Simple Spectrophotometric Methods for the Determination of Sulfamethaxazole in Pharmaceuticals Using FolinCiocalteu and Orcinol as Reagents

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Received 3 September 2008; Accepted 5 October 2008

Abstract: Two simple, sensitive and reproducible spectrophotometric methods (Method A and Method B) were developed for the determination of sulfamethaxazole in bulk and in dosage forms. Method A is based reduction of phosphomolybdic acid present in Folin Ciocalteu reagent by the drug sulfamethaxazole in the presence of sodium carbonate to form a blue colored chromogen having maximum absorption at 760 nm. Method B is based on the diazotization of the drug by sodium nitrite in acidic medium at 5 °C followed by coupling with orcinol to form yellow colored species (λ_{max} 390 nm). Beer's law is obeyed in the range of 5-25 µg/mL for method A and 2-10 µg/mL for method B. Results of analysis were validated statistically and by recovery studies. These methods are successfully employed for the determination of sulfamethaxazole in various pharmaceutical preparations and biological fluids.

Keywords: Sulfamethaxazole (SFZ), Sandell's sensitivity visible, Spectrophotometric determination, Beer's Law.

Introduction

Sulfamethoxazole is a sulfonamide bacteriostatic antibiotic. Sulfonamides are structural analogs and competitive antagonists of *para*-aminobenzoic acid (PABA). They inhibit normal bacterial utilization of PABA for the synthesis of folic acid, an important metabolite in DNA synthesis^{1,2} are used in the treatment of urinary track infections, eye infections and

as a prophylaxis of rheumatic fever³. The drug has been determined by a variety of analytical techniques such as high performance liquid chromatography^{4,5}, high performance thin layer chromatography⁶, gas chromatography⁷, electroanalytical methods⁸⁻¹¹, spectrophotometry¹²⁻¹⁶ and differential scanning calorimetry¹⁷. In the present study the authors had developed two simple and sensitive spectrophotometric methods for the determination of SFZ in pharmaceutical formulations and biological samples.

Experimental

Apparatus

- Systronics UV - Visible Double beam spectrophotometer model 2201.
All the chemicals used were of analytical grade. All the solutions were freshly prepared in distilled water.
- *Folin Ciocalteau reagent*: This reagent is commercially available. The original stock reagent was diluted to 1:2 ratios with water (Method A).
- *20% Sodium carbonate*: Prepared by dissolving 20 g of sodium carbonate in 100 mL of distilled water (Method A).
- *0.1% Sodium nitrite*: Prepared by dissolving 100 mg of sodium nitrite in 100 mL of distilled water (Method B).
- *5N HCl*(Method B)
- *0.2% Orcinol*: Prepared by dissolving 200 mg of orcinol in 100 mL of distilled water (Method B)

Preparation of standard and sample solution

Accurately weighed 100 mg of SFZ was dissolved in 2 mL of 10 M H₂SO₄ 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 method A and 200 µg/mL Method B.

General procedure for the determination of sulfamethaxazole

Method A

Aliquots of the working standard solution of SFZ (100 µg/mL) were transferred into a series of 10 mL calibrated flasks. To all the flasks 1 mL of 1:2 diluted Folin Ciocalteau reagent and 2 mL of 20% sodium carbonate were added. The solution was made up to the mark with distilled water, mixed well and kept aside for 20 min. The absorbance of the blue colored solution was measured at 760 nm against the corresponding reagent blank.

Method B

Aliquots of working standard solution of SFZ solutions (200 µg/mL) were transferred into 10 mL calibrated flasks followed by 2.0 mL 5N HCl to each. After cooling in an ice bath, 2 mL of 1% sodium nitrite was added under swirling. The solutions were allowed to stand for 5 min and then 1 mL of 0.2% orcinol was added. The solution was made up to the mark with distilled water, mixed thoroughly and after 5 min the absorbance was measured at 390 nm against a reagent blank, and the calibration graph was constructed.

Assay of pharmaceutical tablets

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of the SFZ 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 SFZ.

Results and Discussion

Spectral characteristics

Method A involves the reduction of the phosphomolybdic acid by the SFZ in the presence of sodium carbonate. Method B involves the diazotization of SFZ, followed by coupling with orcinol. The absorption spectra of reduced phosphomolybdic acid and SFZ-orcinol complex have absorption maxima at 760 and 390 nm respectively.

Analytical data

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from six replicate samples) were calculated for all the methods and the results are summarized in Table 1. Regression characteristics like standard deviation of slope (b), standard deviation of intercept (a) and % range of error (0.05 and 0.01 confidence limits) and were calculated for all the methods and are shown in Table 1.

Table 1. Optical and regression characteristics, precision and accuracy of the proposed methods.

Parameters	Method A	Method B
$\lambda_{\max}$, nm	760	390
Beer's law limit, $\mu\text{g}/\text{mL}$	5 - 25	2 - 10
Sandell's Sensitivity, $\mu\text{g}/\text{cm}^2/0.001 \text{ abs. unit}$	0.01	0.017
Molar absorptivity, $\text{Litre.mole}^{-1}.\text{cm}^{-1}$	2.53×10^4	1.481×10^4
Stability of Color (hours)	48	24
Regression equation (Y)*		
Intercept (a)	0.013	0.014
Slope(b)	0.0090	0.005
% RSD ^s	1.8	1.72
% Range of errors, 95% confidence limits:		
0.05 significance level	0.67	0.61
0.01 significance level	1.47	1.38

* $Y = a + bx$, where Y is the absorbance and x is the concentration of sulfamethaxazole in $\mu\text{g}/\text{mL}$ \$ for six replicates

Analysis of pharmaceutical preparations

Application of the proposed methods to the determination of SFZ in its dosage forms was successfully made; the results are presented in Table 2. The excellent recoveries obtained indicated the absence of any interference from the excipients.

Table 2. Results of analysis of tablet formulations containing SFZ.

Formulations	Labeled amount, mg	Recovery by reference method*, %	Recovery by proposed methods**, %	
			Method A	Method B
Tablet I	100	99.90	99.95	99.96
Tablet II	100	99.86	99.70	98.60
Tablet III	100	98.76	99.80	99.69

* Reference method was UV method developed in the laboratory.

** Recovery amount was the average of six determinants.

Conclusions

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing SFZ showed no interference from the common excipients. Hence, these methods could be considered for the determination of SFZ in the quality control laboratories.

Acknowledgements

The authors are grateful to the Acharya Nagarjuna University, Guntur and Management of Siddhartha Academy, Vijayawada for their continuous support and encouragement and for providing the necessary facilities.

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