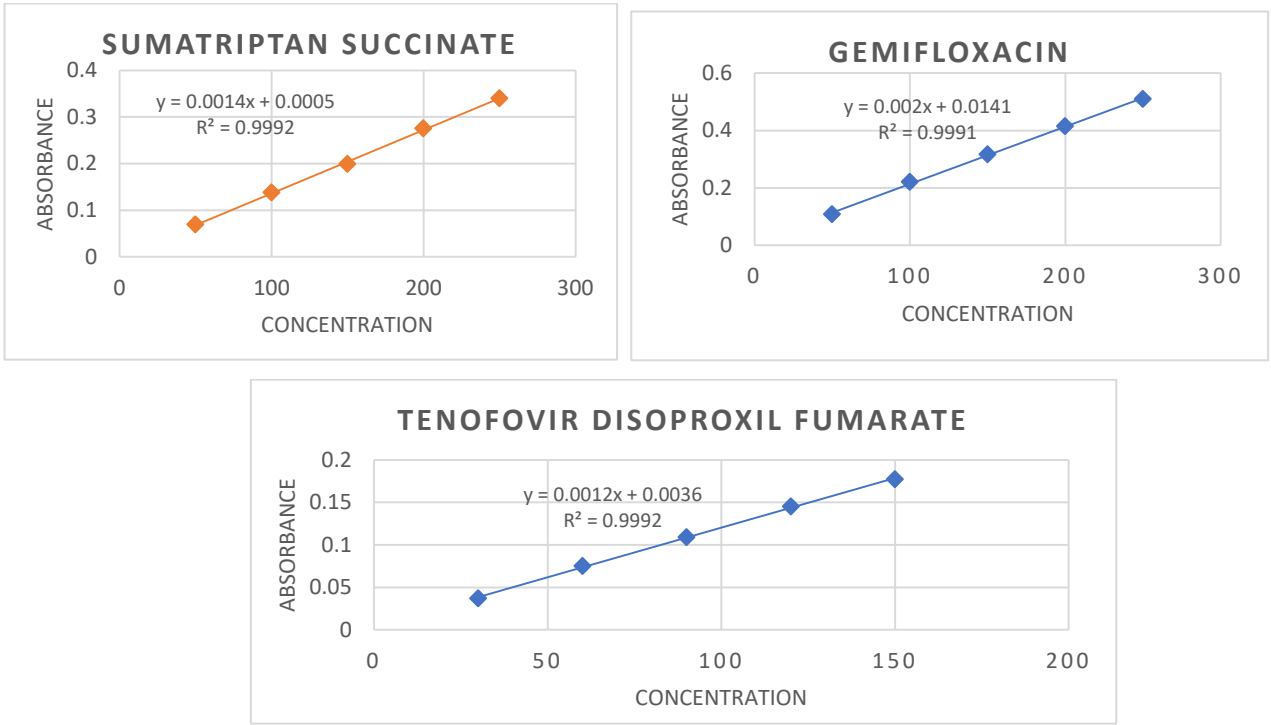


Figure 10: Calibration graph for Sumatriptan Succinate, Gemifloxacin, Tenofovir Disoproxil Fumarate

2. Precision:

The degree of agreement between a set 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 both drugs' lowest, upper, and middle limits were taken and analysed three times on the same day for intra-day precision and three times on three different days for inter-day precision. It was established that the % RSD was less than 2.

Table 5: Interday and Intraday Precision data for Sumatriptan Succinate

	Conc. (µg/ml)	Set 1	Set 2	Set 3	Mean ± SD	%RSD
Intra Day	50 (µg/ml)	0.075	0.076	0.074	0.075 ± 0.001	1.33%
	100 (µg/ml)	0.141	0.142	0.140	0.141 ± 0.0009	0.71%
	150 (µg/ml)	0.206	0.206	0.208	0.206 ± 0.0011	0.56%
	200 (µg/ml)	0.275	0.276	0.277	0.276 ± 0.0008	0.36%
	250 (µg/ml)	0.332	0.334	0.337	0.334 ± 0.002	0.75%
Inter Day	50 (µg/ml)	0.075	0.073	0.074	0.074 ± 0.001	1.35%
	100 (µg/ml)	0.141	0.144	0.145	0.143 ± 0.002	1.45%
	150 (µg/ml)	0.206	0.207	0.204	0.205 ± 0.001	0.74%
	200 (µg/ml)	0.276	0.279	0.277	0.277 ± 0.001	0.55%
	250 (µg/ml)	0.341	0.337	0.332	0.336 ± 0.003	1.34%

Table 6: Interday and Intraday Precision data for Gemifloxacin

	Conc. (µg/ml)	Set 1	Set 2	Set 3	Mean ± SD	%RSD
Intra Day	50 (µg/ml)	0.108	0.109	0.111	0.109 ± 0.001	1.40%
	100 (µg/ml)	0.212	0.215	0.217	0.214 ± 0.002	1.17%
	150 (µg/ml)	0.316	0.314	0.316	0.315 ± 0.0009	0.37%
	200 (µg/ml)	0.403	0.415	0.417	0.411 ± 0.006	1.84%
	250 (µg/ml)	0.510	0.516	0.507	0.511 ± 0.004	0.90%
Inter Day	50 (µg/ml)	0.108	0.110	0.111	0.109 ± 0.002	1.39%
	100 (µg/ml)	0.212	0.217	0.212	0.213 ± 0.002	1.35%
	150 (µg/ml)	0.316	0.319	0.319	0.318 ± 0.001	0.54%
	200 (µg/ml)	0.413	0.417	0.420	0.416 ± 0.007	0.84%
	250 (µg/ml)	0.510	0.511	0.513	0.511 ± 0.001	0.30%

Intraday precision and Interday precision data of Tenofovir Disoproxil Fumarate

Table 71: Interday and Intraday Precision data for Tenofovir Disoproxil Fumarate

	Conc. (µg/ml)	Set 1	Set 2	Set 3	Mean ± SD	%RSD
Intra Day	30 (µg/ml)	0.037	0.038	0.037	0.037 ± 0.0005	1.55%
	60 (µg/ml)	0.075	0.077	0.076	0.076 ± 0.001	1.32%
	90 (µg/ml)	0.109	0.113	0.110	0.110 ± 0.002	1.88%
	120 (µg/ml)	0.147	0.144	0.145	0.145 ± 0.001	1.05%
	150 (µg/ml)	0.177	0.180	0.178	0.178 ± 0.001	0.86%
Inter Day	30 (µg/ml)	0.037	0.036	0.037	0.036 ± 0.005	1.57%
	60 (µg/ml)	0.075	0.077	0.076	0.076 ± 0.001	1.32%
	90 (µg/ml)	0.112	0.114	0.113	0.113 ± 0.001	0.88%
	120 (µg/ml)	0.145	0.142	0.147	0.144 ± 0.002	1.74%
	150 (µg/ml)	0.177	0.181	0.179	0.179 ± 0.002	1.12%

3) Accuracy:

The Accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analysed sample formulation at 80%, 100% and 120% levels. The recovery studies were carried out and percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and given in Table: 8.

Table 8: Accuracy data for formulation of Sumatriptan Succinate (Tablet)

Drug	Standard Concentration (µg/ml)	% Spiked	Conc. Added From formulation (n=3) (µg/ml)	Conc. Recoverd (n=3)	%recovery ± SD (n=3)	%RSD
Sumatriptan Succinate	100 (µg/ml)	80%	80 (µg/ml)	179.58	99.72 ± 0.5	0.40%
	100 (µg/ml)	100%	100 (µg/ml)	199.73	99.86 ± 0.3	0.96%
	100 (µg/ml)	120%	120 (µg/ml)	222.31	101.04 ± 0.8	1.02%

Table 92: Accuracy data for Gemifloxacin Tablet Formulation

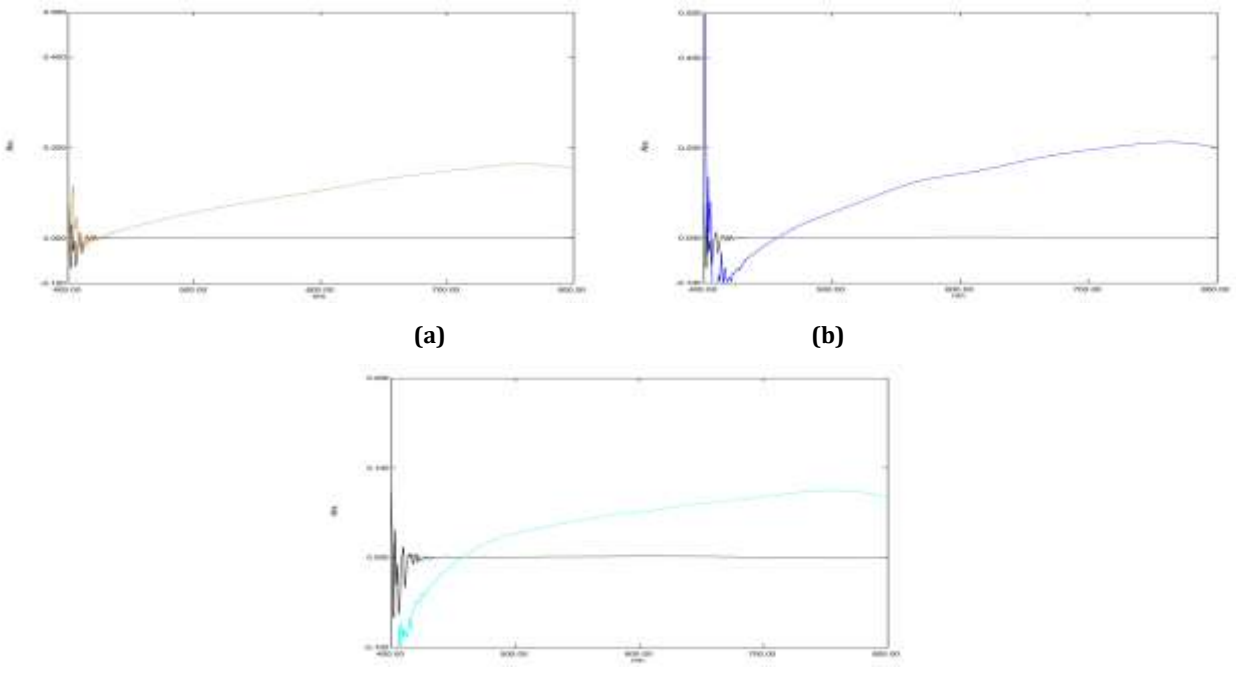
Drug	Standard Concentration (µg/ml)	% Spiked	Conc. Added From formulation (n=3) (µg/ml)	Conc. Recoverd (n=3)	%recovery ± SD (n=3)	%RSD
Gemifloxacin	100 (µg/ml)	80%	80 (µg/ml)	179.85	99.44 ± 0.2	0.14%
	100 (µg/ml)	100%	100 (µg/ml)	199.85	99.92 ± 0.7	0.36%
	100 (µg/ml)	120%	120 (µg/ml)	220.35	100.15 ± 0.8	0.37%

Table10: Accuracy data for Tenofovir Disoproxil Fumarate

Drug	Standard Concentration (µg/ml)	% Spiked	Conc. Added From formulation (n=3) (µg/ml)	Conc. Recoverd (n=3)	%recovery ± SD (n=3)	%RSD
Gemifloxacin	60 (µg/ml)	80%	80 (µg/ml)	107.86	99.81 ± 0.4	0.41%
	60 (µg/ml)	100%	100 (µg/ml)	119.56	99.58 ± 0.3	0.28%
	60 (µg/ml)	120%	120 (µg/ml)	130.27	98.63 ± 0.9	0.93 %

4) Specificity:

The blank and marketed formulations with excipients were used to determine specificity, and a 100 µg/ml solution was generated from the marketed formulation. The specificity of the approach is proven in the graph below, which displays the specific absorbance of sumatriptan succinate at 762 nm. As a result, we can conclude that this strategy is unique.



(a) **(b)**

(C)

Figure 11: Specificity indicating graph of Sumatriptan Succinate (a), Gemifloxacin (b), Tenofovir Disoproxil Fumarate (c)

5) Ruggedness of method:

The developed method's robustness was investigated in two labs and with two distinct cellphones. As indicated in Table 11, the percent RSD for both of these parameters is less than 2 (Sumatriptan Succinate) Table 12 (Gemilfoxacin) Table 13 (Tenofovir disoproxil fumarate)

Table 11: Ruggedness data for Sumatriptan Succinate

Parameter	Mean assay%	SD	%RSD
Lab 1	99.98	0.06	0.07%
Lab 2	100.02		
Smartphone 1	100.16	0.04	0.05%
Smartphone 2	100.19		

Table 12: Ruggedness data for Gemifloxacin

Parameter	Mean assay%	SD	%RSD
Lab 1	99.65	0.02	0.03%
Lab 2	101.23		
Smartphone 1	100.63	0.03	0.03%
Smartphone 2	99.52		

Table 13: Ruggedness data for Tenofovir Disoproxil Fumarate

Parameter	Mean assay%	SD	%RSD
Lab 1	99.96	0.09	0.10%
Lab 2	100.89		
Smartphone 1	101.45	0.03	0.04%
Smartphone 2	99.86		

Estimation of Sumatriptan Succinate using Smartphone application:

The image was obtained using the PhotoMetrix PRO application and sorted by concentration, revealing colour gradients for all three drugs (Figure 13). It was discovered the linear regression equation (Figure 14). Table 17 shows the regression equation data for both methods for all three drugs.

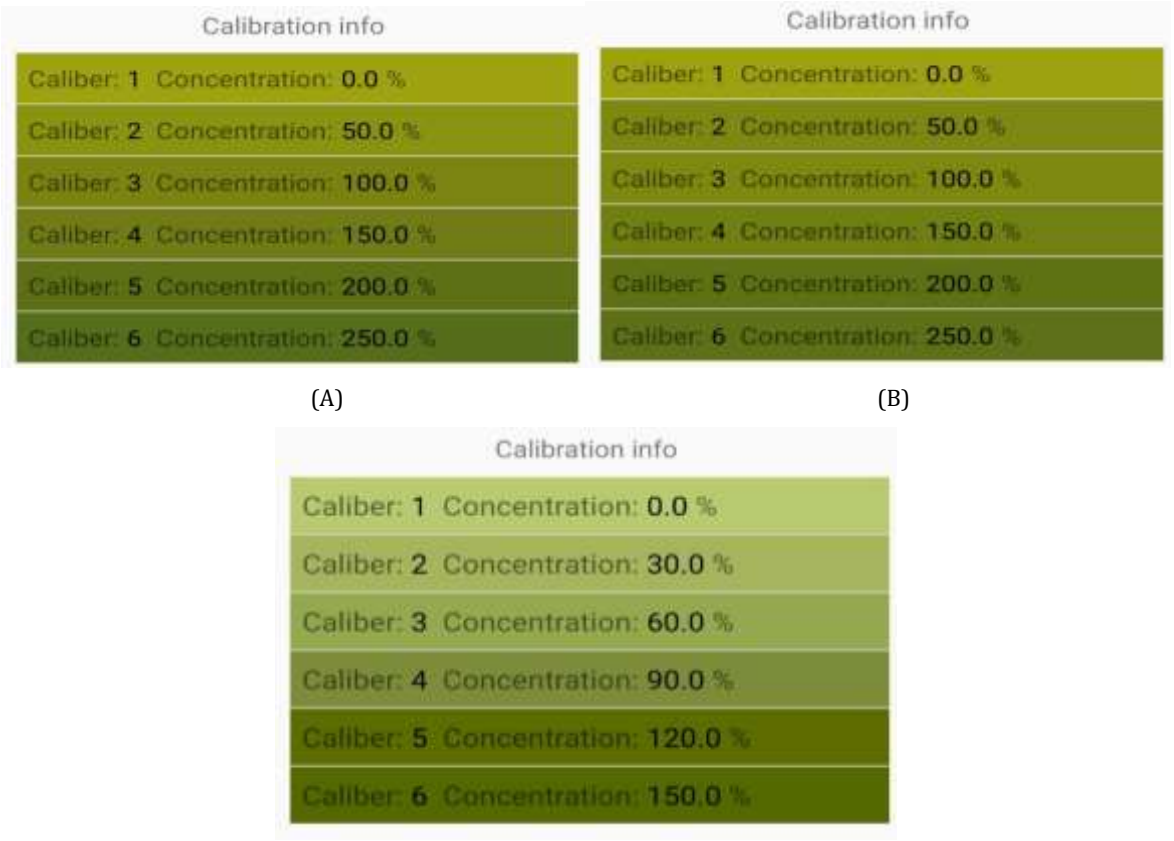
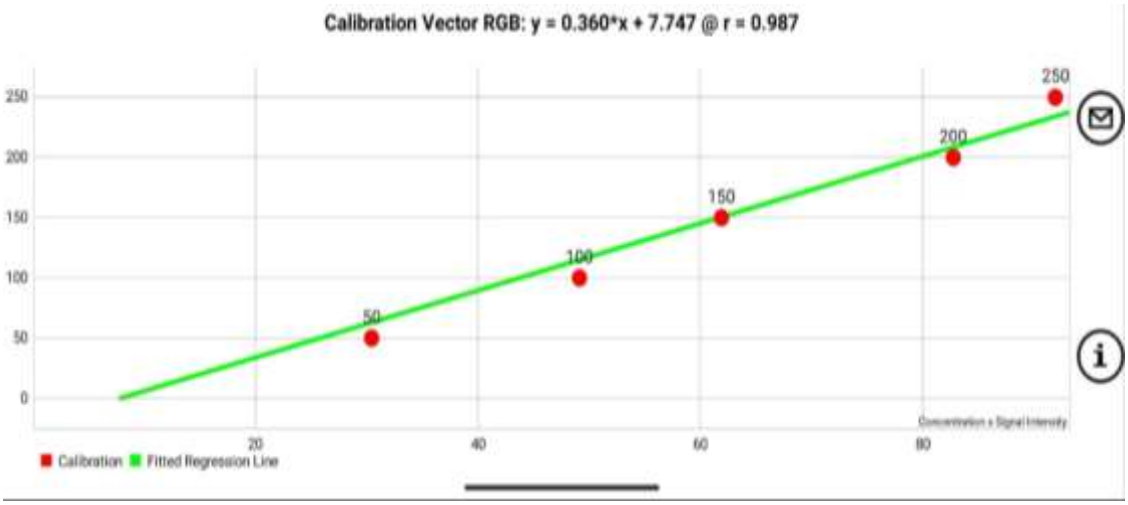
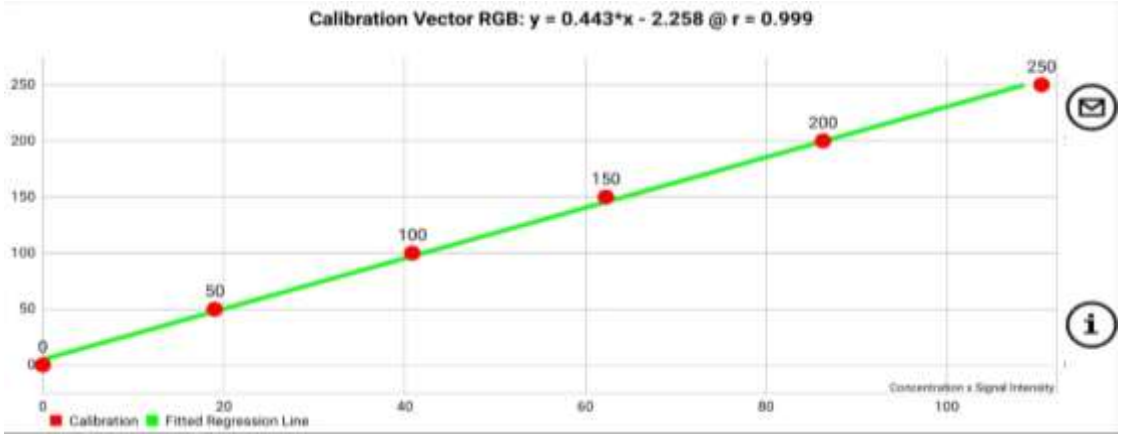


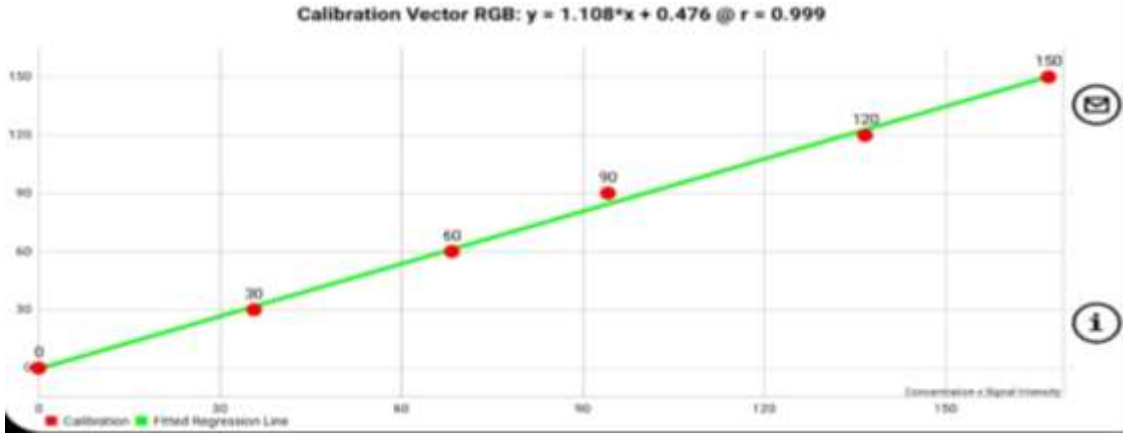
Figure 12: Chart of colour intensity corresponding to the concentration of Sumatriptan Succinate(A), Gemifloxacin (B) and Tenofovir Disoproxil Fumarate(C)



(A)



(B)



(C)

Figure 13: Calibration curve of the Sumatriptan Succinate (A), Gemifloxacin (B) and Tenofovir disoproxil fumarate(C) by Photometrix pro application

Table 143: Regression data for both UV and Photometrix application

Parameter	Drugs	UV Method	Photometric application
Linearity (µg/ml)	Sumatriptan Succinate	50-250	50-250
	Gemifloxacin	50-250	50-250
	Tenofovir Disoproxil Fumarate	30-150	30-150
Regression equation	Sumatriptan Succinate	Y= 0.0013x + 0.0113	Y = 0.360x + 7.747
	Gemifloxacin	Y= 0.002x + 0.0141	Y = 0.443x – 2.258
	Tenofovir Disoproxil Fumarate	Y=0.0012x + 0.0036	Y = 1.108x + 0.476
Slope	Sumatriptan Succinate	0.0013	0.360
	Gemifloxacin	0.002	0.443
	Tenofovir Disoproxil Fumarate	0.0012	1.108
Intercept	Sumatriptan Succinate	0.0113	7.747
	Gemifloxacin	0.0141	2.258
	Tenofovir Disoproxil Fumarate	0.0036	0.476
Correlation Coefficient	Sumatriptan Succinate	0.999	0.987
	Gemifloxacin	0.999	0.999
	Tenofovir Disoproxil Fumarate	0.999	0.999
LOD (µg/ml)	Sumatriptan Succinate	3.5	4.99
	Gemifloxacin	8.25	9.65
	Tenofovir Disoproxil Fumarate	2.75	3.45
LOQ (µg/ml)	Sumatriptan Succinate	11	15.12
	Gemifloxacin	25	28.95
	Tenofovir Disoproxil Fumarate	8.26	10.35

The linearity of Sumatriptan succinate and Gemifloxacin was measured between 50 and 250 g/ml, while Tenofovir Disoproxil Fumarate was measured between 30 and 150 g/ml. Figure 11 depicts the calibration curve and regression equation obtained by the application.

Assay of formulation:

Both methodologies were used to perform the analysis on the marketed formulation. The concentration of sample solutions was approximated as a percent Recovery from a linear regression equation. For both approaches, the assay findings were found to be within an acceptable range and significant. Table 15 shows the results of the assays.

Table15: 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**
Sumatriptan	1	100	99.82	99.82	0.14%	100	99.35	99.35	0.11%
	2	100	99.10	99.10	0.23%	100	98.65	98.65	0.19%
	3	100	100.1	100.1	0.36%	100	99.56	99.56	0.65%
Gemifloxacin	1	100	99.73	99.73	0.08%	100	99.16	99.16	0.37%
	2	100	99.85	99.85	0.06%	100	99.35	99.35	0.33%
	3	100	99.63	99.63	0.13%	100	99.22	99.22	0.11%
Tenofovir disoproxil Fumarate	1	60	59.56	99.26	0.30%	60	59.76	99.60	0.35%
	2	60	59.39	98.98	0.18%	60	59.63	99.38	0.10%
	3	60	59.84	99.73	0.19%	60	59.71	99.51	0.13%

Statistical Comparison of two methods:

To compare the results of the Photometrix application with the UV technique, a paired t-test was performed (two tails). T-stat values were found to be lower than t-critical values, and P values were greater than the applied alpha value (*P>0.05) using a t-test. It means that the procedures have no discernible differences. As a result, the Photometrix programme can estimate Sumatriptan succinate, Gemifloxacin, and Tenofovir Disoproxil Fumarate colorimetrically. The information is presented in Table 16.

Table 164: Applied Pair t-Test Result

Parameters	Drugs	Uv Method	Photometrix PRO
Mean (X)	Sumatriptan Succinate	100.046	100.152
	Gemifloxacin	99.792	99.376
	Tenofovir Disoproxil Fumarate	59.642	59.776
Variance (S^2)	Sumatriptan Succinate	0.10333	0.15052
	Gemifloxacin	0.01392	0.05483
	Tenofovir Disoproxil Fumarate	0.03287	0.01318
Observation (n)	Sumatriptan Succinate	5	5
	Gemifloxacin	5	5
	Tenofovir Disoproxil Fumarate	5	5
Pearson Correlation	Sumatriptan Succinate	0.157242	
	Gemifloxacin	0.852799	
	Tenofovir Disoproxil Fumarate	0.525365	
Hypothesized mean difference	Sumatriptan Succinate	0	
	Gemifloxacin	0	
	Tenofovir Disoproxil Fumarate	0	
Df	Sumatriptan Succinate	8	
	Gemifloxacin	6	
	Tenofovir Disoproxil Fumarate	7	
t stat	Sumatriptan Succinate	-0.470437879	
	Gemifloxacin	3.54766	
	Tenofovir Disoproxil Fumarate	-1.39629	
P (T<=t) one-tail	Sumatriptan Succinate	0.325300597	
	Gemifloxacin	0.006053	
	Tenofovir Disoproxil Fumarate	0.102654	
T Critical one-tail	Sumatriptan Succinate	1.859548038	
	Gemifloxacin	1.94318	
	Tenofovir Disoproxil Fumarate	1.894579	
P (T<=t) two-tail	Sumatriptan Succinate	0.650601194	
	Gemifloxacin	0.012106	
	Tenofovir Disoproxil Fumarate	0.205308	
T Critical two tail	Sumatriptan Succinate	2.306004135	
	Gemifloxacin	2.446912	
	Tenofovir Disoproxil Fumarate	2.364624	

CONCLUSION:

Ammonium metavanadate is an oxidising agent that changes oxidation states from +5 to +3. We're using its oxidation power as a colorimetric reagent to show the importance of colour change in the presence of an acidic medium and heat when reacting with compounds that include sulphur, nitrogen, and phosphorus atoms. For Sumatriptan Succinate, Gemifloxacin, and Tenofovir disoproxil fumarate, the smartphone-based PhotoMetrix PRO software is being used to develop a new and cost-effective colorimetric detection approach. The technique was based on a simple colourant and a quick procedure. The main purpose of this project was to use smartphone-based applications to make colorimetric drug content measurement easier. The approach was also compared to a UV method created using the same reagent and technology, and there were no statistically significant differences in assay findings. In quantitative drug estimation in pharmaceutical dose forms, this revolutionary method can be employed as an alternative to analytical science.

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