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Research

Analytical Method Validation Of Sulfamethoxazole By UV-Spectroscopy

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Abstract

For the quantitative determination of Sulfamethoxazole in a pharmaceutical formulation, a new, straightforward, accurate, quick, precise, reproducible, and cost-effective spectrophotometric approach has been developed and validated in accordance with the ICHQ2 (R1) guideline. The highest absorption wavelength of a spiked Sulfamethoxazole solution was measured over the UV-Visible spectrum. The absorbance was measured at the wavelength of maximum absorbance using several calibration standards of Sulfamethoxazole. Calculations were made to determine the linearity and range of the calibration curve of concentration vs absorbance. QC standards were validated, including accuracy, precision, LOD, LOQ, and robustness. It was found that the maximal wavelength of Sulfamethoxazole was 262 nm with 0.1N NaOH with a regression coefficient of 0.999, the substance followed Beer's Law in the concentration range of 2–10µg/ml. The % recovery was found to be between 99.2 and 100.8%, demonstrating that the developed method was accurate. The method's reproducibility was indicated by less % RSD. The % RSD for intra-day and inter-day precision was determined to be of 0.93 to 1.38 respectively, which is < 2%. The % assay for sulfamethoxazole in formulation was found to be 99.5. According to ICH (International Conference on Harmonization) standards, the analyses' findings have been validated.

Keywords: UV-Visible spectrometry, Sulfamethoxazole, Validation, Linearity, Anti infective.

INTRODUUCTION

Sulfamethoxazole is an isoxazole (1,2-oxazole) compound having a methyl substituent at the 5-position and a 4-aminobenzenesulfonamido group at the 3-position. It has a role as an antibacterial agent, an anti-infective agent, an epitope, an EC 2.5. (fig. 1) The sulfa drugs which are derivatives of sulfanilamide, were the first effective chemotherapeutic agents to be widely used for the cure of bacterial infections in humans. Sulfonamides (Sulfamethoxazole (SMX), sulfadimethoxine, sulfadiazine, etc.) are well known antibacterial drugs. Heavy metals are essential for all forms of life. The combined antibacterial activity of sulfonamides and antimicrobial activity

of heavy metals with a view to establish the relationship and importance of metal–drug interactions have been investigated [1], [2], [3], [4]. Sulfamethoxazole is most often used as part of a synergistic combination with trimethoprim in a 5:1 ratio in co-trimoxazole. Sulfamethoxazole is commonly used as an agent to treat urinary tract infections. In addition, it can be used as an alternative to amoxicillin-based antibiotics to treat sinusitis. It can also be used to treat toxoplasmosis. Sulfamethoxazole is official in IP, USP, BP etc. [5,6]. As per investigation of literature, the UV Spectro-photometric, HPLC analytical method were developed on different wavelength for analysis of Sulfamethoxazole in plasma fluids, Human serum, Plasma and pharmaceutical tablet dosage form or bulk drug samples [7-11]. The rational of this work to develop a simple, accurate, rapid, precise, reproducible and cost effective Spectro-photometric method for the direct quantitative determination of Sulfamethoxazole. There are several dosage forms of sulfamethoxazole available on the market, including chewable tablets, suspension, sustained release capsules, and sustained release tablets [12]. Beer- Lambert law is the main principle governing spectrophotometric quantitative analysis [13-15].

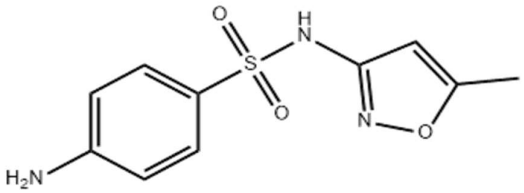

Fig 1: Chemical structure of Sulfamethoxazole

MATERIALS AND METHODS

Materials

SultriCare 80 mg tablets, were procured from market. Solvents like ethanol and sodium hydroxide were purchased from Merck solvents.

Instruments Used

UV-Visible spectrophotometer with UV Win software and make was PG-Instruments. Weighing balances and matching quartz cells with a 1 cm cell path length were utilized along with the mentioned equipment, which had automatic wavelength accuracy of 0.1 nm.

Method development

Standard stock solution preparation

By precisely measuring 10 mg of Sulfamethoxazole and transferring it to a volumetric flask of 10 mL, it was then dissolved in a few mL of ethanol until it was soluble, and the volume was brought up to the desired level with 0.1N NaOH (standard stock solution-1 of 1000 µg/ml). To obtain a concentration of 100 µg/ml from stock 1 solution, pipette off 1 ml, transfer it to a 10ml volumetric flask, and add sodium hydroxide to the desired volume. (Standard Stock Solution-2).

Selection of wavelength for analysis of Sulfamethoxazole

A 10 ml volumetric flask was filled with precisely measured 1 ml of standard stock solution-2, which was then diluted to 10 ml with sodium hydroxide to give a concentration of 10 µg/ml. This stock solution was then used for a first spectral scan in the UV range of 200-400 nm to detect the maximum wavelength and for subsequent dilutions for linearity.

Preparation of serial dilutions

From the standard stock-2 solution, serial dilutions were made to get solutions with concentrations of 2µg/mL, 4µg/mL, 6µg/mL, 8µg/mL, and 10µg/mL. At 285 nm, the absorbance of each solution was measured.

Method validation

According to ICH Q2 (R1) and USP criteria, the suggested technique was validated for a number of parameters, including linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness [19-20].

Linearity

Absorbance was plotted on the y-axis, and concentration was plotted on the x-axis, to create the calibration curve.

Precision

By taking six replicate measurements from the homogeneous solution, repeatability (intraday precision) and intermediate precision (interday precision) for the reference solution (8 µg/mL) were used to determine the method's precision. Three replicates were introduced into the system on the same day to increase the method's accuracy, and after calculating the RSD percentage, the results were expressed as a percentage of the measurement.

LOD and LOQ

The smallest amount of analyte in a sample that can be detected but not necessarily measured as an exact value is the detection limit of an individual's analytical method. The lowest amount of analyte in a sample that can be quantitatively measured with sufficient accuracy and precision is the quantification limit of a specific analytical process.

Robustness

By assessing the 6µg/mL sulfamethoxazole standard solution at various maximums (i.e. ±1nm) of the actual maximum, the robustness of this procedure was ascertained and absorbance was measured.

Stability

Stability of sulfamethoxazole sample solution was determined initially and 24 hours later, the % assay was compared.

Accuracy

The standard addition method was used to assess accuracy. A known quantity of standard solution was added to the sample solution in three different amounts 50%, 100%, and 150% of triplicate—before the solution was tested and the percentage recovery was computed.

Assay

Ten tablets were accurately weighed, the average weight calculated, and they were crushed to a fine powder. The powder equivalent to 10 mg of sulfamethoxazole was dissolved in ethanol with the aid of sonication, and volume was made up using sodium hydroxide up to the mark of 10 ml volumetric flask gives the concentration of 1000µg/ml. The sulfamethoxazole content in its marketed formulation (SultriCare 80 mg) was estimated using pre-validated.

RESULTS AND DISCUSSION

Linearity

The range of the linearity concentration for sulfamethoxazole was 2–10 µg/mL shown in Table 1. Fig 2 and Fig 3 displayed the calibration curve and absorption spectrum respectively. Table 2 displays the results of the calculation of the correlation coefficient, intercept, and slope for sulfamethoxazole.

Table 1: Linearity data of sulfamethoxazole

S.No	Concentration (µg/mL)	Absorbance
1	2.0	0.124
2	4.0	0.242
3	6.0	0.371
4	8.0	0.490
5	10	0.600

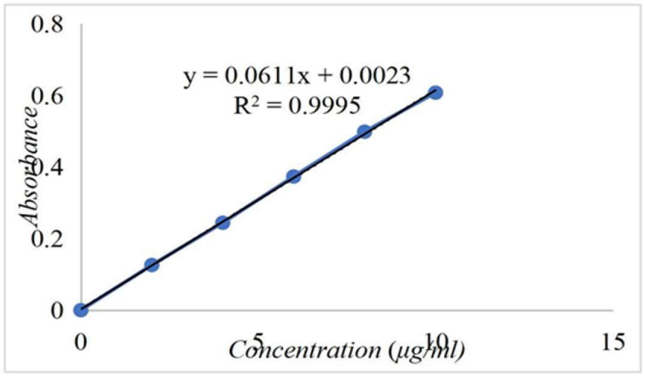

Fig 2: Calibration curve of sulfamethoxazole at 262 nm

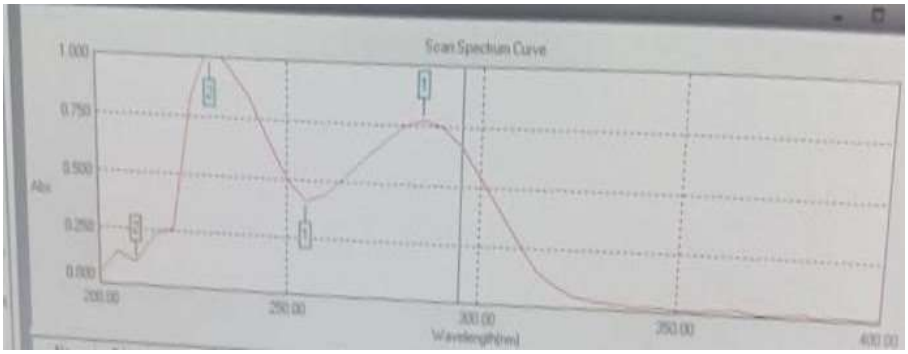

Fig 3: Absorption spectrum of sulfamethoxazole

Table 2: Optical characteristics of sulfamethoxazole

Parameters	sulfamethoxazole
Λmax	285
Slope	0.061143
Linearity	2 to 10µg/ml
Correlation coefficient	0.999
Intercept	0.002286

The calibration curve was plotted, and the correlation coefficient was discovered to be 0.999. Therefore, there was a good correlation between absorbance and concentration.

Precision

In Tables 3 and 4, respectively, the precision data for intraday and interday were displayed.

Table 3: Intraday Precision data of sulfamethoxazole

Concentration (µg/mL)	Absorbance
8.0	0.489
8.0	0.494
8.0	0.496
8.0	0.498
8.0	0.504
8.0	0.508
MEAN	0.4981
STDEV	0.0068
%RSD	1.38

Table 4: Interday Precision data of sulfamethoxazole

S.No	Concentration (µg/mL)	Intraday Absorbance DAY-1	Interday Absorbance DAY-2
1	8.0	0.489	0.488
2	8.0	0.494	0.496
3	8.0	0.496	0.499
4	8.0	0.498	0.495
5	8.0	0.504	0.489
6	8.0	0.508	0.489
MEAN		0.4981	0.4926
STDEV		0.0068	0.0045
%RSD		1.38	0.93

The % RSD for intraday and interday precision was discovered to be 2%. It shows that the technique was precise.

Limit of detection and Limit of quantification

LOD and LOQ was calculated and shown in Table 5

Table 5: LOD and LOQ data

Parameters	sulfamethoxazole (µg/mL)
LOD	0.19
LOQ	0.58

The sulfamethoxazole LOD and LOQ values were found to be 0.19µg/mL and 0.58µg/ml, respectively. It shows the technique was sensitive.

Accuracy

Recovery studies: Recovery studies were conducted by spiking the sample solution with standard solution at concentrations of 50%, 100%, and 150% for three repetitions. The results are presented in Table 6.

Table 6: Accuracy data of sulfamethoxazole

Sample (%level)	Amount Taken (µg/mL)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	Average
50	6	3	8.95	99.4	99.2
50	6	3	8.93	99.2	
50	6	3	8.91	99.1	
100	6	6	12.14	101.1	101.1
100	6	6	12.12	101.0	
100	6	6	12.16	101.3	
150	6	9	15.12	100.8	100.8
150	6	9	15.10	100.6	
150	6	9	15.18	101.2	

A range of 98 to 103% was determined to be the average sulfamethoxazole recovery percentage.

Robustness

Table 7 displayed robustness information.

Table 7: Robustness data of sulfamethoxazole

S.No	Wavelength	Absorbance
1	284	0.310
2	285	0.377
3	286	0.374

The absorbance did not significantly alter when the wavelength changed.

Stability

To check the stability of the solution, a sample solution of sulfamethoxazole with a concentration of 6 µg/mL was taken. In Table 8, stability information was displayed.

Table 8: Stability data of sulfamethoxazole

Time	% Assay
INITIAL	99.5
24 HOURS	99.2

The observed difference in the outcome was less than NMT 2%. It demonstrates the stability of sulfamethoxazole

Assay

Table 9 contains the sulfamethoxazole assay results.

Table 9: Assay of sulfamethoxazole (n=6)

Label claim	Amount found	Assay% ± SD
200mg	199mg	99.5% ± 0.04

Sulfamethoxazole's assay result was 99.5%. It demonstrates that the devised UV-Spectroscopic approach was successful in identifying sulfamethoxazole from tablet dosage form.

CONCLUSION

The developed UV- Spectroscopy method for the estimation of sulfamethoxazole was found to be simple, precise, accurate, robust, economical, and rapid, making it more acceptable and cost-effective. It can also be successfully applied for routine analysis in research institutions and quality control departments.

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