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RESEARCH ARTICLE

Spectrophotometric Method Development and Validation for the Estimation of Mesalazine in Pure and Tablet Dosage Form by UV- Spectrophotometric Method

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ABSTRACT

The objective of the present research work is to develop a simple, accurate, precise and sensitive spectroscopic method was developed for the estimation of Mesalazine in the pure and tablet dosage forms. A simple, rapid and accurate analytical method was developed for the estimation of Mesalazine in bulk and tablet dosage form by UV spectrophotometer and validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantification (LOQ) as per ICH Guidelines. The melting point of Mesalazine (283º C) was recorded to check the identification of the drug. After considering the solubility, 6.8 phosphate buffer was selected as solvent. Mesalazine, 10 µg/ml solution was prepared and scanned in the UV region, from the spectra 330 nm was selected as an analyzing wavelength. Stability of the absorbance at λmax 330 nm was also checked for up to 2 hours and 30 minutes. The optical characteristics such as absorption maxima (nm), beer's law limits (µg/ml)and correlation coefficient (r) were calculated for the method. The analysis of the tablet formulation by proposed method was in good agreement (401 ± 0.4956 mg/tablet) with label claim. The recovery studies were carried out at three different levels, i.e. 120%, 100% and 80%.The low value of % RSD is indicative of the accuracy of the proposed method. The result of recovery study revealed that the commonly encountered excipients and other additives usually present in the dosage form did not interfere with the proposed method. The precision of the proposed method was studied as an intra-day and inter-day analysis. The results obtained in recovery studies will indicate that there is no interference from the excipients used in the formulation.The developed method was validated as per ICH guidelines and was found to be accurate and precise. Thus the proposed method can be successfully applied for the estimation of Mesalazine in pure and tablet dosage form.

KEYWORDS

Mesalazine, UV- Spectrophotometry, Tablet formulation and Validation

INTRODUCTION

Mesalazine also known as mesalamine or 5-aminosalicylic acid (5-ASA) (Figure 1), is an anti-inflammatory drug used to treat inflammatory bowel disease, such as ulcerative colitis and mild-to-moderate Crohn's disease[1]. Mesalazine is a bowel-specific aminosalicylate drug that acts locally in the gut and has its predominant actions there, thereby having few

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systemic side effects. It appears as white amorphous powder, Mesalazine (MEZ) is soluble in hot water and HCl, Insoluble in ethanol[2-3]. It has a melting point of about 283ºC. Mesalazine is not fully understood, it appears to be more topical rather than systemic. Mucosal production of arachidonic acid metabolites, both through the cyclooxygenase pathways, i.e., prostanoids, and through the lipoxygenase pathways, i.e., leukotrienes and hydroxyeicosatetraenoic acids, is increased in patients with chronic inflammatory bowel disease, and it is possible that Mesalazine diminishes inflammation by blocking cyclooxygenase and inhibiting prostaglandin production in the colon. Rapidly and extensively metabolized, mainly to N-acetyl-5-ASA (Ac-5-ASA) in the intestinal mucosal wall and the liver. Ac-5-ASA is further acetylated (deactivated) in at least 2 sites, the colonic epithelium and the liver. Absorption takes place in colon, Half life: 5-6hrs and It is excreted mainly by the kidney as N-acetyl-5 aminosalicylicacid[1]. Several methods have been reported for the quantitative determination of Mesalamine which includes UV[4], Visible[5-6] and HPLC[7-8].

MATERIAL AND METHODS

All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. The pure drug sample of Mesalazine was obtained as a gift sample from Hetero Labs, Hyderabad. Mesacol containing 400 mg of Mesalazine was purchased from Hetero Pharmacy. LABINDIA-3000$^+$ double beam UV-visible spectrophotometer with 1 cm matched quartz cell was used for all spectral measurements.

Preparation of Calibration Graph

The standard stock solution was prepared by dissolving accurately weighed 10 mg of Mesalazine in 6.8 phosphate buffer the volume was made up to 10 ml with 6.8 phosphate buffer in 10 ml volumetric flask (1° Stock solution, 1000 µg / ml), 1 ml of the primary stock solution was diluted to 10 ml with 6.8 phosphate buffer (secondary Stock solution, 100µg / ml). 1 ml of the secondary stock solution was taken in 10 ml standard volumetric flasks diluted to 10 ml with 6.8 phosphate buffer to get the concentration of 10µg/ml. The absorbance of resulting solution was measured against respective blank solution (6.8 phosphate buffer) in the UV region of 200-400 nm, which shows maximum absorbance at 330 nm.

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Mesalazine were transferred into a series of 10 ml standard flasks and volumes were made up to mark with 6.8 phosphate buffer. Eight different concentrations were prepared in the range of 1-10µg/mL and the observance was measured at 330nm against solvent (6.8 phosphate buffer) blank. The obtained absorbance values are plotted against the concentration of Mesalazine to get the calibration graph. The regression equation and correlation coefficient were determined.

Quantification of Mesalazine in Formulation

20 tablets of Mesacol were weighed, pulverized and the powder equivalent to 0.01 mg of Mesalazine was weighed accurately and transferred into a 10 ml standard volumetric flask. The contents were dissolved in 6.8 phosphate buffer. This solution was filtered through Whatsmann filter paper number 40, 1 ml of the above test solution was diluted to 10 ml with 6.8 phosphate buffer to obtain a solution of 100 µg / ml again 1 ml of the above test solution was diluted to 10 ml with 6.8 phosphate buffer in 10 ml standard volumetric flask to produce the concentration 10 µg/ml. Same concentration was repeated six times. The amount of Mesalazine present in the formulation was determined by using slope and intercept values from calibration graph and the results were presented on Table 2.

Interday and Intraday Study

A variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed**.** Intra-day precision was determined by analyzing Mesalazine for three times in the same day at 330nm. Inter-day precision was determined by analyzing the drug daily once for three days at 330 nm.

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Recovery Study of Formulation

In order to ascertain the suitability and reproducibility of the proposed method, known quantities of standard Mesalazine solution were added to previously analyzed samples and the mixtures were analyzed by the proposed method. Aliquots of 0.5 ml of sample drug solution of 100µg/ml were pipetted into each of three 10 ml volumetric flasks. To the first three volumetric flasks (80%, 100%, 120% µg/ml) of standard solution of 100µg/ml was added respectively. The volume was made up to 10.0 ml with 6.8 phosphate buffer solution and the absorbance was measured at 330NM against the reagent blank. The absorbance values were recorded with the help of the standard curve. The total amount and percentage recovery of Mesalazine was determined by using the following formula,

$$\%\,\text{recovery} = \frac{N\Sigma xy - \Sigma x \Sigma y}{N\Sigma x^2 - (\Sigma x)^2} X\ 100$$

Where, N = Number of observations

X = Amount Added in µg/ml

Y = Amount recovered in µg/ml

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of the slope and standard deviation of intercept.

RESULTS AND DISCUSSION

The melting point of Mesalazine (283º C) was recorded to check the identification of the drug. After considering the solubility, 6.8 phosphate buffer was selected as solvent. Mesalazine, 10 µg/ml solution was prepared and scanned in the UV region, from the spectra 330 nm was selected as an analyzing wavelength. Stability of the absorbance at λmax 330 nm was also checked for up to 2 hours and 30 minutes. The optical characteristics such as absorption maxima (nm), beer's law limits (µg/ml) and correlation coefficient (r) were calculated for the method and shown in Table 1.

The analysis of the tablet formulation by proposed method was in good agreement (401± 0.4956 mg/tablet) with label claim. The results from validation studies are shown in Table 2.

The recovery studies were carried out at three different levels, i.e. 120%, 100% and 80%.The low value of % RSD is indicative of the accuracy of the proposed method. The result of recovery study revealed that the commonly encountered excipients and other additives usually present in the dosage form did not interfere with the proposed method. The precision of the proposed method was studied as an intra-day and inter-day analysis. The results from validation studies are shown in Table 3.

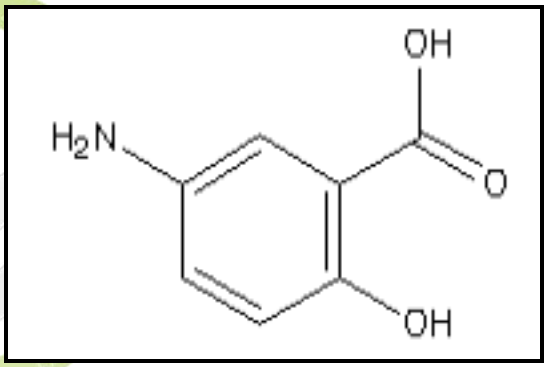

Figure 1: Structure of Mesalazine

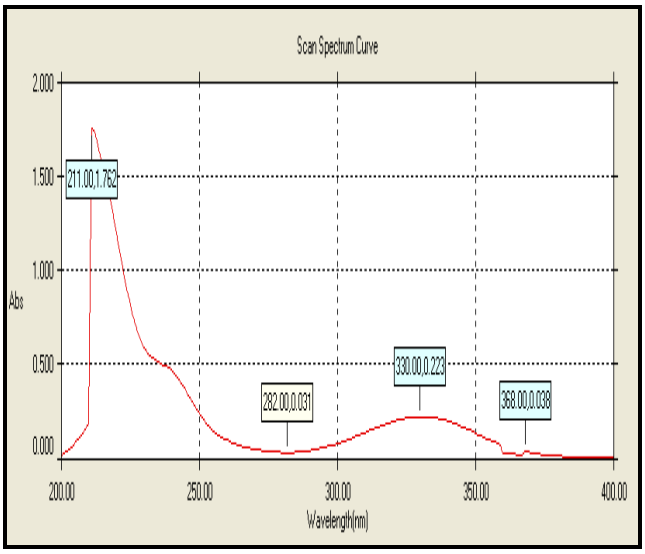

Figure 2: UV- Spectrum of Mesalazine in 6.8 phosphate buffer

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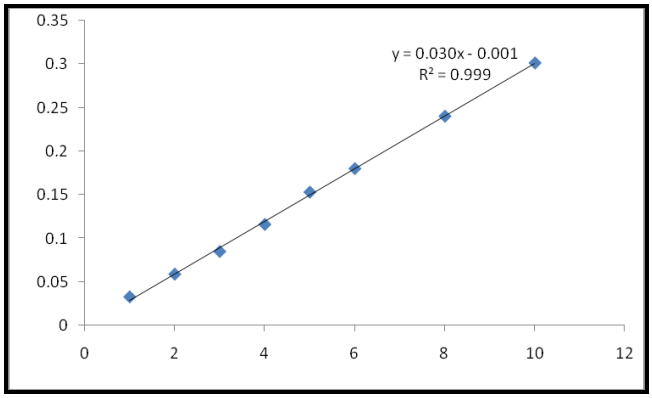

Figure 3: Linearity Curve for Mesalazine

Table 1: Calibration Curve Points of the Proposed Method of Mesalazine

Parameters	Values
λ_{max} (nm)	330
Beer's law limits (µg/ml)	1-10
Regression equation (y=mx+c)	Y = 0.030X-0.001
Slope (m)	0.030
Intercept (c)	- 0.001
Correlation coefficient(r^2)	0.999
% RSD**	< 2
Limit Of Detection (µg/ml)	0.0351
Limit of Quantitation (µg/ml)	0.106

*Y = mx + c, where 'Y' is the absorbance and c is the concentration of Mesalazine in mg/ml

** For six replicates

Table 2: Quantification of Formulation – Mesacol

S.No	Labeled amount (Mg/tab)	Amount found (mg)	Percentage obtained	Average (%)	S.D.	% RSD
1.	400	399	99.70	100.2	0.4956	0.4956
2.		401	100.2			
3.		404	101			
4.		399	99.70			
5.		402	100.5			
6.		401	100.2			

** For six replicates

Table 3: Determination of Accuracy Results for Mesalazine

Brand name	Spiked level	Amount of sample (mcg/ml)	Amount of drug added (mcg/ml)	Amount estimated	Amount recovered	% Recovery ± RSD**
MESACOL	80%	5	4	8.94	3.94	99.33±0.079
MESACOL	100%	5	5	9.94	4.94	99.4±0.041
MESACOL	120%	5	6	10.93	5.93	99.36±0.02

** For six replicates

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CONCLUSION

The procedure described here is simple, rapid, sensitive, selective and cost effective. It is evident from the results that the recommended procedure is well suited for the assay and evaluation of drugs in dosage forms. It can be applied for routine analysis of Mesalazine in drug control laboratories.

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