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Sulfamethoxazole Pharmaceutical Dosage Form Quantitative Estimation Using UV-Visible Spectrophotometric Method

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ABSTRACT:

Sulfamethoxazole, a widely used antibiotic, is often formulated as a combination product with trimethoprim to treat bacterial infections. Accurate and reliable quantitation of sulfamethoxazole is essential for quality control and assurance in pharmaceutical industries. In this study, a simple and cost-effective UV-Visible spectrophotometric method was developed for the quantitative estimation of sulfamethoxazole in pharmaceutical dosage forms.The method involves measuring the absorbance of sulfamethoxazole at 264 nm, which is a characteristic wavelength of the compound. The calibration curve was constructed by plotting the absorbance values against the concentration of sulfamethoxazole in the range of 2-12 µg/mL. The results showed a good linear correlation (r^2 = 0.999) with a limit of detection (LOD) and quantification (LOQ) of 0.5 µg/mL and 1.5 µg/mL, respectively.The method was validated by analyzing commercial pharmaceutical products containing sulfamethoxazole and trimethoprim. The results showed good accuracy and precision, with mean recovery percentages ranging from 98% to 102%. The method was also found to be selective, with no interference from excipients or other components commonly present in pharmaceutical formulations.In conclusion, the proposed UV-Visible spectrophotometric method is a reliable and efficient tool for the quantitative estimation of sulfamethoxazole in pharmaceutical dosage forms. The method can be easily adapted for routine quality control analysis and can help ensure the quality and consistency of sulfamethoxazole-based products.

Keywords: Sulfamethoxazole,UV-Visible Spectrophotometre,Quantitative estimation.

INTRODUCTION :

Sulfamethoxazole (SMX)belongs to Sulphanilamide drug. 4-amino-N-(5- methyl-1, 2-oxazole-3-yl)-benzene sulfonamide is its chemical name. Sulfamethoxazole is an antibacterial medication that is widely used in conjunction with trimethoprime.[1] to treat urinary tract infections or with primary amine to treat Chloroquine-resistant plasmodium falciparum malaria. SMX stands for sulfamethoxazole. They are mostly used to treat urinary infectious illnesses due to their inexpensive cost and strong efficacy against a wide range of gram-positive and gram- negative bacteria. [2-3.] The antibacterial combination of sulfamethoxazole and trimethoprim (TRI), often known as co-trimoxazole, is frequently used in urinary area contaminations, breathing area contaminations, and gastro stomach tract infections. The chemical structure of sulfamethoxazole and trimethoprime is shown in Fig. 1. SMX inhibiting the production of dihydrofolate intermediate binding through dihydropteroate synthetase interferes with the normal bacterial synthesis of folic acid, which inhibits thefolate-depended metabolic process for bacterial growth. [4]

In a literature review, there are several ways for estimating these drugs, including charge transfer complexation.[5] Spectrophotometric approaches outperform other instrumental methods in terms of practicality and cost.[6-8] UV-Visible spectrophotometry[9-1]rapid UPLC [15]spectrophotometry.[16] and flow injection system/HPLC with potentiometry.[17] In recent years, colorimetric sensors have gained increased attention due to their ease of visual observation and simple operations. [18-19] There are no visible spectrophotometric methods for estimating SMX employing O- phenylene diamine (OPDA) by diazotization followed by coupling reaction documented in the literature, which prompted us to create these approaches. Hence, for the first time, we describe few simple, cost-effective, novel methods using OPDA to assay these drugs in bulk samples the goal of this project was to create a simple, sensitive system. and cost- effectiveness UV-Visible spectrophotometric method that could be used determine sulfamethoxazole in bulk drug and pharmaceutical formulations.

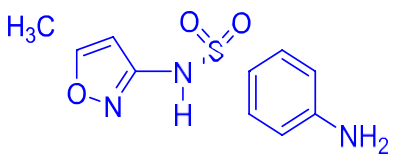


Fig.1. Chemical Structure of Sulfamethoxazole

METHODS OF MATERIALS

Thermo (scientific) GENESYS 10S Visible spectrophotometer was used to perform spectral analysis, and thermofisher reported the results. Normal 10 mm path- length cuvettes are used for analysis. Ultrasonicate (1.3L) was used to sonicate the standard sample and the formulations. ML-T Analytical Balance (RS232) used tomeasure normal and sample products.

CHEMICALS AND REAGENTS

The SMX reference sample was a kind gift from Analog lab, Hyderabad. The GANTANOL (SMX-400mg) formulation was purchased from the local market, M.B Reagent-AR, O -Phenylene diamine Reagent-AR and Distilled water (solvent) was purchased from Brass scientific PVT. Ltd., A.P., INDIA.

Standard stock solution preparation

100 milligrams of the normal medication SMX was accurately weighed and dissolved in 50 mL of diluent water, then transferred to a volumetric flask and sonicated for 5 minutes before being scaled up to 1000 µgm1-1stock solution using the same solvent label. 10 mL of this solution was transferred to a 100 mL volumetric flask and diluted to make a 100 µg ml-1 solution, solution of Sulfamethoxazole concentration.

Selection of method and wavelength

The normal stock solution was additional dilute with milli-Q purely water to attain a concentration of 0.01 litter. each solution was scanned against solvent blank in a UV range (200-400) in 1.0 cm cell. Drug sample overlain spectrum was recorded and the spectrum analysis showed that sulfamethoxazole displays at 259 nm a well-defined λ max. It was found that the overlay spectra for the drug reported at 259 nm are appropriate for the selected drug sample to be λ max.

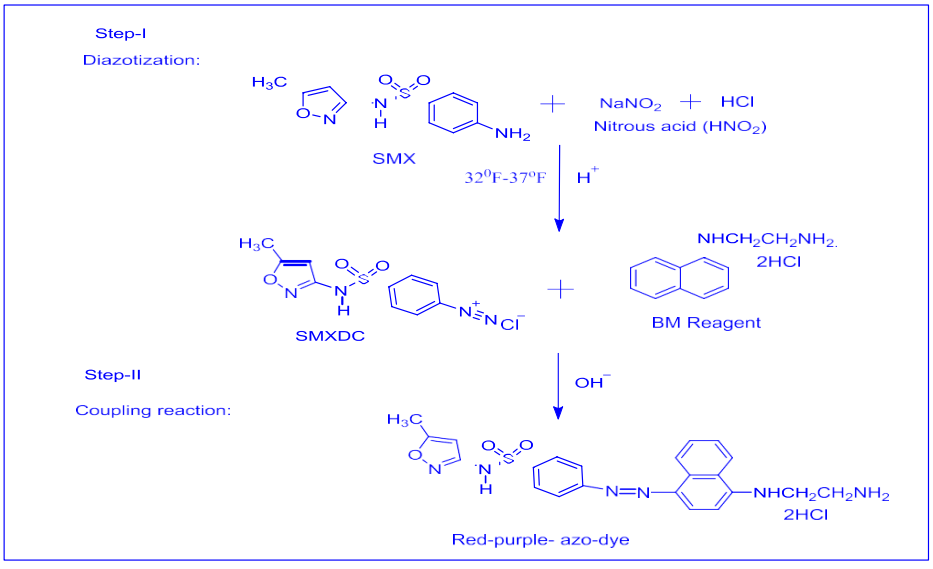
Procedure for formulation:

Twenty tablets were weighed with finally coated, containing the sulfamethoxazole (SMX). In 100 ml of ethanol, a portion of the powder equivalent to 100 mg of sulfamethoxazole was properly measured and dissolved and mixed for about 5 minutes, then filtered, and the ethanol evaporated into a dry state. To make stock solution A, the remaining portion of the solutions was diluted to the mark with ethanol up to 100 ml in a volumetric flask. 10 ml of aliquots were pumped into a 100 ml volumetric flask, and the volume was rendered up to the ethanol level to create the final 100 ml concentration, which was then diluted with ethanol to achieve a concentration of (5 to 30µgml^{-1}). produced as directed above and analysed at the prescribed wavelength of 259 nm, with statistical confirmation. Scheme 1 depicts sulfamethoxazole reaction mechanism with B.M reagent.

Method-A:

Calibration cure Preparation

Fresh aliquots of sulfamethoxazole concentration 0.5 to 3.0 ml were transferred into a series of 10 ml volumetric flasks to provide a final concentration range of (5 to 30 µgml^{-1}). In the presence of acidic condition, 1ml (0.1N) Hcl solution was added to each flask, followed by 1ml (0.1N) sodium nitrite solution. The resultant was mixed and allowed to stand for 5minutes after cooling in an ice bath, at 0 -5^{0}c, to from diazonium chloride solution is known as diazotization. To this solution added 1ml (1%) of urea solution has been applied and regularly shaken to evaporation to nitrogen gas, after added 1ml of (0.1N) NaOH solution for the neutralization. Later the diazonium chloride is coupled with 1ml B.M reagent solution, (N-1-naphylene diamine dihydro chloride), to form Red-Purple color azo-dye complex. The Red Purple color was stable for 21 hrs. At 525 nm, the absorbance was measured against a reagent blank. The calibration curve was used to calculate the amount of Sulfamethoxazole in the sample solution.



Scheme 4.1: Reaction mechanism of sulfamethoxazole with -BM Reagent:

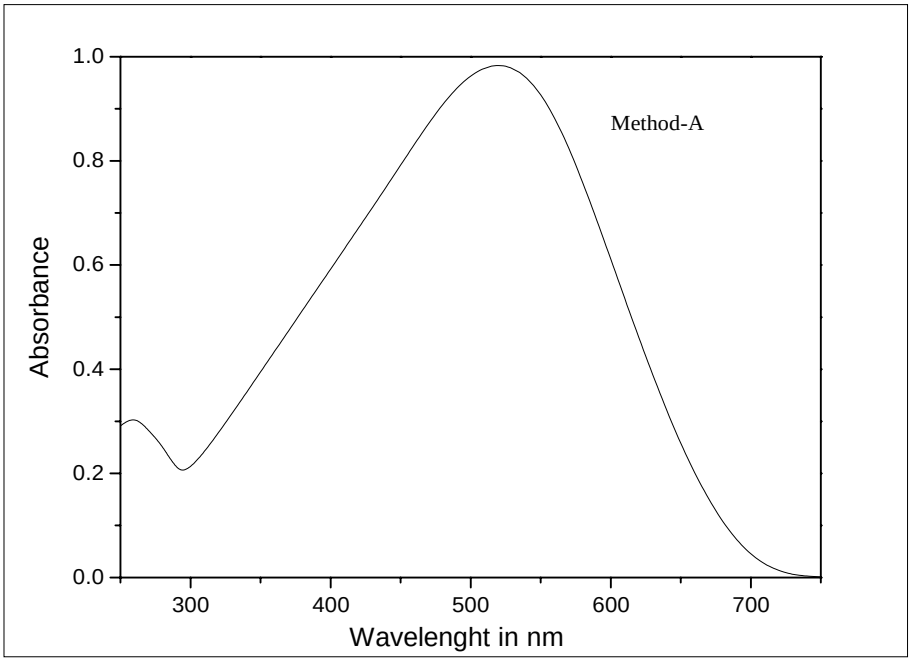


Fig.4.1. Sulfamethoxazole absorption spectrum after treated with B.M reagent

Linearity

By labelling the concentration range of 5-30 µgml-1 sulfamethoxazole and the maximum wavelength of 525 nm sulfamethoxazole, the linear relationship between absorbance and concentration of the drug was observed over the concentration range. The linearity curve was created by plotting the absorbance versus the concentration. A well-correlated linear fit graph was observed for the selected drug sample in the concentration range examined, and the linearity findings were given in Table-1, Fig.3.

Table - 1. The outcomes of linearity for Sulfamethoxazole

S.No	Concentration in µgml^{-1}	Absorbance
1	5	0.197±0.004
2	10	0.394±0.008
3	15	0.592±0.003
4	20	0.789±0.007
5	25	0.987±0.002
6	30	1.184±0.006

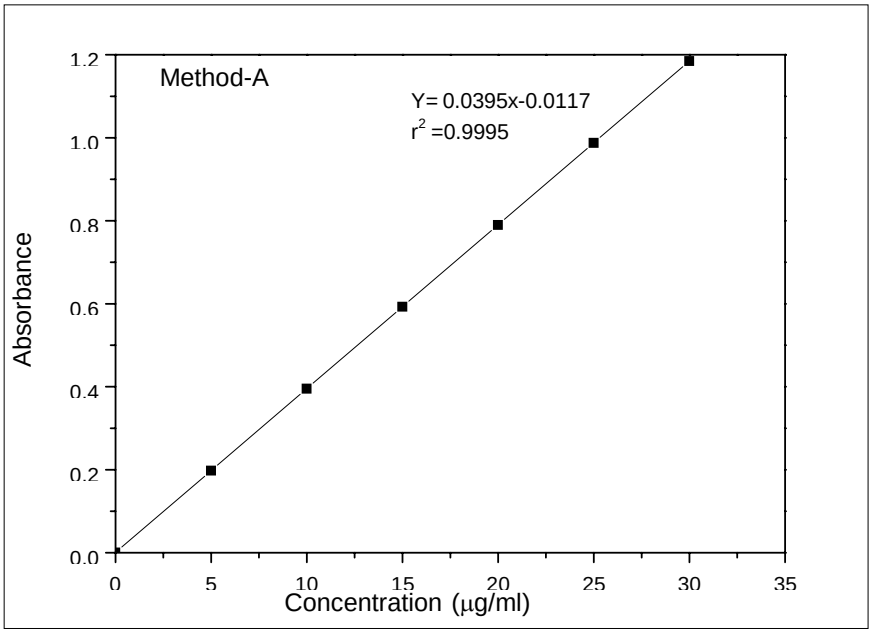


Fig-3. Linearity graph for Sulfamethoxazole

Recovery

Studies of recovery were performed by standard procedure. The method's accuracy was calculated by performing three-level recovery studies (10%, 20%, and 30%). The solution resulting was analyzed in its corresponding wavelength. Using the absorbance values obtained, the percentage recovery and the percentage RSD were determined in each spiked stage. Results were found to be within the 98-102 acceptance limit and less than 2 percent RSD (percentage) percentage. This indicated that the proposed approach was accurate. Table -2 shows the recovery results forSulfamethoxazole.

Table - 2. Findings from studies on recovery

S. No	Amount increased µgml^{-1}	Amount found* µgml^{-1}	% Recovery
1	5	4.97± 0.04	98.40±0.213
2	10	9.89 ± 0.01	98.90±0.422
3	15	14.86 ± 0.03	99.00±0.613
4	20	19.92±0.06	99.60±0.300
5	25	24.88±0.02	99.52±0.101
6	30	29.99±0.01	99.96±0.124

Precision

The relative standard deviation of the absorbance was used to express the system's repeatability and intermediate precision. Sample application and absorbance measurement were calculated by conducting six replicate measurements of the same band using a test solution containing Sulfamethoxazole at 10 µgml^{-1}. The intra-day accuracy of the six replicate solutions was assessed on the similar day, & the inter-day accuracy were tested over three days. The Standard deviation of relative percentage for intra- and inter-day precision sulfamethoxazole were determined to be 0.678 & 0.785, individually. The report exposed the accuracy of the procedure acceptable. Table-3 shows the precision findings for intra-day and inter-day precision, respectively.

Table -3. Precision Results for Sulfamethoxazole

S. No	Intra Day	Inter Day
1	0.394±0.002	0.388±0.006
2	0.395±0.004	0.385±0.007
3	0.397±0.006	0.384±0.002
4	0.399±0.001	0.381±0.003
5	0.401±0.003	0.379±0.001
6	0.402±0.005	0.377±0.004
7	% RSD=0.678	% RSD=0.785

Ruggedness

By assessing the medication solution, ruggedness and robustness were accomplished. with different researchers using the same method. Comparing the discrepancy between two analysts using percent RSD value was found to be 0.253 for sulfamethoxazole in three absorbance replicates. The low RSD value by percent shows the method's roughness. Table- 4 shows the results of the ruggedness test. Although vulnerable to analyst and instrumental variance, the proposed method was proven to be repeatable. We also checked for robustness, and the results are summarised in Table- 5.

Table 4. Ruggedness of the experimental studies.

S.No	Variation	(10 µgml^{-1})
1	Actual	0.394±0.001
2	Analyst to Analyst	0.393±0.004
3	Instrument to instrument	0.397±0.006
4	%RSD	0.253

Values are given in the table are mean SD of three replicate experiment

Table -5. Robustness study of (15 µgml^{-1})

S. No	Change in wave length(+2 nm)	Absorbance	Change in Temp.(0^{0c})	Absorbance
1	520	0.591±0.001	At room temp.	0.529±0.004
2	522	0. 591±0.003	Sun light at Morning	0.595±0.002
3	524	0.589±0.004	Refrigerator	0.599±0.003

Values are given in the table are mean and SD of three replicate experiment

The method's sensitivity

The detection limit (LOD) and qualification limit (LOQ) values have been used to express the sensitivity of the created approach. The values of LOD and LOQ have been determined according to ICH requirements and are listed in Table-6. The regular solution was prepared, and measured the absorption of the prepared solution. The LOD values for Sulfamethoxazole were found to be 0.167µgml^{-1}and 0.250µgml^{-1}respectively. This suggested

the approach could be applicable to the spectrum product at the lowest concentration. The LOQ values were found to be 0.506 µgml^{-1}and 0.759 µgml^{-1}respectively for sulfamethoxazole.

Table -6. Results of Sensitivity studies (LOD and LOQ)

S.No	Parameter	Present method
	Intraday	
1	LOD	0.167
2	LOQ	0.506
	Inter day	
3	LOD	0.250
4	LOQ	0.759

Formulation analysis

Tablet-sample solution (formulation solution) absorbance was registered at 259 nm. The formulation analysis revealed that the system can accurately estimate more than 98 percent of the time, and the findings were determined to be in good agreement

with the label argument values. The percentage assay resulted in a score of 99.85. Table-7 summarises the findings of the formulation analysis. And the description ofthis approach as a whole was summarized in Table- 8.

Table-7. Formulation results for Sulfamethoxazole (SMX)

S. No	Drug	Brand name	Label claim	Amount prepared	Amount found	%assay
1.	SMX	GANTANOL	400 mg	40 mg	39.94 ±0.004	99.85

Table - 8. Analytical performance data for the proposed methods

S.No	Parameter	Present method
1	Wavelength(nm)	525
2	Color	Red-Purple
3	Linearity (µgml^{-1})	5-30
4	Absorptivity l^{-1} mole^{-1} /cm	1.502x10^{4}
5	Shandell's value (µgcm^{-1})	0.0168
6	Regression Equation (y= mx+c)	Y=0.0395x-0.0003
7	Slope (m)	0.0395
8	Intercept (c)	0.0003
9	Correlation Coefficient (r^2)	0.9995
10	Intraday Precession	
	%RSD	0.678

11	Inter day Precession	
	% RSD	0.785
12	Intraday	
	Limit of detection (µgml^{-1})	0.167
	Limit of Quantification (µgml^{-}1)	0.506
13	Inter day	
	Limit of detection (µgml^{-1})	0.250
	Limit of Quantification (µgml^{-1})	0.759

Method-B METHODOLOGY

According to ICH criteria, a UV-visible spectrophotometric method was designed and verified. Distilled water used as solvent, and the absorbance was registered at 469 nm, summarizing the results in Fig. 2.

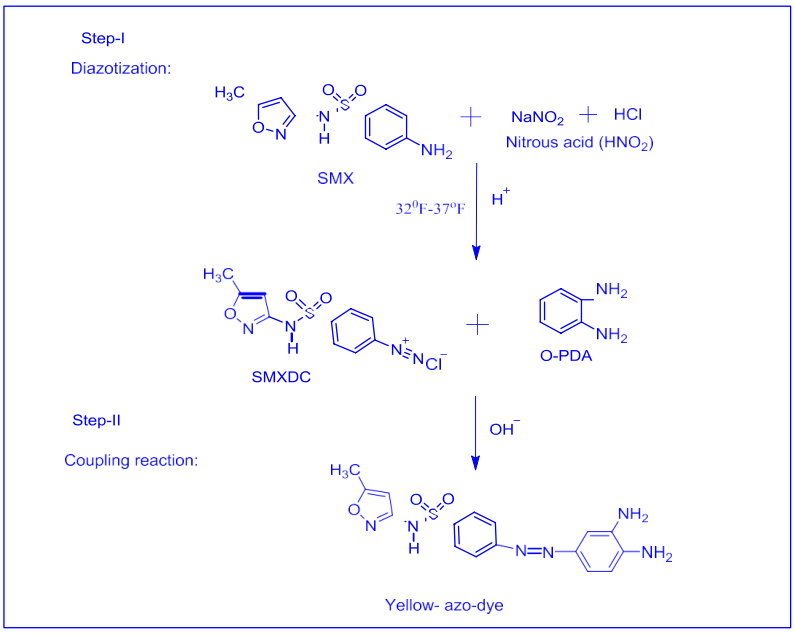
Procedure for Formulations

Twenty tablets weighed and finally coated, containing the sulfamethoxazole (SMX). In 100 ml of ethanol, a carefully measured portion of the powder containing
100 mg of sulfamethoxazole was dissolved and mixed for about 5 minutes, then filtered, and the ethanol evaporated to a dry condition. To make stock solution B, the remaining portion of the solution was diluted to the mark using ethanol up to 100 ml in a standardization flask. To make final 100 ml concentration solution, 10 ml of aliquots were piped into 100 ml volumetric flasks and the volume was rendered up to the ethanol level. The solution was then diluted with ethanol to produce a concentration of (6 to 36 µgml^{-1}) as described above, then analysed at 259 nm with statistically validated results. Scheme 1 depicts sulfamethoxazole reaction mechanism with ortho-phenylene diamine reagents.

Preparation of calibration cure

Fresh aliquots of sulfamethoxazole concentrations of 0.6 to 3.6 ml (6 to 36µ gml^{-1}) were placed into a series of 10 ml volumetric flasks to provide final concentrations of (6 to 36 µgml^{-1}). In each flask, 1ml (0.1N) hydrochloric acid solution was added, followed by 1ml (0.1N) sodium nitrite solution. In the presence of acidic media, the resultant was mixed and allow to stand for 5 minutes, after cooling in an ice bath at 0–5^{0}c, to form diazonium chloride solution known as diazotization. To this solution added 1ml (1%) of urea solution and regularly shaken to evaporate nitrogen gas, after adding 1ml of (0.1N) NaOH Solution for neutralization. Later the diazonium chloride is coupled with 1ml Ortho- phenylene diamine solution to form Yellow coloured azo-dye complex. The coloured species was stable for 23 hrs. At 469 nm, the
yellow chromogen was measured against a reagent blank. The calibration curve was used to calculate the amount of sulfamethoxazole in the sample.

Scheme 4.2: Reaction mechanism of sulfamethoxazole with Ortho phenylene diamine



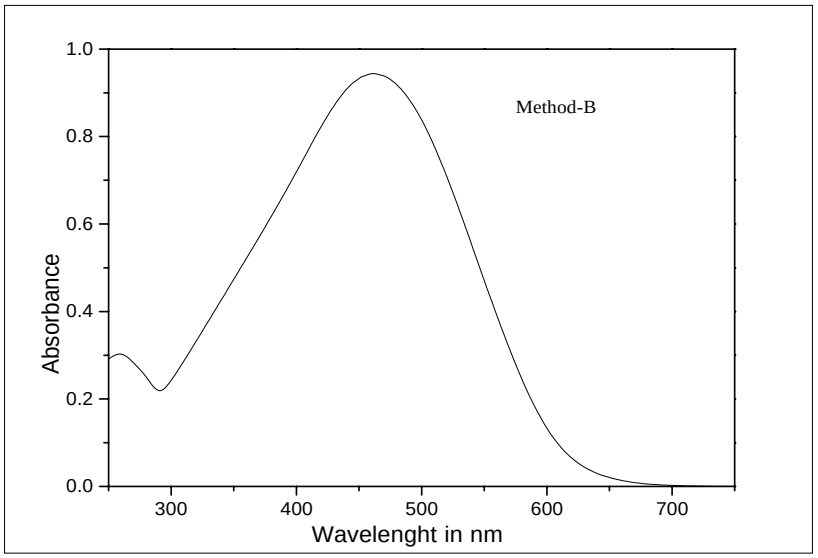


Fig.4.3. Sulfamethoxazole's absorption spectrum after treatment with the O-PDAreagent

Linearity

By labelling the concentration range of the drug, the linear connection between absorbance and concentration of the drug was evaluated over the concentration range. sulfamethoxazole ranges from 6-36 µgml^{-1} react with ortho phenyl diamine (Method-B)and the maximum wavelength of 469 nm sulfamethoxazole. The linearity curve was drawn using the absorbance against concentration obtained. For the selected drugsample in the concentration range tested, a well correlated linear fit graph was observed, and Tab-1, Fig.3 showed the linearity results.

Table - 1. Results of linearity for Sulfamethoxazole

S. No	**Concentration in µgml^{-1}**	**Absorbance**
1	6	0.236±0.006
2	12	0.473±0.003
3	18	0.710±0.002
4	24	0.947±0.004
5	30	1.184±0.006
6	36	1.421±0.003

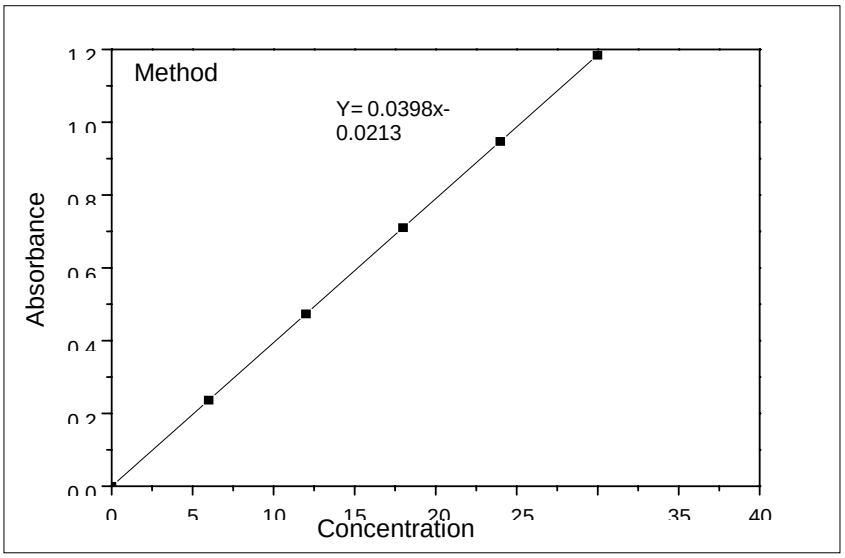


Fig. 4.4 Linearity graph for Sulfamethoxazole

Recovery

Studies of recovery were performed by standard procedure. The method's accuracy was calculated by performing three-level recovery studies (10%, 20%, and 30%). The solution resulting was analysed in its corresponding wavelength. Using the absorbance values obtained, the percentage recovery and the percentage RSD were determined in each spiked stage. Results were found to be within the 98-102 acceptance limit and less than 2 percent RSD (percentage) percentage. This proved that the planned strategy was correct. Tab-2, shows the recovery results for Sulfamethoxazole.

Tab 2. Results obtained from recovery studies

S. No	Amount increased µgml^{-1}	Amount discovered * µgml^{-1}	% Recoverability
	Method-B		
1	6	5.93± 0.02	98.83±0.311
2	12	11.89 ± 0.06	99.08±0.143
3	18	17.97 ± 0.03	99.83±0.410
4	24	23.98±0.01	99.91±0.621
5	30	29.97±0.04	99.90±0.221
6	36	35.96±0.01	99.88±0.108

Precision

The relative standard deviation of the absorbance was used to express the system's repeatability and intermediate precision. Sample application and absorbance measurement were calculated by conducting six replicate measurements of the same band using a test solution containing Sulfamethoxazole at 12µgml^{-1}. The intra-day accuracy of the six replicate solutions was assessed taking place the same day, and the inter-day accuracy was tested over three days. For intra- and inter-day precision sulfamethoxazole, the Standard deviation of Relative % was found to be 0.646 and 0.709 (method-B), respectively. The data revealed that the precision of the approach was acceptable. Table-3 shows the precision results for intra-day and inter-day precisions, respectively.

Table 3. Precision Results for Sulfamethoxazole

S. No	Intra Day	Inter Day
	Method-B	
1	0.474±0.001	0.503±0.004
2	0.476±0.003	0.498±0.006
3	0.479±0.006	0.495±0.001
4	0.480±0.004	0.492±0.007
5	0.482±0.005	0.490±0.003
6	0.483±0.002	0.488±0.001
7	% RSD=0.646	% RSD=0.709

Ruggedness and Robustness

The ruggedness and robustness were achieved by evaluating the drug solution with different researchers using the same method. Comparing the discrepancy between two analysts using % RSD values was found to be 0.422 for sulfamethoxazole in three absorbance replicates, the method's robustness is demonstrated by the low percent RSD figures. Table-4 shows the results of the ruggedness test. The proposed approach, although subject to analyst and instrumental variance, was found to be reproducible.We also screened robustness, and summarized the findings in Table -5.

Table 4. Ruggedness of the experimental studies.

S. No	Variation	(12 µgml^{-1})
	Method-B	
1	Actual	0.473±0.005
2	Analyst to Analyst	0.471±0.003
3	Instrument to Instrument	0.476±0.001
4	% RSD	0.422

Values are given in the table are mean SD of three replicate experiment

Table 5. Robustness study of (12 µgml^{-1})

S. No	Change in wave length (+2 nm)	Absorbance	Change in Temp. (0^{0c})	Absorbance
1	479	0.710±0.003	At room temp.	0.711±0.005
2	481	0. 710±0.002	Sun light at Morning	0.708±0.001
3	483	0.696±0.001	Refrigerator	0.714±0.004

Values are given in the table are mean SD of three replicate experiment

The method's sensitivity

The detection limit (LOD) and qualification limit (LOQ) values have been used to express the sensitivity of the created approach. The values of LOD and LOQ have been determined according to ICH requirements and are listed in Table-6. The regular solution was prepared, and measured the absorption of the prepared solutions. The LOD values for Sulfamethoxazole were found to be 0.257 µgml^{-1}and 0.273 µgml^{-1} (method-B) respectively. This suggested the approach could be applicable to the spectrum product at the lowest concentration. For sulfamethoxazole, the LOQ values were found to be 0.778 µgml^{-1} (method-A) and 0.829 µgml^{-1} (method-B), respectively.

Table -6. Results of Sensitivity studies (LOD and LOQ)

S.No	Parameter	Present method
	Intraday	
1	LOD	0.257
2	LOQ	0.778
	Inter day	
3	LOD	0.273

4	LOQ	0.829

Formulation analysis

Tablet-sample solution (formulation solution) absorbance was registered at 259 nm. The formulation analysis revealed that the system can accurately estimate more than 98 percent of the time, and the findings were determined to be in good

Agreement with the label argument values. The percentage assay resulted in a score of
99.85. The results of the analysis of the formulations were given in Table-7, and the description of this approach as a whole was summarized in Table- 8.

Table7. Formulation results for Sulfamethoxazole (SMX)

S. No	Drug	Brand name	Label claim	Amount prepared	Amount found	%assay
1.	SMX	GANTANOL	400 mg	40 mg	39.94 ±0.004	99.85

Table 8. Analytical performance data for the proposed methods

S. No	Parameter	Method-B
1	Wavelength(nm)	469
2	Color	Yellow
3	Linearity (µgml^{-1})	6-36
4	Absorptivity l^{-1} mole^{-1} cm^{-1}	1.0016x10^{4}
5	Shandell's value (µgcm^{-1})	0.0256
6	Regression Equation (y=mx+ c)	Y=0.0398x-0.0213
7	Slope (m)	0.0398
8	Intercept (c)	0.0213
9	Correlation Coefficient (r^2)	0.9997
10	Intraday Precession	
	%RSD	0.646
11	Inter day Precession	
	% RSD	0.709
12	Intraday	
	Limit if Detection (µgml^{-1})	0.257
	Limit of Quantification (µgml^{-}1)	0.778
13	Inter day	
	Limit if Detection (µgml^{-1})	0.273
	Limit of Quantification (µgml^{-1})	0.829

RESULTS AND DICUSSION

According to ICH guidelines, a UV-visible spectrophotometric method for measuring sulfamethoxazole in tablet dose form has been developed and validated(ICH Committee 2005). As a solvent, filtered water was employed. They measured absorbance at 525 nm (Method-A) and 469 nm (Method-B) (Method-B). The absorbance of Sulfamethoxazole was measured at 525 nm (Method-A) and 469 nm (Method-B), and calibration curves were constructed. The absorptivity values were calculated using the sample wavelength. The absorbance values were measured at a given wavelength. The linearity was found to be within 5-30μgml^{-1} (Method-A) and 6- 36 μgml^{-1} (Method-B) concentration range. Recovery studies determined the exactness of the process. Recovery rate was found to be 98.90 - 99.96 (method-A) and 98.83 – 99.91(method-B) of Sulfamethoxazole. The findings were found to be within the approved 98-100 range and less than 2 %RSD. This indicated that they considered the proposed method to be accurate. Precision experiment was used to test the repeatability of the process. The % of RSD was 0.678 and 0.785 (method-A), 0.646 and 0.709 (method-B) sulfamethoxazole in intra- and inter-day precision, respectively. For six absorbance replicates of Sulfamethoxazole, the percentage of RSD value in ruggedness was found to be 0.253 (method-A) and 0.422 (method-B). The method's robustness is reflected in the tiny percent RSD readings by percent. The proposed approach for routine sulfamethoxazole determination in pharmaceutical formulations was found to be quick, accurate, and fast. In terms of linearity, consistency, precision, specificity, and reproducibility, recovery experiments were conducted to assess the validity and reproducibility of the suggested technique.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict

CONCLUSION :

According to ICH guidelines, a UV-visible spectrophotometric method for evaluating sulfamethoxazole in a single dosage form utilising ethanol as a solvent has been developed and confirmed. The benefits of the proposed method for analytical purposes are fast determination, cost-effectiveness, easy sample preparation, good reproducibility, simple, economical, accurate and practical. Therefore, the proposed method for evaluating sulfamethoxazole may be recommended in routine quality assurance research in pharmaceutical industries.

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