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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TERIFLUNOMIDE TABLETS DOSAGE FORM BY RP-HPLC

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Abstract:
A simple Reverse Phase High Performance Liquid Chromatographic method has been developed and subsequently validated for Teriflunomide tablets. The separation was carried out by using a Buffer : acetonitrile (65:35). The detection was carried out at 250nm. The column was Zorbax Eclipse XDB, C8,150 x 4.6mm, 5µl. The flow rate was selected as 1.5ml/min. The Retention time of Teriflunomide tablets was found to be 6.0. The asymmetry factor or tailing factor of Teriflunomide tablets was found to be 1.2, which indicates symmetrical nature of the peak. The number of theoretical plates of Teriflunomide tablets was found to be 7391, which indicates the efficient performance of the column. These parameters represent the specificity of the method. From the linearity studies, specified concentration levels were determined. It was observed that Teriflunomide tablets were linear in the range of 5% to 150% for the target concentration by RP-HPLC. The linearity range of Teriflunomide tablets 5% to 150% was found to obey linearity with a correlation coefficient of 0.999. The validation of the proposed method was verified by system precision and method precision by RP-HPLC. The %RSD of system suitability for Teriflunomide tablets was found to be 0.25. The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The robustness studies were performed by changing the flow rate, filters and wavelength. The ruggedness study was also performed. The analytical method validation was carried out by RP-HPLC as per ICH guidelines and given below are the tables are the summary of the results.

Keywords: *Teriflunomide, RP-HPLC, Method development, Validation*

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INTRODUCTION:

Teriflunomide is a pyrimidine synthesis inhibitor with anti-inflammatory and immunomodulatory properties used to treat patients with the relapsing-remitting form of multiple sclerosis. Teriflunomide is the active metabolite of leflunomide, and it acts as an immunomodulatory agent by inhibiting pyrimidine synthesis. It is marketed under the name Aubagio® and is indicated for the treatment of multiple sclerosis, specifically relapsing forms. The FDA label states an important warning about the risk of hepatoxicity and teratogenicity for patients using teriflunomide. The exact mechanism by which teriflunomide acts in MS is not known. What is known is that teriflunomide prevents pyrimidine synthesis by inhibiting the mitochondrial enzyme dihydroorotate dehydrogenase, and this may be involved in its immunomodulatory effect in MS.[1-3] IUPAC name is (2Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl) phenyl]but-2-enamide. Molecular formula $C_{12}H_9F_3N_2O_2$. Molecular Weight is 270.2.

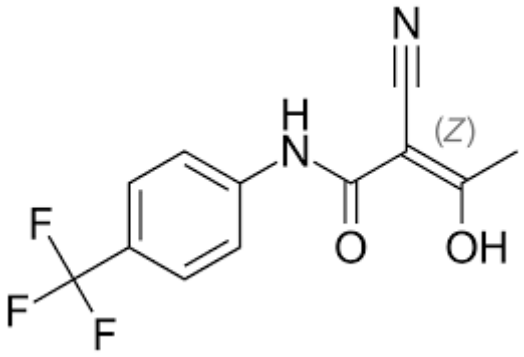

Figure 1: Structure of Teriflunomide

The literature survey disclosed various methods for the estimation of TEF in API, marketed formulations and biological fluids. The detailed information on the various methods available are as follows; chromatographic methods such as HPLC [4, 5], UPLC [6], RP-HPLC [7, 8], LC-MS [9-12]. In all the reported techniques, the overall solvent consumption, cost per analysis and overall time required for analysis were much more. Furthermore, very few HPLC method has been reported so far, for the estimation of TEF in the marketed formulation. Therefore, the current work is directed towards the development of a novel HPLC method for the determination of TEF and its validation according to ICH guidelines.

MATERIALS AND METHODS:

Chemicals and Reagents:

Teriflunomide Gift samples obtained from Hetero labs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 250 nm with column Zorbax Eclipse XDB C8 column (150 x 4.6 mm, 5 µm particle size), dimensions at Ambient temperature. The optimized mobile phase consists of Buffer and ACN (65:35). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Diluent Preparation:

Mobile phase is used as Diluent.

Preparation of Standard solution:

Weigh accurately about 50 mg of Teriflunomide RS/WRS and transfer to a 200 mL volumetric flask. Add 140 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well.

Transfer 10 mL of standard stock preparation into a 50 mL volumetric flask. Dilute to volume with diluent and mix well. (Concentration of about 50 µg/mL of Teriflunomide).

Preparation of Sample solution:

Determine the Average weight using not less than 20 tablets. Weigh and finely powder not less than 20 tablets. Weigh accurately and transfer tablet powder equivalent to about 25 mg into a 100 mL volumetric flask. Add 70 mL of diluent and sonicate for 30 minutes with intermittent shaking. Dilute to volume with diluent and mix well. Centrifuge a portion of the above solution at 3500 rpm for 10 minutes. Transfer

5 mL of the supernatant solution to a 25 mL volumetric flask, dilute to volume with diluent and mix well.
Filter a portion of the above solution through a 0.45 µm PVDF filter after discarding atleast the first 4 mL of the filtrate.
(Sample preparation, concentration of about 50 µg/mL of Teriflunomide).

Procedure:
Equilibrate the column with mobile phase for not less than 30min at a flow rate of 1.0 l/min. Separately inject 10 µl of Blank (diluent), Standard solution (five times) and Sample solution into the chromatographic system. Record the chromatograms and measure the peak responses.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:
To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. The overlay spectrum of Teriflunomide was obtained and the Teriflunomide showed absorbance's maxima at 250 nm. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Zorbax Eclipse XDB C8 column (150 x 4.6 mm, 5 µm particle size), the mobile phase of composition Buffer and ACN (65:35) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation:
The proposed validated method was successfully applied to determine Teriflunomide in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:
Linearity: The linearity study was performed for the concentration of 2.5 µg/ml to 75 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies:
The accuracy was determined by help of recovery study. The recovery method carried out at three level 5%, 50%, 100%, 200%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Teriflunomide and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies:
precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness:
To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 6.

Robustness:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

Forced degradation studies:
The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, The ICH definition of stress testing for the drug product is "studies undertaken to assess the effect to severe conditions on the drug product. Such studies include photo stability testing and specific testing on certain products like metered dose inhalers, creams, emulsions etc. As per FDA guideline "Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods". The results are shown in table 8.

RESULTS AND DISCUSSION:

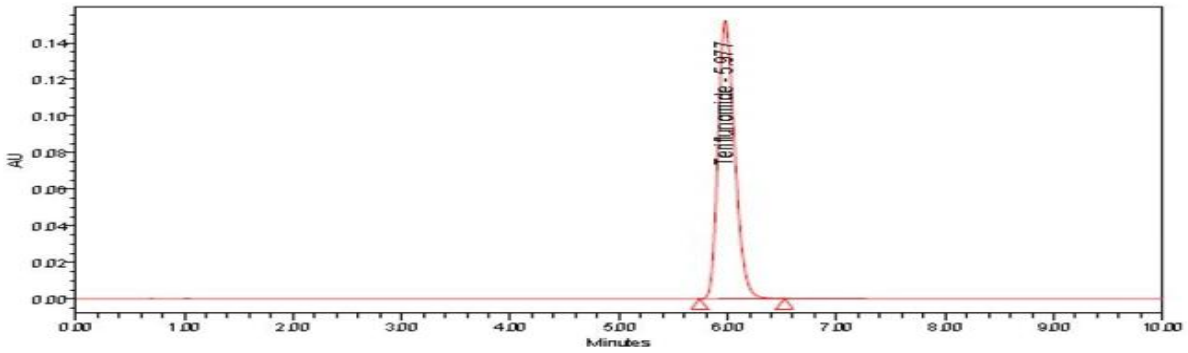

Figure 2: Standard chromatogram

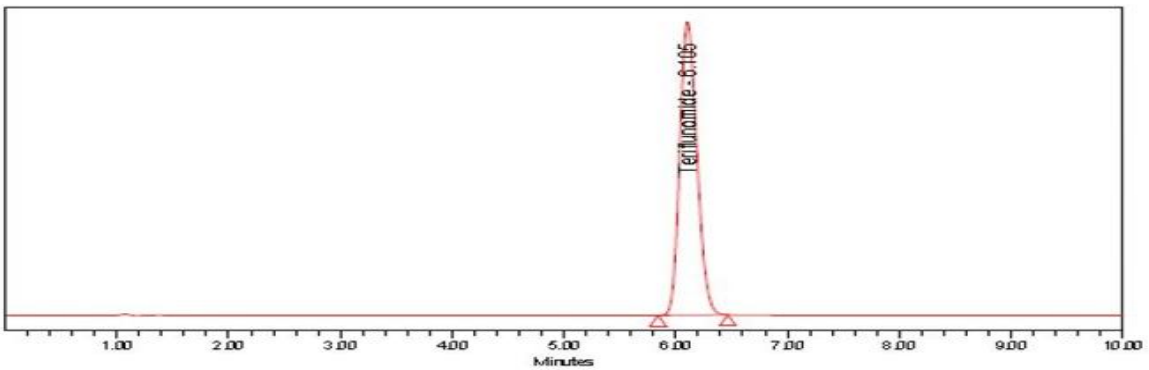

Figure 3: Sample chromatogram

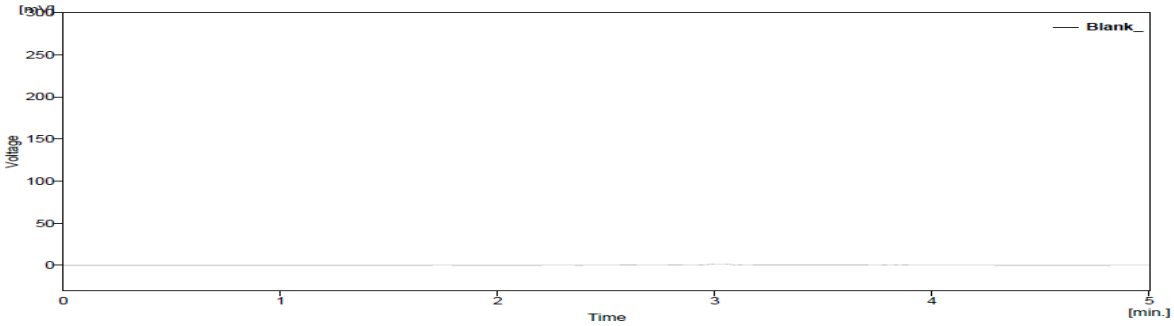

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Injection	Peak Area	USP Plate count	USP Tailing
1	1616310	7147	1.27
2	1617462	7192	1.28
3	1621285	7096	1.28
4	1618228	7215	1.27
5	1610144	7220	1.28
SD	1616686	---	---
% RSD	0.25	---	---

Table 2: Assay results for Teriflunomide

TERIFLUNOMIDE	
std. purity	99.87
Amount found in mg	**2.03**
Assay(%purity)	**101.25**

Table 3: Linearity results of Teriflunomide

Linearity Level	Concentration (µg/mL)	Average Area
L1-5%	2.502	83096
L2-10%	5.004	154525
L3-25%	12.512	414647
L4-50%	25.024	823580
L5-75%	37.537	1241003
L6-100%	50.049	1650624
L7-150%	75.074	2510914

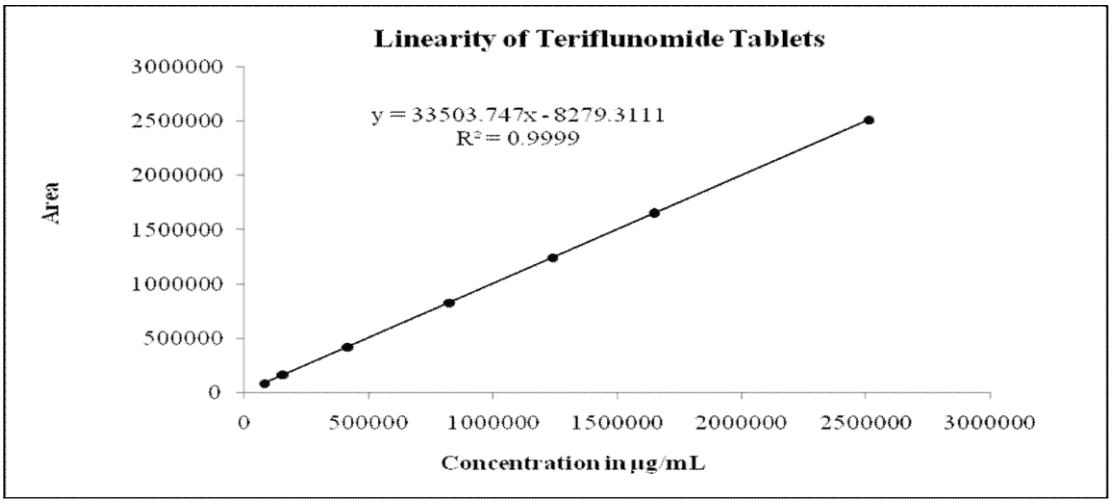

Figure 5: Linearity graph for Teriflunomide

Table 4: Showing accuracy results for Teriflunomide

Sample No.	Theoretical (%)	Mean Peak area	% Recovery	Mean (%) Recovery	% RSD
1	5	79271	99.37	99.63	0.45
2	5	79243	99.37		
3	5	79893	100.15		
1	50	784059	100.65	100.44	0.21
2	50	786797	100.22		
3	50	789985	100.46		
1	100	1589177	101.16	101.05	0.28
2	100	1587887	100.73		
3	100	1593464	101.28		
1	200	3172132	101.92	101.15	0.73
2	200	3155725	101.12		
3	200	3111902	100.43		

Table 5: Precision results for Teriflunomide

Sample No.	Area	%Assay
1.	1570314	**97.87**
2.	1574009	**98.18**
3.	1594849	**99.27**
4.	1590749	**99.07**
5.	1606478	**100.12**
6.	1609080	**100.31**
Mean		**99.13**
Standard Deviation		**0.98848**
% RSD		**0.99**

Table 6. Ruggedness results of Teriflunomide

Injection No.	Analyst-1	Analyst-2
	Peak area	Peak area
1	1616310	1546066
2	1617462	1556162
3	1621285	1552999
4	1618228	1555638
5	1610144	1555822
Mean	1616686	1553337
% RSD	0.25	0.27
Tailing factor	1.27	1.39
Plate count	7147	7861

Table 7: Robustness results for Teriflunomide

Parameters		Retention Time (min)	Mean Peak area (n=5)	%RSD	USP Tailing factor	USP Plate count
Normal Condition (1.0mL/min, 30°C, pH 2.4 Buffer : ACN (650:350)		6.564	1651509	0.61	1.25	7816
Flow Rate Minus	1.35 mL/min	7.292	1846396	0.23	1.26	8080
Flow Rate Plus	1.65 mL/min	5.996	1504834	0.35	1.24	7658
Mobile phase pH Minus	2.2	9.196	1585481	0.23	1.16	9241
Mobile phase pH Plus	2.6	5.683	1684507	0.21	1.33	6907
Column Temperature Minus	25°C	6.775	1677411	0.33	1.25	7756
Column Temperature Plus	35°C	6.144	1666426	0.09	1.25	8.35
Mobile Phase composition Variation 1	Buffer : ACN 670:330	9.215	1648619	0.17	1.23	8693
Mobile Phase composition Variation 2	Buffer:ACN 630:370	5.292	1665191	0.30	1.26	7259

Table 8: Forced degradation study of Teriflunomide

Sample Name	Condition	% Assay	% Degradation	Purity Angle	Purity Threshold
Control Sample	NA	99.77	NA	0.034	0.201
Spike Sample	NA	NA	NA	0.036	0.213
Acid Stress Sample	3 mL 5N HCl, heated on a water bath at 80°C for 3 hours.	78.97	20.80	0.034	0.202
Base Stress Sample	3 mL 0.1N NaOH, heated on a water bath at 60°C for 8 hours.	99.65	0.12	0.034	0.201
Peroxide Stress Sample	3 mL 30% H_2O_2, heated on a water bath at 80°C for 30 minutes.	84.75	15.02	0.033	0.204
UV light Stress Sample	Stressed under UV light for 24 hours.	100.77	NA	0.046	0.204
Heat Stress Sample	Heated in an oven at 105°C for 1 hour and 30 minutes.	98.90	0.87	0.044	0.203

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Teriflunomide in its Tablet form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Teriflunomide in Tablet dosage forms.

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