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Research Article

SPECTROPHOTOMETRIC QUANTITATION OF SULFAMETHOXAZOLE IN BULK DRUG AND PHARMACEUTICAL FORMULATION USING MULTIVARIATE TECHNIQUE

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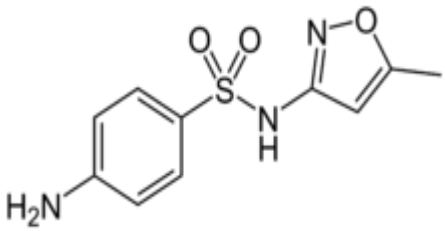
ABSTRACT
A sensitive and accurate UV spectrophotometric method with multivariate calibration technique for the determination of sulfamethoxazole in bulk and pharmaceutical formulation has been described. This technique is based on the use of the linear regression equations by using relationship between concentration and absorbance at five different wavelengths. The results were treated statistically and were found highly accurate, precise and reproducible. The method is accurate, precise (% recovery r =) and linear within the range µg/ml. There was no interference from the excipients. This statistical approach gives optimum results for the eliminating fluctuations coming from instrumental or experimental conditions.

KEY WORDS: UV Spectrophotometry, sulfamethoxazole, pharmaceutical analysis, antimicrobial

INTRODUCTION

Sulfonamides belong to a group of antibacterial drugs, which are often prescribed for treatment of many human and animal infectious diseases. [1] The use of sulfonamides has increased over time especially in combination with trimethoprim (TMP).[2] A number of analytical methods have been described in the literature for the determination of sulfonamides, including the simultaneous analysis of TMP and SMZ. The linear ranges were 2.0–10.0 and 10.0–50.0 mg l_1, and the limits of detection (LOD) were 0.45 and 1.21 mg l_1, respectively. polarographic and voltammetric [3–6] spectrophotometric[7–14] and liquid chromatographic procedures.[15–19].Akay [15] applied reverse phase HPLC with

ultraviolet detection for the simultaneous analysis of TMP and SMZ. This paper describes the application of UV spectral multivariate calibration technique having simple mathematical content for the quantitative determination of sulfamethoxazole in pharmaceutical formulation.



Sulfamethoxazole

THEORY

The basis of this method i.e. multivariate spectral calibration contains the use of linear regression functions obtained five different wavelengths set.[20] This pproach is based on the reduction of multi-linear regression functions to univariate data set, which provides more sensitive determination than the classical method. In case of single wavelength UV spectrophotometry, some errors may occur because of instrumental variations and other sources.

Under optimized conditions the applied statistical method provides considerable resolving power, sensitivity, rapidity and low cost for the quantitative analysis, quality control and routine analysis of subject compounds. The mathematical algorithm of this approach is based on the following summation of multivariate to univariate data sets.

If the absorbance of an analyte (Z) is measured at five wavelengths set (λ = 279.6, 281.6, 283.6, 285.6, 287.6), straight line equation can be written as;

$$A_\lambda = a\times(C_z + k) \rightarrow (1)$$

Where, Aλ represent the absorbance of the analyte

a is the slope and

K is the intercept of linear regression function of the analyte

C_z represents the concentration of analyte.

At five selected wavelengths, the equation system can be summed as

$$A_T = a\times(C_z + b)\times (C_z + c) \times(C_z + d) \times(C_z + e) \times(C_z + K_T) \longrightarrow (2)$$

Which can be simplified to $A_T = C_z(a+b+c+d+e)+K_T \longrightarrow (3)$

Where a, b, c, d, e are the slopes,

A_T & K_T represents the sum of absorbance obtained and sum of intercepts of regression equations at five wave lengths set respectively.

The concentration of the Z analyte in a mixture can be calculated by using –

$C_Z = A_T - K_T / (a+b+c+d+e) \longrightarrow (4)$

EXPERIMENTAL METHODS

Apparatus

All absorption spectra were recorded with a UV-2202 UV/Vis double beam spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells (Systronics).

Reagents and chemicals

API working standard of Sulfamethoxazole was obtained from Natco Pharma Ltd. and test samples were obtained from local market. Methanol is used as solvent.

Procedure

Preparation of Standard Solutions

Standard stock solution of sulfamethoxazole (10 µg/ml) was prepared by dissolving 100mg initially in methanol and then in distilled water. A validation set of 10 solutions in working range of 1-10 µg/ml were freshly prepared and scanned in UV region. The absorption maxima was found at 283.6nm. Absorbance versus concentration was plotted which follows Beer and Lamberts law and gives a straight line. In order to improve this correlation and minimize instrumental fluctuations, absorbance of these solutions were measured over a range of 283.6 nm i.e., 279.6, 281.6, 283.6, 285.6 and 287.6nm.

Preparation of Test Solution

Twenty sulfamethoxazole tablets were powdered in a mortar and an amount equivalent of 10mg of drug was dissolved in methanol which was further diluted in the working concentration range.

METHOD DEVELOPMENT & VALIDATION

The five linear regression functions at the wavelengths 279.6, 281.6, 283.6, 285.6 & 287.6nm for reference standard and tablets were calculated using relationships between the absorbance and concentration. The unknown concentration of sulfamethoxazole in tablets was determined by eq. 4 using the sum of absorbance obtained at above wavelengths for lengths (tables 1 & 2).

The method was validated as per ICH guidelines in order to determine the linearity, precision & accuracy (tables 3 & 4).

Validation of the Method

Linearity

Linearity of the proposed method was verified by analyzing different concentrations in the range of 1-10 µg/ml for Sulfamethoxazole. Each concentration was made in triplicate.

Accuracy

The accuracy of the method was performed by conducting the recovery studies (80, 100 and 120%) of pure drugs from marketed formulation, by standard addition method. The actual and measured concentrations were then compared.

Precision

The intraday precision of the developed method was evaluated by analyzing samples of three different concentrations and the inter day precision was evaluated from the same concentration on three consecutive days; precision was evaluated from the same concentration by three different analysts.

RESULTS AND DISCUSSION

The minimization of the instrumental fluctuations and thus the errors has been observed.

Table 1: Concentration found in Sulfamethoxazole in Gantanol tablets

Concentration (µg/ml)	*279.6nm (λ_1)*	*281.6nm (λ_2)*	*283.6nm (λ_3)*	*285.6nm (λ_4)*	*287.6nm (λ_5)*	*Multi UV**
1	1.01	1.04	1.06	1.08	1.08	0.9
2	2.04	2.07	2.08	2.08	2.07	2.1
3	3.08	3.14	3.17	3.18	3.18	3.0
4	4.04	4.29	4.31	4.30	4.18	4.1
5	5.43	5.47	5.49	5.50	5.48	5.2
6	6.14	6.07	6.10	6.12	6.07	5.8
7	7.10	7.21	7.27	7.27	7.22	7.0
8	8.30	8.34	8.34	8.28	8.22	8.0
9	9.53	9.52	9.49	9.46	9.44	9.1
10	10.30	10.75	10.30	10.27	10.21	10.0

Table 2: Regression characteristics of Sulfamethoxazole

Wavelength (nm)	*Regression equation*	*R^2 Value*
279.6nm	A=0.0607 C_X + 0.0674	0.998
281.6nm	A=0.0678 C_X + 0.0587	0.997
283.6nm	A=0.0667 C_X + 0.0904	0.999
285.6nm	A=0.0638 C_X + 0.0839	0.995
287.6nm	A=0.0643C_X + 0.0717	0.996

Table 3: Accuracy of Sulfamethoxazole

(Standard Addition Technique)

Conc. of drug in formulations(µg/ml)	*Conc. Of pure drug added (µg/ml)*	*Total conc. Of drug found (µg/ml)*	*% Analytical recovery (±SD)*
5	3.6	8.69	100.20 ± 0.024
5	4	9.12	102.50 ± 0.018
5	4.4	9.52	101.15 ± 0.013

Table 4: Precision of Sulfamethoxazole

Repeatability (RSD, n=6)%	0.43	0.98
Precision (RSD)% Intraday (n=6) Interday (n=6)	 0.97 -1.28% 1.69 - 1.72%	 0.73- 1.45 % 1.46 - 1.57%

CONCLUSION

The data obtained for the estimation of Sulfamethoxazole in its drug formulation showed the high level of accuracy and precision after multivariate calibration. Percent recovery and found concentration of the active ingredient in pharmaceutical formulations showed that the amount of drug present is consistent with the label claim.

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