INTRODUCTION

Teriflunomide (fig. 1) is an orally administered, second-generation immunosuppressive/immunomodulatory agent which is an active metabolite of Leflunomide. It acts by competitively inhibiting dihydroorotate dehydrogenase, an enzyme which is responsible for pyrimidine de novo biosynthesis [1-4].

No UV spectrophotometric method has been reported so far, for the estimation of TEF in marketed formulation. Therefore, the current work is directed towards the development of a novel UV spectrophotometric method for the estimation of teriflunomide (TEF) present in the marketed formulation.

MATERIALS AND METHODS

Materials and reagents

Teriflunomide pure drug (API) was procured as a gift sample from Glenmark Ltd., Noida. All the other reagents used were of analytical grade and were procured from specialties private limited, Mumbai, India. Marketed Formulation (Denopsy 14 tablets) was purchased from a local pharmacy shop.
standard solutions in the concentration range of 5-10 μg/ml. The line equation \( y = 0.085x - 0.0223 \) and \( r^2 \) value of 0.9996 demonstrated the good linearity of the method. The calibration curve of linearity and overlay spectra of TEF is depicted in fig. 2 and 3, respectively.

![Fig. 2: Calibration curve of TEF](image)

![Fig. 3: Overlay spectra of TEF](image)

**Table 1: Accuracy study of TEF**

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Quantity of formulation (µg/ml)</th>
<th>Quantity of pure drug added (µg/ml)</th>
<th>% recovery</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>5</td>
<td>4</td>
<td>98.7</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>100</td>
<td>99.25</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>10</td>
<td>98.5</td>
<td>100.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>102.2</td>
<td>100.3</td>
</tr>
<tr>
<td>120</td>
<td>5</td>
<td>11</td>
<td>100.74</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>11</td>
<td>98.01</td>
<td>99</td>
</tr>
</tbody>
</table>

**Accuracy**

Accuracy was assessed in terms of percentage recovery, by spiking the band of formulation with 80%, 100%, 120% of pure drug and then finding out the amount of the drug recovered. The mean percentage recovery was found to be 99.4%, as depicted in table 1.

**Precision**

Intraday and interday precision of the method was assessed by measuring the absorbance of highest concentration six times in the same day and the consecutive day respectively. Precision was reported in terms of %RSD. The %RSD values were found to be less than 2% as depicted in table 2, indicating high precision of the developed method.

**Limit of detection and limit of quantitation**

LOD and LOQ were assessed with the aid of standard deviation (σ) and slope (s) from the calibration curve (n=3), by using the equation \( LOD = 3.3 \sigma/s \) and \( LOQ = 10 \sigma/s \). LOD and LOQ of TEF were found to be 0.38µg/ml and 1.1µg/ml, respectively indicating the good sensitivity of the method towards the analyte. The LOD and LOQ is depicted in table 3.
**Specificity**

The specificity of the method was assessed by comparing the UV absorption spectra and absorbance maxima of standard TEF with the formulation as depicted in fig. 4. The spectra of both standard and formulation showed absorbance maxima value indicating the good specificity of the method.

![Fig. 4: Overlay spectra of TEF](image)

**Robustness**

The robustness was assessed by making small but thoughtful changes in the method parameters such as; scanning wavelength and finding out its effect on the absorbance by calculating %RSD. The % RSD was found to be within 2%, which indicates the reliability of the method. The robustness of the method is depicted in table 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>5-10 μg/ml</td>
</tr>
<tr>
<td>Precision</td>
<td>% RSD = 0.508521</td>
</tr>
<tr>
<td>Intra-day (n = 6)</td>
<td>% RSD = 0.558522</td>
</tr>
<tr>
<td>Accuracy (80%, 100%, 120%)</td>
<td>99.4% (Mean)</td>
</tr>
<tr>
<td>LOD</td>
<td>0.30 μg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.1 μg/ml</td>
</tr>
<tr>
<td>Robustness (n=6)</td>
<td>% RSD = 0.6781</td>
</tr>
</tbody>
</table>

**Analysis of formulation**

The % of the drug found in the formulation from the currently developed method was found to be 99.4%. The absorbance spectra obtained from the formulation (fig. 5) exhibited no interference of the excipients. The close agreement of the percentage of drug found with label claim depicted application of this method for the routine analysis of TEF present in its formulation, as depicted in table 3.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Label Claim (mg)</th>
<th>Quantity found (mg)</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denosy 14</td>
<td>Teriflunomide</td>
<td>Each tablet consists of 14 mg of TEF</td>
<td>13.84</td>
<td>99.4</td>
</tr>
</tbody>
</table>

**CONCLUSION**

A novel simple, rapid and precise UV-Visible spectrophotometric method has been developed for the determination of Teriflunomide in marketed formulation. This validated method can be used by quality control laboratories for the routine quantitative analysis of tablets consisting of Teriflunomide as the additives used in the formulation do not interfere with the analysis. Non-requirement of skilled personnel to operate the instruments involved is an added advantage of this method.

**ACKNOWLEDGMENT**

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**AUTHORS CONTRIBUTIONS**

All the author have contributed equally

**CONFLICT OF INTERESTS**

Declare none

**REFERENCES**


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