DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF TOFACITINIB IN TOFACITINIB 5 MG & 10 MG TABLETS DOSAGE FORM

ABSTRACT

Aim:

The primary objective of the research work is to develop an effective, sensitive, economical, and simple reverse phase HPLC method for the estimation of Tofacitinib in Tofacitinib tablets dosage form.

Study design:

HPLC based quantification studies.

Place and Duration of Study:

Department of Chemistry, Acharya Nagarjuna University, Nagarjuna ~~university~~University, Guntur, Andhra Pradesh, between August 2022 and November 2022.

Methodology:

Estimation of Tofacitinib in Tofacitinib tablets dosage form. The separation was achieved by using a stationary phase ~~was~~ Kromasil C18 (150 x 4.6 mm, 5µ) and the mobile phase consisted of pH 4.0 phosphate buffer and acetonitrile in the ratio of (80:20 volume/volume). The flow rate was 1.5 mL/min. Tofacitinib was detected using UV detector at the wavelength of 215 nm. Column temperature 25°C and sample temperature 25°C and injection volume 20µL, run time was 20 minutes.

Results:

As there is no interference ~~of~~ between blank and placebo at the retention time of Tofacitinib. Degradation study results were shown significant degradation was observed in alkali (base) stress conditions. Hence it can be concluded that Tofacitinib is sensitive to alkali. ~~In order t~~To obtain system precision, a study was conducted with six replicate injections. %RSD was estimated from the peak areas of Tofacitinib found to be 0.16% respectively. ~~Relative~~ The relative standard deviation for method precision was found to be 5mg and 10 mg ~~are~~ is 0.26% and 0.75%. The proposed HPLC method was linear over the range of 24.88-74.64 µg/mL, the correlation coefficient was found to be 1.0000. The accuracy studies were shown as % recovery for Tofacitinib 50% to 150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate. The solution stability of the standard and samples are stable up to 48 hrs on a bench top and refrigerator (2-8°C). The method is robust for changes like flow rate, column oven temperature, pH variation, and the organic phase of the mobile phase. Performed the filter validation for sample solution 0.45 µm PVDF filterers are suitable for filtration.

The method has validated as per ICH guidelines and all the validation parameters are satisfy the ICH Q2 specification acceptance limits.

Conclusion:

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability, and robustness. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Tofacitinib in bulk and pharmaceutical dosage forms.

Keywords: Tofacitinib, Liquid chromatography, Forced degradation, and Validation.

1.0 Introduction

Tofacitinib chemically known as 3-[(3R, 4R) - 4 -methyl-3-[methyl (7H-Pyrrolo [2, pyrimidine-4yl) amino] piperidin-1-yl]-3- oxopropanenitrile. It is an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis [1]. Cytokines work within a complex regulatory network in RA, signaling through different intracellular kinase pathways to modulate the recruitment, activation, and function of immune cells and other leukocytes [2-6]. Several research works elucidated the safety and efficacy of Tofacitinib drug [7-14]. The chemical structure of Tofacitinib was shown in **Figure 1.** [15-16].

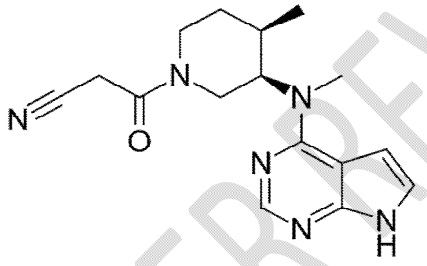

Figure 1. Chemical structure of Tofacitinib

The literature survey reveals that there are no HPLC methods were reported in major pharmacopoeias like USP, EP, JP, and BP. Only a few methods were reported ~~till~~ to date for the estimation of Tofacitinib by using RP-HPLC methods [17-19] and HPTLC [20] methods were reported for the estimation of Tofacitinib in pharmaceutical dosage forms.

Hence we tried to develop stability indicating the HPLC method for Tofacitinib in Tofacitinib in tablets dosage form. The present work describes a simple, stability indicating HPLC method for the determination of Tofacitinib in Tofacitinib in tablets dosage form according to ICH guidelines [21-22].

2.0 Experimental

2.1. Chemicals and Reagents

Analytical-grade Potassium dihydrogen phosphate, Orthophosphoric acid, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide, and water, reagents, and chemicals were procured from Merck Chemicals. Mumbai, India.

2.2. Instrumentation

Agilent HPLC model:1260 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), and Analytical Balance (Metller Toledo Model) were used in the present assay.

Preparation of pH 4.0 phosphate buffer solution

Weighed accurately 1.36 g of potassium dihydrogen orthophosphate into 1000 mL of water sonicated to dissolve and mix~~ed~~ well then pH was adjusted~~-~~ to 4.0 with ortho~~-~~phosphoric acid solution. Filtered through 0.45 µm membrane filter.

Preparation of mobile phase

Prepared a mixture of pH 4.0 phosphate buffer solution and acetonitrile in the ratio of 80:20 (%volume/volume) mixed well and sonicated to degas.

Preparation of diluent

Prepared a mixture of water and acetonitrile in the ratio of 50:50 (%volume/volume) mixed well and sonicated to degas.

Preparation of standard solution

Accurately weighed and transferred 25.7 mg of Tofacitinib working standard into a 50 mL volumetric flask sonicated for 2 minutes to dissolve the contents and made up to the volume with diluent. Further diluted this solution 5 mL in~~-~~to 50 mL volumetric flask and made up the volume with diluent and mixed well. (The concentration of the standard solution containing, Tofacitinib 50 ppm)

Preparation of test solution (for Tofacitinib tablets 5.0 mg)

Weighed accurately 10 tablets (Tofacitinib tablets 5 mg) and transferred (equivalent to 50 mg of Tofacitinib) into 200 mL volumetric flask, added 120 mL of diluent and sonicated for 30 minutes, with intermediate shaking, cool to room temperature and diluted to volume with diluent and mixed well. Filtered the solution through 0.45 µm PVDF syringe filter. Further diluted 5.0 mL of the filtrate solution in~~-~~to 25 mL volumetric flask, diluted to volume with diluent, and mixed well. (The concentration of the solution contains 50.0 µg/mL of Tofacitinib).

Preparation of test solution (for Tofacitinib tablets 10.0 mg)

Weighed accurately 5 tablets (Tofacitinib tablets 5 mg) and transferred (equivalent to 50 mg of Tofacitinib) into 200 mL volumetric flask, added 120 mL of diluent and sonicated for 30 minutes, with intermediate shaking, cool to room temperature and diluted to volume with diluent and mixed well. Filtered the solution through 0.45 µm PVDF syringe filter. Further diluted 5.0 mL of the filtrate solution in~~-~~to 25 mL volumetric flask, diluted to volume with diluent, and mixed well. (The concentration of the solution contains 50.0 µg/mL of Tofacitinib).

Preparation of placebo solution

Weighed accurately and transferred placebo powder (equivalent to 50 mg of Tofacitinib) into 200 mL volumetric flask, added 120 mL of diluent and sonicated for 30 minutes, with intermediate shaking, cool to room temperature and diluted to volume with diluent, and mixed well. Filtered the solution through 0.45 µm PVDF syringe filter. Further diluted 5.0 mL of the~~–~~ filtrate solution in~~–~~to 25 mL volumetric flask, diluted to volume with diluent, and mixed well. (The concentration of the solution contains 50.0 µg/mL of Tofacitinib).

Chromatographic conditions

Chromatographic analysis was performed on Kromasil C18 (150 x 4.6 mm, 5µ) mobile phase ~~consisted~~ consisting of pH 4.0 phosphate buffer and acetonitrile in the ratio of (80:20 volume/volume). The flow rate was 1.5 mL/min, the column oven temperature was 25°C and the sampler cooler

temperature was 25°C, the injection volume was 20 µL, and detection was performed at 215 nm using a photodiode array detector (PDA).

3.0 Method development

UV-spectroscopic analysis of Tofacitinib drug substance ~~was~~ showed ~~that~~ maximum UV absorbance (λmax) at 215 nm respectively.

To develop a suitable and robust HPLC method for the determination of Tofacitinib in Tofacitinib in tablets dosage form, different mobile phases were employed to achieve ~~the~~ a good peak shape. The method development was started with Hypersil BDS C18 (150x4.6mm, 5µm) with the following different mobile phase compositions like ~~that~~ 0.1% orthophosphoric acid buffer and acetonitrile in the ratio of 85:15 volume/volume. It was observed that when Tofacitinib was injected, higher retention time and peak tailing ~~was~~ were not satisfactory. ~~Column~~ The column stationary phase was not suitable for the component. For the next trial change the column from Hypersil BDS to Kromasil C18. The compound Tofacitinib was eluted at void volume and the peak shape was not good.

For the next trial, the mobile phase consisted of pH 4.0 phosphate buffer and acetonitrile in the ratio of 80:20 volume/volume~~-~~ respectively, flow rate 1.5 mL/min, column temperature 25°C, and sampler cooler maintained 25°C. UV detection was performed at 215nm. The compound Tofacitinib was eluted at 10.30 minutes and the peak shape was found to be good. The chromatogram of Tofacitinib standard using the proposed method is shown in **Figure 2.** system suitability results of the method are presented in **Table 1.**

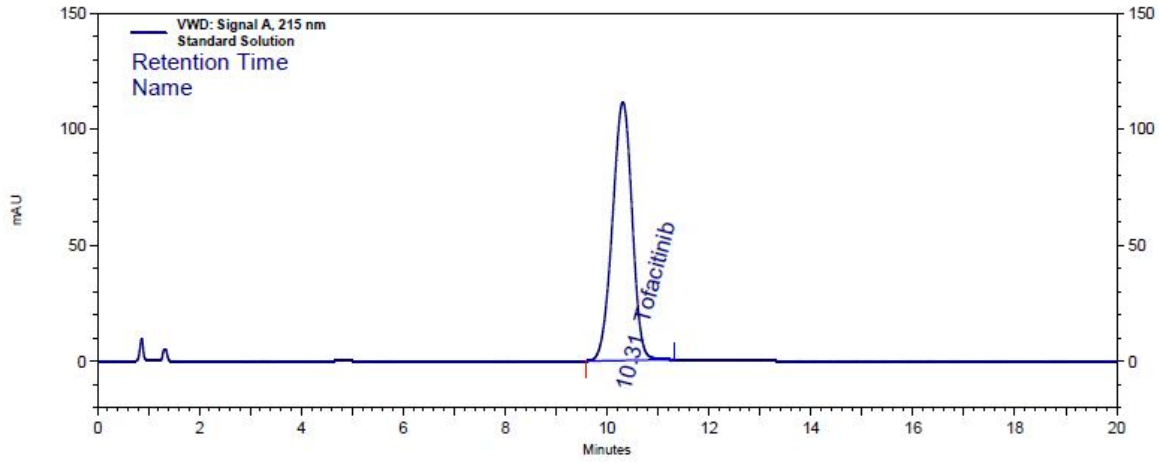

Figure 2. Typical chromatogram of Tofacitinib standard

4.0 Method validation

The developed RP-HPLC method was extensively validated for assay of Tofacitinib in Tofacitinib tablets formulation using the following parameters.

4.1 Specificity & System suitability

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure 3.** showed no peak at the retention time of Tofacitinib peak. This indicates that the diluent solution used in sample

preparation does not interfere ~~in~~ with the estimation of Tofacitinib in Tofacitinib tablets dosage form. Similarly chromatogram of the placebo solution **Figure 4.** showed no peaks at the retention time of Tofacitinib peak. This indicates that the placebo used in sample preparation does not interfere ~~in~~ with the estimation of Tofacitinib in Tofacitinib tablets formulation.

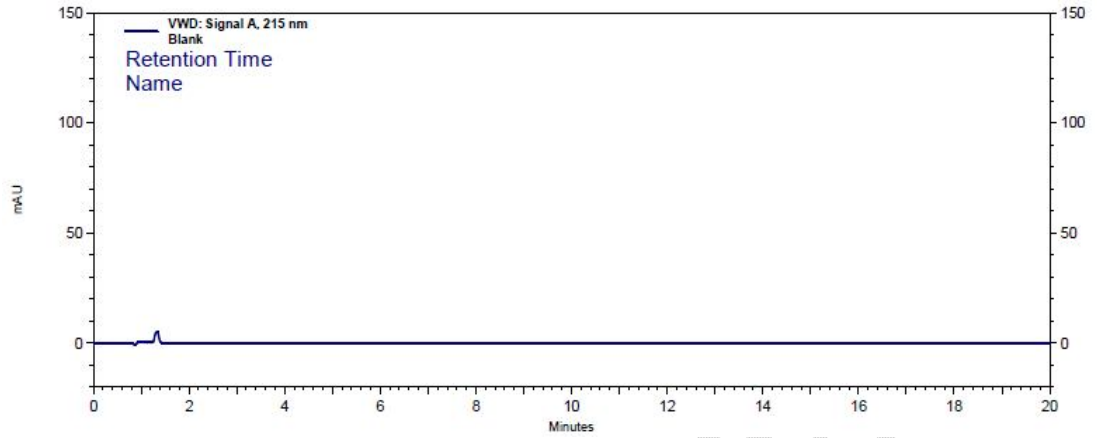

Figure 3. Typical chromatogram blank

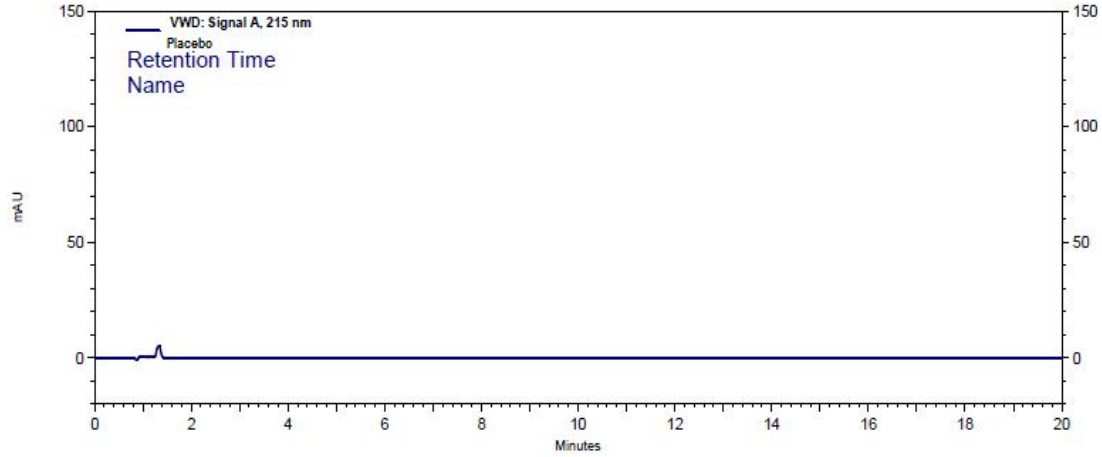

Figure 4. Typical chromatogram placebo

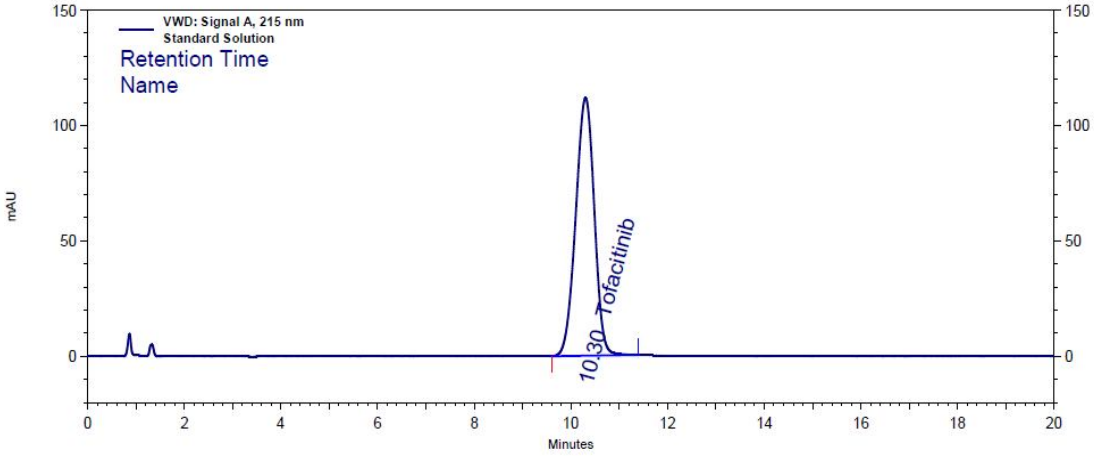

Figure 5. Typical chromatogram standard

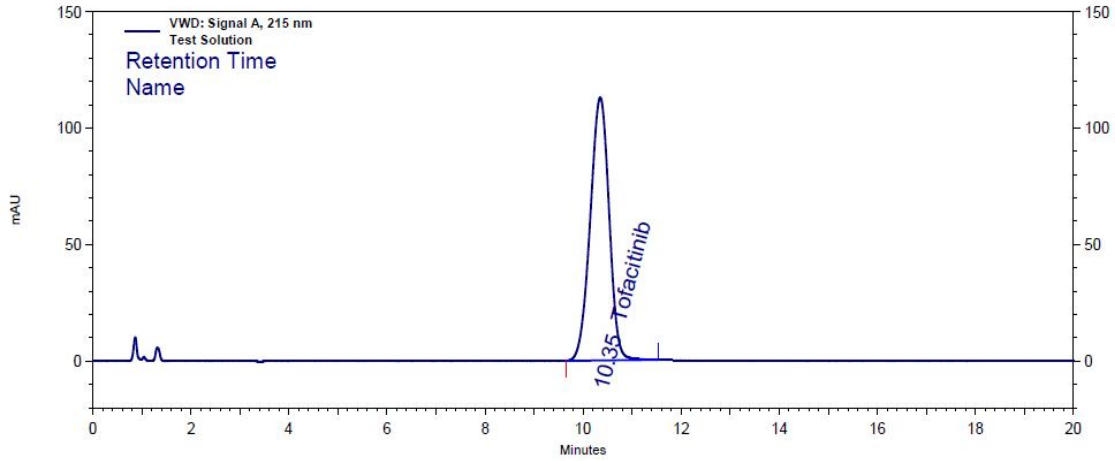

Figure 6. Typical chromatogram sample

Table 1. Specificity results

S.No	Name	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	10.30	No	No
4	Sample solution	10.35	No	No

4.1.1 Force Degradation studies

A study was conducted to demonstrate the effective separation of degradants/impurities from Tofacitinib. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the HPLC system with a PDA detector. ~~Degradation~~ The degradation study results were presented in **Table 2.**

Table 2. Forced degradation results

Stress condition	Degradation condition	%Assay	% Degradation
As such	Controlled sample	101.8	NA
Oxidative degradation	3% H_2O_2 solution 3 hours kept on bench top	99.6	2.2
Acid degradation	0.1 N HCl solution heated at 80ºC for 3 hours	97.2	4.6
Alkali degradation	0.1 N NaOH heated at 80ºC for 3 hours	91.5	10.3
Thermal degradation	60ºC in oven for 7 days	101.7	0.1
Photolytic degradation	Photolytic degradation Exposure to 1.2 million lux hours at 200 watt hours/square meter ultra-violet energy	100.3	1.5

Significant degradation was observed in the alkali (base) stress condition. Hence it can be concluded that Tofacitinib is sensitive to alkali.

4.2 System precision

The standard solution was prepared as per the test method, injected into the HPLC system ~~for~~ six times, and evaluated the % RSD for the area responses. The data were shown in **Table 3.**

Table 3. System precision results

S.No.	No.of injections	Peak area
1	Inj-1	45045979
2	Inj-2	45162156
3	Inj-3	45240687
4	Inj-4	45224016
5	Inj-5	45225412
6	Inj-6	45219371
Average		**45186270**
STDEV		**73860.39865**
% RSD		**0.16**

The relative standard deviation of six replicates standard solution results were found to be within the specification limit i.e.0.16%.

4.3 Method precision

The precision of the test method was evaluated by doing an assay for six samples of Tofacitinib tablets (5 mg and 10 mg) as per the test method. The content in mg and % label claim for Tofacitinib for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table 4. and Table 5.**

Table 4. Method precision data for Tofacitinib tablets (5 mg)

S.No	No. of Preparations	% Assay
1	Preparation 1	100.8
2	Preparation 2	100.3
3	Preparation 3	100.7
4	Preparation 4	101.1
5	Preparation 5	100.7
6	Preparation 6	100.9
Average		**100.8**
SD		**0.2665**
%RSD		**0.26**

Table 5. Method precision data for Tofacitinib tablets (10 mg)

S.No	No. of Preparations	% Assay
1	Preparation 1	100.1
2	Preparation 2	99.4
3	Preparation 3	100.4
4	Preparation 4	101.3
5	Preparation 5	100.5
6	Preparation 6	101.4
Average		**100.5**
SD		**0.7521**
%RSD		**0.75**

Overall and individual % of Assay are complying as per test method specification. The relative standard deviation of six assay preparations 5mg and 10 mg ~~are~~ is 0.26% and 0.75%.

4.4 Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of the analyte. The linearity of response for Tofacitinib was determined in the range of 50% to 150 % (24.88-74.64 µg/mL for Tofacitinib). The calibration curve of the analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r^2] was found to be 1.000. Therefore the HPLC method was found to be a linear standard curve that ~~were~~ was calculated and given in **Figure 7.** to demonstrate the linearity of the proposed method. From the data obtained which is given in **Table 6.** and the method was found to be linear within the proposed range.

Table 6. Linearity studies for Tofacitinib

S.No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 50%	24.8811	22630203
2	Linearity at 75%	37.3217	33940308
3	Linearity at 100%	49.7623	45252405
4	Linearity at 120%	59.7147	54352901
5	Linearity at 150%	74.6434	67878609
Correlation coefficient (r^2)			1.0000
Intercept			- 2627.2858
Slope			909648.6341
% Y-intercept			-0.06

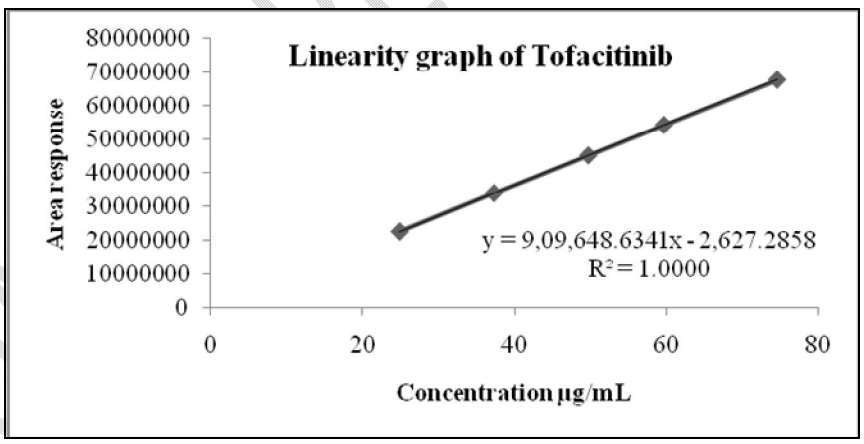

Figure 7. Calibration curve for Tofacitinib

4.5 Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples of Tofacitinib at 50% to 150% of the target concentration level. The recovery samples were prepared in triplicate preparations on Tofacitinib API spiked to placebo~~,~~ and analyzed as per the proposed method for each concentration level except 50% and 150 %. The above samples were chromatographed and the percentage recovery of each sample was calculated for the amount added. Evaluated the precision of

the recovery at each level by computing the relative standard deviation of six preparations for 50% and 150% level recovery samples results. The data obtained which given in **Table 7.** and the method was found to be accurate.

Table 7. Recovery studies for Tofacitinib

% Level	µg added	µg found	% Recovery	Mean % Recovery	% RSD
50% level-1	25.1243	25.4414	100.90	100.5	0.43
50% level-2	25.1514	25.3095	100.45		
50% level-3	25.4144	25.4281	100.04		
100% level-1	50.1145	70.4922	100.54	100.2	0.26
100% level-2	50.8982	70.9631	100.09		
100% level-3	50.1693	70.2264	100.08		
150% level-1	75.1854	75.1144	99.93	100.1	0.10
150% level-2	75.1479	75.2574	100.10		
150% level-3	75.3354	75.4593	100.12		

4.6 Solution stability of analytical solutions

Solution stability standards and sample solutions ~~was~~ were established at various conditions such as bench top at room temperature and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of initially prepared standard and sample solutions with freshly prepared standard solutions.

Table 8. Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.01	1.01
48hrs	1.00	1.02

Table 9. Results for solution stability of sample at room temperature

Time Interval	%Assay	%Assay difference
Initial	100.8	NA
24hrs	100.6	0.2
48hrs	101.6	0.8

Table 10. Results for solution stability of sample in Refrigerator

Time Interval	%Assay	%Assay difference
Initial	100.8	NA
24hrs	100.9	0.1
48hrs	101.3	0.5

Standard and sample solutions are stable for 48 hours when stored at room temperature and 2-8°C.

4.7 Robustness studies

To validate the method's robustness the chromatographic performance at changed conditions was evaluated compared to the nominal conditions of the method. ~~Standard~~ The standard solution was injected at each of the following changed conditions.

Table 11. Robustness studies Results

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ± 10%	1.7 mL	3559	1.1	0.11
	1.3 mL	3196	1.0	0.10
Temperature variation ± 5°C	30°C	3640	1.2	0.09
	20°C	3178	1.1	0.39
pH variation ± 0.2	4.2	3431	1.0	0.07
	3.8	3320	1.2	0.16
Mobile phase Variation ± 10%	88:22	3797	1.3	0.06
	92:8 v/v	3273	1.1	0.04

~~Method~~ The method is robust for changes like flow rate, column oven temperature, pH variation, and the organic phase of the mobile phase.

4.8 Filter validation

Performed the filter validation for sample solution, one portion of the solution was centrifuged and the other portion of the solution was filtered through 0.45 µm PVDF and 0.45 µm Nylon filters.

Table 12. Results for Filter validation

S.No.	Filter details	Area Response	% Assay	~~Difference~~ The difference when compared to Centrifuged
1	Centrifuged Sample	45965214	100.7	**NA**
2	0.45 µm PVDF Filtered Sample	45099392	101.1	**0.4**
3	0.45 µm Nylon Filtered Sample	44870597	98.5	**-2.1**

Filter validation parameter was established. Based on the above results and observations 0.45 µm PVDF filterers are suitable for filtration.

5.0 Discussion

RP-HPLC method for estimation of Tofacitinib in Tofacitinib tablets dosage form was developed and validated as per ICH guidelines. A simple, accurate, and reproducible reverse phase HPLC method was developed for the estimation of Tofacitinib in Tofacitinib tablets dosage form. The optimized method consists of a mobile phase ~~consisted~~ consisting of pH 4.0 phosphate buffer and acetonitrile in the ratio of (80:20 volume/volume) with Kromasil C18 (150 x 4.6mm, 5µ) column. The retention time of Tofacitinib was found to be 10.30 minutes. The developed method was validated as per ICH Q2A (R1) guideline.

As there is no interference ~~of~~ between blank and placebo at the retention time of Tofacitinib. Degradation study results were shown significant degradation was observed in alkali (base) stress conditions. Hence it can be concluded that Tofacitinib is sensitive to alkali.

~~In order t~~To obtain system precision, a study was conducted with six replicate injections. %RSD was estimated from the peak areas of Tofacitinib found to be 0.16% respectively.

The proposed HPLC method was linear over the range of 24.88-74.64 µg/mL, the correlation coefficient was found to be 1.0000. ~~Relative~~ The relative standard deviation for method precision was found to be 5mg and 10 mg ~~are~~ is 0.26% and 0.75%.

The accuracy studies were shown as % recovery for Tofacitinib 50% to 150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

The solution stability of the standard and samples are stable up to 48 hrs on a bench top and refrigerator (2-8°C). The method is robust for changes like flow rate, column oven temperature, pH variation, and the organic phase of the mobile phase.

Performed the filter validation for sample solution 0.45 µm PVDF filterers are suitable for filtration.

6.0 Conclusion

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability, and robustness. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Tofacitinib in bulk and pharmaceutical dosage forms.

Ethical approval

It is not applicable.

Abbreviations

USP: United States Pharmacopeia

EP: European Pharmacopoeia

JP: Japanese Pharmacopoeia

BP: British Pharmacopoeia

API: Active pharmaceutical ingredients

HPLC: High-Performance Liquid Chromatography

HPTLC: High-Performance Thin-Layer Liquid Chromatography

RT: Retention Time

ICH: International Council on Harmonization

SD: Standard Deviation

RSD: Relative Standard Deviation

7.0 REFERENCES

1. http://www.rheumatology.org/I-Am-A/Patient-Caregiver/Diseases-Conditions/ Rheumatoid -Arthritis

2. Maria A Lopez-Olivo, Huifang Lu, Jean H Tayar. Review of Tofacitinib in rheumatoid arthritis. Clin. Invest. (Lond.). 2015; 5: 23–38.

3. Kirandeep Kumar, Sonesh Kalra, Sandeep Kaushal. Systematic Review of Tofacitinib: A New Drug for the Management of Rheumatoid Arthritis. Clinical Therapeutics. 2014; 36: 1074-1086.
4. Sohita Dhillon. Tofacitinib: A Review in Rheumatoid Arthritis. Drugs. 2017; 77: 1987-2001.
5. Roy Fleischmann, Joel Kremer, Yoshiya Tanaka, David Gruben, Keith Kanik, Tamas Koncz et.al. Efficacy and safety of Tofacitinib in patients with active rheumatoid arthritis: review of key Phase 2 studies. International Journal of Rheumatic Diseases. 2016; 19: 1216–1225.
6. J.A. Hodge, T.T. Kawabata, S. Krishnaswami, J.D. Clark et.al. The mechanism of action of Tofacitinib – an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis. Clin Exp Rheumatol. 2016; 34: 318-328.
7. Stanley B Cohen, Yoshiya Tanaka, Xavier Mariette, Jeffrey R Curtis et.al. Long-term safety of Tofacitinib for the treatment of rheumatoid arthritis up to 8.5 years: integrated analysis of data from the global clinical trials. Annals of the rheumatic diseases. 2017; 76.
8. Castaneda, Oswaldo M, Romero, Felix J, Salinas, Ariel Citera. Safety of Tofacitinib in the Treatment of Rheumatoid Arthritis in Latin America Compared With the Rest of the World Population. Journal of Clinical Rheumatology. 2017; 23: 193-199.
9. S Perez-Baos, J Barrasa, P Gratal, A Larrañaga-Vera, I Prieto-Potin, G Herrero-Beaumon. Tofacitinib restores the inhibition of reverse cholesterol transport induced by inflammation: understanding the lipid paradox associated with rheumatoid arthritis. British Journal of pharmacology. 2017; 174: 3018-3031.
10. Brian Feagun. Update on Tofacitinib for Inflammatory Bowel Disease. Gastroenterology hepatol. 2016; 12: 572-574.
11. Naoki Iwamoto, Sosuke Tsuji, Ayuko Takatani, Toshimasa Shimizu et.al. Efficacy and safety at 24 weeks of daily clinical use of Tofacitinib in patients with rheumatoid arthritis. Plos one. 2017; 1-5.
12. Eun Bong Lee, Roy Fleischmann, Stephen Hall, Bethanie Wilkinson, John D. Bradley et.al. Tofacitinib versus Methotrexate in Rheumatoid Arthritis. The New England Journal of Medicine. 2018; 26: 2377-2386.
13. Yong Chen, Fang Yuan Gong, Zhen Jun Li, Zhe Zhou et.al. A study on the risk of fungal infection with Tofacitinib (CP-690550), a novel oral agent for rheumatoid arthritis. Scientific Reports. 2017; 7: 1-9.
14. S Menon, R Riese, R Wang, CW Alvey, H Shi, W Petit, S Krishnaswami. Evaluation of the Effect of Tofacitinib on the Pharmacokinetics of Oral Contraceptive Steroids in Healthy Female Volunteers. Clin Pharmacol Drug Dev. 2016; 5: 336-342.
15. http://pubchem.ncbi.nlm.nih.gov/compound/9926791
16. http://www.drugbank.ca/drugs/DB08895
17. Sampath Kumar Reddy, V.S Nagaraju, S.T. Rajan, S Eshwaraiah et.al. Stability indicating HPLC method for the quantification of Tofacitinib citrate and its related substances. Der pharma chemical. 2014; 6: 11-19.
18. A. S. K. Shankar, B. Datchayani, N. Balakumaran, Mohammed Rilwan, R.Subaranjani. Development of a Validated Reverse Phase Liquid Chromatographic Assay-Method for determination

of Tofacitinib in pure form and in Physical Admixtures. Research Journal of Pharmacy and Technology. 2017; 10: 223-226.
19. S Vijay Kumar, Vinay Dhiman, KK Giri, K Sharma, M Zainuddin, R Mullangi. Development and validation of a RP-HPLC method for the quantitation of Tofacitinib in rat plasma and its application to a pharmacokinetic study. Biomed Chromatogr. 2015; 29: 1325-1329.
20. NV Thakariya, SB Ezhava. Stability Indicating HPTLC Method for Determination of Tofacitinib Citrate. Der Pharma Chemica. 2017; 9:12-18.
21. ICH guidelines, for stability testing of new drug substances and products Q1A (R2), 2004.
22. ICH guidelines for validation of analytical procedures: text and methodology Q2 (R1) 2005.