Research Article

SPECTROFLUOROMETRIC DETERMINATION OF SULPHAMETHOXAZOLE 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.

Keywords: Sulphamethoxazole, Spectrofluorometry, Formulation, Estimation.

INTRODUCTION

Sulfonamides were the first chemotherapeutic agents effective for the treatment of bacterial infections in man[1] and are now available as widely used pharmaceutical products in medicine and veterinary practices. The pharmaceutical products containing these compounds are usually accompanied by others that increase their efficiency. Examples are the couples of sulfaquinixaline/ pyrimethamine, sulphadiazine/ trimethoprim and sulfamethoxazole/ trimethoprim. In the co-trimoxazole combination, sulphamethoxazole, sulphonamide with a structure similar to the of p-aminobenzoic acid (PABA) a template for folic acid, uses the woods-fields theory of competitive antagonism against PABA, and thus inhibits the incorporation of PABA into folic acid2.Trimethoprim on the other hand prevents the reduction of dihydrofolste to tetrahydrofolate, which is essential for one carbon transfer reaction crucial for the synthesis of bacterial DNA3. While the mammalian cell relies on the ready-made and available folic acid, the synthetic step is necessary for the buildup of the bacterial of the two agents for their synergistic activity has been found useful, and works satisfactorily to reduce both the toxicity of the individual agents and chances for the possible resistance of the organism to the agents, thus enhancing their therapeutic effect.

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[2]. For routine analysis where the amylate to appear together with other compounds, separation techniques are always required; HPLC [3-8] is the most frequently used. The analytical detection is usually carried out by spectrophotometric methods based on Bratton-Marshall reaction [9] in direct or derivative mode [10] to determine the total content of sulfonamides in urine and honey without free treatment of the samples. Also, this methodology has been coupled to Flow injection analysis (FIA) [11-13] to simplify the procedures the problem to determine the content of SMZ in pharmaceutical matrices is the normal interference caused by trimethoprim (TMP). The drug added as enhancer of its efficiency. Therefore, the determination of the SMZ in presence of TMP is a usual analytical problem in quality control of the pharmaceutical industry. Methods involving fluorescence spectrometry have been widely applied to analytical problems which require highly sensitive detection in [14]. The sensitivity and selectivity of fluorometric methods have encouraged their wide spread use in the analysis of numerous organic compounds, and many methods for generating strongly fluorescent derivatives have been devised. A fluorometric method has been proposed [15] involving diazotization of the amine contained in the sulfa methoxazole moiety and subsequent oxidation of the compound to a fluorescent trial.

At present, the official USP method for the analysis of the combination sulphamethoxazole-trimethoprim drug in pharmaceutical preparation commonly utilized, is the high pressure liquid chromatography (HPLC) [5] which for a small scale industries, is expensive and hence not appropriate. The objective of the present work was to develop a simple, sensitive and selective spectrofluorimretic method which could be used to avoid the overlapping spectral bands of the components.

MATERIALS AND METHODS

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.

Validation

Method validation [16-22] 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.

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.

Stability

Problems of stability are usually encountered with these compounds, mainly at ambient temperature. The stability of sulphamethoxazole was verified concentrations were measured for 6h in the interval of 1 h and found that the differences are within the limit.

RESULTS AND DISCUSSION

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 co-relation co-efficient higher than in all the curves assayed in pure form. The values are presented in table 1.

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

The stability studies of solution were carried out as described in method and the values were presented in table 3. From the above data it shows that there are no significant differences while the final sample solution was kept for 6hrs at ambient temperature.

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

The mean of the three different recovery studies were presented in table 4. In that overall mean of the recovery studies were 100.15%. The drug sulfamethoxazole in formulation was well identified under this condition. There is no interference is observed in different blank samples of sulfamethoxazole.

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 shows the regression analysis of calibration curve for sulfamethoxazole in pure form showed a linear relationship between the intensity of fluorescence and the concentration with correlation. Correlation co-efficient was found to be higher than 0.999 in all the curves assayed. Figure 2 shows the absorption spectrum of sulphamethoxazole, figure 3 shows fluorescence spectrum of sulfamethoxazole at 260nm as excitation and 365 as emission.
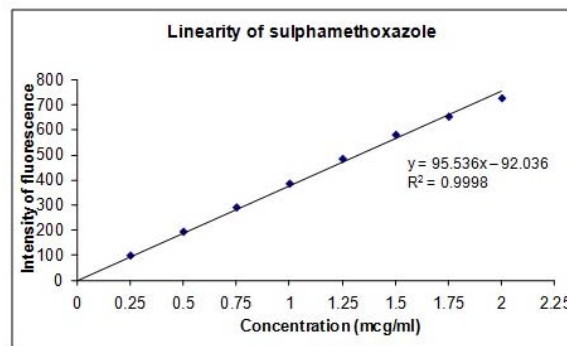

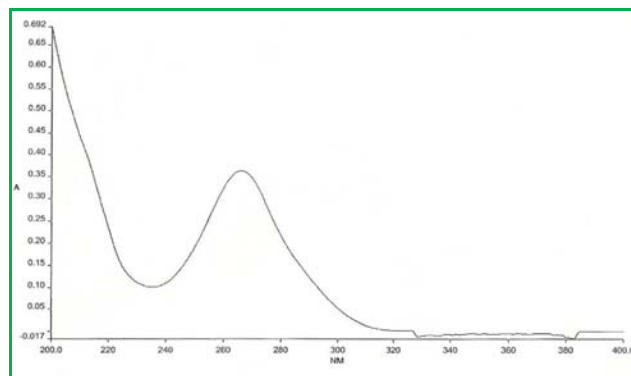**Figure 1:** Calibration curve of Sulphamethoxazole

Figure 2: Absoption spectrum of Sulphamethoxazole

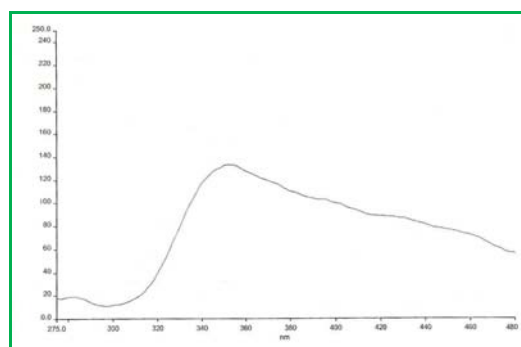**Figure 3:** Fluorescence spectrum of Sulphamethoxazole

The LOD determined as the amount drug was found to be 3.223×10^{-2} µg/ml and the LOQ was determined as the lowest concentration was found to be 9.75×10^{-1} µg/ml in formulation.

The result of the interferences study showed that no interference of any component with the drug has been proved and was found from the recovery of sulphamethoxazole was 100.63 ± 0.78, $100.0\ 4 \pm 0.77$ and 99.79 ± 0.31. This indicates the absence of interferences of any component with drug.

Ruggedness was performed as described in method by two different analysts, the method could not be repeated in a different laboratory or using different equipment and their results were prescribed in table 5. Robustness was performed as described in method and the results were prescribed in table 6. From the robustness data it has been proved that there are no significant changes when the drug analyzed indifferent wavelength.

Table 5: Ruggedness

Analyst 1 Intensity of fluorescence (nm)	Analyst 2 Intensity of fluorescence (nm)
385.0	385.5
384.9	385.8
385.6	384.7

Table 6: Robustness

Solvents	Intensity of fluorescence (nm)
Qualigens	385.5
Fishers	384.3

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 9.75×10^{-1} µg/ml and 3.223×10^{-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.

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