Heterocyclic Letters
Vol. 15| No.1|75-82|Nov-Jan|2025
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

RAPID STABILITY INDICATING HPLC METHOD FOR THE ESTIMATION OF ORGANIC IMPURITIES OF TERIFLUNOMIDE IN PHARMACEUTICAL DOSAGE FORMS OF TABLETS AND DRUG SUBSTANCES

D. Manjunath Naik[a], V. Krishna Murthi Naik[b] and K. SudhakarBabu[a*]

Department of Chemistry,
Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India-515003.
degavathmanjunath.111@gmail.com

Abstract
A simple, accurate, precise method was developed for the estimation of the Organic Impurities of Teriflunomide in a tablet dosage form and ooptimized separation was achieved on a Symmetry C18 column (250 mm×4.6 mm; 5 µm) using mobile phase composition of water, acetonitrile and Triethylamine in the ratio of 65:35:0.5 (v/v/v), pH 6.0, at a flow rate of 1.0 mL/min in isocratic elution. UV detection was carried out at a wavelength of 250 nm. The temperature was maintained at 50°C. Well-resolved peaks were observed with high numbers of theoretical plates, lower tailing factor and reproducible relative retention time. The method was validated and all the validation parameters were found to be within the acceptance limits.

Key words: Teriflunomide, Phenyl isoxazole, 4-(trifluoromethyl) aniline, stress degradation, RP-HPLC method development, Validation.

Introduction
Teriflunomide (trade name Aubagio, marketed by Sanofi) is an Immunosuppressive Agent. Teriflunomide was investigated as a medication for multiple sclerosis (MS) and act by inhibiting pyrimidinede novo synthesis by blocking the enzyme dihydro-orotateDehydrogenase [I,III, IV&V]. A literature search confirms that there is no method reported for the simultaneous estimation of Teriflunomide and its organic impurities in quantitatively like Phenyl isoxazole and 4-(trifluoromethyl) aniline in pharmaceutical dosage forms of Tablets or in drug substance. Hence the present work aimed to develop a simple stability indicating RP-HPLC method for the separation and quantification of Teriflunomode and its organic impurities like Phenyl isoxazole impurity and 4-(trifluoromethyl) aniline. The main aim of this method was to determine and validate the Teriflunomide and its impurities based on International Conference on Harmonization guidelines [II]. This method was made use of a reproducible procedure for the quantitative analysis of drug samples as the bulk drug and in tablet dosage forms. The designed method was considered as an advisable to develop precise, accurate, simple RP-HPLC method.

Material and Methods

Chemicals, reagents and instruments:

Teriflunomide, Ortho phosphoric acid (H3PO4), Triethylamine (C6H15N), Di-potassium hydrogen phosphate (K2HPO4), Potassium hydroxide (KOH), Acetonitrile and Milli-Q water. Symmetry C-18 250 x 4.6mm, 5µm column, and HPLC instrument equipped with UV-VIS spectrophotometer & PDA detector.

Mobile phase and solutions preparation:

Preparation of Mobile phasese:

Mixed 650 mL of water, 350 mL of acetonitrile and 5 mL of triethyl amine, the pH of solution adjusted to 6.0with diluted orthophospharic acid.

Preparation of Diluent:

Weighted 0.87 g of Di potassium hydrogen phosphate and dissolved in 500 mL of water, and adjusted the pH of the solution to 10.0 with diluted potassium hydroxide solution.Mixed the two solutions(buffer and acetonitrile) in the ratio of 65:40 (%) respectively.

Standard Preparation:

Accurately Weighted and transferred 32 mg of Teriflunamide standard into a 100 ml clean dry volumetric flask, added few of acetonitrile to dissolve the material by sonication and made up to the final volume with diluent.

Further pipetted out 1 mL stock solution into 100 mL of volumetric flask and diluent to volume with diluent. (3.2 µg/ml of Teriflunomide).

Sample Preparation:

Accurately Weighted equivalent weight of 80 mg teriflunamide tablet powder transferred into a 50 ml volumetric flask,added20 ml of acetonitrile and sonicated for 30 min, further the volume was made up with diluent and filtered the solutionwith suitable filter (1600 µg/ml of Teriflunomide).

Degradation studies:

Oxidation:

Accurately Weighted 80 mg of teriflunomide tablet powder was and transferred into 50 mL of volumetric flask, added 20 mL of acetonitrile, sonicated to 30 min, and added10% hydrogen peroxide (H_2O_2).Thesolutionswere kept on bench top for 24 hours. For HPLC study, the resultant solution was diluted to obtain solution which was injected into the system and the chromatograms were recorded to assess the stability of sample.

AcidDegradation Studies:

Accurately Weighted 80 mg of teriflunomide tablet powder was transferred into 50 mL of volumetric flask,added 20 mL of acetonitrile, sonicated for 30 min and added 10ml of 1Nhydrochloride acid. Thesolutionswere kept at 60°C for 24 hours.After degradation, neutralized with 1N of NaOH. For HPLC study, the resultant solution was diluted to get sample solution, this solution wasinjectedintothe HPLC system and the chromatograms wererecordedto get thestabilityofsample.

AlkaliDegradationStudies:

Accurately Weighed80 mg of teriflunomide tablet powder was transferred into 50 mL of volumetric flask,added 20 mL of acetonitrile, sonicated to 30 min, and added 10ml of 1NSodium hydroxide. Thesolutionswere kept at 60°C for 24 hours.After degradation neutralized with 1N of HCl.For HPLC study,theresultantSolutionwasdiluted to obtain samplesolution,this solution wasinjectedintothe system and the chromatograms were recorded to assessthestabilityofsample.

Thermal DegradationStudies:

Accurately Weiged 80 mg of teriflunomide tablet powder was transferred into 50 mL of

volumetric flask, added 20 mL of acetonitrile, sonicated to 30 min, and diluted the solution.For HPLC study, the resultant Solution was diluted to obtain sample solution, this solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

PhotoStabilitystudies:

Accurately Weighed 80 mg of teriflunomide tablet powder was transferred into 50 mL of volumetric flask, added 20 mL of acetonitrile, sonicated to 30 min, and diluted the solution. To the HPLC study, the resultant Solution was diluted to obtain sample solution, this solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

HumidityDegradation Studies:

Accurately Weighed 80 mg of teriflunomide tablet powder was transferred into 50 mL of volumetric flask, added 20 mL of acetonitrile, sonicated to 30 min, and diluted the solution. To the HPLC study, the resultant Solution was diluted to obtain sample solution, this solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

Results and Discussion:

With the progress of International Conference on Harmonization (ICH) guidelines, the determination of a stability-indicating related substance method has developed into more clearly and obligatory. The guidelines necessary for handling of forced degradation studies under different conditions, like acid, base, photo, oxidation, heat and humidity followed by separation of drugs from degradation products Hence for the necessity of separation of several components through the study of stability samples, HPLC has gained reputation in stability studies due to its specificity, sensitivity and high-resolution capacity. The work planned in this research was subjected towards the study of the chromatographic actions of the samples of stress degradation of teriflunamide and its impurities in the tablet dosage formulation. The stability indicating study of present drug has not reported so far in the literature as per our knowledge and motivated us to develop an RP-HPLC- PDA stability indicating test where the degradation products were resolved from the integral drugs.

Method development

The standard drug solution containing 3.2 µg/mL concentrations of both impurities and Teriflunomide was initially used for method development studies, the resultant chromatogram revealed in the Figure 2 and the standard chromatogram was shown in Figure 3.

Method validation

The method was validated as per ICH guidelines. The different validation parameters which were performed are following: linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness, degradation studies and the stability indicating capability.

System suitability test:

System suitability was evaluated with freshly prepared standard solutions. Six replicate standard solution injections were performed and calculated the % RSD for retention time and peak area. Other parameters theoretical plates and tailing factor were measured. System suitability results were tabulated in table 1 and table 2. % RSD values were within the limit 5%.

Linearity:

Linearity parameter was evaluated with standard and its organic impurities solutions by preparing six different concentrations. Linearity levels are LOQ, 25%, 50%, 75%, 100%, 125%, 150% concentrations. All six linearity solutions were injected into the HPLC system

and calculated the correlation coefficient values. Correlation coefficient was calculated for concentration versus peak area. Results were obtained in table 2 &3. Results were satisfactory, correlation coefficient values were above 0.99.

Precision:

Precision was performed for system precision for six replicate standard injections and method precision six replicate sample preparations by spiking of organic impurites. Six replicate solutions were carried out as per the test procedure mentioned in the materials and method section. Peak area, method precision % of RSD results were calculated and tabulated in table 4. Precision results were satisfactory and % RSD values were below 5 %.

Acceptance Criteria: The % RSD should not be more than 5%

Accuracy:

Accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out by different concentration of organic impurities added to the sampleLOQ, 50 %, 100 % and 150 % were evaluated. Accuracy recovery and % RSD were calculated and tabulated in table 5. % of recovery results were between 80 % to 120 % and % RSD values were below 10.0 %.

Robustness:

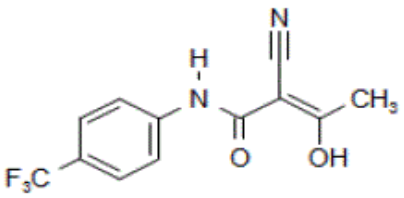
Robustness of the method was performed with flow rate, mobile phase pH, temperature variations evaluated. System suitability was conducted to check the variation changes and results were found satisfactory.

Limit of detection and limit of quantification:

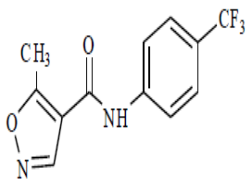
Limit of detection (LOD) is the least concentration of analyte in a sample that can be identified but not quantified. Limit of quantification (LOQ) is defined as least concentration of analyte in a sample that can be estimated with tolerable precision, accuracy and reliability by a specified method under affirmed experimental conditions. The LOD were found to be 0.015 and 0.06 for Teriflunomide and Phenyl isoxazole respectively.The LOQ were found to be 0.051 and 0.210 for Teriflunomide and Phenyl isoxazole respectively.

Degradation Studies:

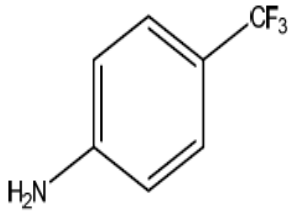
Degradation studies are acid, base, peroxide, thermal, humidity and Photolight conditions were evaluated. Further all stress results and force degradation results were tabulated in the table 6 and the resultant chromatogram revealed in the Figure 4 to 6.



(a)



(b)



(c)

Figure 1: Chemical structures of (a) Teriflunomide (b) Phenyl isoxazole impurity (c) 4-(trifluoromethyl) aniline

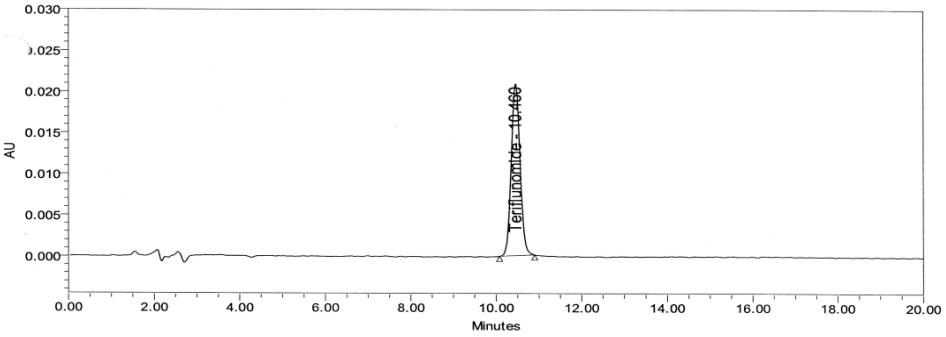


Figure 2: Standard chromatogram of Teriflunimode.

Table 1: System suitability results

Teriflunomide				
Injections	**Retention Time(min)**	**Area**	**USP Plate Count**	**USP Tailing factor**
1	10.458	284158	10973	1.21
2	10.450	284978	10078	1.21
3	10.459	283985	10017	1.21
4	10.451	285146	10180	1.22
5	10.458	284562	10909	1.21
6	10.456	285148	10321	1.22
Mean		284663		
Std. Dev.		508.45		
% RSD		0.2		

Table 2: Linearity concentration table

Linearity level	**Teriflunomide**		**Phenyl isoxazole impurity**	
	Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
LOQ	0.051	4510	0.215	10547
25 %	2.005	195456	2.001	129341
50 %	4.109	385451	3.945	255145
75 %	6.122	585252	5.987	397841
100 %	8.147	785974	7.994	525870

150 %	12.101	1133514	12.178	784578
Correlation coefficient	0.999		0.999	

Table 3: Linearity concentration 4-(trifluoromethyl) aniline

Linearity level	**4-(trifluoromethyl) aniline**	
	Concentration (µg/mL)	**Peak area**
LOQ	24.158	2900
25 %	26.958	3712
50 %	53.158	7512
75 %	80.025	11921
100 %	107.153	15165
150 %	160.481	23147
Correlation coefficient	0.999	

Table 4:Method precision results

Method precision		
S. No	**Phenyl isoxazole impurity**	**4-(trifluoromethyl) aniline**
1.	0.510	0.012
2.	0.511	0.012
3.	0.512	0.011
4.	0.513	0.011
5.	0.512	0.012
6.	0.511	0.012
Mean	0.512	0.0117
S.D	0.00105	0.00051
%RSD	0.2	4.4

Table 5: Accuracy results

Accuracy (%Recovery)			
S. No	**Recovery level**	**Phenyl isoxazole impurity**	**4-(trifluoromethyl) aniline**
1.	LOQ-1	98.1	95.1
2.	LOQ-2	97.5	92.5
3.	LOQ-3	99.3	93.1
4.	50%-1	99.0	91.0

5.	50%-2	98.1	92.1
6.	50%-3	98.5	90.5
7	100%-1	99.9	98.5
8	100%-2	99.4	99.4
9	100%-3	100.2	99.0
10	150%-1	101.5	97.5
11	150%-2	100.1	96.1
12	150%-3	99.2	98.2

Table 6: Degradation results

Stress condition	**% Amount remaining**	**% Amount degraded**	**Peak Purity**	
			Purity Angle	**Purity Threshold**
Normal	100.9	0.046	2.458	6.547
Acid	101.6	0.061	2.458	6.585
Base	102.1	0.038	1.478	8.781
oxidation	87.9	14.051	0.267	1.409
Thermal	102.1	1.201	2.160	5.487
Photolytic	101.1	0.131	2.845	5.182
Humidity	101.4	0.047	3.845	6.894

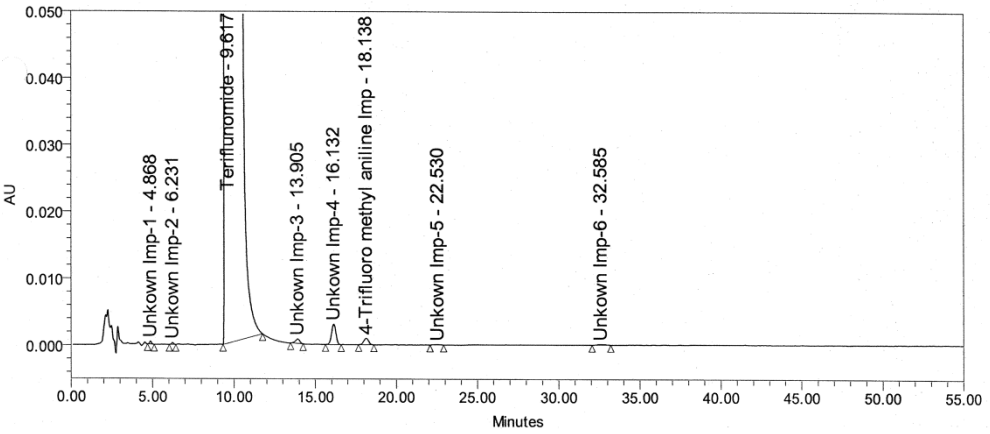


Figure 3: Acid degradation chromatogram of Teriflunimide.

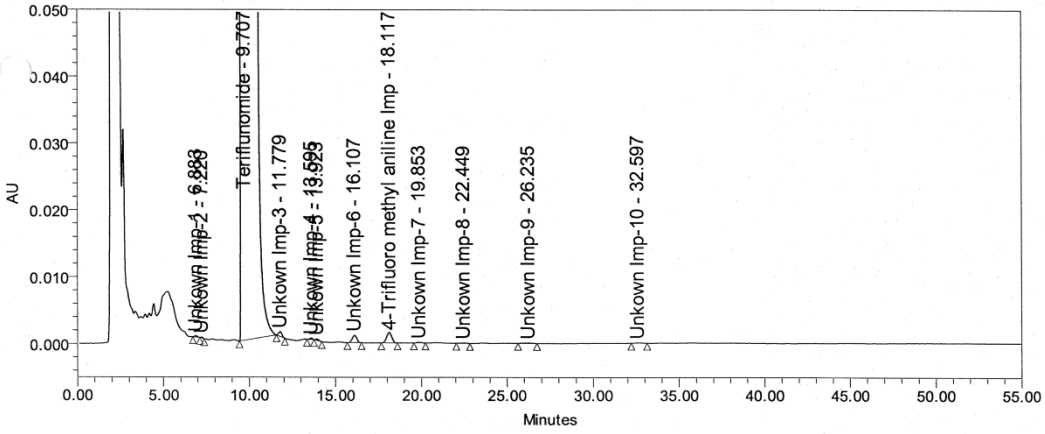


Figure 4: Base degradation chromatogram of Teriflunimide.

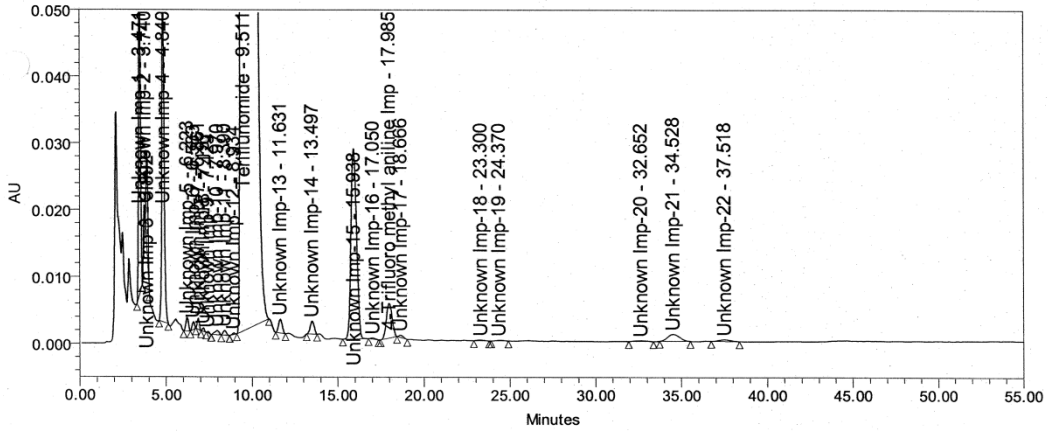


Figure 5: Oxidation degradation chromatogram of Teriflunimide.

Results and Discussions:

The current study describes new and simple, reliable, economic elution RP-HPLC-PDA method for the estimation of Teriflunomide. The forced degradation studies were conducted for the Teriflunoide by using several degradation conditions like oxidation, acidic, alkali, thermal, and photolytic conditions and proposed method was effectively employed from the resolution of employed samples peaks. To our present knowledge, no such detailed and stability indicating method has been presented for the impurities of this drug. The developed method finished use of PDA as a tool for peak integrity and purity confirmation. Therefore the proposed study method can be used for quantification of teriflunamide impuries in bulk and pharmaceutical dosage form. Finally, this method was carefully validated; as a result, it can be suggested for routine analysis and for testing quality through stability studies of the drugs.

Acknowledgement

The authors thankful to the Department of chemistry, S. K. University, Ananthapuramu, India for their support and encouragement.

References

I Bruneau JM, Yea CM, Spinella-Jaegle S, Fudali C, Woodward K, Robson PA, Sautès C, Westwood R, Kuo EA, Williamson RA, Ruuth E. Purification of human dihydro-orotate dehydrogenase and its inhibition by A77 1726, the active metabolite of leflunomide. The Bioch J., 1998; 336(2): 299–03.

II International Conference on Harmonization (ICH); Validation of analytical procedures: Methodology, Q2B (CPMP/ICH/281/95), 1995. Available at: http://www.ich.org.

III Marriott J, O'Connor P. Emerging therapies in relapsing-remitting multiple sclerosis. Rev Recent Clin Trials, 2010; 5(3): 179–88.

IV O'Connor PW, Li D, Freedman MS, Bar-Or A, Rice GP, Confavreux C, et al .A Phase II study of the safety and efficacy of Teriflunomide in multiple sclerosis with relapses. Neurology, 2006; 66(6): 894–900.

V Osiri M, Shea B, Robinson V, Suarez-Almazor M, Strand V, Tugwell P, Wells G. Leflunomide for treating rheumatoid arthritis. Cochrane database of syst rev (Online), 2003; (1): CD002047.

Received on December 20, 2024,