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NEW SMARTPHONE BASED COLORIMETRIC METHOD DEVELOPMENT AND VALIDATION OF SODIUM DODECYL SULPHATE IN BULK AND DOSAGE FORM

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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 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. The colorimetric method based on a smartphone and the UV method were both based on the detection of colour intensity as concentration increased. The developed method uses methylene blue as a dye to form a complex with SLS in a 1:1 ratio. The developed method exhibits good linearity ranging from 1-2µg/ml. With increasing the concentrations of API, the colour intensity increases. Samples were detected at 653 nm using UV-visible spectroscopy. Photometrix PRO software was used to analyse using mobile phone. A comparative study was done Using statistical tool i.e two paired test on both procedures conventional colorimetry and novel mobile phone analysis, the results show that both are equally significant.

KEYWORDS: UV spectrophotometry, Smartphone based colorimetry, Photometrix PRO, RGB Histogram.

INTRODUCTION

Pharma industry is excipient based industry, every final dosage form(tablet, capsule, powder etc) has only some amount of API content where as a large part of that dosage form consist of

excipients which are used to impart specific properties to the final product. One such excipient is Sodium lauryl sulphate/Sodium dodecyl sulphate (SLS).

It is sodium alkyl sulfate consisting of 12 carbon tail attached to a sulfate group. As a result of its hydrocarbon tail and its anionic "head group", it has amphiphilic properties that allow it to form micelles at low concentration which make it highly useful in wide range of products. It is used in cleaning and hygiene products, as wetting agent in dental preparations and other oral solid dosage forms, foaming and leathering agent in shampoo and soaps, tablet lubricants and as a releasing agent in suppositories and pessaries are some of the well-known uses of SLS in pharma industry. SLS market size was valued over 590 million USD in 2020 and it is expected to grow at rate of 3.8% in 2021 to 2027 driven by the rapidly increasing demand for household detergents & cleaners, personal care and industrial cleaning agents.

The assay of SLS is officially done by titrimetric method using methylene blue against Benzethonium Chloride.[1] However there are no UV-visible spectrophotometric method that is officially approved as SLS does not give absorbance at UV as well as in visible range. Though having a wide range of use there are very few analytical methods to analyze it accurately and reliably, not many efforts done in the development of an easy, fast and accurate analytical method of SLS. Many articles demonstrated the formation of cationic dye and SLS complex to analyze in visible light range. In this method complex formation is done between cationic dye Methylene blue and alkyl surfactant i.e., SLS and the complex is extracted in chloroform. (Colorimetric quantitation of trace amounts SLS in the presence of nucleic acid and protein). The extracted complex is then analyzed in visible range using UV-visible Spectro photo meter.

The basic principle behind the UV spectroscopy is absorption of visible and UV radiation (200– 400 nm) is associated with excitation of electrons, in both atoms and molecules, from lower to higher energy levels. Since the energy levels of matter are quantized, only light with the precise amount of energy can cause transitions from one level to another will be absorbed.[1] UV spectrophotometric methods based on principle of additivity and absorbance, recording and mathematical processing absorption spectra of standard solutions and sample solutions in same way or differentl The basic principle behind the UV spectroscopy is absorption of visible and UV radiation (200– 400 nm) is associated with excitation of electrons, in both atoms and molecules, from lower to higher energy levels. Since the energy levels of matter are quantized, only light with the precise amount of

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The UV-visible spectroscopy is based on principle of absorbing visible and UV radiation associated excitation of electron from lower energy state to higher state which is quantified in a sufficiently diluted solution which follow Beer-Lambert's law only light with precise can cause transition from one stage to another. The result is an absorbance spectrum that is in proportion with the concentration of the substance present in the solution.[2]

The fundamental data of a spectrophotometer displays the percentage of light shining on a specimen that is reflected or transmitted by it. A single value for a given wave length can be acquired, or values can be determined to cover the complete visible range. The question of what wave-length 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 part or all of the visible range. If the curve is steep and the absorption bands are thin and sharp, the points may need to be obtained at every millimicron with the narrowest spectral band possible.[3]

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. The light source in biochemistry analysers based on colorimetric approach is a tungsten halogen lamp. To achieve the required wavelength, the lamp must be modified with filters or a monochromator.[4]

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.[5]

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).[6]

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.[7]

Colorimetry is a technique for calculating the quantitative value of colours that is often employed in biological research. When a material binds with colour-forming chromogens, it produces colour. Differences in colour intensity lead to differences in light absorption.

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.[8]

This procedure necessitated the use of sophisticated tools. The goal of this study is to establish a simple, cost-effective method for estimating SLS. The method uses Methylene blue as a complex forming agent, which reacts with SLS to produce a SLS-dye complex having blue colour. Photo Metrix-PRO application captured and analysed the data image.

MATERIAL AND METHOD

Chemicals and Reagents

Methylene blue solution IP, Sodium Lauryl Sulphate, double distilled water, Chloroform

Apparatus and Applications

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

Preparation of methylene blue solution IP

Dissolve 150mg of methylene blue in 100 ml of ethanol and dilute with ethanol to produce 250ml.

Preparation of standard stock solution

Weigh about 10mg of SLS and transferred into a previously calibrated 100ml volumetric flask. The final volume was made up to the mark using double distilled water to obtain the standard stock solution of 100µg/ml concentration.

Method development

UV-Vis. spectroscopy

Selection of wavelength for SLS Methylene blue complex

Blank preparation

Black sample was prepared by taking 10 ml of double distilled water(DDW) without SLS in pre-calibrated 10 ml volumetric flask, 0.2 ml of Methylene blue solution IP was added and hand shaking was done to mix well.it was transferred to 25 ml separating funnel, 10 ml of chloroform was added. Shaking was done for 1 min. upon settling the chloroform layer was separated and used as blank.

Preparation of working stock solution

The working stock solution was prepared by dilution of the standard stock solution with DDW to obtained 1.8 ppm solution of SLS in 10 ml of volumetric flask, 0.2 ml of Methylene blue was added to that and shacked well. Transfer the solution to a 25 ml volumetric flask and 10 ml of chloroform was added and shanking was done for 1 min. upon settling the chloroform layer was separated and scanned against blank to select wavelength.

Wavelength selection

Wavelength was selected using chloroform layer of working stock solution against blank, the solution was scanned across the range 400-800nm. SLS methylene blue complex was found to have a maximum absorbance wavelength at 655nm. Prepare a calibration curve using the working solution, ranging from 1-2µg/ml, and construct a linear regression equation **Error! Reference source not found.**

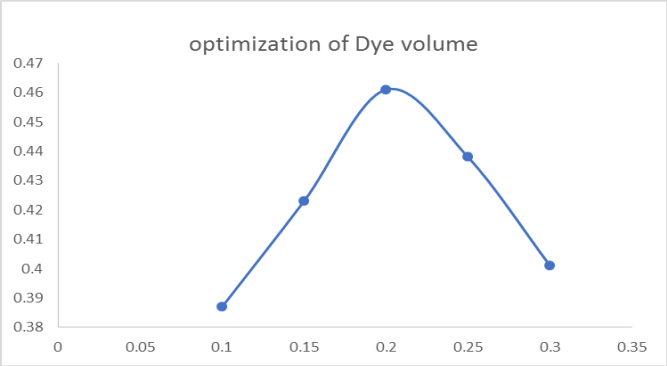
Method optimization

Optimization of regent volume

Take 10 ml of working stock solution, add different ml of methylene blue (0.1,0.15,0.2,0.25,0.3 ml) apply little shaking to mix the dye and form complex, transfer it to a 25 ml separating funnel, add 10 ml chloroform shake it by hands for 1 min. Separate the Chloroform layer and absorbance reading taken at 653 nm, as shown in table 1.

Table 1: Optimization of reagent quantity.

Sr. No.	quantity of reagent	Observation
1	0.1	0.387
2	0.15	0.423
3	0.2	0.461
4	0.25	0.438
5	0.3	0.401



Optimization of shaking time

Take 10 ml of working stock solution in 10ml volumetric flask add 0.2 ml of methylene blue and mix it well with a little shaking transfer it to a 25 ml separating funnel and add 10 ml of chloroform & shake it for 1,2,3,4,5 min, take absorbance 653 nm. Table 2.

Table 2: Optimization of shaking time.

Time	Absorbance
1	0.462
2	0.466
3	0.465
4	0.46
5	0.463

As there was no major change appeared in absorbance at different shaking time, to make method easy to perform 1 min shaking time was selected for further development.

Optimization conditions

Table 3: Optimization conditions.

Parameter	Optimized value
Concentration of Methylene blue	As per IP
Volume of methylene blue	0.2 ml
Shaking time	1 min
Temperature	Room temperature

Preparation of calibration graph for SLS

Different aliquots from stock solutions are taken in 10 ml volumetric flask to obtained sample solution from 1 ppm to 2 ppm range (1,1.2,1.4,1.6,1.8,2 ppm) (1 ppm = 1 µg/ml). Then add 0.2 ml methylene blue in 10 ml of sample solution mix it well then transfer to a 25ml separating funnel, add 10 ml chloroform. Shake for 1 min then upon settling separate chloroform layer and observe it under visible region at 653 nm.

Estimation of sls 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 1.

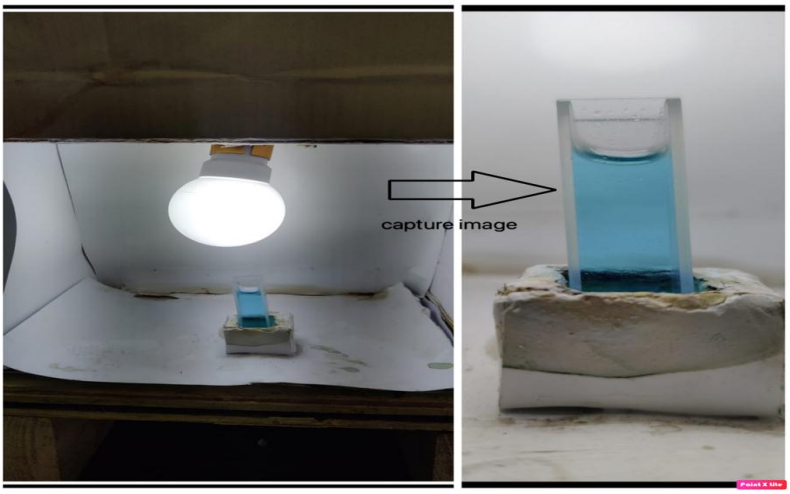


Figure 1: Experimental set up.

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. Using univariate and multivariate analysis, this programme processes and displays the results. For the best results, many smartphone types were used. Figure 2 depicts the steps for utilising the PhotoMetrixPRO application.

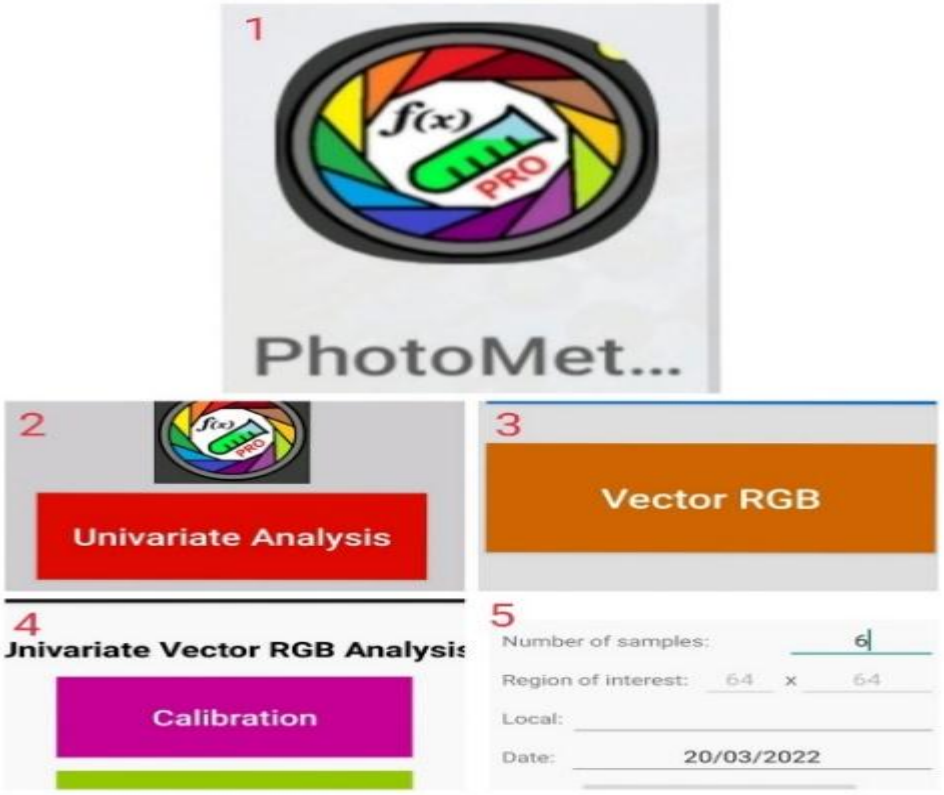


Figure 2: Steps for run the photometrix pro application.

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 1-3 ug/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

Absorbance of SLS-methylene blue complex was linear with the concentration range of 1-2µg/ml at 653 nm, by obeying Beer's law (Figure 3). A calibration curve was plotted between concentration Vs absorbance (Error! Reference source not found.). The plot was found to be linear.

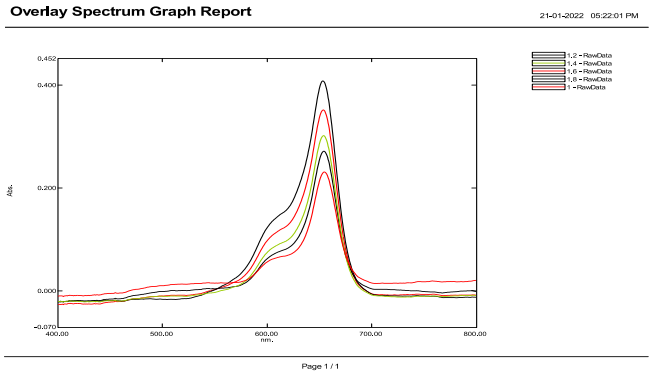


Figure 3: Overlay UV spectra of SLS.

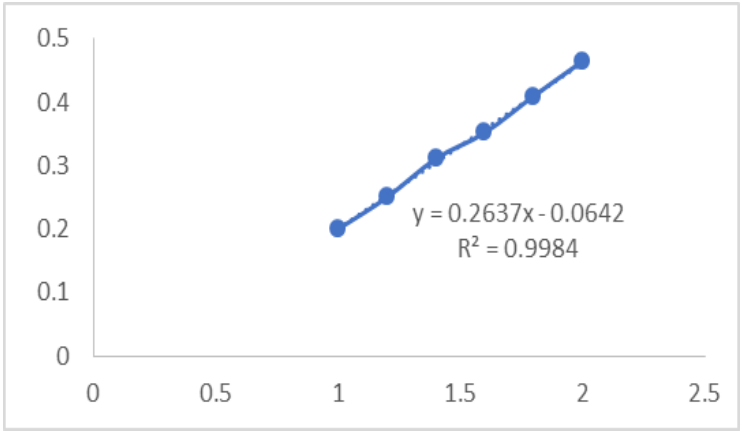


Figure 6: Linearity graph.

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. The percent RSD (Table 4 & Table 4) was calculated and determined to be less than 2.

Table 3: Intraday precision of SLS.

	Concentration(µg/ml)	Mean ± SD (n = 3)	% RSD
Intraday	1	0.209 ± 0.00173	0.83
	1.2	0.254 ± 0.00503	1.98
	1.4	0.306 ± 0.00577	1.88
	1.6	0.354 ± 0.0057	1.63
	1.8	0.413 ± 0.0057	1.4
	2	0.459 ± 0.0057	1.26

Table 4: Interday precision of SLS.

	Concentration(µg/ml)	Mean ± SD (n = 3)	% RSD
Interday	1	0.208 ± 0.0023	1.11
	1.2	0.247 ± 0.0040	1.63
	1.4	0.3 ± 0.0045	1.48
	1.6	0.353 ± 0.0057	1.63
	1.8	0.413 ± 0.0057	1.4
	2	0.455 ± 0.0041	0.91

3. Accuracy

The accuracy of the method was determined by recovery experiment. A known quantity of pure drug was added to pre-analyzed sample formulation at 80%, 100%, 120% levels. The recovery studies were carried out and the percentage recovery and the percentage standard deviation of the percentage recovery were calculated and given in table 5.

Table 5: Accuracy data of formulation.

	Conc. From formulation (µg/ml)	% Spiked	Standard conc. Added (µg/ml)	Concentration recovered (µg/ml)	%Recovery ± SD. (n=3)	%RSD
SLS	1	80	0.8	0.8	100.16% ± 1.457	1.45
	1	100	1	0.967	98.83% ± 0.0057	0.59
	1	120	1.2	1.15	98.04% ± 0.0208	1.80

4. Analysis of the marketed formulation

The assay of **different formulations** was determined and concentration found to be 1.8 %, 1.4% and 2% which falls within the acceptance criteria (1-2%) (Table 6).

Table 6: Assay results of different marketed formulations.

Marketed Formulations	Quantity found (%W/W)
Formulation 1 (Tooth paste)	1.8
Formulation 2 (Tooth paste)	1.4
Formulation 3 (Dental powder)	2

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 (dispersible tablet). The specificity of the method is demonstrated in following Figure 4 in which graph shows the specific absorbance at 652 nm of SLS. Hence, it can be concluded that this method is specific.

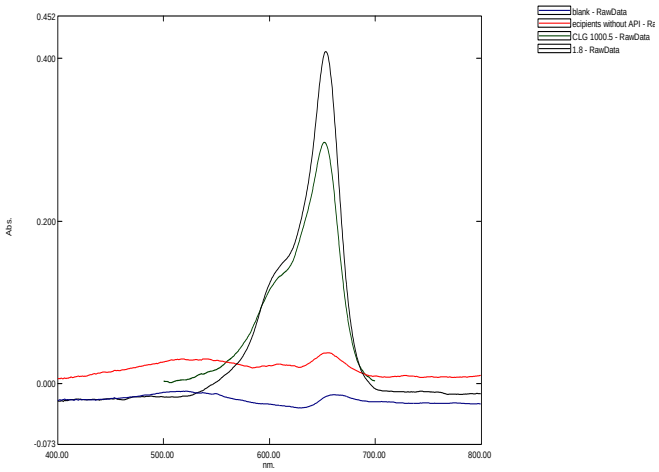


Figure 4: Specificity indicating graph.

Estimation of SLS using smartphone application

By using PhotoMetrix PRO application the image was captured and according to concentration (Figure 5). The linear regression equation was observed (Figure 6). Correlation coefficient (r^2) for PhotoMetrix PRO 0.989 and 0.998 for UV vis spectrophotometry respectively. Regression equation data for both method Table 6.

Figure 5: Chart of colour intensity corresponding to the concentration of SLS.

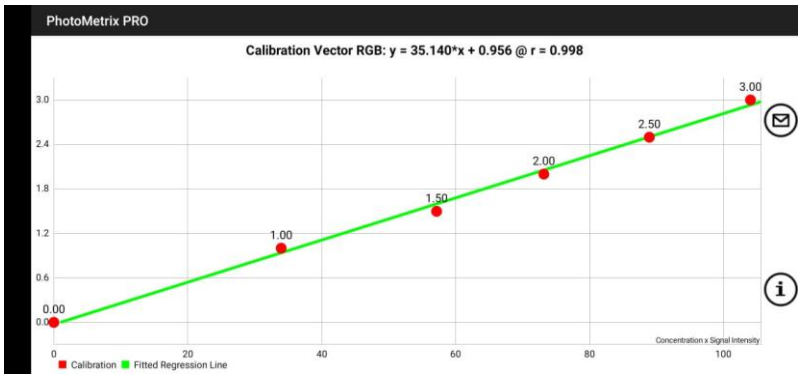


Figure 6: Calibration curve of SLS by photometrix pro application.

Table 6: Regression data for both UV and photometrix application.

Parameter	UV Method	Photometric application
Linearity (µg/ml)	1-2	1-3
Regression equation	y = 0.2637x - 0.0642	y=35.140x + 0.956
Slope	0.2637	35.140
Intercept	0.0642	0.956
Correlation coefficient	0.998	0.998
LOD (µg/ml)	0.043	0.2904
LOQ(µg/ml)	0.115	0.88

The linearity of the standard SLS was taken in the range of 30-150 µg/ml. The calibration curve and regression equation generated by the application was shown in the **Error! Reference source not found.** The concentration of sample solution was found to be 100.66 µg/ml (Nominal 100 µg/ml). The % assay was found to be 100.66 % which is within the acceptance criteria.

Assay of formulation

The assay was performed on the different marketed formulation 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 7.

Table 7: Assay results of different formulation for both the methods.

	UV		Photometrix	
	Quantity of SLS in %w/w	% RSD	Quantity of SLS in %w/w	% RSD
Formulation 1	1.8	1.70	1.65	0.61
Formulation 2	1.4	1.5	1.48	0.78
Formulation 3	2	0.58	1.88	1.11

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 SLS using colorimetry. Table 8 displays the information.

Table 8: Applied Pair t-Test Result.

	UV method	Photometrix PRO
Mean (X)	1.79	1.63
Variance (s^2)	0.0003	0.001
Observations (n)	5	5
Pearson Correlation	0.13693064	
Hypothesized mean difference	0	
df	6	
t stat	9.922779	
P (T<=t) one-tail	3.03E-05	
t Critical one-tail	1.94318	
P (T<=t) two-tail	6.05E-05	
t Critical two ail	2.446912	

CONCLUSION

The smartphone-based PhotoMetrix PRO application is used to develop a novel and cost-effective colorimetric detection method for SLS. 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 dose forms, this unique method can be utilised as an alternative to analytical science.

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