ORIGINAL ARTICLE

High Performance Liquid Chromatographic Studies of Teriflunomide: Method Development and Validation for Drug Development and Formulation

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Abstract

Aim: There is an unmet analytical need to develop a rapid, robust, and precise method for teriflunomide in drug development and research in pharmaceutical formulations. The main spotlight of this research work is to develop a precise, simple, and accurate method for the application of analytical research development of teriflunomide and its pharmaceutical formulations. **Materials and Methods:** The quantification and analytical validation of teriflunomide was developed with a stationary phase XBridge column C18 (4.6 mm × 250 mm, 5 µm) and LC1120 Agilent high performance liquid chromatographic instrument equipped with variable wavelength detector at 294 nm and Acetonitrile: Buffer containing 20 mM of Ammonium acetate and 5 mL glacial acetic acid with pH adjustment 4.48 (60:40, v/v) was used as mobile phase passed at a flow rate 1 mL/min. Elution takes place at a retention time of 2.853 min. **Results and Discussion:** Validation of the method was performed as per International Council for Harmonisation guidelines, which shows linearity concentration range from 10 to 60 µg/mL amid correlation coefficient = 0.999 with Y = 78737 × +47703 regression equation was obtained. Accuracy of the proposed method was established to 99.03–99.13% with relative standard deviation (% RSD) values <2%. Method precision, system precision, and reproducibility % RSD value were 0.14%, 0.23%, and 0.14%, respectively. The limit of detection and limit of quantitation of teriflunomide were 0.06 µg/mL and 0.2 µg/mL, correspondingly. The intermediate precision was carried out and the results of Teriflunomide were achieved <2% RSD. Robustness was performed by deliberate changes in wavelength and flow rate and the results of robustness were achieved <2% RSD. **Conclusion:** The developed method was precise, simple, and accurate for the application of analytical research development of teriflunomide and its pharmaceutical formulations.

Key words: Teriflunomide, high performance liquid chromatography, detector, international council for harmonisation guidelines

INTRODUCTION

Teriflunomide is an active metabolite of leflunomide, which inhibits pyrimidine synthesis and act as an immunomodulatory drugs applicable for the treatment of patients with multiple sclerosis.[1] Its IUPAC name was (2Z) - 2 – cyano – 3 – hydroxyl – N - [4- (trifluoromethyl) phenyl] but - 2 -enamide was revealed in [Figure 1]. According to the literature review, a few methods were reported for the quantification of teriflunomide, which include high performance liquid chromatographic (HPLC),[2-15] ultraviolet spectroscopic,[16-20] and liquid chromatographic-mass spectroscopic methods.[21-28] This research was intended

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to establish a new and simple reversed-phase-HPLC validated method for quantification of teriflunomide as per International Council for Harmonisation (ICH) guidelines.[29]

MATERIALS AND METHODS

Chemicals

Teriflunomide active pharmaceutical ingredient was attained from Rochem, India. Ammonium acetate, Glacial acetic acid, Water, and Acetonitrile (Merck Chemical Company, HPLC-Grade) were used as the solvent system. Denopsy® tablet contains teriflunomide 7 mg is procured from Natco Pharma Limited, India.

Instruments

Agilent LC-1120 HPLC, attached with VWD detector and EZ Chrome software. An XBridge C18 (4.6 mm × 250 mm, 5 µm) column was employed. Sample weighing, pH detection, and sonication were performed by a Shimadzu electronic balance, Global pH Meter, and Equitron Sonicator.

Chromatographic conditions

Stationary phase was XBridge C18 (4.6 mm × 250 mm, 5 µm) column with a mobile phase contains Acetonitrile: Buffer containing 20 mM of Ammonium acetate and 5 mL glacial acetic acid with pH adjustment 4.48 (60:40, v/v) be selected and passed with a 1.0 mL/min flow rate at 294 nm was delivered. A 20 µL injection volume was used and the run time was observed to be 10 min and shows a 2.853 min retention time.

Mobile phase preparation

HPLC grade 1000 mL water was added with 1.54 g of Ammonium acetate and 5 mL glacial acetic acid, with pH adjustment 4.48, and sonicated followed by vacuum filtration through 0.22 µm filter. HPLC-grade acetonitrile was mixed with the above solvent in a ratio of 60:40 (v/v) and sonicated for 10 min followed by vacuum filtration through 0.22 µm filter, which produce diluent.

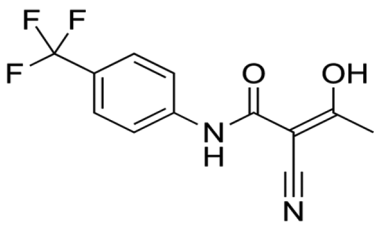

Figure 1: Teriflunomide structure

Standard stock solution preparation

7 mg teriflunomide were moved to a 100 mL volume flask and dissolved up to the mark with diluents to attain a concentration of 70 µg/mL.

Preparation of sample solution

Twenty tablets of Denopsy® (7 mg teriflunomide) were mashed to fine powder and 7 mg teriflunomide equivalent weight powder was kept in a 100 mL volume flask and dissolved with diluents. Further sonicated 30 min followed by vacuum filtration through 0.22 µm filter. Diluents were added up to mark to obtain a concentration of 70 µg/mL. Then 14.3 mL was transferred to a 100 mL volumetric flask and diluents were added up to the mark to attain concentration of 10 µg/mL. 20 µL from the above solution was taken for injection into the HPLC to obtain the chromatogram. From the chromatogram, peak areas were measured and revealed in [Figures 2 and 3].The % assay is calculated by comparing sample with the standard chromatogram peak areas and the result was revealed in [Table 1].

RESULTS AND DISCUSSION

System suitability

50 µg/mL six replicates of teriflunomide were injected for system suitability check and outcomes were revealed in [Table 2].

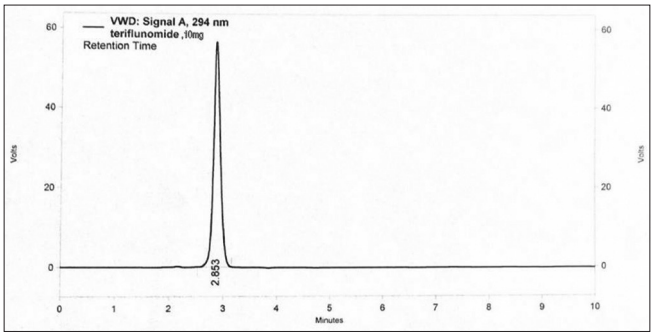

Figure 2: Standard chromatogram of teriflunomide

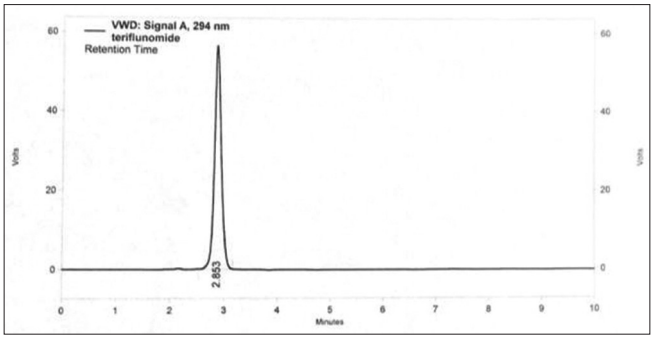

Figure 3: Sample chromatogram of teriflunomide

Specificity

Blank solutions were injected into the HPLC and the chromatogram was revealed in [Figure 4]

Linearity

Aliquots of 1.43, 2.86, 4.29, 5.72, 7.15, and 8.58 mL were acquired from a 70 µg/mL standard solution and moved into 10 mL volume flasks individually, and diluents were added up to the mark to obtain a concentration range from 10 to 60 µg/mL. An injection of each 20 µL above solution was injected to the HPLC to obtain the chromatogram. From the chromatogram, retention time and peak area were recorded, and a graph was plotted against concentrations, is revealed in [Figure 5 and Table 3].

Table 1: Teriflunomide assay

Drug	Denopsy® labeled claim in (mg)	Amt. found in (mg)	Percentage labeled claim±%RSD (*n*=3)
Teriflunomide	7	6.97	99.62±0.30

Table 2: Teriflunomide system suitability

Parameters (*n*=6)	Teriflunomide
Retention time in minutes	2.853
Theoretical plate number	2388
Tailing/asymmetry factor	1.2

Table 3: Teriflunomide linearity

Concentration (µg/mL)	Peak areas
10	8343840
20	15905887
30	23228939
40	31276209
50	39000404
60	47927017

Accuracy

It was determined by the standard addition method at a level of 50%, 100%, and 150% and the outcomes were revealed in [Table 4].

Precision

Precision method

20 µg/mL of teriflunomide homogenous sample was injected 6 times and the relative standard deviation (%RSD) meant for peak areas of 6 repeated injections were determined as reported in [Table 5].

Precision system

20 µg/mL teriflunomide was injected 6 times and the % RSD meant for peak areas of 6 repeated injections were determined as reported on [Table 6].

Intermediate precision (ruggedness)

20µg/mL teriflunomide was injected 6 times in different days and labs. % RSD meant for peak areas of 6 repeated injections were determined as mentioned in [Table 7].

Limit of quantitation (LOQ) and limit of detection (LOD)

LOQ and detection were estimated through the formula LOD = 3.3 × SD/S and LOQ = 10 × SD/S, correspondingly.

Table 4: Teriflunomide accuracy

Level (%)	Added amount (µg/mL)	Found amount (µg/mL)	Recovery%	Statistical data
50	10	9.91	99.1	Mean - 99.07
50	10	9.89	98.9	S.D - 0.15
50	10	9.92	99.2	%RSD - 0.15
100	20	19.86	99.3	Mean - 99.13
100	20	19.82	99.1	S.D - 0.15
100	20	19.8	99.0	%RSD - 0.15
150	30	29.67	98.9	Mean - 99.03
150	30	29.7	99.0	S.D - 0.15
150	30	29.76	99.2	%RSD - 0.15

SD: Standard deviation, % RSD: Relative standard deviation

Table 5: Teriflunomide precision method

Teriflunomide	
Concentration in µg/mL	**Assay (%)**
20	100.4
20	100.5
20	100.3
20	100.5
20	100.2
20	100.5
Average	100.4
Standard deviation	0.1376
% RSD	0.14

% RSD: Relative standard deviation

Table 6: Teriflunomide precision system

Teriflunomide	
Concentration in µg/mL	**Peak areas**
20	15372804
20	15383846
20	15305921
20	15318895
20	15317581
20	15306546
Average	15334266
Standard deviation	34727.07
% RSD	0.23

% RSD: Relative standard deviation

Table 7: Teriflunomide precision intermediate

Conc. in µg/mL	Lab-A (% assay)-HPLC-A day 1	Lab-B (% assay)-HPLC-B day 2
20	100.23	100.41
20	100.09	100.12
20	100.31	100.00
20	100.00	100.23
20	100.16	100.39
20	100.02	100.41
Average	100.1	100.3
Standard deviation	0.1226	0.1731
% R.S.D	0.12	0.17
Precision intermediate within laboratories variations		
Lab-A (% Assay)-HPLC-A		**Lab-B (% Assay)-HPLC-B**
Average	100.1	Average 100.3
S D	0.1226	S D 0.1731
% RSD	0.12	% RSD 0.17
Reproducibility between the labs assay %		
Average	100.2	
S D	0.14	
% R.S.D	0.14	

SD: Standard deviation, % RSD: Relative standard deviation, HPLC: High performance liquid chromatographic

Table 8: Teriflunomide validation

Parameters	Teriflunomide	
Linearity (µg/mL) with range	10–60	
Slope (m)	78737	
Intercept (y)	47703	
Correlation coefficient (r^2)	0.999	
LOD in µg/mL	0.06	
LOQ in µg/mL	0.2	
Precision method (% R.S.D; *n*–6)	0.14	
Precision system (% R.S.D; *n*–6)	0.23	
Precision intermediate (% R.S.D; *n*-24)	Lab-A	Lab-B
	0.12	0.17
Reproducibility (% R.S.D; *n*–48)	0.14	
Accuracy in %	99.03–99.13	
Robustness (% R.S.D; *n*–6)	Flow rate less	Flow rate more
	0.02	0.05
	Wavelength less	Wavelength more
	0.10	0.15

LOD: Limit of detection, LOQ: Limit of quantitation

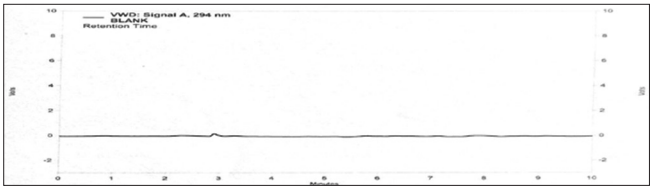

Figure 4: Blank chromatogram

Where, SD: Standard Deviation of response, that is, Y-intercept and S: Slope of calibration curve and shown in [Table 8].

Teriflunomide robustness

A deliberate change in wavelength ±2 nm and flow rate of ±10% were made to evaluate the robustness of the method and the outcomes are presented in [Table 9].

Numerous mobile phase mixtures were approached for method optimization. Suitable separations with excellent peak symmetry for teriflunomide were acquired at 294 nm. Teriflunomide was found to be 2.853 min retention time with 10–60 µg/mL linearity range, having $R^2 = 0.999$. Accuracy of the proposed method was established to 99.03–99.13% with % RSD values <2%. Method precision, system precision, and reproducibility % RSD value were 0.14%, 0.23% and 0.14%, respectively. LOD and LOQ values of teriflunomide were 0.06 µg/mL and 0.2 µg/mL, respectively. Robustness studies % RSD values was <2%.

Table 9: Teriflunomide robustness by modify in flow rate and mobile phase

Factors	Average peak areas (*n*-3)	Standard deviation	Percentage RSD	Retention time	Theoretical plates
0.9 mL/min flow rate	15339264	2718.5	0.02	2.880	2921
Actual flow rate 1 mL/min	15334480	4680.0	0.03	2.824	2688
1.1 mL/min flow rate	15262893	7784.5	0.05	2.627	2861
292 nm wavelength	15178718	15496.5	0.10	2.613	2821
Actual 294 nm wavelength	15347725	1942.5	0.01	2.824	2866
296 nm wavelength	15327921	22941.5	0.15	2.900	2781

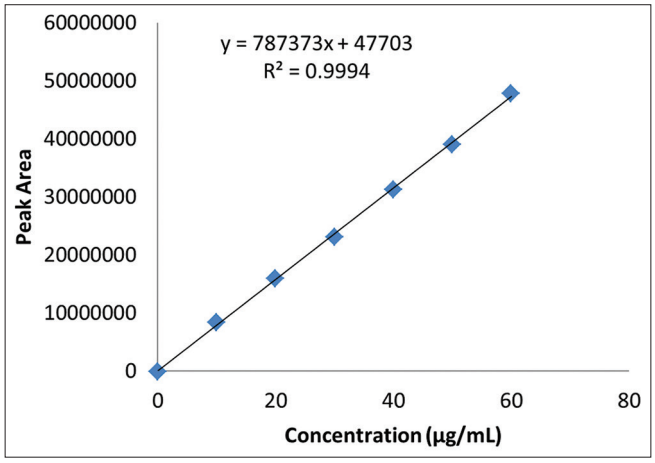

Figure 5: Teriflunomide linearity range graph

CONCLUSION

The quantification and the validation for teriflunomide in pharmaceutical formulation were performed through HPLC as stated by the ICH guidelines. 10–60 µg/mL linearity, having a correlation coefficient value of 0.999, was achieved for teriflunomide. 99.03–99.13% recovery of the drug was achieved, which was within the acceptance criteria. Less than 2% RSD of precision was achieved, which confirmed the developed method was precise, simple, and accurate for the application of analytical research development of teriflunomide and its pharmaceutical formulations.

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