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Determination of Sulfadoxine in Pharmaceutical Formulations by Dual Wavelength Spectrophotometry Using *Methylene blue*

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Abstract: Simple extractive Spectrophotometric method is described for the determination of Sulfadoxine in pharmaceutical formulations. These methods are based on the formation of ion association complexes of the Sulfadoxine with basic Methylene blue. The limits of detection and quantification are also reported for both methods. Intra-day and inter-day precision and accuracy of the developed methods were evaluated as per the current ICH guidelines. The methods were successfully applied to the assay of Sulfadoxine in its tablet formulation and the results were compared with those of a reference method by calculating the Student's t-value and F-value.

Key words: Sulfadoxine · UV Spectroscopy · Methylene blue

INTRODUCTION

Sulfadoxine is chemically 4-amino-*N*-(5,6-dimethoxypyrimidin-4-yl) benzenesulphonamide. Sulfadoxine is an ultra-long-lasting sulfonamide often used in combination with pyrimethamine to treat or prevent malaria [1]. Literature survey reveals the estimation of sulfadoxine in pharmaceutical formulations by various Spectrophotometry [2-6], Liquid chromatography [7-9] and spectrofluorimetry [10] methods. Author of the article and his research team has developed a UV Method development different pharmaceutical dosage form [11-25]. The methods utilize Methylene blue [26], which has successfully been used for the sensitive spectrophotometric determination. The present work deals with the development of four simple, low cost and sensitive Spectrophotometric methods for the quantitative estimation of sulfadoxine in bulk and pharmaceutical Preparations Using Methylene blue.

MATERIAL AND METHODS

Instrument used for the present study was UV-Vis double beam spectrophotometer with 1cm matched pair quartz cell. All chemicals used were of analytical reagent grade. Sulfadoxine are the commercial tablet formulations labelled to contain 500 mg per tablet.

Preparation of Stock Solution and Working Solution: Standard stock solution of Sulfadoxine was prepared by dissolving 10mg of Sulfadoxine in 10ml of Methylene blue and distilled water (80:20) which gives 50 µg/ml. One ml of this stock solution was taken and was diluted up to 10 ml by using Methylene blue and distilled water (80:20) to produce a concentration of 100 µg/ml solution. From the above Methylene blue 10 ml was transferred into 100 ml volumetric flask and volume was made up to the mark with methanol to make 50µg/ml. Then the sample was scanned with UV-Vis Spectrophotometer in the range 200-400nm against Methylene blue and distilled water (80:20) as blank and the wavelength corresponding to maximum absorbance was noted which is its λ max i.e. at 522 nm.

Assay for Tablets: An amount of finely ground tablet powder equivalent to 500 mg of Sulfadoxine was accurately weighed into a beaker; 100 mL of Methylene blue was added and stirred for 20 min and warmed. Then, the content was transferred to a 100 mL calibrated flask, the beaker was washed with water and the washings were also transferred to the flask and the volume was diluted with water to the mark, mixed well and filtered using a Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (50 µg mL^{-1} Sulfadoxine) was diluted appropriately to get 5 and 35 µg mL^{-1} concentrations for

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analysis by method. The assay of the tablets was completed according to the general procedure.

Dual Wavelength Spectrophotometry: In this method [27] two wavelengths were selected for each drug in a way so that the difference in absorption is zero for one drug at a time. As per spectrum, we found that absorption of Sulfadoxine was same in 465 and 496 nm. The entire mixed standard was scanned at these selected wavelengths and a calibration curve was plotted between absorbance difference and the respective concentrations. The value of coefficient of correlation was 0.9994 Sulfadoxine. The sample solutions were measured at selected wavelengths and the values of difference in absorbance were extrapolated on the working standard curve to get the concentration.

Method Validation: The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for specificity, linearity, precision (repeatability and intermediate precision), accuracy, short term stability and system suitability. Standard plots were constructed with seven concentrations in the range of 10-50 µg/mL prepared in triplicates to test linearity. The peak area of Sulfadoxine was plotted against the concentration to obtain the calibration graph.

The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicates injections of freshly prepared Sulfadoxine test solution in the same equipment at a concentration value of 100 % (20 µg/mL) of the intended test concentration value on the same day. Method accuracy was tested (% recovery and % RSD of individual
measurements) by analyzing sample of Sulfadoxine at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Sulfadoxine recovered in the samples. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The %RSD was found and lying with in ±2.0.This showed that the precision of the methods are satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods.

RESULTS AND DISCUSSION

A linear correlation was found between absorbance at λ max and concentration of Sulfadoxine. The graphs showed negligible intercept and are described by the equation:

Table 1: Analytical and regression parameters of the proposed methods.

Parameter	Methylene blue
Beer's law limits	10-50
λ max, nm	522 nm
Molar absorptivity	8.2×10^2
Sandell sensitivity	0.3190
Limit of detection	1.64
Limit of quantification	1.07
Regression equation *	
(a) Intercept	3.553
(b) Slope	0.9543
Correlation coefficient, (r)	0.9996
LOD	0.186
LOQ	0.325

Table 2: Accuracy of the proposed method

Sample	Label Claim	Estimated amount (mg/tab)	Spike Level (%)	Amount of Drug Added	Amount of Drug recovered	% Recovery	RSD (% n=5)
Method	500	501.08	80	10	500.87	100.33	0.762
			100	15	499.08	99.97	0.311
			120	20	500.04	100.07	0.098

Table 3: Intraday, Interdays, data of tablet formulation.

		Interday precision % COV		
Sample	Intra day precision % COV (n=6)	Day 1[a]	Day 2[a]	Day 3[a]
Method	1.7453	1.326	0.801	0.663

COV: Coefficient of variance

$$Y = a + bX$$

(Where Y = absorbance of 1^{-cm} layer of solution; a = intercept; b = slope and X = concentration in µg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and r1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of Dual wavelength Spectrophotometry are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [28] are also presented in Table. 1 and reveal the very high sensitivity of the methods. To evaluate the accuracy and precision of he methods, pure drug solution at three different levels (within the working limits) was analysed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision for the methods. For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 1.9-2.5% and represent the best appraisal of the methods in routine use. The linearity of the methods were found to be 10-50 µg/ml. The optical characteristics such as correlation co-efficient, slope, intercept, olar absorptivity, sandel's sensitivity LOD and LOQ were calculated and are shown in Table 1. To study the precision of the method the analysis of formulation was carried out for six times. The % RSD values were found to be 0.548. A known amount of standard drug material was added with pre-analysed formulation in different levels. The amount of drug recovered was calculated and the percentage recovery was found to be in the range of 99.96% - 101.14%.

CONCLUSION

The methods that were developed for the determination of Sulfadoxine in the presence of each other are based on analytical techniques. Dual wavelength Spectrophotometry were validated and found to be simple, sensitive, accurate and precise. In spite of the low content of Sulfadoxine, the methods were successfully used to estimate the amount of Sulfadoxine present in tablet formulations without the need for addition of standard Sulfadoxine. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed samples and the mixtures were analyzed by the proposed methods. To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Six samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively.

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