A Novel High-Performance Thin-Layer Chromatography Method for the Quantitative Determination of Clofazimine

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ABSTRACT SUMMARY
A novel and sensitive high-performance thin-layer chromatographic (HPTLC) method with densitometry was developed and validated for the estimation of antileprotic drug, clofazimine (CFZ). Prior literature reports show only use of HPLC and UV spectrophotometric techniques for the estimation of CFZ. This method is sensitive, accurate, reproducible, cost-effective and fast.

INTRODUCTION
Clofazimine is an anti-mycobacterial agent used as an adjuvant therapy in treatment of leprosy. In addition to its primary antimicrobial activity, CFZ also possesses anti-inflammatory/immunosuppressive properties which emphasize the reported therapeutic efficacy of clofazimine in various non-microbial, chronic inflammatory disorders, predominantly cutaneous in origin like discoid lupus erythematosus, pustular psoriasis, Melkerson-Rosenthal syndrome, necrobiosis lipoidica, granuloma annulare and various types of cancers. It is a substituted rimenophenazine bright-red dye originally developed in 1954 for the treatment of tuberculosis. Currently, CFZ is given to patients in oral regimens of 100 to 300 mg, one to three times a day for 3 months to 4 years, depending on the disease and the response of the patients. Following administration of 300 mg per day of CFZ, less than 1% of metabolites are recovered in the urine within a 24 h period. It is excreted slowly, largely in the unchanged form. The objective of the present study was to develop HPTLC method in order to generate better sensitivity and evade the tedious and prolonged sample preparation methods necessarily performed with reported HPLC methods. Therefore, the method finds greater application while performing quantification of CFZ during pharmacokinetic studies and otherwise.

EXPERIMENTAL METHODS
Preparation of CFZ standard stock solution: Stock solution was prepared by weighing CFZ (10 mg). Weighed powder was accurately transferred to a volumetric flask of 10 mL and dissolved in and diluted to the mark with chloroform to obtain a standard stock solution of CBZ (1000 μg/mL). Further dilution was prepared of strength of 10 μg/mL in chloroform as working solution.

Sample application: Samples were applied to the plates as bands 6mm wide, 10mm from the bottom, by means of a pressurized nitrogen gas (150 kg/cm²) through Camag automatic TLC sampler 4 fitted with a 100μL syringe. The rate of application and the distance between the tracks varied depending on the type of analysis. Linear ascending development, with one step mobile phase consisting of toluene/ethyl acetate/methanol/glacial acetic acid (6:3:1:0.1 v/v) was performed in a twin-trough glass chambers (20 cm×10 cm) obtained from Camag, with tightly fitting lids and previously saturated with the mobile phase for 10 min at room temperature (25±0.5°C) and relative humidity (60±5%). After the sample application, the TLC plate was developed for a distance of 8 cm (migration time of 20 min) in mobile phase, immediately dried at 60±0.5°C before densitometry analysis. The bands were visualized in Camag UV cabinet at 286 nm.

Method validation: Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) for sensitivity, accuracy, precision, repeatability and robustness. Linearity of the method was evaluated by constructing calibration curves at five concentration levels. Calibration curves were plotted over a concentration range of 10–100 ng/spot. The calibration curves were developed by plotting peak area versus concentrations (n =3) with the help of the winCATS software (version 1.4.2). The inter-day accuracy and precision of the assay were assessed by the average relative percentage deviation (%DEV) from the nominal concentration and the relative standard deviation (RSD) values, respectively, based on the reported guidelines. Precision (%RSD) and accuracy (%DEV) were calculated by the following equations:

\[
\% \text{RSD} = \frac{\text{standard deviation}}{\text{average calculated concentration}} \times 100
\]

\[
\% \text{DEV} = \frac{1 - \text{average calculated concentration/nominal concentration}}{100}
\]

Limit of detection (LOD) and Limit of Quantification (LOQ) were calculated using the following formulae:

\[
\text{LOD} = \frac{3.3 \times \text{Standard Deviation of the y-intercept}}{\text{Slope of calibration curve}}
\]

\[
\text{LOQ} = \frac{10 \times \text{Standard Deviation of the y-intercept}}{\text{Slope of calibration curve}}
\]

Repeatability studies were performed with quality control samples. These samples at three different concentration levels (80 and 100 ng/band) were applied as 12 tracks on TLC plate on the same
RESULTS AND DISCUSSION

Regression by chromatography of CFZ followed equation: \( Y = 47.24x + 379.8 \)

Table 1: Linear range, correlation coefficient, LOD and LOQ for CFZ

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>10-100 ng/spot</td>
</tr>
<tr>
<td>Correlation coefficient (as per area)</td>
<td>0.999</td>
</tr>
<tr>
<td>Limit of Detection (ng/band)</td>
<td>2.59</td>
</tr>
<tr>
<td>Limit of quantification (ng/band)</td>
<td>7.86</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
</tr>
<tr>
<td>Specificity (Rf)</td>
<td>0.25±0.02</td>
</tr>
</tbody>
</table>

Table 2: Intra-day and inter-day precision of the method

<table>
<thead>
<tr>
<th>Nominal Concentration (ng/band)</th>
<th>Calculated concentration (ng/band)</th>
<th>Precision (%RSD)</th>
<th>Accuracy (%Dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>19.48</td>
<td>3.42</td>
<td>-3.55</td>
</tr>
<tr>
<td>40</td>
<td>40.64</td>
<td>1.01</td>
<td>2.65</td>
</tr>
<tr>
<td>80</td>
<td>76.35</td>
<td>1.09</td>
<td>0.34</td>
</tr>
</tbody>
</table>

In repeatability studies, CFZ applied in two concentration levels (80 and 100 ng/band) on 12 tracks showed identical peaks with respect to position, intensity and also formed parallel lines on the TLC plate (Figure 1). Further, the RSD of Rf values for each concentration on the plate was <0.01%.

CONCLUSION

This study for the first time indicates the competent way for the planar chromatographic estimation of clofazimine samples. A novel HPTLC-densitometry method described over here is simple, rapid, specific, and reproducible for the estimation of clofazimine. Further, this method after appropriate modifications (if required) can further be used for the estimation of clofazimine in biological fluids/tissues in various pathological conditions.

REFERENCES


ACKNOWLEDGMENTS

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