ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Gresiofulvin in Tablet dosage form. Chromatography was carried on Spherisorb CN (250 x 4.6) mm; 5µm) analytical column using mobile phase ACN : 5% THF IN WATER (30:70) at a flow rate of 01.0 ml/min. The detection was carried out at 293 nm. The retention time of Gresiofulvin is found to be 3.760. Correlation co-efficient for Gresiofulvin was found to be 0.999. Assay result of marketed formulation was found to be in 101.04%. The proposed method was validated with respect to linearity, accuracy, precision, selectivity, and robustness. Recovery was found in the range of 98.09 %– 98.67 %. Statistical Analysis proves that the developed methods were successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEY-WORDS: Gresiofulvin, RP-HPLC, Mobile phase, Validation, Analytical method development

INTRODUCTION:

The IUPAC name of the Gresiofulvin drug is (2S,6'R)- 7-chloro- 2',4,6-trimethoxy- 6'-methyl- 3H,4'H-spiro [1-benzofuran- 2,1'-cyclohex[2]ene]- 3,4'-dione, with molecular formula and molecular weight C_{17}H_{17}ClO_6 and 352.766 g/mol respectively. The molecular structure of the drug is given in Fig.1.

The antifungal effects of Gresiofulvin are believed to be due to inhibition of fungal cell mitosis and nuclear acid synthesis. It also binds to and interferes with the function of spindle and cytoplasmic microtubules by binding to alpha and beta tubulin. It binds to keratin in human cells, and then once it reaches the fungal site of action, it binds to fungal microtubules thus altering the fungal process of mitosis.

Gresiofulvin is Official in Indian Pharmacopoeia (2007), US Pharmacopoeia 37 (NF 32). However no stability indication method has been reported till date for the estimation of Gresiofulvin using the RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Gresiofulvin in Pharmaceutical dosage form using UV spectrophotometry. The proposed method are optimized and validated as per ICH guidelines.

Materials and methods

Materials

HPLC Thermo separation Product TSP UV 2000. Gresiofulvin was obtained as a gift sample by Bhumi Pharmaceuticals, Vadodara, and Gujarat, India. The commercial fixed dose Grisorid (250 mg) was procured from local market. All solvents (HPLC grade) were obtained from Merck Chemicals.
Figure 1: Chemical structure of Gresiofulvin

Methods

Working Standard preparation

Take 50 mg equivalent of Gresiofulvin reference standard and dilute up to 50 ml with ACN: Water 5% THF (30:70). Filter through 0.45 µm cellulose acetate filter paper. Pipette out 2.5 ml of this solution in the 25 ml volumetric flask and make the volume with ACN: Water 5% THF (30:70), Sonicate it for 15 minutes, now this is used as working standard solution.

Sample preparation

Take 50 mg equivalent tablet powder of Gresiofulvin reference standard and dilute up to 50 ml with ACN: Water 5% THF (30:70). Pipette out 2.5 ml of this solution in the 25 ml volumetric flask and make the volume with ACN: Water 5% THF (30:70), Sonicate it for 15 minutes, Again pipette out 2 ml of this solution and make the volume up to 10 ml in the volumetric flask. Filter the solution through Whatmann filter paper no. 41. This solution was used as sample solution.

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters:

Pumps: Mode of chromatography: Reversed Phase Chromatography

Mode of Elution: Isocratic

Flow Rate: 1.0 ml/min

Oven: Oven Temperature: 30° ± 2°C

Detector: Type: DAD detector

Wavelength: 293 nm

Column: Spherisorb CN (250 x 4.6) mm ; 5µm

Sample Volume: 20 µl

Run time: 10 min

Mobile Phase: ACN : 5% THF IN WATER (30:70)

Diluent: Methanol

System Suitability Parameters:

Retention time: 3.760

Asymmetry: 1.63

Theoretical plates: 4276

Linearity and Range (n=3):

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 10-50 µg/ml (10, 20, 30, 40, 50 µg/ml) for Gresiofulvin. The plot of peak area against concentration was plotted. Correlation coefficient and regression line equations were calculated. Linearity range was established through consideration of required practical range and according to each drug concentration present in the pharmaceutical product, to give accurate, precise and linear results.

Precision

Repeatability

Repeatability was determined by analyzing standard solution of Gresiofulvin having the concentration 20 µg/ml. Scanned these solutions six times in a day. The results were reported in terms of % RSD (relative standard deviation).

Intradays Precision

The intra-day precision of the proposed method was determined by measuring the corresponding responses 3 times on the same day for 3 different concentration of Gresiofulvin (20, 30 and 40 µg/ml). The results were reported in terms of % RSD.

Interday Precision

The inter-day precision of the proposed method was determined by measuring the corresponding responses on 3 different days over a period of 1 week for 3 different concentration of Gresiofulvin (20, 30 and 40 µg/ml). The results were reported in terms of % RSD.
Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of Griseofulvin by the Standard addition method. Known amount of standard solutions of DIC (10, 20 and 30 μg/ml) were spiked to a pre-quantified sample solution of Griseofulvin (20 μg/ml). Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the respective calibration curves.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of y-intercept of calibration curve (σ) and average of slope (S) of the calibration curve.

\[
\text{LOD} = 3.3 \times \sigma / \text{s},
\]

\[
\text{LOQ} = 10 \times \sigma / \text{s}
\]

Robustness

The robustness was studied by analyzing the sample of griseofulvin by deliberate variation in the method parameters. The change in the response was noted. Robustness of the method was studied by changing different experimental conditions like temperature of column by ± 2°C, Flow rate by ±0.2 ml/min, Mobile phase by ± 2 %.

RESULT AND DISCUSSION

VALIDATION PARAMETER

Linearity and Range

Linear correlation was obtained between peak area and concentration of Griseofulvin in the range of 10-50 μg/ml. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r). The overlay chromatogram is presented in Figure 2. The linearity data are presented in Table 1. Calibration curve is presented in Figure 3. The optical and regression characteristics are listed in Table 2.
Table 4: Intraday Precision data for Gresiofulvin from tablet formulation

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Mean peak Area (μV.s) ± S.D (n = 3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3479356 ± 23717.18</td>
<td>0.682</td>
</tr>
<tr>
<td>30</td>
<td>5020159 ± 24244.72</td>
<td>0.483</td>
</tr>
<tr>
<td>40</td>
<td>6628112 ± 24525.92</td>
<td>0.370</td>
</tr>
</tbody>
</table>

Intraday precision

The data for intraday precision for Gresiofulvin is shown in Table-4. The % RSD for intraday precision was found to be between 0.370 – 0.682%.

Table 5: Intraday Precision data for Gresiofulvin from tablet formulation

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>Mean peak Area (μV.s) ± S.D (n = 3)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3462107 ± 6922.43</td>
<td>0.1999</td>
</tr>
<tr>
<td>30</td>
<td>5064708 ± 41967.50</td>
<td>0.8236</td>
</tr>
<tr>
<td>40</td>
<td>6688854 ± 1463.36</td>
<td>0.0219</td>
</tr>
</tbody>
</table>

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Gresiofulvin was found to be 98.09 - 98.67%. The results are shown in Table-6. Recovery greater than 98 % with low SD justifies the accuracy of the method.

Table 6: Recovery data for Gresiofulvin from tablet formulation

<table>
<thead>
<tr>
<th>% Level of recovery</th>
<th>Amount of drug taken (μg/ml)</th>
<th>Amount of drug added (μg/ml)</th>
<th>Total amount found (μg/ml) ± SD (n=3)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>20</td>
<td>10</td>
<td>29.76 ± 0.54</td>
<td>98.09%</td>
</tr>
<tr>
<td>100%</td>
<td>20</td>
<td>20</td>
<td>39.68 ± 0.19</td>
<td>98.41%</td>
</tr>
<tr>
<td>150%</td>
<td>20</td>
<td>30</td>
<td>49.60 ± 0.29</td>
<td>98.67%</td>
</tr>
</tbody>
</table>

Limit of detection and limit of quantification

The Limit of detection (LOD) and Limit of quantitation were found to be 0.01 and 0.03 μg/ml respectively.

Robustness

The method is found to be robust as the results were not significantly affected by slight variation in composition of mobile phase, pH of mobile phase and flow rate of the mobile phase (Table-7).

Table 7: Robustness data for Gresiofulvin from tablet formulation

<table>
<thead>
<tr>
<th>SR NO.</th>
<th>Area at Flow rate (ml/min)</th>
<th>Area at ACN: THF (28°C)</th>
<th>Area at Temp eratu re</th>
<th>Area at Temp eratu re</th>
<th>Area at Flow rate (ml/min)</th>
<th>Area at ACN: THF (32°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3713</td>
<td>3456</td>
<td>4408</td>
<td>3683</td>
<td>3529</td>
<td>3529</td>
</tr>
<tr>
<td>2</td>
<td>3711</td>
<td>3465</td>
<td>4392</td>
<td>3689</td>
<td>3526</td>
<td>3528</td>
</tr>
</tbody>
</table>

Applicability of the method

The proposed RP-HPLC method was successfully applied for determination of Gresiofulvin in tablet dosage form. The percentage was found to be satisfactory, which is comparable with the corresponding label claim amount (Table-8).
**Table 8:** Application of RP-HPLC method to Gresiofulvin tablet formulation

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Drug</th>
<th>Label Claim (mg)</th>
<th>Amount found (mg)</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grisorid</td>
<td>Gresiofulvin</td>
<td>250</td>
<td>247.43</td>
<td>101.04%</td>
</tr>
</tbody>
</table>

**CONCLUSION**

In estimation of Gresiofulvin in tablet dosage form, separation was achieved on Spherisorb CN (250 x 4.6) mm ; 5µm at 30°C temperature by using a mobile phase ACN : 5% THF IN WATER (30:70) at a flow rate of 1.0 ml/min and UV detection for Gresiofulvin was carried out at 293 nm. Data suggests that peak purity index of the drug was found to be greater than 0.990, so there is no co-elution of any degradation products with main peaks and the results obtained were found within the acceptance criteria. Results of the validation for Gresiofulvin of the above method were linear in the range of 10-50 μg/ml. The % recovery was found to be 98.09 % - 98.67%. The results of the precision study indicate that the proposed method shown good repeatability with a % RSD of 0.567. Similarly %RSD from the intraday precision data was found to be 0.370% - 0.682% and %RSD from the Interday precision data were found to be 0.0219% - 0.8286%. Absolute difference between mean assay values of method precision and intermediate precision was found to be less than 2.0 %. Robustness is performed by making changes in flow rate, Mobile phase composition and temperature. The assay obtained after proposed changes compared with the assay obtained in normal conditions. According to the acceptance criteria difference in the assay should not be more than 2%. The results obtained are well within the acceptance criteria. The % assay results of 101.04% for Gresiofulvin indicates that the proposed method was successfully utilized for the estimation Gresiofulvin in Tablet dosage forms. Hence, the method can be termed as robust. Since the results are well within the limit of acceptance criteria for all validation parameters, therefore the method can be considered as validated and suitable for intended use. So, the proposed stability indicating RP-HPLC assay method can be successfully applied for the estimation of Gresiofulvin in tablet dosage form.

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