Original Article

STEP TOWORDS THE FORMULATION OF INJECTABLE HYDROGEL: PREFORMULATION STUDIES OF TERIFLUNOMIDE

Phalke Pallavi L*[1], Patel Dipti H[1]

[1]Department of Pharmaceutics, Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Parul University, At P.O Limda, Tal-Waghodia, Dist- Vadodara-391760, Gujrat, India.
Email: falkepallavi@gmail.com

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Abstract

Purpose: The aim of formulation of this hydrogel is to treat the osteoarthritis which is one of the common inflammatory autoimmune disorders of joints. The use of conventional drugs like NSAIDs having various side effects.
The aim of our research was to develop a formulation of injectable hydrogel containing teriflunomide. This study will be helps us for the development of stable, robust and therapeutically effective dosage form.

Method: Solubility of Teriflunomaide was determined by using various solvents. By using infrared spectrum and absorption maxima the purity of sample was checked. With the help of UV spectrophotometer standard UV curve was developed. Drug excipients compatibility studies were performed using Fourier transform infrared spectroscopy, differential scanning colorimetry, partition coefficient and also short term drug solution stability studies were performed.

Result: Teriflunomide is soluble in methanol & 0.1 N NaOH on the other hand poorly soluble in distilled water, phosphate buffer (pH 7.4) and 0.1 N HCl. Absorption maxima was observed at 292nm and infrared spectrum was showing the characteristic peaks. The standard curve obtained was linear with correlation coefficient below 1. Log P value obtained at 2.78 it confirms that teriflunomide is having both hydrophilic & hydrophobic nature. After the entire short term stability studies drug was found to be stable in short term stability studies.

Conclusion: The studies like melting point, FTIR etc suggested that drug selected for the formulation was found to be pure. Partition coefficient values suggested that it is having both hydrophilic and hydrophobic nature. Results obtained from short term stability studies were suggested that drug is stable showing no degradation on phosphate buffer which confirmed by no shift of λ max.

Keywords: Teriflunomide, Drug characterization, Drug-excipients compatibility studies, Preformulation.

Introduction [1, 2, 3]

Preformulation testing is the first step in the rational development of dosage forms of the drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to formulator in developing stable and bio available dosage forms. The selected drug TEF was characterized for the physicochemical and spectral properties. Preformulation studies are generally carried out before going for the actual formulation and dosage form development. Preformulation studies are one of the major parts of the development of dosage form because the physicochemical properties of the drug and excipients may affect the

properties of the resulting product. TEF is the newest DMARD for the treatment of the disease and leads to the inhibition of dihydroorotate dehydrogenase (DHODH). Teriflunomide is BCS Class two drug with half life of 18 to 19 days. After oral administration of teriflunomide, maximum plasma concentrations are reached, on average, in 1-4 hours. After a single IV dose, teriflunomide has a total body clearance of 30.5 ml/h.

37.5% is eliminated in the feces and 22.6% in urine.

Characterization and Identification of Teriflunomide [4, 5, 6]

TEF (99.82% pure) was procured from Intas Pharmaceuticals Ltd., India. The drug was characterized for the following parameters.

Physical Characterization

An Organoleptic property such as color, odor, taste and appearance of Teriflunomide was observed.

Identification of Teriflunomide:

Identification of drug sample was carried out by solubility study, melting point determination and UV-spectroscopy.

BCS Solubility study of Teriflunomide [7, 8]

The solubility of drug in various solvents was determined by using shake flask method. Excess amount of API can added into 250ml conical flask containing different types of media such as 0.1N HCl, pH 7.4 Phosphate buffers, distilled water, methanol and acetonitrile the shaking process was carried for 24 hours by keeping the conical flask on rotator shaker at 200 rpm. A portion drug dissolved was filtered through (0.45µm) and concentration of drug in the filtrate was determined by UV spectrophotometer at 292 nm .

Melting point determination [9]:

Melting point of Teriflunomide was determined by melting point apparatus using capillary method. Few amount of sample is place in capillary tube which having 1mm diameter with one end is closed. The capillary is placed in melting point apparatus and start heating, when sample starts melting at that time temperature is note down.

Spectrophotometric Identification of Teriflunomide [10]:

I) Determination of calibration curve for Teriflunomide:

Selection of Solvent:

Diluent: Methanol/ Phosphate buffer saline pH 7.4/ Acetonitrile/ 0.1N HCl

A. In Methanol

Accurately weighed quantity of TRF, 5 mg each, was dissolved in 100 ml methanol separately to prepare stock solution of concentration 50 µg/ml. The stock solution of TEF was suitably diluted with methanol in the range of

2-10 µg/ml respectively. Λmax was determined with 10 µg/ml concentration. All concentrations were analyzed by UV-spectrophotometer, at λmax 292 nm. Calibration curve was plotted by placing absorbance on Y-axis and concentration on X-axis.

B. In phosphate buffer saline pH 7.4

Accurately weighed quantity of TEF, 5 mg each, was dissolved separately in 100 ml phosphate buffer saline (PBS) to prepare stock solution of concentration 50 µg/ml. Stock solution of TEF was suitably diluted with PBS in the range of 2 to 20 µg/ml and 2-10 µg/ml respectively. λmax was determined with 10 µg/ml concentration. All concentrations were analyzed by UV spectrophotometer, at λmax 292 nm. Calibration curve was plotted by placing absorbance on Y-axis and concentration on X-axis.

C. In Acetonitrile

Accurately weighed quantity of TEF, 5 mg each, was dissolved in 100 ml Acetonitrile separately to prepare stock solution of concentration 50 µg/ml. Stock solution of TEF was suitably diluted with Acetonitrile in the range of 2 to 20 µg/ml and 2-10 µg/ml respectively λmax was determined with 10 µg/ml concentration. All concentrations were analyzed by UV spectrophotometer, at λmax 292 nm. Calibration curve was plotted by placing absorbance on Y-axis and concentration on X-axis.

D. In 0.1N HCl

Accurately weighed quantity of TEF, 5 mg each, was dissolved in 100 ml 0.1N HCl separately to prepare stock solution of concentration 50 µg/ml. The stock solution of TEF was suitably diluted with 0.1N HCl in the range of 10-30 µg/ml respectively. λmax was determined with 10 µg/ml concentration. All concentrations were analyzed by UV spectrophotometer, at λmax 292 nm. Calibration curve was plotted by placing absorbance on Y-axis and concentration on X-axis API solutions (10µg/ml) in methanol was scanned in UV spectrophotometer (Shimadzu 1800) with 10mm quartz cuvettes over wavelength range 200-400 nm to get the wavelength of maximum absorption (λmax).

II) Determination of absorption maxima of Teriflunomide (λmax) [11]:

Accurately Weigh Teriflunomide dissolved in methanol to obtained solution of 10 µg/ml solution. UV spectrum was recorded in the wavelength between 200-400 nm ranges using UV spectrophotometer against blank methanol. Wavelength for maximum absorbance was recorded.

Drug-Excipient compatibility study

Fourier Transform infrared (FTIR) spectroscopy [12]

FTIR spectra of TEF were obtained by using FTIR-spectrophotometer (PerkinElmer Spectrum 65 FT-IR). The method used for study is pressed KBr pellet method and the ratio of sample is should be 1:100, where 1 is a part of drug sample and 100 is a part of KBr. In order to take spectra, 4-5 mg of samples were triturated with potassium bromide and compressed to form pellet. The scanning range was 400-4000cm^{-1} at ambient temperature and the absorption bands were compared with the mentioned standards peaks/standard reported values.

Determination of thermal behavior by differential scanning calorimetry [13,14]

Drug was assessed by carrying out thermal analysis [13]. It was performed with Mettler, automatic thermal analyzer system equipped with cooling accessory. Sealed and perforated aluminum pans were used for the experiments of the sample. Accurately weighed (5mg) TRF and physical mixture of TRF and excipients were separately placed in standard aluminium pans and sealed with a lid. Temperature calibration was performed using indium as standard. The entire samples were run at scanning rate of 100C/min from 50 to 3000C.

Partition coefficient of Teriflunomide [15, 16]

The partition coefficient was calculated by taking Octanol and water (10 ml each) in a separating funnel followed by addition of 10 mg TEF (analyzed separately). The funnel was shaken vigorously for 15 min and it was then clamped in stand and kept for 24 hrs for effective partitioning. After 24 hrs, sample from water was withdrawn, diluted and the absorbance was measured by UV spectrophotometer at 292 nm.

At equilibrium, the ratio of unionized drug distributed between organic and aqueous phase is defined as partition coefficient and calculated by the formula given below:

$P_{o/w} = (C_{Octanol} / C_{water})$ at equilibrium, where, $P_{o/w}$ is partition coefficient; $C_{octanol}$is concentration of drugs in octanol; $C_{water}$is concentration of drugs in water.

Short-term Drug Solution Stability Study [17, 18]

Short-term drug solution stability of TRF (20µg/ml) in phosphate buffer saline pH 7.4 was performed at low temperature and room temperature for seven days and the resultant samples were analyzed by UV-Spectrophotometer at 292 nm.

Result of Preformulation Studies for Teriflunomide (TRF)

Physical characterization

Table 1:- Quantity and purity of Teriflunomide

Supplied By	**Intas Pharmaceuticals**
Quantity	100gm
Purity (Assay)	99.7%

Drug specifications were provided by the respective pharmaceutical company. The assay was performed for confirmation of purity of the drug by the quality control department of the industry. The purity was found to be 99.7 % for the TRF. Similar purity was also reviewed from the literature [19, 20, 21]. Both drugs were found to have required purity to conduct the studies for the formulation design and development.

Organoleptic Properties of TFR [22, 23, 24]:

Organolepic characterization revealed that the teriflunomide was the white crystalline odourless powder. Taste of the teriflunomide was reviewed in literature which revealed that the taste of TRF was tasteless. The organoleptic properties were shown in following table.

Table 3:- Organoleptic Properties of TFR

Properties	**Observations**
Color	White

Odor	Odorless
Taste	Tasteless
Appearance	Crystalline Powder

Solubility study of Teriflunomide [25, 26, 27]

The saturation solubility of pure drugs was calculated in different solvents on UV spectrophotometer by using calibration equation of drugs in those particular solvents. Teriflunomide is soluble in methanol and 0.1N NaOH, poorly soluble in distilled water, Phosphate Buffer and 0.1N HCl.

Solubility of TRF was determined in various solvents and the results are summarized in table. Solubility studies revealed that the TRF was soluble in methanol and at alkaline pH (0.1N NaOH). This study can support the fact that TRF could be the possible suitable candidate for the drug delivery system which are meant for the release of drug in intestine where favorable pH for solubility is existing.

Table 4: Preliminary solubilities in different solvents

Solvent	**Conclusion**
Phosphate Buffer (pH 7.4)	Poorly soluble
Water	poorly soluble
Methanol	soluble
0.1N HCl	Poorly soluble
0.1N NaOH	soluble
DMSO	Soluble

Solubility was also determined precisely in various solvents and at various pH. Solvents like water, DMSO, phosphate buffer (pH 7.4) and aqueous solvent at acidic and basic pH were used to determine the solubility of TRF. This study revealed that the TRF has got excellent solubility in DMSO subsequently in alkaline pH and even in phosphate buffer (pH 7.4). The data obtained during solubility study is tabulated and shown in following table and figure respectively.

Fig.1: Graphical presentations of solubility of TRF in different solvent

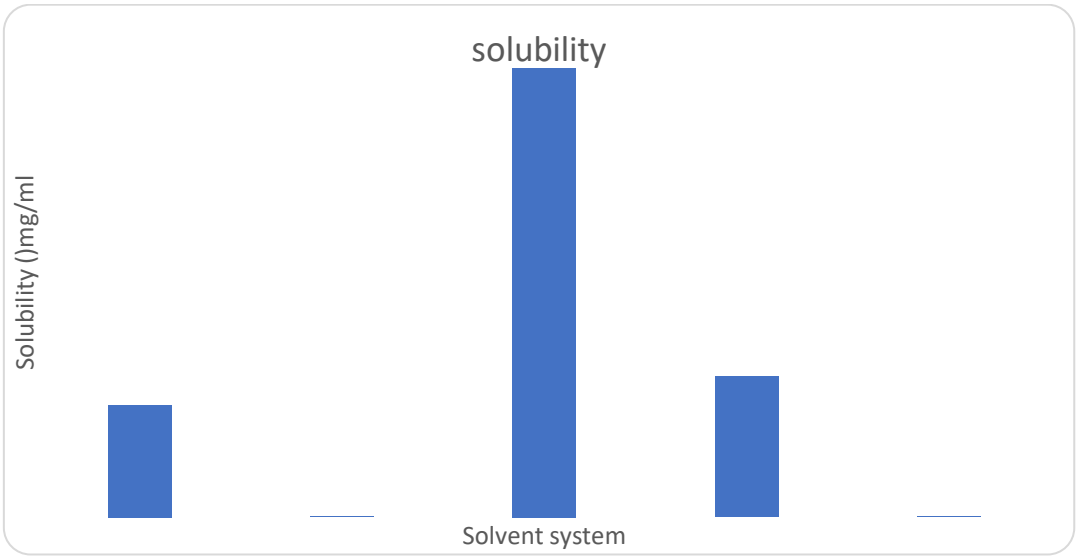


Table 5: Solubility of Teriflunomide (Teriflunomide is weakly acidic with pKa 3.1 at room temperature and having a pH dependent solubility)

Solvent	Reference values (Drug bank)
Phosphate Buffer (pH 7.4)	8 mg/ml at pH 7.6 (Soluble)
Water	0.0124 mg/ml (Practically insoluble in water)
DMSO	32 mg/ml (Freely soluble)
0.1N HCl	0.02 µg/ml (Poorly soluble)
0.1N NaOH	10 mg/ml (Soluble)

Melting point Determination [28, 29]

Melting point of API was found to be, which is in range as given in literature (227-231˚C). Hence the drug can be stated as pure. Melting point of TRF was found to be 230.4±1.2˚C. [Mehta et al., 2017]

Table 6: Melting point Determination

Sr. No	API	Melting Point (°C)	Mean ±SD
01	TFR	230.4	230.4±1.2
02		231.4	
03		229.4	

Drug identification by UV-Spectrophotometric analysis [30,31]

Determination of Absorption maxima (λmax):

Calibration curves of TRF were carried out in methanol, Phosphate Buffer (pH 7.4), 0.1N HCl and Acetronitrile. The absorption maxima in the given medium shown below in table 6.3. While calibration plot of TRF with regression values (r^2) in above mentioned solvents showed good linearity in the concentration range of 2-10µg/ml in all the media

Table 7: λmax of Teriflunomide in different solvents

S. NO.	Solvent	λmax (nm)
1.	Methanol	292.0
2.	PBS saline (pH 7.4)	291.6
3.	0.1 N HCl	291.2
4.	Acetonitrile	291.2

The λmax of TEF in different solvents were found to be at 292 nm as shown by fig.(a-d). The reported value of UV λmax for TEF is 292 nm.

Fig.2: The λmax of TRF in (a) methanol, (b) PBS pH7.4, (c) 0.1N HCl, (d) Acetonitrile

Fig.2(a): λmax in Methanol

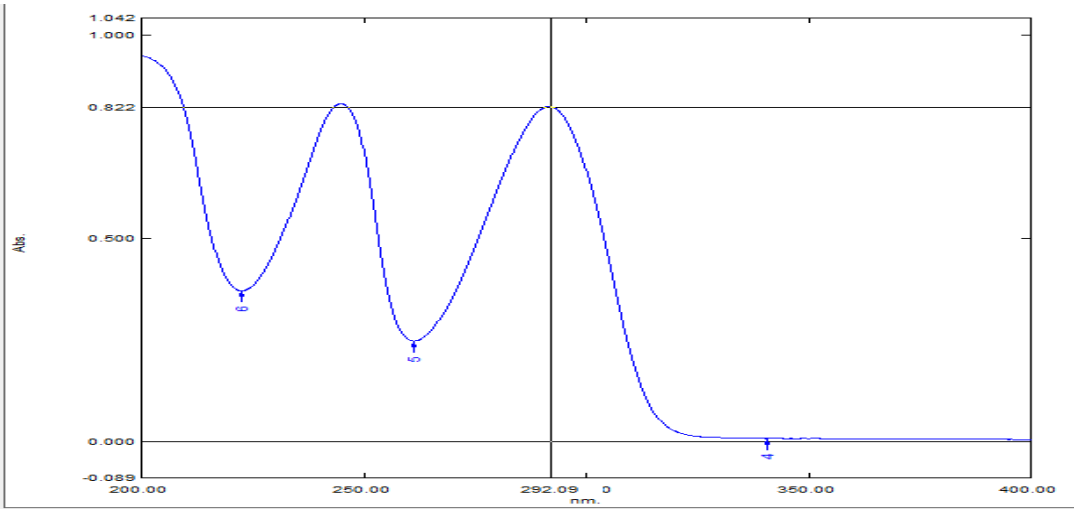


Fig.2(b): λmax in PBS saline (pH 7.4)

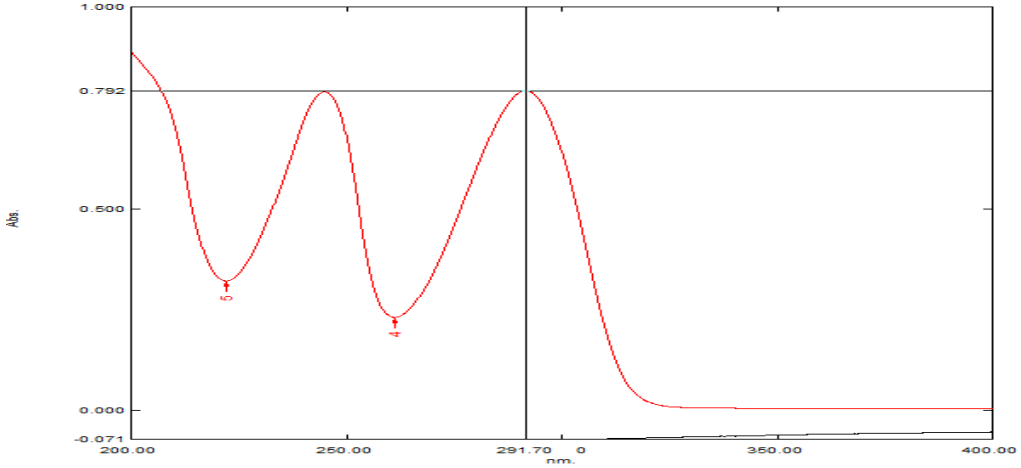


Fig.2(c): λmax in 0.1N HCl

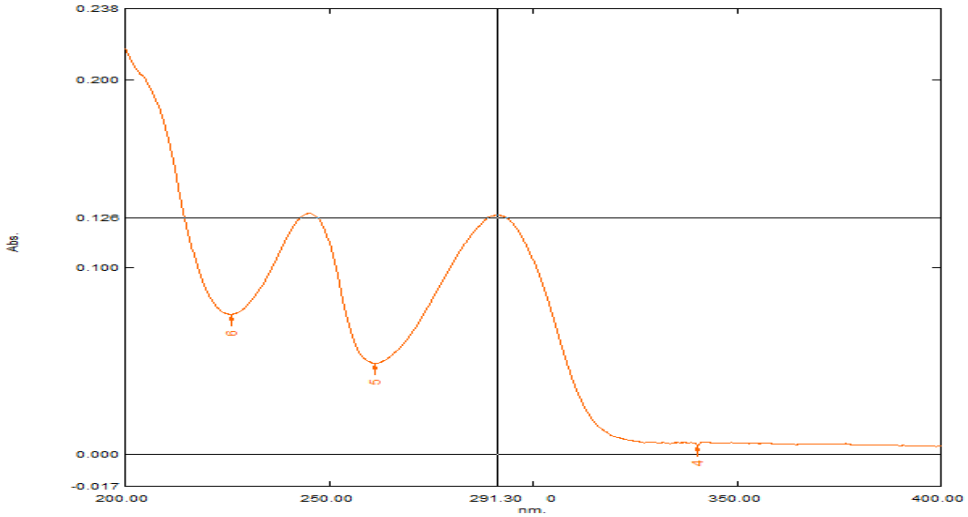
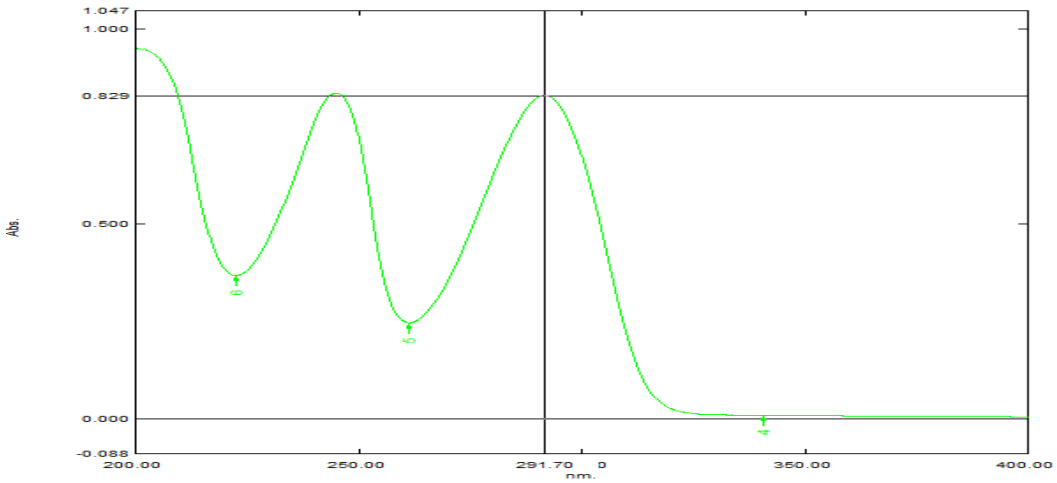


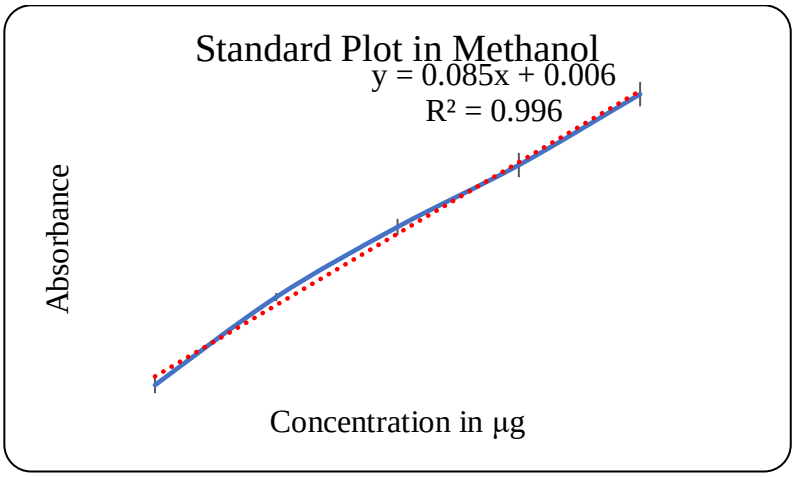
Fig.2(d): λmax in Acetonitrile



Calibration curve:

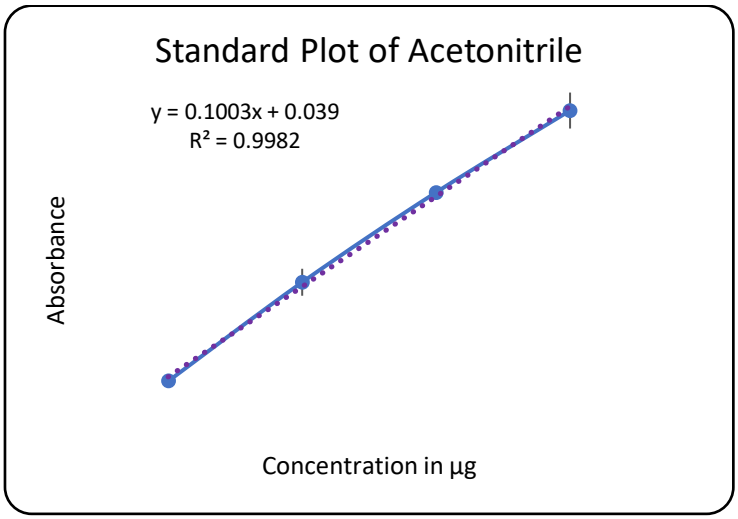
(a) UV Calibration curves of TEF in Methanol

Conc (µg/ml)	absorbance
2	0.14±0.02
4	0.356±0.01
6	0.528±0.02
8	0.68±0.03
10	0.854±0.03



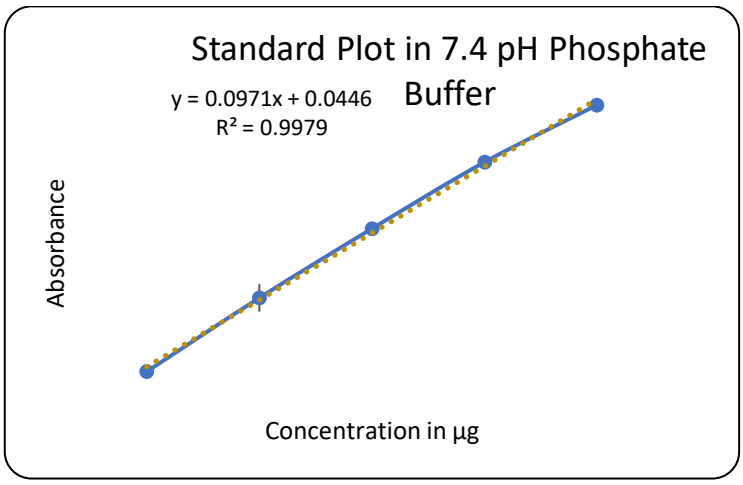
(b) UV Calibration curves of TEF in Acetonitrile

Conc (µg/ml)	Absorbance
2	0.232±0.01
4	0.458±0.03
6	0.652±0.01
8	0. 974±0.04



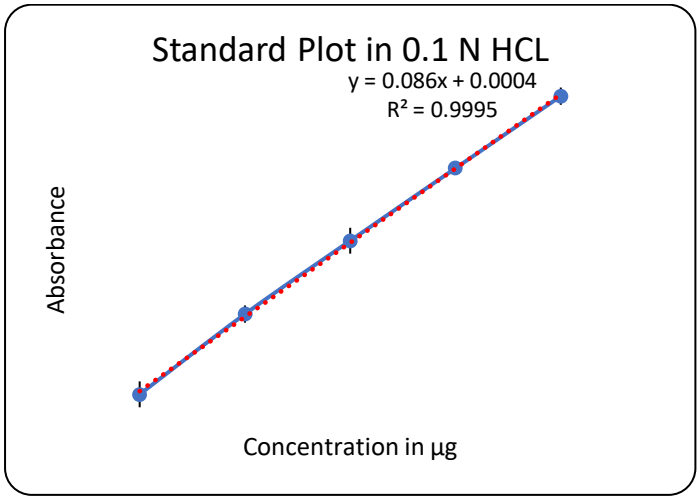
(c) UV Calibration curves of TEF in PBS 7.4

Conc (µg/ml)	absorbance
2	0.225±0.01
4	0.439±0.04
6	0.64±0.01
8	0.823±0.02
10	0.999±0.01



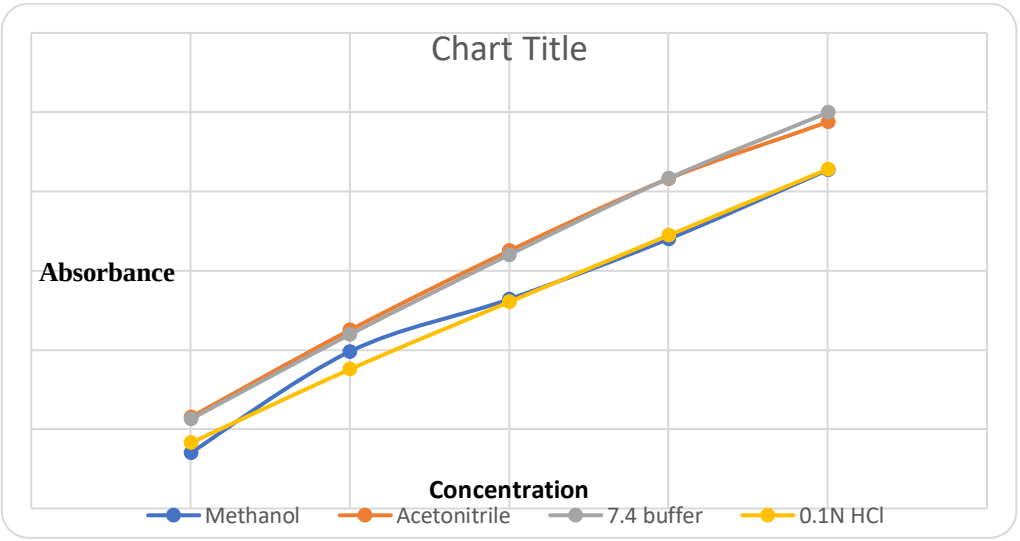
(d) UV Calibration curves of TEF in 0.1N HCl

Conc (µg/ml)	absorbance
2	**0.165±0.03**
4	**0.351±0.02**
6	**0.521±0.03**
8	**0.689±0.01**
10	**0.856±0.02**



Concentration	Methanol	Acetonitile	7.4 buffer	0.1N HCl
0	0	0	0	0
2	0.14	0.232	0.225	0.165
4	0.356	0.458	0.439	0.351
6	0.528	0.652	0.64	0.521
8	0.68	0. 974	0.823	0.689
10	0.854	00	0.999	0.856

Fig.3: UV Calibration curves of TEF in (a) methanol, (b) Acetonitrile, (c) pH 7.4 PBS, (d) 0.1N HCl



The solution containing different concentration of TFR was prepared in different solvents and scanned at 292 nm by using UV spectrophotometer. Graph of absorbance Vs. concentration was plotted and found to be linear over the range of 2-10 µg /ml indicating its compliance Lambert's-Beer's law.

Drug-Excipient Compatibility Study [32, 33]:

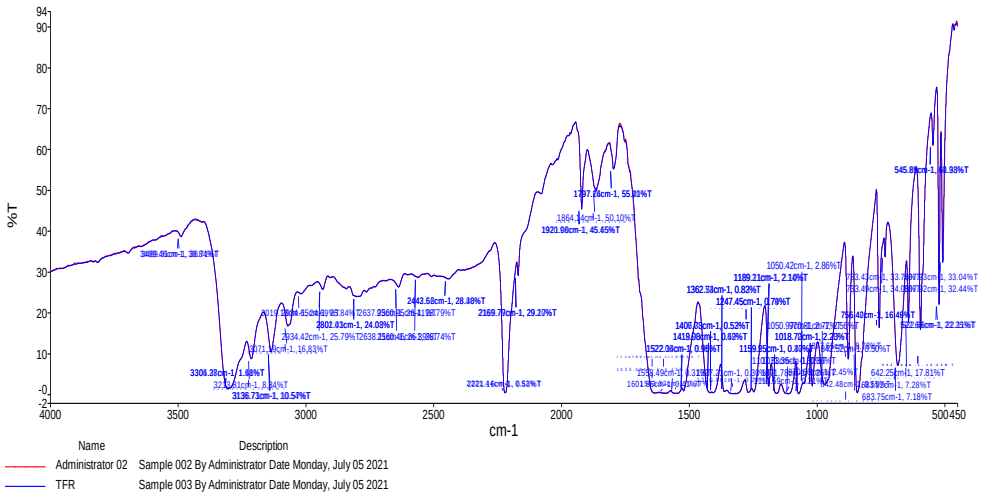
Fourier transforms infrared spectroscopy (FTIR)

The absorption bands in spectra were observed for TRF drug in the region of 450- 4000 cm^{-1} . The major peaks of TRF drug confirmed and the fingerprint region present of different functional hydroxyl, nitrile and amide groups. The characteristic peaks of TRF sample in fig. 6.2e were obtained at 3324.20 cm^{-1} (-NH- stretch of acetamide group), 3136.39 cm^{-1} (-O-H stretch of hydroxyl group), 2221.13 cm^{-1} (-C≡N group), 1634.75 cm^{-1} (-C=O), 1419.67 cm^{-1} (-C-N stretching), 1325.15 cm^{-1} (-CF-bond), 610-746.48 cm^{-1}(aromatic-C-H bending) (table 6.2).

However, the results of IR study of physical mixture of TRF with excipients (TG-18) in fig. b showed that all the excipients used in the formula were compatible with the presence of characteristic peaks of TRF.

Fig.4: FT-IR of (a) TFR (b) TFR+TG-18

(a) FT-IR of TFR



(b) FT-IR of TFR+TG-18

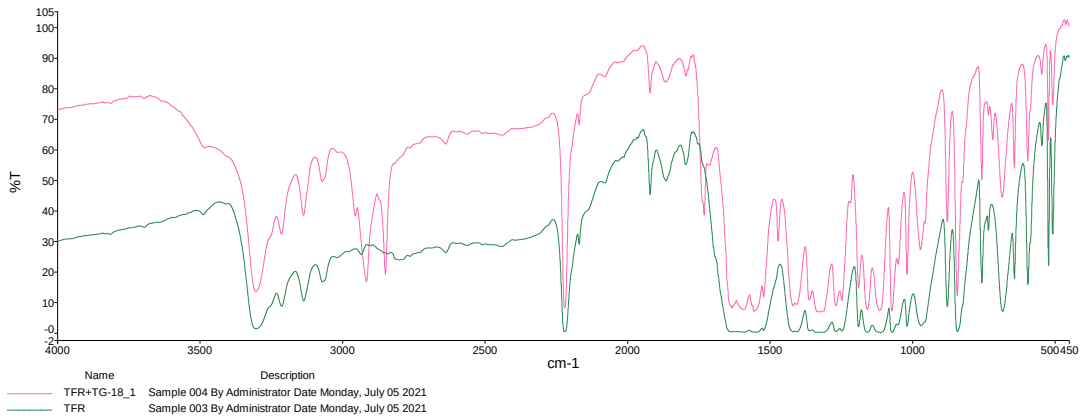


Table 8: FTIR peaks of Teriflunomide Standard and Observed

Name of Groups	**Peaks ranges of TRF (cm-1)**	**Observed Peaks TRF (cm-1)**	**Intensity**
-NH stretch of acetamide (O=C-NH-	3100-3350	3213	Strong
-OH stretch of Hydroxyl group	3350-3600	3489	Medium
-C=O of acetamide(O=C-NH-)	1700-1800	1797	Medium
-C-F Halide (CF3)	1400-1000	1320	Strong
(-C-N stretching), Nitrile group	2200-2300	2221	Strong
Nitrile group (−C ≡N)	2000-2300	2221	Strong
Aromatic -C-H Stretching	3000-3100	3071	Medium
Aromatic -C-H Def	600-700	642	Strong

Partition-Coefficient [34, 35]:

Log P value obtained at 2.78±0.39 confirmed that TRF is having both hydrophilic and hydrophobic nature. This study revealed that the actives may be suitable candidates for the formulation to be prepared during investigation.

Drug Solution Stability in Phosphate buffer saline (pH 7.4)[31]

Stability Data showed in the Table confirmed that drug solution in the phosphate buffer is stable as there is no Shift in λmax from 292nm, indicated that no degradation takes place.

Table 9: Drug Solution Stability in Phosphate buffer saline (pH 7.4)

Storage condition (10 µg/ml in phosphate buffer saline pH 7.4)	**Shift in λmax from 292nm (TEF)**					
	Day 1		Day 2		Day 7	
		TEF		TEF		TEF
4°C protected from light		292 292 292		292 292 292		291.5 292 292
4°C unprotected from light		292 292 292		292 292 292		291.5 291.5 291.5

Room temperature protected from light		292 292 292		292 292 292		292 292 292
Room temperature unprotected from light		292 292 292		292 292 292		291.5 291.5 292

Competing Interests:

Authors do not have any conflict of interest.

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Author Contributions:

Both the authors are contributed for this work.

Ethics Approval:

This paper does not have any animal study.

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