IJPSR (2013), Vol. 4, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 September, 2012; received in revised form, 06 November, 2012; accepted, 22 December, 2012

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING ASSAY METHOD OF MESALAMINE BY USING DIFFERENT STRESS DEGRADATION CONDITIONS

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Keywords:

Mesalamine, UV spectrophotometer, Stress degradation studies, Validation

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ABSTRACT

A simple, sensitive, highly accurate UV spectrophotometric method has been developed for the determination of mesalamine in bulk and tablet dosage form. Solution of mesalamine in distilled water shows maximum absorbance at 330 nm. Beer's law was obeyed in the concentration range of 2-16 µg/ml with the slope, intercept, correlation coefficient, detection and quantitation limits were also calculated. The proposed method has been applied successfully for the analysis of the drug in pure and in its tablets dosage forms. Result of percentage recovery and placebo interference shows that the method was not affected by the presence of common excipients. The method was validated by determining its sensitivity, accuracy and precision which proves suitability of the developed method for the routine estimation of mesalamine in bulk and solid dosage form. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. Mesalamine was subjected to stress degradation under different conditions recommended by ICH. The samples generated were used for degradation studies using the developed method.

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 dilute HCl and alkali hydroxides, Mesalamine is available in tablet dosage forms and is an official drug of USP. A chemical structure of mesalamine as shown in **(Figure 1)** [1-7]

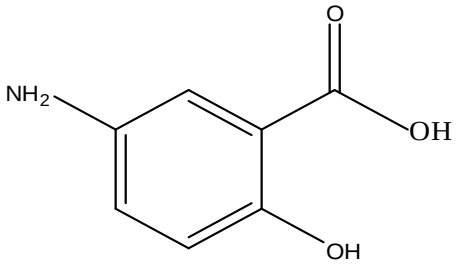

FIGURE 1: CHEMICAL STRUCTURE OF MESALAMINE

The aim of this work is to develop and validate an analytical method by using UV-Vis spectrophotometer for the estimation of pyrimethamine in bulk and pharmaceutical dosage forms and also perform degradation studies on the drug as per ICH guidelines using the proposed method.

MATERIAL AND METHODS: Mesalamine sample was obtained from Lupin Pharmaceuticals. The instrument used for the present study was UV-Vis double beam Shimadzu Corporation, high speed scanning spectrophotometer. The solvent used was distilled water (AR grade), NaOH (AR grade), HCl (AR grade) and H_2O_2 (AR grade). These chemicals were purchased from Merck Chemicals (Mumbai, India).

UV Method Development [8-10]:

1. **Preparation of Stock Solution:** Standard stock solution of mesalamine was prepared by dissolving 10 mg of mesalamine in sufficient quantity of distilled water and make up the volume up to 100 ml, which gives 100 µg/ml solutions.

2. **Preparation of Working Solution:** From the above stock solution 0.8 ml was transferred into 10 ml volumetric flask and volume make up with distilled water to give 8µg/ml. Then sample was scanned with UV-Vis spectrophotometer in the range 200-400 nm and the wavelength corresponding to maximum absorbance was noted at 330 nm **(Figure 2).**

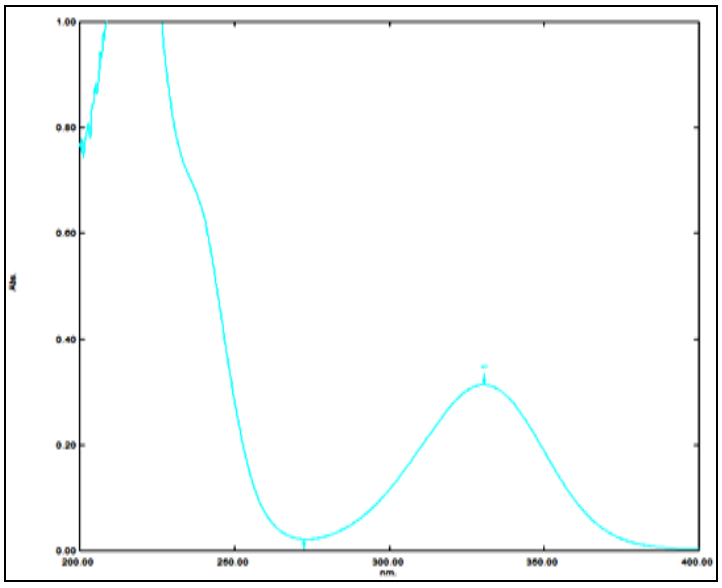

FIGURE 2: λ_{max} OF MESALAMINE SHOWING AT 330 nm

Preparation of Calibration Curve: 0.2-1.6 ml of 100 µg/ml solutions were taken and diluted up to 10 ml using distilled water to produce 2-16 µg/ml solutions respectively. Then graph was plotted taking concentration on x-axis and absorbance on y-axis which shows a straight line **(Figure3).**

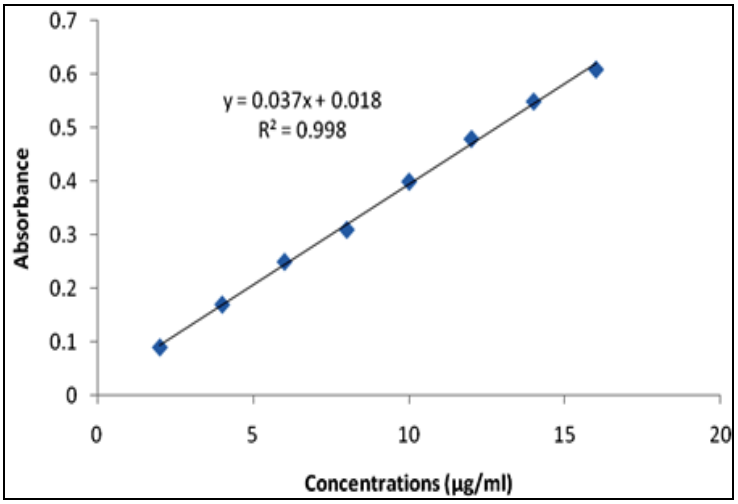

FIGURE 3: CALIBRATION CURVE OF MESALAMINE

Method validation [11-13]:

1. **Linearity:** Various aliquots were prepared from the stock solution (100µg/ml) ranging from 2-16 µg/ml. The samples were scanned in UV-Vis Spectrophotometer against distilled water as blank. It was found that the selected drug shows linearity between the ranges of 2-16 µg/ml **(Figure 3 and Table 1)**

TABLE 1: OPTICAL CHARATERISTICS

Beer's law limit(µg/ml)	2-16µg/ml
Correlation coefficient	0.998
Regression equation(Y*)	0.037x - 0.018
Slope(a)	0.037
Intercept(b)	0.018

2. **Precision:** Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study six different solutions of same concentration 8 µg/ml were analyzed three times in a day and the absorbance was noted. In the interday variation studies, solution of same concentration 8 µg/ml were analyzed three times for the three consecutive days and the absorbance result mean, standard deviation and % RSD was calculated and given in **(Table 2 and 3)**

TABLE 2: INTER-ASSAY PRECISION

Concentration (µg/ml)	%RSD			Average %RSD
	Day 1	Day 2	Day 3	
8	1.176%	1.178%	1.176%	1.178%

TABLE 3: INTRA-ASSAY PRECISION

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Average %RSD
8	0.31	0.31	0.30	
8	0.32	0.31	0.31	
8	0.31	0.31	0.30	
8	0.31	0.32	0.31	1.374%
8	0.30	0.31	0.32	
8	0.31	0.30	0.31	
%RSD	1.178%	1.178%	1.766%	

3. **Accuracy:** Solutions were prepared in triplicate at levels 80%, 100%, and 120% of test concentration using mesalamine working standard as per the method and taken absorbance of each solution in triplicate **(Table 4).**

TABLE 4: ACCURACY READING OF MESALAMINE

No. of Preparation	Concentration (µg/ml)		%Recovery	Statistical results		
	Formulation	Pure drug		Mean	SD	%RSD
S_1: 80%	10	8	99.63%			
S_2: 80%	10	8	99.74%	99.75%	±0.0036	1.178%
S_3: 80%	10	8	99.89%			
S_4:100%	10	10	99.87%			
S_5:100%	10	10	100.15%	99.92%	±0.0034	1.178%
S_6:100%	10	10	99.74%			
S_7:120%	10	12	100.10%			
S_8:120%	10	12	99.89%	99.99%	±0.0054	1.766%
S_9:120%	10	12	100%			

4. **Robustness:** Robustness of the method was determined by carrying out the analysis under different temperature condition. The respective absorbance of 8 µg/ml was noted and the result was indicated as % RSD **(Table 5).**

TABLE 5: ROBUSTNESS

Sr. no.	Concentration (µg/ml)	Absorbance	
		Room temperature	18 °C
1	8	0.31	0.30
2	8	0.31	0.32
3	8	0.30	0.31
4	8	0.31	0.30
5	8	0.32	0.31
6	8	0.31	0.30
7	MEAN	0.31	0.30
8	SD	±0.00365	±0.00447
9	%RSD	1.177%	1.490%

5. **Ruggedness:** Ruggedness of the method was determined by carrying out the analysis by different analyst. The respective absorbance of 8 µg/ml was noted then result indicated as % RSD **(Table 6).**

TABLE 6: RUGGEDNESS

Sr. no.	Concentration (µg/ml)	Absorbance		
		Analyst 1	Analyst 2	Analyst 3
1	8	0.31	0.32	0.31
2	8	0.31	0.31	0.30
3	8	0.30	0.31	0.31
4	8	0.31	0.30	0.31
5	8	0.31	0.31	0.32
6	8	0.31	0.30	0.31
7	MEAN	0.31	0.308	0.31
8	SD	±0.00365	±0.00307	±0.00363
9	%RSD	1.178%	0.998%	1.170%

6. **Limit of Detection (LOD):** The limit of detection (LOD) was determined from solutions of different concentrations ranging from 0.1-0.5 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value **(Table 7).**

7. **Limit of Quantification (LOQ):** The LOQ is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated using formula **(Table 7).**

Stress Degradation Studies [14-17]:

1. **Acidic Degradation:** 1 ml of stock solution of Mesalamine, and 5 ml of 2 N HCl was added in 10 ml of volumetric flask and the volumetric flask was kept at room temperature. After 3 hours, solution neutralized and diluted with distilled water up to 10 ml and the dilution was done to achieve the appropriate concentration (8 µg/ml) **(Figure 4)**

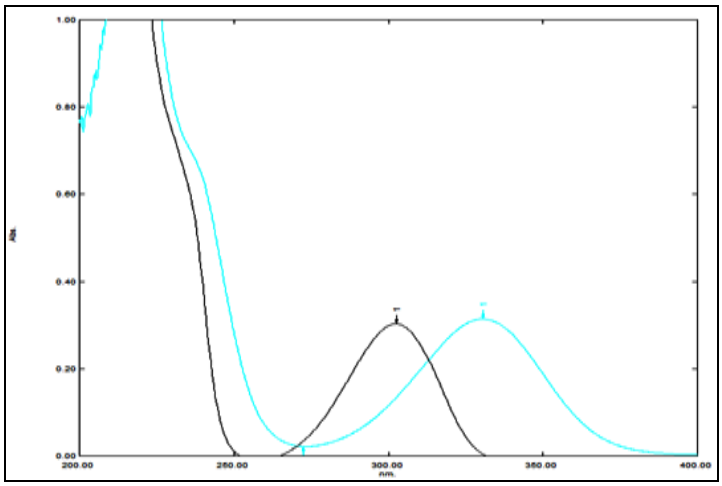

FIGURE 4: COMPARISON BETWEEN STANDARD MESALAMINE (8 µg/ml) AND ACID DEGRADED SAMPLE OF MESALAMINE (8 µg/ml)

2. **Alkali Degradation:** 1 ml of stock solution of Mesalamine and 5 ml of 2.5 N NaOH was added in 10 ml of volumetric flask and the volumetric flask was kept at room temperature. After 3 hours, solution neutralized and diluted with distilled water up to 10 ml and the dilution was done to achieve the appropriate concentration (8 µg/ml) **(Figure 5)**

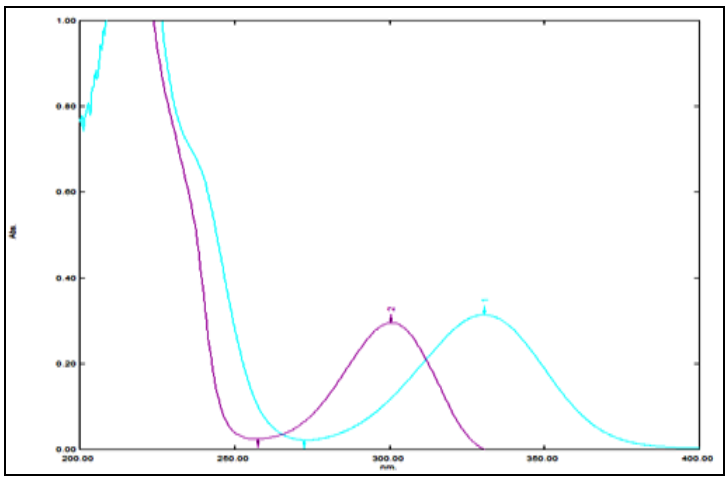

FIGURE 5: COMPARISON BETWEEN STANDARD MESALAMINE (8 µg/ml) AND ALKALI DEGRADED SAMPLE OF MESALAMINE (8 µg/ml)

3. **Dry Heat Induced Degradation:** Mesalamine sample was taken in a petriplate and exposed to a temperature of 50°C for 3 hours in an oven. After 3 hours, 10 mg of the sample was diluted with distilled water up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration (8µg/ml) **(Figure 6)**

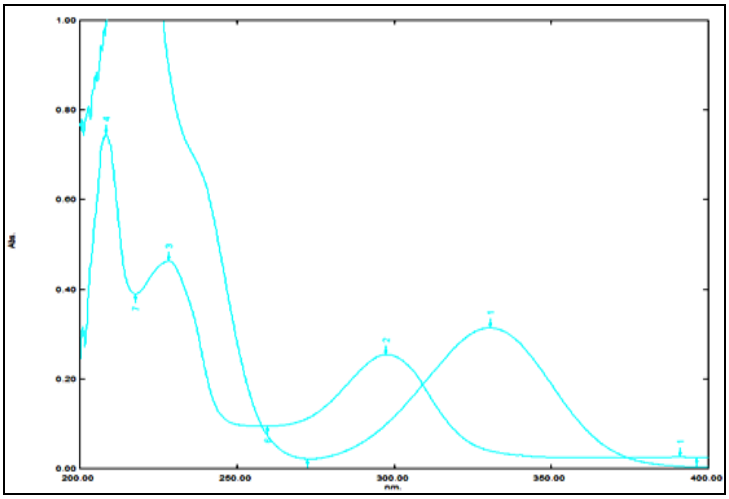

FIGURE 6: COMPARISON BETWEEN STANDARD MESALAMINE (8 µg/ml) AND DRY HEAT DEGRADED SAMPLE OF MESALAMINE (8 µg/ml)

4. **Oxidation:** 1 ml of the stock solution of Mesalamine and 5 ml of 6 % w/v of hydrogen peroxide added in 10 ml of volumetric flask and volumetric flask was kept at normal condition for 3 hour. After 3 hours, solution diluted with distilled water up to 10 ml and the dilution was done to achieve the appropriate concentration (8 µg/ml) **(Figure 7)**

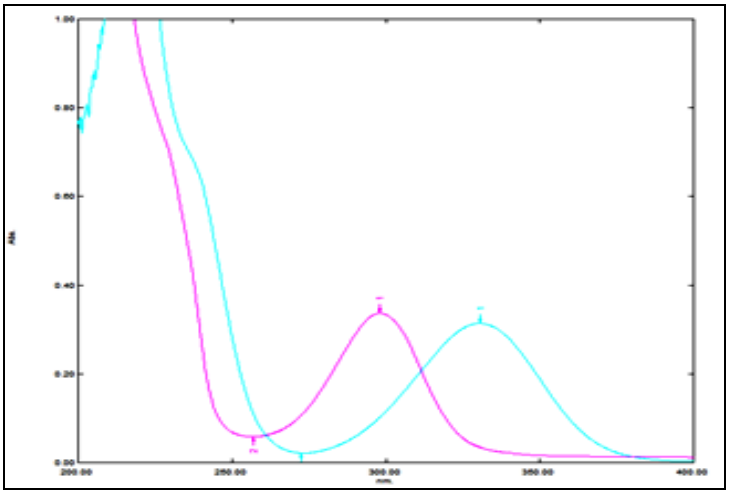

FIGURE 7: COMPARISON BETWEEN STANDARD MESALAMINE AND (8µg/ml) AND OXIDISED SAMPLE OF MESALAMINE (8 µg/ml)

5. **Photo-Degradation:** Mesalamine sample was taken in a petriplate and exposed to a shorter & longer (254 & 366nm respectively) in UV chamber for 3 hrs. 10 mg of the sample was diluted with distilled water up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration (8 µg/ml)

6. **Sunlight Degradation:** Mesalamine sample was taken in a petriplate and exposed to sunlight for 3 hrs. 10 mg of the sample was diluted with distilled water up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration (8µg/ml)

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%, recoveries of the drug, indicating that the method was accurate. The method was also found to be specific. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2 %. The stress degradation studies showed that Mesalamine undergoes degradation in acidic, oxidation, alkaline and dry heat (67.75%, 54.84%, 64.52%, and 45.17% respectively).

TABLE 7: SUMMARY OF VALIDATION PARAMETERS

Parameter	Result
Linearity indicated by correlation coefficient	0.998
Precision indicated by % RSD	1.275%
Accuracy indicated by % recovery	1.178%
Limit of Detection	0.83 µg/ml
Limit of Quantification	2.54 µg/ml
Range	2-16 µg/ml
Linear regression equation	0.037x + 0.018
Robustness indicated by % RSD	1.177%

TABLE 8: RESULT OF STRESS DEGRADATION STUDIES

Stress condition	Time	Observation	% Degradation
Acidic Degradation	RT for 3hours	λmax shifted	67.75%
Alkali Degradation	RT for 3hours	λmax shifted	64.52%
6% Hydrogen Peroxide	RT for 3hours	λmax shifted	54.84%
Dry Heat 50°C	RT for 3hours	λmax shifted	45.17%
Sunlight	For 3 hours	λmax not shifted	0
Photodegradation	For 3hours	λmax not shifted	0

CONCLUSION: The proposed method found to be simple, economical, and selective. The method does not require heat treatment, expensive reagents therefore 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 in bulk and pharmaceutical formulation and percentage degradation.

The proposed method is also useful for determination of Mesalamine stability in sample of pharmaceutical dosage form.

ACKNOWLEDGEMENTS: Authors are grateful to the Lupin Pharmaceuticals for providing the gift samples of pure drug.

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How to cite this article:
Gatkal SH, Mhatre PR, Chopade VV and Chaudhari PD: Development and Validation of a Stability indicating assay method of Mesalamine by using different Stress Degradation conditions. *Int J Pharm Sci Res*. 2013; 4(1); 401-406.