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A Novel Stability Indicating Chromatographic Method Development And Validation For The Quantification of Tofacitinib In Pure And Its Dosage Form

Prathyusha Naik C.N*[1], K.B. Chandra Sekhar[2]
[1]*Research Scholar, Research and Development, Jawaharlal Nehru Technological University, Anantapur 515002, AP, India.*
[2]*Professor and Director, JNTUA- Oil Technological and Pharmaceutical Research Institute, Jawaharlal Nehru Technological University, Anantapur 515002, AP, India.*
Corresponding Author: Prathyusha Naik C.N

Abstract: *The present study was focused to develop a novel, selective, reliable and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method for the detection and quantification of Tofacitinib in pure form as well as in its dosage form. The chromatographic method was carried out using isocratic elution programme on C_{18} Phenomenex Luna (250 x 4.6 mm x 5µm) column with a mobile phase proportion of water and Methanol in the ratio of 50:50 (% v/v). The flow rate was set to 1.0 ml/min with 20 µl injection volume. The eluted components were monitored at 254 nm and ambient column oven temperature was maintained. The developed analytical method was validated according to the ICH guidelines.*
The developed method was also subjected to various stress conditions like acidic and alkaline hydrolysis, oxidation, photolysis and thermal degradation. The method showed linearity across the concentration range of 10- 60 µg/ml. Limit of detection and quantification was found to be 1.45 and 4.40 µg/ml respectively. The developed method is specific, precise, accurate, robust and stability indicating which can be successfully applied for routine analysis, quality control analysis and also suitable for stability analysis of assay of Tofacitinib in pure form and its formulation as per the regulatory requirements.
Keywords: *Tofacitinib, RP-HPLC, Stress condition*

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I. Introduction

Tofacitinib (TFB)[1] is a drug of the januskinase (JAK) inhibitor class[2], discovered and developed by Pfizer.[3] Chemically, it is (3R,4R)-4-Methyl-3-(methyl-7H-pyrrolo[2,3-d] pyrimidin-4-ylamino)-ß-oxo-1-piperidinepropanenitrile,2-hydroxy-1,2,3-propanetri carboxylate (1:1). It is currently approved for the treatment of rheumatoid arthritis (RA) in the United States and Russia, and is being studied for treatment of psoriasis, inflammatory bowel disease, and other immunological diseases, as well as for the prevention of organ transplant rejection. It is an inhibitor of the enzyme janus kinase 3 (JAK3), which interferes with the JAK-STAT signaling pathway, and transmits extracellular information into the cell nucleus, influencing DNA transcription [4]. The chemical structure of Tofacitinib Citrate is shown in (Fig.1).

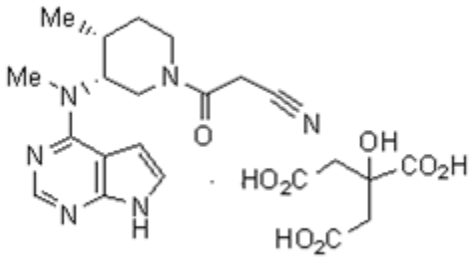


Fig-1: Chemical structure of Tofacitinib Citrate

From thorough literature only one method have been reported in single analyte includes liquid chromatography [5] method. The current study was aimed to develop a simple, accurate, precise and robust validated stability indicating cost effective method using RP-HPLC and was extended for its formulations and bulk samples than existing one.

II. Materials And Methods

Tofacitinib (TFB) working standard was obtained from MSN Laboratories Private Limited, Hyderabad, India. HPLC grade methanol and AR grade of hydrochloric acid and hydrogen peroxide were purchased form SD fine chem. Ltd, Mumbai. HPLC grade water and AR grade Orthophosphoric acid was purchased from Merck, Mumbai and sodium hydroxide from Molychem, Mumbai.

A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) chromatographic system, the output signal was monitored and processed using LC solutions as chemstation, a UV/VIS PDA detector (SPD-20AT) and an LC-20AD solvent delivery module was used during the method development and validation. A Phenomenex Luna C18 250 x 4.6 mm, 5 µm, column was used as stationary phase.

Preparation of Standard solutions:

Working standard solution was prepared by weighing 10 mg of drug in 10 ml volumetric flask and sufficient amount of Methanol was added to dissolve it and final volume was made up to 10 ml (Stock A; 1000 µg/ml). Various aliquots from stock A were prepared to obtain concentrations between 10-60 µg/ml, which were further diluted using mobile phase.

III. Results And Discussion

Method validation

The developed method was validated as per ICH Q2 guidelines [6] for various validation parameters viz specificity, selectivity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness. Degradation of TFB was performed under neutral, acidic, basic, oxidative, thermal and photolytic stress conditions [7-11].

Linearity test solutions for the assay method were prepared from working TFB stock solution at six concentration levels from 50 to 150% of assay analyte concentration (10- 60µg/ml). The peak area versus concentration data was treated by least-squares linear regression analysis as shown in table 1.

Table 1 Validation parameters determined during method validation for the detection and quantification of Tofacitinib

Validation Parameter	Tofacitinib
Linearity Range	10-60µg/ml
Regression equation	Y= 46121x + 50032
Correlation coefficient (r^2)	0.999
LOD (µg/ml)	1.45
LOQ (µg/ml)	4.40
Precision (% RSD)	0.87
Intermediate Precision (%RSD)	1.58
Assay (%)(± % RSD)	100.17 ±0.43
Recovery (%) (± % RSD)	99.95 ± 1.40

***The validation parameters were determined from the analysis of six standard solutions (n = 6) over a concentration range of 10.0– 60.0 µg/ml. %RSD: Percentage relative standard deviation. LOD: Limit of detection, LOQ: Limit of quantification.**

The precision of the related substances method verified by repeatability and by intermediate precision. Repeatability was checked by injecting six individual preparations of Tofacitinib sample and % RSD of area for each concentration was calculated. The intermediate precision of the method was also evaluated using different analyst and performing the analysis on different days. Precision of assay method was evaluated by carrying out six independent assays of real sample of Tofacitinib at 40µg/ml level against qualified reference standard and % RSD was found not more than 2.0 % and were therefore deemed as sufficient proof of the precision of this method. The % RSD for repeatability and intermediate precision was shown in table 1.

LOD and LOQ for sample were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at LOQ level by injecting six individual preparations of TFB and the % RSD of the area was calculated. The values of LOD and LOQ were reported in table 1.

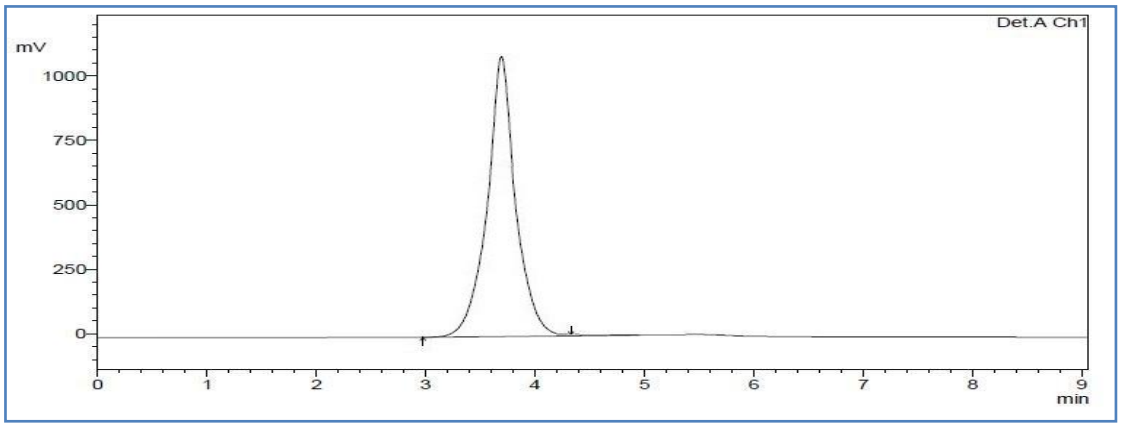
Accuracy of the assay method was evaluated in triplicate using three concentration levels 50, 100 and 150µg/ml on real sample. Standard addition and recovery experiments were conducted on real sample to determine accuracy of the related substance method. Study was carried out in triplicate using three concentration levels. The percentage of recoveries for TFB was calculated and from the result obtained it can be concluded that the method delivers accurate results. The results of recovery studies were depicted in table 1.

Robustness was determined for the developed method, experimental conditions were deliberately altered. The flow rate of the mobile phase was 1.0 ml/min, to study the effect of flow rate was changed by 0.1 units. The effect of the column temperature was studied at 30^{o}C and 40^{o}C instead of 35^{o}C. Slight change in these parameters is not much effected and which shows the method is robust.

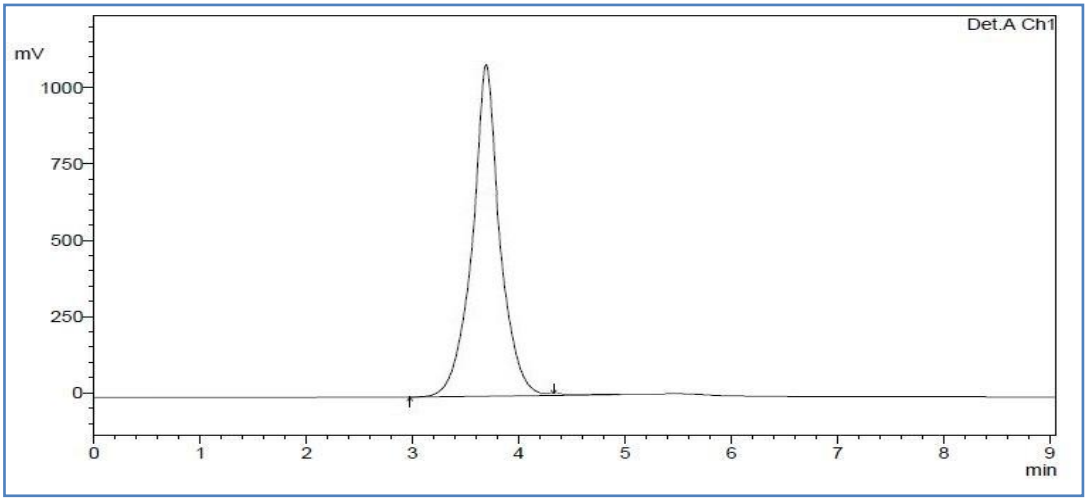
Solution stability of TFB in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h. The same sample solutions were assayed for 12-h interval up to the study period.

Specificity is the ability of the method to measure the analyte response. It was tested by analysing the prepared sample solutions which contained typical formulation with excipients. Chromatograms obtained for Tofacitinib standard and sample solutions were shown in Fig.2. Stress studies were performed for Tofacitinib formulation to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted with a stress condition of UV light (254 nm), temperature (70^{o}C), acid (0.1M HCl), base (0.1M NaOH) and oxidation (3.0% H_2O_2) to evaluate the ability of the proposed method to separate Tofacitinib from its degradation product. For heat and photolytic studies, study period was 10 days. Whereas for hydrolytic, acid, base and oxidation, it was 24 h. Peak purity test was carried out for the Tofacitinib peak by using PDA detector in stress samples.

Tofacitinib was found to slight degrade in oxidation (3.0%v/v H_2O_2) and mild degradation was observed in thermal stress conditions shown in Fig.3. TFB was found to be stable in all conditions conditions shown in Fig. 4. Assay studies were carried out for stress samples against Tofacitinib qualified working standard. The mass balance (% assay + % degradants) results are presented in Table 2. The purity and assay of Tofacitinib was unaffected by degradation products and thus confirms the stability-indicating power of the developed method.



(a)



(b)

Fig. 2: Chromatograms obtained for Tofacitinib during specificity testing with chromatogram (a) showing the peak obtained for Tofacitinib from a standard solution and (b) showing the peak obtained for Tofacitinib detected from a formulation

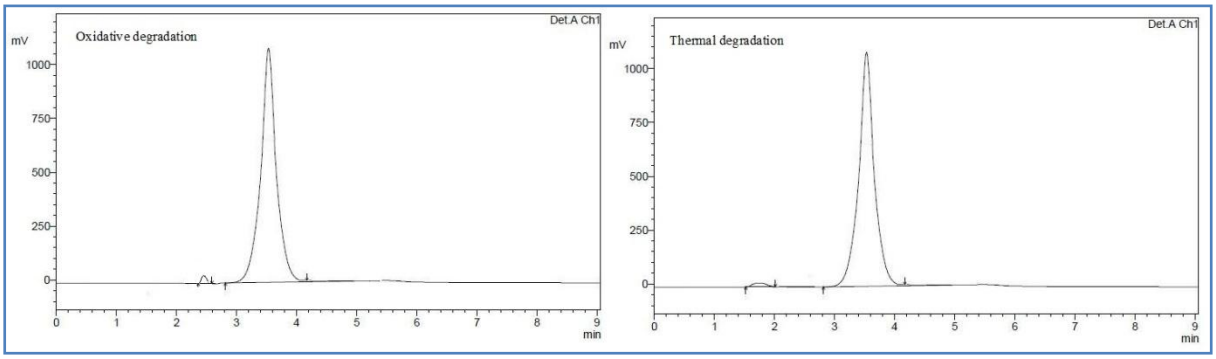


Fig.3: Typical chromatograms of Tofacitinib forced degradation samples (Oxidative (3.0%H_2O_2) and Thermal studies)

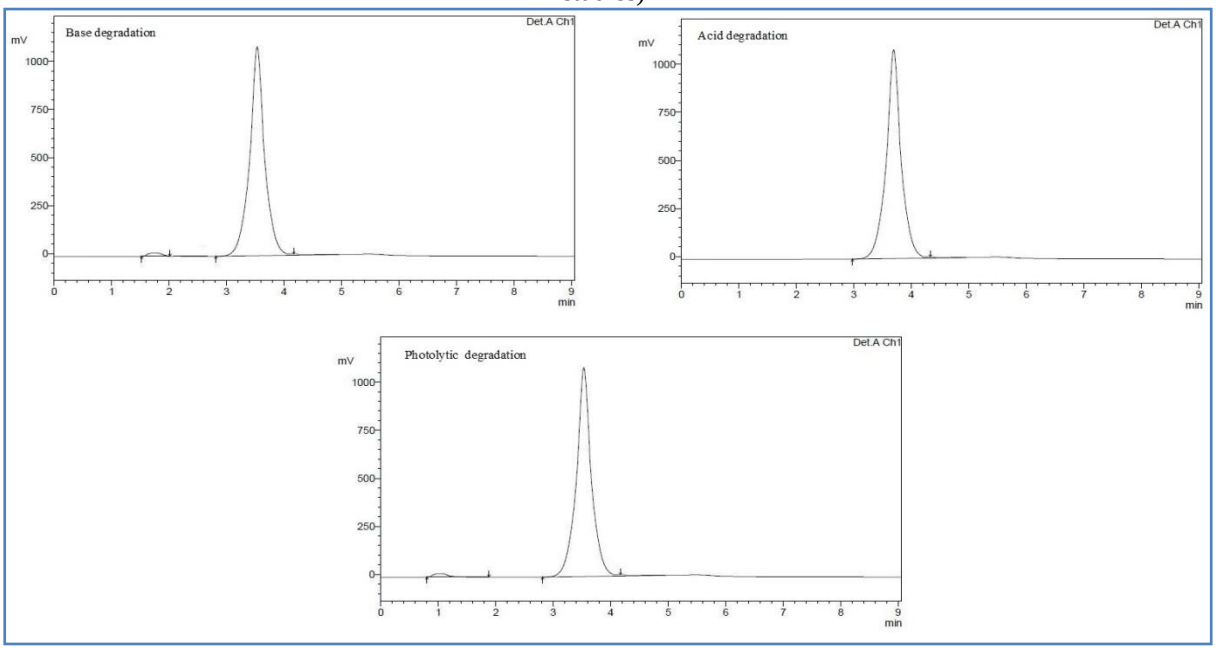


Fig. 4: Typical chromatograms of Tofacitinib forced degradation samples (Acid, Base and Photolytic studies)

Table 2: Summary of forced degradation studies

Stress Condition	**Tofacitinib**	
	% Purity	**% Degradation**
Acid degradation	98.6	1.4
Base degradation	97.4	2.6
Peroxide degradation	88.2	11.8
Thermal degradation	92.7	7.3
Photolytic degradation	98.1	1.9

IV. Conclusion

A new specific stability indicating RP-HPLC method was developed for the estimation of TFB in the pharmaceutical dosage form and validated according to ICH guidelines. The method was found to be specific for the detection of all possible impurities in the dosage form under various conditions and accurate, precise and robust for the assay of TFB in dosage forms.

Acknowledgement

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