Acta Biomedica Scientia

e - ISSN - 2348 - 2168
Print ISSN - 2348 - 215X

www.mcmed.us/journal/abs

Research Article

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TERIFLUOMIDE IN TABLET DOSAGE FORM

K.Dinesh Babu[1*], D.Babu Ananth[2], C.N.Arul Anand Raj[1]

[1]Department of Pharmaceutical Analysis, College of Pharmacy, Mother Theresa Post and Research Institute of Health Sciences, Puducherry-605003, India.
[2]Department of Pharmaceutical analysis, Edayathangudy.G.S Pillay College of Pharmacy, Nagapattinam- 611002, Tamilnadu, India.

ABSTRACT

The drug analysis plays an important role in the development, manufacture and therapeutic use of drugs. Standard analytical procedure for newer drugs or formulation may not be available in Pharmacopoeias; hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid. Hence, it is proposed to improve the existing method and to develop a new method and validate for the estimation of Terifunomide in pharmaceutical dosage form. 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 has been 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. From the linearity studies, specified concentration levels were determined. It has been 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 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.

Keywords :-

Access this article online	
Home page: http://www.mcmed.us/journal/abs DOI: http://dx.doi.org/10.21276/abs.2018.5.2.17	**Quick Response code**

Received:25.10.18	**Revised**:12.11.18	**Accepted**:15.12.18

INTRODUCTION

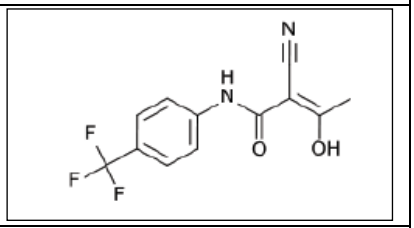
Teriflunomide is an Immunosuppressive Agent, a medication for multiple sclerosis (MS) and act by inhibiting pyrimidine synthesis by blocking the enzyme dehydrogenase. The drug was approved by the FDA on September 13, 2012 and in the European Union on August 26, 2013. Extensive literature survey reveals that few HPLC and LC-MS/MS methods are available for estimation of Teriflunomide individually and in combination with other drugs. There is no stability indicating analytical methods were reported for estimation of Teriflunomide. Hence, the objective of the work is to develop and validate simple, rapid, sensitive and accurate stability indicating HPLC method for the estimation of Teriflunomide tablet dosage form [1-3].

The study involves in gathering the background information about physic chemical properties and to determine if special handling/ treatment of sample are needed. From the physiochemical property select detector parameters, it was aimed to calculate approximately separation parameters/ isocratic or gradient mode, perform forced degradation experiments to challenge method, Optimization separation conditions, Summarize

Corresponding Author **K.Dinesh Babu** Email: - dinesh.dinpharmaus@gmail.com

methodology and finalize documentation, analysis of marketed formulations and validate method[4-6].

DRUG PROFILE [7-9]

Proper Name	:	Teriflunomide
Synonyms	:	Teriflunomide
Structure	:	
Molecular formula	:	$C_{12}H_9F_3N_2O_2$
IUPAC name	:	(Z)-2-Cyano-3-hydroxy-but-2-enoic acid-(4-trifluromethyl phenyl)-amide (or) 2-Cyano-3-hydroxy-N-[4-(trifluromethyl)phenyl]-2(Z)-butenamide
Molecular weight	:	270.21 g/mol
CAS No.	:	163451818
Melting point	:	229 - 232°C
Description	:	White to almost white powder
Solubility	:	Sparingly soluble in acetone; slightly soluble in methylene chloride; very slightly soluble in acetonitrile; insoluble in water, ethanol and isopropyl alcohol.
pH (1% in water)	:	3.19
Storage	:	Store at controlled room temperature, 15 to 30°C.
Category	:	Teriflunomide is the active metabolite of leflunomide, and it acts as an immunomodulatory agent by inhibiting pyrimidine synthesis.

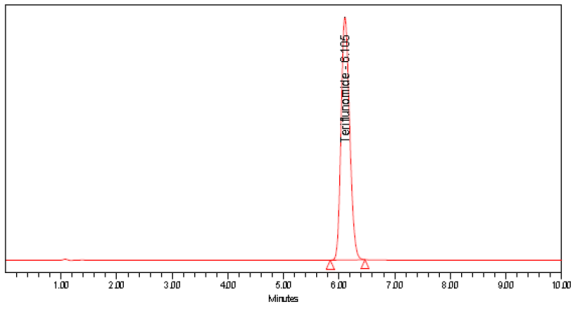
MATERIALS AND METHODS

1. Method development [10]

The objective of this experiment was to optimize the assay method for estimation of Teriflunomide tablets based on the trails made. 3 trials were performed among which the optimized method is mentioned bellow

Trial (Optimized Method):

Buffer preparation	:	20Mm of Potassium dihydrogen orthophosphate buffer pH 2.40, filter through 0.45µm membrane filter and degas.
Mobile Phase	:	Buffer and ACN (65:35). Sonicated to degas.
Diluent	:	Water : ACN (30 : 70)
Chromatographic conditions:		
Column	:	Zorbax Eclipse XDB C8 column (150 x 4.6 mm, 5 µm particle size)
Column temperature	:	30°C
Sample temperature	:	5°C
Elution mode	:	Isocratic
Flow rate	:	1.0 ml/min
Injection volume	:	10µl
Detector wave length	:	250nm
Run time	:	10 min.
Seal wash	:	90:10 (Water: ACN)
Needle wash	:	10:90 (Water: ACN)
System Suitability	:	USP Tailing Factor – NMT 2.0 and Plate count - NLT 2000.
Conclusion	:	The peak was observed with good tailing and good shape, with plate count above 2000 (10721) and tailing factor below 2 (1.3). And this method was finalized for assay of Teriflunomide Tablets.



Peak Results

	Name	Vial	Injection	RT	Area	USP Plate Count	USP Resolution	USP Tailing	Int Type
1	Teriflunomide	2	1	6.105	1664517	7391		1.19	BB

System suitability:

The theoretical plates value for the Teriflunomide peak from standard solution should be NLT 2000.

Tailing factor for Teriflunomide peak should be NMT 2.0

The % RSD for the Teriflunomide peak for 5 replicate injections of standard solution should be NMT 2.0

Calculations:

Quantity of Teriflunomide Present in the tablet as % of labelled amount:

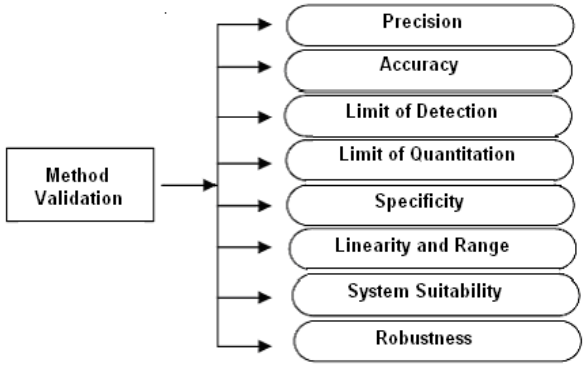
$$\frac{A_T}{A_S} \times \frac{Ws}{200} \times \frac{10}{50} \times \frac{100}{W_T} \times \frac{25}{5} \times \frac{P}{100} \times \frac{AW}{L} \times 100$$

A_T	:	Peak area of Teriflunomide from the chromatogram of the assay preparation
A_S	:	Mean peak area of Teriflunomide from the chromatogram of the standard preparation.
Ws	:	Weight of Teriflunomide working standard taken, in mg
W_T	:	Weight of tablet powder taken, in mg
P	:	Potency of Teriflunomide working standard used in percent on as is basis
L	:	Label claim in mg
AV	:	Average weight of tablet in mg

METHOD VALIDATION [18-21]

The word "Validation" means "Assessment" of validity or action of proving effectiveness.

ICH defines validation as "establish the documented evidence which provides a high degree of assurance that a specific process will consistently produce a product of predetermined specifications and quantity attributes".



Analytical method validation for Teriflunomide tablets

RESULTS AND DISCUSSION

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 column C8, Injection volume of 10 µl is injected and eluted with the Mobile phase (Buffer and ACN, in the ratio of 65:35) which was pumped at a flow rate of 1.5 ml at 250 nm. The peak of Teriflunomide was found well separated at 6.0 min. 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. The results are found to be complying with the acceptance criteria for each of the parameter. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity and hence it is suitable for routine analysis as well as for stability analysis [11].

CONCLUSION

A HPLC method for Teriflunomide tablets was developed and validated in tablet dosage form as per ICH guide lines. The results of this validation are as summarized in the report. The results are found to be complying with the acceptance criteria for each of the parameter. Waters Alliance HPLC (Empower software with PDA detector) with Zorbax Eclipse XDB, C8,150 x 4.6mm, 5µ column, Injection volume of 10 µl is injected and eluted with the Mobile phase (Buffer and ACN, in the ratio of 65:35) which was pumped at a flow rate of 1.5 ml at 250 nm. The peak of Teriflunomide was found well separated at 6.0 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity and hence it is suitable for routine analysis as well as for stability analysis.

REFERENCES

1. Ewing GW. (1960) *Instrumental Methods of Chemical Analysis*, McGraw Hill Publishing Company Ins., 2nd Ed., 3.
2. Hohat H. Willard, Lunne L. Merrit, John A. (1999) Instrumental methods of analysis, 7th edition New Delhi: CBS Publishers.
3. International Conference on Harmonization, 1996. Validation of Analytical Procedures: Methodology, Federal Register, 1-8.
4. International Conference on Harmonization, (1995) Draft Guideline on Validation of Analytical Procedures, Definitions and Terminology, Federal Register, 26, 11260.
5. Lloyd R, Snyder, Joseph J, Kirkland, Joseph L, Glajesh. (1997) Practical HPLC method development. 2nd edition, pp 1-14.

6. Sharma BK. (1994) Instrumental Methods of Chemicals Analysis. 13th edition, Goel Publisher House, Meerut, 7.
7. Skoog DA, West DM and Holler FJ. *Fundamentals of Analytical Chemistry*, Saunders College Publishing, New York, 6th Ed., 713.
8. Synder KL, Krikland JJ and Glajch JL. (1983) Practical HPLC Method Development 2nd Edn, Wiley-Interscience Publication, USA, 653-661.
9. *United State Pharmacopoeia*, (2000) Vol. I & II, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 1923.
10. *United State Pharmacopoeia*, (2000) Vol. I & II, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 2149.
11. Vijay Malik, Drugs and Cosmetics Act 1940, 16th Edn., Eastern Book Company, Lucknow, 5.

Cite this article:
Dinesh Babu K, Babu Ananth D, Arul Anand Raj CN. RP-HPLC analytical method development and validation of terifluomide in tablet dosage form. *Acta Biomedica Scientia*, 2018;5(2):123-126. DOI: http://dx.doi.org/10.21276/abs.2018.5.2.17