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Stability Indicating HPTLC Method for Determination of Mesalamine

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Abstract
Mesalamine is an anti-inflammatory agent used for treating ulcerative colitis and mild to moderate Crohn's disease. It is used clinically for the treatment of conditions such as diarrhea, rectal bleeding and stomach pain. It belongs to class of drugs known as amino salicylates. It is available in the market as granules, tablets, capsule, rectal suspensions, enema and suppositories in different strengths. A simple, rapid validated stability indicating HPTLC method for Mesalamine has been successfully developed. This method is based on HPTLC separation followed by UV detection at227nm. The separation was carried on Merck TLC aluminium sheets pre-coated with Silica Gel $60F_{254}$ using Ethyl acetate: Methanol: Ammonia as a Mobile Phase. Mesalamine gave well defined and sharp peak at R_f0.40± 0.02. Calibration curve was linear in range 250-1250ng/band. Stress degradation was carried out as per ICH Q1A(R2). The study included hydrolysis at different pH, oxidation, thermal and photolytic stress conditions. This method can be applied to determination of stability of Mesalamine. The suitability of this HPTLC method for quantitative determination of Mesalamine was proved by validation in accordance with requirements of ICH guidelines Q2A(R1).

Keywords
Mesalamine, Stability-Indicating HPTLC, Validation.

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INTRODUCTION:
Chemically, Mesalamine is 5-aminosalicyclic acid which is an anti-inflammatory agent, more specifically classified as amino salicylates in NSAID drugs [1]. It is used clinically for the treatment of ulcerative colitis, diarrhea, rectal bleeding, and stomach pain [2-3]. It is available in the market as granules, tablets, capsule, rectal suspensions, Enema, suppositories in different strengths. It is official in IP/USP/ BP. [4-6]. There are number of UV method [7], HPLC methods [8-12], UPLC methods [13-14], and HPTLC method [15-16], reported for estimation of Mesalamine. There are four stability-indicating methods reported in literature so far; two by HPLC & two by HPTLC technique. The results of stress degradation studies reported in these, vary a lot. Hence, the main objective of my research work was to confirm the stress degradation study results. Stress degradation studies were carried out as per ICH Q1A(R2) guidelines [17] and ICH Q1B guidelines [18] method was validated as per ICH Q2(R1) guidelines [19].

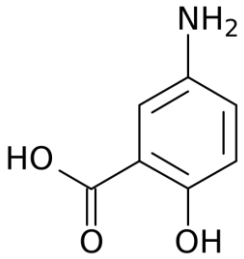

Fig. 1: Structure of Mesalamine

MATERIALS AND METHODS:

INSTRUMENTATIONS:

HPTLC system: Camag TLC system (Muttenz, Switzerland)
Sample applicator: Linomat 5 (Camag, Switzerland)
Scanner: TLC scanner 3 (Camag, Switzerland)
Data processor: winCATS (version 1.4.3.6336)
Development chamber: Camag twin trough chamber (20 x 10 cm), (10 x 10cm)
Syringe for application: Camag Linomat Syringe 695.0014 (100 µL) (Hamilton, Switzerland)
Shimadzu balance (Model AY-120)
Newtronic Photostability Chamber (Model NEC103RSPI):
1) UV Light up to 200 watt hours/square-meter
2) Fluorescent Light (1.2 million LuxHours.)

Chromatographic conditions:

- **Stationary phase:** Merck HPTLC aluminium plates (10×10 cm, 0.2mm thick), Pre-coated with silica gel 60F_{254}.
- **Mobile Phase:** Ethyl acetate: Methanol: Ammonia (7:3:0.1 v/v/v).
- **Densitometric scanning:** At 227nm.
- **Saturation time:** 15 minutes.

Chemicals and reagents

Ethyl acetate99.5% AR/ACS, Methanol AR, Ammonia AR was purchased from LOBA Chemie Pvt. Ltd. Mumbai, India.

EXPERIMENTAL WORK:

Preparation of Standard stock solution:

Standard stock solution of Mesalamine was prepared by dissolving 25mg of drug in 25ml of diluent which contains 1N HCL (8.5% of desired volume), Methanol (2% of desired volume) and then diluted with water upto 25ml to get concentration of 1000µg/ml. From the standard stock solution, working standard solution was prepared by further diluting it with methanol containing 50µg/ml of Mesalamine.

Selection of analytical wavelength

The standard solution of Mesalamine of concentration 10µg/ml was prepared using methanol and scanned over the wavelength range 200nm to 400nm by using UV-Visible spectrophotometer. The detection wavelength 227 nm was selected for further studies.

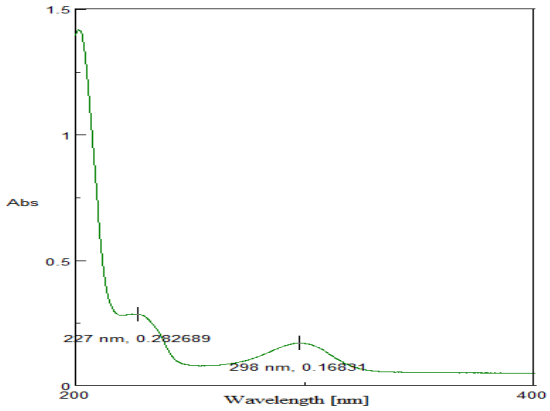

Fig. 2: UV Spectrum of Mesalamine (10µg/ml)

Chromatographic conditions:

Initially, mobile phase optimization trials were conducted using various solvents in different proportions. Optimized mobile phase was Ethyl acetate: Methanol: Ammonia (7:3:0.1 v/v). TLC plates pre-coated with silica gel 60 F_{254}, of dimension 10 cm × 10 cm with 250µm layer thickness were used as stationary phase. TLC plates were pre-washed with methanol and dried. The standard solution of Mesalamine was spotted on the dried, pre-coated TLC plate as a band with 6mm width. The chromatographic development was carried out by

using mobile phase with 15 minutes chamber saturation time and run up to distance 95 mm. Densitometric scanning was performed at 227nm. The standard densitogram of Mesalamine (500ng/band) is shown in the figure.

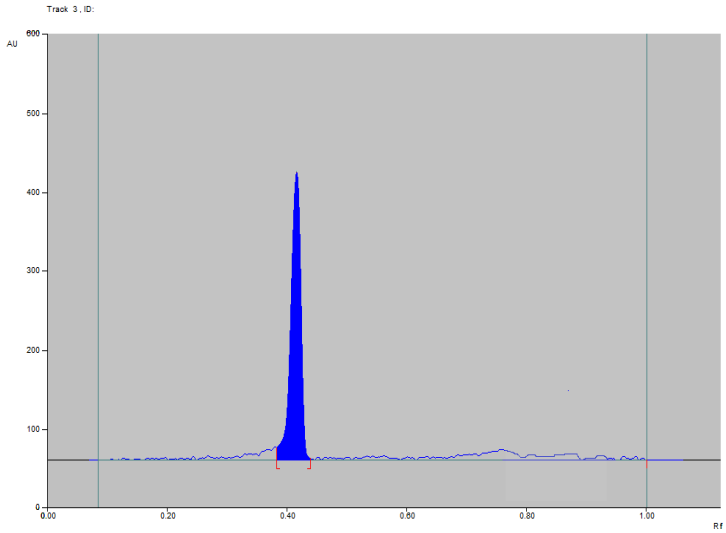

Fig. 3: Densitogram of Mesalamine (500ng/band) R_f0.40 ± 0.02

Stress Degradation Study of Bulk Drug:

Stress degradation studies were carried out to provide evidence on how the quality of drug varies under various stress conditions like of Hydrolysis under acidic/ basic/ neutral condition, Oxidation, Dry heat and Photolysis. Optimization of stress conditions was done by changing strength of reagent and duration of exposure to get 10-30 % degradation. The stress degradation study was carried out as per ICH Q1A (R2) and Q1 B guidelines.

Optimization trials

Initially trials were conducted using various normalities of HCl and NaOH by keeping the sample solution overnight. For the thermal study sample was heated at 70^0C for 2 hours to 5 hours andfor oxidation, trials were conducted using 3% $H_2O_2$by keeping the sample for 1 hour. It was observed that drug gets degraded partially.

Optimized stress conditions

Working Standard Solution Preparation:

From the Standard stock solution of 1000µg/ml, working standard was prepared containing 500µg/ml of Mesalamine by diluting it with methanol.

Alkaline hydrolysis

1ml of working standard solution containing Mesalamine (500µg/ml) was mixed with 1ml of 0.2N NaOH, and volume was made with methanol up to 10ml, kept for 84 hours. Average 77.66 % of Mesalamine was recovered with peak of degradation.

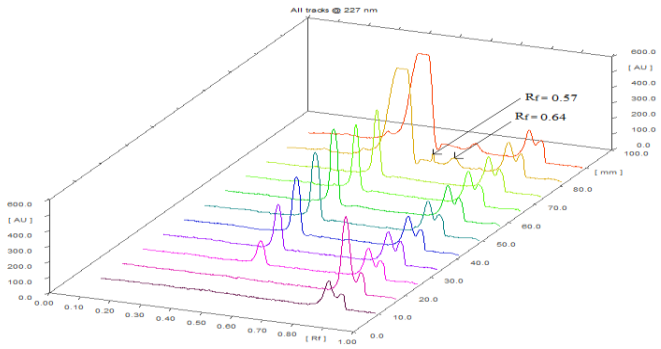

Fig. 4: 3D densitogram of sample after alkaline hydrolysis (0.2N NaOH) kept for 84 hours and densitogram of Mesalamine (Track 1: MeOH Blank, Track 2: 0.2N NaOH Blank, Track 3 to 7: 250-1250ng/band Standard Linearity (250, 500, 750, 1000, 1250 ng/band), Track 8 and 9: Stress sample of 0.2N NaOH (kept for 84 hours) – 1000ng/band Track

10 and 11: Stress sample of 0.2N NaOH (kept for 84 hours) – 10,000ng/band spotting.
Two peaks were observed at R_f0.57 & 0.64indicating products of degradation. To confirm this, alkaline hydrolytic solution at higher concentration was spotted. A well resolved peak for the product of degradation was obtained at R_f0.57 & 0.64. It has different UV Spectrum as compared to Mesalamine, as seen in Fig.5.

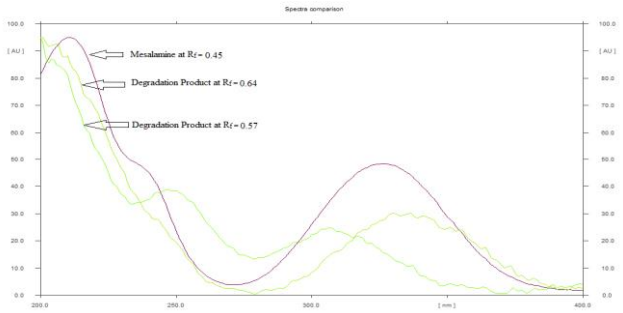

Fig.5: Spectral Scanning done for track of alkaline hydrolysis sample for peaks at R_f 0.45, 0.57, 0.64

Acid hydrolysis
1ml of working standard solution containing Mesalamine (500µg/ml) was mixed with 1ml of 0.5N HCL and volume was made with methanol up to 10ml, refluxed for 2 hours. Average 90.13 % of Mesalamine was recovered with well resolved peak of degradation product.

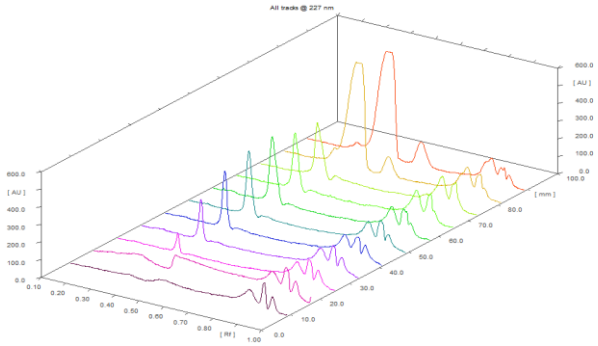

Fig. 6: 3D densitogram of sample after acid hydrolysis (0.5N HCL) refluxed for 2 hours and densitogram of Mesalamine (Track 1: MeOH Blank, Track 2: 0.5N HCL Blank, Track 3 to 7: 250-1250ng/band Standard Linearity (250, 500, 750, 1000, 1250 ng/band), Track 8 and 9: Stress sample of 0.5N HCL (Refluxed for 2 hours) – 1000ng/band Track 10 and 11: Stress sample of 0.5N HCL (Refluxed for 2 hours) – 10,000ng/band spotting.
A well resolved peak for the product of degradation was obtained at R_f0.54, which hasdifferent UV Spectrum as compared to Mesalamine, as seen in Fig.7.

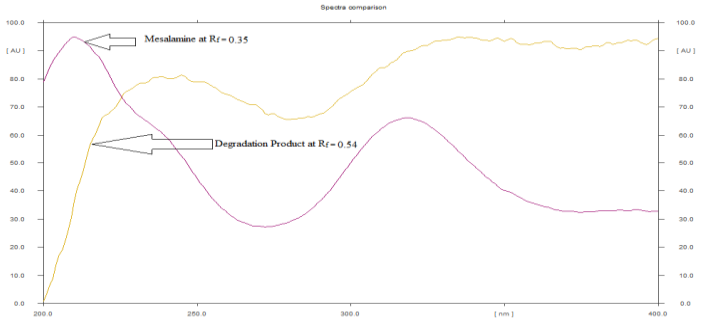

Fig. 7: Spectral Scanning done for acid hydrolysis for peak at R_f0.35, 0.54

Degradation under neutral condition (Neutral hydrolysis)

1ml of working standard solution containing Mesalamine (500µg/ml) was mixed with 1ml of double distilled water, and volume was made with methanol up to 10ml, kept for 48 hours. Average 75.78 % of Mesalamine was recovered with well resolved peak of degradation product.

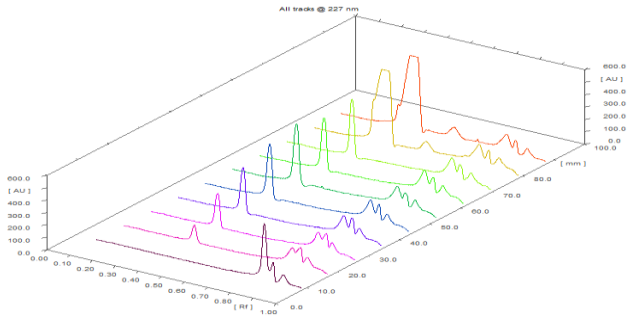

Fig. 8: 3D densitogram of sample after neutral hydrolysis kept for 48 hours and densitogram of Mesalamine (Track 1: MeOH Blank, Track 2 to 6: 250-1250ng/band Standard Linearity (250, 500, 750, 1000, 1250 ng/band), Track 7 and 8: Stress sample of Neutral (kept for 48 hours) – 1000ng/band, Track 9 and 10: Stress sample of Neutral (kept for 48 hours) – 10,000ng/band spotting.

A well resolved peak for the product of degradation was obtained at R_f0.58, which has different UV Spectrum as compared to Mesalamine, as seen in Fig.9.

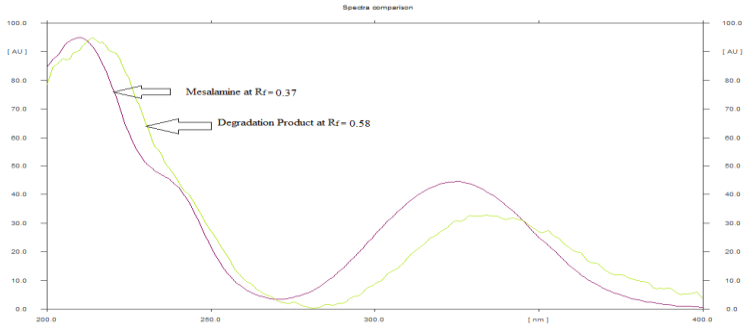

Fig. 9: Spectral Scanning done for neutral hydrolysis for peak at R_f0.37, 0.58

Oxidative degradation

1ml of working standard solution containing Mesalamine (500µg/ml) was mixed with 1ml of 1% H_2O_2v/v, and volume was made with methanol up to 10ml, kept for 30 minutes. Average 78.43 % of Mesalamine was recovered with no peak of degradation.

Photo-degradation studies

Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux Hours. Sample was weighed, dissolved and diluted to get (50µg/ml) as final concentration was applied to TLC plate. After the photo degradation study under UV light 99.94 % and Fluorescence light 91.02 % Mesalamine was recovered with no peak of degradation.

Degradation under dry heat (Thermal hydrolysis)

Dry heat study was performed by keeping the drug in hot air oven at 70°C for 5 hours. Sample was weighed, dissolved and diluted to get (50µg/ml) as final concentration was applied to TLC plate. Average 85.72 % of Mesalamine was recovered with peak of degradation.

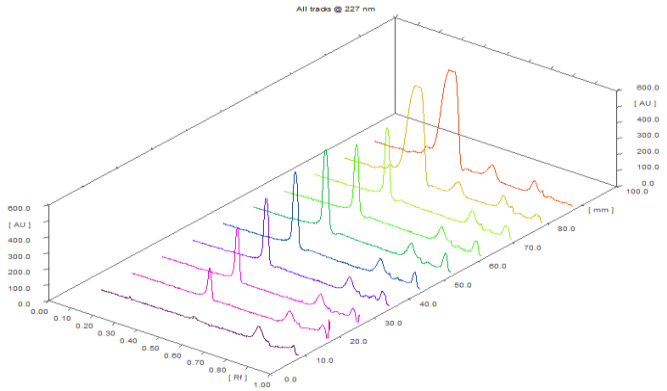

Fig. 10: 3D densitogram of sample after thermal hydrolysis kept at 70° C for 5 hours and densitogram of Mesalamine (Track 1: MeOH Blank, Track 2 to 6: 250-1250ng/band Standard Linearity (250, 500, 750, 1000, 1250 ng/band), Track 7 and 8: Stress thermal hydrolysis (kept at 70° C for 5 hours) – 1000ng/band, Track 9 and 10: Stress thermal hydrolysis (kept at 70° C for 5 hours) – 10,000ng/band spotting.

A well resolved peak for the product of degradation was obtained at R_f0.60, which has different UV Spectrum as compared to Mesalamine, as seen in Fig.11.

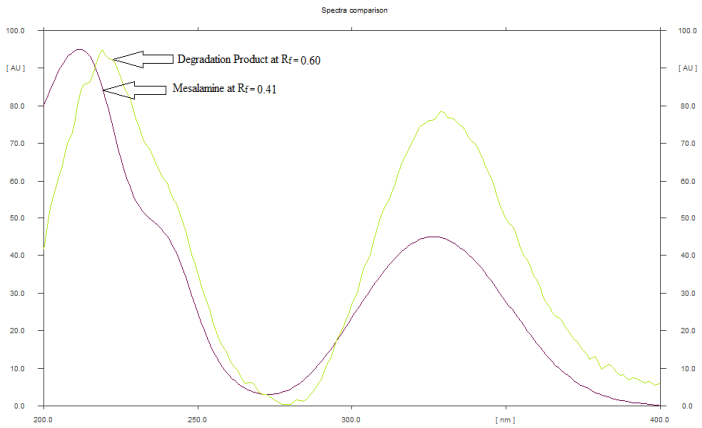

Fig. 11: Spectral Scanning done for thermal hydrolysis for peak at R_f0.41, 0.60

Result of forced degradation studies:

Table 1: Summary of stress degradation of Mesalamine

Stress Degradation Condition	% Recovery	R_f of degradation product	Peak Purity r(s,m)	Peak Purity r(m,e)
Alkaline hydrolysis (0.2N NaOH) kept for 84 hours	77.66	0.57 0.64	0.993598 0.998395	0.987483 0.984421
Acid hydrolysis (0.5N HCL) refluxed for 2 hours	90.13	0.54	0.997444	0.998162
1% H_2O_2 v/v kept for 30 minutes	78.43	Not found	0.994079	0.991484
Photo--degradation studies (UV, 200-watt hours/square meter & Fluorescence 1.2 million Lux. Hours)	For UV. Light: 99.94 For Fluorescence: 91.02	Not found Not found	0.993396 0.996384	0.994548 0.999347
Neutral hydrolysis kept for 48 hours	75.78	0.58	0.998796	0.994860
Thermal hydrolysis (70° C for 5 hours)	85.72	0.60	0.999138	0.994683

METHOD VALIDATION

The developed method was successfully validated according to the ICH Q2 (R1) guidelines

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.9840, indicating the non-interference of any other peak of degradation product or impurity.

Linearity

Linearity was observed for the range of concentrations 250 -1250ng/band of Mesalamine. Calibration curve was obtained as peak area vs. amount spotted. The equation was found to be y = 7.5045x + 2160.1 and correlation coefficient (R^2) was found to be 0.9716.

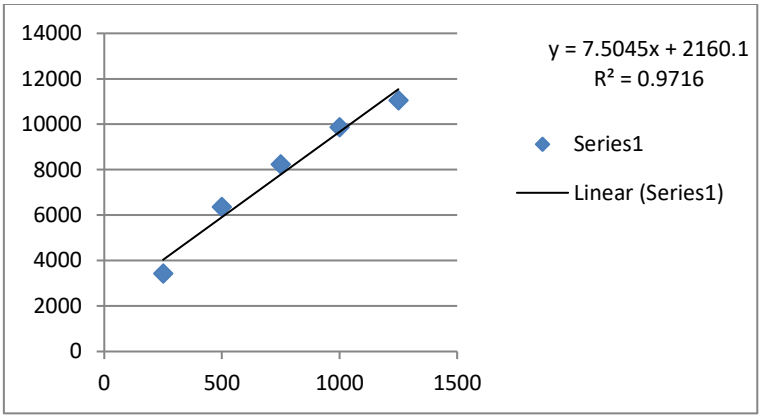

Fig. 12: Calibration curve of mesalamine ranging from 250-1250ng/band

Range - 250-1250ng/band.

Assay

10 tablets were accurately weighed and powdered. From the powder, an amount equivalent to 25mg of Mesalamine was accurately weighed and transferred to 25 ml volumetric flask. Diluent was added and then diluted with water, sonicated for 15 min, and solution was filtered. Further dilutions were made using methanol to get the final concentration50µg/ml from which 500ng/band was applied on the TLC plate. Assay was calculated by extrapolation from standard curve which was found to be96.08%.

Accuracy

To determine the accuracy of the method, recovery studies were carried out by addition of standard drug to pre-analyzed sample solution in triplicate 80%, 100% and 120%. The concentration of sample chosen was in500ng/band. The recovery percentage of the drug was calculated by slope and intercept of the linearity plot of drugs. The results obtained for accuracy is shown in the table.

Table 2: Recovery studies of Mesalamine

Level	Amount (ng/band)	Area (AU) (Average)	Amount Recovered(ng/band)	%Recovery
80	900	10123.4	892.4831	99.16479
100	1000	11029.2	994.7178	99.47178
120	1100	12028.5	1107.506	100.6823

Precision

The precision of the method was demonstrated by intra-day and inter-day studies. For precision evaluation, 6 Replicates of one standard solution of fixed concentrations were analyzed on the same day in order to record any intra-day variations in the results and percentage RSD was calculated. For the inter-day,6 Replicates of one standard solution of fixed concentrations were analyzed on three consecutive days and percentage RSD was calculated.

Table 3: Intra-day & Inter-day precision studies of Mesalamine

Concentration (ng/band)	Conditions	Mean area (Average)	Standard deviation	% RSD
250	Intra-day	2630.083	14.80668	0.56
250	Inter-day	2826.317	25.5308	0.90

*** Average of 6 determinations**

Limit of detection (LOD) and Limit of Quantitation (LOQ):

*** LOD and LOQ based on the Standard deviation of the Response and the Slope:**

LOD and LOQ were calculated as 3.3σ /S and 10σ /S respectively.

Where, σ= the standard deviation of the lowest response of linearity and

S = average slope of the calibration curve.

The value for LOD was found to be33.341ng/band and for LOQ the value was found to be 101.033ng/band.

LOD based on visual evaluation 100ng/band.

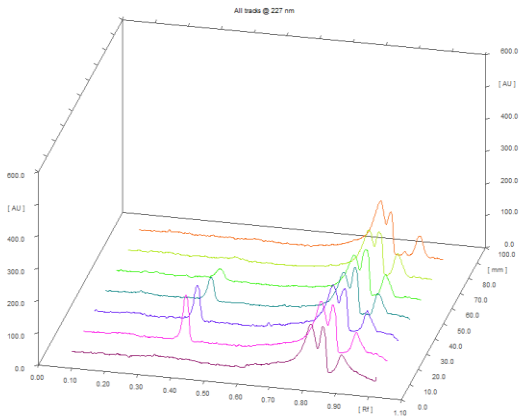

Fig.13: LOD based on visual evaluation (Track 1: MeOH Blank, Track 2: 250ng/band, Track 3: 200ng/band, Track 4: 150ng/band, Track 5: 100ng/band, Track 6: 50ng/band, Track 7: 25ng/band)

The Maximum Noise area = 75.2.

LOD based on S/N ratio 31.085ng/band.

Concentration (ng/band)	R_f	Area
250	0.39	2607.7
200	0.39	2065.1
150	0.40	1522.1
100	0.39	974.0

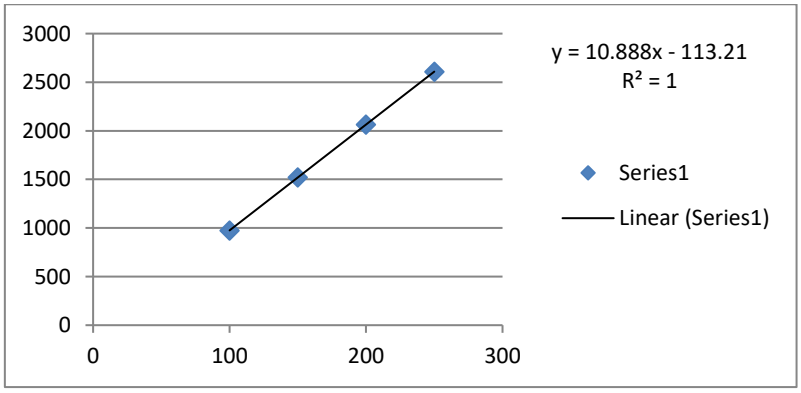

Fig. 14: Calibration curve ranging from 250-100ng/band

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which saturation time (15mins) ± 5min.., changing the Mobile phase composition, changing the Time from spotting to development (immediate), changing detection wavelength and the effects on the peak area was noted. The %RSD values of all robustness parameters were examined and found to be within the limit of 2%, showed that the proposed method was robust.

Table 4: Summary of validation studies

Sr. no.	Validation parameters	Mesalamine
1.	Linearity	y = 7.5045x + 2160.1 R^2= 0.9716
2.	Range	250-1250ng/band
	Precision	(% RSD)
3.	A)Inter-day	0.90
	B)Intra-day	0.56
	Accuracy	(% Recovery)
4.	80%	99.16479
	100%	99.47178
	120%	100.6823
5.	LOD	Based on the Standard deviation of the Response and the Slope:33.341ng/band Based on visual evaluation100ng/band. LOD based on S/N ratio 31.085ng/band.
6.	LOQ	Based on the Standard deviation of the Response and the Slope: 101.033ng/band.
7.	Specificity	Specific
8.	Robustness	Robust

DISCUSSION

The methods available in literature based on Stability indicating HPTLC method for estimation of Mesalamine, report that Mesalamine is relatively unstable to stress degradation conditions of Acid, Base, oxidation, dry heat. In the HPTLC method reported by Saroj H Gatkal, it was reported that Mesalamine stock solution was prepared using only Methanol as a diluent, but the main problem of Mesalamine is its Solubility and is poorly soluble in Methanol. Hence, we have used Diluent for preparation of stock solution which contains 1N HCL (8.5% of desired volume), Methanol (2% of desired volume) and then diluted with water. We have optimized the strength of the reagent and time of reflux. Our study confirms that the degradation is within the limit of 10-30% degradation. Thus, we have developed stability-indicating HPTLC method for the determination of Mesalamine wherein the stress conditions were optimized to achieve 10-30% degradation in Oxidation, Hydrolysis at different pH, Thermal and Photolytic conditions.

CONCLUSION

A simple and rapid method was developed and validated. And the degradation product was observed in hydrolysis at different pH (Acid, Base, Neutral conditions) and Thermal Hydrolysis within the limit of 10-30%. The method has been successfully validated according to ICH Q2R1 Guidelines. This method can be used for routine stability monitoring of Mesalamine.

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