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Visible Spectrophotometric Determination of Sulfamethaxazole in Pharmaceutical Formulations

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Two simple, precise, accurate, costy effective and rapid spectrophotometric methods (mehod **A** and method **B**) were developed for the determination of sulfamethaxazole (SFX) in its bulk and dosage forms. The method **A** involves formation of chloroform extractable yellow colour ion pair complex of the drug with bromocresol green in acidic medium. Method **B** is based on the formation of ternary complex between drug, ammomium thiocyanate and ammonium molybdate in presence of ascorbic acid and HCl. The coloured complexes shows absorbance maxima at 430 and 690 nm for methods **A** and **B**, respectively. Beer's law is obeyed in the range of 2-10 µg/mL for both methods **A** and **B**. Results of analysis for all the methods were validated statistically and by recovery studies.

Key Words: Sulfamethaxazole, Sandell's sensitivity, Ternary complex, Ion pair complex Beer's law.

INTRODUCTION

Sulfamethaxazole is a sulfonamide bacteriostatic antibiotic. Sulfonamides are structural analogs and competitive antagonists of *p*-amino benzoic acid (PABA). They inhibit normal bacterial utilization of *p*-amino benzoic acid for the synthesis of folic acid, an important metabolite in DNA synthesis[1,2]. These are used in the treatment of urinary track infections, eye infections and as a prophylaxis of rheumatic fever[3]. The drug has been determined by a variety of analytical techniques such as high performance liquid chromatography[4,5], high performance thin layer chromatography[6], gas chromatography[7], electro analytical methods[8-11], spectrophotometry[12-16] and differential scanning calorimetry[17]. By exploiting the various functions groups in the sulfamethaxazole the authors had developed two simple and sensitive spectrophotometric methods for the determination of sulfamethaxazole in pharmaceutical formulations and biological samples.

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EXPERIMENTAL

Elico UV-visible double beam spectrophotometer model SL-156. All the chemicals used were of analytical grade. All the solutions were freshly prepared in distilled water.

Reagents for method **A**: (a) 0.2 % bromocresol green: prepared by dissolving 200 mg of bromocresol green in 100 mL of distilled water. (b) 0.1N HCl. (c) Chloroform (analytical grade)

Reagents for method **B**: (a) 0.003 M ammonium molybdate; (b) 10 % ammonium thiocyanate in distilled water; (c) 10 % ascorbic acid in distilled water; (d) 5 % sodium lauryl sulphate in distilled water; (e) 3 M HCl.

Preparation of standard and sample solution: Accurately weighed 100 mg of sulfamethaxazole was dissolved in 2 mL of 10 M H_2SO_4 and made up to 100 mL with distilled water to give a concentration of 1 mg/mL. The final concentration was brought to 100 µg/mL for both methods **A** and **B**.

Assay procedure for the determination of sulfamethaxazole

Method A: Into a series of 100 mL separating funnels, aliquots of drug sample (0.2-1.0 mL) was added. 1 mL of 0.1N HCl and 2 mL of 0.2 % bromocresol green was added to each funnel and mixed well. The funnels were shaken vigorously with 5 mL of chloroform for 2 min and then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a 50 mL beaker, dried over anhydrous sodium chloride and transferred to a 10 mL volumetric flask. Then the combined extract was made up to the mark with chloroform and mixed well. The absorbance of the organic phase was measured at 430 nm against a reagent blank similarly prepared. The standard calibration curve was prepared to calculate the amount of the drug.

Method B: Appropriate volumes of the standard solution containing (20 -100 µg) of sulfamethaxazole, 2.5 mL of 0.003 M ammonium molybdate solution, 1 mL of 3 M HCl, 0.5 mL of 10 % ascorbic acid solution and 2 mL of 10 % ammonium thiocyanate were added in heating tubes. Left for 15 minutes until complete formation of Mo (V)-SCN complex, 1.5 mL of 5 % sodium lauryl sulphate was added, the mixture was homogenized by shaking, immersed in a water bath at 50 ºC for 0.5 h, then cooled to room temperature. The mixture was transferred into a 10 mL volumetric flask then diluted to volume with distilled water and the absorbance was measured at λ_{max} 690 nm against a blank. The standard calibration curve was prepared to calculate the amount of the drug

Assay of pharmaceutical tablets: Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of the sulfamethaxazole was dissolved in water and filtered. The filtrate was made up to 100 mL and appropriate aliquots of the drug solution were treated as described above for the determination of sulfamethaxazole.

RESULTS AND DISCUSSION

The method **A** is based on the formation of ion-pair complex between the positively charged drug and anionic dye bromocresol green. Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction. The method **B** is based on the fact that an ion-pairs is formed between the sulfamethaxazole and molybdenum(V) thiocyanate binary complex through the protonated nitrogen atom of the drug. The reduction probability of Mo(VI) may occur by ascorbic acid or SCN^- in acidic media and react with thiocyanate to form a red binary Mo(V) SCN^- complex, non extractable with chloroform.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity for these methods are presented in Table-1. The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Parameters	Method **A**	Method **B**
λ_{max} (nm)	430	690
Beer's law limit (µg/mL)	2 – 10	2 – 10
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.028	0.036
Molar absorptivity (L mol^{-1} cm^{-1})	2.880×10^4	1.696×10^4
Stability of color (h)	12	8
Regression equation (Y)*		
Intercept (a)	0.013	0.008
Slope (b)	0.003	0.0024
% RSD**	0.86	1.23
% Range of errors (95 % confidence limits):		
0.05 significance level	0.726	1.02
0.01 significance level	1.075	1.52
Correlation coefficient	0.9988	0.9987

*Y = a + bx, where Y is the absorbance and x is the concentration of Sulfamethaxazole in µg/ mL; **for six replicates

The accuracy of these methods was ascertained by comparing the results obtained with the proposed and reference methods in the case of formulation are presented in Table-2. As an additional check on the accuracy of these methods, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed formulation and percent recovery values obtained are listed in Table-2. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

Conclusion

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the

TABLE-2
RESULTS OF ANALYSIS OF TABLET FORMULATIONS
CONTAINING SULFAMETHAXAZOLE

Formulations	Labeled amount (mg)	Recovery by reference method* (%)	Recovery by proposed methods (%)**	
			Method **A**	Method **B**
Tablet I	100	99.90	99.89	99.75
Tablet II	100	99.86	99.90	98.65
Tablet III	100	98.76	99.74	99.79

*Reference method was UV method developed in the laboratory.
**Recovery amount was the average of six determinants.

reproducibility and accuracy of the methods. Analysis of the authentic samples containing sulfamethaxazole showed no interference from the common excipients. Hence, these methods could be considered for the determination of sulfamethaxazole in the quality control laboratories.

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