IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2020 Vol.:18, Issue:2

A Review on Polymorphism of Teriflunomide and Its Absolute Configuration by Single Crystal X-Ray Diffraction

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Cheddi Srinivasa Rao, Vangala Ranga Reddy*, Duddu Swapna, Tallapaneni Venkateswarlu, Kota Satyanarayana, Gopal Vaidyanathan

Natco Pharma Ltd, Natco Research Centre, B-13, Industrial Estate, Sanath Nagar, Hyderabad. 500 018, India.

Submission: 23 April 2020
Accepted: 01 May 2020
Published: 30 May 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Single Crystal; Torsion angle; Micronization; Particle size; Degree of crystallinity

ABSTRACT

Teriflunomide is a recently developed orally active immunosuppressant used in the treatment of multiple sclerosis. The polymorphic screening studies of API in different single and binary solvents reveals the existence of a single polymorph. The structural configuration of Teriflunomide API was confirmed as Z around the double bond from the torsion angle C4-C3-C2-O1, -1.0(2)°. The micronization of API does not impact the thermodynamic and kinetic behavior. The physical properties of Teriflunomide API were thoroughly characterized by solid-state characterization techniques like Powder X-ray diffraction (PXRD), Differential Scanning Calorimetry (DSC), Dynamic Vapor Sorption (DVS) and Microscopy (PLM) techniques.

INTRODUCTION

Teriflunomide (**Figure no. 1**) belongs to a novel class of orally active drugs used in the treatment of an orphan disease multiple sclerosis [1]. Chemically it is (*Z*)-2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl) phenyl]-2-butenamide. Teriflunomide is the active metabolite of leflunomide [2], a disease-modifying agent used in the treatment of rheumatoid arthritis and psoriatic arthritis. It acts by inhibiting *tyrosine kinase*. Teriflunomide belongs to BCS class-II which shows low solubility and high permeability. Several newer APIs are poorly water-soluble, adding to the challenge of designing an optimal formulation. There are various ways to improve drug substance solubility's, such as chemical modification (e.g., prodrugs), use of co-solvents or other excipients (surfactants, phospholipids), and manipulation of particle size/morphology. Particle size reduction through milling or micronization is the most commonly used technique to increase specific surface area and thereby leading to enhanced dissolution and thus impact bioavailability. It is also important to conduct thorough studies to understand the degree of crystallinity and the stability of micronized API which generates an amorphous phase during the micronization process which can cause severe changes in thermodynamic and kinetic properties of the molecule [3].

The stereochemistry across the double bond i.e. *E* or *Z* is very important for the activity of Teriflunomide. The ultraviolet and nuclear magnetic resonance spectra are mostly used tools to characterize stereochemistry across the double bond. However, in the case of Teriflunomide, we could not get any meaningful conclusions due to the non-feasibility of *E* isomer synthesis. In the present study, we attempt and succeed in growing single crystal to establish structural configuration across the double bond of teriflunomide. The physical characteristics of micronized API and the stability studies were carried to understand the stability of API. Further, the API was thoroughly characterized by solid-state characterization techniques like powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), dynamic vapor sorption (DVS) and particle size analysis are employed to develop a stable and efficient drug product [4].

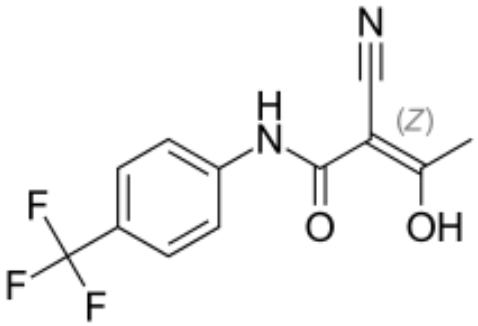


Figure No. 1: (*Z*)-2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl) phenyl]-2-butenamide

MATERIALS AND METHODS

Materials:

Synthesis of Teriflunomide: Teriflunomide was synthesized using a patented procedure developed by NATCO Pharma Ltd., [5].

Polymorph screening in single and binary solvents:

Teriflunomide (1.0 g) was taken in a conical flask and a minimum amount of solvents such as ethyl acetate, acetonitrile, tetrahydrofuran, methanol, and acetone was added to dissolve the sample at 40 °C. The clear solution was left for 24hrs at 25 °C. The separated solid mass was filtered and dried at RT. Teriflunomide (1.0 g) was taken in a conical flask and a minimum amount of solvents such as dimethylformamide and acetic acid was added to dissolve the sample at 40 °C. Then add anti-solvent such as methanol and water till the precipitation was observed. The precipitated samples were left for 24 hrs at 25 °C. The separated solid mass was filtered and dried at RT.

Single Crystal Preparation:

Teriflunomide (10 g) was dissolved in ethyl acetate (80 mL) at 40° C and filtered to remove any particulate matter. The filtered solution was left at 25 °C for 24 hr. The separated crystals were filtered and a suitable crystal was selected by observing the shape and size in a microscope. The un-fractured crystal was mounted to Single Crystal X-ray diffractometer as shown in **Figure no. 2**.

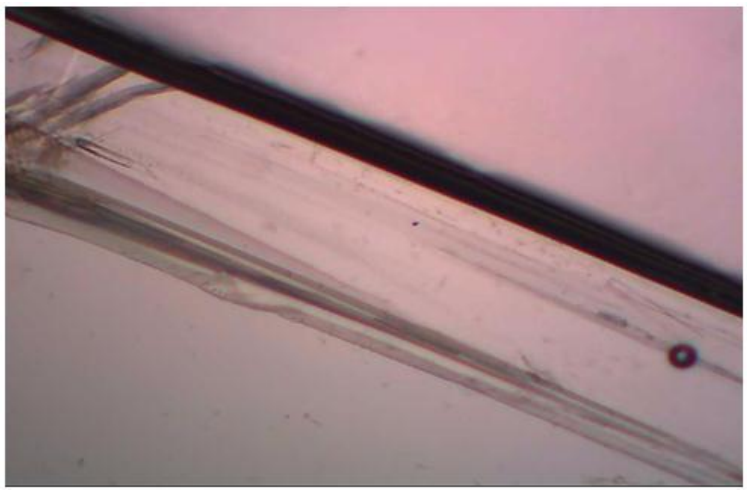


Figure No. 2: Microscopic image of teriflunomide single crystal at 40X

METHODS

X-ray Powder Diffraction (PXRD):

The Powder X-ray Diffractograms were obtained by PANalytical, X'Pert Pro diffractometer. A silicon standard was used to check the instrument peak position. The sample was exposed to CuKα1 (Å) (1.5406) radiation with a voltage and current of 45 (kV) and 40 (mA) respectively. The X-ray powder diffraction was collected with a scan range of 3.0° to 50.0° 2θ. Silica standard was used to check the 2-theta peak position.

Differential Scanning Calorimetry (DSC):

The thermal profiles were produced using a Mettler Toledo DSC. Approximately 3.9 mg of the sample was weighed into 40 µL alumina pans. Samples were heated from 30°C to 300 °C, at a rate of 10 °C/min in an N_2 flow of 40 mL/min. An Indium standard was used to check the instrument performance.

Dynamic Vapor Sorption (DVS):

The isotherms were produced using an SMS DVS analyzer. Typically a sample size of about 40 mg was loaded into the sample pan and the sample was analyzed on DVS automated sorption analyzer at an isothermal temperature of 25 °C. The absorption of relative humidity was increased from 0% to 90% RH with an increment of 10% RH. The description of relative humidity was then decreased similarly to accomplish a full cycle.

Microscopy:

Labomed Digital Trinocular Microscope was used to analyze the shape of the crystals grown. The field and aperture diaphragms were adjusted to ensure the optimum contrast and depth of the field.

Particle size determination by Microscope using particle size analyzer:

The microscopic images were produced using Carl Zeiss optical Microscope at 40X magnification and particle size was determined by using ipvPClass particle size analyzer software 2.0.

Single-crystal X-ray structure analysis:

The crystal data was collected at 100 K on a Bruker D8 QUEST instrument with an IμS Mo micro source (λ = 0.7107 A°) and a PHOTON-100 detector. The raw data frames were reduced and corrected for absorption effects using the Bruker Apex 3 software suite programs [6]. The structure was solved using the intrinsic phasing method and further refined with the SHELXL [7] program and expanded using Fourier techniques. Anisotropic displacement parameters were included for all non-hydrogen atoms. N bound H atoms and O bound H atoms were located in difference Fourier maps and their positions and isotropic displacement parameters were refined. All C bound H atoms were positioned geometrically and treated as riding on their parent C atoms [C-H = 0.96 Å, and U_{iso} (H) = 1.5U_{eq}(C) for methyl H atoms]. The final refinement converged to R= 0.0599 (Rw= 0.1768) for 2515 independent reflections with I≥ 2σ (I) and R= 0.0723 (Rw= 0.1833) for all unique reflections. The goodness of fit S was 1.107.

RESULTS AND DISCUSSION

Crystallization studies (Screening for Polymorphic forms):

Polymorphic screening of Teriflunomide involves recrystallization of the drug substance from single and binary solvents to identify and to acquire the most stable or novel polymorph. The polymorphic form obtained from individual and combination of solvents was evaluated by X-ray and thermal (DSC) techniques. The observations from crystallization studies are tabulated below in Table-1 and relevant diffraction patterns and thermal data are shown from **Figure no. 3** to **Figure no. 6**.

Table No. 1: Observations from the re-crystallization studies

Recrystallization Solvent		Identification by PXRD	DSC Melting peak Temperature (°C)
Fig. 3	**Fig. 4**		
Acetone	DMF + MeOH	Unique diffraction under X-ray for all the samples during re-crystallization in different solvents	The same thermal behavior of all the samples resulted in the melting point at about 230 °C.
Acetonitrile	DMF + H_2O		
THF	AcOH + H_2O		
Methanol	-NA-		

The above studies reveal the fact that the Teriflunomide API shows no propensity to re-crystallize-out in different single and binary solvents due to its molecular rigidity and leads to single polymorph [8].

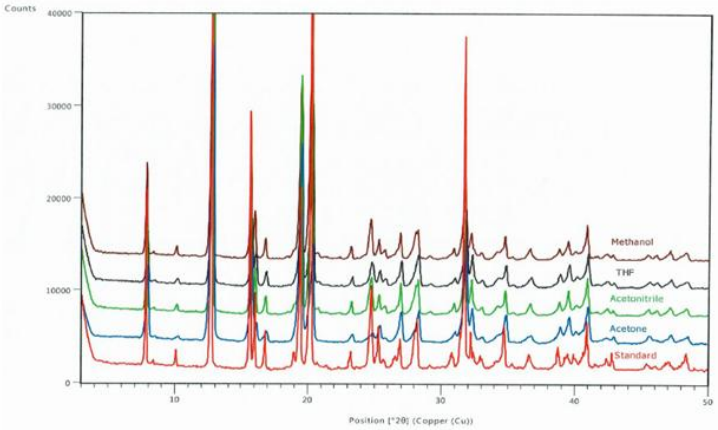


Figure No. 3: Overlaid PXRD pattern of Teriflunomide API recrystallized from different solvents

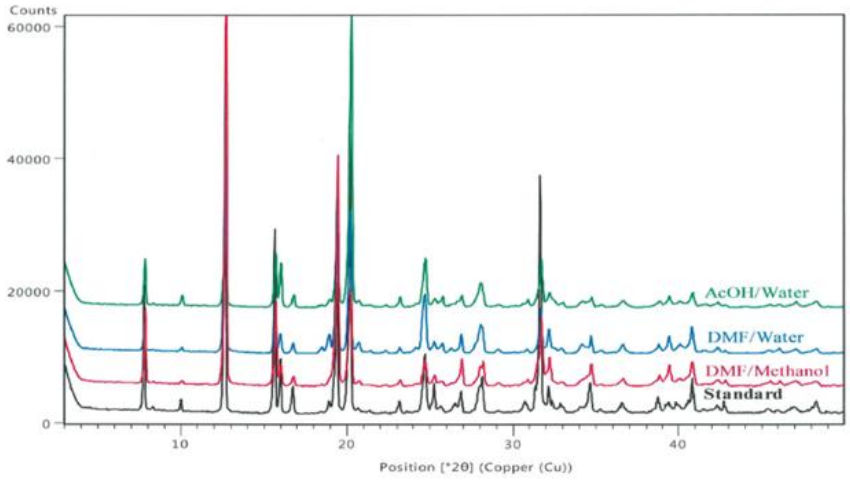


Figure No. 4: Overlaid PXRD pattern of Teriflunomide API recrystallized from different binary solvents

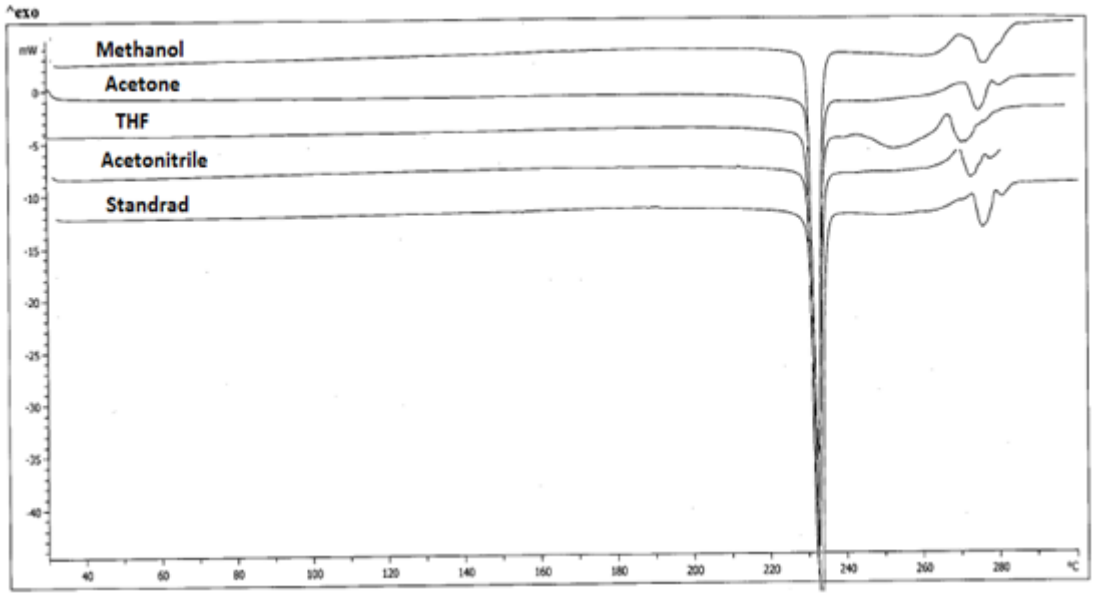


Figure No. 5: Overlaid DSC thermogram of Teriflunomide API recrystallized from different solvents

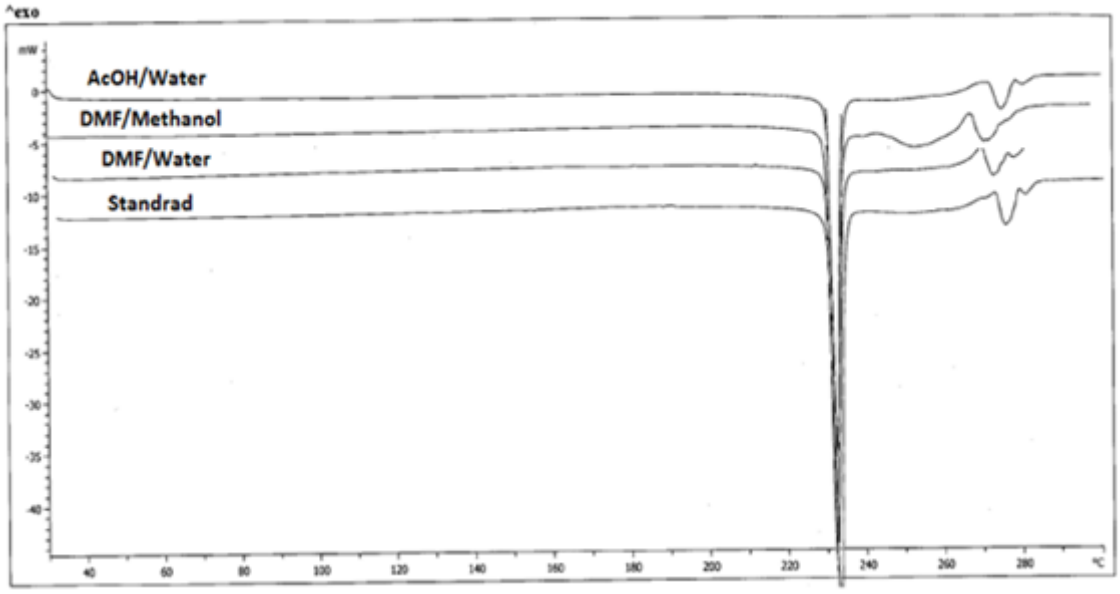


Figure No. 6: Overlaid DSC thermogram of Teriflunomide API recrystallized from different binary solvents

Micronization:

As the drug substance corresponds to BCS Class-II, the reduction of particle size is one of the approaches to achieve the desired solubility of the solid form. We selected the same approach by modifying the particle size/morphology for improving solubility which enhances dissolution and bioavailability of the material [9]. However, the micronized material was thoroughly characterized by PXRD, DSC, and Microscopy.

Characterization of micronized API:

Microscopy:

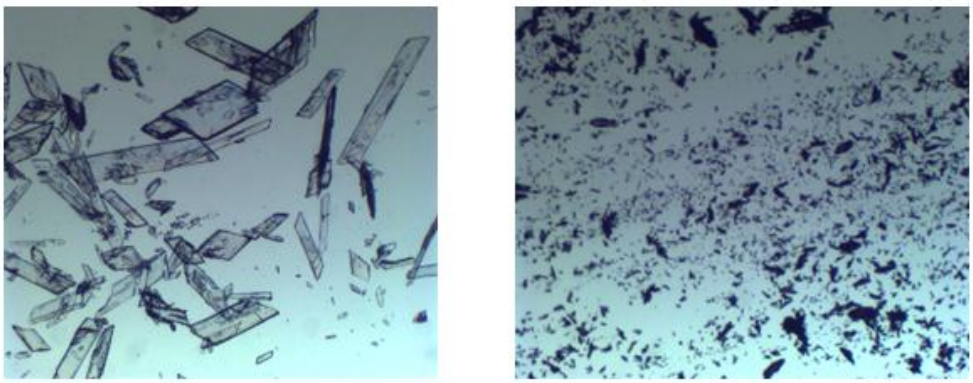


Figure No. 7: Morphology of Teriflunomide API batch sample before and after Micronization

The morphology of un-milled material was having rod-shaped particles and after micronization, the morphology is fine irregular particles. Further, the typical particle size of the sample before and after micronization was determined by obtaining an adequate number of images using ipvPClass particle size analyzer software. The D[90] of particle size before and after micronization was about 224 and 20 µm respectively.

PXRD and DSC:

PXRD pattern of the micronized and un-micronized batch samples show the variation in peak intensities and resolutions. However, both the patterns are closely matching with each other and the corresponding overlaid diffractogram is shown in Fig. 8.

The variations in peak intensities and peak resolutions/size are due to particle orientation within the sample and the particle size as the sample shows typical rod shape morphology.

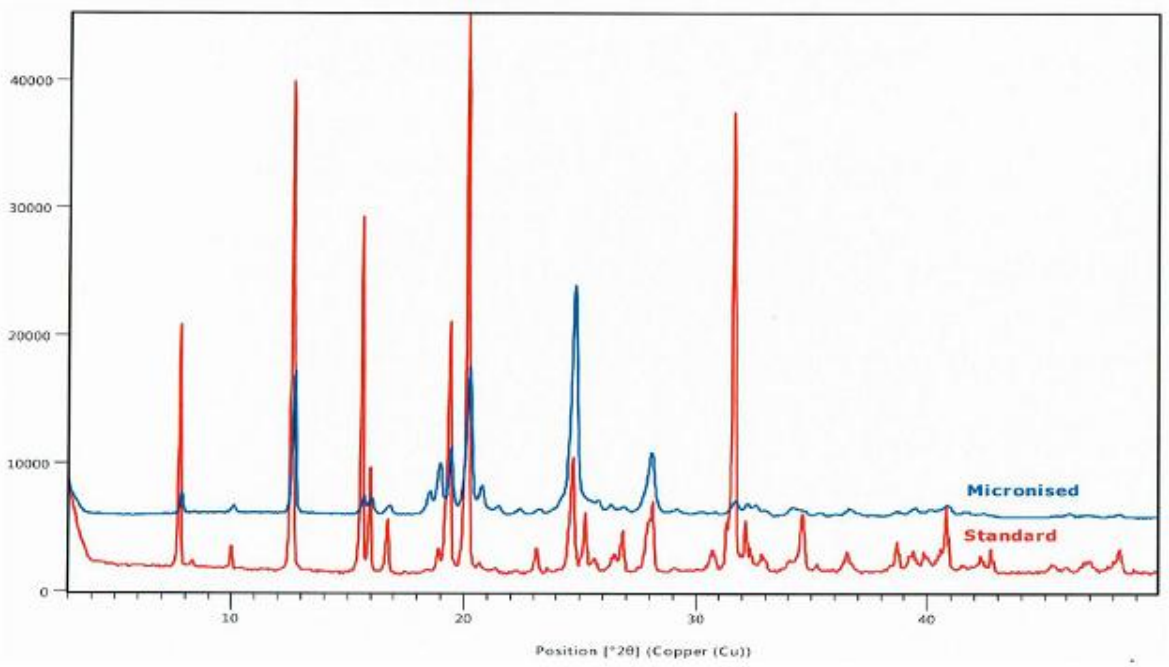


Figure No. 8: Overlaid X-ray diffractogram of Teriflunomide API batch sample before and after Micronization

It is important to understand the crystal defects, during micronization which results in the generation of amorphous nature in the sample. The generation of significant amorphous phase can cause severe changes in the thermodynamic and kinetic properties of molecule [3]. So, the estimation of the degree of crystallinity of Teriflunomide API before and after the micronization process is essential. The degree of crystallinity was calculated using high score plus software with constant background method [10]. The studies revealed that the micronized batch shows less degree of crystallinity (70%) than the un-micronized sample (92%).

The DSC thermogram of the micronized and un-micronized batch sample shows a similar melting point at about 230 °C and no further thermal transitions were observed before the melting point indicates the thermal stability of the material.

Hygroscopicity of Micronized API:

The generation of amorphous material in micronized API leads to enhance the hygroscopic nature. The hygroscopic study of micronized API was performed by using the DVS instrument. The DVS isotherm of micronized Teriflunomide drug substance is shown in Fig-9. The sorption isotherm shows that the % moisture uptake by the API is stable up to 80% RH and a sudden increase in % moisture uptake of 1.0% w/w was observed from 80-90% RH which could be due to generation of amorphous material during micronization. The same

phenomenon was not observed in un-micronized samples. According to the EP, the micronized API belongs to non-hygroscopic material based on the classification of hygroscopicity of Pharmaceutical materials as there is no significant moisture uptake by the micronized API at 80% RH.

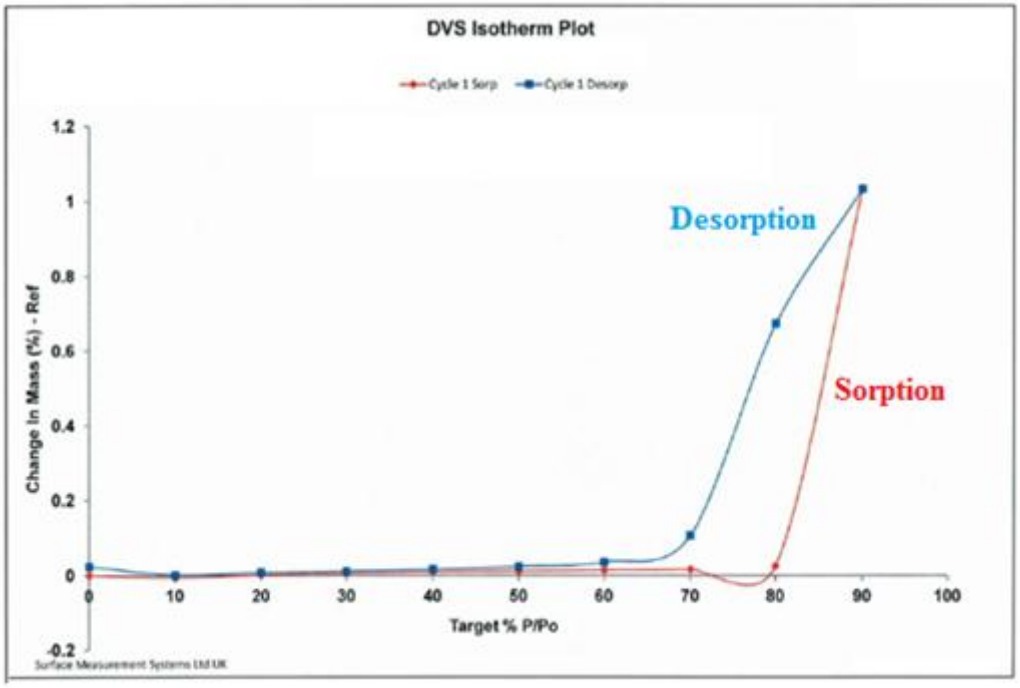


Figure No. 9: DVS isotherm plot of Teriflunomide Micronized sample

Besides hygroscopicity, the physical stability of the solid drug must be adequately characterized. To accomplish this, the stability studies were conducted for the micronized batch samples at long term stability i.e. 25°C/60% RH for 24M and accelerated stability i.e. 40°C/75% RH for 6M as per the as per ICH Q1A(R2) guideline [11]. The stability of the solid form of teriflunomide drug substance was monitored by diffraction and thermal studies during the specified stability storage conditions. The studies reveal the fact, that there is no significant change in the physical properties as shown in Figure 10.

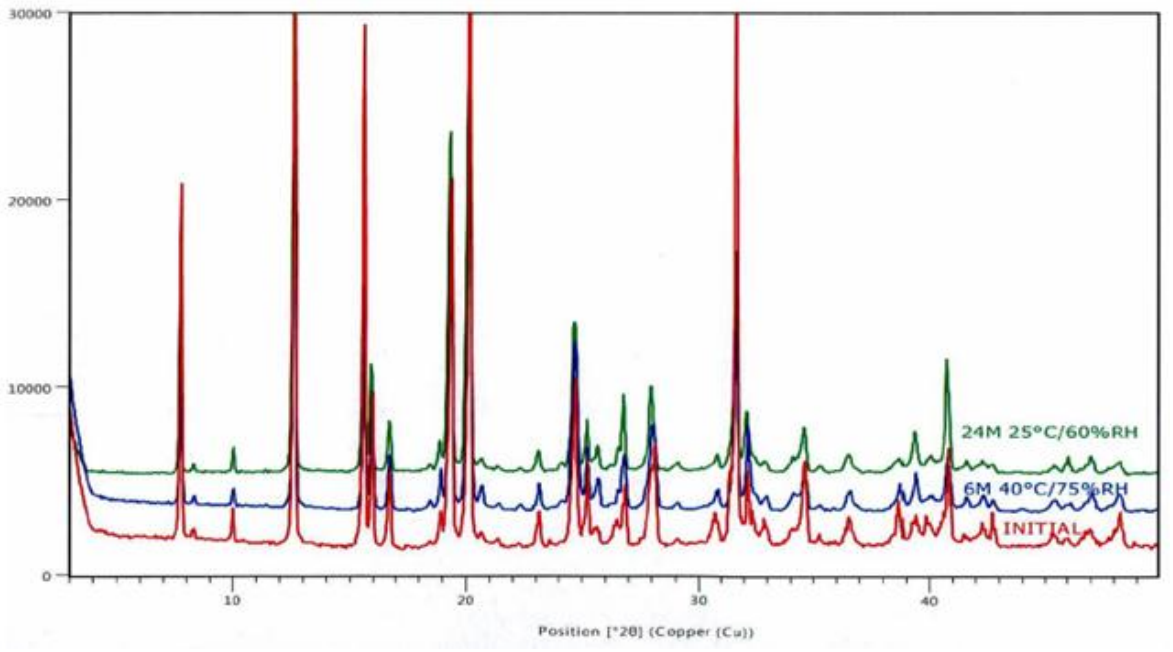


Figure No. 10: Overlaid PXRD pattern of Teriflunomide API stored at 40°C/75%RH, 6M and 25°C/60%RH, 24M

Crystallographic Data of API:

Single crystal X-ray crystallography is the most powerful structural method for the determination of the 3D structures of molecules. While the results of a routine diffraction experiment readily provide an unambiguous determination of the relative configuration of all stereogenic centers in the molecule, determination of absolute configuration is more challenging [12]. The structural configuration was studied from single-crystal data by growing the crystal and the parameters are refined using SHELXTL-PLUS software. The structural refinement parameters are as follows.

Empirical formula	:	$C_{12}H_9F_3N_2O_2$
Formula weight	:	270.21
Temperature	:	100(2) K
Wavelength	:	0.71073 Å
Crystal system	:	Triclinic

Space Group	:	$P\bar{1}$	
Unit cell dimensions	:	a = 4.71560(10) Å	α = 102.2130(7)°
	:	b = 10.7756(3) Å	ß = 96.8940(9)°
	:	c = 11.5674(3) Å	γ = 93.2840(8)°
Volume	:	568.25(2) $Å^3$	
Z	:	2	
Density (Calculated)	:	1.579 Mg/m^3	
Absorption coefficient	:	0.142mm^{-1}	
F(000)	:	276	
crystal size	:	0.400 x 0.330 x 0.120 mm^3	
θ range for data collection	:	2.349 to 27.500°	
Index ranges	:	-6<=h<=4, -13<=k<=13, -15<=1<=15	
Reflections collected	:	9307	
Independent reflections	:	2515 [R(int) = 0.0282]	
Completeness to θ = 25.242°	:	96.5%	
Refinement method	:	Full-matrix least-squares on F^2	
Data / resu.aints / parameters	:	2515 / 0 / 217	
Goodness-of-fit on F^2	:	1.107	
Final R indices [l>2σ(l)]	:	R1 = 0.0599, wR2 = 0.1768	
R indices (all data)	:	R1 = 0.0723, wR2 = 0.1833	

Largest diff. peak and hole	:	0.462 and -0.321 e. $Å^{-3}$
Measurement	:	Bruker D8 QUEST PHOTON-100 Detector
Software Used	:	SHELXTL-PLUS

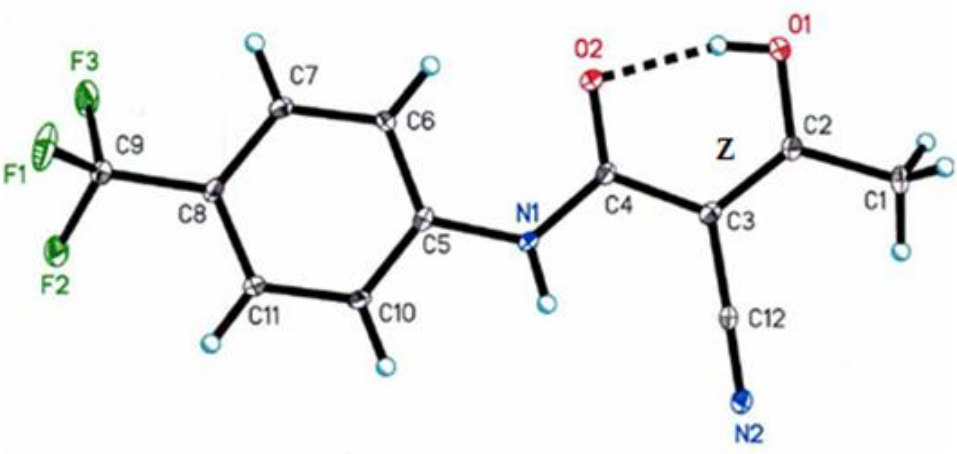


Figure No. 11: ORTEP diagram of Teriflunomide Single crystal (30% ellipsoid)

The ORTEP diagram shows the displacement of ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii in Fig-11. The dotted line indicates the intramolecular hydrogen bond within the molecule. The molecular structure around the double bond is *Z*-isomer, which confirmed by the torsion angle C4-C3-C2-O1, -1.0(2) °. The intramolecular hydrogen bonding between O_2 and O_1 stabilizes the molecule for the lower energy associated with the internal energy. This indicates that the structure of Teriflunomide is rigid that cannot exhibit polymorphism and the same was confirmed from polymorphic studies.

The simulated PXRD pattern generated from single-crystal data is matching with the powder pattern of Teriflunomide API synthesized by NATCO as shown in Fig. 12.

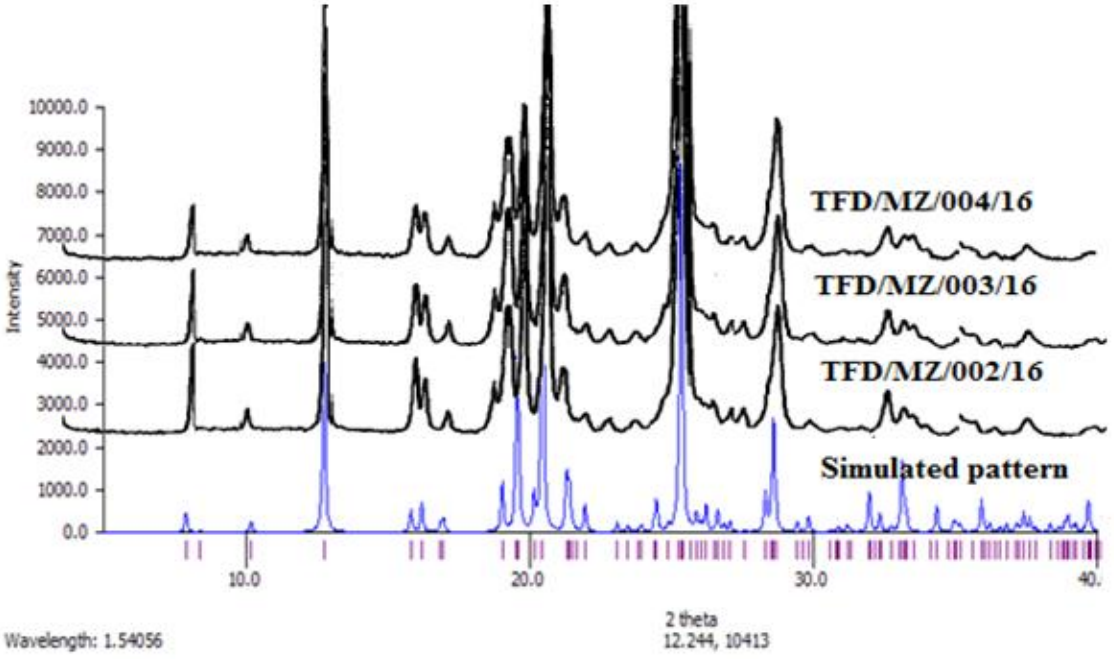


Figure No. 12: Overlay of micronized batch samples with simulated X-ray diffractogram

CONCLUSION

Teriflunomide API doesn't exhibit polymorphism and no occurrence of polymorphic transformation was observed during the long term and accelerated stability studies. The single-crystal studies unequivocally establish Z configuration around the double bond from the torsion angle C4-C3-C2-O1, -1.0(2)°. The intramolecular hydrogen bonding shows that the Teriflunomide API is rigid and doesn't show molecular confirmation of the drug.

ACKNOWLEDGMENT

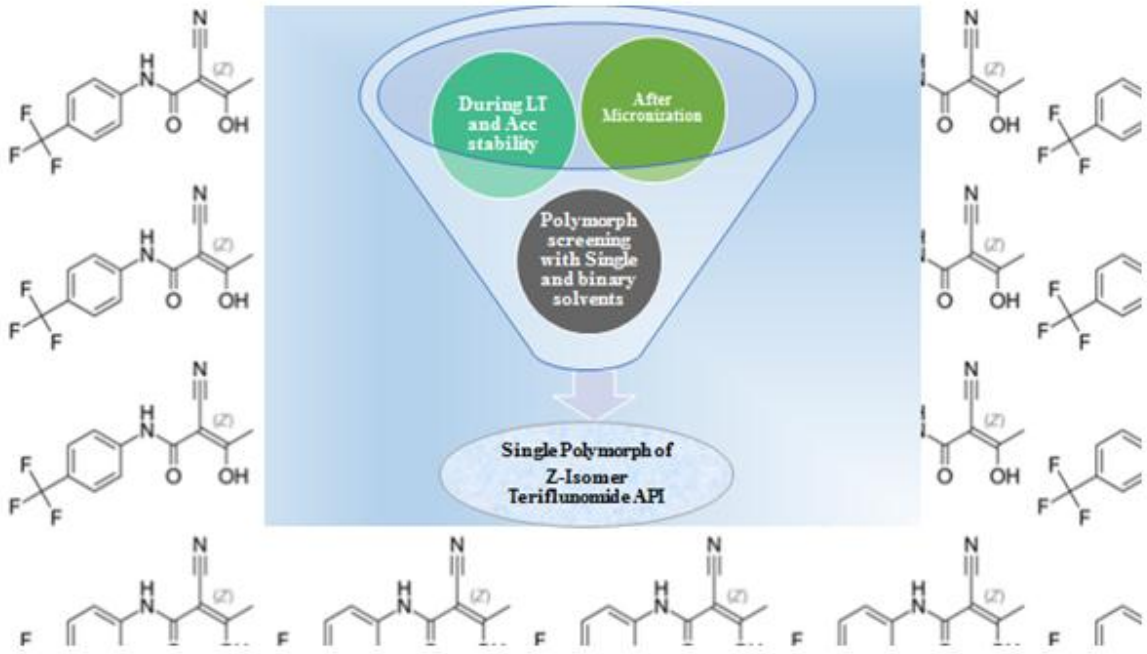
The authors acknowledge the management of NATCO Pharma Ltd., Natco Research Centre for permitting to publish the work.

REFERENCES

1. Miller. A. E: Teriflunomide in multiple sclerosis: an update. Neurodegenerative Disease Management, 2016; 7: 1
2. Magne, D., Mézin, F., Palmer, G., Guerne, P. A., 2006: The active metabolite of leflunomide, A77 1726, increases proliferation of human synovial fibroblasts in presence of IL-1β and TNF-α. Inflammation Research. 55, 469–475
3.Anne MarieHealy: Pharmaceutical solvates, hydrates, and amorphous forms: A special emphasis on cocrystals Volume 117, 1 August 2017

4. Anthony R. West Jacob: Solid State Chemistry and its applications, Chichester, West Sussex, UK: Wiley, 2014
5. Satyanarayana K, T. Venkateshwarlu, D. Swapna. Application ID 6909/CHE/2015.
6. Bruker: APEX3, SAINT and SADABS: Bruker AXS, Inc., Madison, Wisconsin, USA 2016.
7. Sheldrick G. M. Crystal structure refinement with SHELXL, Acta Crystallography 2015; C71: 3-8.
8. Ella M. King: Impact of Rigidity on Molecular Self-Assembly, Langmuir 2019, 35, 48, 16062-16069, Publication Date: October 14, 2019
9. Jaehwi Lee: Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution, and bioavailability. In Asian Journal of pharmaceutical sciences 2014, 9: 304-316.
10. Characterization of Crystalline and Partially Crystalline Solids by X-ray Powder Diffraction (XRPD) European Pharmacopeia: 9.0, 2.9.33, and USP 41 <941>.
11. Guidance for Industry: Q1A (R2) Stability Testing of New Drug – FDA
12. Determination of absolute configuration using X-ray diffraction, Volume 28, Issue 10, 15 October 2017, Pages 1304-1313.

Graphical Abstract:



	***Dr. Vangala Ranga Reddy – Corresponding Author*** *Deputy General Manager-ARD* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Cheedi Srinivasa Rao*** *Deputy Manager-ARD* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Duddu Swapna*** *Executiver-CRD* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Tallapaneni Venkateswarlu*** *Manager-CRD* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Dr. Kota Satyanarayana*** *Vice President-CRD* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Dr. Gopal Vaidyanathan*** *Vice President-ARD & DQA* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*