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Development and Validation of Stability Indicating RP-HPLC Method for Quantitative Estimation of Tofacitinib in Tofacitinib Tablets Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The main intention of the research work is to develop an effective, sensitive, economical and uncomplicated reverse phase HPLC technique 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 Nagar, 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 Kromasil C18 (150 x 4.6 mm, 5µ) and the mobile phase

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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 meddling between blank and placebo at the retention time of Tofacitinib. Degradation study results were shown significant degradation was observed in alkali (base) stress condition. Hence it can be concluded that Tofacitinib is sensitive to alkali. To obtain system exactitude, a study was conducted with six replicate injections. %RSD was estimated from the peak areas of Tofacitinib found to be 0.16% respectively. The relative standard deviation for method exactitude was found to be 5 mg and 10 mg is 0.26% and 0.75%. The proposed HPLC technique 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% to150% 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 upto 48 hrs on a bench top and refrigerator (2-8°C). The technique 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 technique has authenticated as per ICH instructions and all the validation parameters are satisfy the ICH Q2 specification acceptance limits.

Conclusion: The urbanized technique 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; validation.

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

1. 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 Fig. 1. [15-16].

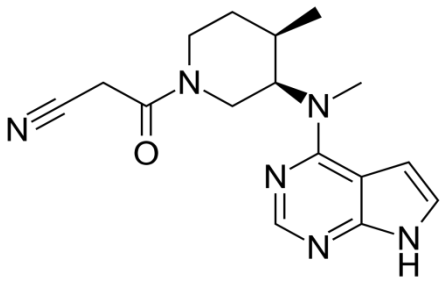

Fig. 1. Chemical structure of Tofacitinib

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 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. 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.

2.2.1 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 well then pH was adjusted to 4.0 with ortho phosphoric acid solution. Filtered through 0.45 µm membrane filter.

2.2.2 Preparation of mobile phase

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

2.2.3 Preparation of diluent

Prepared a mixture of irrigate and cyanomethane in the proportion of 50:50 (%volume/volume) mixed well and sonicated to degas.

2.2.4 Preparation of standard solution

Weighed correctly and transferred 25.7 mg of Tofacitinib working standard into a 50 mL volumetric thermos sonicated for 2 minutes to dissolve the contents and made upto the quantity with diluent. Further diluted this solution 5 mL in to 50 mL volumetric thermos and made up the quantity with diluent and mixed well. (The concentration of the standard solution containing, Tofacitinib 50 ppm)

2.2.5 Preparation of test solution (for Tofacitinib tablets 5.0 mg)

Weighed correctly 10 tablets (Tofacitinib tablets 5 mg) and transferred (equivalent to 50 mg of Tofacitinib) into 200 mL volumetric thermos, added 120 mL of diluent and sonicated for 30 minutes, with intermediate shaking, cool to room temperature and diluted to quantity 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 thermos, diluted to quantity with diluent, and mixed well. (The concentration of the solution contains 50.0 µg/mL of Tofacitinib).

2.2.6 Preparation of test solution (for Tofacitinib tablets 10.0 mg)

Weighed correctly 5 tablets (Tofacitinib tablets 10 mg) and transferred (equivalent to 50 mg of Tofacitinib) into 200 mL volumetric thermos, added 120 mL of diluent and sonicated for 30 minutes, with intermediate shaking, cool to room temperature and diluted to quantity 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 thermos, diluted to quantity with diluent, and mixed well. (The concentration of the solution contains 50.0 µg/mL of Tofacitinib).

2.2.7 Preparation of placebo solution

Weighed correctly and transferred placebo powder (equivalent to 50 mg of Tofacitinib) into 200 mL volumetric thermos, added 120 mL of diluent and sonicated for 30 minutes, with intermediate shaking, cool to room temperature and diluted to quantity 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 thermos, diluted to quantity with diluent and mixed well. (The concentration of the solution contains 50.0 µg/mL of Tofacitinib).

2.2.8 Chromatographic conditions

Chromatographic analysis was executed on Kromasil C18 (150 x 4.6 mm, 5µ) mobile phase consisting of pH 4.0 phosphate buffer and acetonitrile in the proportion of (80:20 volume/volume). The run velocity was 1.5 mL/min, the column oven temperature was 25°C and the sampler cooler temperature was 25°C, the inoculation volume was 20 µL, and detection was implemented at 215 nm using a photodiode array detector (PDA).

3. METHOD DEVELOPMENT

UV-spectroscopic analysis of Tofacitinib drug substance demonstrated utmost UV absorbance (λmax) at 215 nm respectively.

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

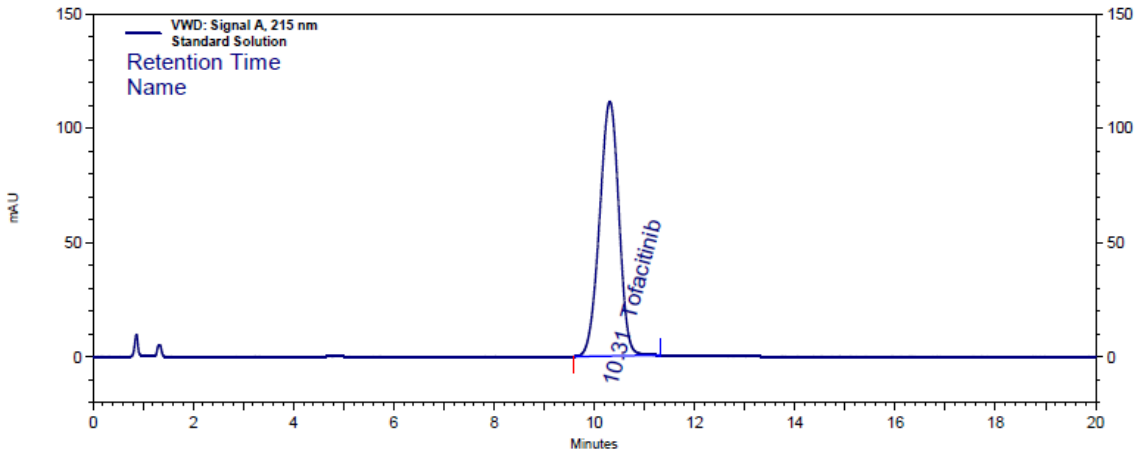

Fig. 2. Typical chromatogram of Tofacitinib standard

For the subsequently trial the mobile segment consisted of pH 4.0 phosphate buffer and acetonitrile in the proportion 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 establish to be good. The chromatogram of Tofacitinib standard using the proposed method is shown in Fig. 2. System suitability results of the technique are presented in Table 1.

4. METHOD VALIDATION

The urbanized RP-HPLC technique was extensively authenticated for assay of Tofacitinib in Tofacitinib tablets formulation using the following parameters.

4.1 Specificity and System Suitability

4.1.1 Blank and Placebo interference

A study to established the meddling of blank and placebo were conducted. Diluent and placebo were infused into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution Fig. 3. showed no peak at the retention time of Tofacitinib peak. This specify that the diluent solution used in sample preparation does not interfere with the inference of Tofacitinib in Tofacitinib tablets dosage form. Similarly chromatogram of the placebo solution Fig. 4. showed no peaks at the retention time of Tofacitinib peak. This indicates that the placebo used in sample preparation does not interfere with the inference of Tofacitinib in Tofacitinib tablets formulation.

4.1.2 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. The degradation study results were presented in Table 2.

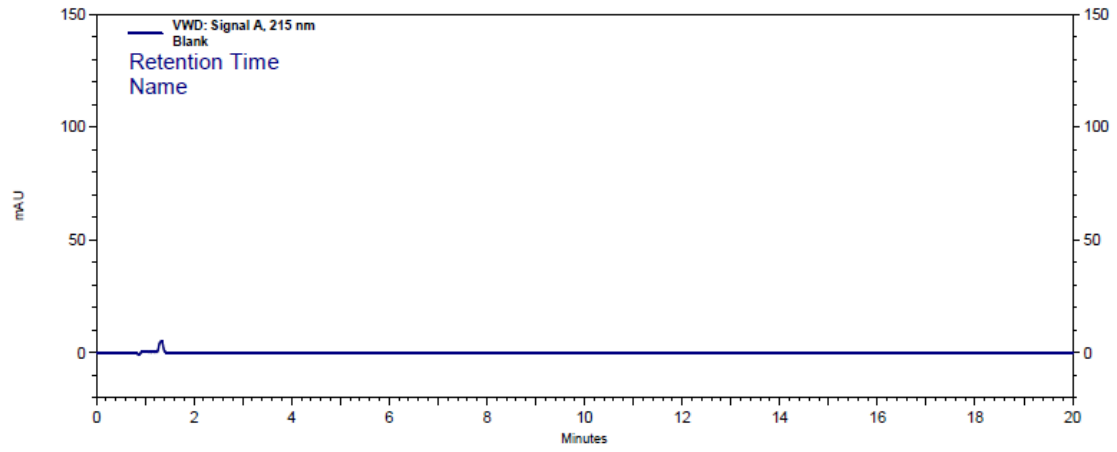

Fig. 3. Typical chromatogram blank

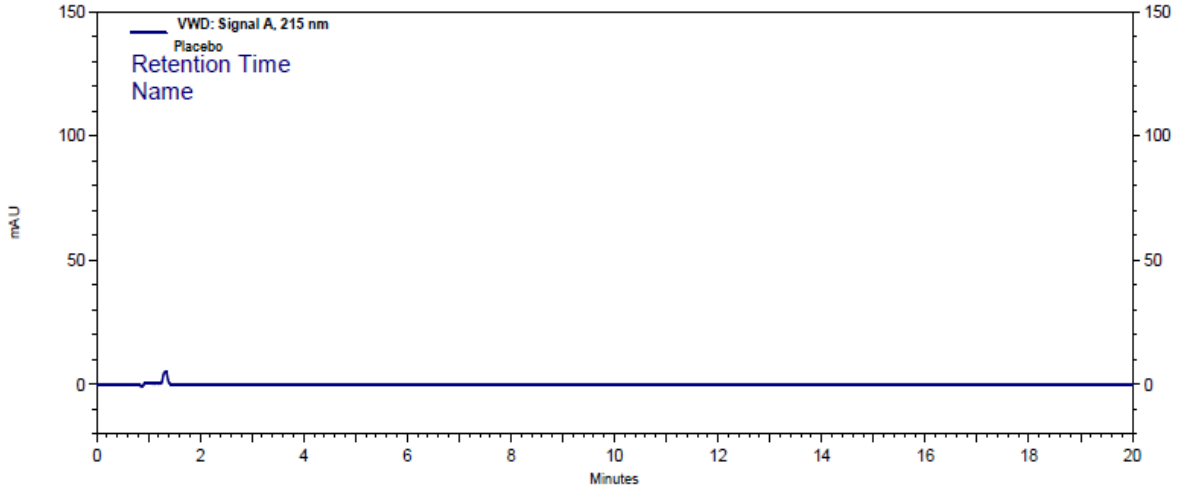

Fig. 4. Typical chromatogram placebo

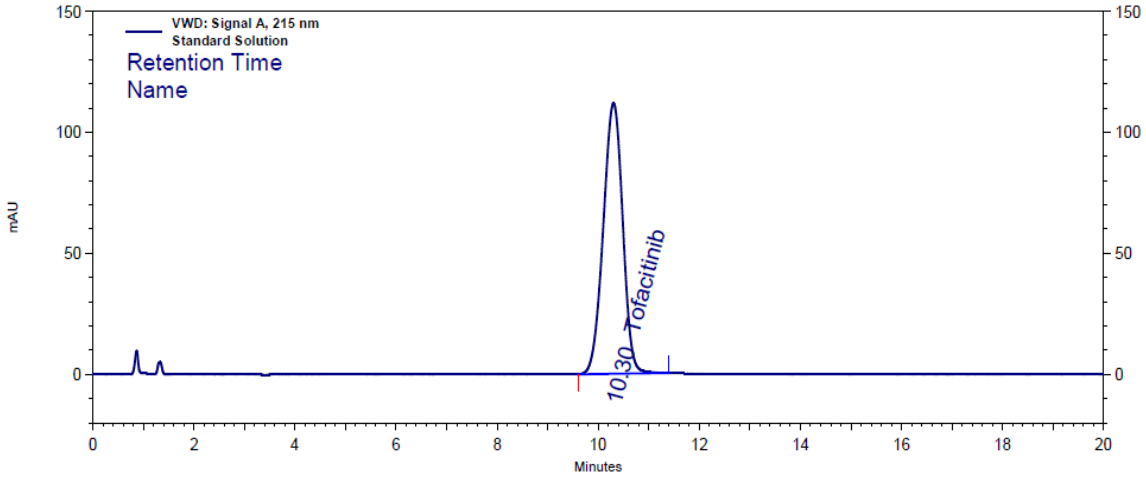

Fig. 5. Typical chromatogram standard

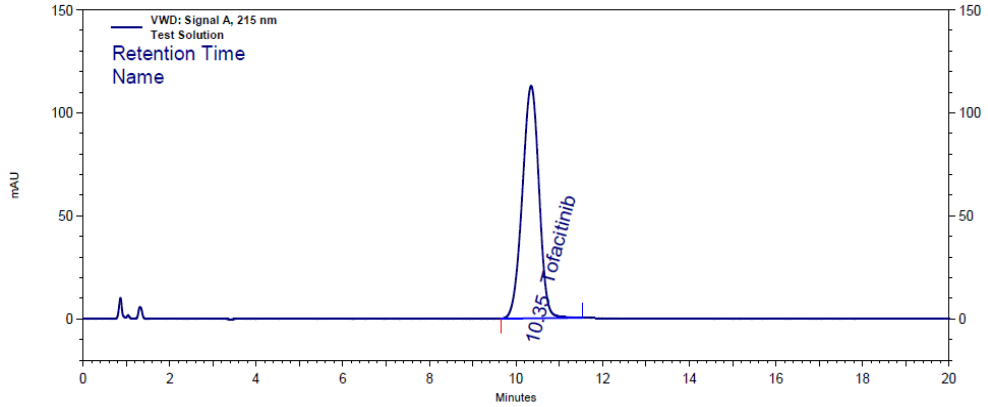

Fig. 6. Typical chromatogram sample

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

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

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**

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 organized as per the test technique, infused into the HPLC system six times, and evaluated the % RSD for the vicinity responses. The data were shown in Table 3.

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

4.3 Method Exactitude

The exactitude of the test technique was evaluated by doing an assay for six samples of Tofacitinib tablets (5 mg and 10 mg) as per the test technique. 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.

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

4.4 Linearity of Detector Response

The linearity of an analytical technique 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 technique was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r^2] was establish to be 1.000. Therefore the HPLC technique was establish to be a linear standard curve that was calculated and given in Fig. 7 to demonstrate the linearity of the proposed technique. From the data obtained which is given in Table 5 and the technique was establish to be linear within the proposed range.

4.5 Accuracy

The accuracy of the test technique was demonstrated by preparing recovery samples of Tofacitinib at 50% to 150% of the target concentration level. The recovery samples were organized in triplicate arrangements on Tofacitinib API spiked to placebo, and analyzed as per the recommend technique for apiece attentiveness level except 50% and 150%. The above samples were chromatographed and the percentage recovery of apiece sample was calculated for the amount added. Evaluated the exactitude of the recovery at apiece level by computing the relative standard deviation of six preparations for 50% and 150% level recovery samples consequences. The data obtained which given in Table 6 and the technique was establish to be accurate.

4.6 Solution Stability of Analytical Solutions

Solution constancy standards and sample solutions were established at an assortment of circumstances such as bench top at room temperature and in refrigerator 2-8°C. The constancy of standard and sample solutions was establish by assessment of initially prepared standard and sample solutions with freshly prepared standard solutions.

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

4.7 Robustness Studies

To authenticate the technique robustness the chromatographic performance at distorted circumstances was assessed evaluated to the ostensible conditions of the technique. The standard solution was infused at each of the following altered circumstances.

The technique is robust for altered like flow rate, column oven temperature, pH variation, and the organic phase of the mobile phase.

Table 4. Method precision data for Tofacitinib tablets

S. No	No. of Preparations	% Assay (5 mg)	% Assay (10 mg)
1	Preparation 1	100.8	100.1
2	Preparation 2	100.3	99.4
3	Preparation 3	100.7	100.4
4	Preparation 4	101.1	101.3
5	Preparation 5	100.7	100.5
6	Preparation 6	100.9	101.4
Average		**100.8**	**100.5**
SD		**0.2665**	**0.7521**
%RSD		**0.26**	**0.75**

Table 5. 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

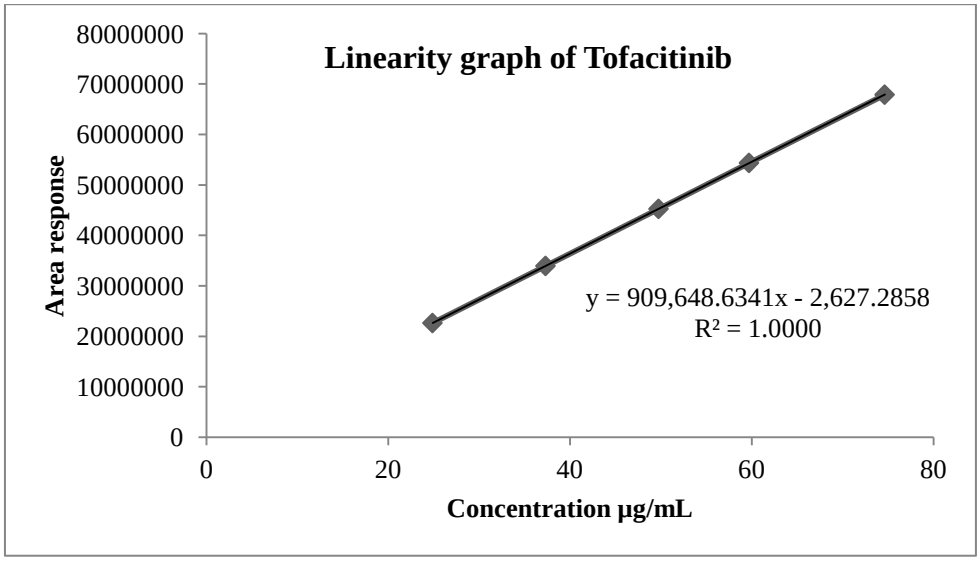

Fig. 7. Calibration curve for Tofacitinib

Table 6. 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		

Table 7. 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 8. 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 9. 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

Table 10. 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^{o}C	30^{o}C	3640	1.2	0.09
	20^{o}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 v/v	3797	1.3	0.06
	92:8 v/v	3273	1.1	0.04

Table 11. Results for Filter validation

S. No.	Filter details	Area Response	% Assay	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**

4.8 Filter Validation

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

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

5. DISCUSSION

RP-HPLC technique for inference of Tofacitinib in Tofacitinib tablets dosage form was urbanized and authenticated as per ICH instructions. A simple, accurate and reproducible reverse segment HPLC technique was urbanized for the inference of Tofacitinib in Tofacitinib tablets dosage form. The optimized technique consists of a mobile segment consisting of pH 4.0 phosphate buffer and acetonitrile in the proportion of (80:20 volume/volume) with Kromasil C18 (150 x 4.6mm, 5µ) column. The retention time of Tofacitinib was establish to be 10.30 minutes. The urbanized technique was authenticate as per ICH Q2A (R1) guideline.

As there is no meddling flanked by blank and placebo at the retention time of Tofacitinib. Degradation study consequences were shown noteworthy degradation was observed in alkali (base) stress conditions. Hence it can be concluded that Tofacitinib is sensitive to alkali.

To achieve system exactitude a study was conducted with six replicate inoculations. %RSD was estimated from the zenith vicinity of Tofacitinib found to be 0.16% respectively.

The proposed HPLC technique was linear over the assortment of 24.88-74.64 µg/mL, the correlation coefficient was establish to be 1.0000. The relative standard deviation for technique exactitude was originate to be 5 mg and 10 mg is 0.26% and 0.75%.

The accuracy studies were exposed as % recovery for Tofacitinib 50% to150% level. The limit of % recovered revealed is in the range of 98 and 102% and the consequences obtained were establish to be within the limits. Hence the technique was establish to be accurate.

The solution constancy of the standard and samples are steady able to 48 hrs on a bench top and refrigerator (2-8°C). The technique is robust for modify 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. CONCLUSION

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

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Available: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. Hodge JA, Kawabata TT, Krishnaswami S, Clark JD, 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. Available:http://pubchem.ncbi.nlm.nih.gov/compound/9926791
16. Available:http://www.drugbank.ca/drugs/DB08895
17. Sampath Kumar Reddy, Nagaraju VS, Rajan ST, Eshwaraiah S, et al. Stability indicating HPLC method for the quantification of Tofacitinib citrate and its related substances. Der Pharma Chemical. 2014;6:11-19.
18. Shankar ASK, Datchayani B, Balakumaran N, Mohammed Rilwan, Subaranjani R. 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. Vijay Kumar S, Vinay Dhiman, Giri KK, Sharma K, Zainuddin M, Mullangi R. 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. Thakariya NV, Ezhava SB. 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.

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