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RP HPLC - METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TERIFLUNOMIDE TABLET

PANDALA ARCHANA [1], DR.G. SURESH KUMAR [2]

[1,2] Department of Pharmaceutical Analysis, Sri Indu Institute of Pharmacy in Ibrahimpatnam, Hyderabad.

mail id: pandalaarchana1@gmail.com

ABSTRACT: Stability of the pharmaceutical product is most important, so that work is carried out to develop a new, simple, precise, accurate, validated stability indicating RP-HPLC method for estimation of Teriflunomide in its tablet dosage form. In this method 0.25% v/v Trifluoroacetic acid in Water, Acetonitrile and Methanol (30:50:20 % v/v) was used as a mobile phase and Ace C18 (250*4.6, 5 µm) column was used for the separation of drug with other degraded product. The flow rate 1.5 mL/min, detection wavelength 250 nm and 250C column temperature was used. The retention time for Teriflunomide was found to be 4.02 minute. The developed method meets all the acceptance criteria for the validation of analytical method as per the ICH guideline. A simple, precise, and accurate stability indicating RP-HPLC method was developed for estimation of Teriflunomide in pharmaceutical dosage form. Validation parameter proves that method is repeatable, sensitive, and selective for the analysis of Teriflunomide. Based on this evidence the method can be stated as highly economical and it is recommended for routine analysis and stability studies.

Keywords: Teriflunomide, RP-HPLC, Stability studies, photolytic degradation, validation

INTRODUCTION

Teriflunomide is approved and relatively newer chemical entity for the treatment of multiple sclerosis. Therefore, it is thought of interest to study the quality of marketed formulation of Teriflunomide. The literature review also revealed that no analytical method is reported for the chemical quantification of Teriflunomide by RP-HPLC, However the innovators has revealed the composition of mobile phase yet the ratio of components and other crucial chromatographic conditions are not mentioned. So, it is thought of interest to explore & develop the rapid, precise and accurate method for the estimation of Teriflunomide in its marketed dosage form using Reverse Phase HPLC method. Although few methods are reported for the quantitate determination of Teriflunomide in human plasma by using LC-MS. We developed simple, rapid, stability indicating & sensitive HPLC Method for the estimation of Teriflunomide and perform Forced Degradation of Teriflunomide according to ICH guidelines. In this study, we validate developed HPLC method as per ICH guidelines [1-3].

MATERIALS

Materials: Teriflunomide was procured as gift sample from Amneal Pharmaceuticals Ltd, Ahmedabad, Gujarat, India. Hydrochloric acid, triethylamine, and sodium hydroxide were obtained from AR Grade, Merck chem. Ltd, India. Methanol, hydrogen peroxide and acetonitrile were procured from HPLC Grade, Merck chem. Ltd, India.

METHOD (Table 1)

Table 1: Method development trials for Teriflunomide [4]

Trial no.	Mobile phase	Ratio	Flow rate(ml/min)	Retention time(min)	Column	Comment
1	Water:Acetonitrile	50:50	1.0	3.8	X-bridge C18 (150*4.6 mm, 5µm)	Doublet peak
2	Water:Methanol	50:50	1.0	2.8	X-bridge C18 (150*4.6 mm, 5µm)	Doublet blunt peak
3	Water: Methanol	50:50	1.0	2.8	Zorbax C18 (150*4.6 mm, 5µm)	Peak shape not proper (Fronting)
4	Water:Acetonitrile:Methanol	40:30:30	1.5	0.9	Ace C8 150*4.6 mm, 5 µm	Sharp peak but hump observed at RT 5min
5	Acetonitrile:Methanol	50:50	1.5	2.0	Ace C18 150*4.6 mm, 5 µm	Negative base line pattern, Peak shape improper
6	Water:Acetonitrile:Methanol	30:50:20	1.5	4.0	Ace C18 250*4.6 mm, 5 µm	Tailing observed
7	0.25% Triethylamine buffer:Acetonitrile:Methanol	30:50:20	1.5	4.0	Ace C18 250*4.6 mm, 5 µm	Sharp Peak, Optimum Retention time

Forced degradation study [5, 6]

Degradation conditions:

Acid degradation: Sample was exposed in 5 ml 0.1N HCl & 1 N HCl at 80°C for 1 hours.

Alkali degradation: Sample was exposed in 5 ml 0.1N NaOH & 1 N NaOH at 80°C for 1 hours.

Peroxide degradation: Sample was exposed in 30% H2O2 at 80°C for 1 hours.

Thermal degradation: Sample was exposed to 80°C for 2 hours.

Photo degradation: Sample was exposed to sun light for 4 hours.

Forced degradation standard stock preparation: An accurately weighed quantity (100 mg) of Teriflunomide was transferred in 100 ml volumetric flask, dissolved in diluents with aid of sonication and diluted to volume with diluent to make 1000 µg/ml Teriflunomide standard stock solution.

Photolytic degradation: 5 ml of forced degradation standard stock solution was exposed to sun light for 4 hours. Then dilution was made in such way that final concentration for Teriflunomide (100 µg/ml) was achieved at same procedure followed.

Validation of analytical procedure: Solution containing 50, 100, 150, 200 µg/mL Teriflunomide were prepared from standard stock solution. Prepared solutions were analyzed as per the proposed method. Five replicate analyses were carried out. The mean area with its standard deviation and % relative standard deviations of the peak areas were calculated. Mean area AUC against concentration were plotted to obtain the calibration curve. Regression equation, co-relation coefficients were computed from calibration curves [7].

Preparation of linearity stock solution:

Transfer an accurately quantity of about 100 mg of Teriflunomide to a 100 ml volumetric flask add 70 ml diluent and sonicate to dissolve. Make volume up to the mark with diluent and mix.

Method precision [8]

Repeatability: The precision of the instrument was checked by repeated injection (n=6) of standard solutions of Teriflunomide under the same chromatographic condition and measurement of peak area, retention time. The RSD value should not be more than 2%.

Intraday precision: Aliquots of 2.0, 3.0 and 4.0 ml of API solution of Teriflunomide were transferred to a series of 20 ml volumetric flask. The volume was adjusted up to mark with diluent to get 100, 150 and 200 µg/ml solution of Teriflunomide.

Interday precision: Aliquots of 2.0, 3.0 and 4.0 ml of Teriflunomide solutions were transferred to a series of 20 ml volumetric flask. The volume was adjusted up to mark with diluent to get 100, 150 and 200 µg/ml solution of Teriflunomide. The chromatogram of these solutions were taken three times in three different days.

Specificity: Specificity of an analytical method was assessed by defining its ability to measure accurately and specificity the analyte of interest without interference from blank. the analyte should have no interference from other extraneous components and be well resolved from them. Diluent was taken as blank and analyzed as per proposed method. Peak purity of main peak was determine using PDA detector.

Assay: Weighed and powdered 20 tablets. Powder equivalent to 100 mg of Teriflunomide was taken into 100.00 ml volumetric flask. 70 ml of diluent was added and sonicated for about 15 minutes with intermittent shaking. Allow it to come to room temperature, diluted up to the mark with diluent and mixed. Centrifuge the portion of this solution at 3000 RPM for 5 minutes. 5.00 ml of the clear supernatant was pipette into 50.00 ml volumetric flask, diluted up to the mark with diluent and mixed (100 µg/ml) [9].

RESULT AND DISCUSSION

In the fourth trial Mobile phase composition used was water: Acetonitrile: Methanol (30:50:20 % v/v). The peak was eluted at optimum retention time but tailing was observed, so in the next trial water is replaced by 0.25% v/v Trifluoracetic acid in water. Based on the conclusion obtained from the above trials in various mobile phase compositions and various columns: Column: ACE C18 (250*4.6 mm, 5µm), Mobile Phase: Buffer (0.25 % Trifluoacetic acid): Acetonitrile: Methanol (30:50:20 % v/v), Injection Volume: 10 µL, Run time: 10 minutes (Figure 1). From the forced degradation study, it can be concluded that Teriflunomide is highly prone to oxidative degradation. Optimum degradation was observed in acid and alkali treatment. Thermal and photolytic degradation is very low **[6] (Table 2, 3; Figure 2-5).**

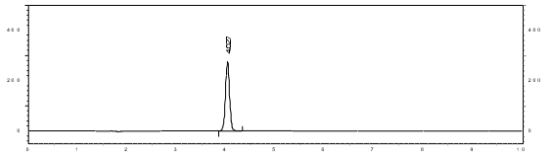
The data for intraday precision of Teriflunomide are presented in **Table 4.** Range of % RSD was found to be 0.1121 – 0.2519 % for Teriflunomide.

The data for interday precision of Teriflunomide are presented in **Table 5** Range of % RSD was found to be 0.2633 – 0.6313 % for Teriflunomide [10].

Sample ID: Teriflunomide

Data Descriptin: Buffer ACN: Metha no l (30 :50 :20) (AceC18 - 250 mm) Vial No.:

Injection Volume:



Name	Retention Time	Area	Asymmetry	Theoretical plates (DAB)	Resolution (DAB)	Area Percent	Capacity factor
Teriflunomide	4.027	3212741	1.03	11903	0.00	100.000	0.00000
Totals		3212741				100.000	

Figure 1: Development of optimized trial chromatogram of Teriflunomide

Table 2: Forced degradation summary of Standard preparation

Stress condition	Teriflunomide		
	Area	R.T.	% Degradation
As Such	3212741	4.027	-
Acid 1 N HCl	2619760	4.027	18.213
Acid 0.1 N HCl	2706630	4.027	15.898
Alkali 1 NaOH	2785419	4.027	13.159
Alkali 0.1 NaOH	3233760	4.027	-
Peroxide	1451789	4.027	55.335
Thermal	3092920	4.027	4.068
Photolytic	2994160	4.027	2.234

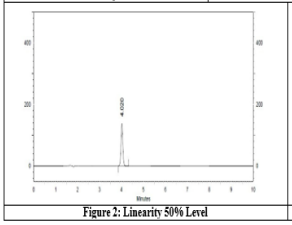


Figure 2: Linearity 50% Level

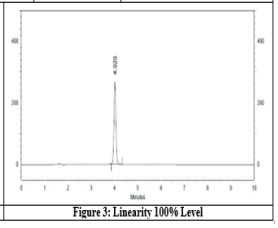


Figure 3: Linearity 100% Level

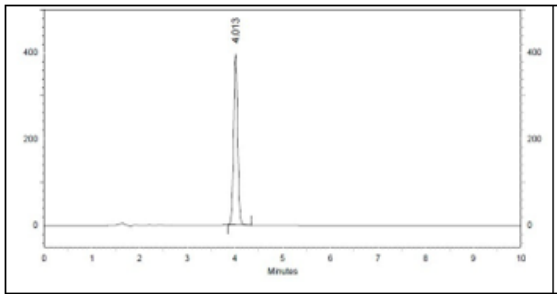


Figure 4: Linearity 150% Level

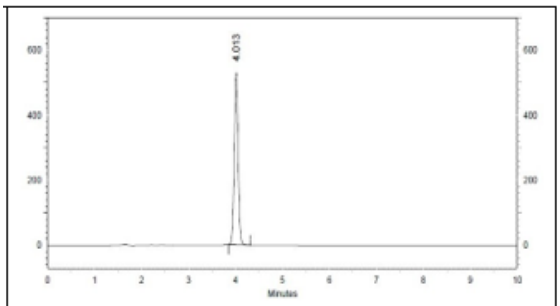


Figure 5: Linearity 200% Level

Table 3: Data of Repeatability (n=6)

Sr. No.	Area
1	3294801
2	3297916
3	3300581
4	3306102
5	3306629
6	3310321
Mean	3302725
SD	5913.671516
% RSD	0.18

Table 4: Intraday precision for Teriflunomide

Sr No.	Conc. (µg/ml)	Area Mean ± SD (n=3)	%RSD
1	100	3290624 ± 3688.21	0.1121
2	150	4736357 ± 7376.56	0.1557
3	200	6420674 ± 16175.41	0.2519

Table 5: Interday precision for Teriflunomide

Sr No.	Conc. (µg/ml)	Area Mean ± SD (n=3)	%RSD
1	100	3295456 ± 8675.21	0.2633
2	150	5015642 ± 31665.37	0.6313
3	200	6434393 ± 34696.75	0.5392

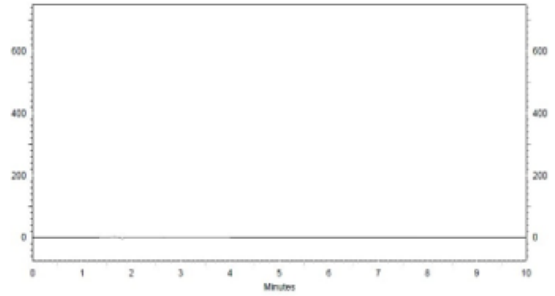


Figure 6: Specificity Diluent

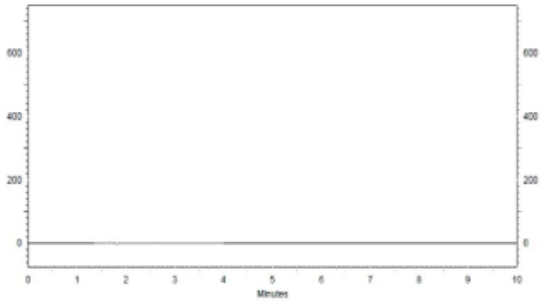


Figure 7: Specificity placebo

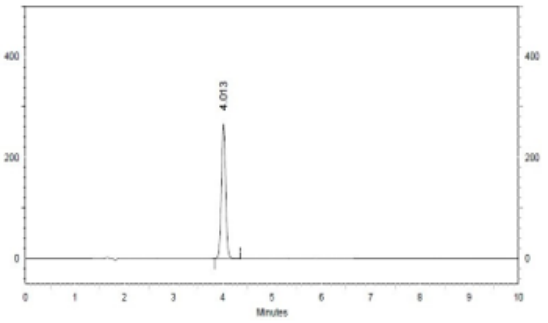


Figure 8: Specificity Standard

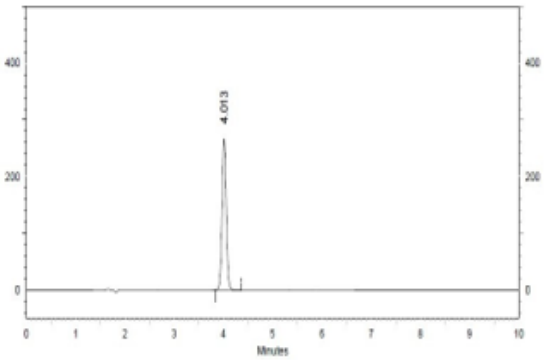


Figure 9: Specificity Sample

Table 6: Assay of marketed formulation

Brand	Label Claim (mg)	Taken concentration (µg/ml)	Recoverd concentration (µg/ml)	% Mean recovery ± SD

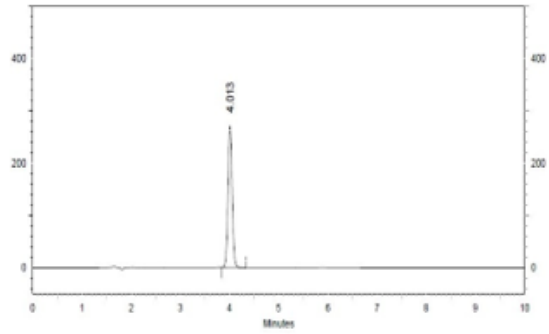


Figure 10: Teriflunomide Tablet sample set-I

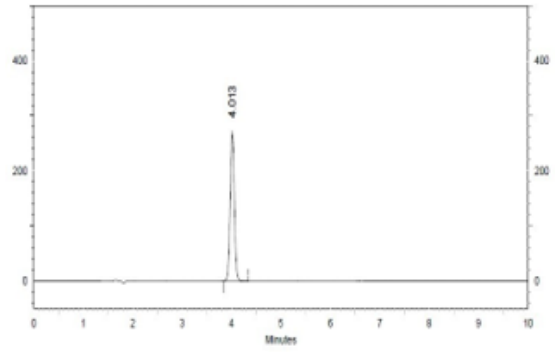


Figure 11: Teriflunomide Tablet sample set-II

CONCLUSION

The simple, sensitive, accurate, rapid, and reliable Stability Indicating Analytical Method has been developed for determination of teriflunomide in pharmaceutical formulation. In order to investigate the stability of drug, a stress testing of drug sample by exposing it to variety of force degradation conditions has been recommended. Teriflunomide was subjected to stress degradation under different condition recommended by international conference on harmonization (ICH). The higher percentage of recovery study indicates that there is no interference of excipients in the presence of formulation. The stability study indicates appreciable changes were observed by treating the drug with acidic, oxidative, and thermal condition.

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Conflict of Interest: There is no any conflict of interest

Ethical Clearance: NIL

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