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Teriflunomide Quantitative Assessment in Pharmaceutical Tablet Formulations and Bulk Using a Validated UV-Visible Spectrophotometric Method

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Abstract: A simple, rapid, accurate, precise and economical UV–Visible spectrophotometric method was developed and validated for the quantitative estimation of teriflunomide, an immunomodulatory agent, in bulk active pharmaceutical ingredient and finished dosage form. Teriflunomide showed maximum absorbance at 245 nm in methanol, which was selected as the analytical wavelength. The approach was assessed, for linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ) in accordance with the validation principles described in ICH Q2(R1). A linear response was obtained over 2–12 µg/mL, with a coefficient of determination (R^2) of 0.9998. Recovery at 80%, 100% and 120% levels ranged from 99.38% to 100.25%, with a mean recovery of 99.81%. Repeatability, intraday and interday precision showed %RSD values of 0.56%, 0.74% and 0.82%, respectively. The calculated LOD and LOQ were 0.18 and 0.55 µg/mL, respectively. Robustness and ruggedness studies produced consistent assay results under small deliberate changes in wavelength and between analysts/instruments. The technique was effectively used to a marketed tablet formulation, giving an assay of 99.50% of label claim. The proposed method offers a practical and economical alternative for routine quantitative quality-control analysis of Teriflunomide where chromatographic methods are not required.

Keywords: *Teriflunomide; UV–Visible Spectroscopy; Analytical Method Validation; Linearity; Accuracy; Precision; Assay; Pharmaceutical Analysis.*

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I. INTRODUCTION

Teriflunomide is an orally active immunomodulatory drug used in the management of relapsing forms of multiple sclerosis. It is the active metabolite of leflunomide and exerts its principal pharmacological action through selective and reversible inhibition of mitochondrial dihydroorotate dehydrogenase (DHODH), thereby affecting the proliferation of activated T and B lymphocytes. Because the drug is therapeutically important, reliable analytical procedures are required to establish the identity, strength and quality of bulk drug substances and finished dosage forms. [1]

Analytical method validation provides documented evidence that an analytical procedure is fit for its intended purpose. Important traits are robustness, linearity, accuracy, and precision. and analytical sensitivity. UV–Visible spectrophotometry remains attractive for routine pharmaceutical analysis because it is comparatively simple, rapid, economical and requires less solvent and sample preparation than many chromatographic procedures. [2]

Published analytical work on teriflunomide has predominantly employed RP-HPLC, RP-UHPLC and stability-indicating chromatographic approaches. These procedures provide valuable specificity and impurity information but can require specialized columns, mobile phases, longer preparation or higher-cost instrumentation. The current study therefore focuses on a simple.UV–Visible spectrophotometric procedure suitable for routine quantitative estimation of teriflunomide. [5–8]

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II. LITERATURE REVIEW

Kashid et al. reported development and proof of an RP-UHPLC technique for teriflunomide active pharmaceutical ingredient. The method provided satisfactory linearity, precision and accuracy, but required chromatographic instrumentation and specialized operating conditions [5].

Koppisetty et al. developed a validated RP-HPLC assay method for quantitative separation of teriflunomide and its process-related impurities. The chromatographic approach provided improved separation capability for impurity-related analysis, although it involved column-based instrumentation and mobile-phase requirements [6].

Boltia et al. reported validated chromatographic methods for determination of teriflunomide and investigated its intrinsic stability. Their work demonstrated the usefulness of chromatographic techniques for degradation and stability-related investigations [7].

Dadi et al. described a quality-by-design-assisted RP-HPLC method for estimation of teriflunomide and process impurities. The study further demonstrates the importance of chromatographic methods when simultaneous drug-substance and impurity control is required [8].

The literature indicates that chromatographic procedures provide important advantages for impurity profiling and stability-indicating analysis. The present study therefore positions UV–Visible spectrophotometry as a simpler routine assay approach rather than as a replacement for stability-indicating chromatographic methods [5–8].

III. MATERIALS AND METHODS

➢ *Chemicals and Reagents*

Teriflunomide working standard was used as the reference material. Methanol of analytical grade and freshly made distilled water were used as reported in the source study. A commercially available teriflunomide tablet formulation was procured from a local pharmacy for assay evaluation. All reagents were of suitable analytical purity. [3,4]

➢ *Instrumentation*

Spectral measurements were performed using a double-beam UV–Visible spectrophotometer (Systronics UV-2202, India) with matched 1-cm quartz cells. The instrument was subjected to appropriate wavelength and baseline checks before use. An analytical balance of suitable sensitivity was used for weighing. [3,4]

➢ *Preparation of Standard Stock Solution*

precisely weighed ten milligrams of teriflunomide working standard was transferred to a 100 mL volumetric flask. After adding about 70 milliliters of methanol, the mixture of solution was sonicated for 10 min to ensure dissolution. The volume was made up to the mark with methanol to obtain a 100 µg/mL stock solution.

➢ *Determination of λmax*

One millilitre of the 100 µg/mL stock solution was diluted to 10 mL with methanol to obtain a 10 µg/mL working solution. Using methanol as blank, the solution was scanned from 200 to 400 nm in a 1-cm quartz cell. The maximum absorbance was observed at 245 nm in methanol; therefore, The analytical wavelength was chosen to be 245 nm.

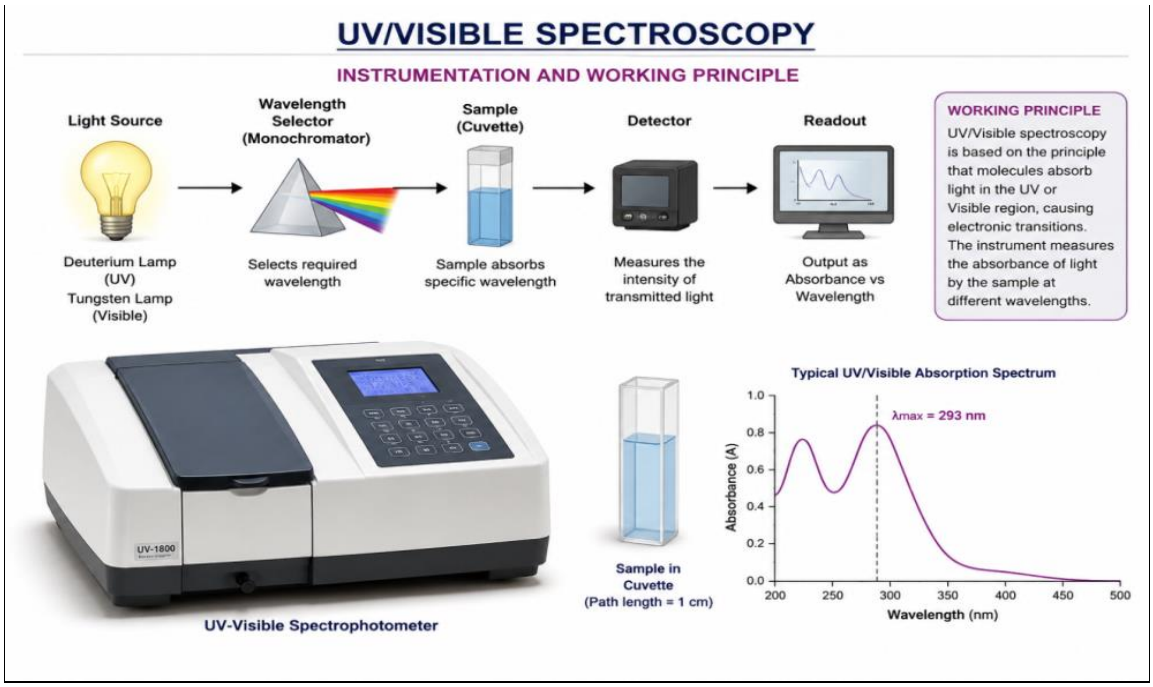

Fig 1. Representative UV–Visible Spectral Scan and Analytical Wavelength Selection as Presented in the Source Study

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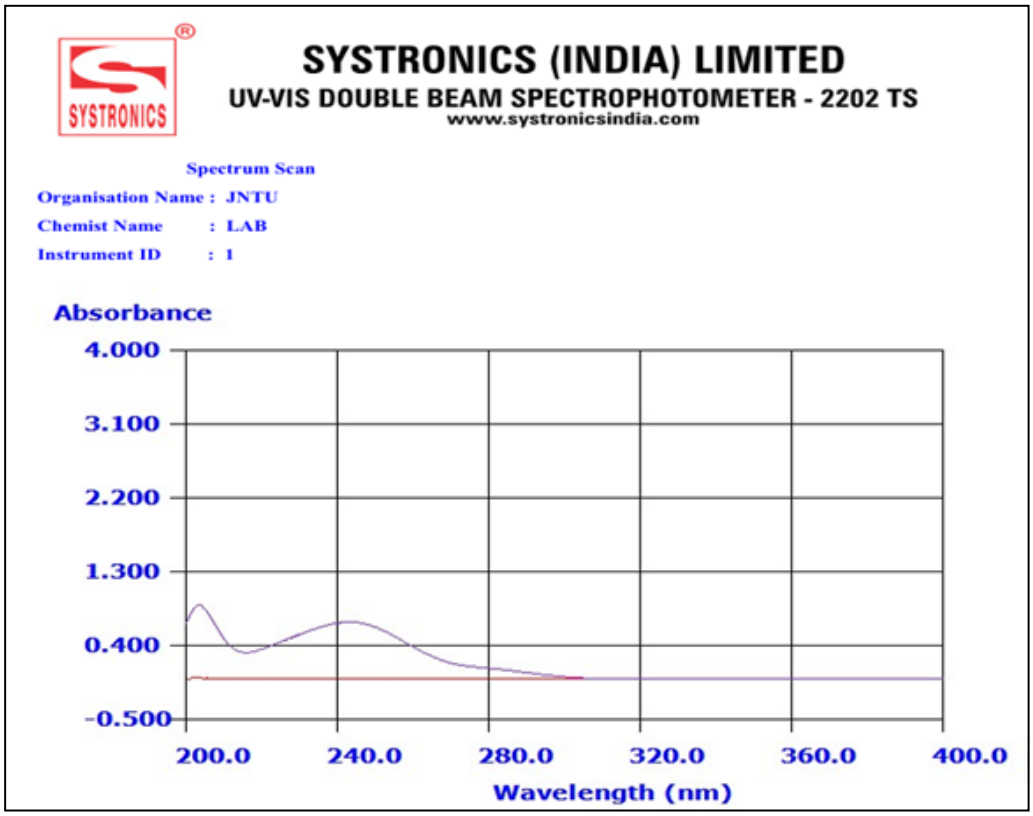

Fig. 2. The Drug Exhibited Maximum Absorbance at 245 nm in Methanol

➢ *Preparation of Calibration Standards*

Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of the 100 µg/mL stock solution were transferred separately into 10-mL volumetric flasks and diluted to volume with methanol to obtain 2, 4, 6, 8, 10 and 12 µg/mL, respectively. Absorbance was measured at 245 nm against methanol blank. A calibration plot was prepared by plotting concentration against absorbance.

Table 1. Calibration Data for Teriflunomide.

Concentration (µg/mL)	Absorbance
2	0.152
4	0.301
6	0.452
8	0.604
10	0.751
12	0.901

The supplied calibration observations gave a linear regression equation of $y = 0.0748x + 0.0012$, with $R^2 = 0.9998$. The response increased proportionally with concentration across 2–12 µg/mL, supporting quantitative application within the studied range.

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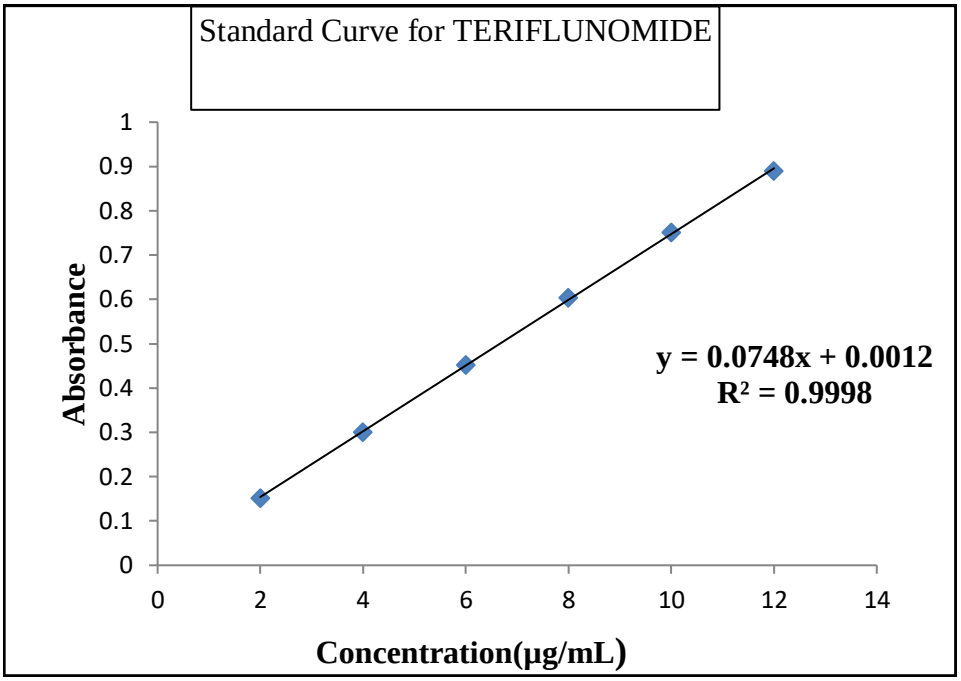

Fig.3: Linearity Curve for Teriflunamide

➢ *Sample Preparation and Assay*

Twenty commercially available tablets were weighed. And finely powdered. 10 milligrams of Teriflunomide in powder form were added to a 100 milliliter volumetric flask. After adding around 70 mL of methanol, the mixture was sonicated for 20 min. The solution was cooled, diluted to volume with methanol and filtered through Whatman No. 41 filter paper. After adding around 70 mL of methanol, the mixture was sonicated. Absorbance was measured at 245 nm against methanol blank. Assay was calculated by comparison with a standard solution of corresponding concentration.

IV. RESULTS

➢ *Linearity*

Linearity was assessed over 2–12 µg/mL using the calibration solutions. Regression analysis was performed using concentration as the independent variable and absorbance as the response.

➢ *Accuracy*

Accuracy was evaluated by the standard-addition technique at 80%, 100% and 120% levels. Known quantities of standard were added to pre-analyzed sample solution and the percentage recovery was calculated.

Table 2. Accuracy/Recovery Results.

Level (%)	Amount added (µg/mL)	Amount recovered (µg/mL)	Recovery (%)
80	6.4	6.36	99.38
100	8.0	8.02	100.25
120	9.6	9.58	99.79

➢ *Precision*

Repeatability was assessed using six replicate measurements of an 8 µg/mL solution under the same conditions. Intraday precision was assessed using three concentrations within the linear range measured three times on the same day. Interday precision was assessed on three consecutive days. Results were expressed as percentage relative standard deviation (%RSD).

Table 3. Precision Results.

Precision Study	Mean Absorbance	%RSD
Repeatability	0.604	0.56
Intraday precision	0.603	0.74
Interday precision	0.605	0.82

➢ *LOD and LOQ*

LOD and LOQ were calculated from the standard deviation of the response and the slope of the calibration curve using: [2] $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$ where σ is the standard deviation of the response/intercept and S is the slope of the calibration curve. Using the reported slope (0.0748) and σ (0.0041), the source study reported LOD = 0.18 µg/mL and LOQ = 0.548 µg/mL (rounded to 0.55 µg/mL).

➢ *Robustness*

Robustness was evaluated by deliberately varying the analytical wavelength around the selected condition. The reported assay values at 243, 245 and 247 nm were 99.34%, 99.50% and 99.42%, respectively.

Table 4. Robustness Results Reported in the Source Study.

Parameter	Condition	Assay (%)
Wavelength	243 nm	99.34
Wavelength	245 nm	99.5
Wavelength	247 nm	99.42

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➢ *Ruggedness*

Ruggedness was assessed by comparing assay results obtained by two analysts and by two UV spectrophotometers under the same procedure. The reported results were 99.50% and 99.32% for Analyst I and II, respectively, and 99.50% and 99.41% for instruments I and II, respectively.

V. DISCUSSION

➢ *Spectral Characteristics*

The UV spectrum of teriflunomide in methanol showed a distinct maximum at 245 nm. Selection of this wavelength provides a direct and convenient measurement point for routine assay. Methanol served as both the solvent and blank in the reported procedure. [3,4]

➢ *Linearity*

The calibration data showed a progressive increase in absorbance from 0.152 at 2 µg/mL to 0.901 at 12 µg/mL. The coefficient of determination calculated from the supplied observations was approximately 0.9998, indicating an excellent linear relationship. The concentration range therefore provides a suitable working interval for quantitative estimation under the stated conditions.

➢ *Accuracy*

Recovery results at 80%, 100% and 120% levels were 99.38%, 100.25% and 99.79%, respectively, with a reported mean recovery of 99.81%. The recovery values fall within the 98–102% acceptance interval stated in the source study and support the accuracy of the proposed procedure.

➢ *Precision*

The %RSD values for repeatability, intraday precision and interday precision were 0.56%, 0.74% and 0.82%, respectively. All were below 2%, demonstrating low variability under repeatability and intermediate-precision conditions.

➢ *Robustness and Ruggedness*

Small wavelength changes produced assay results of 99.34–99.50%, showing limited influence of the deliberate variation. Likewise, assay results obtained by two analysts and two instruments were closely comparable. These observations support the operational reliability of the procedure under the studied laboratory conditions.

➢ *Sensitivity*

The reported LOD of 0.18 µg/mL and LOQ of 0.55 µg/mL demonstrate that the method can detect and quantify teriflunomide at concentrations substantially below the routine assay working level. The sensitivity is adequate for the stated quantitative range.

➢ *Assay of Marketed Tablets*

The marketed tablet sample was analyzed at a final concentration of 8 µg/mL. The reported assay was 99.50% of label claim, indicating satisfactory drug content for the tested formulation. Because the study is based on UV absorbance, the procedure is most appropriate for assay where a stability-indicating impurity profile is not the primary analytical objective. [3,4]

VI. CONCLUSION

A simple, rapid and economical UV–Visible spectrophotometric method was developed for quantitative estimation of teriflunomide and validated using the reported performance characteristics. The method exhibited linearity over 2–12 µg/mL with $R^2 \approx 0.9998$, mean recovery of 99.81%, precision below 2% RSD, and reported LOD and LOQ of 0.18 and 0.55 µg/mL. Robustness and ruggedness studies showed consistent results, and the assay of the marketed tablet was 99.50% of label claim. The method is therefore suitable for routine assay and quality-control applications in laboratories equipped with UV–Visible spectrophotometry. For impurity profiling, forced-degradation studies and stability-indicating applications, chromatographic procedures remain preferable.

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