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Research Article

A VALIDATED ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ASSAY DETERMINATION OF MESALAMINE

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ABSTRACT

Objective: The objective of current study is to develop and validate an ultra fast, simple, precise, accurate and specific chromatographic method for the determination of Mesalamine in pure form and in pharmaceutical formulations.

Methods: An Ultra Performance Liquid Chromatograph instrument and BEH C18 Waters Acquity column were used for Assay determination of Mesalmine. Buffer was prepared by using 1.36g of potassium dihydrogen phosphate and 2.02g of sodium-1-heptane sulphonate in 890mL of water and adjusted the pH of the solution to 2.2 with diluted ortho phosphoric acid. The mobile phase was prepared by mixing of buffer, Methanol and Acetonitrile in the ratio of 890:80:30 (v/v/v) respectively. The flow rate of 0.8ml min^{-1} was set with an isocratic program, the temperature of column compartment maintained at 40°C and Ultra violet detection done at 230nm wavelength. The Mesalamine peak eluted at about 1.2 minutes and then run time was set as about 2 minutes.

Results: The correlation coefficient (≥ 0.999) shows the linearity of response against concentration over the range of 30ppm to 90ppm. The observed result shows that the method is rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

Conclusion: The developed and validated ultra performance liquid chromatographic method is suitable for assay determination of Mesalamine in pure and pharmaceutical formulations which is more useful with respect to less analysis time.

Keywords: Liquid Chromatography, Mesalamine

INTRODUCTION

Mesalamine (United States Adopted Name) is also known as Mesalazine (International Nonproprietary Name / British Approved Name), or 5-aminosalicylic acid (5-ASA) or chemically designated as 5-amino-2-hydroxy benzoic acid. Mesalamine is an anti-inflammatory drug used to treat inflammatory bowel disease, such as ulcerative colitis [1] and mild-to-moderate Crohn's disease [2]. Local intestinal flora split sulfasalazine into sulfapyridine and 5-aminosalicylate [3]. The chemical formula of Mesalamine is $C_7H_7NO_3$ with the molecular weight of 153.135g mol^{-1} and molecular structure was shown in Fig: 1. A number of analytical methods have been developed for the analysis of Mesalamine in pure form and pharmaceutical dosage forms. These methods include spectrophotometry [4, 5, 6] and high-performance liquid chromatography (HPLC) combined with UV [7, 8, 9] fluorescence [10, 11] and electrochemical (EC) [12] detections. But as such there is no validated method established by using ultra performance liquid chromatographic system with less analysis time. Mesalamine is usually present at trace levels in a complex biological matrix, and the potentially interfering endogenous substances (which are usually present at higher concentrations than the drug) need to be removed before the analysis. According to the pKa values of carboxyl and amino groups (2.30 and 5.69, respectively), solubility of Mesalamine increases at pH‹2 and pH›5.5 and is reduced in 2.0 to 5.5 pH interval [13]. The complication in separation and detection of Mesalamine is due to high polarity nature and amphoteric properties of the molecule. As such there is no validated method available with short run time of about 2 minutes to determine the Mesalamine. A key benefit of the method was less run time which will also save the solvents consumption.

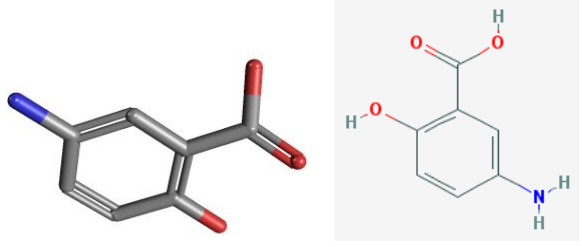

Fig. 1: Molecular Structure of Mesalamine

MATERIALS AND METHODS

Analytical reagent grades of Potassium dihydrogen orthophosphate and Sodium 1-octane sulphonate were used for buffer preparation and diluted phosphoric acid was used for pH adjustment of the buffer to 2.2. The water used for the buffer, standard and sample preparations was purified by Milli Q water purification system (Millipore) which was equivalent to highly pure HPLC grade water. The HPLC grade solvents of Methanol and Acetonitrile were used for the mobile phase preparation. Membrane filter with 0.22 µm size (Millipore) was used for the mobile phase filtration and degassed under vacuum. The Mesalamine standard material used for the Assay determination of Mesalamine in extended release tablets and 3-Amino Salicylic acid was used to ensure system suitability requirement of chromatographic analysis. HPLC grade Methanol and 1N Hydrochloric acid were used to dissolve Mesalamine standard material, formulated sample and further diluted with HPLC grade water. The complete analysis was carried out by using "Class A" volumetric glassware for accuracy.

Equipment

An Acquity UPLC system manufactured by Waters which consist of Photo Diode Array (PDA) detector, Quaternary solvent manager, Sample manager, column heating compartment was used for assay determination of Mesalamine. UPLC instrument was controlled by Waters Empower chromatographic software. A Waters Acquity BEH C18, 50 x 2.1mm, column with particle size of 1.7µm was used as stationary phase for chromatographic separation and determination of Mesalamine. Sartorius semi micro analytical balance was used for all weighing, Thermo orion pH meter was used for buffer pH adjustment, Bandelin sonicator used to dissolve the standard, sample and were centrifuged by using Hermle centrifuge machine.

Standard and Sample Preparation

The Mesalamine standard stock solution was prepared by dissolving an accurately weighed amount of Mesalamine standard in 1N Hydrochloric acid (5% of desired volume), Methanol (1% of desired volume) and then diluted with water to achieve 0.6 mg mL^{-1} concentration, then the standard stock solution further diluted with water to achieve final concentration of 0.06 mg mL^{-1}. The system

suitability solution was prepared by using same pattern of diluents which were used for standard preparation to achieve the final concentration of 0.06 mg mL^{-1} and 0.0011 mg mL^{-1} of Mesalamine and 3-Amino salicylic acid respectively. The sample solution of Mesalamine in pure form was prepared by dissolving an accurately weighed amount of Mesalamine sample in 1N Hydrochloric acid (5% of desired volume), Methanol (1% of desired volume) and diluted with water to achieve 0.6 mg mL^{-1} concentration, then the sample stock solution was further diluted with water to achieve final concentration of 0.06 mg mL^{-1}. The sample preparation of Mesalamine in pharmaceutical formulations was a critical step as it involves the extraction of Mesalamine from other formulated compositions. The Mesalamine pharmaceutical dosage units were taken in to 500ml volumetric flask which contains 100ml of methanol and it was kept under sonication for about 10minutes to disperse the formulated dosage units, then added 200ml of 1N Hydrochloric acid further kept under sonication for about 45 minutes or till the dosage units dissolves completely with intermediate shaking and then dilution made up to 500ml volume with 1N Hydrochloric acid. A portion of above formulated dosage solution was centrifuged at 4000 RPM for 10 minutes. Pipettes 5ml of centrifuged solution in to a 1000ml volumetric flask, further diluted and made up to the mark with water.

Chromatographic Conditions

An Ultra Performance Liquid Chromatograph (UPLC) was used to carry out the chromatographic analysis. The Mesalamine was separated from its process impurities and formulated impurities by using a BEH C18, 50 x 2.1mm, 1.7 µ, Waters Acuity column. An isocratic pumping program with a flow rate of 0.8 ml $minute^{-1}$ at a column temperature of 40°C temperature resulted the retention time of Mesalamine at about1.2 minutes and hence run time of chromatographic analysis was fixed as about 2 minutes. The absorbance of Mesalamine was measured at 230nm of UV wavelength. The injection volume set as 5µL. For strong needle wash, water and Methanol in the ratio of 10:90 v/v, weak needle wash, water, methanol and acetonitrile in the ratio of 60:30:10 v/vv and for seal wash, water and methanol in the ratio of 70:30 v/v was used along with sample loop option as partial loop with needle overfill. Buffer was prepared by using 1.36g of potassium dihydrogen phosphate and 2.02g of sodium-1-heptane sulphonate in 890mL of water and adjusted the pH of the solution to 2.2 with diluted ortho phosphoric acid. The mobile phase was prepared by mixing of buffer, Methanol and Acetonitrile in the ratio of 890:80:30 (v/v/v) respectively.

Method Validation

The method was verified to determine the system suitability parameters and validated for specificity, repeatability, linearity, accuracy, range, ruggedness and robustness as per the International Conference on Harmonization (ICH) guidelines [14, 15].

Specificity

Specificity study was conducted to demonstrate the interference of placebo, and impurities from analyte peak of Mesalamine. The study was extended to ensure the effective separation of degradation peaks of formulation ingredients from the retention time of Mesalamine and its impurities. The Assay test was performed on Placebo equivalent to the amount present in test preparation as per test method to demonstrate the interference of placebo retention time with Mesalamine retention time. To demonstrate the impurity interference, Prepared impurity mix solution at 0.3% concentration level of test concentration and injected into the chromatographic system as per test method. Also prepared standard solution by spiking impurity mix solution with analyte (Mesalamine) at 0.3% of test concentration and injected into the chromatographic system as per test method. Run the chromatogram for three times of retention time of main analyte peak and observed the retention time interference of impurities with Mesalamine retention time. The drug product and ingredients were exposed to acid hydrolysis, base hydrolysis, peroxide oxidation, water degradation, sun light, UV light, Heat and humidity stress conditions to induce degradation [16].

Repeatability

Performed the repeatability of test method by preparing six sample solutions by using drug product (Mesalamine extended release tablets 1.2 g) and calculated the % assay and % relative standard deviation from six sample preparations.

Linearity

To demonstrate the method linearity for Mesalamine, prepared five standard solutions of Mesalamine with concentration ranging from 50 % to 150 % of the target standard concentration (60µg/mL) and injected into the chromatographic system. The correlation coefficient between the linearity levels and Bias at target concentration (60µg/mL) was calculated.

Accuracy

To demonstrate the Accuracy of method, prepared sample solutions by using drug product (Mesalamine extended release tablets 1.2 g) ranging from 40% to 160% (40%, 80%, 100%, 120% and 160%) of the target test concentration as per test method. Prepared six sample solutions at lower and higher levels and three sample solutions at intermediate levels and calculated the % recovery at each level.

Range

The analytical method range was determined based on lower concentration level (40%) and higher concentration level (160%) of the target test concentration (60µg/mL). The sample solutions with known concentrations at 80%, 100% and 120% levels were injected in to the chromatographic system and verified the range of method.

Ruggedness

To demonstrate ruggedness of test method, conducted system-to-system variability on two systems by different analyst using the different column on different day by preparing six sample solutions using drug product (Mesalamine Extended release tablets 1.2 g) and analyzed as per the test method. The results were calculated for % assay and % relative standard deviation from six sample preparations. The study includes bench top stability of mobile phase, Mesalamine standard and sample solutions, refrigerator stability on Mesalamine standard, sample and system suitability solutions.

Robusness

To demonstrate the method robustness, checked the system suitability parameters by injecting standard and system suitability solution, by using two mobile phases, one containing 90% of the method organic phase (Acetonitrile/Methanol) and other contains 110% of the method organic phase (Acetonitrile/Methanol), checked with the flow rates of 0.7 mL/min, 0.8 mL/min and 0.9 mL/min, checked at 35°C, 40°C and 45°C of various column oven temperature and also checked with the mobile phase having buffer of pH 2.0, 2.2 and 2.4 and then evaluated system suitability parameters under the various test conditions as mentioned above.

RESULT AND DISCUSSION

An ultra performance reverse phase chromatographic technique was used to determine the % of Mesalamine present in the drug product or drug substance. Ion pair reagent of sodium-1-heptane sulphonate was used in buffer preparation to improve the resolution and avoid the other substances co elution at same retention time in reverse phase chromatography. The phosphate buffer with pH 2.2 was found more appropriate for asymmetric peak shape, robust resolution and for better separation of Mesalamine from its impurities, placebo. The pH of the buffer solution was selected as 2.2 based on pKa values of carboxyl and amino groups as 2.30 and 5.69 respectively. The buffer strength selected to reduce load on column and to achieve good symmetric peak. The presence of organic solvents in the mobile phase was studied. Methanol and Acetonitrile were used as an organic modifier. The C18 stationary phase was used for Mesalamine separation as it shows polar nature. When BEH C18 column used as a stationary phase, the method is more selective, sensitive and achieved well separation of Mesalamine from its impurities and degrades within a short run time and also more

rugged with respect to the variations in the mobile phase compositions.

The concentration of Mesalamine was established according to the absorbance at wavelength 230nm which was given good recovery through out its range of the analytical method. The utilization of advanced chromatographic technique such as Ultra Performance Liquid Chromatograph resulted precise, accurate method with less run time which benefits to quality control or routine testing of Mesalamine. The required selectivity, separation and symmetry of Mesalamine peak was achieved within the short run time of 2 minutes. The developed analytical method was subjected for further validation as per the current ICH guidelines to use for its intended purpose. The observed values of system suitability parameters such as tailing factor for Mesalamine peak, relative standard deviation of the area response of Mesalamine and the resolution between Mesalamine and 3- Amino salicylic acid peaks are found satisfactory against described acceptance criteria. The results are as shown in Table 1.

Table 1: System Suitability results of Mesalamine peak

Parameter	Observed Results	Acceptance criteria
Tailing Factor	1.0	Not more than 2.0
Resolution	3.7	Not less than 2.0
% RSD of five replicates	0.1%	Not more than 2.0%

The calculated results of % relative standard deviation from the six different preparations of Mesalamine drug product evidenced that the method was precise. Repeatability study results as shown in Table 2.

Table 2: Results of Repeatability study

S. No.	Preparation No.	% Assay of Mesalamine
1	Preparation No - 1	99.5%
2	Preparation No - 2	97.9%
3	Preparation No - 3	97.9%
4	Preparation No - 4	98.8%
5	Preparation No - 5	97.7%
6	Preparation No - 6	99.4%
	Average % Assay of Mesalamine	98.5%
	% Relative Standard Deviation	0.8%

Specificity study of the analytical method was demonstrated the well separation of Mesalamine peak from its impurities and placebo interference. As there was no peaks of impurity, placebo were eluted at the same retention time of Mesalamine peak and the degradation study of all exposed samples were found spectrally pure as evidenced that the purity threshold was greater than the purity angle of Mesalamine peak and there was no Flag in purity results.

The results of specificity study with degradation impurities are as shown in Table 3.

Table 3: Specificity study with degradation impurities

Stress condition	Purity angle	Purity Threshold	Purity Flag
Acid hydrolysis	0.430	0.925	No
Base hydrolysis	0.383	0.903	No
Peroxide oxidation	0.391	0.947	No
Water stress	0.374	0.907	No
Heat stress	0.432	0.957	No
Humidity stress	0.401	0.924	No
Sunlight stress	0.440	0.944	No
UV light stress	0.389	0.934	No

The above values described by using the Empower 2 software with waters PDA detector.

The relative standard deviation of Mesalamine content in six different preparations was found less than 2.0% during repeatability test and it was evidenced that the method is more precise. The recovery results of method was found between 97.0% and 103.0% throughout its concentration levels of 40%, 80%, 100% 120% and 160% of target concentration (60µg/mL) which was demonstrated the accuracy of analytical method throughout its range as shown in Table 4.

Table 4: Results of Recovery (Accuracy) study

S. No.	Level in %	Mean % Recovery
1	40%	99.77%
2	80%	99.93%
3	100%	98.57%
4	120%	98.03%
5	160%	98.00%

The observed correlation coefficient results (r^2 = 0.9999) from the plotted calibration curve with the Mesalamine peak area versus concentration throughout the range of about 30µg/mL to 90µg/mL evidenced that the method was linear throughout its concentration range. The data of regression analysis of the calibration curves are shown in Table 5 and Linearity curve for Mesalamine was shown in Fig: 2.

Table 5: Linearity results of concentration and peak area

S. No.	Linearity Level	Concentration (µg/ml)	Peak Area
1	50%	30.0045	444621
2	75%	45.0068	667070
3	100%	60.0090	897934
4	125%	75.0113	1118954
5	150%	90.0135	1337501

The resulted correlation coefficient from above data is 0.9999

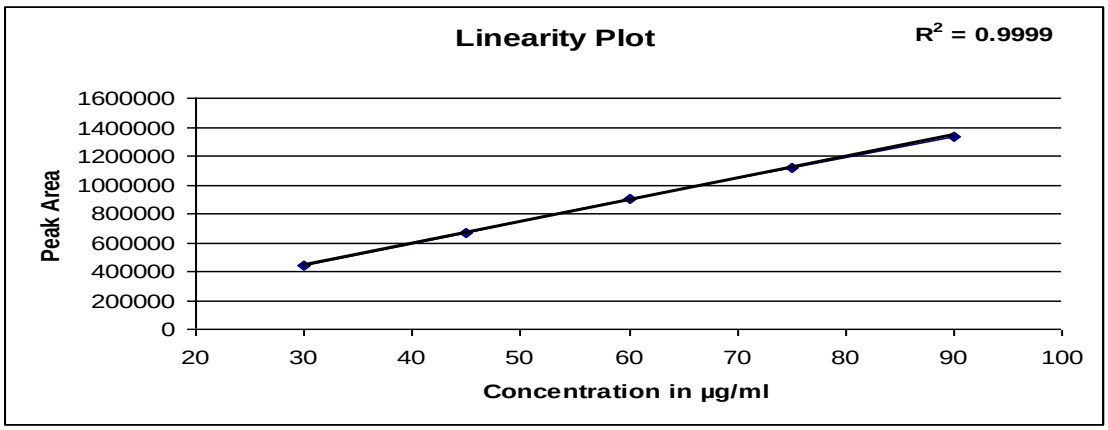

Fig. 2: Linearity Curve for Mesalamine

The Chromatograms generated for Blank, system suitability, standard and sample of Mesalamine by using above validated method on an Ultra performance liquid chromatograph system were shown in Fig: 3, Fig: 4, Fig: 5 and Fig: 6 respectively. The above data proved that the analytical method developed and validated is more precise, accurate, robust and rugged throughout its range and can be more useful for commercial applications with unique advantage of less analysis time and cost effective.

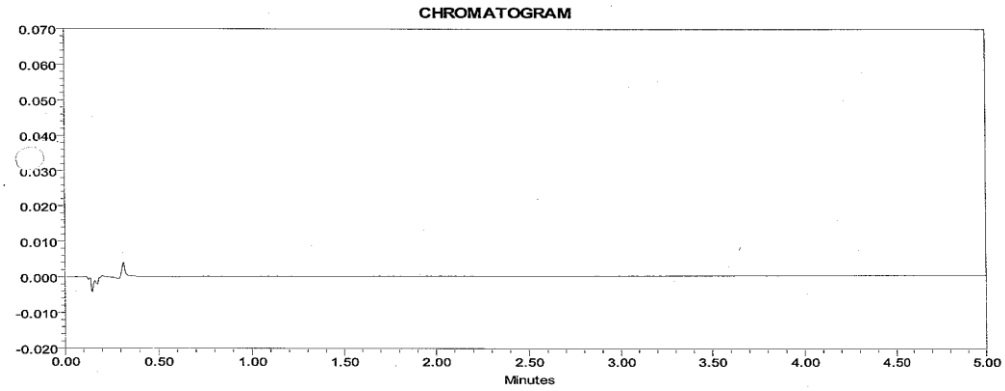

Fig. 3: Specimen Chromatogram of Blank Injection

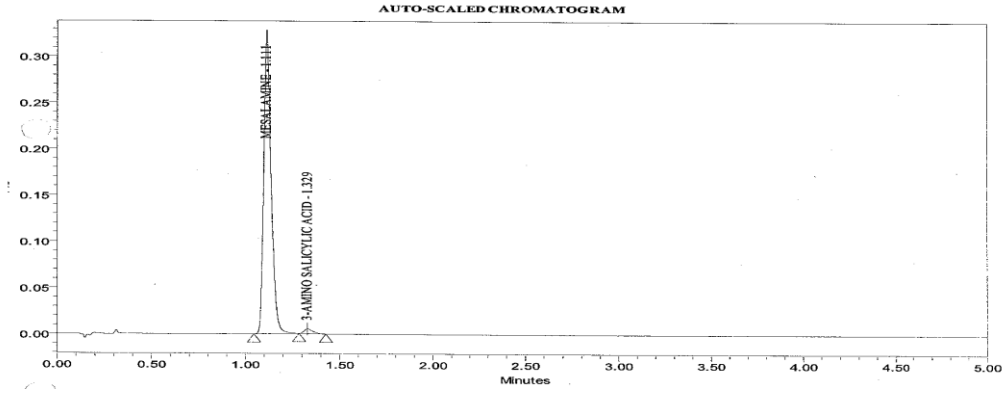

Fig. 4: Specimen Chromatogram of System Suitability Injection

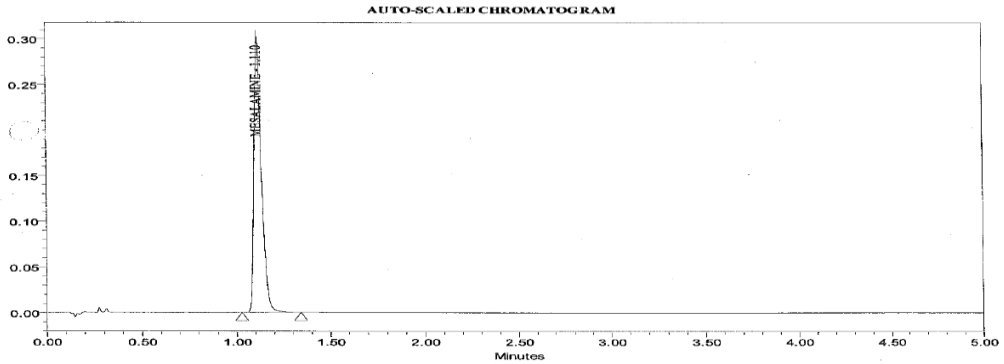

Fig. 5: Specimen Chromatogram of Standard Injection

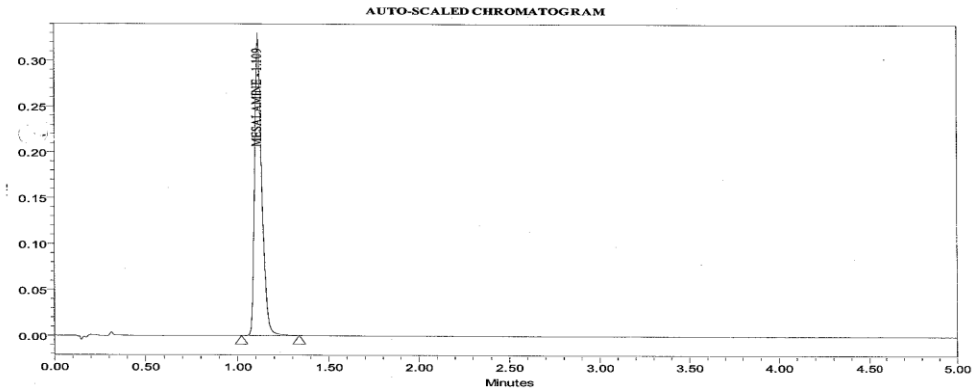

Fig. 6: Specimen Chromatogram of Sample Injection

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