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Original Article

# Application of UV spectrophotometric method for easy and rapid estimation of sulfasalazine in pharmaceutical formulation (suspension)

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## ABSTRACT

**Introduction**: The present research work is about the development of a simple, economic, accurate, quick and reproducible UV spectrophotometric method for sulfasalazine (SFZ) estimation in the pharmaceutical formulation (suspension) and also when it is in the bulk. **Materials and Methods**: Water and methanol was used in proportion of 1:1 for the preparation of stock solution. Different solutions of SFZ were prepared by diluting the stock solution with water. **Results**: The solution was prepared and determine at wavelength $\lambda_{max}$ 359 nm. The drug was determined at maximum wavelength ($\lambda_{max}$ 359 nm). When the drug concentration is in the range of 2–20 µg/ml it obeys Beers law with line equation y=0.073x+0.003 and correlation coefficient of 0.992. Results obtained were validated statistically and by recovery study method. **Conclusion**: The result of analysis was validated according to ICH guidelines and this proposed method can be used for the routine analysis of sulfasalazine suspension formulation.

**Key words**: Sulfasalazine, UV spectrophotometry, Validation, Coefficient, Statistically.

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## INTRODUCTION

***Sulfasalazine*** is used in various diseases of ulcerative colitis.[1] Sulfasalazine actually decreases the symptoms associated with bowel diseases; like abdominal pain, infection, inflammation, diarrhea and bleeding in the rectum. The mechanism of action of Sulfasalazine (SFZ) and its metabolites, is unclear, but it shows anti-inflammatory and immune-modulatory properties in clinical trials. Autoradiographic studies shows that SFZ when given in higher concentration accumulates in serous fluid, in intestine and in the liver.[2] Major therapeutic effect of SFZ and its metabolites is in ulcerative colitis.[3] Orally administered drug shows < 15% bioavailability. SFZ metabolized to SP and 5-ASA by intestinal bacterias. SP shows good absorption properties as compared to 5-ASA.

In order to formulate a drug molecule it is important to analyze the procedure and to develop a simple, accurate, precise, sensitive and reproducible method for the estimation of drug samples.[4] Our main objective is to develop and to validate UV spectrophotometric method according to ICH guideline. Valid measurements will be obtained through validation. Quality, reliability and consistency of analytical methods can be obtained through method validation results.[5] Absorbance and concentration relationship was described by Beer's law. It states that the monochromatic light's intensity decreases as the number of absorption molecules increases.[6]

No method has been developed up till now in order to analyze SFZ in the pharmaceutical formulation or in the bulk sample. Soit was the need to develop the simplest method for the quantitative determination of SFZ. The present work is to develop the spectrophotometric methods and validation according to the guidelines of ICH.

## MATERIALS

Sulfasalazine raw material was obtained from Sigma Aldrich of USA while Methanol of Merck KGA, Germany and sulfasalazine suspension of Azulfidine was obtained. Analytical grade reagents were used in this procedure. UV/Visible Spectrophotometer (double beam) (ShimadzuUV-1600) and analytical balance of Shimadzu (AUW220D) were used.

## METHODOLOGY

### Preparation of standard stock solutions

Take 5 ml of methanol in a volumetric flask of 10 ml. Add 10 mg sulfasalazine in it. Dissolve it and add quantity sufficient water in it to prepare stock solution containing 1 mg/ml of SFZ.

### Preparation of calibration curve

Take 500 ml volumetric flasks and transfer aliquots of 1-10 ml portion of stock solutions into them and then add quantity sufficient distilled water upto the mark. At 200-400 nm solutions were scanned using the blank. The $\lambda_{max}$ found was 359 nm against blank. The calibration curve was plotted by making different concentrations. For determine calibration curve different dilutions were prepared i.e. 2 µg/ml, 4 µg/ ml, 6 µg/ ml, 8 µg/ ml, 10 µg/ ml , 12 µg/ ml , 14 µg/ ml , 16 µg/ ml, 18 µg/ ml and 20 µg/ ml, table 2 shows the optical characteristics.

### Preparation of sample solution

SFZ suspension that was available in the market analyzed by the proposed method. Take commercially available SFZ in 10 ml volumetric flak that is equivalent to 10 mg of SFZ. Now take 5 ml of methanol and add it into volumetric flask. Mix it well and leave the solution for 15 min, Now add water Q.S to 10 ml. Take Whatman filter paper #41 and filter the solution. In this way different solutions were prepared having concentrations of 10 µg/ ml, 15 µg/ ml and 20 µg/ ml by diluting with distilled water. Absorbance of all the solutions was then determined against blank. Standard calibration curve was used to determine drug content in the preparation. Table 3 shows the concentration of the drug determined

**Table 1: Dilution of sulfasalazine and its absorbance for calibration curve**

| standard ID | concentration(mcg) | Absorbance |
|---|---|---|
| 1 | 2 | 0.160 |
| 2 | 4 | 0.339 |
| 3 | 6 | 0.484 |
| 4 | 8 | 0.537 |
| 5 | 10 | 0.696 |
| 6 | 12 | 0.861 |
| 7 | 14 | 1.046 |
| 8 | 16 | 1.145 |
| 9 | 18 | 1.376 |
| 10 | 20 | 1.500 |
| y= | mx+c | |
| slope | 0.073 | |
| intercept | 0.003 | |

**Table 2: Summary of the UV method validation**

| Calibration parameters | |
|---|---|
| Absorption maxima | 359 |
| Linearity range mcg/ml | 2 mcg-20 mcg |
| Standard regression equation | y=0.073x +0.003 |
| Correlation coefficient | 0.992 |
| Limit of quantification | 0.41 |
| Limit of detection | 0.13 |
| Beer's law limit | 2-20 µg/ml |

**Table 3: Estimation of sulfasalazine in suspension formulation**

| Label claim | Conc. prepared (µg/ml) | Conc. recovered (µg/ml) | Amount found (mg/ml) | % Label claim | Mean ± SD (n=3) |
|---|---|---|---|---|---|
| 250 mg/ml | 10 | 9.88 | 247 | 98.8 | 97.73 |
| | | 9.79 | 244 | 97.6 | |
| | | 9.68 | 242 | 96.8 | |
| | 15 | 14.78 | 246 | 98.4 | 98.4 |
| | | 14.67 | 244 | 97.6 | |
| | | 14.87 | 248 | 99.2 | |
| | 20 | 19.79 | 247 | 98.8 | 98.4 |
| | | 19.64 | 245 | 98.0 | |
| | | 19.69 | 246 | 98.4 | |

**Table 4: Determination of precision**

| Sample number | Assay of sulfasalazine as % of labeled amount | |
|---|---|---|
| | Analyst 1(intraday precision) | Analyst 2(intraday precision) |
| 1 | 99.72 | 97.51 |
| 2 | 99.34 | 98.48 |
| 3 | 98.23 | 96.43 |
| 4 | 97.33 | 97.57 |
| 5 | 96.67 | 96.54 |
| Mean | 98.258 | 97.306 |
| SD | 1.293433415 | 0.84316665 |

**Table 5: Determination of accuracy**

| ingredient | amount mcg/ml | level of addition % | added amount mcg/ml | total amount mcg/ml | recovery % |
|---|---|---|---|---|---|
| SFZ (suspension) | 10 | 80 | 8 | 18 | 17.8 |
| | 10 | 100 | 10 | 20 | 19.6 |
| | 10 | 120 | 12 | 22 | 21.5 |

by this method.

## RESULT AND DISCUSSION

### Linearity

2-20 µg/ml concentrations of SFZ were used to verify the linearity of the response of the drug. The calibration curve was drawn by taking concentration of the drug on x-axis and absorbance on y-axis and linear regression analysis was then applied as shown in Table 1. Following equation of calibration curve was obtained for sulfasalazine

$$y = 0.073x + 0.003$$

In these concentrations, linear curve was found while r2 (correlation coefficient) was 0.992 as shown in Figure 2.

### Precision

Six assay were performed with SFZ test samples and method precision assay (also known as intraday precision) was carried out. The inter day precision also known as intermediate precision was evaluated by two different analysts, two different systems and different days in the same laboratory. By using two different analysts the relative standard deviation (SD) and assay values obtained were 1.29, 98.25, and 0.84, 97.30, respectively as shown in Table 4.

### Accuracy (recovery test)

Accuracy is the percent of recovered amount by assay from a known added amount. For the measurement of accuracy data three concentration levels were carried out as lower, intermediate and higher concentration from standard solutions and analyzed. The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e., 80%, 100% and 120%. Result of recovery studies are reported in Table 5.

### Robustness and stability of solutions

By changing the reagents and equipments the robustness of the spectrophotometric method was evaluated. The stability of SFZ solutions was determined at different storage conditions for 5 days.

### Determination of active ingredients in suspension

For the determination of SFZ in suspension, validated method was used by performing assay of six bottles. Results were shown in Table 3 which tells that concentration of drug in suspension samples was according to the requirements (97.73%-98.4% of the label claim).

### Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is defined as is the lowest concentration of a substance that an analytical process can reliably distinguished from the absence of that substance. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability (ICH guideline Q2B, 2005) (Table 2). The LOD and LOQ were calculated as:

$$LOD = 3.3 \times \frac{SD\ intercept}{s}$$

$$LOQ = 10 \times \frac{SD\ intercept}{s}$$

Where, S = slope of the linearity curve (0.073x), SD = standard deviation of y-intercept

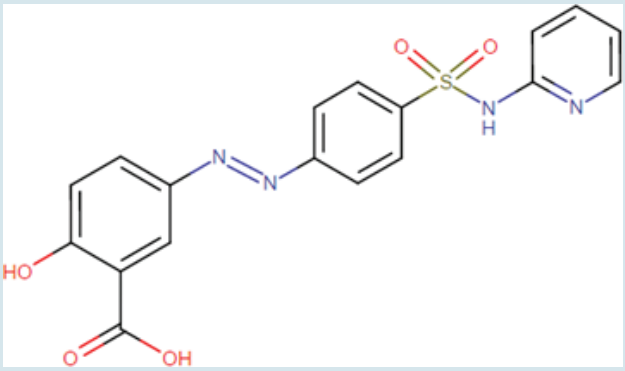


**Figure 1:** Chemical structure of sulfasalazine (Drug Bank).

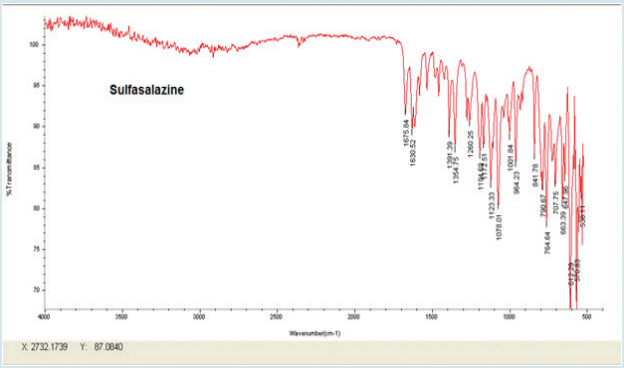


**Figure 2:** FTIR of Sulfasalazine.

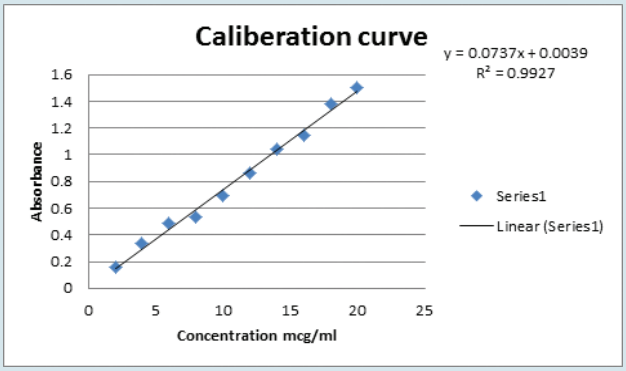


**Figure 3:** Calibration Curve of Sulfasalazine.

## DISCUSSION

For SFZ suspension UV-spectrophotometric method was developed. UV-Spectrophotometric methods the commonest and routinely used method for analyzing pharmaceutical dosage forms and does not require any complex and complicated preparations of the sample. Significantly low standard error, standard deviation, and coefficient of variance were obtained for sulfasalazine while maximum absorbance was obtained at 359 nm. In 2-20 µg/ml of concentration range, 10 Point calibration curve

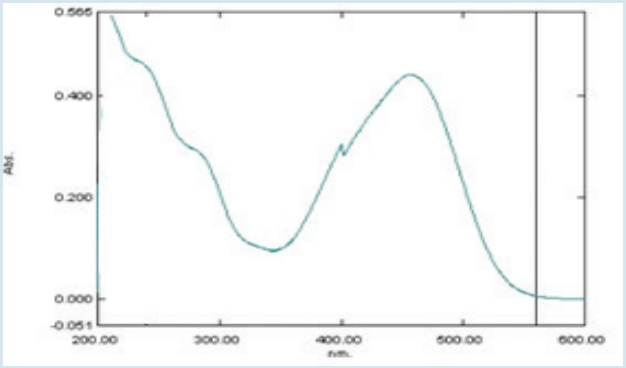


**Figure 4:** UV spectrum of sulfasalazine.

data was drawn, which obeys Beer's law. 0.992 correlation co-efficient was found which is according to the requirement that it should be less than 0.990. The proposed method for SFZ is very sensitive as indicated by limit of detection (LOD) and limit of quantification (LOQ) values i.e.0.13 µg/ml and 0.41 respectively. In SFZ suspension formulations 97.73 ± 1.0 to 98.40 ± 0.4 assay values were obtained. Assay values thus obtained were also according to the requirements which shows that the proposed method does not show any excipients interaction with the formulation. The standard deviation (SD), coefficient of variance and standard error (SE) were significantly low and precision results were also within the required acceptable range.

## CONCLUSION

This methodwas validated for quantification Sulfasalazine suspension 250 mg / 5 ml for oral use. This analytical methodology was developed according to the guidelines of the law. The proposed spectrophotometric method is economical, quick and easy to perform. This method can be used in industry for quality control determination and also in pharmacy for compounding purposes as it is safe and reliable method. The method was validated as per the guidelines laid by ICH. The results of the validated tests were found to be satisfactory and therefore this method can be applied successfully for routine quality control analysis of sulfasalazine in bulk and pharmaceutical formulation (suspension).

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

No conflict of interest.

## ABBREVIATIONS USED

**SFZ:** Sulfasalazine; **SP:** Sulfapyridine; **BD:** Bowel disease; **5-ASA:** 5-aminosalicylic acid; **ICH:** International Conference on Harmonisation.

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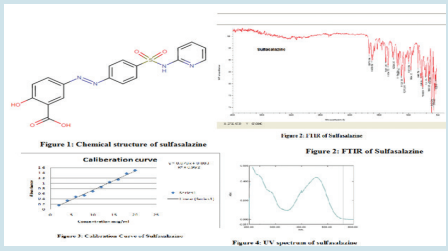
### PICTORIAL ABSTRACT




### SUMMARY

- The present research work discusses the development of a UV estimation method for sulfasalazine. Simple, accurate, cost efficient, and reproducible spectrophotometric method has been developed for the estimation of Sulfasalazine in bulk and pharmaceutical dosage form.

### ABOUT AUTHORS

**Yasir Mehmood:** Is working as Quality Control Manager in National pharmaceutical industry. His current research interests are formulation development of different dosage form, clinical study design and different health related issues. He is also reviewer and editorial member of some journals.