Research Article

QUANTITATIVE METHOD DEVELOPMENT AND VALIDATION OF SULFASALAZINE IN TABLET DOSAGE FORM BY UV-SPECTROSCOPY

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ABSTRACT

A simple and reproducible UV spectrophotometric method for the quantitative determination of sulfasalazine in tablet dosages form was developed and validated in the present work so that amount of the drug metabolized and excreted unchanged from urine can be calculated. The parameters linearity, precision, accuracy, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. UV spectrophotometric method was performed at 354 nm. It is found that the selectivity and sensitivity of method to be in desirable range. The samples were prepared in ethanol. The method obeys Beer's Law in concentration ranges employed for evaluation. The content of sulfasalazine in tablet dosage form was determined. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantitation of sulfasalazine in dosages form. The linear concentration ranges were 2-25 µg/mL, (p value 0.927, Co-relation coefficient = 0.989).

Keywords: UV spectrophotometer, Sulfasalazine tablet, future body fluid analyser.

INTRODUCTION

Sulfasalazine is a sulfa drug[1]. It is a derivative of mesalazine. It is formed by combination of sulfapyridine with salicylate by an azo bond. It can be abbreviated by SSZ[2]. Sulfasalazine and its metabolite 5-aminosalicylic acid (5- ASA) are poorly absorbed from gut so its main mode of action is believed to be inside the intestine. Sulfasalazine is used for the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease[3]. Sulfasalazine can reverse the scarring associated with cirrhosis of the liver. Myofibroblasts cells contribute to scar tissue in a diseased liver; these cells also secrete proteins that prevent the breakdown of the scar tissue. Sulfasalazine retard this secretion. It also helps in healing cirrhosis of the liver[4].

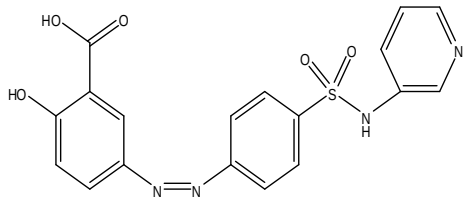

Figure 1: Chemical structure of Sulfasalazine

It is so commonly used drug but, till today no quantitative determination has been performed by using UV spectrophotometry for the same, so in the present research paper a novel work has been performed to interpret the amount of drug used, metabolized and excreted in unchanged form from the biological fluids.

UV absorption spectroscopy is one of the best methods for determination of amount of the organic compounds. Peaks observed for standard is compared with that of sample by measuring the absorbance at specific wavelength.

This determination is based on Beer's law which is as follows.

$A = \log I_0 / I_t = \log 1/T = -\log T = abc = \varepsilon bc$

Where ε is extinction co-efficient, c is concentration, and b is the length of the cell that is used in UV spectrophotometer[5].

MATERIALS AND METHODS

Apparatus

A LAB INDIA UV-3000 UV/VIS Spectrophotometer was used with 1cm match quartz cell.

Materials and Reagents

All the reagents and solvents were of analytical grade. Methanol is used as a solvent (Mw-32.64g/mol) of Merck specialties private Ltd. The drug selected for study were procured from Ipca laboratories ltd. (INDIA) and the structure of the drug is given below-

Preparation of standard solution

Equivalent to 500 mg of pure sulfasalazine was weighed accurately and transferred into a clean 100 ml volumetric flask. 50 ml of methanol was added and sonicated for 5 min and then make up to the volume with methanol to prepare working standard solution (5mg/ml). The above solution was filtered through Whatman filter paper and the filtrate was collected. The prepared standard solution is of 5000 µg/ml. From this, 1 ml was pipette out and put in the 100 ml volumetric flask. Dilution of this used as stock solution of 50 µg/ml. From this stock solution we

prepare different dilutions of 2, 4,6,10,12,14,16,20,22,25 µg/ml concentrations.

Preparation of sample solution

Equivalent to 500 mg of sulfasalazine was weighed accurately, from the crushed 20 tablet powder and a stock of 50 µg/ml. and further dilution of 2, 4,6,10,12,14,16,20,22,25 µg/ml concentrations were prepared as discussed in preparation of standard.

Selection of analytical wavelength

Standard solution of sulfasalazine (10 µg/ml) was scanned in the range of 200 to 500 nm for the determination of wavelength having maximum absorbance. Sulfasalazene showed 354 nm as the wavelength having maximum absorbance.

Table 1: Absorbance of pure sulfasalazine and marketed preparation (tablet) at 354 nm wavelength

Concentration (µg/ml)	Absorbance, pure (nm)	Absorbance, marketed (nm)
2	0.078	0.102
4	0.182	0.162
6	0.255	0.249
10	0.411	0.412
12	0.495	0.504
14	0.587	0.576
16	0.666	0.632
20	0.889	0.779
22	0.940	0.889
25	1.115	1.110

Table 2: Validation parameters

Parameter	Result
Absorption maxima(nm)	354 nm
Linearity range(µg/ml)	2-25µg/ml
Standard regression equation	Y=0.041x-0.002
Correlation cofficient	0.989

General procedure

These selected analytical wavelength were used for the determination of absorbance of the pure drug and marketed sample by the help of different dilutions of 2, 4, 6, 10, 12, 14, 16, 20, 22, 25 µg/ml concentrations. These dilutions of standard and test analyzed for three times and prepared the calibration curve.

RESULTS

Linearity

Linearity was obtained between 2-25µg/ml concentration and absorbance. The equation of calibration curve obtained was Y=0.041x-0.002.The correlation was 0.989 shown in above figure.

Analysis of Variance (Anova)

Anova method for making simultaneous comparisons between two or more means had been used. This is a statistical method that yields values that can be tested to determine whether a significant relation exists between variables.

Solution – Let us take the hypothesis that there is marked effect of concentration on absorbance.

Null Hypothesis: H_0: $\mu_1=\mu_2$, Alternative Hypothesis: H_1: $\mu_1\neq\mu_2$

Tests of Between-Subjects Effects

Interpretation of Anova Table

Since the p value (0.927) is more than 0.05 (5% level of significance). Hence concentration in the marketed preparation is, as labeled.

Table 3: Test whether on Increasing Concentration, Absorbance Increases or Not of the Pure and Marketed Formulation

Concentration (µg/ml)	Standard Drug (Abs)	Marketed Drug (Abs)
2	0.078	0.102
4	0.182	0.162
6	0.255	0.249
10	0.411	0.412
12	0.495	0.491
14	0.587	0.582
16	0.666	0.661
20	0.889	0.832
22	0.940	0.919
25	1.110	1.064

Table 4: Dependent Variables

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.001[a]	1	0.001	0.009	0.927
Intercept	6.146	1	6.146	54.787	0.000
concentration	0.001	1	0.001	0.009	0.927
Error	2.019	18	0.112		
Total	8.166	20			
Corrected Total	2.020	19			

Df = degree of freedom, F = ANOVA variable

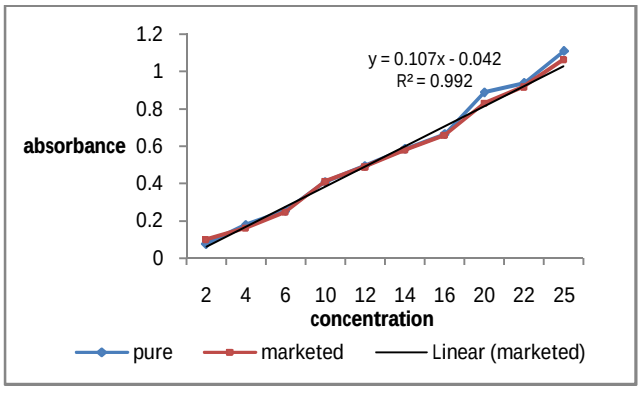

Figure 2: Absorbance of pure sulfasalazine and marketed preparation (tablet) at 354 nm wavelength

DISCUSONS

Attempt has been made to develop rapid sensitive, economic, precise and accurate analytical method for sulfasalazine in pure and pharmaceutical dosage form. The proposed method is based on UV spectophtometeric

absorption using methanol as solvent, maximum absorbance was found to be at 354 nm. Beer's law was obeyed in concentration ranging from 2-25µg/ml. The correlation coefficient values were above 0.989 which shows that absorbance was linear with concentration. The optical characteristics such as beer's law limit, correlation coefficient and analysis of variance were calculated and validated. To study interference of various excipient recoveries was done for formulation. It showed that there is no interference of excipient on the pure drug. From all the validation parameters, the develop method was found to be simple, economic and accurate. Hence proposed method could be effectively applied for analysis of sulfasalazine in bulk and formulated tablet dosage form.

CONCLUSION

A spectrophotometric method for quantifying sulfasalazine in pure and tablet form has been developed and validated. This method is selective, precise, accurate, reproducible and linear over the concentration range studied. This method is simple and suitable for the determination of sulfasalazine in formulations without interference from excipient or from common degradation products in IPQC (In- Process quality control) and pharmacokinetic studies. The same developed method can be very easily simulated with biological fluids to quantify that how much drug has been metabolised and how much is excreted unchanged.

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