Research Article

Development and Validation of Stability Indicating HPTLC Method for Determination of Mesalamine as Bulk Drug and In Pharmaceutical Formulation

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ABSTRACT
A sensitive, selective, precise and stability indicating high performance thin layer chromatographic method of analysis of Mesalamine both as a bulk drug and in formulations was developed and validated in pharmaceutical dosage form. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of Toluene: Methanol: Ethyl Acetate (6.5:2.5:1 v/v). This system was found to give compact spots for Mesalamine (Rf value of 0.63±0.2). Mesalamine was subjected to acid and alkali hydrolysis, oxidation, photodegradation and dry heat treatment also the degraded products were well separated from the pure drug. Densitometric analysis of Mesalamine was carried out in the absorbance mode at 244 nm. The linear regression data for the calibration plots showed good linear relationship with R² = 0.998 in the concentration range of 200-600 ng/band. The method was validated for precision, accuracy, and recovery. The limits of detection and quantization were 2.62 and 7.94 ng per spot, respectively. The drug undergoes degradation under acidic, alkaline conditions, oxidation and dry heat treatment. Peaks of degraded product were resolved from the standard drug with significantly different Rf values. This indicates that the drug is susceptible to acid hydrolysis, alkaline hydrolysis, oxidation, dry heat degradation. Statistical analysis proves that the method is reproducible and selective for the estimation of the said drug. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

Keywords: Mesalamine, HPTLC, validation, stability-indicating, degradation.

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. (fig. no. 1)

Fig. 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.

MATERIALS AND METHODS
Mesalamine sample was obtained from Lupin Pharmaceuticals. The solvent used Methanol (AR grade), Toluene(AR grade), Ethyl Acetate(AR grade), glacial acetic acid (AR grade), NaOH (AR grade), HCl (AR grade) and H2O2 (AR grade). These chemicals were purchased from Merck Chemicals (Mumbai, India).

Equipment
Camag HPTLC system consisting Linomat 5 applicator, camag TLC scanner 3 and

Vol. 2 (2) Apr-Jun 2013 www.ijpcsonline.com 998
WinCATS software V-1.4.4 was used for chromatographic separation. Spotting of samples was done by using Hamilton microliter syringe.

**Preparation of Standard stock solution**

Standard stock solution of Mesalamine was prepared by dissolving 10 mg of drug in 100 ml of methanol to get concentration 100µg/ml. 2 ml standard stock solution of Mesalamine was then diluted in 10 ml methanol to get working standard solution 20 µg/ml. (100 ng/5µl) From the stock solution 2, 4, 6, 8, and 10µl were applied on TLC plate, at a distance 10mm from both x-axis and y-axis.

**Validation of Analytical Method**

**Linearity**

A stock solution of Mesalamine (100 ng/5µl) was prepared in methanol. Different volumes of stock solution as 2, 3, 4, 5, and 6µl were spotted on TLC plate to obtain concentration of 200, 300, 400, 500 and 600ng/band of Mesalamine, respectively. The data of peak area versus drug concentration were treated by linear least-square regression analysis. The response for the drug was found to be linear in the concentration range 200–600ng/band. The calibration curve is shown in (fig.no.2)

![Fig. 2: Calibration curve for Mesalamine](image)

Regression Equation: \( y = 5.036x + 680.4 \)

Coefficient of correlation: 0.998.

**Precision**

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intraday studies, 3 different concentrations 200, 400, and 600ng/band of standard stock solution were spotted in triplicate and were analyzed. The percentage RSD was calculated.

**Table 1: Intra-day precision studies for mesalamine**

<table>
<thead>
<tr>
<th>CONC. (ng/band)</th>
<th>PEAK AREA</th>
<th>MEAN</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 3</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1657</td>
<td>1652</td>
<td>1698</td>
<td>1669</td>
</tr>
<tr>
<td>400</td>
<td>2712</td>
<td>2764</td>
<td>2743</td>
<td>2739.66</td>
</tr>
<tr>
<td>600</td>
<td>3671</td>
<td>3654</td>
<td>3665</td>
<td>3663.33</td>
</tr>
</tbody>
</table>

**Table 2: Inter-day precision studies for mesalamine**

<table>
<thead>
<tr>
<th>CONC. (ng/band)</th>
<th>PEAK AREA</th>
<th>MEAN</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1654</td>
<td>1658</td>
<td>1650</td>
<td>1654</td>
</tr>
<tr>
<td>400</td>
<td>2719</td>
<td>2726</td>
<td>2735</td>
<td>2726.66</td>
</tr>
<tr>
<td>600</td>
<td>3669</td>
<td>3673</td>
<td>3665</td>
<td>3669</td>
</tr>
</tbody>
</table>

**Accuracy**

To check the accuracy of the method, recovery studies were carried out by over spotting standard drug solution to pre-analyzed sample solution at three different levels 80, 100, and 120 %. Basic concentration of sample chosen was 300 ng/band of Mesalamine bulk drug to which 240, 300, and 360 ng/band of Mesalamine tablet were added by over spotting. The areas were noted after development of plate. The drug concentrations of Mesalamine were calculated by using regression equation. The results obtained are shown in Table 3.

**Table 3: Recovery studies of mesalamine**

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>CONC. (ng/band)</th>
<th>AREA</th>
<th>MEAN</th>
<th>RECOVERED CONC.</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>300+240</td>
<td>3390</td>
<td>3367.333</td>
<td>533.544</td>
<td>98.80</td>
</tr>
<tr>
<td>100%</td>
<td>300+360</td>
<td>3345</td>
<td>3671</td>
<td>3670.333</td>
<td>593.790</td>
</tr>
<tr>
<td>120%</td>
<td>300+360</td>
<td>3952</td>
<td>3968.667</td>
<td>652.952</td>
<td>98.93</td>
</tr>
</tbody>
</table>
**Limit of Detection (LOD)**

LOD is calculated from the formula: -

\[
LOD = \frac{3.3 \sigma}{S}
\]

Where,

\( \sigma \) = the standard deviation of the response for the lowest conc. in the range

\( S \) = the slope of the calibration curve.

**LOD = Mesalamine: 2.62 ng/band**

**Limit of Quantification (LOQ)**

The quantitation limit (QL) may be expressed as:

\[
Q L = \frac{10 \sigma}{S}
\]

**LOQ = Mesalamine: 7.94 ng/band**

**Range**

Mesalamine: 200 – 600 ng/band.

**Specificity**

The densitogram was studied for interference at the Rf of Mesalamine. Lack of interfering peaks in the blank at the Rf of the Mesalamine drug was taken as indication of the specificity of the method. The spectra of standard and sample at corresponding Rf matched exactly, indicating absence of other interference at that Rf.

**Table 4: Summary of validation parameters**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>VALIDATION PARAMETER</th>
<th>MESALAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity Equation</td>
<td>Y=5.036x + 680.4, ( R^2=0.998 )</td>
</tr>
<tr>
<td>2</td>
<td>Accuracy (% mean recovery)</td>
<td>98.80-98.93%</td>
</tr>
<tr>
<td>3</td>
<td>LOD</td>
<td>2.62 ng/band</td>
</tr>
<tr>
<td>4</td>
<td>LOQ</td>
<td>7.94 ng/band</td>
</tr>
<tr>
<td>5</td>
<td>Precision (% RSD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intra day</td>
<td>0.901%</td>
</tr>
<tr>
<td></td>
<td>Inter day</td>
<td>0.215%</td>
</tr>
</tbody>
</table>

**Stress degradation Studies**

Densitogram of Mesalamine

Methanol was used as a solvent for solution preparation. Stationary phase was aluminium HPTLC plate (20×10cm) precoated with silica gel F254. Mobile phase Toluene: Methanol: Ethyl Acetate (6.5:2.5:1v/v) was used. Standard stock solution of Mesalamine 5µl (400ng/band) was applied on TLC plate. The retention factor of Mesalamine was 0.63 ± 0.2. The typical densitogram of working standard solutions is as shown in (fig.no.3)

![Fig. 3: The typical densitogram of working standard (400 ng/band)](image-url)
Acidic hydrolysis
To 8 ml of stock solution, 1 ml of 2.3 N HCl was added. The volume was made up to 10 ml with methanol (0.4 µg/5 µl). This mixture then was kept for 3 hours for 30°C. 5 µl of resultant solution (400 ng/band) was then applied on TLC plate along with respective blank in adjacent track and the densitogram were developed. The results obtained are shown in fig. 4.

Alkaline hydrolysis
To 8 ml of stock solution, 1 ml of 2.2 N NaOH was added. The volume was made up to 10 ml with methanol (0.4 µg/5 µl). This mixture then was kept for 3 hours for 30°C. 5 µl of resultant solution (400 ng/band) was then applied on TLC plate along with respective blank in adjacent track and the densitogram were developed. The results obtained are shown in fig. 5.
Oxidation
8 ml of stock solution, 1 ml of 6 % H₂O₂ was added. The volume was made up to 10 ml with Methanol (0.4µg/5µl). This mixture then was kept for 3 hours at RT. 5µl of resultant solution (400 ng/band) was applied on TLC plate along with respective blank in adjacent track and the densitogram were developed. The results obtained are shown in fig. 6.

Fig. 6: Densitogram of Mesalamine (300ng/band, at Rf = 0.63) after oxidative degradation product of Mesalamine was observed at Rf 0.62 and degraded product was found at Rf 0.43.

Degradation under dry heat
Dry heat studies were performed by keeping drug sample in oven (50°C) for a period of 3 hours. 10mg of exposed drug was weighed accurately and transferred to a 100 ml of volumetric flask and dissolved in methanol, the volume was made up with methanol to get conc. of 100µg/ml. 8 ml standard stock solution of Mesalamine was then diluted in 10 ml methanol to get working standard solution (0.4µg/5µl). 5µl of resultant solution (400ng/band) was then applied on TLC plate along with respective blank in adjacent track and the densitogram were developed. The results obtained are shown in fig. 7.

Fig. 7: Densitogram of Mesalamine (400ng/band, at Rf = 0.63) after dry heat degradation product of Mesalamine was observed at Rf 0.61 and degraded product was found at Rf 0.51.

Photo-degradation studies
Long UV-Degradation at 366 nm
The photochemical stability of the drug was studied by exposing the drug sample to long UV (366nm) light for 3 hour 10 mg after exposure, accurately weighed 10 mg of drug in 100 ml of methanol to get concentration 100µg/ml. 8 ml standard stock solution of Mesalamine was then diluted in 10 ml methanol to get working standard solution (0.4µg/5µl). 5µl of resultant solution (400ng/band) was then applied on TLC plate.

Short UV-Degradation at 254 nm
The photochemical stability of the drug was studied by exposing the drug sample to short UV (254nm) light for 48 hour 10 mg after exposure, accurately weighed 10 mg of drug in 100 ml of methanol to get concentration 100µg/ml. 8 ml standard stock solution of Mesalamine was then diluted in 10 ml methanol to get working standard solution (0.4µg/5µl). 5µl of resultant solution (400ng/band) was then applied on TLC plate.

Table 5: Summary of stress degradation studies of bulk drug

<table>
<thead>
<tr>
<th>S. No</th>
<th>Stress degradation parameter</th>
<th>Peak area</th>
<th>% degradation</th>
<th>Rf of degraded product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Initial</td>
<td>2712</td>
<td>-</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>Acid Degradation</td>
<td>1976</td>
<td>27.14</td>
<td>0.64</td>
</tr>
<tr>
<td>3</td>
<td>Alkali Degradation</td>
<td>2064</td>
<td>23.90</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>Oxidative Degradation</td>
<td>2158</td>
<td>20.43</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>Dry Heat Degradation</td>
<td>2121</td>
<td>21.80</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>Long UV</td>
<td>2717</td>
<td>no degradation</td>
<td>0.63</td>
</tr>
<tr>
<td>7</td>
<td>Short UV</td>
<td>2719</td>
<td>no degradation</td>
<td>0.63</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
HPTLC method was validated as per ICH guidelines. The developed method was found to be linear within the range of 200 – 600ng/band with R²=0.998. The accuracy of method was determined at 80, 100, 120% level. The % recoveries were found to be 98.80%, 98.96%, and 98.93% within the limit of 98% to 102%. The LOD and LOQ were found to be 2.62 ng/band and 7.94 ng/band indicating the sensitivity of the method. The developed method was found to be precise as the % RSD values for intraday and inter-day were found to be less than 2%. The summary of validation parameters of proposed HPTLC method is shown in table 4. The stress degradation studies showed that Mesalamine undergoes degradation in acid, base, oxidation, dry heat, (27.14%, 23.90% 20.43% 21.80%). Summary of the results of stress degradation studies of Mesalamine are shown in the table 5.

CONCLUSION
The proposed methods are precise, specific, accurate, and stability-indicating ones. Mesalamine can be determined in bulk and pharmaceutical formulation and percentage degradation. ICH guidelines were followed throughout the study for method validation and stress testing, and the suggested method can be applied for quality control and routine analysis.

ACKNOWLEDGEMENT
Authors are greatful to the Ayushakti Ayurved Pvt. Ltd., Palghar for providing instrumentation and necessary facilities to carry out the research work.

REFERENCES


