



Spectrophotometric Determination of Mesalamine by PDAC and NQS Reagents in Bulk and Tablet Dosage Form

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Received on: 15-09-2010; Revised on: 14-10-2010; Accepted on: 12-12-2010

ABSTRACT

Two simple and sensitive visible spectrophotometric methods for the assay of mesalamine in bulk and pharmaceutical formulations have been carried out. Method A (λ_{max} , 475nm) is based on the reaction of mesalamine with 1,2-naphthoquinone-4-sulphonate (NQS) in the presence of sodium hydroxide. Method B (λ_{max} , 524nm) based on a condensation reaction between mesalamine and acidic solution of *p*-dimethyl amino cinnamaldehyde (PDAC) to form instant reddish brown colored product. All variables have been optimized and the reaction mechanisms are presented. Regression analysis of the Beer's plots showed good correlation in the concentration ranges 1.0-16.0 $\mu\text{g}/\text{m}^3$ and 5.0-25.0 $\mu\text{g}/\text{m}^3$ for methods A and B respectively. Recovery studies for mesalamine were found to be 96.6% and 98.0%. The %RSD values were found to be 0.447% and 0.395% when reacted with NQS and PDAC respectively. No interference is observed from excipients and the proposed method was statistically validated.

Key words: Mesalamine, Spectrophotometric method, Para dimethyl amino cinnamaldehyde (PDAC), 1,2-Naphthoquinone-4-sulphonate (NQS)

INTRODUCTION

Mesalamine (5-aminosalicylic acid, 5-ASA) (MEZ) (Figure 1) is used for its local effects in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease^{1,2}. 5-amino salicylic acid has been shown to be a potent scavenger of reactive oxygen species that play a significant role in the pathogenesis of inflammatory bowel disease, inhibition of natural killer cell activity, inhibition of antibody synthesis, inhibition of cyclo-oxygenase and lipoxygenase pathways and impairment of neutrophil function^{3,4}. Literature reveals that that very few methods were developed for the estimation of mesalamine in pure and pharmaceutical dosage form. A HPLC method adopted by the British Pharmacopoeia⁵ (BP) is based on the mobile phase containing glacial acetic acid, methanol and methyl isobutyl ketone (10: 40: 50 v/v). A HPLC method available in United States Pharmacopoeia (USP)⁶ is based on the mobile phase containing tetrabutyl ammonium hydrogen sulphate as an ion-pairing agent, which shortens column life. Moreover, mobile phase preparation requires tedious procedures. A simple UV spectrophotometric method was developed for the determination of MEZ in pure and its pharmaceutical formulations^{7,8}. Simple colorimetric estimation of MEZ using PDAB, Gibb's, MBTH reagent was performed⁹. Determination of 5-aminosalicylic acid in pharmaceutical formulations by square wave voltammetry at pencil graphite electrodes¹⁰. Determination of 5-aminosalicylic acid related impurities by micellar electrokinetic chromatography with an ion-pair reagent¹¹. A simple HPLC method was reported for simultaneous assay of 5-aminosalicylic acid and its metabolite in human plasma was reported¹². High performance liquid chromatography, 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) and nitrosation method was developed for the quantization of MEZ in coated tablets¹³.

In this study, we developed simple and sensitive spectrophotometric method for MEZ using 1, 2-naphthoquinone-4-sulphonic acid sodium salt (NQS) as a chromogenic reagent which resulted in the formation of coloured complex (method A). Second method involves condensation reaction of MEZ with *p*-dimethyl amino cinnamaldehyde (PDAC) resulting in colour complex (method B).

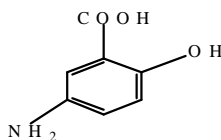
MATERIALS AND METHODS

Equipment

Spectral and absorbance measurements were made on an SHIMADZU UV-1700 series by using 1cm quartz cells. SHIMADZU electronic balance was used for weighing the samples.

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Mesalamine
(Fig. 1)

Fig 1: Structure of mesalamine

Chemicals and reagents

MEZ, USP, pharmaceutical grade, was obtained as a gift sample from Sun Pharmaceutical's Mumbai, India. All the chemicals are of analytical reagent grade of Merck Pharmaceuticals. Double distilled water was used to prepare all solutions. Freshly prepared solutions were always employed for the proposed work. 0.01 mole L⁻¹ sodium hydroxide solution was prepared by dissolving 2.0 g in 500 m³ of water. 1.0 mole L⁻¹ Hydrochloric acid solution was also prepared.

Preparation of NQS solution

NQS solution 0.5%(w/v) was prepared by dissolving 500.0 mg into a 100 m³ volumetric flask and the solution is protected from light during use.

Preparation of PDAC solution

PDAC solution (0.5%w/v) was prepared by dissolving 500.0 mg of PDAC in 100 m³ volumetric flask using methanol.

Standard solution of the drug

A stock solution of mesalamine was prepared by dissolving required quantity in double distilled water (1 mg/m³). Working standard solution (100 $\mu\text{g}/\text{m}^3$) was prepared by suitable dilution of stock standard solution with distilled water for method A and method B.

MEASUREMENT PROCEDURE

Method A

Into a series of 10.0m³ volumetric flasks, 1.0 m³ portions of NQS solution and 1.0 m³ sodium hydroxide were placed. An appropriate volume of 100 $\mu\text{g}/\text{m}^3$ working standard solution of the drug (0.1-1.6 m³) was added to each volumetric flask and left for the reaction to occur between the drug and the reagent. The volume was made to the mark with water. The absorbance of the solutions was measured at 475 nm against a reagent blank. The standard calibration curve (Fig. 2) was prepared to calculate the amount of the analyte drug in unknown samples.

Fig.2: Calibration graph for mesalamine on reaction with NQS reagent

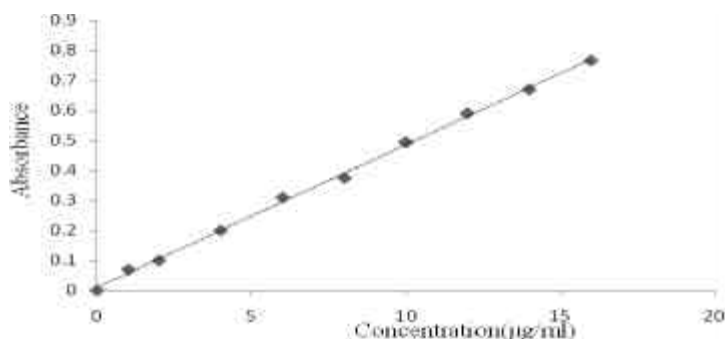


Fig.3: Calibration graph for mesalamine on reaction with PDAC reagent

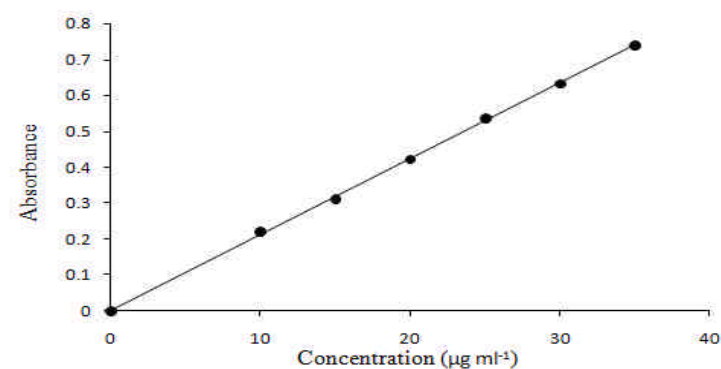


Fig 4: Absorption spectrum of MEZ on reaction with PDAC and NQS

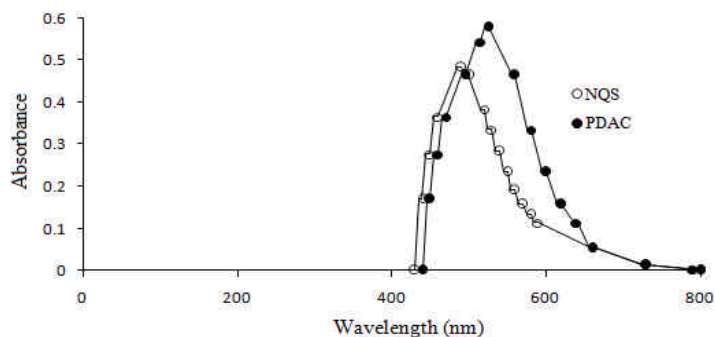


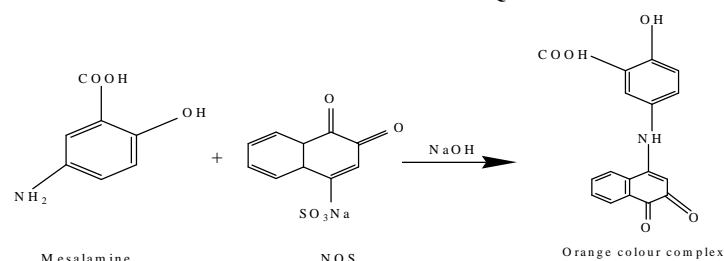
Table 1: Optical characteristics

| S.No | Parameters | Method A (NQS) | Method B (PDAC) | Reference method |
|------|--|----------------------|---------------------------------------|---|
| 1. | λ_{max} | 475 nm | 524 nm | 440nm |
| 2. | Beer's law limits ($\mu\text{g}/\text{m}^3$) | 1.0-16 | 5.0-25.0 ($\mu\text{g}/\text{m}^3$) | 50.0-500.0 ($\mu\text{g}/\text{m}^3$) |
| 3. | Molar absorptivity ($\text{l}/\text{mol}/\text{cm}$) | 7.0061×10^3 | 3.065×10^3 | 3.744×10^2 |
| 4. | Sandell's sensitivity | 0.0104 | 0.030 | 0.415 |
| 5. | Regression equation (Y) | | | |
| 6. | Slope, b | 0.047 | 0.021 | 0.0237 |
| 7. | Intercept, c | 0.010 | 0.002 | 0.0041 |
| 8. | Correlation coefficient (R) | 0.998 | 0.998 | 0.998 |
| 9. | % Relative standard deviation | 0.447 | 0.395 | 0.143 |
| 10. | Limit of detection ($\mu\text{g}/\text{m}^3$) | 0.154 | 0.7 | 0.699 |
| 11. | Limit of quantification ($\mu\text{g}/\text{m}^3$) | 0.468 | 2.23 | 1.84 |

Method B

Into a series of 10.0m³ volumetric flasks, 0.5 m³ of PDAC (0.5 %) reagent, 1 m³ of 0.1mole L⁻¹ hydrochloric acid was placed. An appropriate amount of 100 $\mu\text{g}/\text{m}^3$ standard drug solution (1.0-5.0 m³) was added to each volumetric flask and left

Scheme 1: Reaction mechanism of MEZ with NQS



Scheme 2: Reaction mechanism of MEZ with PDAC

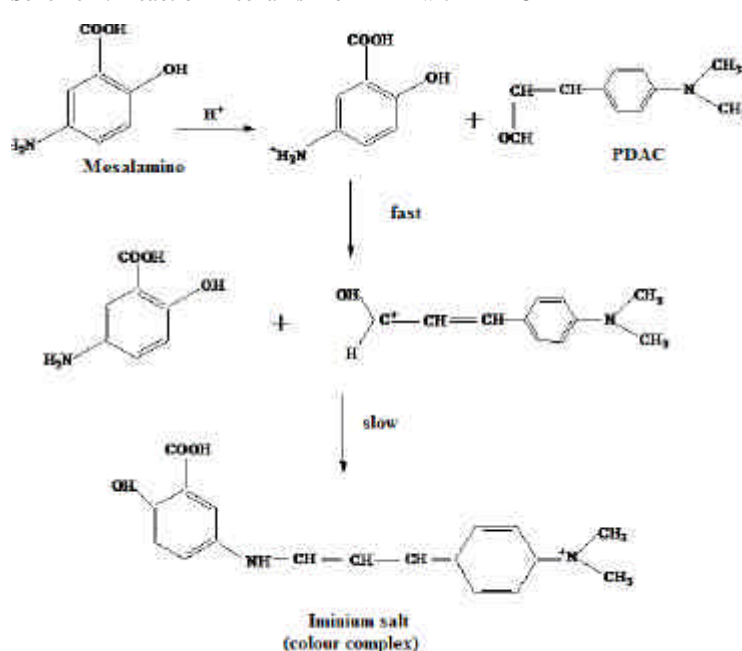


Table 2: Validation parameters of MEZ for method A and B

| Parameters | Method A | Method B | Reference method |
|-------------------|------------|-------------|------------------|
| Recovery | 96.6% | 98.0% | 99.49% |
| Interday analysis | 99.12±0.96 | 100.33±0.52 | 99.58±0.02 |
| Intraday analysis | 99.58±0.82 | 100.53±0.65 | 99.58±0.02 |

for 2 min latter volume was made to the mark with water. The absorbances were measured at 524 nm against a reagent blank. The standard calibration curve (Fig. 3) was prepared to calculate the amount of the analyte drug in unknown samples.

Procedure for measuring MEZ in dosage form

The content of five tablets was crushed using the mortar and pestle and the powder equivalent to 100.0 mg of active ingredient was taken. Concentration of 100 $\mu\text{g}/\text{m}^3$ for methods A and B was prepared with suitable dilutions and finally adjusted with water. And the solutions were analyzed as per the procedure.

RESULTS AND DISCUSSION

Spectral characteristics

In case of method A, absorption spectrum of the reddish-brown color (MEZ-NQS) complex is shown in Fig. 3 with a maximum absorbance at 475 nm. The complex formation was completed immediately after all reagents were added, no heating or standing time was needed. The color complex is stable for at least 24 h at room temperature (25 °C) as determined by the proposed method.

Similarly in case of method B, absorption of the reddish brown color due to the condensation reaction between PDAC in presence of the acidic medium gave a maximum absorbance at 524nm. The color complex formation took about 2 min for the complete formation of reaction product and the color formed was stable with time at room temperature (25 °C) as determined by the proposed method.

Effect of NQS concentration

The reaction revealed that it depends upon the concentration of NQS (Scheme 1). The absorbance of the solution increased as the NQS concentration increased. Higher NQS concentrations up to 1.0% (w/v) had no effect on the absorption values. The experiment was carried out using 0.5% (w/v).

Effect of PDAC concentration

The reaction revealed that it depends upon the concentration of PDAC (scheme 2). The studies showed that 0.5% (w/v) concentration gave good results.

Effect of solvent

The PDAC is soluble in most of the organic solvents (methanol, DMSO, acetonitrile) and very slightly soluble in water. So the PDAC was dissolved in methanol in order to get the clear solution of PDAC.

Effect of acidity

To have the condensation reaction acidic medium is necessary. Different acidic mediums were tested like hydrochloric acid and sulphuric acid all prepared in concentration range of 0.01-0.15 mole L⁻¹. Best results were obtained in case of hydrochloric acid in the concentration of 0.1 mole L⁻¹, so the experiment was carried out with the same concentration.

Linearity and range

The limits of the Beer law, the molar absorptivity and the Sandell's sensitivity, regression equation, and correlation coefficient were determined for the proposed methods A and B (Table 1). A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range of 1.0-16.0 $\mu\text{g}/\text{m}^3$ in case of method A and 5.0-25.0 $\mu\text{g}/\text{m}^3$ in case of method B for mesalamine in the final measured volume of 10m³. Regression analysis of Beer's plot λ_{max} revealed a good correlation $R^2 = 0.998$ for both methods A and B. The graph showed negligible intercept and were described by the regression equations $y_1 = 0.047x + 0.010$, $y_2 = 0.021x + 0.002$ for method A and B respectively (where y is the absorbance of a 1cm cell, 0.047 and 0.021 are the slopes, 0.010 and 0.002 are the intercepts and x is the concentration of the mesalamine in $\mu\text{g}/\text{m}^3$). The high molar absorptivity of the resulting colored complex indicates the high sensitivity of the methods A and B.

VALIDATION OF THE METHOD

Six tubes containing varying volumes of MEZ stock solution, (0.01-1.6 and 1.0-5.0 m³) with respective to methods A and B were prepared. 1.0 m³ of 0.5% w/v NQS was added to each of these tubes. Then 1.0m³ of 0.05 mole⁻¹ sodium hydroxide was also added. Then the volume is made up to the mark. The absorbance readings of each of the mixtures of both the drug mixtures were then recorded at 475nm for method A and 0.5m³ of PDAC and 1.0m³ of hydrochloric acid are added and finally volume is made up to the mark. This process was repeated three times and on each occasion fresh stock solutions of MEZ solution was used. The average absorbance reading was obtained from the determinations, and used to generate the calibration curves. Linear regression analysis was used to calculate the slope, intercept and coefficient of determination (R^2) of each calibration line. The limit of detection (LOD) was computed from the calibration graphs using the equation $3.3 s/s$ where s is the standard deviation of three blank determinations and s is the slope of the calibration curve. The limit of quantification (LOQ) was calculated as $10 s/s$.

Precision

The precision of the method was determined by replicate analysis of five separate

solutions of five separate solutions of the working standards at two concentration levels of each drug. Relative standard deviations are also calculated which indicates good precision of the proposed methods.

Robustness and ruggedness

Robustness was examined by evaluating the influence of a small variation of the method variables including the concentration of analytical reagent and the pH of the sodium chloride solution. It was found that small variations in these variables did not affect the method significantly. This was an indication of the reliability of the proposed method during its routine application for the investigated drugs. The ruggedness was tested by applying the proposed method of analysis for both the drugs using the same operational conditions. Results obtained from inter-day RSD and within-day RSD variations were found to be reproducible and are represented in the Table 2.

CONCLUSION

Both the methods A and B were simple, sensitive and reliable with good precision and accuracy. The proposed methods are specific while estimating the commercial formulations without interference of excipients and other additives. Hence, proposed two methods can be used for the routine spectrophotometric determination of mesalamine in pure samples and pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Sun pharmaceuticals, India for supplying the gift samples of MEZ. Authors also extend their thanks to the Principal, JSS College of Pharmacy, Mysore for providing the necessary facilities to carry out the present work.

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Source of support: Nil, Conflict of interest: None Declared