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UV-Visible Spectrophotometric Method for Quantitative Estimation of Sulfamethoxazole in Pharmaceutical Dosage Form

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ABSTRACT

A simple UV–visible spectrophotometric method was developed and validated for the quantitative estimation of sulfamethoxazole in tablet dosage forms in accordance with ICH guidelines (ICH, 2005). The method employs filtered water as solvent and measurement of absorbance at 525 nm, with calibration curves showing good linearity over the concentration range of 5–30 µg mL−1μg mL−1. Absorptivity values were calculated at the selected wavelength. Accuracy, evaluated through recovery studies, showed percent recovery between 98.90 and 99.96% with %RSD values below 2%, confirming the reliability of the method. Precision studies (intra- and inter-day) yielded a %RSD of 0.678, while ruggedness assessment using six replicate absorbance measurements produced a %RSD of 0.253, indicating good robustness. All validation parameters met ICH acceptance criteria, demonstrating that the proposed method is simple, rapid, accurate, precise, and suitable for routine quality control analysis of sulfamethoxazole in pharmaceutical tablet formulations.

Keywords: UV–visible spectrophotometric method, sulfamethoxazole, Accuracy, Precision, ICH guidelines

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INTRODUCTION

Sulfamethoxazole (SMX) belongs to Sulphadiazine drug. Chemically, 4-amino-N-(5- methyl-1, 2-oxazole-3-yl)-benzene sulfonamide having the molecular formulae $C_{10}H_{11}N_3O_3S$. Sulfamethoxazole is an antibacterial medication[1,2,3] that is widely used in conjunction with trimethoprime to treat urinary tract infections or with primary amine to treat Chloroquine-resistant plasmodium falciparum malaria. Sulfamethoxazole is a sulfonamide antimicrobial that inhibits bacterial growth by interfering with folic acid synthesis, which is essential for bacterial cell survival and replication[4]**.** 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. The antibacterial combination of sulfamethoxazole and trimethoprim, often known as co-trimoxazole[5], is frequently used in urinary area contaminations, breathing area contaminations.

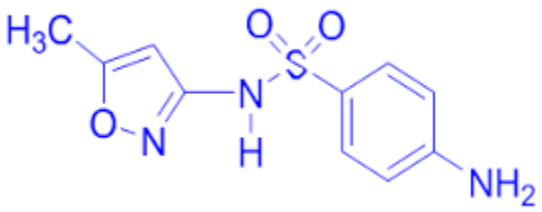

Figure 1: Structure of Sulfamethoxazole

In a literature review, there are several ways for estimating these drugs, including charge transfer complexation. Spectrophotometric approaches outperform other instrumental methods in terms of practicality and cost. UV-Visible spectro photometry[6,7], rapid UPLC spectrophotometry and flow injection system/ HPLC[8] with potentiometry. In recent years, colorimetric sensors have gained increased attention due to their ease of visual observation and simple operations. 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[9]. 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[10-15]

MATERIALS AND METHOD

Thermo (scientific) GENESYS 10S Visible spectrophotometer was used to perform spectral analysis, Ultrasonicate (1.3L) was used to sonicate the standard sample and the formulations. ML-T Analytical Balance (RS232) used to measure 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 μgml^{-1}stock 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 μgml^{-1} solution.

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 λmax. 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. The maximum obtained wavelength for the selected drug was used using spectrophometric method to estimate sulfamethoxazole.

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 SMX 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 upto 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 analyzed at the prescribed wavelength of 259 nm, with statistical confirmation.

Preparation of Calibration Curve:

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 to30 $\mu g ml^{-1}$). 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°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.

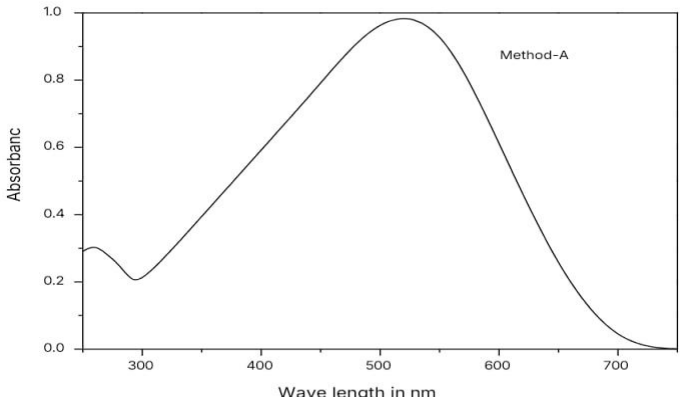

Figure 2: Sulfamethoxazole absorption spectrum after treated with B.M reagent

RESULTS:

Linearity:

By labelling the concentration range of 5-30 $\mu g ml^{-1}$ 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, Figure 3.

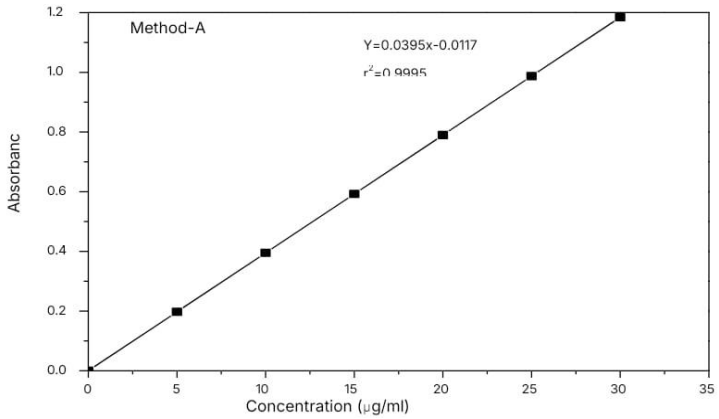

Figure 3: Linearity graph for Sulfamethoxazole

Table 1: The outcomes of linearity for Sulfamethoxazole

S. No	Concentration(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

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 98-102acceptance limit and less than 2 percent RSD (percentage) percentage. This indicated that the proposed approach was accurate. Table -2 shows the recovery results for Sulfamethoxazole.

Table 2: Recovery data of Sulfamethoxazole

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 at10 µgml^{-1}. The intra-day accuracy of the six replicate solutions was assessed on the similar day, & the inter-day accuracy was tested over three days. The Standard deviation of relative percentage for intra- and inter-day precision sulfamethoxazole was 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
	%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 shown in Table- 5.

Table 4: Ruggedness data of the Sulfamethoxazole

S. No	Variation	(10µgml^{-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 Sulfamethoxazole (15 µgml^{-1})

S. No	Change in Wave length	Absorbance	Change in Temp.	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

DISCUSSION:

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) The absorbance of Sulfamethoxazole was measured at 525 nm 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} concentration range. Recovery studies determined the exactness of the process. Recovery rate was found to be 98.90 - 99.96 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 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. 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.

CONCLUSION:

A UV–visible spectrophotometric method for the estimation of sulfamethoxazole in tablet dosage forms was developed and validated in accordance with ICH guidelines (ICH, 2005). Filtered water was used as the solvent, and the absorbance of sulfamethoxazole was measured at 525 nm. Calibration curves constructed over the concentration range of 5–30 μg mL−15–30 μg mL−1 demonstrated good linearity. Absorptivity values were calculated at the selected wavelength. Accuracy was evaluated by recovery studies, with percent recovery ranging from 98.90 to 99.96%, falling within the accepted range of 98–100% and with %RSD values below 2%, confirming method accuracy. Precision studies (intra- and inter-day) showed %RSD of 0.678 for sulfamethoxazole, indicating good repeatability. Ruggedness, assessed using six replicate absorbance measurements, yielded a %RSD of 0.253, further evidencing method robustness. Overall, the method complied with ICH criteria for linearity, accuracy, precision, ruggedness, and reproducibility.

The results demonstrate that the proposed UV–visible spectrophotometric method for the determination of sulfamethoxazole in tablet dosage forms is simple, rapid, and reliable. The method showed excellent linearity over 5–30 μg mL−15–30 μg mL−1, with high accuracy (98.90–99.96% recovery), and low %RSD values in precision, ruggedness, and robustness studies, all within ICH-acceptable limits. These findings confirm that the method is suitable for routine quality control analysis of sulfamethoxazole in pharmaceutical formulations and can be confidently applied for regular assay in industrial and laboratory settings.

REFERENCE:

1. Brunton LL, Hilal-Dandan R, Knollmann BC. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 14th ed. New York: McGraw-Hill Education; 2023.
2. Katzung BG, Vanderah TW. Basic and Clinical Pharmacology. 16th ed. New York: McGraw-Hill Education; 2021.
3. Sweetman SC, editor. Martindale: The Complete Drug Reference. 40th ed. London: Pharmaceutical Press; 2020.
4. Drug Bank. Sulfamethoxazole. Toronto (ON): Drug Bank; 2024. Available from: https://go.drugbank.com
5. Sohrab I, M.R., Fathabadi and A.H. Nour, Simultaneous Spectrophotometric determination of sulfamethoxazole and trimethoprim in pharmaceutical preparations by using multivariate calibration methods. J. Appl. Che. Res.,3:47-52,2010.
6. Islam, T., S. Ferdous, P. Jain and H.M. Reza, Method development and validation of baclofen mouth dissolving tablets by UV spectroscopy. EUR. J. Applied Sci., 5:7-11: 2013.
7. Bana, T., G. Yadav and R. Dud he. Development and validation of oseltamivir phosphate API by UV-spectrophotometer. Global J. Pharmacology., 7: 294- 297:2013.
8. Wang JC, Zhang Q, Cai DF. Stability indicating validated HPLC method for analysis of berberine hydrochloride and trimethoprim in the pharmaceutical dosage form. J Chemistry 2013:1-9.
9. Shamsa F, Amani L. Determination of sulfamethoxazole and trimethoprime in pharmaceuticals by visible and UV spectrophotometry. Iran J pharm Res 2016: 1:31-6.
10. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 6th ed. Belmont: Cengage Learning; 2007.

11. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. 5th ed. Mumbai: Himalaya Publishing House; 2007.
12. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. New Delhi: CBS Publishers; 2002.
13. Vogel AI. Textbook of quantitative chemical analysis. 6th ed. Harlow: Pearson Education; 2000.
14. Connors KA. A textbook of pharmaceutical analysis. 3rd ed. New York: Wiley-Interscience; 1997.
15. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's pharmacology. 8th ed. London: Elsevier Churchill Livingstone; 2016.