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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF TOFACITINIB

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ABSTRACT: A simple and precise RP-HPLC method was developed and validated for the quantification of Tofacitinib. Chromatography was carried out on Phenomenex Luna C18 (250 x 4.6mm, 5µm) column using a mobile phase was methanol: water (45:55% V/V) at a flow rate of 1.0mL/min. The analyte was monitored using a UV detector at 254 nm. The retention time was found to be 4.35 minutes. The proposed method was found to be linear in the concentration range of 15-90 µg/mL with a correlation coefficient of 0.999. The mean recovery was found to be 99.24 %. The developed method has been validated according to ICH guidelines and found to be selective, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the estimation of Tofacitinib in routine quality control analysis.

KEYWORDS: Tofacitinib, RP-HPLC, Mobile phase, ICH guidelines, Validation

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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 [20]. 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 [1-5]. Several research works elucidated the safety and efficacy of Tofacitinib drug [6-13]. Chemical structure of Tofacitinib was shown in fig no.1 [18-19].

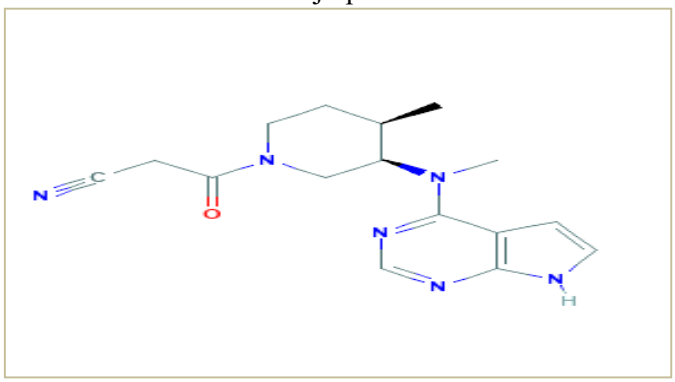

Fig.1 Structure of Tofacitinib

Extensive literature survey revealed that only two methods have been reported for the quantification of Tofacitinib by using HPLC methods [14-15], Bio Analytical Works [16] and HPTLC [17]. The main aim of the present research work to develop a linear, accurate, precise, robust and cost-effective method for the estimation Tofacitinib accordance with ICH guidelines (Q2R1).

2. MATERIALS AND METHODS

Instrument used

The liquid chromatographic system consists of shimadzu LC Solutions- 20 AD UFLC with PDA detector, binary pump and septum injector valve with 20 µl fixed loop. The analytes were monitored at 254 nm. Chromatographic analysis was performed on Phenomenex Luna C18 ODS column having 250 mm× 4.6 mm i.d. and 5µm particle size.

Materials used

API of Tofacitinib was procured from varun herbals, Hyderabad, India. Water was distilled and purified with the Merck Millipore system. HPLC grade methanol was purchased from Merck (India) Ltd., Mumbai, India.

Chromatographic Conditions

The Phenomnex Luna C_{18} column ODS (250 x 4.6mm, 5µm) equilibrated with mobile phase Methanol and Water in the ratio of 45:55 (v/v) was used and the flow rate was maintained at 1.0 mL/min. Detection wavelength with UV detector at 254 NM, and the injection volume was 20 µL and the run time was kept 10 min.

Preparation of Mobile Phase

A mixture of methanol and water in the ratio of 45:55% v/v was prepared and used. Before proceeding to analysis mobile phase was sonicated, filtered and degassed by 0.45 µ membrane filter.

Preparation of Standard Stock Solution

10 mg of the Tofacitinib pure drug was weighed and transferred into 10mL volumetric flask and add 10mL of mobile phase (1000µg/mL concentration). From this stock solution various aliquots are prepared and injected.

Assay Procedure

10mg of the Tofacitinib bulk drug was weighed accurately and transferred into 10mL volumetric flask and make up to volume by using mobile phase. The solution was sonicated for 5 mins.

3. RESULTS AND DISCUSSION

System Suitability

The results obtained from validation of the method and system suitability studies are summarized in Table no 1.

Specificity

Specificity data was shown in Table no 2. Chromatogram was shown in fig 2.

Linearity

Tofacitinib follows linearity in the concentration range of 15-90 µg/mL. The result was shown in Table no 3 and fig. 3.

Accuracy

The accuracy of the method studied at three different concentration levels, i.e. 50%, 100%and 150% showed affordable % recoveries in the range of 99 - 102.20 % for Tofacitinib. The results were shown in Table no 4.

Precision

The precision study was evaluated on the basis of % RSD value. The %RSD was found to be less than 2%. Results of precision study are shown in Table no 5.

Detection and quantification limits

LOD & LOQ results were shown in table no 6.

Robustness

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The content of the drugs were not adversely affected by these changes as evident from the low values of % relative standard deviation (less than 2 %). The results were shown in table no 7.

Table No. 1: Results of System Suitability

Parameter	Result	Acceptance Limit
Retention time (Rt)*	4.35 min	--
Resolution factor*	NA	--
Number of theoretical plates (N)*	2561	More than 2000
Tailing factor (T)*	1.26	Less than 2
* Number of injections: 6 replicates		

Table No.2: Specificity Data

Sr. No.	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	R_t:4.35 min	λ_{max} : 254 nm

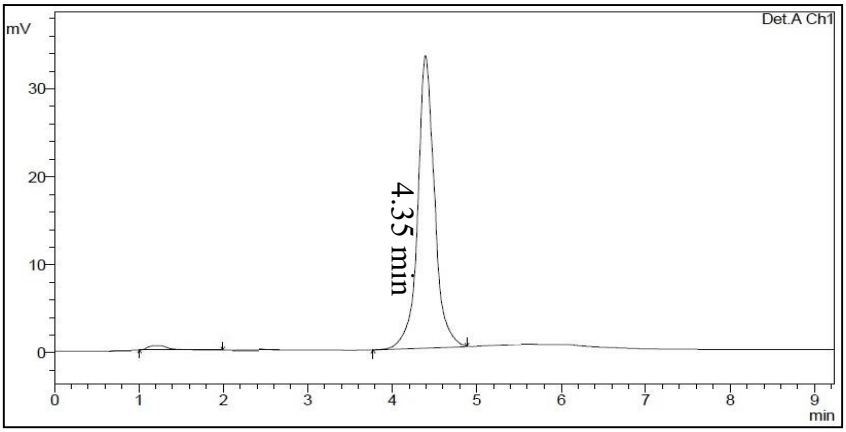

Fig 2: Chromatogram showing specificity

Table No.3: Results of Linearity and range

Sr. No	Concentration (µg/mL)	Peak Area
1	15	186542 µg/mL
2	30	370651 µg/mL
3	45	570465 µg/mL
4	60	731654 µg/mL
5	75	921640 µg/mL
6	90	1120465 µg/mL

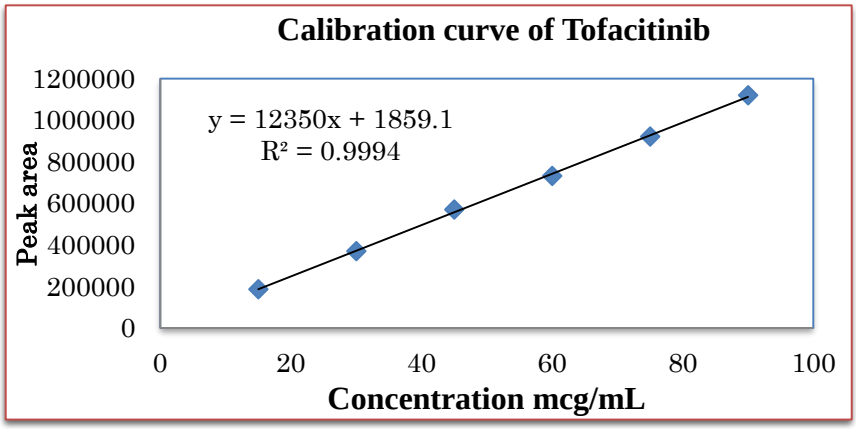

Fig 3. Calibration curve for Tofacitinib

Table No.4: Results of Accuracy

Spiked Concentration (µg/mL)	Peak area	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery	% Mean Recovery
30	365052	30.01	30.16	100.47	100.58
	370151		30.58	101.88	
	361054		29.83	99.37	
60	741347	60.03	61.25	102.02	102.21
	750642		62.02	103.30	
	736064		60.81	101.29	
90	1110216	90.05	91.73	101.86	101.09
	1102132		91.06	101.11	
	1093231		90.32	100.30	

Table No. 5: Results of intraday and interday precision

Sr.No.	Intraday precision Area	Interday precision Area
1	731564	750321
2	730156	731324
3	741347	742106
4	750642	721640
5	736064	729465
6	731064	731506
Mean	736806.2	734394
Std Dev	7271.838	9290.4
%RSD	0.98	1.26

Table No.6: Results of LOD&LOQ

Sr. No	Parameter	Slope	Standard Deviation	Value µg/mL
1	Limit of Detection	12350	8321.2	**2.22**
2	Limit of Quantification			**6.73**

Table No.7: Results for Robustness

Sr. No.	Control	Flow rate (±10%)		Temperature((± 5°C)	
		0.9mL/min	1.1mL/min	30 °C	40 °C
1	741640	750642	731324	731324	730156
2	729465	736064	742106	752106	741347
3	731506	721064	720640	721640	750642
Mean	734203.7	735923.33	731356.7	735023.33	740715
SD	5323.89	12075.57	8763.48	12709.78	8375.30
%RSD	0.72	1.64	1.19	1.72	1.13

Table No.8: Summary and validation parameters for RP-HPLC

Sr. No	Parameter	Result	Acceptance criteria
1	Retention time	4.35 min	$k' > 2$
2	Tailing factor	Less than 2	$A_s < 2$
3	Theoretical plate	More than 2000	$N > 2000$
4	Linearity range µg/mL	15-90µg/mL	-
5	Slope	12350	-
6	Intercept	8321.2	-
7	Correlation coefficient	0.999	>0.999
8	Intraday precision	0.98	NMT 2%
9	Interday precision	1.26	NMT 2%
10	%Recovery	101.29	98%-102%
11	Limit of detection	2.22	-
12	Limit of quantification	6.73	-
13	%Assay	99.24%	98%-102%

4. CONCLUSION

The proposed RP-HPLC method for the quantification of Tofacitinib was found as accurate, precise, reliable, economic and robust. The method has several advantages which include simple mobile phase composition, simple and improved selectivity as well as sensitivity. The existing methods for determination of Tofacitinib were either costly or having more run time. The present method has been found to be adequately robust and cost effective can be used for quantification of Tofacitinib in bulk form. The method was validated as per ICH guidelines.

CONFLICT OF INTEREST

Author has no any conflict of Interest.

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