Research Article

Development and Validation of Stability Indicating HPLC Assay Method for Determination of Mesalamine in Bulk Drug and Tablet Formulation

Saroj H. Gatkal*, Priti R. Mhatre, Vitthal V. Chopade, Prvin D. Choudhari
Department of Quality Assurance Techniques, Modern College of Pharmacy Nigdi, Pune-411044, Maharashtra, India.
***Corresponding author's E-mail:** saroj.gatkal@gmail.com

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ABSTRACT

The objective of the current study was to develop simple, precise and accurate stability indicating by Gradient RP-HPLC assay method and validated for determination of Mesalamine in solid pharmaceutical dosage forms. Gradient RP-HPLC separation was achieved on an analytical C18 R column (250 mm × 4.6 mm i.d., 5 µm particle size) using mobile phase of Water: Methanol (80:20 v/v) at a UV detector. The drug was subjected to acid degradation, alkaline degradation, oxidation, photolysis and heat to apply stress condition. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 10-60 µg/ml with a correlation coefficient 0.999. The method was also found to be robust as indicated by the % RSD values which are less than 2%. The stress degradation studies showed that Mesalamine undergoes degradation in acid, base, oxidation, dry heat (2.1%, 1.86%, 1.60%, 1.33% respectively). Degradation products produced as a result of stress studies did not interfere with detection Mesalamine and the assay can thus be considered stability indicating.

Keywords: Mesalamine, Stability indicating assay validation, RP-HPLC, chromatographic analysis.

INTRODUCTION

Mesalamine is chemically (5-amino-2hydroxybenzoic acid) is an anti-inflammatory agent, structurally related to the salicylates, which is active in inflammatory bowel disease and active ulcerative colitis. 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, Mesalamine is available in tablet dosage forms and is an official drug of USP. (figure 1).

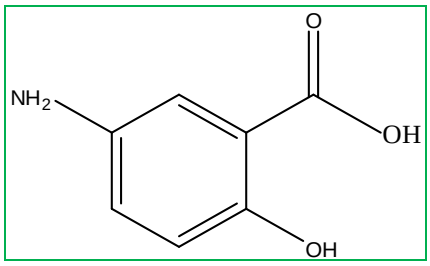

Figure 1: Structure of Mesalamine

The mechanism of action of this drug remains uncertain. 5-ASA has been shown to be a potent scavenger of reactive oxygen species that play a significant role in the pathogenesis of inflammatory bowel disease, inhibition of natural killer cell activity, inhibition of antibody synthesis, inhibition of cyclo-oxygenase and lipoxygenase pathways and impairment of neutrophil function[1-2].

MATERIALS AND METHODS

Mesalamine sample was obtained from Lupin pharmaceuticals. Mesalamine tablet was purchased from local market. The solvent used Water (HPLC grade), Methanol (AR grade), NaOH (AR grade), HCl (AR grade), H_2O_2 (HPLC grade), OPA (HPLC grade). These chemicals were purchased from Merck Chemicals (Mumbai, India).

Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out on a C-18, column on the working standard solution of Mesalamine (40µg/ml). Initially, trials were carried out using Water and Methanol in various proportions along with varying pH, to obtain the desired system suitability parameters. After several trials, Water: Methanol (pH adjusted to 3.8 with Ortho Phosphoric acid) (80: 20 v/v), was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.[3-8]

Preparation of Standard stock solution

10mg of Mesalamine reference standard was weighed accurately and transferred in 100ml volumetric flask. Drug was dissolve in Water: Methanol (80: 20 v/v) and volume was made up to 100ml with same solvent. So as to get the concentration 100µg/ml. 4ml standard stock solution of Mesalamine was then diluted in 10ml Water: Methanol (80: 20 v/v) to get working standard solution 40µg/ml.

Preparation of mobile phase

Mobile phase was prepared by mixing Water:Methanol (pH adjusted to 3.8 with Ortho Phosphoric acid) (80: 20 v/v), filtered through 0.45µ membrane filter paper and then sonicated on ultra sonic water bath for 30min.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done using Water: Methanol (80: 20 v/v) and scanned over the range of 200 - 400nm and the spectra was obtained. It was observed that Mesalamine showed considerable absorbance at 330 nm (Figure 2).

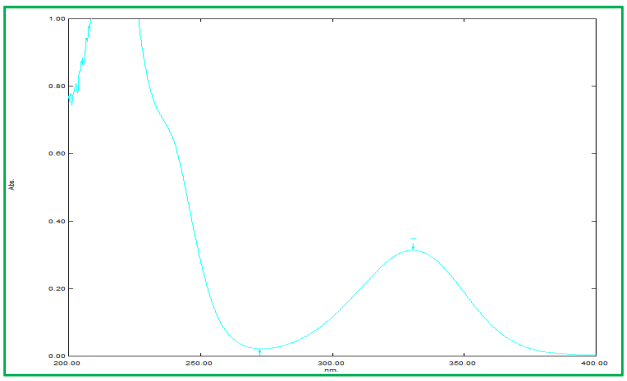

Figure 2: UV spectrum of Mesalamine (40µg/ml) in Water: Methanol (80: 20 v/v)

Chromatogram of Mesalamine

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Mesalamine was injected to get the chromatogram. The retention time for Mesalamine was found to be 5.92 ± 0.02 min. Chromatogram of Mesalamine is shown in (Figure 3)

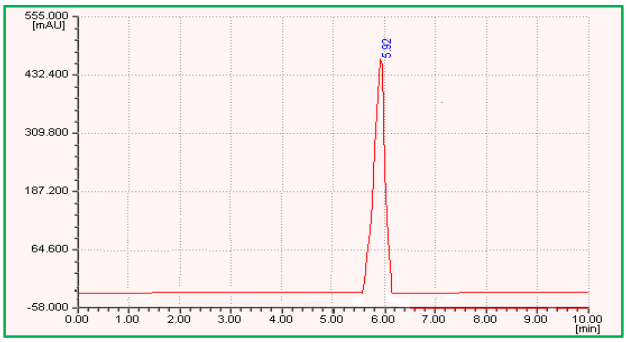

Figure 3: Representative Chromatogram of Mesalamine (40µg/ml, RT = 5.92)

Degradation Studies

The International Conformance on Harmonization (ICH) guidelines entitled stability testing of new drug substance and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies of the Mesalamine using the proposed method[8-12].

Acidic hydrolysis

4ml of working standard solution was mixed with 2ml of 2N HCl and kept for 3 hours. After 3hours solution was neutralized with NaOH then solution was diluted to 10ml with Water: Methanol (80:20) and injected in stabilized chromatographic conditions. Under this condition, degradation was observed (RT = 4.12) (table 1 & figure 4).

Alkaline hydrolysis

4ml of working standard solution was mixed with 2ml of 1.6N NaOH and kept for 3 hours. After 3 hours solution was neutralized with HCl then solution was diluted to 10ml with Water: methanol (80:20) and injected & degradation was observed (RT = 3.87) (table 1 & figure 5).

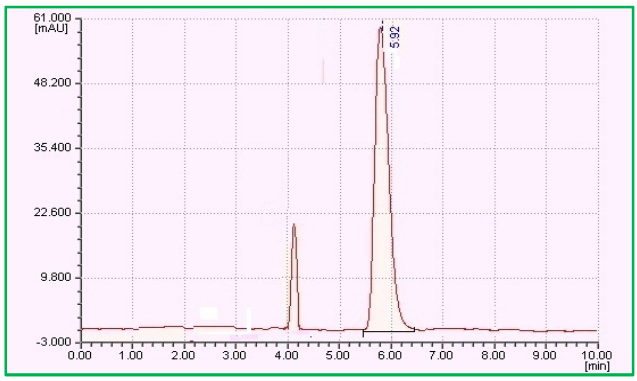

Figure 4: Chromatogram of Mesalamine (40µg/ml) after acidic hydrolysis induced degradation with degradation product at RT 4.12

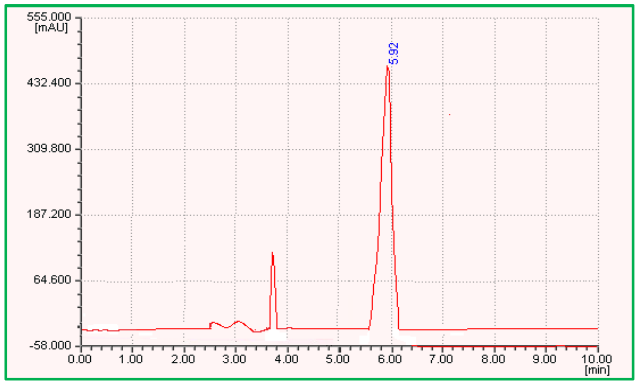

Figure 5: Chromatogram of Mesalamine (40µg/ml) after alkali hydrolysis induced degradation with degradation product at RT 3.87

Oxidation

4ml of working standard solution was mixed with 3ml 6% solution of H_2O_2. The solution was diluted to 10ml with Water: Methanol (80:20 v/v) and refluxed for 3 hours. The solution was injected in stabilized chromatographic conditions. (RT =6.72) (Table 1 & Figure 6).

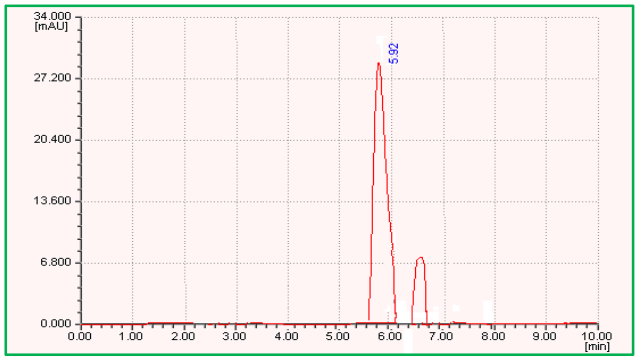

Figure 6: Chromatogram of Mesalamine (40µg/ml) after oxidative degradation with degradation product at RT 6.72

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (50ºC) for a period of 3 hours. 10mg of exposed drug was weighed accurately and transferred to a 100ml of volumetric flask and dissolved in Water: Methanol (80:20v/v), the volume was made up with Water:

Methanol (80:20v/v) to get conc. of 100µg/ml. 4ml standard stock solution of Mesalamine was then diluted in 10ml Water: Methanol (80:20v/v) to get working standard solution 40µg/ml. The solution then injected in stabilized chromatographic conditions. (table 1 & figure7).

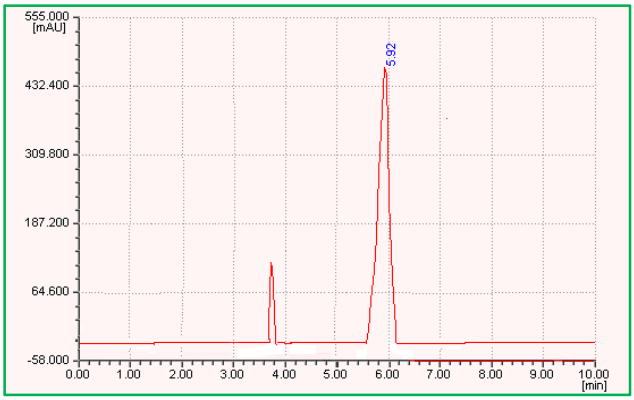

Figure 7: Chromatogram of Mesalamine (40µg/ml) after dry heat degradation with degradation product at RT 3.85

Photo-degradation studies

Long UV-Degradation at 366nm

The photochemical stability of the drug was studied by exposing the drug sample to long UV (366nm) light for 48 hour 10mg after exposure, accurately weighed 10mg of drug in 100ml of methanol to get concentration 100µg/ml. 4ml standard stock solution of Mesalamine was then diluted in 10 ml Water: Methanol (80:20 v/v) to get working standard solution 40µg/ml and was then injected in stabilized chromatographic conditions No degradation peaks were obtained.

Short UV-Degradation at 256nm

The photochemical stability of the drug was studied by exposing the drug sample to short UV (256nm) light for 48 hour 10mg after exposure, accurately weighed 10mg of drug in 100ml of methanol to get concentration 100µg/ml. 4ml standard stock solution of Mesalamine was then diluted in 10ml methanol to get working standard solution 40µg/ml and was then injected in stabilized chromatographic conditions. No degradation peaks were obtained.

Validation of Analytical methods

The validation for HPLC method development was performed using parameters like Linearity, Precision, Accuracy, Limit of detection (LOD), Limit of quantification (LOQ) and Robustness.

Linearity

The standard stock solution containing 100µg/ml of Mesalamine to prepare range of standard solutions containing six different concentrations of analyte. The linearity of the relationship between peak area and concentration was determined by analyzing six standard solutions over the concentration range 10-60µg/ml. The results obtained are shown in (table 2&7). The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure 8).

Table 2: Linearity studies of Mesalamine

Concentration (µg/ml)	Peak Area
10	44770.6
20	56796.7
30	66895.3
40	76584.8
50	86611.4
60	97685.2

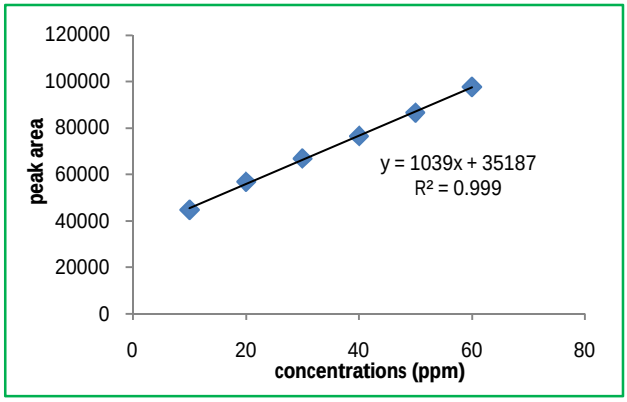

Figure 8: Calibration curve for Mesalamine

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the inter day studies, 3 different concentrations 30, 40 and 50µg/ml were injected in stabilized chromatographic conditions and were analyzed in triplicate. The percentage RSD was calculated. The result obtained for intraday variations are shown in (table 3 & 7). In the inter day variation studies, 30, 40 and 50µg/ml were injected in stabilized chromatographic conditions and were analyzed. This procedure was repeated once a day for three consecutive days. The percentage RSD was calculated. The result obtained for interday variations are shown in (table 4& 7).

Accuracy

To check accuracy of the method, recovery studies were carried out by mixing standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 30 µg/ml of Mesalamine bulk drug solution to which 24, 30 and 36 µg/ml of Mesalamine tablet solution was added. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of Mesalamine were calculated by using linearity equation. The results obtained are shown in (table 5 & 7).

Table 1: Summary of stress degradation studies of Mesalamine bulk drug

Stress deg. Parameter	Peak Area	Percent Degradation	RT of deg. Product
Initial	76584.8	-	-
Acid (2N HCl Kept for 3 hrs)	74984.1	2.1%	4.12
Alkali (1.6N NaOH Kept For 3 hrs)	75566.2	1.86%	3.87
6% H_2O_2 Kept For 3 hrs	75364.3	1.60%	6.72
Dry Heat at (50ºC for 3 hrs)	75890.7	1.33%	3.85
Long UV 366 nm	76581.7	-	-
Short UV 254 nm	76587.3	-	-

Table 3: Intra-day precision studies for Mesalamine

Concentration (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
30	66895.3	66882.6	66876.5	66884.8	9.591142	0.01434
40	76584.8	76578.6	76597.3	76586.9	9.52523	0.01243
50	86611.4	86656.7	86632.5	86633.53	22.66767	0.02616

Table 4: Inter-day precision studies for Mesalamine

Concentration (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
30	66885.3	66892.6	66876.1	66884.67	8.268212	0.01236
40	76574.1	76588.2	76587.3	76583.2	7.893668	0.01030
50	86621.4	86656.7	86642.4	86640.17	17.75566	0.02048

Table 5: Recovery Studies of Mesalamine

Level	Std Sample Conc. (µg/ml)	Area	Mean	Recovered Conc.	% Recovery
80	30 + 24	90871.3	90875.72	53.598	99.25
		90768.1			
		90987.8			
100	30 + 30	97285.5	97238.73	59.722	99.53
		97139.1			
		97291.6			
120	30 + 36	104329.3	104010.1	66.239	100.36
		105224.8			
		102476.2			

Table 6: Robustness Studies of Mesalamine

	Tailing factor	Peak area			Mean	SD	% RSD
		Trial 1	Trial 2	Trial 3			
Flow Rate							
0.8	1.74	76566.9	76559.4	76571.8	76566.03	6.24526	0.00815
0.9	1.59	76584.8	76578.6	76597.3	76586.9	9.52523	0.01243
1.0	1.35	76597.5	76656.4	76653.9	76635.93	33.30771	0.04346
Mobile Phase composition							
(78:22)	1.72	76590.1	76578.6	76563.8	76577.5	13.18446	0.01721
(80:20)	1.64	76584.8	76578.6	76597.3	76586.9	9.52523	0.01243
(82:18)	1.33	76599.4	76592.5	76586.7	76592.87	6.357935	0.00830
pH of Mobile Phase							
3.7	1.43	76576.4	76569.0	76594.2	76579.87	12.95273	0.01691
3.8	1.67	76584.8	76578.6	76597.3	76586.9	9.52523	0.01243
3.9	1.72	76558.3	76577.8	76595.1	76577.07	18.41096	0.02404

Limit of Detection (LOD)

LOD is calculated from the formula:

$$DL = \frac{3.3\,\sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. in the range

S = slope of the calibration curve.

LOD =Mesalamine: **0.0302 µg/ml**

Limit of Quantification (LOQ)

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

LOQ = Mesalamine: **0.0916µg /ml.**

Range

Mesalamine: **10-60µg/ml**

Robustness

Robustness was performed by injecting the Mesalamine standard solution in to the HPLC by altering the flow rate, changing the pH and changing the composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in (table 6 & 7).

Table 7: Summary of validation parameters of Mesalamine

Validation Parameter	Mesalamine
Linearity Equation	Y=1039x - 35187
(r^2)	0.999
Range	10 – 60µg/ml
Precision (% RSD)	
Intraday	0.01764%
Inter day	0.01438%
Accuracy (% recovery)	99.25%, 99.53%, 100.36%
LOD	0.0302µg/ml
LOQ	0.0916µg/ml
Specificity	Specific
Robustness indicated by %RSD	0.021347 %

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (99.25% to 100.36%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 99.25% to 100.36%. The LOD and LOQ were found to be 0.0302µg/ml and 0.0916µg/ml indicating the sensitivity of the method. The method was also found to be robust as indicated by the % RSD values which are less than 2%. The summary of validation parameters of proposed spectrophotometric method is shown in Table 7. The stress degradation studies showed that Mesalamine undergoes degradation in acid, base, oxidation, dry heat (2.1%, 1.86%, 1.60%, and 1.33% respectively). Summary of the results of stress degradation studies of Mesalamine are shown in the Table 1.

CONCLUSION

All the above factors lead to the conclusion that the proposed method as accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of Mesalamine bulk and pharmaceutical formulation and percentage degradation. The proposed method is also useful for determination of Mesalamine stability in sample of pharmaceutical dosage forms.

REFERENCES

1. Tripathi KD: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd. Sixth Edition, 2010, 787-788.
2. Yadav AV and Yadav VB: Improvement of physicochemical properties of mesalamine with hydrophilic carriers by solid dispersion method. Research J. Pharma and Tech, 1, 2008, 324-328
3. Dunge A, Sharda N, Singh B and Singh S, Establishment of inherent stability of Stavudine and development of a validated stability-indicating HPLC assay method, Journal of Pharmaceutical and Biomedical Analysis, 37, 2005, 1115–1119.
4. Kumar R., Rao G. and Naidu P., Stability Indicating RP-LC Method for Determination of RasagilineMesylate in Bulk and Pharmaceutical Dosage Forms, International Journal of Applied Biology and Pharmaceutical Technology, 2, 2002, 247-259.
5. Dunge A, Sharda N, Singh B, Singh S, Validated specific HPLC method for determination of Zidovudine during stability studies, Journal of Pharmaceutical and Biomedical Analysis, 37, 2005, 1109-1114.
6. Trabelsi H, Bouabdallah S, Bouzouita K andSafta F,Determination and degradation study of Haloperidol by high performance liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, 29, 2002, 649-657.
7. Vignaduzzo S., Castellano P. and Kaufman T., Method development and validation for the simultaneous determination of Meloxicam and Pridinolmesylate using RP-HPLC and its application in drug formulations, Journal of Pharmaceutical Biomedical Analysis., 46, 2008, 219-25.
8. Chopade V., Tembhurkar N., JadhavS. and Chaudhari P., Development And Validation of A Stability Indicating Assay Method of Mefloquine HCl By Using Different Stress Degradation Conditions, Journal Of Pharmacy Research, 5, 2012, 2631-2635.

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