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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR MESALAMINE

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ABSTRACT

A simple, specific, accurate, cost effective & time efficient reversed phase high performance liquid chromatographic method was developed for the determination of Mesalamine, using RP -18 (4.6 mm X 2.5 cm) column and a mobile phase composed of methanol: water (50:50 v/v) at flow rate 0.5 ml/min. The retention time of Mesalamine was found to be 3.070 min which is much less than the other method used for the estimation of Mesalamine. Linearity was established for Mesalamine in the range 20 – 50 µg / ml. The percentage recovery of Mesalamine was found to be in the range 99.77%.The proposed method is precise, accurate, selective and rapid for the determination of Mesalamine for QC level.

Key words: Mesalamine (MSZ), RP-HPLC, validation.

INTRODUCTION

Mesalamine (5-aminosalicylic acid) is an anti-inflammatory agent, structurally related to the salicylates, which is active in inflammatory bowel disease and active ulcerative proctitis. It is a tan to pink crystalline powder, relatively insoluble in chloroform, ether, n-hexane and ethyl acetate and freely soluble in dil.HCl and alkali hydroxides[1, 2]. Mesalamine is available in tablet dosage forms (400 mg) and is an official drug of USP. Literature survey reveals that, some study about HPLC determination of Mesalamine and its degradation metabolite in plasma[3, 4, 5, 6, 7], determination of 5-aminosalicylic acid in pharmaceutical formulation by differential pulse voltammetry[8]. Quality evaluation of this drug through the application of various analytical methods is attracting much attention. The use of buffer itself adds to the cost of analysis as it involves extensive washing of the columns using considerable amount of HPLC grade solvents. The time required for washing the column also adds to the cost of analysis. This additional cost is also reflected in the cost of the drug. The present method has been developed excluding the use of buffer which makes it highly cost effective with respect to use of solvents as well as time of analysis. A RP – 18 column (4.6 mm X 2.5 cm,) and a mobile phase composed of methanol: water (50:50v/v) at a flow rate of 0.5 ml/ min was used. The retention time of Mesalamine was found to be 3.070 min respectively.

EXPERIMENTAL

Reagents and materials

Reference standard of Mesalamine was procured from WALASA PHARMACEUTICALS, GOA. The tablet formulations MESACOL was procured from local market. Water, methanol, were of HPLC grade and purchased from Merck, India. Membrane Filters P/N – M47N45, 47 mm, 0.45µ were obtained from MZ ANALYSENTECHNIK.

Preparation of standard stock and sample solution

Standard stock solution of Mesalamine was prepared by dissolving accurately weighed 100 mg of Mesalamine in 100 ml of aqueous methanolic solution (50:50) by a Trans-o-sonic Sonicator for 10 minutes to get concentration of 1000µg/ml solutions.Then the solution is filtered by whatmann filter paper No.41. From this 5ml was taken and diluted to 50ml with that solvent to get 100µg/ml. 20 tablets are weighed and grounded to fine powder. An accurately weighed quantity of powder sample equivalent to 100mg of Mesalamine was dissolved with 100ml of solvent by an ultrasonicator. The extract was filtered by whatmann filter paper. From the solution 5ml was taken and diluted to 50ml with the solvent to get 100µg/ml. Further dilution of (20µg/ml) was made and the solution is then filtered by Hypodermic syringe filter.

Table 1: Calibration Curve of standard Drugs

Sl No.	Concentration(µg/ml)	Peak Area
1	20	0.18955
2	30	0.29956
3	40	0.39438
4	50	0.47664

Assay

10 µl of standard and sample were injected into an injector of liquid chromatography, from the peak area of Mesalamine amount drug in samples were computed. The values are given in Table 2.

Chromatography

Chromatographic separation was performed on a Jasco HPLC system consisting of Jasco PU – 2089 pump, Jasco UV 2010 plus photo diode array detector. Rheodyne injection syringe with 20 µl loop volume and windows based chrompass software. An ODS C-18 RP- column (Intersite 4.6 mm X 2.5 cm,) was used for separation . The elution was carried out isocratically at flow rate of 0.5 ml / min using methanol: water (50: 50 v/v) as mobile phase. The run time was 10 min. Before analysis both mobile phase and sample solutions were degassed by sonication and filtered through 0.2µm filter. The analytes were monitored at 220 nm. The analytes were identified by comparison of retention times obtained from sample and standard solutions. The work was performed in an air conditioned room maintained at 25 ± 2°C.

Table 2: Analysis of formulation

Formulation	Labeled Claimed (mg)	Amount Found (mg)*	Drug Content (%)	S.D.	R.S.D
Formulation -1	400	410.693	102.67%	0.5989	0.1458
Formulation -2	400	389.586	97.39	0.2361	0.0606

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

Column chemistry, solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so the tablet components were free from interference from the solvent and from excipients. Other criteria, for example time required for analysis, appropriate *k* range for eluted peaks, assay sensitivity, solvent noise, and use of the solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. Columns containing different stationary phases, the final choice giving satisfactory resolution and run time was the 25 cm × 4.6 mm, C18 reversed-phase column. A series of aqueous mobile phases containing methanol, Acetonitrile and water solutions of different volume fractions were also tested. The best results were obtained by use of 50:50 (*v*/*v*) methanol-water. The flow rate was determined by testing the effect of different flow rates on peak area and resolution; 0.5 mL min^{-1} was found to be optimum. All experiments were performed at ambient temperature. The appropriate wavelength for simultaneous determination of Mesalamine is 220nm in 50:50 (*v*/*v*) methanol:water. Under the optimum chromatographic conditions, the retention times obtained for Mesalamine was found to be 3.070 min, which was shown in (Fig.1). Retention factor, tailing factors and number of theoretical plates are reported in Table 3. The number of plates (*N*) is a measure of column efficiency; which shows the high separation efficiency of the column used.

Validation of the Method

The method was validated for linearity, accuracy, precision, repeatability, selectivity, and specificity.

All validation studies were performed by replicate injection of sample and standard Solutions.

Table 3: Property of Mesalamine

Chromatographic parameters	Result
Retention time (min)	3.070
Tailing factor, T	1.02
Number of theoretical plates, N	3333.59

Linearity

Several aliquots of standard of Mesalamine was taken in different 10 ml volumetric flasks and diluted up to the mark with water and methanol such that the final concentration of Mesalaminee is 20- 50 µg / ml. Evaluation of two drug was performed with PDA detector at 220 nm, peak area recorded for all the peaks and are given in the Table 1. Slope and intercept value for calibration curve was Y = 0.0097X + 0.0002(R^2 = 0.9982) for Mesalamine.

Accuracy

The accuracy of the method was confirmed by studying recovery at three different concentrations, 80, 100, and 120% of those expected, in accordance with ICH guidelines, by replicate analysis (*n* = 6). Standard drug solutions were added to a pre analyzed sample solution and percentage drug content was measured. The results from study of accuracy are reported in Table. 4. From these results it was clear that the method enables very accurate quantitative estimation of Mesalamine in tablet dosage form, because all the results were within acceptable limits, i.e. COV < 2.0% and S.D. < 1.0.

Table 4:

Drug	Amount taken (µg)	Amount added		Recovery* (%±S.D)	Cov* (%)
		%	µg/ml		
Mesalamine	20	80	16	104.45±0.3431	0.3381
		100	20	99.77±0.30005	0.30074
		120	24	100.24±0.2217	0.2211

Precision, LOD and LOQ

Precision was studied both intra-day and inter-day. Six replicate sample solutions were prepared from the stock solution. For study of intra-day precision the concentrations of the two drugs were measured three times on the same day at intervals of 1 hr. In the inter-day study the drug concentrations were measured on three different days. The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank and *S* is the slope of the calibration plot. The results are reported in Table. 5 and 6 .

Table 5:

DRUG	LOD µg mL^{-1}	LOQ µg mL^{-1}
MESALAMINE	0.8078	2.4479

Table 6:

Formulation	Intraday precession* (RSD %)	Interday precession* (RSD %)		
		Day 1	Day 2	Day 3
Mesalamine	0.9234	0.3734	0.3443	0.3327

Selectivity and Specificity

The selectivity of the method was checked by injecting solutions of Mesalamine. It was observed that sharp peak for Mesalamine was obtained at retention time 3.070 min. The specificity of the method was assessed by comparing chromatograms obtained from drug standards with that obtained from tablet solutions. The retention time of the drug standards and the drugs from sample solutions was same, so the method was specific. The method was also selective because there was no interference from excipients in the tablets.

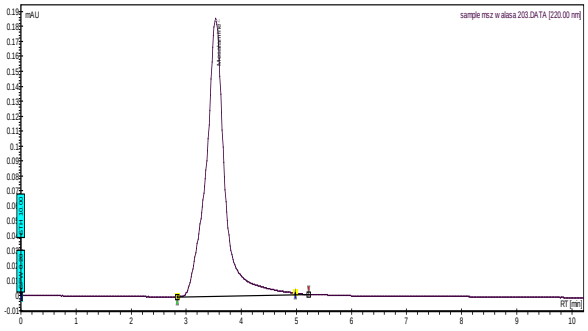

Fig.1:

CONCLUSION

A new, rapid reversed-phase HPLC method has been developed for analysis of Mesalamine in a tablet formulation. It is shown above that the method was accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The run time is relatively short, i.e. 10 min, which enables rapid quantitation of many samples in routine and quality-control analysis of tablet formulations. The same solvent was used throughout the experimental work and no interference from any excipient was observed. These results show the method could find practical application as a quality-control tool for analysis of Mesalamine from their dosage forms in quality -control laboratories.

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