EUROPEAN JOURNAL OF BIOMEDICAL
AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 2
Year: 2025

METHOD DEVELOPMENT AND VALIDATION FOR RP-UHPLC OF TOFACINIB ESTIMATION IN TABLET DOSAGE FORM

Manjunath S. Y.*, MD. Parveena, Ravindar Bairam and P. Balakrishanaiah

Department of Pharmaceutical Chemistry and Analysis.

***Corresponding Author: Manjunath S. Y.**

Department of Pharmaceutical Chemistry and Analysis.

Article Received on 09/12/2024 **Article Revised on 30/12/2024** **Article Published on 20/01/2025**

ABSTRACT

For Tofacitinib, a novel analytical technique for the UHPLC method was developed, refined, and then used on pharmaceutical dosage forms. To achieve a suitable chromatographic separation, several mobile phase systems were made and employed; however, the suggested mobile phase, which included buffer, acetonitrile, and methanol in the ratio 40:40:20, provided superior sensitivity and resolution. Thermo Scientific's UV detector (4.6mm x 3mm x 5µm) was used to make the detection at 290 nm. In terms of retention durations and theoretical plates, the optimal flow rate for tofacitinib was determined to be 0.7 ml out of the various flow rates that were examined. Tofacitinib has a retention time of 2.129. Tofacitinib's asymmetry factor, also known as the tailing factor, was determined to be 1.4, indicating that the peak is symmetrical. Retention duration, tailing factor, capacity factor, and theoretical plate count were among the system suitability metrics that were computed. Tofacitinib's theoretical plate count was approximately 2210, indicating the column's effective operation. The method's specificity is shown by these factors. Visual examination of the peak area plot as a function of analyte concentration was used to assess the linearity range; the relevant calibration graphs are displayed in the figure, and the results are displayed in the table. The designated concentration range was established based on the linearity studies. Tofacitinib was shown to be linear for the target concentrations between 50% and 150%. System precision and technique precision were used to confirm the validity of the suggested approach. Tofacitinib's system and method precision percentages were tabulated. In order to conduct placebo interference tests, the standard was injected first, followed by the placebo and the standard. At the RT of the analyte peak, they showed no signs of placebo interference. By altering the flow rate and conducting filter validation tests on various filter types, robustness experiments were conducted. The suggested approach was confirmed to be resilient after the analytical data and filter validation results were tabulated. Two analysts and a different system were used to conduct the investigation on ruggedness. The developed method is found to be robust because the results were tabulated and found to be within limitations.

KEYWORDS: Method Development, Validation, Tofacitinib, Tablet Dosage, RP-UHPLC.

INTRODUCTION

The intracellular enzymes known as Janus kinases, which are involved in signaling pathways that impact hematopoiesis and immune cell function, are inhibited by tofacitinib. The FDA has approved it for the treatment of moderate to severe rheumatoid arthritis in patients who are intolerant to methotrexate or who do not respond well to it. Tofacitinib is being researched for the treatment of psoriasis and has also been explored in clinical trials for preventing organ transplant rejection in addition to rheumatoid arthritis.[1-4] Along with more severe immunologic and hematological side effects, known side effects include headache and nausea. Pfizer markets tofacitinib under the Xeljanz trademark.3-[(3R,4R)] is the IUPAC designation for [methyl({7H-pyrrolo[2,3-d]pyrimidin-4--yl})amino]piperidin-1-yl]-4-methyl-3-.Three-oxopropanenitrile. C16H20N6O is the molecular formula. 312.6 is the molecular weight.

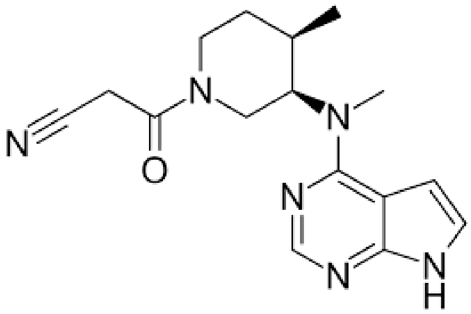

Figure 1: Structure of Tofacitinib.

he 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[5-7] and HPTLC[8] 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.[9-10] The methods reported by the use of reverse phase high-performance liquid chromatography (RP-HPLC) mostly involve the gradient mode of analysis, which makes analysis complex. Hence, the current work aims to develop an accurate, specific, stability-indicating, isocratic method for the estimation of Tofacitinib in bulk and tablet form.

MATERIALS AND METHODS

Chemicals and Reagents: Tofacitinib Gift samples obtained from MSN laboratories. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 290 nm with column Thermo scientific (4.6mmX 3mm,5µm), dimensions at Ambient temperature. The optimized mobile phase consists of pH 5.3 Buffer: Acetonitrile: Methanol (40:40:20 v/v). Flow rate was maintained at 0.7 ml/min.

Selection of Diluent

Diluent is selected initially based on solubility of the substance. Tofacitinib showed high solubility in methanol and water in the ratio of 50:50 and used as diluent.

Detection method & Selection of wavelength (λ max)

Known concentration of Tofacitinib working standard was taken and dissolved in methanol and water (50:50) such that the standard solution contains about 1mg/ml. Placebo and blank solutions were also prepared. All these solutions were scanned between 200 to 400nm using HPLC. From the UV spectrum at the drug shows good absorbance 290 nm. After reviewing the chromatograms and peak purity chromatograms a wavelength of 290 is selected as the optimum wavelength for this drug.

Optimization of mobile phase

On the basis of retention property study results of the drug and reviewing the results "phosphate buffer at pH 5.3" is decided as the buffer preparation to be used.

Selection of pH of the buffer

pH" plays very an important role in achieving the chromatographic separation as it controls the elution properties by controlling ionization characteristics.

At pH 5.3 peak shape, peak tailing and theoretical plate count was found to be satisfactory and hence 3.5 is decided as the pH of the Buffer.

Mobile phase composition

Many trials on composition of Buffer, Acetonitrile & methanol were made to decide the ultimate composition of the mobile phase.

After reviewing many trials good peak shape, retention time, tailing factor, theoretical plates are obtained with the mobile phase composition Buffer: ACN: Methanol (4:4:2). Hence it was finalized.

Selection of flow rate

Flow rate selection is done depending on the retention time, peak symmetry, hence many trials were made to decide the flow rate and 0.7 ml /min was found to be acceptable as the peak is sharp. Hence 0.7 ml/ min flow rate was selected for this project.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.7 ml/min for 30 minutes to equilibrate the column at ambient temperature. The overlay spectrum of Tofacitinib was obtained and the Tofacitinib showed absorbance's maxima at 290 nm. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Thermo scientific (4.6mmX 3mm,5µm) column, the mobile phase of composition pH 5.3 Buffer: Acetonitrile: Methanol (40:40:20 v/v) was allowed to flow through the column at a flow rate of 0.7 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Tofacitinib in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity: The linearity study was performed for the concentration of 49.85 µg/ml to 149.55 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Tofacitinib and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

RESULTS AND DISCUSSION

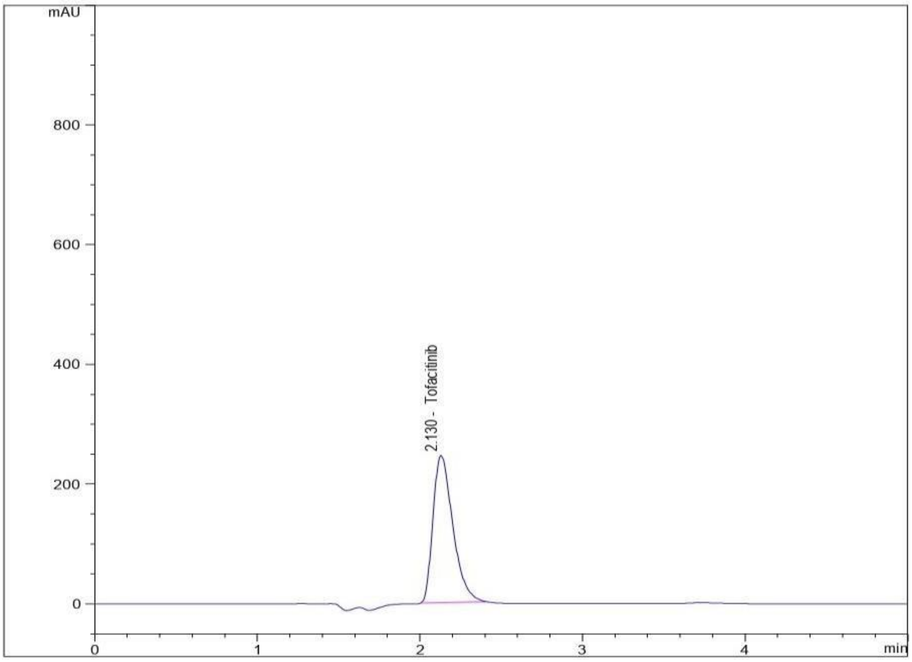

Figure 2: Standard chromatogram.

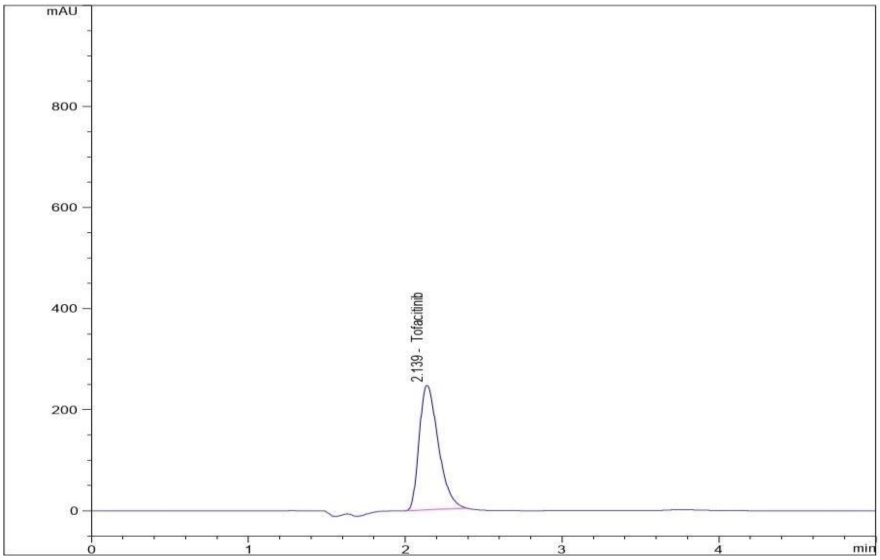

Figure 3: Sample chromatogram.

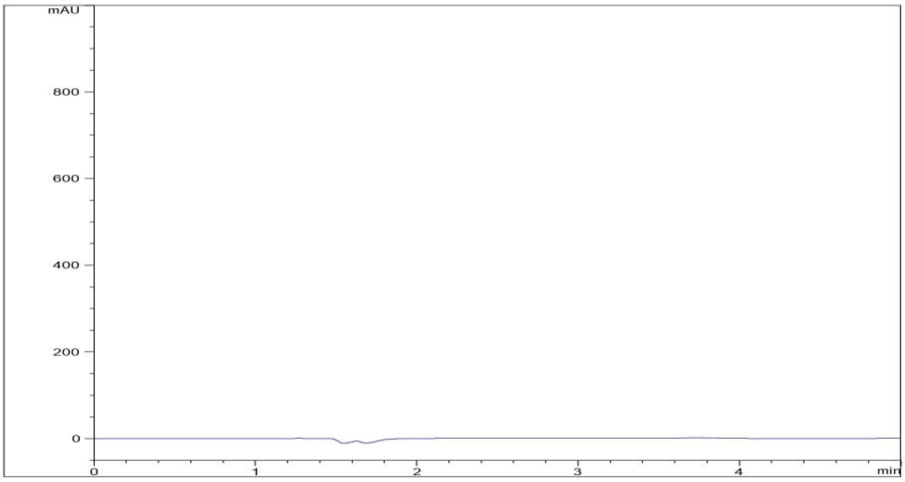

Figure 4: Blank chromatogram.

Table 1: System suitability parameters.

No of injection	Area	RT	Theoretical plates
Standard injection-01	2090.937	2.129	2673
Standard injection-02	2096.766	2.130	2841
Standard injection-03	2096.895	2.132	2798
Standard injection04	2089.145	2.130	2737
Standard injection-05	2091.971	2.139	2719
Mean	2094.479	2.132667	
SD	0.003983	4.538515	
% RSD	0.186775	0.216689	

Table 2: Assay results for Tofacitinib.

LABEL CLAIM	AVERAGE WEIGHT	AREA	PERCENTAGE ASSAY
5mg	151.53mg	2101.160	99.70%

Table 3: Linearity results of Tofacitinib.

Concentration (mcg/ml)	Concentration (%)	Areas
49.85	50	1077.940
74.78	75	1577.896
99.70	100	2096.160
124.63	125	2575.177
149.55	150	3157.028

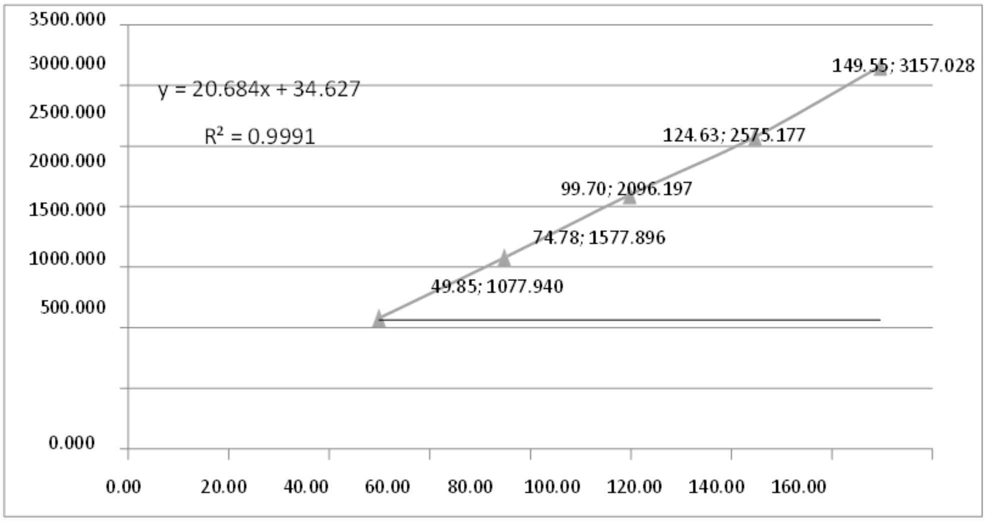

Figure 5: Linearity graph for Tofacitinib.

Table 4: Showing accuracy results for Tofacitinib.

Spiked Level	Concentation in ppm	% Recovery	%Recovery mean
50%	50	99.40	**99.46%**
	50	99.80	
	50	99.20	
100%	100	99.60	**99.66%**
	100	99.80	
	100	99.60	
150%	150	98.20	**98.86%**
	150	99.20	
	150	99.20	
	Mean	99.28	
	SD	0.480	
	%RSD	0.484	

Table 5: Precision results for Tofacitinib.

No. of Sample	% Assay
	Tofacitinib Tablets 5mg
01	99.60
02	99.80
03	99.60
04	99.60
05	99.80
06	99.80
Mean	**99.70**
SD	**0.1095**
% RSD	**0.1099**

Table 6: Ruggedness results of Tofacitinib.

No. of Sample	% Assay
	Tofacitinib Tablets 5mg
01	100
02	99.80
03	99.80
04	100.20
05	99.80
06	99.80
Mean	**99.90**
SD	**0.17**
% RSD	**0.17**

Table 7: Robustness results for Tofacitinib.

Parameter	RT			Area		
	Mean	Std dev	% RSD	Mean	Std dev	% RSD
Low flow variation 0.5mL/min	2.400	0.0016	0.068	2254.222	0.4595	0.02
High flow variation 0.9mL/min	1.908	0.0009	0.046	1888.341	1.33	0.07
Low Wavelength variation 288	2.132	0.0015	0.069	2150.522	1.083	0.05
High Wavelength variation 292	2.130	0.0012	0.054	2133.551	1.53	0.07

CONCLUSION

A simple, reproducible and efficient reverse phase Ultra High Performance Liquid Chromatography (RP-UHPLC) method has been developed for estimation of Tofacitinib in its tablet dosage form. Separation was done by using mobile phase consists of Phosphate buffer (pH 5.3): Acetonitrile: methanol (40:40:20, v/v). Chromatography separations were carried out on Thermo scientific (4.6mmX 3mm,5µm) at a flow rate of 0.7ml/min and UV detection at 290nm and the retention time for Tofacitinib is 2.129 minutes. The linear dynamic response was found to be in the concentration of 50µg-150µg/ml. The slope, intercept and Correlation coefficient was found to be 0.9991 respectively. Proposed methods were found to be simple, accurate, precise and rapid and could be used for routine analysis.

REFERENCES

1. Papp KA, Krueger JG, Feldman SR, Langley RG, Thaci D, Torii H, Tyring S, Wolk R, Gardner A, Mebus C, Tan H, Luo Y, Gupta P, Mallbris L, Tatulych S: Tofacitinib, an oral Janus kinase inhibitor, for the treatment of chronic plaque psoriasis: Long-term efficacy and safety results from 2 randomized phase-III studies and 1 open-label long-term extension study. J Am Acad Dermatol, May 2016; 74(5): 841-850. doi: 10.1016/j.jaad.2016.01.013. Epub 2016 Feb 19.
2. BusinessWire: U.S. FDA Approves Pfizer’s XELJANZ® (Tofacitinib) for the Treatment of Active Polyarticular Course Juvenile Idiopathic Arthritis.
3. BioSpace: XELJANZ for the treatment of Ankylosing Spondylitis.
4. FDA Approved Drug Products: Xeljanz (Tofacitinib) Oral Solution, Tablets, and Extended Release Tablets.
5. 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.
6. 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.
7. 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.
8. Thakariya NV, Ezhava SB. Stability indicating HPTLC method for determination of Tofacitinib citrate. Der Pharma Chemica, 2017; 9: 12-18.
9. ICH guidelines, for stability testing of new drug substances and products Q1A (R2), 2004.
10. ICH guidelines for validation of analytical procedures: text and methodology Q2 (R1), 2005.