



SPECTROPHOTOMETRIC DETERMINATION OF MESALAZINE IN BULK AND TABLET DOSAGE FORMS BASED ON DIAZOCOUPILING REACTION WITH RESORCINOL

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ABSTRACT

A novel spectrophotometric method for the assay of mesalazine in bulk and dosage form has been developed. The method is based on the diazotization of mesalazine followed by its coupling with resorcinol to form a red colored azo dye. The colored product formed absorbed visible region strongly and optimal detector response was obtained at a wavelength of 490 nm. The analytical parameters and their effects on the reported method are investigated. Beer's law was obeyed in the concentration range of 2-20 µg/ml. The proposed method was directly and easily applied to the tablet dosage forms. Results obtained from the analysis of commercial preparations with the proposed method are in good agreement with those obtained with the reported spectrophotometric method.

Keywords: Mesalazine, Resorcinol, Spectrophotometric analysis, Beer's law.

INTRODUCTION

Mesalazine (MSZ), chemically known as 5-amino-2-hydroxybenzoic acid (Fig. 1), is an anti-inflammatory drug used to treat and also maintain the remission of mild to moderate ulcerative colitis or Crohn's disease¹⁻⁴. MSZ is thought to be the major therapeutically active part of the sulfasalazine molecule in the treatment of ulcerative colitis. MSZ is produced by bacterial action on sulfasalazine in the colon⁵. The mechanism of action of MSZ is unknown, but appears to be topical rather than systemic. Mucosal production of arachidonic acid metabolites (both through the cyclooxygenase pathways and through the lipoxygenase pathways) and hydroxyeicosatetraenoic acids is increased in patients with chronic inflammatory bowel disease, and it is possible that MSZ diminishes inflammation by blocking cyclooxygenase and inhibiting prostaglandin production in the colon⁶. MSZ acts as a scavenger of oxygen-derived free radicals⁷, which are produced in greater numbers in patients with inflammatory bowel disease.

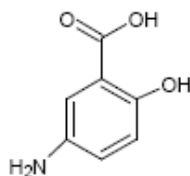


Figure 1: Structure of Mesalazine

The therapeutic importance of MSZ has necessitated the development of analytical methods for its determination in dosage forms in compliance with good manufacturing standards. The drug is officially listed in British Pharmacopoeia⁸ and United States Pharmacopoeia⁹ which describes a HPLC procedure for its assay. HPLC method adopted by the British Pharmacopoeia and United States

Pharmacopoeia is based on the mobile phase containing glacial acetic acid, methanol & methyl isobutyl ketone and tetrabutylammonium hydrogen sulphate as an ion-pairing agent, respectively. Detailed survey of literature for MSZ revealed potentiometric titration¹⁰, micellar electrokinetic chromatography¹¹, differential pulse voltammetry¹², HPLC^{10, 13-19} and LC/MS/MS²⁰ techniques for its determination in pharmaceutical dosage forms and biological fluids. The above mentioned techniques are sensitive but require sophisticated equipment, tedious sample preparation and laborious clean up procedure prior to analysis. Visible spectrophotometry, because of its simplicity, speed, sensitivity, reasonable accuracy and precision, and cost-effectiveness continues to be the preferred technique in laboratories of developing and underdeveloped nations and also avoid interference from common analysis.

In the literature few spectrophotometric methods have been reported for its determination. MSZ has been determined in the visible region based on the reaction with Bratton-Marshall reagent²¹, *p*-dimethylamino benzaldehyde²¹, Gibb's reagent²¹, potassium iodate & potassium iodide²² and 1, 1-diphenyl-2-picrylhydrazyl radicals¹⁰. The reported spectrophotometric methods suffer from one or the other disadvantage such as poor accuracy and precision, use of relatively expensive reagents, carcinogenic reagents, preparation of buffers, heating, narrow range of linear response and low molar absorptivity. Therefore, there is a need for a simple, cost effective and sensitive spectrophotometric method for the determination of MSZ in pharmaceutical dosage forms.

The present communication reports a novel spectrophotometric method for the determination of MSZ in bulk and tablet dosage form. The proposed



method is based on the formation of red colored azo dye by the diazotization of MSZ followed by coupling with resorcinol in alkaline medium having absorption maximum at 490 nm. The proposed method has been successfully applied to the determination of MSZ in tablet dosage form. The proposed method is free from the disadvantages of interference of the excipients normally found along with MSZ in tablet dosage form and does not involve use of carcinogenic reagents or expensive reagents or heating steps. Further, the method was found to possess adequate accuracy and precision.

MATERIALS AND METHODS

Instrumentation

An ELICO double beam model SL 159 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

Materials and Reagents

All the reagents were of analytical grade and all solutions were prepared in double distilled water. Aqueous solutions of 1% (w/v) sodium nitrite (Sdfine-Chem limited, Mumbai), 2% (w/v) sulphamic acid (Merck, Mumbai), 0.5% (w/v) resorcinol (Sdfine-Chem limited, Mumbai), 1 N hydrochloric acid solution (Fisher Scientific, Mumbai), and 4 M sodium hydroxide (Merck, Mumbai) were prepared.

MSZ was kindly provided by Matrix laboratories, Hyderabad, India, and was used as received. Commercial dosage form of MSZ such as Asacol (Win medicare, New Delhi), Meseacol (Sun Max pharma limited, Ahmedabad) and Salofalk GR (Germen remedies, Mumbai) containing 400 mg, 400 mg and 250 mg of active ingredient were purchased locally and included in the present study.

Stock solution of MSZ was prepared by dissolving 100 mg of MSZ in 50 ml of distilled water in a 100 ml volumetric flask and volume was made up to the mark with distilled water (1 mg/ml). The stock solution was diluted stepwise with the same solvent to obtain working standard solution of concentration 100 µg/ml.

General Procedure for the Assay of MSZ

Fresh aliquots of MSZ ranging from 2-20 µg/ml were transferred into a series of 10 ml volumetric flasks. A volume of 1.0 ml of sodium nitrite (1%) was added to each flask followed by 1.0 ml of HCl (1N) and cooled in an ice bath. After 5 min, 1.0 ml of sulphamic acid (2%) was added to each flask. Then, volumes of 0.5 ml of resorcinol (0.5%) and 1 ml of NaOH (4M) solutions were added. The contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored azo dye was measured at 490 nm against the corresponding reagent blank. The colored azo dye was stable upto 3 hours. A calibration graph was constructed and the corresponding regression equation was computed to obtain the concentration of MSZ.

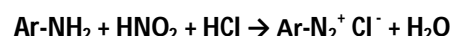
Assay of MSZ in Tablet Dosage Form

To minimize a possible variation in the composition of the tablets, the mixed contents of 5 tablets were weighed and grounded. The powder equivalent to 100 mg of MSZ was stirred well with 50 ml of water and filtered through Whatman No. 1 filter paper. The residue was washed with water for complete recovery of the drug. The filtrate was transferred to a 100 ml volumetric flask and volume was made up to mark with distilled water. The percentage recovery of the drug was calculated from the corresponding linear regression equation.

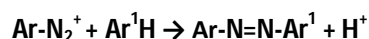
RESULTS AND DISCUSSION

Chemistry and Nature of the Colored Species

Primary aromatic amine upon treatment with nitrous acid in an ice-cooled solution forms diazonium salt²³.



Under proper conditions diazonium salts react with certain aromatic compounds to yield products of general formula Ar-N=N-Ar¹, called as azo compounds and the reaction is called as diazo coupling reaction²³.



The proposed method involves the diazo-coupling reaction of diazonium salt of MSZ with resorcinol in alkaline medium. MSZ was treated with sodium nitrite solution at 0-5°C in acidic medium, resulting in the formation of diazonium chloride. The excess nitrate is removed with sulphamic acid. The diazonium chloride is coupled with resorcinol to form a red colored azo dye in an alkaline medium having λ_{max} at 490 nm (Figure 2). The red color azo dye is stable for about 3 hours. The possible reaction scheme is given in Figure 3.

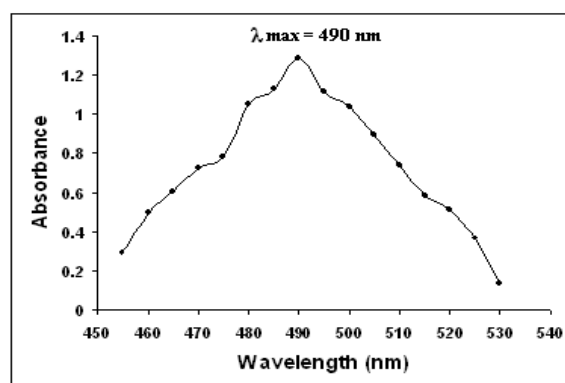


Figure 2: Absorption spectra of Azo dye derivative of MSZ with Resorcinol

Optimization of Reaction Conditions

Experimental variables, such as the concentration of sodium nitrite, HCl, sulphamic acid, resorcinol & NaOH were optimized. The optimum conditions for the color development of the proposed method were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following

experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

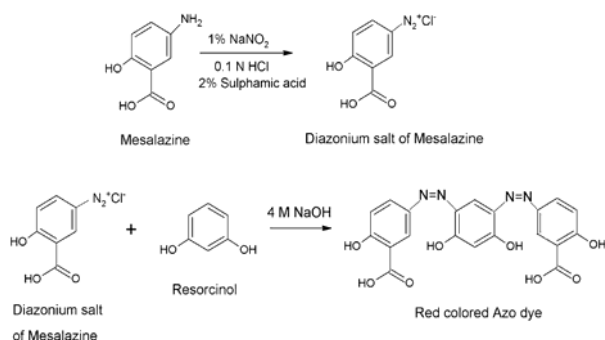


Figure 3: Proposed reaction scheme for the formation of Azo dye derivative of MSZ with Resorcinol

Effect of Temperature on Diazotization

Diazotization and coupling reactions were found to be temperature dependent. Diazotization was carried out at 0-5°C and coupling reactions was carried out at room temperature. Above 5°C (for diazotization) and 30°C (for coupling), there was a decrease in intensity of the color.

Effect of Acid Concentration

The effect of acid strength on the diazotization reaction was studied using different volumes (0.2-2.0 ml) of 1 N HCl, and the maximum absorbance was observed with 1 ml of 1 N HCl. Above this range, a decrease in the absorbance was observed. Therefore, the optimum acid strength for the diazotization was fixed as 1 ml of 1 N HCl.

Effect of Sodium Nitrite and Sulphamic Acid

The effect of adding various amounts of sodium nitrite solution on absorbance of 10 µg/ml MSZ was examined. The concentration of sodium nitrite was varied between 0.2-2.0 ml of 1% aqueous sodium nitrite. The results showed that 1 ml of 1% sodium nitrite gave maximum absorbance. Thus, 1 ml of 1% sodium nitrite was chosen for the procedure. The excess of nitrite could be removed by the addition of 1 ml of 2% sulfamic acid. An excess of sulphamic acid has no effect on color.

Effect of Concentration of Resorcinol

To optimize the concentration of coupling agent, different volumes (0.1-1.5 ml) of 0.5% resorcinol were added to the mixture under study. It was found that 0.5 ml of resorcinol solution was sufficient for maximum and stable color development. There was a decrease in absorbance at lower concentration of 0.5% resorcinol, whereas no change in absorbance was observed at higher concentration. So 0.5 ml of 0.5 % resorcinol solution was optimized.

Effect of Sodium Hydroxide Concentration

The effect of NaOH concentration on the absorbance was studied; volumes from 0.2-2.0 ml of 4 M NaOH solutions were examined. The investigations showed that 1 ml of

NaOH gave maximum absorbance, therefore 1 ml of 4 M NaOH was chosen for the procedure.

Stability of the Colored Species

After diluting the reaction solution, it was found that the absorbance of the chromogen formed remained stable for at least 3 hours. This allowed the processing of large batches of samples, and their comfortable measurements with convenience. This is increasing the convenience of the method as well as making the method applicable for large number of samples.

Method Validation

Analytical Data

The optical characteristics such as Beer's law limits, Sandell's sensitivity and molar absorptivity were calculated for the proposed methods and the results are summarized in Table 1. Regression analysis of the Beer's law plot at their λ_{\max} revealed a good correlation. Graphs of absorbance *versus* concentration are described by regression equation $Y = mx + c$ (where Y is the absorbance, m is the slope, x is the concentration of drug in µg/ml and c is the intercept) obtained by least squares method. The results were summarized in Table 1.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by equations²⁴: $LOD = \delta/3/s$ and $LOQ = \delta/10/s$, respectively, where δ is the standard deviation of blank and s is slope of calibration. The results (Table 1) indicating proposed method was highly sensitive.

Table 1: Analytical parameters for the assay of MSZ

Parameters	Values
λ_{\max} (nm)	490
Beer's Limit (µg/ml)	2-20
Molar Absorbivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	1.569×10^4
Sandell's sensitivity ($\mu g \cdot cm^{-2}/0.001$ absorbance unit)	0.0097
Stability of colored products (hours)	3
Regression equation ($Y = mx + c$) ^{§§}	
Slope (m)	0.1253
Intercept (c)	-0.0097
Coefficient correlation (r)	0.9991
LOD (µg/ml)	0.0368
LOQ (µg/ml)	0.111
Standard deviation [§]	0.0014
Relative standard deviation (%)	0.682
% Range of error (Confidence Limits)	
0.05 level	± 0.5701
0.01 level	± 0.8436

^{§§} $Y = mx + c$, where Y is the absorbance and x is the concentration of drug in µg/ml. [§]Average of six determinations.

Precision and Accuracy

The precision and accuracy of the proposed method was determined by carrying out six replicate determinations of fixed concentration of MSZ, within Beer's limit, by the proposed method. The calculated standard deviation and relative standard deviation for the proposed method showed that the precision was good (Table 1). The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and concentrations taken for MSZ. The results obtained are compiled in Table 1 and shown that the accuracy is good.

Recovery Studies

The validity and reliability of the proposed method was assessed by the recovery studies via standard addition method. The recovery studies were carried out by adding a fixed concentration of bulk sample of MSZ to the pre-analyzed formulation and the total concentration was once again determined using the proposed method. The % recovery of the added pure drug was calculated by the formula²⁵:

$$\% \text{ Recovery} = [(C_t - C_s) / C_a] \times 100$$

Where C_t is the total drug concentration measured after standard addition; C_s , drug concentration in the formulation sample; C_a , drug concentration added to formulation. The results (Table 2) revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed analytical method (Table 2). The closeness of the recoveries suggests a lack of interference from tablet excipients and thereby establishing some degree of selectivity.

Table 2: Recovery by the standard addition method

Amount of drug in tablet (mg)	Amount of drug added (mg)	Mean % of Recovery (n=5)	± SD
400	10	101.15	± 0.642
250	10	99.57	± 0.349

Robustness

The robustness of the proposed method relative to each operational parameter was challenged. The operational parameters investigated were as follows:

- Diazotization temperature: 5°C ± 1°C
- Coupling temperature: 27°C ± 3°C
- Volume of 1% sodium nitrite: 1 ml ± 0.2 ml
- Volume of 1 N HCl: 1 ml ± 0.1 ml
- Volume of 0.5% resorcinol: 0.5 ml ± 0.1 ml
- Volume of 4 M NaOH: 1 ml ± 0.2 ml

The robustness of the proposed method was assessed by analyzing the content of MSZ in tablet dosage form (Asacol, containing 400 mg of active component) under variable experimental conditions. A sample solution

containing 15 µg/ml of the drug was prepared and assayed five times by the proposed method. The results showed a mean percent recovery of 99.89, with relative standard deviation of 1.25%. The values of mean recovery and relative standard deviation represent good reliability of the proposed method. Thus, the operational conditions of the proposed method are robust.

Application of the Proposed Method to the Tablet Dosage Forms Containing Mesalazine

The proposed method was applied to the determination of MSZ in commercially available tablets. The same samples were analyzed, simultaneously by the proposed method and the reference method. The results were compared statistically by applying Student's t-test for accuracy and the variance ratio F-test for precision with results from the reference method²¹ at a 95% confidence level. The calculated t-test and F-values (Table 3) did not exceed the tabulated values of 2.77 and 6.39, respectively, indicating no significant difference between the proposed method and the reference method in terms of accuracy and precision.

Table 3: Determination of mesalazine in tablet dosage form

Brand name of tablet	Labeled amount (mg)	% Found ± S.D [§]	
		Reference Method ^{§§}	Proposed Method
Asacol	400	100.08 ± 0.675	99.73 ± 0.914 %RSD = 0.916 t = 1.23 F = 1.52
Meseacol	400	99.95 ± 0.769	99.69 ± 0.830 %RSD = 0.832 t = 2.09 F = 4.36
Salofalk GR	250	98.98 ± 0.709	100.05 ± 0.901 %RSD = 0.900 t = 1.87 F = 2.74

[§]Average value of five determinations; Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39; ^{§§}involving Bratton-Marshall reagent.²¹

CONCLUSION

A simple, economical, selective and sensitive spectrophotometric method was developed and validated. The proposed method does not require heat treatment, expensive reagents and sophisticated instruments. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of the authentic samples containing MSZ showed no interference from the common excipients. Hence, this method could be considered for the determination of MSZ in the quality control laboratories.

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