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# A validated RP-HPLC assay method for Tofacitinib in pharmaceutical drug products

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**Abstract:** This study aimed to establish and validate a reliable RP-HPLC assay method for the quantification of tofacitinib (TFC), a janus kinase (JAK) inhibitor, in pharmaceutical formulations. The newly developed method exhibits simplicity, specificity, precision, and sensitivity. Experimental procedures utilized a Shimadzu Prominence 20A HPLC system equipped with a Inertsil ODS 3V C18 column (5µm particle size, 4.6 X 250 mm dimensions). The mobile phase, consisting of 0.05M ammonium acetate buffer at pH 5.0 and acetonitrile (65:35 v/v) in isocratic mode with a flow rate of 1.0 mL/min, facilitated accurate detection of the Tofacitinib peak at 230 nm wavelength. Comprehensive validation, including assessments of linearity, accuracy, precision, and robustness, was conducted in accordance with ICH requirements. The results demonstrated satisfaction, with a retention time ( $t_R$ ) of approximately 5.3 minutes. The imperative need for a swift and efficient RP-HPLC method for analyzing TFC led to the successful development and validation of this technique. Consequently, the RP-HPLC method has undergone thorough validation, establishing it as a user-friendly and trustworthy means for Tofacitinib analysis.

**Keywords:** Tofacitinib; RP-HPLC; tablet; validation; assay method. © 2023 ACG Publications. All rights reserved.

# **1. Introduction**

Tofacitinib (refer to Figure S1 in the supporting information, CAS no: 477600-75-2) emerges as a novel, powerful, and selective Janus kinase (JAK1/JAK3) inhibitor, approved for treating rheumatoid arthritis (RA) in the US [1-3]. Beyond its established application for RA, clinical trials have explored Tofacitinib's efficacy in treating psoriasis and preventing organ transplant rejection. Noteworthy adverse effects encompass serious immunological and hematological side effects, along with reported incidents of headache and nausea [2-4].

The Tofacitinib molecule lacks a monograph in the EP, USP, BP, or Indian Pharmacopoeia. While the literature reveals various analysis methods, their limitations are detailed in the following paragraph. Consequently, a novel, straightforward, and reproducible method has been developed, utilizing readily available materials in any standard drug laboratory.

Upon reviewing the literature, it was observed that LC-MS-MS methods [5-8] have been documented for the determination of Tofacitinib in rat or human plasma. However, it is noteworthy that only a limited number of approaches employed RP-HPLC.

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Prathyusha Naik and colleagues devised a stability-indicating method for Tofacitinib employing RP-HPLC [6]. However, this method exhibited unsatisfactory resolution in the FDA-approved 0.1 N HCl solution, the dissolution medium for the relevant tablet. Another approach, developed by Sampath Kumar Reddy Govind et al. [4], relies on a retention time of approximately 12 minutes for Tofacitinib, leading to a time-intensive analysis of formulations containing the compound. It is worth noting that this method is most suitable for quantifying known impurities in Tofacitinib, as the preferred retention time in HPLC assay methods typically falls between 3 and 6 minutes.

Considering the drawbacks associated with existing methods, such as the complexity of techniques like LC-MS-MS and issues like late retention time and ineffectiveness in dissolution media, the development and validation of an RP-HPLC method for Tofacitinib formulations were deemed valuable. This method aims to enable the analysis of TFC efficiently within a short timeframe, accommodating various dissolution media and different excipients. Notably, the proposed method is both economical and efficient, meeting ICH criteria without compromising quality. In this study, Tofacitinib quantification was achieved via RP-HPLC using cost-effective solvents and a straightforward methodology. A forced degradation study was conducted to assess the stability of tofacitinib in pharmaceuticals and derivatives, providing essential data for determining its shelf life under different conditions. The current work outlines the quantitative analysis of Tofacitinib post various stability indicating studies, and the method's validation according to ICH criteria attests to its accuracy, precision, and robustness. Consequently, this technique can be reliably employed to assess the stability and release of Tofacitinib.

### 2. Experimental

## 2.1. Materials and Methods

The pure sample of Tofacitinib Citrate was generously provided by MSN Laboratories Private Limited, located in Telangana, India. For the analysis, high-performance liquid chromatography (HPLC) grade solvents were exclusively utilized. 3X distilled water obtained from tap water by Elga Labwater, Purelab Classic system, methanol (HPLC Gradient Grade, J.T Baker, suitable for laboratory, research, or manufacturing use, from Norway), and acetonitrile (HPLC Far UV/ Gradient Grade, J.T Baker, suitable for laboratory, research sourced from China). Analytical grade reagents, essential for the analysis, encompassed ammonium acetate (Sigma Aldrich-Emsure/ACS, Reag. Ph Eur, Germany), 30% hydrogen peroxide (Sigma Aldrich-Emprove, Ph Eur, BP, USP, Germany), triethylamine (Sigma Aldrich for synthesis, Belgium), 37% hydrochloric acid (Isolab Chemicals, Germany), sodium hydroxide (Fluka Ph Eur, BP, NF, E524, pellets, 98-100.5%), 100% glacial acetic acid (Sigma Aldrich, Emprove, Ph Eur, BP, JP, USP, E 260, Germany), and 85% o-phosphoric acid (Sigma Aldrich Emsure, ACS, ISO, Reag. Ph Eur, Switzerland). To facilitate a comparative analysis with the test tablets, the original drug Tofacitinib tablets "Xeljanz 5 mg Filmtabletten (manufactured by Pfizer Manufacturing Deutschland, Batch Number: DH7942)" were acquired. The analytical standard employed in the study was Sigma-Aldrich Tofacitinib citrate with a purity of  $\geq$  98% (HPLC). This standard was identified by the product code PZ0017-25MG and the batch number 0000068911.

The experiments were conducted using the Shimadzu Prominence HPLC, featuring a variable detector (SPD20A) and a pump (LC20AT). Prior to analysis, solutions underwent degassing through the Prominence degasser DGU-20A. A stainless steel Inertsil ODS 3V C18 column, with dimensions of 4.6 x 250 mm, a particle size of 5  $\mu$ m, and a stationary phase of octadecyl silane bound to the silica surface, was employed, with the column temperature set at 30°C. Ultrasonication was facilitated using the Advantage Lab ultrasonicator (AL-0412). The mobile phase, a crucial component, was prepared by blending acetonitrile and 0.05M ammonium acetate buffer of pH 5.0 in a ratio of 35:65 v/v. Prior to usage in the study, the mobile phase underwent filtration through an RC membrane filter with a size of 0.45  $\mu$ m and degassing via an ultrasonic sonicator. The flow rate of the mobile phase was consistently maintained at 1.0 mL/min, and the injection volume was set at 10  $\mu$ L. A variable wavelength detector was employed for wavelength scanning, with a selected wavelength of 230 nm.

2.2. Validation of the Proposed Method

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The validation of the method adhered to the guidelines outlined by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the United States Food and Drug Administration (FDA). Parameters assessed included specificity, linearity, accuracy, precision, and robustness.

#### 2.2.1. Preparation of Standard Stock Solution

The standard stock solution was prepared as follows: Tofacitinib citrate standard, equivalent to 10 mg of TFC, was weighed and transferred to a 20 mL volumetric flask. Subsequently, 10 mL of diluent (water: methanol in a 50:50 v/v ratio) was added, and the mixture was sonicated for 10 minutes in an ultrasonic bath. Then, diluent was added to achieve a concentration of 500  $\mu$ g/mL. The resulting stock solution was appropriately diluted, thoroughly mixed, and employed for the assessment of all TFC validation parameters at the required concentrations (50  $\mu$ g/mL Tofacitinib).

#### 2.2.2. Preparation of Sample Solution

Twenty TFC tablets (comprising TFC Test Product 5mg and XELJANZ 5 mg) were weighed, and their average weight was determined. Five tablets, equivalent to 25 mg of tofacitinib, were introduced into a 500 mL volumetric flask. Subsequently, 200 mL of diluent was added to the sample, and the solution was sonicated for 20 minutes in an ultrasonic bath. Finally, diluent was added to the sample to generate a concentrated solution of 50  $\mu$ g/mL. All solutions underwent filtration through a 0.45  $\mu$ m PTFE membrane syringe filter and were subsequently transferred to an HPLC vial.

#### 2.2.3. Specificity

A specificity test was conducted to evaluate the method's ability to measure the active ingredient of interest in the sample. For the selectivity test, the mobile phase, placebo, standard, and sample solutions were introduced into the HPLC system, and the resolution between peaks was measured, considering the nearest eluting peak. The acceptance criteria include the following: No peaks should appear in the retention time of tofacitinib due to the blank, mobile phase, or placebo in both the standard and sample solution chromatograms. There should be a demonstration of no overlapping with other peaks in the standard and sample chromatograms. The standard and sample chromatograms should exhibit similarity.

Additionally, for the selectivity test (Table 1 and Figure 1), the sample and placebo solutions were exposed to the following stress conditions and subsequently injected:

- Temperature (80°C) 3 days
- Temperature and Humidity (40°C and 75%RH) 7 days
- Photostability 8 days

The resulting chromatograms were analyzed to assess their effectiveness.

#### 2.2.4. Linearity and Range

Linearity is crucial to demonstrate that sample solutions fall within a concentration range where the analyte response is directly proportional to concentration. To establish this, various TFC solutions were prepared from standard stock solutions spanning concentrations of 10.00-60.00  $\mu$ g/mL. Subsequently, six distinct concentrations (10, 20, 30, 40, 50, and 60  $\mu$ g/mL) were analyzed. To assess linearity, the peak area underwent least square regression analysis, resulting in a calibration equation with slope, y-intercept, and correlation coefficient (r2).

#### 2.2.5. Accuracy

The accuracy of the method is defined by the closeness between the measured concentration of the analyte and its actual concentration. To validate the accuracy of the method in measuring TFC, nine samples with three different concentration levels (40, 50, and 60  $\mu$ g/mL) were created by adding varying amounts of the active substance to a placebo. Three samples were prepared for each concentration level,

resulting in a total of nine. Following this, the percentage recovery, and relative standard deviation (RSD) were calculated for each of the sample duplicates.

#### 2.2.6. Precision

Precision refers to the degree of closeness between results. Intra-day and inter-day precision were established using six assay determinations at 100% concentration levels (50  $\mu$ g/mL). For intermediate precision, six distinct sample solutions were analyzed on consecutive days. Method precision was evaluated by computing Relative Standard Deviation (RSD) results, and the RSD of combined results obtained by both analysts was calculated to assess intermediate-precision results.

#### 2.2.7. Robustness

A robustness analysis was conducted to investigate the effect of small, deliberate modifications on the chromatographic settings of TFC. Adjustments of 10% in flow rate and a 10°C shift in column temperature were implemented. Both sample and standard solutions were evaluated for each variation, and all system suitability parameters were assessed under each modification.

# 3. Results and Discussion

#### 3.1. Method Development of TFC

The primary objective of this study was to establish and validate an RP-HPLC method for the analysis of TFC in a pharmaceutical formulation. Various methods were explored during the development process. However, after conducting all the necessary optimizations, it was determined that using a C18 column as the stationary phase and an acetonitrile: 0.05 M ammonium acetate buffer (35:65 v/v) as the optimal mobile phase, with a flow rate of 1.0 mL/min, proved to be most effective. Parameters such as a wavelength of 230 nm and a column temperature of 30°C were incorporated into the analysis. The total run time was set at 10 minutes, and the measured TFC retention time (tR) was approximately 5.3 minutes. Figure 1 illustrates the chromatogram of TFC.



Figure 1. Chromatogram of TFC working standard showing  $t_R$  5.29 min. ( $C_{Tofa} = 50 \mu g/mL$ )

#### 3.2. Method Validation

The HPLC equipment met the criteria outlined in the system suitability test, with the results for the TFC peak summarized in Table 2.

#### 3.2.1. Specificity

The specificity results indicate that the chromatographic peaks in the mobile phase, placebo, standard, and sample solutions chromatograms did not interfere with each other (refer to Supporting Information Figure S2). Both standard and sample chromatograms displayed similarities, confirming the specificity of the method. Additional information, including a peak purity graphic, can be found in the supporting information (Figure S3).

### 3.2.2. Degradation Behavior of the Drug Product-Stress Studies

TFC underwent evaluation under conditions of humidity, temperature, and photodegradation. The observation table for the stress study conducted in these conditions is presented in Table 1. Analysis of the HPLC chromatograms collected from various stress settings revealed that TFC remained unaffected in terms of its assay.

Stress Conditions	Peak Area of Tofacitinib	Assay (%)
Temperature (80°C)	1090228	101.5
Temperature and Humidity (40°C - 75% RH)	1063006	98.2
Photostability	1076601	99.4

# Table 1 Desults of strass studie

#### *3.2.3. Linearity and Range*

The linearity of the analytical procedures was assessed by injecting pure TFC at six different concentration levels, ranging from 10 to 60 µg/mL. The slope, Y-intercepts, and correlation coefficient of each TFC concentration were determined by plotting peak area against concentration. The results from the linearity studies statistically demonstrate a significant correlation between peak area and concentration. The linear equation for TFC was established as y = 22,142,454.76x - 2686.5 (refer to Figure S4 in the supporting information). The TFC method exhibited a linear concentration range from 10 to 60  $\mu$ g/mL, with regression coefficients of 0.9996.

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Parameter		HPLC assay		
Retention time	$e(t_R)$	5.27±0.02 min		
No. of theoret	ical plate (N)	4087		
Tailing factor	$(A_s)$	1.40		
Range		10-60 µg/mL		
Linearity (Reg	ression equation)	y = 22142454.76x - 2686.50		
Intercept (SD)		-0.24		
Correlation co	efficient	$r^2 = 0.9996$		
	80% level <sup>a</sup> (mean± SD)	99.87±1.21%		
Accuracy	100% level <sup>a</sup> (mean± SD)	98.56±1.57%		
	120% level <sup>a</sup> (mean± SD)	101.93±1.24%		
Intermediate precision <sup>b</sup> (mean± SD and RSD%)		99.07±0.51% and 0.52		
Intermediate precision <sup>c</sup> (mean± SD and RSD%)		98.61±0.66% and 0.67		
Repeatability <sup>b</sup>	(mean $\pm$ SD and RSD%)	99.81±0.54% and 0.55		
Repeatability <sup>c</sup>	(mean± SD and RSD%)	100.14±1.21% and 1.21		
Limit of dedec	ction (LOD)	$1.22 \mu g/mL$		
Limit of quant	ification (LOQ)	4.06 µg/mL		

<sup>a</sup> Replicates of three determinations.

<sup>b</sup> Replicates of six determinations of TFC Test Product.

<sup>c</sup> Replicates of six determinations of XELJANZ 5 mg Film Tablet Standard deviation (SD) and Relative standard deviation (RSD)

3.2.4. Accuracy

The accuracy of the analytical method was assessed by verifying the consistency between the true value and the experimental value. For this purpose, three concentrations (40, 50, and 60  $\mu$ g/mL) from various ranges of the TFC standard curves were selected. TFC recovery was examined in triplicate for drug sample concentrations at 80%, 100%, and 120% levels. Recovery experiments were conducted to examine the impacts of excipients commonly used in pharmaceutical drug formulations. The recovery of TFC in the sample demonstrated a good level of quantitative accuracy. The accuracy results are presented in Table 3.

**Tofacitinib Recovery (%)** Sample No 80%-1 100.85 80%-2 100.25 80%-3 98.52 100%-1 99.88 100%-2 98.97 100% - 396.82 120%-1 100.59 120%-2 102.19 120%-3 103.02 SD\* 1.88 RSD\* 1.88% **Confidence Interval** 100.12±1.07%

 Table 3. Results of the determination of the accuracy of the method

\*Standard deviation (SD) and Relative standard deviation (RSD)

#### 3.2.5. Precision

In accordance with the ICH regulations on precision studies, the results of the repeatability and inter-precision studies were observed to be satisfactory in relation to both results and inter-assay RSD. The results are shown in Table 2.

#### 3.2.6. Robustness

Upon intentional adjustments to chromatographic parameters, including flow rate and column temperature, all analytes were effectively separated, and the order of elution remained constant. Specifically, the flow rate was modified to 0.9 mL/min and 1.1 mL/min, respectively, and the column temperature was adjusted to 20°C and 40°C, respectively (Figure 2). Data on retention time, observed peak area, and RSD were reported within acceptable limits. The robustness results are presented in Table 4.

Robustness parameters	t <sub>R</sub> Peak		Assay + SD%	RSD%	
	(minute)	area*	1100 a g = 0 D / V	IGD /V	
Flow rate					
Flow rate (0.9 mL/min)	5.8	1167339	99.66±0 <b>.</b> 79	0.79	
Flow rate (1.1 mL/min)	4.8	958321	100.30±1.68	1.67	
Column Oven					
Temperature (20°C)	5.3	1036302	99.39±0 <b>.</b> 52	0.52	
Temperature (40°C)	5.3	1047346	100.63±0.06	0.06	

**Table 4.** Evaluation data for robustness study of tofacitinib

\*Results of three replicates, t<sub>R</sub> Retention time, SD Standard deviation, RSD Relative standard deviation





Figure 2. HPLC chromatograms were obtained under various TFC robustness conditions; TFC tablet exposed to Flow rate (0.9 mL/min) (A) and working standard (B), TFC tablet exposed to Flow rate (1.1 mL/min) (C) and working standard (D), TFC tablet exposed to Temperature (20°C) (E) and working standard (F), TFC tablet exposed to Temperature (40°C) (G) and working standard (H).

# 3.3. Assessment of Uncertainty of the Method

During the method validation process, uncertainty contributions were measured and evaluated in accordance with the guidelines provided by EURACHEM and GUM documents [9, 10]. The primary

sources of uncertainty include the uncertainty related to the purity of the standard sample ( $u_{std}$ ), calibration curve slope ( $u_{cal}$ ), sample recovery ( $u_{rec}$ ), and repeatability ( $u_{rep}$ ).

$$u = \sqrt{(u_{std})^2 + (u_{cal})^2 + (u_{rec})^2 + (u_{rep})^2}$$
(Eq. 1)

To determine the expanded uncertainty at a 95% confidence level, the combined uncertainty obtained from Equation 1 is multiplied by the coverage factor (k=2). Table 5 provides a summary of the combined and expanded uncertainty results obtained from this validation. Detailed explanations of the uncertainty calculations can be found in the supporting information (Appendix 1).

Table 5. Results	s of combined	and expanded	uncertainty
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Analyte	<b>U</b> <sub>std</sub>	<b>u</b> <sub>cal</sub>	u <sub>rec</sub>	u <sub>rep</sub>	Combined uncertainty	Expanded uncertainty
Tofacitinib	1.15	0.97	0.02	0.01	1.50	3.01

In this study, we have successfully developed and validated an RP-HPLC assay method following ICH criteria for the routine quality control analysis of tofacitinib tablet formulations. The method exhibits specificity in quantifying TFC. The accuracy, assessed through the standard addition method, revealed a range of 96.8% to 103.0%, affirming the method's capability to quantify TFC even in the presence of excipients used in the formulation. The precision and intermediate precision standard deviations, ranging from 0.54 to 0.51, showcase the repeatability and reproducibility of the TFC method. Comparing our method with literature studies (see Table 6), it is evident that our approach yields meaningful results and effectively responds to low concentrations. Ruggedness and robustness studies indicate that the proposed method's test results remain unaffected by operational and environmental variables. To further validate the method's ability to selectively quantify TFC in the presence of its degradation products, forced degradation studies were conducted under humidity, temperature, and photochemical conditions. Subsequent analysis using the proposed method confirmed the stability of TFC under these conditions.

Accuracy %	racy Precision LOD LOG 6 % (µg/mL) (µg/m		LOQ (µg/mL)	Reference
99.95	100.17	1.45	4.40	[6]
101.29	99.24	2.22	6.73	[7]
100.28	100.31	0.18	0.54	[8]
 100.12	99.81	1.22	4.06	Current study

Table 6. Comparison of accuracy, precision, LOD and LOQ results with literature studies

### 4. Conclusions

In conclusion, a reverse-phase high-performance liquid chromatographic method for the quantification of Tofacitinib has been successfully developed and validated using an Inertsil ODS 3V C18 column (250 mm x 4.0 mm, 5 $\mu$ m) and a mobile phase consisting of acetonitrile and 0.05M ammonium acetate buffer at pH 5.0 (35:65 v/v). This method eliminates time-consuming chemical operations, offering a significantly easier and quicker alternative. The validated isocratic HPLC method demonstrates specificity, linearity, precision, and accuracy. It proves to be a valuable tool for the routine analysis of identifying and quantifying Tofacitinib.

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# **Supporting Information**

Supporting information accompanies this paper on <u>http://www.acgpubs.org/journal/journal-of-</u> <u>chemical-metrology</u>

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# References

- [1] S.V. Kumar, V. Dhiman, K.K. Giri, K. Sharma, M. Zainuddin and R. Mullangi (2015). 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.* **29**, 1325-1329.
- [2] Vakar, R. Mazumder, S. Padhi, K.S. Tiwari and P. Kinjal (2021). Development of colon targeting tablet of a JAK inhibitor to combat chronic ulcerative colitis: a novel approach for local drug delivery, *Indian J. Pharm. Educ Res.* **55**, 414-427.
- [3] S.K.R. Govind, V.S. Nagaraju Ch, S.T Rajan, S. Eshwaraiah, Kishore M and I.E. Chakravarthy (2014). Stability indicating HPLC method for the quantification of tofacitinib citrate and its related substances, *Der Pharma Chemica.* **6**, 11-19.
- [4] (NIH), N.I.o.H., Pubchem, National Library of Medicine. 2004, National Library of Medicine: 8600 Rockville Pike, Bethesda, MD 20894.
- [5] T. Kaleswararao and D. Suryakala (2021). RP-HPLC Method development and validation for cleaning residue determination of tofacitinib citrate in tofacitinib tablets, *J. Pharmaceut. Res. Int.* **33**, 262-276.
- [6] C.N.P. Naik and K.B.C. Sekhar (2018). A novel stability indicating chromatographic method development and validation for the quantification of Tofacitinib in pure and its dosage form, *IOSR J. Appl. Chem.* **11**, 33-37.
- [7] B.S.S. Kiran and S. Raja (2018). RP-HPLC method development and validation for the quantification of tofacitinib, *J. Life Sci. Res.* **4**, 646-653.
- [8] N. Thakariya and S.B. Ezhava (2017). Development and validation of UV spectrophotometric method for the estimation of tofacitinib citrate, *Pharma Sci. Monitor.* **8**, 401-408.
- [9] V.J. Barwick and S.L.R Ellison (2000). VAM Project 3.2.1 Development and Harmonisation of Measurement Uncertainty Principles Part (d): Protocol for uncertainty evaluation from validation data. LGC/VAM/1998/088.

A C G publications

[10] S.L.R. Ellison and A. Williams (2012). Eurachem/CITAC guide: Quantifying uncertainty in analytical measurement, 3rd ed. ISBN 978-0-948926-30-3 Available from www.eurachem.org



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