Original Article

# NOVEL UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF TERIFLUNOMIDE IN TABLET DOSAGE FORM

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## ABSTRACT

**Objective**: The current work is intended towards the development of a novel, simple, and precise UV spectrophotometric method for the estimation of teriflunomide (TEF) present in the marketed formulation.

**Methods**: Acetonitrile was used as asolvent and the absorbance of the drug was measured at the absorbance maxima of TEF, UV 284 nm.

**Results**: Calibration curve plotted in concentration range 5-10 µg /ml exhibited excellent linear relationship with line equation y = 0.0858x-0.0223 and $r^2$ value of 0.9996. The method was found to comply all the validation parameters as per the ICH guideline indicating the sensitivity of the method towards analyte.

**Conclusion:** The method can be used satisfactorily for the routine analysis of TEF present in marketed formulation.

**Keywords:** UV spectrophotometric, Teriflunomide, Marketed formulation, ICH guideline, Sensitivity

## 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 bio-synthesis [1-4].

Literature survey disclosed some chromatographic methods such as HPLC [5-8], LC-MS [9-12] for the quantitative estimation of TEF present in API, marketed formulation and biological fluids.
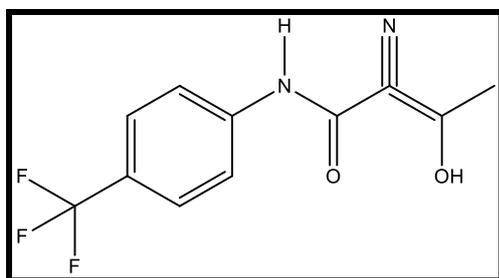


**Fig. 1: Teriflunomide**

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 TEF in marketed formulation and its validation according to ICH guidelines [13, 14].

## 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.

### UV instrumentation

Shimadzu UV-1800, a double-beam UV-Visible spectrophotometer along with 1 cm identical quartz cuvettes was used to measure the absorbance. UV Probe PC software was used to control the instrument and to analyze the data obtained.

### Preparation of standard solution

Stock solution comprising 100 µg/ml of TEF was prepared by using acetonitrile as a diluent. 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml of this solution was pipetted out in five different 10 ml volumetric flask and the volume was made up, to get a working solution consisting of 5,6,7,8,9 and 10 µg/ml.

### Preparation of sample solution (Formulation)

As specified in the label, each tablet contains 14 mg of TEF. Weight of 10 tablets was noted and crushed using mortar and pestle, the quantity of powder corresponding to 10 mg of teriflunomide was taken in a 10 ml volumetric flask and the volume was made up with acetonitrile. Required dilutions were made to get a final concentration of 10 µg/ml.

### Method validation

ICH Q2A, ICH Q2B guidelines were followed for the validation of the method to evaluate the parameters: Linearity, accuracy, precision, the limit of detection (LOD) and limit of quantification (LOQ), specificity and robustness.

## RESULTS AND DISCUSSION

### Optimization of spectrophotometric conditions

Ideal solvent for the method was selected after several experimental trials. Solvents like water, methanol, ethanol, isopropanol and acetonitrile used initially. Because of better solubility and reproducible readings of absorbance maxima, acetonitrile was used for the method development. The absorbance maxima for the method was carefully chosen after obtaining the absorbance spectra of standard and sample (formulation) solution of TEF.

### Linearity

The calibration curve (fig. 2) was plotted between absorbance versus concentration. The linearity of TEF was constructed by scanning six

standard solutions in the concentration range of 5-10 µg/ml. The line equation y = 0.0858x-0.0223 and r² 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.
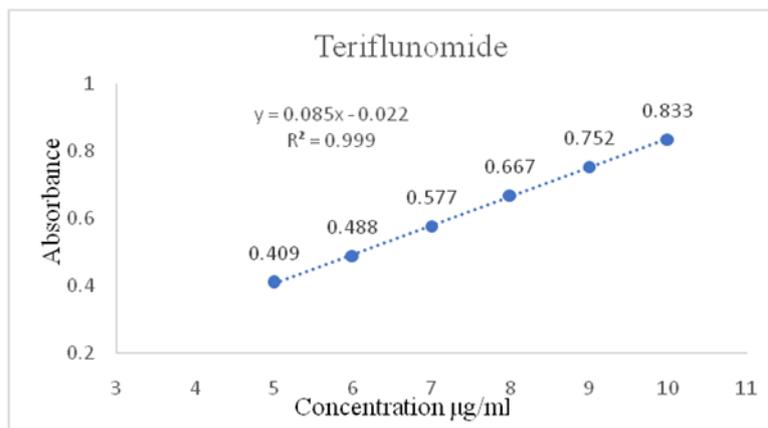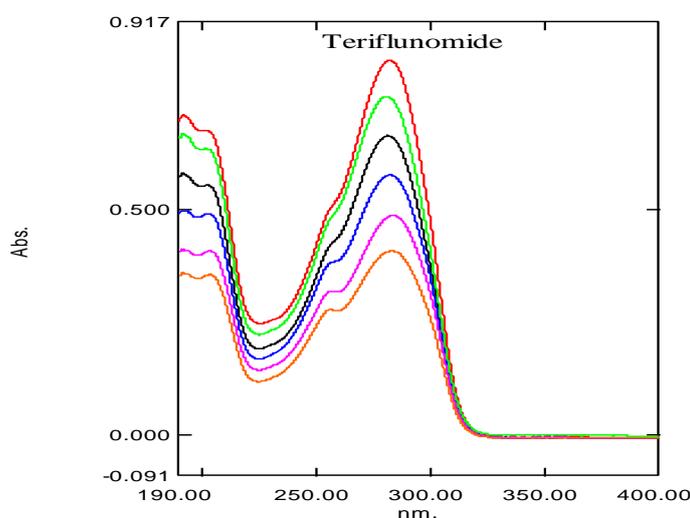


**Fig. 2: Calibration curve of TEF**



**Fig. 3: Overlay spectra of TEF**

**Table 1: Accuracy study of TEF**

| Level of recovery | Quantity of formulation (µg/ml) | Quantity of pure drug added (µg/ml) | % recovery | Mean |
|---|---|---|---|---|
| 80 | 5 | 4 | 98.7 | 99.3 |
| | 5 | 4 | 100 | |
| | 5 | 4 | 99.25 | |
| 100 | 5 | 10 | 98.5 | 100.4 |
| | 5 | 10 | 102.2 | |
| | 5 | 10 | 100.3 | |
| 120 | 5 | 11 | 100.74 | 98.6 |
| | 5 | 11 | 98.01 | |
| | 5 | 11 | 99 | |

**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 σ/s and LOQ = 10 σ/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.
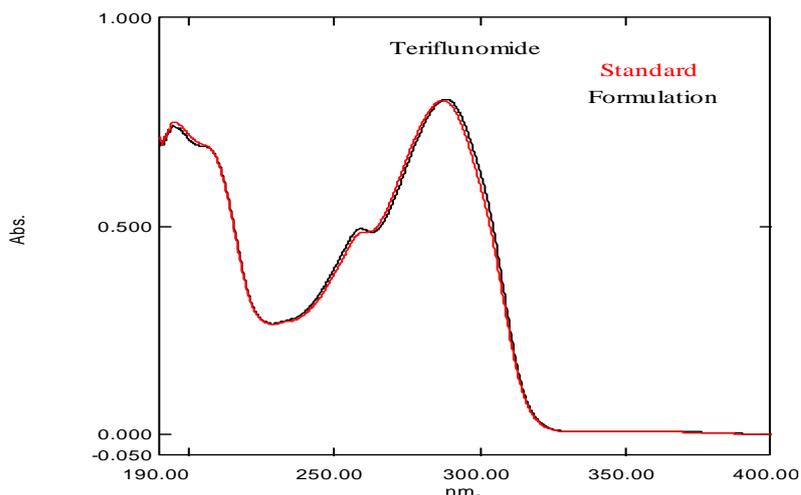


**Fig. 4: Overlay spectra of TEF**

## 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 2: Overall validation report of the method**

| Parameters | | Values |
|---|---|---|
| Linearity | | 5-10 µg/ml |
| Precision | Inter-day (n=6) | % RSD = 0.508521 |
| | Intra-day (n = 6) | % RSD = 0.558522 |
| Accuracy (80%, 100%, 120%) | | 99.4% (Mean) |
| LOD | | 0.38 µg/ml |
| LOQ | | 1.1 µg/ml |
| Robustness (n=6) | | % RSD = 0.6781 |

## 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 3: Analysis of formulation**

| Formulation | Drug | Label Claim (mg) | Quantity found (mg) | % label claim |
|---|---|---|---|---|
| Denopsy 14 | Teriflunomide | Each tablet consists of 14 mg of TEF | 13.84 | 99.4 |

## 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

## AUTHORS CONTRIBUTIONS

All the author have contributed equally

## CONFLICT OF INTERESTS

Declare none

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