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High performance liquid chromatographic and ultra violet spectroscopic determination of mesalamine in pharmaceutical formulations

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ABSTRACT

A simple, reliable and reproducible reverse phase HPLC and UV-spectroscopic methods were developed for the determination of mesalamine present in pharmaceutical dosage forms. The HPLC analysis was performed using Hypersil ODS C_{18} (0.46cm ×25cm, 5mm) column from Shimadzu in isocratic mode, with mobile phase methanol: 10mM tetra butyl ammonium hydrogen sulphate (50:50%v/v) at a flow rate of 1ml/min with UV detection at 210 nm. In this HPLC method, zonisamide is used as internal standard. The retention times were 2.267min and 3.833min for mesalamine and zonisamide respectively. UV-spectroscopic analysis was performed at 298 nm. Extraction of mesalamine from tablet was carried out using phosphate buffer (pH- 3.6). The linearity range was found to be 1-50 µg/ml for both HPLC and UV- methods. The results, obtained by the two methods in pharmaceutical preparation were compared. There were no significant difference between the mean values and the precision. These validated methods are suitable for the determination of mesalamine in pharmaceutical tablet formulations.

KEYWORDS

Mesalamine;
RP-HPLC;
UV spectrophotometer;
Recovery studies.

INTRODUCTION

Mesalamine, 5-Amino-2-hydroxybenzene-1 carboxylic acid, is used, either orally or rectally, as an antiinflammatory agent in treating ulcerative colitis[1] and, to a lesser extent, Crohn's disease[2]. Mesalamine is a white to pinkish crystaline powder. The antiinflammatory actions of mesalamine are believed to be secondary to, at least in part, the inhibition of arachidonic acid in the bowel mucosa by the enzyme cyclooxygenase[3]. Inhibition of cyclooxygenase effectively diminishes the production prostaglandins, thereby reducing colonic inflammation[4]. Mesalamine also interferes with leukotriene synthesis, possibly by inhibiting the lipoxygenase enzyme. Inhibition of colonic mucosal sulfidopeptide leukotriene synthesis and chemotactic stimuli for polymorphonuclear leukocytes also may occur. Mesalamine inhibits accumulation of thromboxane A and superoxide formation in the rectal mucosa, which may contribute to its antiinflammatory action[5].

A survey of literature revealed that a few analytical methods were reported for the determination mesalamine in biological samples[6-17]. In the present investigation an attempt was made to develop a simple, sensitive and accurate UV and RP-HPLC methods for the analysis of mesalamine in bulk and its pharmaceutical dosage forms and the results obtained were compared statiscally.

EXPERIMENTAL

Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20 µl Injection of sample loop (manual), and SPD 10A VP UV-visible detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Hypersil ODS C_{18} (46 mm × 25 cm, 5mm) column was used for the separation.

Standards and chemicals

Mesalamine and zonisamide used as an internal standard (IS) were gifts obtained from Torrent pharma limited (India) and Cipla pharma Limited (India). MESACOL (SUN pharma Ltd, India) containing 400 mg of mesalamine, are purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Methanol of HPLC grade was purchased from Ranbaxy Fine Chemicals Ltd (New Delhi, India); Tetra butyl ammonium hydrogen sulphate was purchased from (Himedia Laboratories pvt. Ltd., Mumbai, India).

The 10mM tetra butyl ammonium hydrogen sulphate was prepared by dissolving 1.6977gms of tetra butyl ammonium hydrogen sulphate in 500ml of double distilled water. The mobile phase used in this study was a mixture of 10mM tetra butyl ammonium hydrogen sulphate and methanol 50:50 %v/v. The contents of the mobile phase were filtered before use through a 0.45µ membrane filter.

Spectral and absorbance measurements were made with Elico SL-159 UV-Visible spectrophotometer by using 1cm quartz cells. Afcoset ER 200A electronic balance was used for weighing the samples. Phosphate buffer (pH- 3.6) was prepared by dissolving 0.9gms of anhydrous disodium hydrogen phosphate and 1.298gms of citric acid monohydrate in one liter of double distilled water. The λ_{max} was determined from UV spectrum of mesalamine in phosphate buffer (pH- 3.6) in the range of 200-400nm and it was found to be 298.0nm.

Chromatographic conditions

The mobile phase was a mixture of 10mM tetra butyl ammonium hydrogen sulphate and methanol 50:50 %v/v. The stationary phase is a Hypersil ODS C18, 4.6mm × 250 mm, 5µm column. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1ml/min. The column temperature was maintained at 23±1^{0}C. The eluents were monitored at 210nm using UV-detector. The identification of the separated mesalamine and zonisamide were confirmed by running the chromatograms of the individual compounds under identical conditions.

Method development

Different solvent systems were used to develop sensitive and reliable high performance liquid chromatographic and ultra violet spectroscopic method. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, analysis time, and sensitivity of the assay, preparatory steps and use of same solvent system for extraction of the drug from the formulation for the estimation of the drug content.

Preparation of standard drug solutions and construction of standard curve

For HPLC method

Stock solution of mesalamine (1 mg/ml) was prepared by dissolving 25 mg of mesalamine in 25 ml of volumetric flask containing 10 ml of methanol and 10ml of 10mM tetra butyl ammonium hydrogen sulphate. The solution was sonicated for about 10 min and then made up to volume with mobile phase. Daily working standard solutions of mesalamine was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25mg of zonisamide in 10 ml of methanol and 10ml of 10mM tetra butyl ammonium hydrogen sulphate, sonicated for 20min. then made up to the volume with mobile phase. Calibration standards were prepared by taking suitable aliquot's of the working standard solutions in six different 10ml volumetric flasks to yield concentrations in the range of 1-50µg / ml. To the above solutions 20µg /ml of zonisamide (internal standard) was added and the final volume was made up to the mark. Calibration curve was plotted between peak area ratios of drug vs. internal standard against concentration of the drug as shown in figure 1.

UV method

The stock solution was prepared by dissolving 10 mg of mesalamine in 10 ml of phosphate buffer (pH-

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3.6), sonicated for 10 minutes. Daily working standard solutions of mesalamine was prepared by suitable dilution of the stock solution with phosphate buffer (pH-3.6). Suitable aliquots of this working standard solution was transferred separately into a series of 10 ml volumetric flasks and diluted to 10 ml with phosphate buffer (pH- 3.6) to yield concentrations in the range of 1-50µg/ml.The absorbances of the resulting solutions were measured at λ_{max} 298 nm against reagent blank. A standard graph was drawn by plotting concentration of drug on X-axis and the corresponding absorbance values on Y-axis as shown in figure 2.

The characteristic parameters of the calibration equation for the proposed HPLC and UV methods are shown in TABLE 1.

Preparation of sample solutions

1. HPLC method

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of mesalamine was extracted with methanol and 10mM tetra butyl ammonium hydrogen sulphate in a 25ml volumetric flask using ultra sonicator. This solution was filtered through 0.45µm filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in triplicate. The results were shown in TABLE 2.

TABLE 1: Characteristic parameters of the calibration equation for the proposed HPLC and UV methods for the determination of mesalamine

Parameter	HPLC-method (210nm)	UV-method (298nm)
Calibration range (µg/ml)	1-50	1-50
Detection limit (µg/ml)	0.328	0.293
Quantitation limit (µg/ml)	0.983	0.879
Regression equation*		
Slope(b)	0.1825	0.0242
Standard deviation of the slope(S_b)	0.608	0.134
Intercept (a)	0.1494	0.0062
Correlation coefficient(r^2)	0.9991	0.9998
Precision (%RSD#)		
Intra day (n=6)	0.836	0.97
Inter day (n=6)	1.37	1.54

*Y= a + bC where C is the concentration of mesalamine and Y is the peak area, # %RSD is Percent relative standard deviation of the Precision, of six samples of same concentration

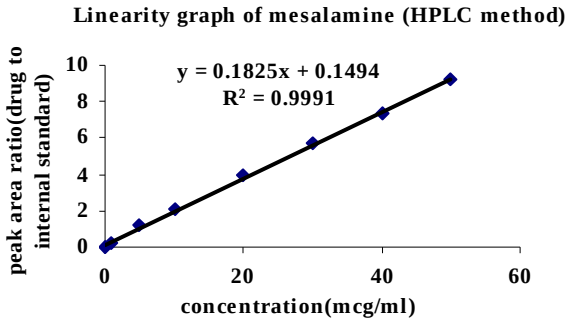

Figure 1: Calibration curve of Mesalamine (HPLC-method)

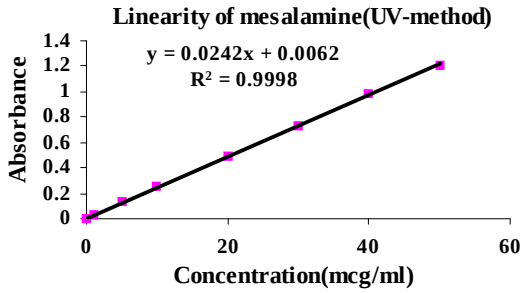

Figure 2: Calibration curve of Mesalamine (UV- method)

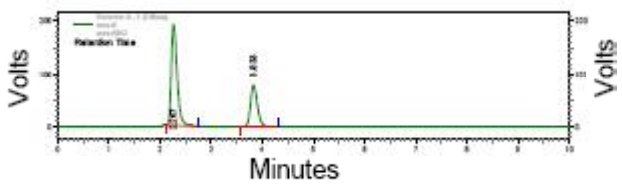

Figure 3: A typical chromatogram of Mesalamine in formulation

UV method

The suitable aliquot's (sample solution 100µg/ml) were diluted in phosphate buffer (pH- 3.6) so as give a concentration of 10µg/ml. Absorbance values were recorded at 298nm against reagent blank. The drug content and the absorbance values were calculated by us-

TABLE 2: Amount of Mesalamine in tablet dosage forms by proposed HPLC and UV- methods

Formulation	Labeled amount (mg)	HPLC method*			UV- method*		
		Mean ±s.d (amount mg recovered)	%Drug recovered	% RSD	Mean ±s.d (amount mg recovered)	%Drug recovered	% RSD
Mesacol	400	398.04±0.298	99.51±0.0745	0.0749	395.58±1.144	98.895±0.286	0.289

*Each value is average of three determinations ± standard deviation.

TABLE 3: Accuracy readings for the proposed HPLC and UV methods for the determination of Mesalamine

Amount of standard drug added to the pre analyzed (10µg/ml) formulation	HPLC method*		UV- method*	
	% Recovery (mean ± S.D)	% RSD	% Recovery (mean ± S.D)	% RSD
8	101.51±0.27	0.266	101.39±0.1159	0.1143
10	98.143±0.2802	0.2855	99.3253±0.2637	0.2655
12	99.34±0.2095	0.2109	98.6757±0.3026	0.3067

*Each value is average of three determinations ± standard deviation

TABLE 4: System suitability parameters of mesalamine

Sl.no.	Parameter	Value
1	Resolution	3.28
2	Capacity factor	2.37
3	Theoritical plates	2959
4	Tailing factor	0.924
5	HETP	0.071×10^{-5}
6	Asymmetry	1.09

ing the standard calibration curve. The results were shown in TABLE 2.

RESULTS

Method validation[18-19]

1. Linearity

The calibration curve was obtained (1-50µg/ml for both HPLC and for UV-methods). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by least square method.

2. Precision

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six replicates of standard solutions were made and the response factor of drugs and percentage relative standard deviation (%RSD) were calculated. In the inter day variation studies, six replicates of standard solutions were made for three consecutive days and response factor of drugs and percentage relative standard deviation were calculated. From the data obtained, the developed RP-HPLC and UV methods were found to be precise.

3. Accuracy

The accuracy was determined by the recovery of known amount of mesalamine reference standard added to the samples at the beginning of the process. An accurately weighed amount of powder equivalent to 10 mg of mesalamine was transferred to 100ml volumetric flask and dissolved in mobile phase(for HPLC-method) and in phosphate buffer (pH- 3.6)(for UV-method) respectively to make final concentration of 100µg/ml. 1.0ml of this solution was transferred into 10mll volumetric flasks containing 0.8, 1.0, 1.2 ml of mesalamine standard solution (100µg/ml) and reagent blank is added to make up the volume to give a final concentration of 18, 20 and 22µg/ml. All the solutions were triplicated and assayed. The percentage recovery of added mesalamine standard was calculated and the data was shown in TABLE 3.

4. Limit of detection (L.O.D) and limit of quantification (L.O.Q)

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation for UV and signal to noise ratio in case of HPLC method.

5. System suitability

For system suitability for HPLC method, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), HETP, capacity factor (k^1), plates per meter and peak symmetry of samples. The results were shown in TABLE 4.

6. Robustness

The percent recovery of mesalamine was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for mesalamine was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

DISCUSSION

HPLC method

The chromatographic method was optimized by

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changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. A non-polar C-18 analytical chromatographic column was chosen as the stationary phase for the separation and determination of mesalamine. The separation of peaks was dependent on the composition of the mobile phase. The developed method was used based upon its ability to detect and quantify mesalamine with the use of high-performance liquid chromatographic system equipped with UV-Visible detector.

Under the presently prescribed conditions, the recovery studies of mesalamine were found to be from 99.14 to 101.51% respectively. This method is very useful for determination of mesalamine in pharmaceutical dosage forms, clinical studies and pharmacokinetic studies. The observation of %RSD less than 2.0 for both intra- and inter-day measurements indicates high degree of precision. In the present method, we have established a linearity range of 1-50µg /ml; this linearity range covers all the strengths of mesalamine. Hence this method can be applied for quantifying the low levels of mesalamine in pharmaceutical dosage forms and other pharmacokinetic studies.

UV method

From the optical characteristics of the proposed method, it was found that mesalamine obeys linearity within the concentration range of 1-50µg/ml. It was found that the % RSD is less than 2%, which indicates that the method has good reproducibility. From the results shown in accuracy TABLE 2, it was found that the percentage recovery values of pure drug from the preanalyzed solution of formulation were in between 98.68-101.39%, which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method. The study was made to test ruggedness of the method through the interday and intraday analysis of samples. Results obtained confirmed ruggedness of the method. The developed method was found to be accurate, precise, reproducible and stable, which indicated that this method can be used for the routine analysis of mesalamine in bulk and the pharmaceutical dosage forms.

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