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Sensitive Spectrophotometric Method for the Determination of Mesalamine in Bulk and Pharmaceutical Formulations

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ABSTRACT

A simple and sensitive spectrophotometric method was developed and validated for the determination of mesalamine in bulk and pharmaceutical formulations. The method was based on the reaction of drug with the mixture of potassium iodate and potassium iodide. The method was linear in the range of 15-50µg/ml. The absorbance was measured at 487nm. The method was validated with respect to accuracy, precision, specificity, ruggedness, and robustness, limit of detection and limit of quantitation. Thus a simple and precise method has been developed.

Keywords: Mesalamine, Potassium iodide, Potassium iodate, Spectrophotometric method, Tri iodate.

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]. Despite the fact that it has been used for over 50 years, the mechanism of action of this drug remains uncertain. 5-ASA 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 Pharmacopoeia (BP) is based on the mobile phase containing glacial acetic acid, methanol and methyl isobutyl ketone (10: 40: 50 v/v) [5]. A HPLC method available in United States Pharmacopoeia (USP) is based on the mobile phase containing tetrabutylammonium hydrogen sulphate as an ion-pairing agent, which shortens column life. Moreover, mobile phase preparation requires tedious procedures [6]. The spectrophotometric method was developed for the determination of MEZ in pure and its pharmaceutical formulations [7-8]. Very few HPLC methods were developed for

simultaneous determination of 5-aminosalicylic acid and its metabolite in human plasma [9] and nitrosation method for the quantization of MEZ in coated tablets [10].
In this present study, we developed simple and sensitive spectrophotometric method for MEZ using potassium iodate and potassium iodide mixture in both bulk and pharmaceutical formulations.

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.

Materials and Reagents

MEZ, USP, pharmaceutical grade, was obtained as a gift sample from Sun Pharmaceutical's Mumbai, India. Pharmaceutical formulations of MEZ used for the study are obtained from the local market. All other 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.

Standard MEZ Solution

A stock solution of MEZ (500µg/ml) was prepared by dissolving 50 mg in 100ml volumetric flask with double distilled water. The stock solution (500µg/ml) was used to prepare the further dilutions and volume is made up to the mark with water.

METHODS

Procedure for the determination of MEZ

Aliquots of stock solution (500µg/ml) were pipette into as series of 10ml volumetric flask. To each flask, 2.2ml of potassium iodide and 3.0ml of potassium iodate were added and diluted to volume with distilled water. The reaction was allowed to proceed at room temperature. The absorption spectrum of MEZ was done and it showed 487nm as the maximum absorption point (Fig.2). The calibration curve was constructed by plotting absorbance against the initial concentration of MEZ. The linearity range or Beer's range follows in the range between 15 to 50µg/ml (Fig.3). The content of MEZ was calculated either from the calibration graph or corresponding regression equation and found that the absorbance is stable over considerable period of time.

Procedure for the determination of MEZ in pharmaceutical formulation

Ten tablets are taken and finely powdered and a quantity of powder equivalent to 50mg of MEZ was dissolved in distilled water and then passed through the Whatt man filter paper, then the resultant stock solution is also analysed as per the procedure. The nominal content of the tablet preparation was calculated from method for determination of MEZ as it contains –COOH group in its moiety. Keeping this in mind, a mixture of potassium iodide and iodide was allowed to react with MEZ which yielded iodine. Then the liberated iodine reacted with the excess of iodide ion resulting in the formation of triiodide ion (Scheme 1)

Optimization

The different parameters affecting the development process were extensively studied to determine the optimum conditions for the assay procedures. The optimum values of the variables

were maintained throughout the determination process. Optimisation of spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimised via a number of preliminary experiments. The effect of concentration of potassium iodate and potassium iodide was studied and found that 3.0ml of (1 x 10^{-1}M) potassium iodate and 2.0ml of (1.5 x 10^{-1}M) potassium iodide were giving good results.

Effect of the concentration of potassium iodate

The effect of the volume of potassium iodate on the absorbance of the product was studied in the range of 0.5ml to 5.0ml. The absorbance increased with increase in the volume of the potassium iodate and became constant at 3.0ml. Further addition of potassium iodate did not show much change in the absorbance of the solution and therefore, 3.0m of 1 x 10^{-1}M potassium iodate was chosen as an optimum value.

Effect of the concentration of potassium iodide

The effect of the volume 1.5 x 10^{-1}M potassium iodide on the absorbance of the product was studied in the range of 0.5ml to 5.0ml, keeping the constant concentration of MEZ (40µg/ml). The maximum absorbance was obtained with 2.0ml; further addition caused significant change on the absorbance. But upon optimization it was found that 2.0ml was giving good results. Thus, 2.0ml of 1.5 x 10^{-1}M potassium iodide was used throughout the experiment.

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 potassium iodide and potassium iodate method (Table 1). A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range of 15- 50µg/ml for MEZ in the final measured volume of 10ml. Regression analysis of Beer's plot λ_{max} revealed a good correlation R^2 = 0.998 for the proposed method. The graph showed negligible intercept and were described by the regression equations *y*= 0.015 x + 0.002. (where *y* is the absorbance of a 1cm cell, 0.015 is the slope, 0.010 and 0.002 is the intercept and x is the concentration of mesalamine in µg/ml). The high molar absorptivity of the resulting colored complex indicates the high sensitivity of the proposed method.

Validation of the method

Six tubes containing varying volumes of MEZ stock solution, (0.15ml-0.5ml). Then 3.0ml 1 x 10^{-1}M potassium iodate and 2.0ml of 1.5 x 10^{-1}M potassium iodide was also added. Then the volume is made up to the mark. The absorbance of the resultant solution was then recorded at 487nm. 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 σ/s where σ is the standard deviation of three blank determinations and s is the slope of the calibration curve. The limit of quantisation (LOQ) was calculated as 10 σ/s.

Accuracy

The accuracy was ascertained by recovery studies using the standard addition method. The proposed method was used for estimation of MEZ from tablets after spiking with additional pure drug. The amount of MEZ was determined and compared with the proposed method. (Table 2)

Precision

The precision of the method was determined by replicate analysis of five separate solutions of the working standards at two concentration levels of each drug. At two concentrations intraday and inter day precision studies are performed for two consecutive days. Relative standard deviation was also calculated and was found to be 0.4024 which indicates good precision of the proposed method.

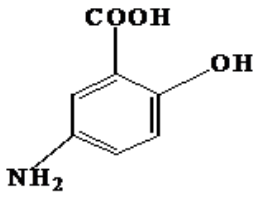
Robustness and ruggedness

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. The ruggedness was tested by applying the proposed method of analysis 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.

Stability of the oxidized product

Under the optimum conditions, the reaction between potassium iodide and potassium iodate takes about 15min at room temperature for the product to get oxidized, and the absorbance no longer changed significantly. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remains stable for at least 4 hours. This increased the convenience of the methods as well as made it applicable for large number of samples.

Fig 1: Structure of mesalamine



Mesalamine

Fig.2: Absorption spectra of MEZ with potassium iodide and potassium iodate

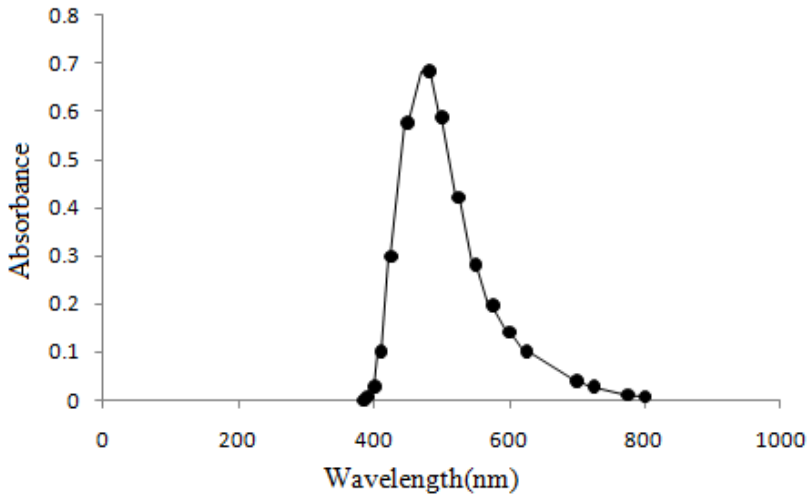
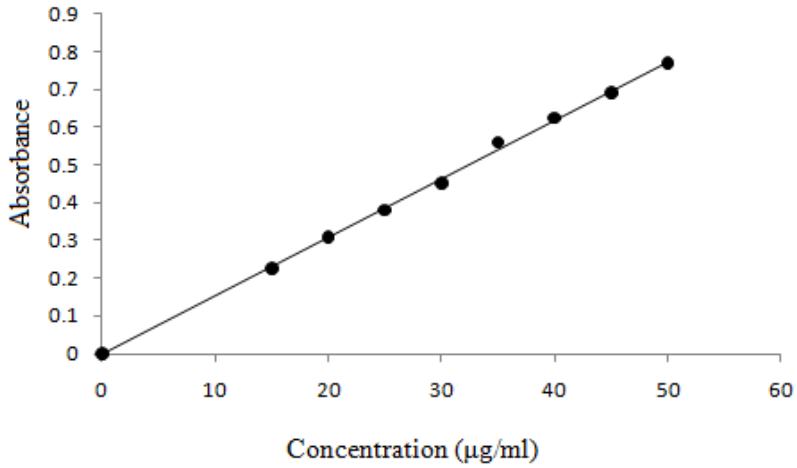
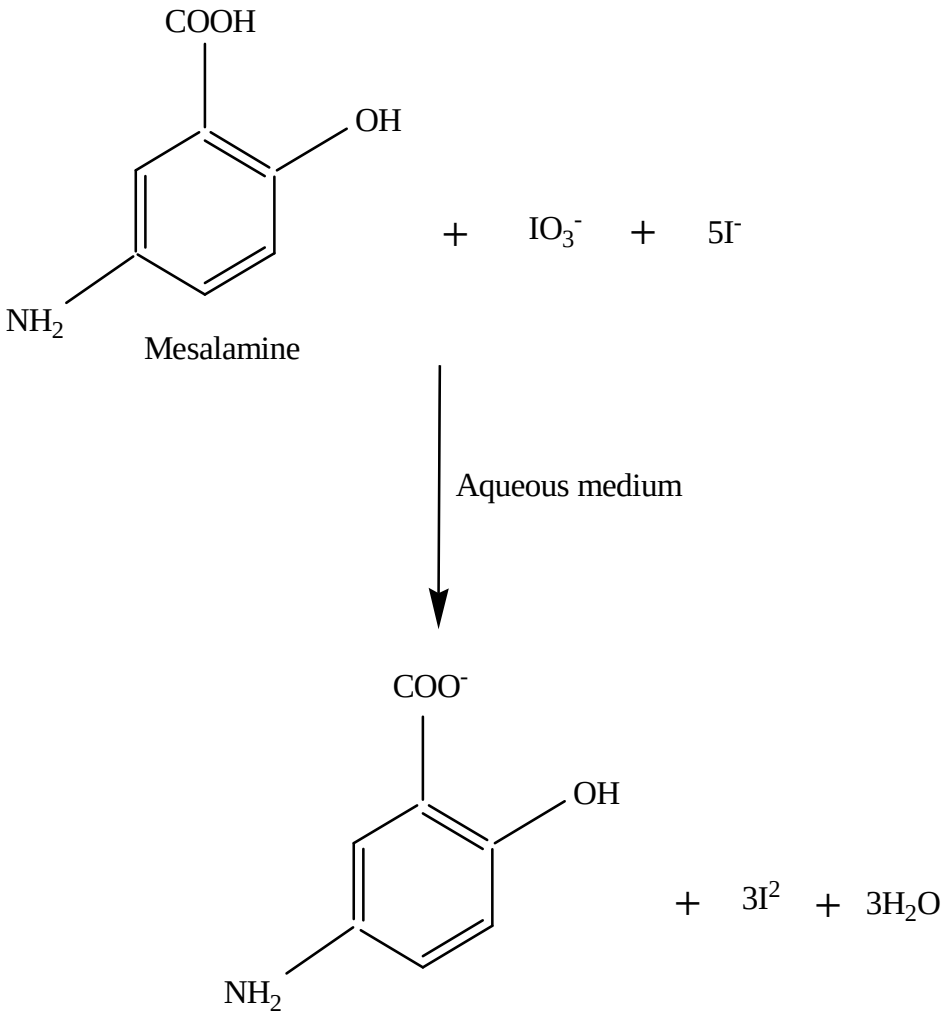


Fig.3: Calibration graph for MEZ (15-50µg/ml)



Scheme :1 Reaction between potassium iodide and potassium iodide:



$I_2 + I^- \longrightarrow I_3^-$ (Triiodide ion)

Fig.4: Graph showing the effect of volume of potassium iodate on the absorbance of the solution

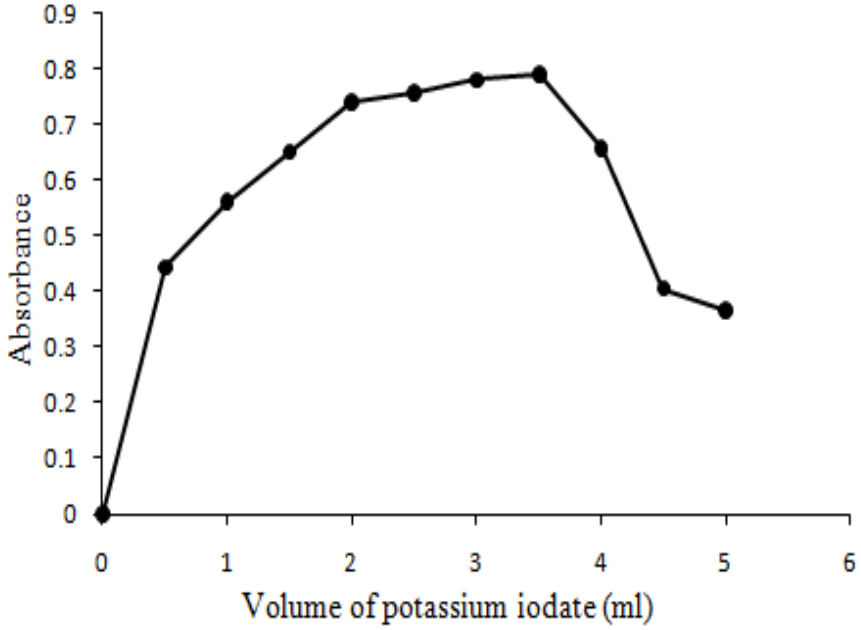


Fig.5: Graph showing the effect of potassium iodide on the absorbance of the solution

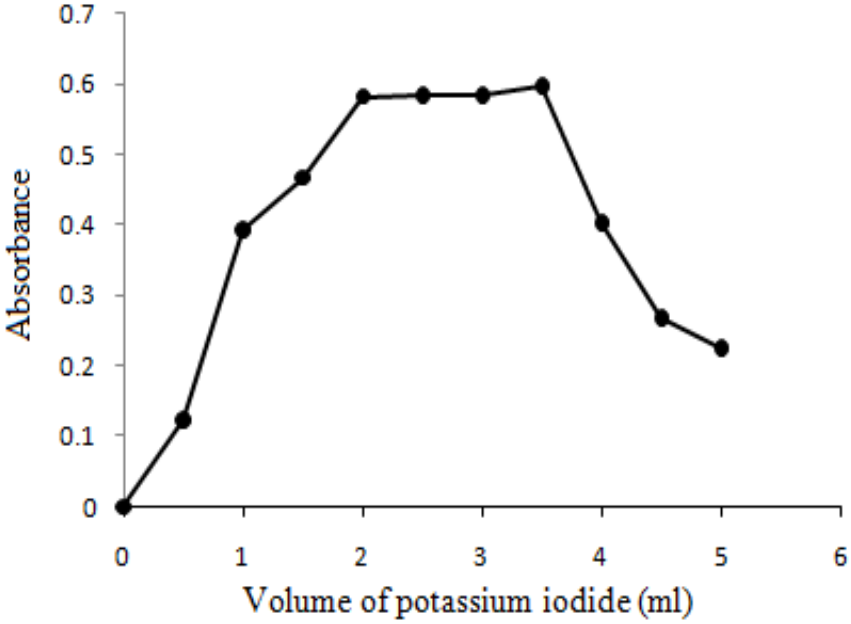


Table 1: Optical characteristics of MEZ

S.NO	Parameter	Values
1.	λ_{max}/ nm	487
2.	Beers law limits (µg/ml)	15-50
3.	Molar absorptivity (1 /mol/cm)	0.001958
4.	Correlation coefficient (R)	0.998
5.	Sandell's sensitivity(ng cm^{-2})	1.5 X 10^{-5}
6.	Regression equation (y)	
7.	Slope, *b*	0.015
8.	Intercept, *c*	0.002
9.	Relative standard deviation%	0.4024
10.	Limit of detection (µg/ml)	0.5808
11.	Limit of quantification(µg/ml)	1.76

Table 2: Recovery of MEZ in pharmaceutical formulations

Formulation	Labeled amount (mg)	Amount added (mg)	Recoverey by the reference method(%)	Recovery by proposed method
Mesalamine	100	5.0	98.895	99.30
	100	10.0	95.025	99.30
	100	15.0	94.567	99.30

RESULTS AND DISCUSSION

Spectral Characteristics:

Oxidation of MEZ was attempted in the present study for the development of spectrophotometric method. The present method is based on the reaction between potassium iodate, potassium iodide and drug. The absorption spectra of the colours chromogen formed between mesalamine and the mixture of potassium iodide and iodate have absorption maxima at 487nm.

Chemistry of the coloured chromogens

In the present method the drug reacts with potassium iodide and iodate at normal room temperature to give a reddish brown coloured product. It has been reported in the literature (12) that iodine is formed as a result of the interaction of a mixture of iodide and iodate with inorganic or organic acid.

$$(5\ I^- + IO^-_3 + 6\ H^+ \rightarrow 3\ H_2O + 3\ I_2)$$

In aqueous medium, the iodide ions react with the liberated iodine to yield triiodide ion

$$(I_2 + I^- \rightarrow I3^-)$$

which detected in UV detector at 353 nm. So the same principal was applied to MEZ as it contains –COOH group in its moiety. Keeping this in mind, a mixture of potassium iodide and iodate was allowed to react with MEZ which yielded iodine. Then the liberated iodine reacted with the excess of iodide ion resulting in the formation of triiodide ion (Fig:2).

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of mesalamine in pure form and in pharmaceutical dosage forms.

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