INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TERIFLUNOMIDE IN TABLET

M. S. Kalshetti*, Vidyasree Kumbhar, Priti Reure, Shraddha Jamakhandi
Dept. of Pharmaceutical Quality Assurance, D.S.T.S Mandal's College of Pharmacy, Solapur.

ARTICLE INFO

Article history
Received 27/05/2022
Available online
10/06/2022

Keywords
UV Method,
UV Spectrophotometric,
Teriflunomide.

ABSTRACT

Teriflunomide is the active metabolite of leflunomide. It is an immunomodulatory agent used in the treatment of multiple sclerosis. UV spectrophotometric method has been developed for the estimation of teriflunomide in tablets using methanol as solvent. Teriflunomide has shown λ max at 293 nm. The method has been validated according to ICH Q2 (R1) guidelines. A linear response is observed (R2=0.9995) in the range of 4-20 µg/ml, with recovery (100.68%) and precision (% RSD is 0.07).This method is simple and suitable for routine pharmaceutical analysis.

Corresponding author
M. S. Kalshetti
Dept. of Pharmaceutical Quality Assurance,
D.S.T.S Mandal's College of Pharmacy, Solapur,
Bijapur Road,
Solapur- 413004 (MS)
smkcops@gmail.com

*Please cite this article in press as **M. S. Kalshetti** et al. Uv Spectrophotometric Method for the Estimationof Teriflunomidein Tablet. Indo American Journal of Pharmaceutical Research.2022:12(05).*

INTRODUCTION

Teriflunomide [(Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]but-2-enamide)] is an oral immune-suppressive/immunomodulatory agent (Fig. 1). Teriflunomide (TEF) is the active metabolite of leflunomide [1]. It is useful in the treatment of multiple sclerosis. Multiple Sclerosis is a demyelinating disorder that affects the central nervous system. The immune system attacks the myelin sheath of the nerve fiber, causing inflammation, which destroys nerve cell processes, altering electrical messages in the brain. TEF acts by inhibiting pyrimidine de novo synthesis by blocking the enzyme dihydroorotate dehydrogenase, the enzyme involved in the synthesis of pyrimidine. The drug was approved by the USFDA and EMA [2].

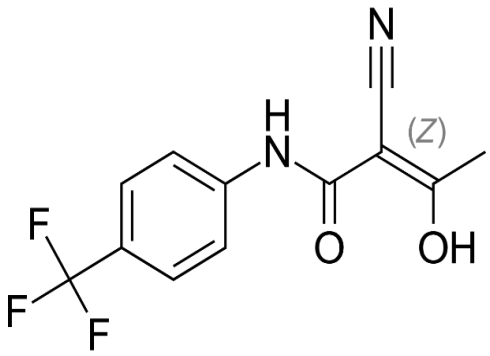

Fig. 1 Teriflunomide.

A literature survey reveals that only a single method is reported for teriflunomide by UV spectrophotometry in acetonitrile at 284 nm [3]. Some other methods are also reported such as LC-UV [4-12], LC-MS [12], LC-MS/MS [13], HPTLC [14-15]. So, there is a need for a UV spectrophotometric method using methanol for the estimation of teriflunomide in the tablet formulation. Therefore, the current work is to develop a validated simple, and precise UV spectrophotometric method for the estimation of teriflunomide in tablet formulation.

MATERIALS AND METHODS

Materials

Teriflunomide was obtained from Biocon Ltd., Bangalore, as a gift sample. Methanol was obtained from Loba Chemie Pvt. Ltd., Mumbai. Denopsy tablet containing 14 mg (Natco pharma Ltd.) purchased locally.

Instruments

UV -Visible double beam spectrophotometer (Systronics-2201), Electronic balance (Shimadzu AY220) were used.

Methods

Selection of solvent

Solubility of TEF was checked in different solvents like methanol, ethanol, acetonitrile, and distilled water.

Preparation of standard stock solution

10 mg of teriflunomide was transferred to a 10 ml volumetric flask, dissolved in methanol, and volume was made up to the mark with methanol to get 1000 µg/ml solution. 0.8 ml of the above solution was diluted to 20 ml with methanol (40 µg/ml).

Determination of absorption maxima

Teriflunomide solution (16 µg/ml) was scanned in the range of 200-400 nm in UV-VIS Spectrophotometer to determine the absorption maxima.

Preparation of sample solution

10 tablets were weighed and powdered. Powder equivalent to 70 mg teriflunomide was weighed and transferred to a 50 ml volumetric flask, dissolved in methanol, and volume was made up to the mark with methanol (1400 µg/ml). This solution was sonicated and filtered using Whatman filter paper. 1 ml of filtrate was diluted to 10 ml to get 140 µg/ml solution, and 1 ml of the resulting solution was diluted to 10 ml with methanol to get a final concentration of 14 µg/ml and the absorbance of the solution was measured at 293 nm.

Method validation

Validation of the method was carried out as per the ICH guidelines [16] for the following parameters.

Linearity and Range

4,8,12,16, and 20 µg/ml of teriflunomide solutions were prepared by diluting a standard stock solution of 40 µg/ml with methanol. The absorbance of these solutions was recorded at 293 nm. The graph was plotted between absorbance and concentration. The correlation coefficient of TEF was calculated.

Precision

The precision of an analytical procedure is defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

The absorbance of teriflunomide solutions (16 µg/ml) was determined and the same was repeated six times on the same day (repeatability) and three consecutive days (intermediate precision).

Accuracy

Accuracy of the developed method was carried out by performing a recovery study using the standard addition method, in which the standard drug was spiked at three different levels (80%, 100%, and 120%) to the pre-analyzed formulation. The amount of TEF was calculated at each level and % recoveries were calculated.

$$\% \text{ Recovery} = \frac{\text{recovered value}}{\text{spiked value}} \text{ X } 100$$

Robustness

The robustness of the developed method is its capacity to remain unaffected by small but deliberate variations in method parameters. The robustness of the method was determined by measuring the absorbance of 16 µg/ml TEF solution at 292 nm, 293 nm, and 291 nm i.e., ± 1nm.

RESULTS AND DISCUSSION

Selection of solvent

Teriflunomide is insoluble in water, and soluble in methanol and acetonitrile. Methanol is selected as the solvent.

Determination of absorption maxima

The teriflunomide solution (16 µg/ml) showed λ_{max} 293 nm (Fig. 2).

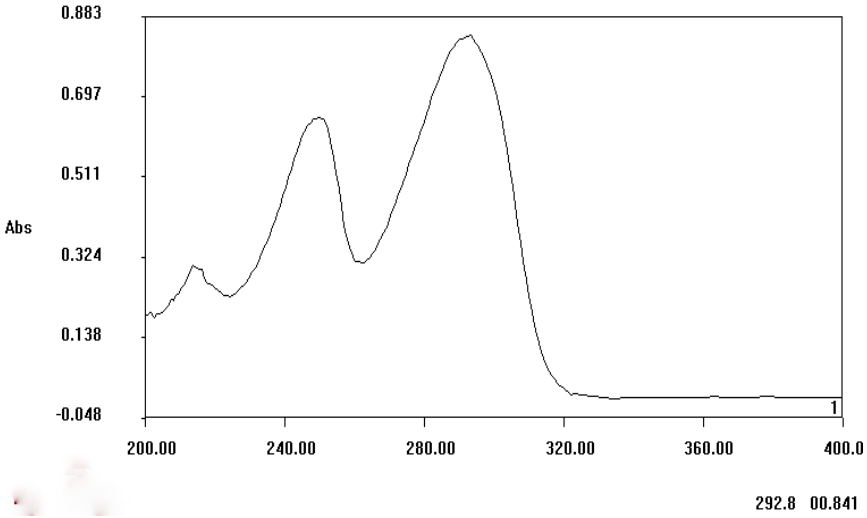

Fig. 2 UV scan of Teriflunomide.

Method validation

Linearity and Range

Linearity concentrations and absorbances are displayed in (Table 1). Absorbance v/s concentration of TEF graph is plotted (Fig. 3). The method is linear over the range 4-20 µg/ml with correlation coefficient of 0.9995 and regression equation as y = 0.0578x + 0.141. The overlay of UV spectra is shown in (Fig. 4).

Table 1 Linearity.

Conc.(µg/ml)	Absorbance
4	0.375
8	0.597
12	0.832
16	1.079
20	1.29

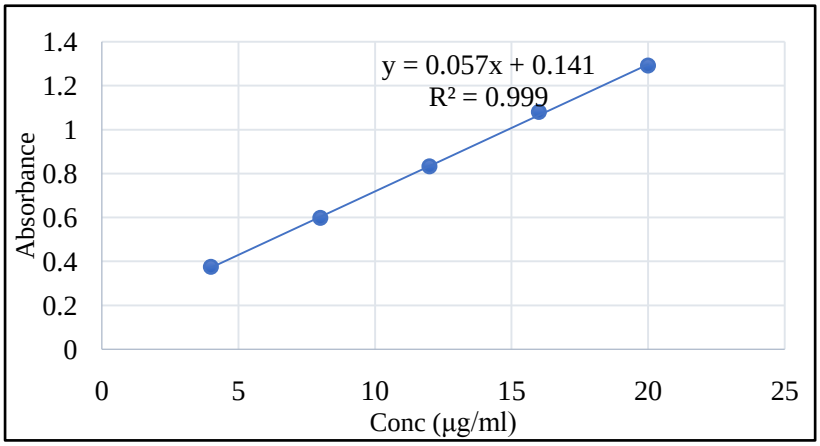

Fig. 3 Linearity graph of teriflunomide.

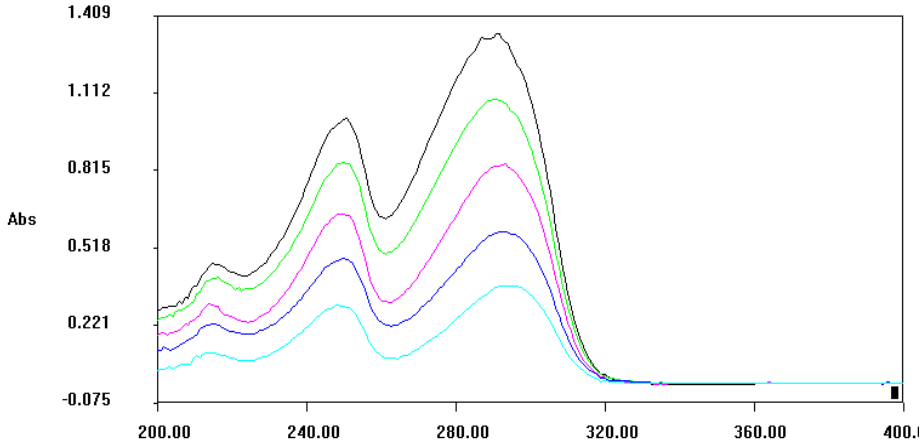

Fig. 4 Overlay of UV spectra of 4-20 µg/ml solutions of TEF.

Precision

The absorbance of six experiments and % RSD (< 2) for repeatability and intermediate precision is shown in Table 2.

Table 2 Results of repeatability and intermediate precision.

Sr. No.	Absorbance (Repeatability)	Absorbance (Intermediate precision)		
		Day 1	Day 2	Day 3
1	1.101	1.109	1.16	1.173
2	1.100	1.11	1.157	1.173
3	1.099	1.11	1.157	1.170
4	1.1	1.113	1.157	1.148
5	1.1	1.111	1.158	1.144
6	1.101	1.108	1.157	1.144
Mean	1.10	1.11	1.16	1.15
SD	0.001	0.002	0.001	0.015
%RSD	0.09	0.18	0.09	1.30

Accuracy

Accuracy is between 100.12 - 101.71 % as shown in Table 3.

Table 3 Results of recovery.

Sr. No.	Level of % Recovery	Amount Added (µg/ml)	Amount found (µg/ml)	% Recovery
1.	80	6.4	6.51	101.71
2.	100	8.0	8.01	100.12
3.	120	9.6	9.62	100.20

Robustness

The robustness of the method was studied by calculating % the relative standard deviation, which was found to be less than 2% (Table 4). The variation in the wavelength showed similar results.

Table 4 Robustness.

Sr. no.	Wavelength (nm)	Absorbance	Mean ± SD	%RSD
1.	292	1.119		
2.	293	1.113	1.112 ± 0.007	0.629
3.	294	1.105		

Assay of teriflunomide tablet

The developed method is applied for the estimation of TEF in the tablet. The result of the assay is shown in (Table 5). Teriflunomide tablet contains 101.71 % of the stated amount of Teriflunomide $C_{12}H_9F_3N_2O_2$.

Table 5 TEF content in tablet.

Formulation	Drug	Label claim (mg)	Amount of drug found (mg)	% Content
DENOPSY tablet	Teriflunomide	14	14.24	101.71

CONCLUSION

The simple, cost-effective UV spectrophotometric method is developed and validated in compliance with ICH guidelines for the estimation of teriflunomide. This method is accurate, precise, sensitive and linear in the range of 4-20 µg/ml. The analytical method can be used for routine analysis of teriflunomide in the formulation in quality control, research, and analytical laboratories.

ACKNOWLEDGEMENT

The authors are thankful to Biocon Ltd., Bangalore for providing a gift sample of teriflunomide. The authors are thankful to D.S.T.S. Mandal's College of Pharmacy, Solapur for providing the facilities required for the research work.

CONFLICT OF INTEREST

The authors declare no Conflict of interest.

REFERENCE

1. PubChem [internet]. Bethesda (MD): National Library of Medicine (US), National Centre for Biotechnology Information; 2004 Teriflunomide; [cited 2022 April 10]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Teriflunomide
2. Drug bank online [internet] Teriflunomide; [cited 2022 May 10]. Available from: https://go.drugbank.com/drugs/DB08880
3. Vishwas TS, Gurupadayya BM. Novel UV spectrophotometric method for the determination of Teriflunomide in tablet dosage form. Int J Curr Pharm Res. 2019;11(5):81-84.
4. Patel KN, Patel KK, Prajapati LM, Karen HD and Patel CN. Quantitative analytical method development and validation for Teriflunomide in Pharmaceutical dosage form. World J Pharm Pharm Sci. 2020;9(5):1064-1079.
5. Bhavya M, Pravin P and Yatin G. Development and validation of stability indicating RP-HPLC method for estimation of teriflunomide in active pharmaceutical ingredient. J Pharm Innov. 2017;6(9):440-449.
6. Upadhyay PS, Dr. Vinay CD, Bhumi P, Jaymin P. Development and validation of stability Indicating assay Method for estimation of Teriflunomide in tablet dosage form. Indo Am J Pharm Sci. 2018;5(04):3209-3219.
7. Sobhani K, Garrett DA, Liu DP, Rainey PM. A Rapid and Simple High-Performance Liquid Chromatography Assay for the Leflunomide Metabolite, Teriflunomide (A77 1726), in Renal Transplant Recipients. Am J Clin Pathol.2010;133:454-457.
8. Patel K, Dr. Patel C, Patel M and Dr. Patel MM. Development and validation of stability indicating HPLC method for estimation of teriflunomide in API and Pharmaceutical dosage form. World J Pharm Pharm Sci. 2017;6(12):780-800.
9. Sushma G, Sridevi P, Bhagavan RM and Vinutha K, Shaik A. Method development and validation for the determination of related substances in teriflunomide by RP-HPLC method. World J Pharm Pharm Res. 2019;8(12):882-891.

10. Dr. Dutt KR, Mannam S, Illendula S. A new analytical method development and validation for the determination of teriflunomide in bulk form and marketed pharmaceutical dosage form. Int J Pharm Anal Res. 2021;10(2):129-137.
11. Babu DK, Babu DA, Arul Anand Raj CN. RP-HPLC analytical method development and validation of terifluomide in tablet dosage form. Acta Biomedica Sci. 2018;5(2):123-126.
12. Suneetha A and Rajeswari KR. Comparision of LC-UV and LC-MS methods for simultaneous determination of teriflunomide, dimethyl fumarate and fampridine in human plasma: application to rat pharmacokinetic study. Biomed Chromatogr. 2016
13. Suneetha A and Rajeswari KR. Estimation of Teriflunomide Along with Concomitant Drugs in Different Biological Matrices Using LC-MS/MS. Int J Pharm Sci Res. 2015;6(5):1915-1923.
14. Karmankar S, Tajne M. Development of Validated Stability Indicating HPTLC Method for the Estimation of Teriflunomide in Bulk and Tablet Dosage Form. Curr Pharm Anal. 2020;16(7):814-822.
15. Vishwas TS, Gurupadayya BM, Rupshee J. Novel HPTLC-Densitometric method for the estimation of Teriflunomide in tablet dosage form. Int J Pharm Pharm Sci. 2019;11(6):80-84.
16. Validation of Analytical Procedures: text and methodology Q2 (R1) [Internet], International Council for Harmonization, Geneva, November 2005. Available from: https://database.ich.org/sites/-default/files/-Q2%28R1%29%20Guideline.pdf

5487847845122**0515**