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Research Article

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF MESALAMINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Mesalamine in bulk and tablet dosage form. Isocratic elution at a flow rate of 1.2 mL/min was employed on an Xterra ODS C18 column (250 mm x 4.6 mm I.D., 5 µm particle size) at ambient temperature. The mobile phase consisted of phosphate buffer pH 6.8:methanol (60:40, v/v). The UV detection wavelength was 235 nm and 20 µL of sample was injected. The retention time for Mesalamine was 2.172 min. The % recovery was within the range between 98.0 % and 101.3 %. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Mesalamine in bulk samples and its formulations.

Keywords: Mesalamine, phosphate buffer, methanol and HPLC.

INTRODUCTION

Mesalamine (Fig. 1) also known as Mesalazine or 5-amino salicylic acid (5-ASA), is an anti-inflammatory drug used to treat inflammatory bowel disease, such as ulcerative colitis and mild-to-moderate Crohn's disease[1]. Mesalamine is a bowel-specific amino salicylate drug that acts locally in the gut and has its predominant actions, thereby having few systemic side effects. As a derivative of salicylic acid, Mesalamine is also thought to be an antioxidant that traps free radicals, which are potentially damaging byproducts of metabolism[2]. Mesalamine is considered the active moiety of Sulfasalazine, which is metabolized to Sulfapyridine and Mesalamine[3]. Literature survey revealed that a few analytical methods have been reported for the determination of Mesalamine in pure drug, pharmaceutical dosage forms and in biological samples using spectrophotometry[4-7], HPLC[8-11], UPLC[12] and LC-MS[13] either in single or in combined forms. The aim of the present work is to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of Mesalamine in bulk and in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination of Mesalamine in bulk and tablet dosage forms.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade methanol and water were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Potassium dihydrogen phosphate and sodium hydroxide of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. The working standard of Mesalamine was provided as gift sample from Pharma Train, Hyderabad, India. Mesalamine tablets were purchased from local market.

Instrumentation and analytical conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra ODS C18 column (250 mm x 4.6 mm; 5 µm), a 2695 binary pump, a 10 µL injection loop and a 2487 dual absorbance detector and running on Waters Empower

software. Isocratic elution with phosphate buffer:methanol (60:40, v/v, pH 6.8 adjusted with sodium hydroxide) was used at a flow rate of 1.2 mL/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use. The UV spectrum of Mesalamine was taken using an Elico SL-159 UV-Visible spectrophotometer.

Stock and working standard solutions

Accurately weigh and transfer 10 mg of Mesalamine working standard into a 10 mL volumetric flask, add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.1 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter. The calibration curve was plotted with the five concentrations of the 6-14 µg/mL working standard solutions. Chromatogram was recorded thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of Mesalamine tablets

Weigh 20 Mesalamine tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Mesalamine into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.1 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter. An aliquot of this solution was injected into HPLC system. Peak area of Mesalamine was measured for the determination.

Procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines[14]. The method was validated for linearity, precision, accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 6-14 µg/mL prepared in triplicates to test linearity. The peak area of Mesalamine was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Mesalamine test solution in the same equipment at a concentration value of 100 % (10 µg/mL) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of Mesalamine was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Mesalamine at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Mesalamine recovered in the samples. Sample solution short term stability was tested at ambient temperature (20 ± 10^0C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSIONS

Selection of the detection wavelength

The UV spectra of Mesalamine in 60:40, v/v mixture of phosphate buffer and methanol was scanned in the region between 200 and 400 nm and shows λmax at 235 nm.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Mesalamine is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of phosphate buffer and methanol was selected as mobile phase and the effect of composition of mobile phase on the retention time of Mesalamine was thoroughly investigated. The concentration of methanol and buffer were optimized to give symmetric peak with short run time (Fig. 2). A short run time and the stability of peak asymmetry were observed in the ratio of 60:40, v/v of phosphate buffer and methanol. It was found to be optimum mobile phase concentration.

Validation of the method

Linearity

Five points calibration graphs was constructed covering a concentration range 6-18 µg/mL (Three independent determinations were performed at each concentration). Linear relationships between the peak area signals of Mesalamine the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Precision

The validated method was applied for the assay of commercial tablets containing Mesalamine. Sample was analyzed for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, and was found to be 101.31% showing that the content of Mesalamine in tablet formulations confirmed to the content of requirements (95–105%) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on three consecutive days (n=3) indicated a RSD of 0.35. This indicates good method precision.

Accuracy

The data for accuracy were expressed in terms of percentage recoveries of Mesalamine in the real samples. The mean recovery data of Mesalamine in real sample were within the range of 98.0 and 101.3%. The mean %RSD was 99.9% satisfying the acceptance criteria for the study. It was proved that there is no interference due to excipients used in tablet formulation. Hence the accuracy of the method was confirmed. The results are furnished in Table 2.

Stability

The stability of Mesalamine in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 10^0C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that Mesalamine is stable in standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor, HETP and number of theoretical plates were also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Mesalamine in tablet formulation. The results are furnished in Table 3.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Mesalamine in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 5 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of Mesalamine in pharmaceutical dosage form.

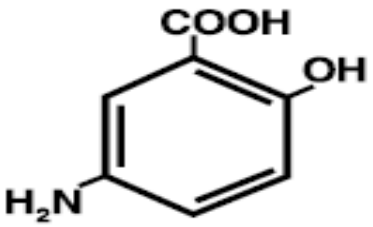

Fig. 1: Chemical structure of Mesalamine

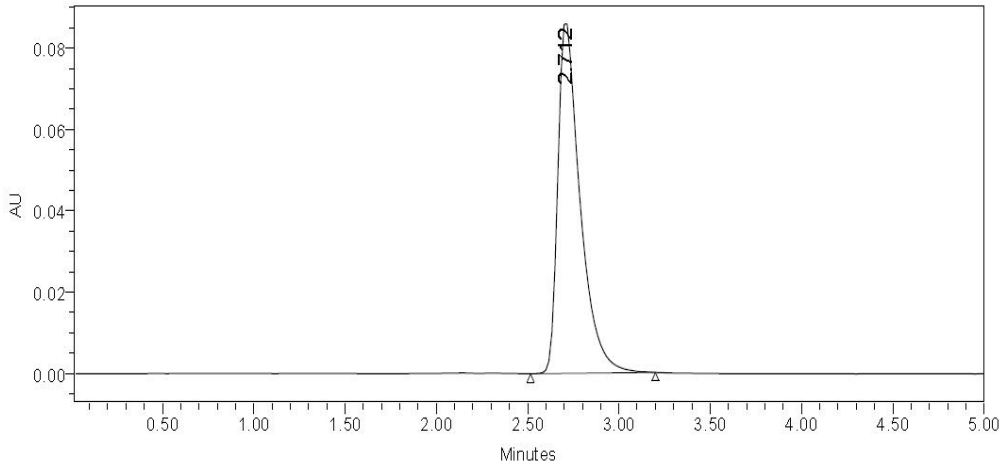

Fig. 2: Typical chromatogram of Mesalamine

Table 1: Linearity of Mesalamine

S. No.	Linearity level	Concentration	Area
1	I	6 µg/mL	370872
2	II	8 µg/mL	547634
3	III	10 µg/mL	732631
4	IV	12 µg/mL	896161
5	V	14 µg/mL	1076176
Correlation Coefficient			0.999

Table 2: Recovery studies of Mesalamine

%Concentration (at specification level)	Area	Amount added (mg)	Amount found (mg)	%Recovery	Mean recovery
50%	746569	5.27	5.3	101.3%	99.9%
100%	1403315	10.0	10.0	100.4%	
150%	1987020	14.5	14.2	98.0%	

Table 3: System stability parameters

S. No.	Parameters	Values
1	λmax (nm)	235
2	Beer's law limit (µg/mL)	6-14
3	Correlation coefficient	0.999
4	Retention time (min)	2.712
5	Theoretical plates	2398
6	Tailing factor	1.68
7	Limit of detection (µg/mL)	0.03
8	Limit of quantification (µg/mL)	0.10

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