



Original article

Validation of stability indicating RP-HPLC method for the estimation of mesalamine in bulk and tablet dosage form

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ABSTRACT

Introduction: Stability of pharmaceutical product may be defined as, the capacity of a particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications.

Method: The present paper deals with the development of a stability indicating reverse phase HPLC with UV–Visible detector method for the determination of mesalamine using phenomenex RP-C18 (250 × 4.6 mm, packed with Luna 5 μ) column. A mobile phase consisting of methanol: water (50:50) was employed in this study. The flow rate was kept at 0.9 ml/min and the injection volume was 20 μl. The separation was performed at ambient temperature. Eluents were monitored by UV detector set at 230 nm.

Results: The developed method was statistically validated for the linearity (20–50 μg/ml) and the results of precision (≤2%), accuracy, robustness, specificity, LOD (0.19 μg/ml) and LOQ (1.8 μg/ml) are well within the limits. Over all % Recovery was found to be 99.72%.

Conclusion: The specificity of the method was ascertained by force degradation studies by acid hydrolysis, alkali hydrolysis and degradation by oxidation. The degraded products were well resolved from the analyte peak with significant difference in their RT values.

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1. 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} UPLC¹² and LC-MS¹³ either in single or in combined forms.

Stability of pharmaceutical product may be defined as, the capacity of a particular formulation in a specific container or closure

system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. Stability of a drug can also be defined as, the time from the date of manufacture and packing of the formulation until its chemical and biological activity is not less than a predetermined label of potency and its physical characteristics have not changed appreciably or deleteriously.¹⁴

The ICH guideline requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances.¹⁵ It is stated that testing should include the effect of temperature, humidity (where appropriate), oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values. An ideal stability indicating method is one that quantifies the drug per se and also resolves its degradation products.¹⁶

2. HPLC instrumentation and conditions

2.1. Instrumentation and analytical conditions

The analysis of the drug was carried out on an HPLC system equipped with a reverse phase phenomenex RP-C18 (250 × 4.6 mm, packed with Luna 5 μ) column, a 20 μl injection loop and a UV detector set at 230 nm and running on A-4000 software. Isocratic

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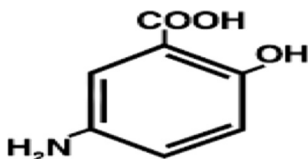


Fig. 1. Chemical structure of mesalamine.

elution with flow rate of 0.9 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 SL-159 UV–Visible spectrophotometer.

2.2. Chemicals and reagents

Mesalamine (API) was provided by Taj Pharmaceuticals, Mumbai, India. Methanol and water of HPLC grade were purchased from Rankem (Ranbaxy Fine Chemicals Ltd.), Delhi. Hydrogen peroxide I.P. (3%) solution was purchased from Desai Chemicals, Visakhapatnam and commercial formulation of mesalamine [Asacol] was purchased from local medicine store (Apollo Pharmacy, Visakhapatnam).

2.3. Stock and working standard solutions

Accurately weighed 10 mg of mesalamine working standard was taken into a 10 ml volumetric flask, added with 7 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 0.1 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45 μm filter. The calibration curve was plotted with the seven concentrations ranging from 20 to 50 $\mu\text{g}/\text{ml}$ working standard solutions. Chromatogram was recorded thrice for each dilution. Calibration solutions were prepared daily and analyzed immediately after preparation.

2.4. Assay of mesalamine tablets

Twenty mesalamine tablets were weighed and the average weight was calculated. Accurately sample equivalent to 10 mg of mesalamine was weighed and transferred into a 100 ml volumetric flask. Fifty milliliters of diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same diluent. Mixed well and filtered through 0.45 μm filter. Further 0.1 ml was pipette out of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45 μm filter. An aliquot of this solution was injected into HPLC system. Peak area of mesalamine was measured for the determination.

2.5. Forced degradation studies

The drug was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. The various degradation pathways studied are acid hydrolysis, basic hydrolysis and oxidative degradation.

2.6. Acid hydrolysis

An accurately weighed 10 mg of pure drug was transferred to a clean and dry round bottom flask. 30 ml of 0.1 N HCl was added to it

and it was refluxed in a water bath at 60 °C for 4 h. After reflux drug was solubilized using methanol and allowed to cool to room temperature. The sample was then neutralized using 2 N NaOH solution and final volume of the sample was made up to 100 ml with water to prepare 100 ppm solution. Finally it was injected into the HPLC system against a blank run.

2.7. Basic hydrolysis

An accurately weighed 10 mg of pure drug was transferred to a clean and dry round bottom flask. 30 ml of 0.1 N NaOH was added to it and it was refluxed in a water bath at 60 °C for 4 h and allowed to cool to room temperature. The sample was then neutralized using 2 N HCl solution and final volume of the sample was made up to 100 ml with water to prepare 100 ppm solution. Finally it was injected into the HPLC system as above.

2.8. Oxidation with (3%) H₂O₂

Accurately weighed 10 mg of pure drug was taken in a clean and dry 100 ml volumetric flask. 30 ml of 3% H₂O₂ and a little solvent was added to it to make it soluble and then kept as such in dark for 24 h. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the HPLC system as above.

3. Method validation¹⁷

3.1. Linearity and calibration curve

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighings of synthetic mixtures of the test product components, using the proposed procedure.

3.2. Accuracy

Accuracy was best determined by the standard addition method. Previously analyzed samples of mesalamine API were added with standard drug solutions and are analyzed by the proposed method. Recovery (%) and RSD (%) were calculated for each concentration.

3.3. Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH guidelines. Repeatability of sample injection was determined as intra day variation and intermediate variation was determined by measurement of inter day variation. For these determinations, three concentrations of the solutions of mesalamine API were used.

3.4. Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small but deliberate variations in method parameters”. The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, % organic solvent strength and buffer concentration etc. To determine the robustness of the method experimental

conditions are purposely altered and chromatographic characters are evaluated. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1 ml/min), temperature (± 2 °C), wavelength of detection (± 2 nm) and water content in mobile phase ($\pm 2\%$) were studied to determine the robustness of the method.

3.5. Limit of detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (S_a), which may be related to LOD and the slope of the calibration curve, b , by

$$\text{LOD} = 3S_a/b$$

3.6. Limit of quantitation (LOQ)

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represents the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$\text{LOQ} = 10S_a/b$$

Where, S_a is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs and b is slope of the corresponding calibration curve.

3.7. Specificity

The specificity of the method was determined by exposing the drug sample to acidic (0.1 N HCl), basic (0.1 N NaOH) and oxidizing (3% H_2O_2) stress conditions. The resulting solutions were then analyzed and the analyte peak was evaluated both for peak purity and for resolution from the nearest eluting peak.

3.8. Stability

Stability of mesalamine API was determined after storage of the drug solution for 24 h at room temperature (25 ± 2 °C).

4. Results and discussion

4.1. Optimization of chromatographic conditions

The chromatographic conditions were optimized by different means i.e. using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for sample preparation etc. and finally the prescribed method is

Table 1
Linearity and range.

Conc.($\mu\text{g/ml}$)	Area (mv)
20	1069
25	2045
30	3025
35	4012
40	5123
45	6028
50	7145

accepted. Significance of mobile phase implies the present method is successfully established without use of any buffer. There is no interference of degradants in the retention of mesalamine (Tables 6 and 7).

Table 2
Results of recovery study.

Amount of drug added (μg) to analyte	Recovery from formulation		
	Mean amount (μg) found ($n = 6$)	Mean % recovery	% RSD
32	31.89	99.67	0.297
40	39.87	99.69	0.365
48	47.90	99.81	0.242

Table 3
Results of intra-assay and inter-assay.

Conc. of mesalamine ($\mu\text{g/ml}$)	Observed Conc. of mesalamine ($\mu\text{g/ml}$) by the proposed method			
	Intra-day		Inter-day	
	Mean ($n = 6$)	% RSD	Mean ($n = 6$)	% RSD
20	19.93	0.810	19.26	0.907
30	29.56	0.92	29.27	0.744
50	49.83	0.64	49.01	0.287

Table 4
Result of robustness test.

Change in parameter	% RSD ($n = 6$)
Flow (0.8 ml/min)	0.29
Flow (1.0 ml/min)	0.12
Temperature (27 °C)	0.29
Temperature (23 °C)	0.43
Wavelength of detection (228 nm)	0.59
Wavelength of detection (232 nm)	0.42

Table 5
Assay of mesalamine tablet.

Brand name of tablet	Amount of drug considered (mg)	Mean (\pm SD) amount (mg) found by the proposed method ($n = 6$)	% Mean assay
Asacol (800 mg)	100	99.91 (± 0.8)	99.91

Table 6
Peak results for forced degradation studies.

Stress conditions	Mesalamine API		Degraded product 1		Degraded product 2	
	Rt	Area	Rt	Area	Rt	Area
0.1 N HCl	2.534	7029.21	–	–	–	–
0.1 N NaOH	2.534	8012.23	1.361	84.56	3.12	93.63
3% H_2O_2	2.534	9421.26	2.035	2395.80	–	–

Table 7
Results of force degradation studies of mesalamine API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass balance (%)
Acid hydrolysis (0.1 N HCl)	4 h	100	0.00	100
Basic hydrolysis (0.1 N NaOH)	4 h	96.92	3.89	96.95
Oxidation (3% H_2O_2)	24 h	79.72	20.27	79.65

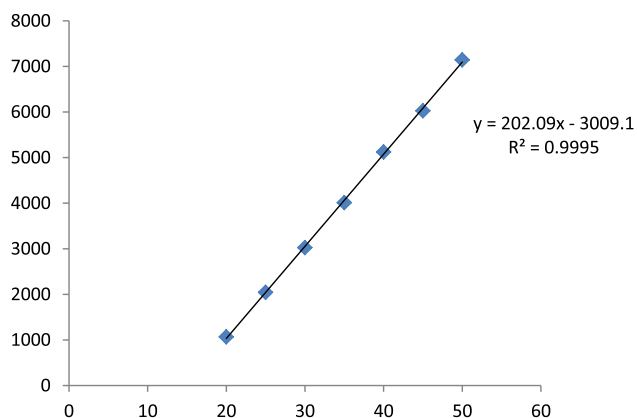


Fig. 2. Calibration curve of mesalamine API.

4.2. Method validation

4.2.1. Accuracy: recovery study

The recovery of the method, determined by adding a previously analyzed test solution with additional drug standard solution, was 99.72%. The values of recovery (%) and RSD (%) listed in Table 2 indicate the method is accurate.

4.2.2. Precision: intra-assay and inter-assay

The intra and inter day variation of the method was carried out and the high values of mean assay and low values of standard deviation and % RSD (% RSD < 2%) within a day and day to day variations for mesalamine revealed that the proposed method is precise (Table 3).

4.2.3. Linearity and range

The calibration curve showed good linearity in the range of 20–50 µg/ml, for mesalamine API with correlation coefficient (r^2) of 0.99 (Fig. 2; Table 1). A typical calibration curve has the regression equation of $y = 202.09x - 3009.1$ for mesalamine.

4.2.4. Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1 ml/min), Temperature (± 2 °C), Wavelength of detection (± 2 nm) and water content in mobile phase ($\pm 2\%$) studied to determine the robustness of the method are also in favor of (Table 4, % RSD < 2%) the developed RP-HPLC method for the analysis of mesalamine API.

4.2.5. LOD and LOQ

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 0.19 µg/ml and 1.8 µg/ml respectively.

4.2.6. Specificity and stability in analytical solution

The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the mesalamine API was stable in solution form up to 72 h at 25 °C.

The results of linearity, precision, inter and intra day assays, robustness, LOD, LOQ, specificity and stability in analytical solution established the validation of the developed RP-HPLC method for analysis of mesalamine.

4.2.7. Forced degradation studies

The results of the forced degradation studies were given in Table 6 and Figs. 4–6. In all the three stress conditions, there is significant change in peak area but not in retention time of mesalamine API. Well separation of the degraded product from the parent peak shows the method is stability indicating. Fig. 3 shows the chromatogram of pure mesalamine API.

4.2.8. Assay of mesalamine in dosage form

Assay was performed by using the regression equation ($y = 202.09x - 3009.1$) obtained from the standard curve of mesalamine API. Results obtained are given in Table 5. The assay of containing mesalamine was found to be 99.91% as per the method (Table 7).

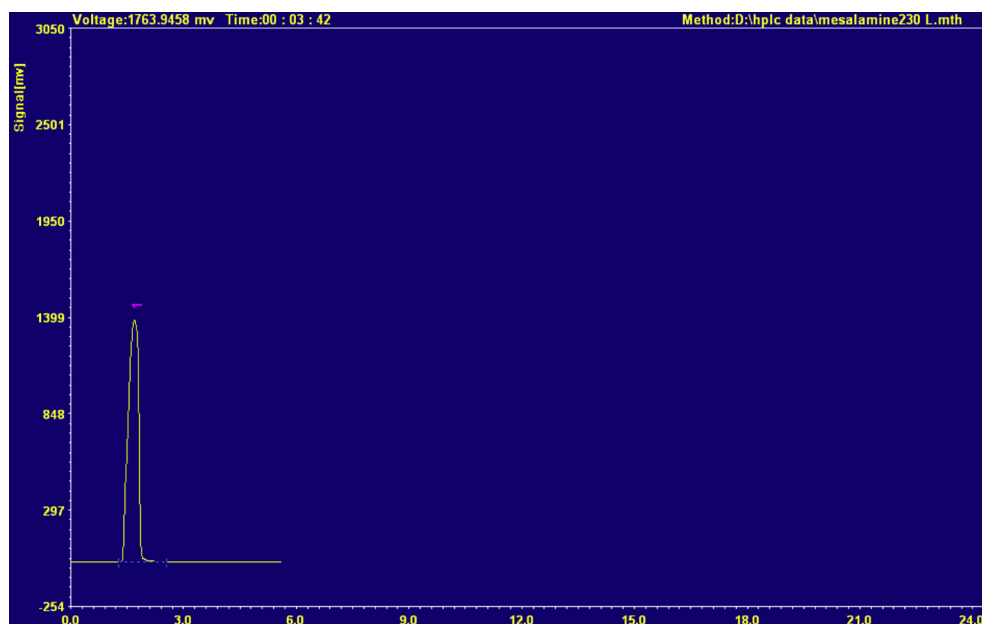


Fig. 3. HPLC spectrum of mesalamine (50 ppm, R_t 2.534 min).

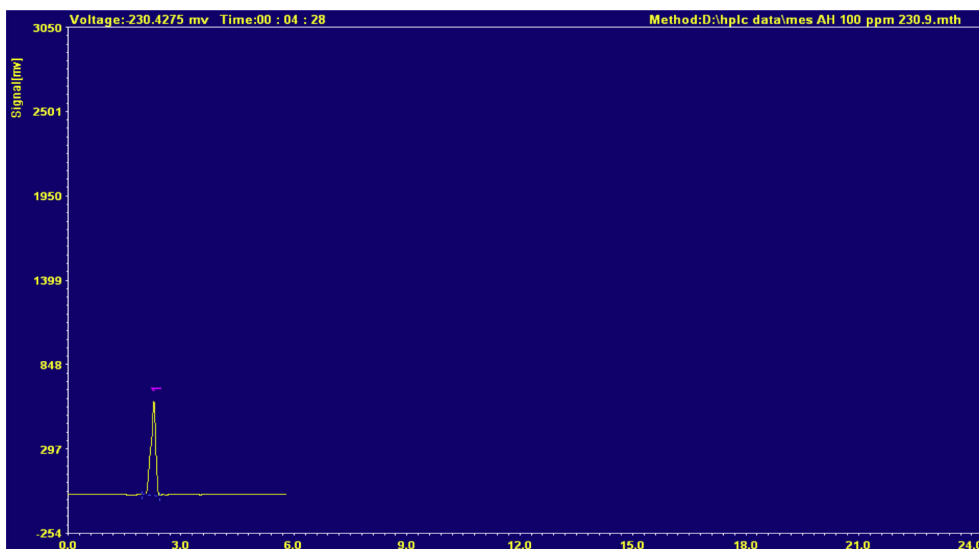


Fig. 4. Chromatogram showing the acid degraded products.

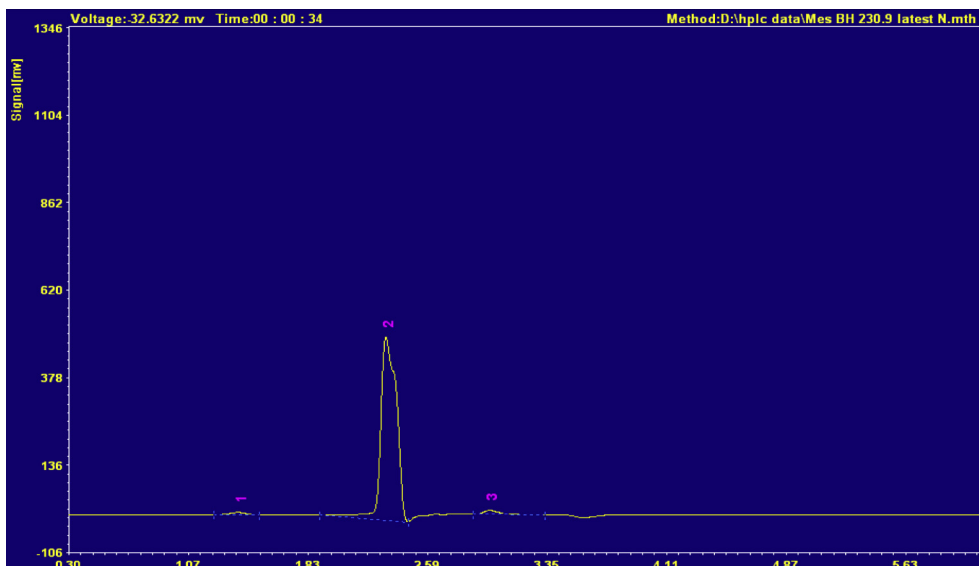


Fig. 5. Chromatogram showing the base degraded products.

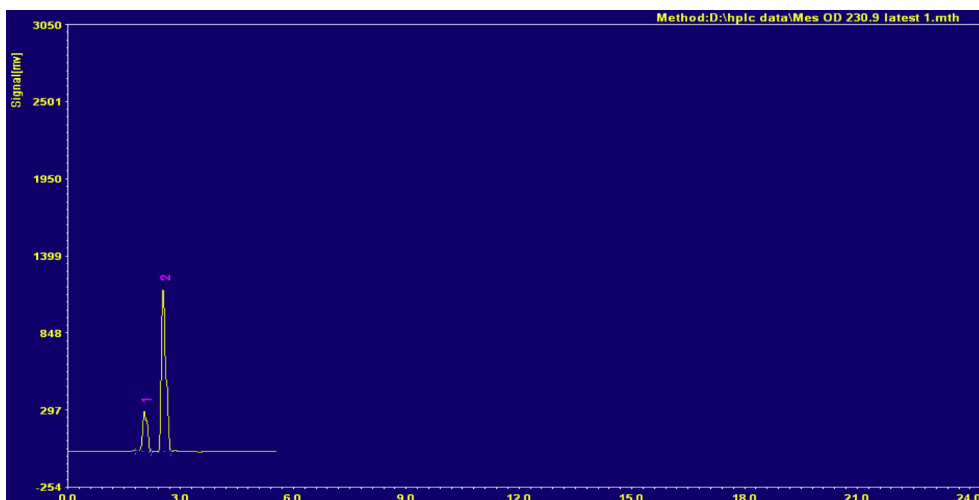


Fig. 6. Chromatogram showing mesalamine, degraded product and H_2O_2 .

5. Conclusion

A sensitive and selective stability indicating RP-HPLC method has been developed and validated for the analysis of mesalamine. Based on peak purity results, obtained from the analysis of force degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of mesalamine indicated that the developed method is specific for the estimation of mesalamine in presence of degradation products. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

Even though no attempt has been made to identify the degraded products, proposed method can be used as a stability indicating method for assay of mesalamine in commercial formulations.

Conflicts of interest

All authors have none to declare.

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