SPECTROFLUOROMETRIC DETERMINATION OF SULPHAMETOXAZOLE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, robust and selective and sensitive spectrofluorometric method has been developed for the determination of

sulphamethoxazole in pharmaceutical formulations. The method was based on the scanning of methanolic solution of the drug and

methanolic solution of formulation. The fluorescent product formed which was measured after excitation at 385nm. The method

showed high sensitivity with linearity range from 0.25 to 2.0 µg/ml. The lower limit of detection (LOD) was found to be 3.223×10^{-2}

µg/ml and the limit of quantization (LOQ) was determined as the lowest concentration was found to be 9.75×10^{-1}

µg/ml. The variables that affected the reaction were carefully studied and optimized. The proposed method was applied successfully for the

determination of sulphamethoxazole in pharmaceutical formulations. The percentage recovery is ±S.D (n=9) were 100.63 ± 0.78,

100.04 ± 0.77 and 99.79± 0.31 for Pharmaceutical formulation.

INTRODUCTION

- Sulfonamides were the first chemotherapeutic agents effective for the treatment of bacterial infections in man and are now available as widely used pharmaceutical products in medicine and veterinary practices.
- Sulphamethoxazole [N’-(5-methylisoxazole -3-yl) sulfanilamide] (SMZ) is the most widely used sulfonamide for the control of bacterial diseases. The official method is a non selective potentiometric titration with sodium nitrite based on the reaction of the aromatic amine group

MATERIALS

- For the development process we used UV-visible spectrophotometer (PerkinElmer Lambda 25), Spectrofluorimeter (PerkinElmer LS55), Sonicater (Branson 2510), Electronicbalance (precise 92sm-202A). Methanol\ (HPLC grade), water- double distilled water, pure sulphamethoxazole has been obtained as gift sample and the drug was used as such for further analysis. Formulations were purchased from the local pharmacies and used for analysis.

PREPARATION OF STANDARD SOLUTION

- An accurately weighed amount (25mg) of sulphamethoxazole was quantitatively transferred into a 50mL calibrated flask, dissolved in methanol and made up the volume. Then 0.2ml of the above solution was pipetted out and using micro-pipette transferred in to a 10mL standard flask diluted with methanol. The concentration of the working standard solution was 10µg/ml

PREPARATION OF SAMPLE SOLUTION

- Equivalent to 12.5mg of sulphamethoxazole was weighed accurately, from the crushed tablet powder and transferred into a clean 25ml standard flask. 15ml of methanol was added and solicited for 5minutes and then made up to the volume with methanol.The above solution was filtered through whatman filter paper and the filtrate was collected. From the above filtrate 0.2ml was pipetted out using micro-pipette and transferred into a 10ml standard flask, which then was made up the volume with methanol and mixed well. Further dilutions were carried out to get 1µg/ml concentration

PRECISION AND ACCURACY

- Method validation regarding reproducibility was achieved by replicate injection of extracted standard solution at low, medium and high concentration levels, where intensity of fluorescence was measured in comparison to the intensity of fluorescence of the standard.
- Intermediate precision study was conducted during routine operation of the system over a period of six consecutive days. Statistical evaluation revealed relative standard deviations at different values of six replicates. Within-day repeatability was studied by six replicate at three concentration levels.
- Accuracy was estimated as the deviation to the observed mean concentration from actual concentration and found to be less than 2% for all the concentration. The procedure which was stated in 2.8 was done in addition of 50%,75%,100% of drug as average along with the tablet powder and further dilution was made to get 1.0µg/ml concentration. These solutions were used for further analysis to perform recovery studies.

- Validation

Method validation was performed in terms of specificity and selectivity, precision and accuracy, linearity and stability.

- Linearity and range

Calibration standards of sulphamethoxazole, covering the range 0.25-2.0µg/ml were prepared with the suitable dilution made from sulphamethoxazole stock solution. The calibration curves were obtained by plotting the intensity of fluorescence against of concentration of sulphamethoxazole. The slope and intercept of the calibration line were determined by linear regression using the least squares method

- Specificity and selectivity

The interference from endogenous compounds was investigated by the analysis of six different blank matrices

RESULTS

- Validation
- Calibration standards for sulfamethoxazole covering the range of 0.25-2.0µg/ml were prepared by the method mentioned above and the serial dilutions were made with methanol. The calibration curve was obtained by plotting the intensity by fluorescence of the sulfamethoxazole versus analyte concentration. The slope and intercept of the calibration like was determined by linear regression using the least square method. The data was presented in table 1 and the calibration curve was presented in fig 1. Regression analysis of the calibration curve showed a linear relationship between the intensity of fluorescence of sulphamethoxazole and the concentration with corelation co-efficient higher than in all the curves assayed in pure form.

Table 1: Linearity profile of sulfamethoxazole

Sl. No	Concentration (µg/ml)	Intensity of fluorescence
1	0.25	97
2	0.5	195
3	0.75	290
4	1.0	385
5	1.25	475
6	1.5	570
7	1.75	670
8	2.0	766

- The precision was carried out as described in method and the results were presented in table 2. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision values hence; the developed method can be used to analyze the sulfamethoxazole in tablet formulation. The mean of the precision value is 100.66%. This value was obtained from 99.61-101.8.The regression equation was found to be y = 95.119x -92.786

Table 2: Analysis of tablet formulation

S. No.	Weight of tablet powder (mg)	Intensity of fluorescence	Weight of drug to be present (mg)	Amount of drug found	% drug content
1	16.2	122	12.36	12.3	99.61
2	16.9	135	12.90	13.08	101.4
3	16.8	134	12.82	13.06	101.8
4	16.5	127	12.59	12.5	99.32
5	16.5	128	12.39	12.72	101.05
6	16.7	131	12.74	12.84	100.82

Table 3: Stability of the solution

Time (hrs)	Intensity of fluorescence
0	385.5
1	384.8
2	385.0
3	385.2
4	384.9
5	384.6
6	384.8

Table 4: Recovery study of sulphamethoxazole

Level added (%)	Pure or drug added (mg)	Mean as recovery
50	6.5	100.63±0.78
75	13.0	100.04±0.77
100	19.5	99.79±0.31

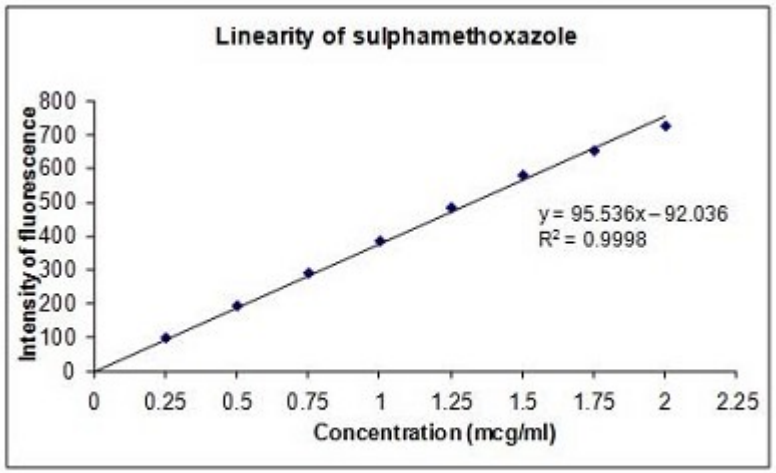

Figure 1: Calibration curve of Sulphamethoxazole

APPLICATION OF THE PROPOSED METHOD TO ANALYSIS OF SULPHAMETHOXAZOLE

- It is evident from the above-mentioned results that the proposed methods gave satisfactory results with sulphamethoxazole in bulk. Thus, its tablets were subjected to the analysis of their contents from the active ingredient by the proposed methods and the official (potentiometric titration) method. The tablet content, as percentage, was 100.63 ± 0.78, 100.04 ± 0.77 and 99.79 ± 0.31. These results were compared with those obtained from the official method by statistical analysis with respect to the accuracy and precision. No significant differences were found between the calculated and theoretical values.

CONCLUSION

- A spectrofluorometric method for quantifying sulphamethoxazole in formulation has been developed and validated. The linear range of the proposed spectrofluorometric method was 0.25- 2.0 µg/ml. The assay is selective, precise, accurate and linear over the concentration range from 0.25- 2.0 µg/ml, the concentration of sulphamethoxazole used for the precision study is 1.0 µg/ml in formulations could be precisely quantified and detected was approximated 9.75 x 10-1 µg/ml and 3.223 x 10-2 µg/ml respectively. Also, the proposed method involved spectrofluorometric measurements with comparable analytical performance devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. Therefore, these methods can be recommended for the routine analysis of sulphamethoxazole in quality control and clinical laboratories