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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TOFACITINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Dr. Narasimha Rao B. V.*[1], Dr. Prasada Rao M.[1], Udaya Krishna Veni A.[2], Lavanya D.[3], Ambika G.[4], Manasa J.[5], Dharshan M.[6], Swetha M.[7]

[1]Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh.

[2]Assistant Professor, Department of Pharmaceutical Analysis, SS and N College of Pharmacy, Narasaraopet, Andhra Pradesh.

[3-7]IV/IV B. Pharm Student, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.

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***Corresponding Author: Dr. Narasimha Rao B. V.**
Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh.
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ABSTRACT

This work developed and validated a reverse phase high performance liquid chromatography method for estimating tofacitinib in bulk and pharmaceutical formulations. In statistically planned studies, a number of method aspects were changed, such as mobile phase ratio and column type, to assess how these factors affected the chromatographic separation of tofacitinib. The separation was carried out on a Phenyl HexylColumn (150 x 4.6 mm and 5µm) at room temperature under isocratic conditions at a flow rate of 1.0 mL/min using 0.1% formic acid pH 2.9: Acetonitrile in a ratio of 50:50 (v/v). A PDA detector operating at 287 nm for a total of 6 minutes made the detection with the retention time of 3.521 minutes. Calibration curves were linear between 2.75 and 16.5 µg/mL. The calculated LOQ of 0.98µg/mL and the observed LOD of 0.32µg/mL show how sensitive the developed technique is. The %RSD being less than 2 validated the robustness and ruggedness of the approach. The assay % for formulation analysis was 99.36. Consequently, this method was frequently used to analyze tofacitinib in bulk and pharmaceutical formulations.

KEYWORDS: Tofacitinib, Method Development, Calibration curve, Robustness and Ruggedness.

INTRODUCTION

Ankylosing spondylitis, ulcerative colitis, moderate-to-severe rheumatoid arthritis, psoriatic arthritis, and polyarticular course juvenile idiopathic arthritis are among the chronic inflammatory diseases that are treated with tofacitinib, a JAK inhibitor.[1,2] It interferes with the JAK-STAT signaling system, which carries extracellular information into the cell nucleus and affects DNA transcription, by inhibiting the enzymes janus kinase 1 (JAK1) and janus kinase 3 (JAK 3).[3-5]

For the estimation of tofacitinib in bulk and pharmaceutical formulation, only a few analytical (more run and retention time) and bioanalytical procedures have been described up to this point. Fig. 1 depicts the chemical structure of tofacitinib.

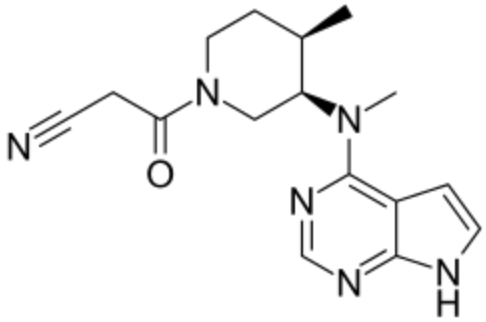

Fig. 1: The Chemical structure of Tofacitinib.

MATEIALS AND METHODS

Chemicals and Reagents

The working standard drug tofacitinib (99.30% purity) was obtained from Dr. Reddy's Laboratories, Hyderabad, Telangana. The formulation dosage form having brand name Tofajak-In XR 11mg containing 11 mg of tofacitinib, was purchased from local Pharmacy. HPLC grade Methanol, Water and Acetonitrile were purchased form Merk chemicals private limited, Mumbai. Formic acid used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of Mobile Phase

A 50:50% v/v mixture of acetonitrile and 0.1% formic acid (P^H 2.9) was made. The mobile phase was sonicated for 15 minutes to remove dissolved gases, and then it was filtered through a 0.45 µm nylon filter before being used.

Preparation of standard drug solution

11 mg of the tofacitinib were carefully weighed and added to a 10 ml volumetric flask. It was then completely dissolved in 7mL of acetonitrile using an ultrasonicator. After adjusting the final volume in the volumetric flask with the same solvent, the solution was filtered using a 0.45µm Nylon filter. A 1100 µg/mL standard stock solution was obtained. The concentrations (11 µg/mL) required for method development and validation parameters were prepared from the stock (1100 µg/mL) solution of tofacitinib using mobile phase as a diluent.

Preparation of formulation solution

Ten tofacitinib tablets (11 mg according to the label) were carefully weighed and pulverised into a fine powder. 11 mg of tofacitinib precisely weighed tablet powder, was added to a 10 mL volumetric flask. About 7 mL of acetonitrile was added to the flask, and the mixture was sonicated for 15 minutes to ensure complete drug extraction from the tablet

matrix. The same diluent was used to adjust the volume once the solution had cooled to room temperature. The resultant solution was thoroughly mixed to produce a sample stock solution with a concentration of 1100 µg/Ml. The sample stock solution was filtered using a 0.45 µm nylon filter, and the first few mL of the filtrate were thrown away.

1.0 mL of the filtered sample stock solution was carefully pipetted into a 10 mL volumetric flask and diluted to volume with the diluent to produce a working sample solution of 110 µg/mL. Additional dilutions were made from the working sample solution using the same diluent in order to achieve the required concentration 11 µg/mL for assay and validation testing.

METHOD DEVELOPMENT

Selection of Wavelength

The PDA detector was used to scan reference solutions containing 11 µg/mL in order to choose an appropriate wavelength. The maximum wavelength that was acquired was chosen as the appropriate wavelength for the detection.

Table 1: Optimized Chromatographic Conditions.

Parameter	Condition
Mobile Phase	0.1% formic acid pH 2.9: acetonitrile 50:50
Column	Phenyl HexylColumn (150 x 4.6 mm and 5µm)
Flow Rate	1.0 ml/min
Wavelength	287nm
Injection Volume	20 µL
Temperature	30°C
Run time	6min

METHOD VALIDATION

The method was validated in terms of specificity, system suitability, LOD & LOQ, linearity, accuracy, precision, ruggedness, and robustness in compliance with the ICH requirements. Validation was carried out using duplicate injections of the sample and standard solutions into the column.[6]

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

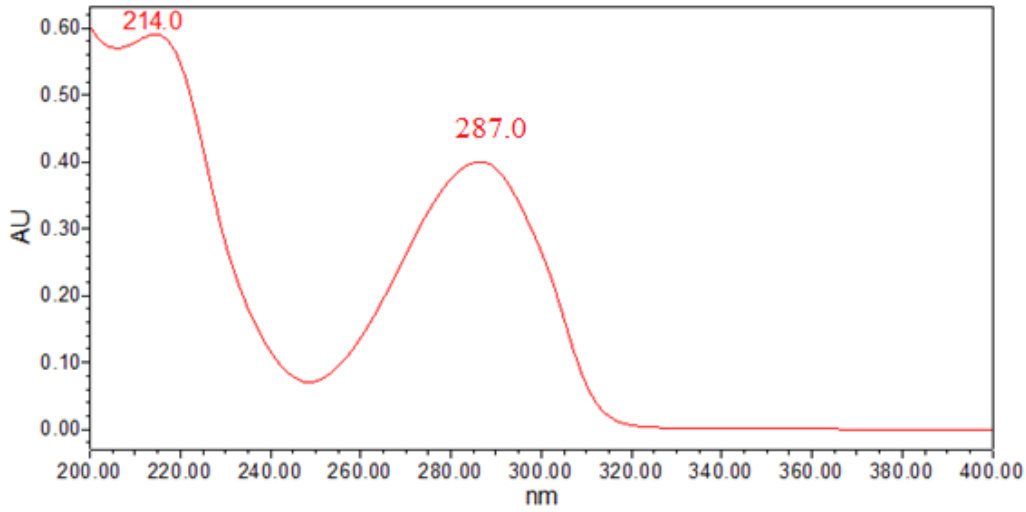

Fig. 2: UV Spectra of Tofacitinib.

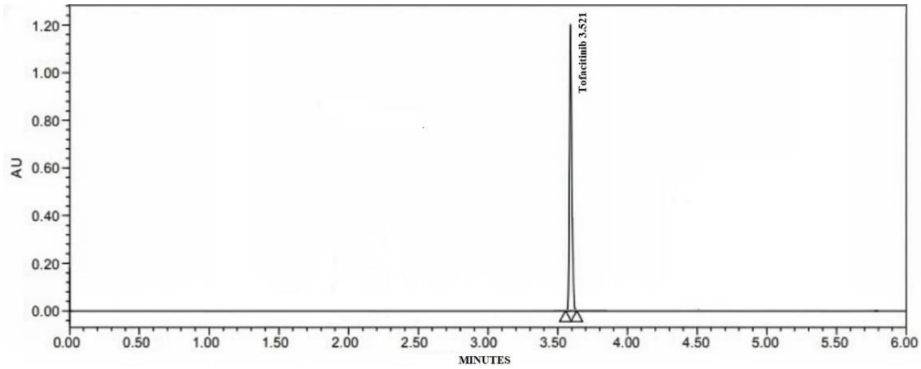

Fig. 3: Optimized Chromatogram of Tofacitinib.

Table 2: Results for Optimized Chromatogram.

S.NO	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Tofacitinib	3.521	5036	1.25

METHOD VALIDATION

Specificity

No inference of diluent & Placebo

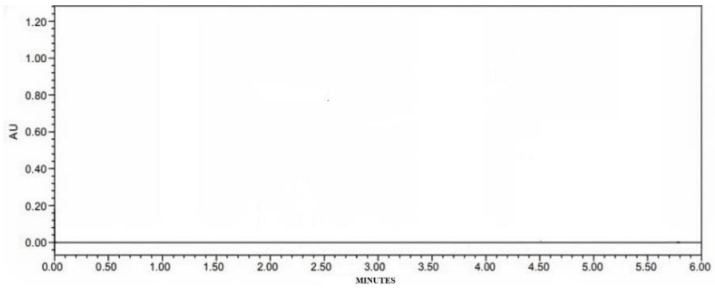

Fig. 4: Chromatogram of Blank.

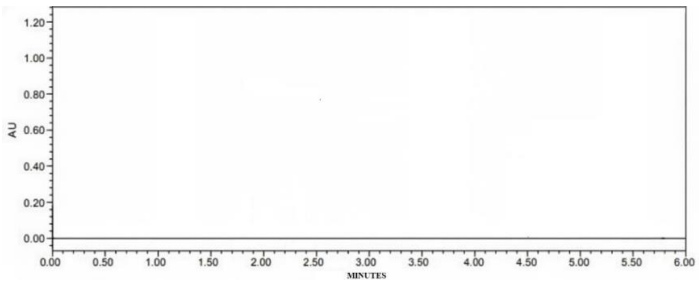

Fig. 5: Chromatogram of Placebo.

Linearity

Table 3: Results for Linearity.

S. No	Level	Tofacitinib	
		Concentration in µg/mL	Peak Area
1	Level 1	2.75	145632
2	Level 2	5.5	289754
3	Level 3	8.25	432876
4	Level 4	11	578945
5	Level 5	13.75	721564
6	Level 6	16.5	865342

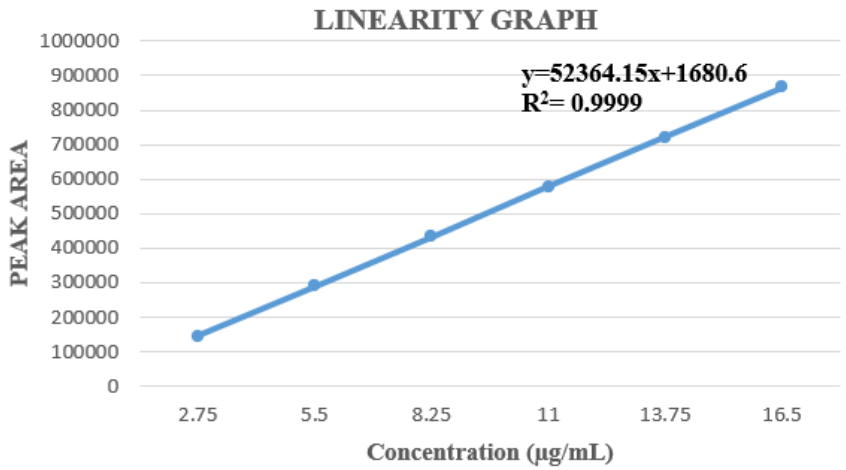

Fig. 6: Linearity graph for Tofacitinib.

LOD & LOQ

Tofacitinib 's LOD was found to be 0.32µg/mL, while its LOQ was determined to be 0.98µg/mL.

Precision

The system and method precision percent RSDs for tofacitinib were found to be 0.19 and 0.48, respectively. The % RSD was found to be within the permissible range of less than 2 for both system and method precision. As a result, the established process was said to be precise.

Table 4: Results for Precision.

S.NO	Injection	System Precision		Method Precision	
		Retention Time	Peak Area	Retention Time	Peak Area
1	Injection-1	3.517	579862	3.523	582967
2	Injection-2	3.520	580163	3.525	583569
3	Injection-3	3.521	578956	3.518	578125
4	Injection-4	3.519	579628	3.521	578998
5	Injection-5	3.522	580259	3.519	578692
6	Injection-6	3.520	576998	3.516	575695
Mean		3.519833	579311	3.520333	579674.3
STD		0.001572	1118.384	0.003037	2758.865
%RSD		0.04	0.19	0.09	0.48

Accuracy

The recovery percentage was found to vary between 99.27 and 100.14% (Table 5**)**. At 50%, 100%, and 150% spiking levels, the percentage RSD was found to be within the permissible range for tofacitinib. The results showed that the suggested procedure was accurate, with an acceptance limit of 98–102% and a percentage RSD of less than two.

Ruggedness (Intermediate Precision)

A percentage RSD of less than two has to be used to describe ruggedness. In the developed approach, the percentage RSD for tofacitinib was 0.2 (Table 6**)**. The ruggedness of the process is validated by results that fall within the allowed range.

Robustness

The percentage change of tofacitinib in the devised technique was found to be within the permissible range of less than 2 (Table 7**)**. As a result, it was demonstrated that when the analytical conditions were slightly altered, the suggested approach was appropriate for the analysis of tofacitinib. This shows that the results are unaffected by even minor changes to the analytical conditions.

Table 5: Results for Accuracy

Recovery Level	Concentration in µg/ml			Amount Found	% Recovery	% RSD
	Target	Spiked	Total			
50%	5.5	2.75	8.25	8.19	99.27	0.09
	5.5	2.75	8.25	8.21	99.51	
	5.5	2.75	8.25	8.20	99.39	
100%	5.5	5.5	11	10.93	99.36	0.07
	5.5	5.5	11	10.95	99.54	
	5.5	5.5	11	10.94	99.45	
150%	5.5	8.25	13.75	13.71	99.70	0.25
	5.5	8.25	13.75	13.69	99.56	
	5.5	8.25	13.75	13.77	100.14	

Table 6: Results for Ruggedness.

S.NO	Injection	Retention Time	Peak Area
1	Injection-1	3.519	577986
2	Injection-2	3.521	579165
3	Injection-3	3.518	577268
4	Injection-4	3.523	580159
5	Injection-5	3.518	577356
6	Injection-6	3.522	579952
Mean		3.520167	578647.7
STD		0.001951	1173.509
%RSD		0.06	0.2

Table 7: Results for Robustness.

S. No	Condition	Tofacitinib		
		Retention Time	Peak Area	% Change
1	Standard	3.521	579069	--
2	+MP (55:45)	3.520	578962	0.01
3	-MP (45:55)	3.523	579258	0.03
4	+Flow Rate 1.2ml/min	3.517	572685	1.10
5	-Flow rate 0.8ml/min	3.522	578864	0.04
%RSD		0.07	0.47	

Assay

Tofacitinib's assay percentage in formulation analysis was 99.36%. As a result, it was demonstrated that the approach was appropriate for regular analysis of tofacitinib in both bulk and formulations.

Table 8: Results for Formulation.

S. No	Drug	Brand	Label Claim	Peak Area	Amount Found	% Assay
1	Tofacitinib	Tofajak-In XR	11 mg	575260	10.93mg	99.36

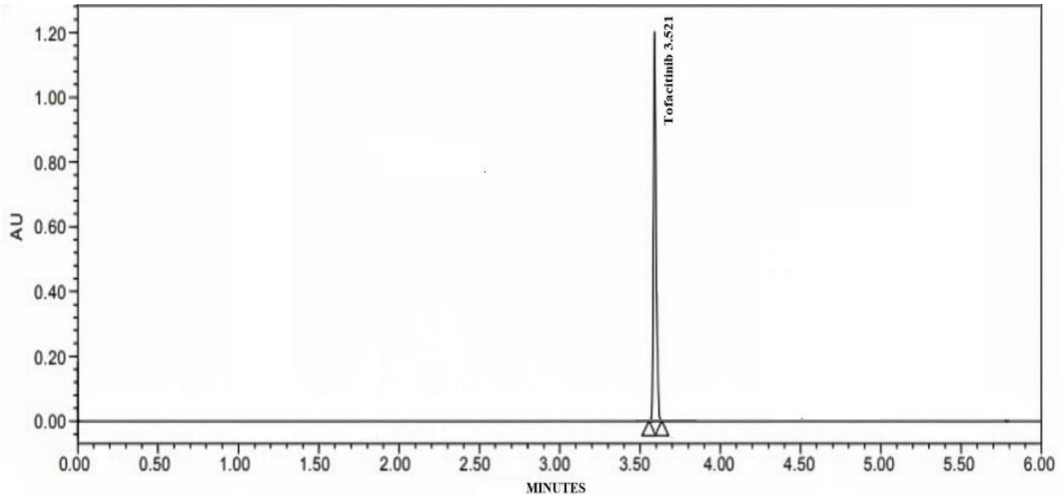

Fig. 7: Chromatogram of Formulation.

CONCLUSION

Prior to the start of this work, there was some information in the literature regarding the LC determination of tofacitinib with high run time and retention time, which were time-consuming and restricted to pharmaceutical formulations containing only 5 and 10 mg. To fill the research gap left by the current analytical and bioanalytical methods for assessing tofacitinib in pharmaceutical formulation (11 mg label claim) and bulk drug, the author developed a sensitive, accurate, and precise RP-HPLC approach with optimum run time and acceptable retention time. The recommended RP-HPLC method for the tofacitinib test was shown to be suitable for routine quantitative analysis following validation. The HPLC method saved time while preparing the standard and sample and did away with the requirement for time-consuming extraction. The low standard deviation data show the exceptionally high precision of the new approach. The linearity, accuracy, specificity, and precision values were found to be within acceptable ranges. The chromatogram's lack of extra peaks showed that there was no conflict between the tablet's common excipients. Thus, it is shown that the devised RP-HPLC method is straightforward, linear, precise, sensitive, and reproducible. The new approach is therefore simple to use and offers a fast analytical time for routine quality monitoring of tofacitinib in pharmaceutical bulk and formulations. The results indicate that the suggested approach has good precision and accuracy.

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