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Development and Validation of RP-HPLC Method for Estimation of Tofacitinib in Bulk Form

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ABSTRACT : A simple, accurate, precise, and robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Tofacitinib in bulk drug form. Chromatographic separation was achieved using a C18 column with a suitable mobile phase consisting of an aqueous buffer and organic solvent in optimized proportions. The analysis was carried out at an appropriate flow rate, and detection was performed using a UV detector at the selected wavelength. Tofacitinib exhibited a well-resolved peak with satisfactory retention time and peak symmetry. The developed method was validated according to International Council for Harmonisation (ICH) guidelines for various validation parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated excellent linearity over the selected concentration range with a correlation coefficient close to unity. Recovery studies confirmed the accuracy of the method, while precision studies showed low relative standard deviation values, indicating good repeatability and intermediate precision. The robustness of the method was established by introducing small deliberate variations in chromatographic conditions without significantly affecting the results. The developed RP-HPLC method was found to be simple, reliable, cost-effective, and suitable for routine quality control analysis of Tofacitinib in bulk pharmaceutical materials.

KEYWORDS : Tofacitinib, RP-HPLC, Method Development, Method Validation, Bulk Drug Analysis, ICH Guidelines, Quantitative Estimation, Accuracy, Precision, Linearity, Quality Control, Pharmaceutical Analysis.

INTRODUCTION : Tofacitinib is an orally active Janus kinase (JAK) inhibitor that has gained significant importance in the treatment of various autoimmune and inflammatory disorders, including rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, and juvenile idiopathic arthritis. By selectively inhibiting JAK1 and JAK3 enzymes, Tofacitinib modulates intracellular signalling pathways involved in immune response and inflammation, thereby reducing disease progression and improving patient outcomes. Due to its widespread therapeutic application and increasing pharmaceutical production, the development of reliable analytical methods for its quantitative estimation is essential. Quality control of pharmaceutical substances requires accurate, precise, and reproducible analytical techniques to ensure the safety, efficacy, and consistency of drug products. Among various analytical methods, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely employed techniques because of its high sensitivity, selectivity, accuracy, and suitability for routine pharmaceutical analysis. RP-HPLC offers efficient separation and quantification of drug compounds in bulk materials and dosage forms within a relatively short analysis time.

Several analytical techniques have been reported for the determination of Tofacitinib, including spectrophotometric, chromatographic, and bioanalytical methods. However, there remains a need for a simple, rapid, cost-effective, and validated RP-HPLC method that can be conveniently applied for routine quality control analysis of Tofacitinib in bulk drug substances. Method validation is a critical step in analytical method development to demonstrate that the method is suitable for its intended purpose. According to the guidelines of the International Council for Harmonisation (ICH Q2(R2)), validation parameters such as specificity, linearity, accuracy, precision, robustness, detection limit, quantitation limit, and system suitability must be evaluated. Therefore, the present study aims to develop and validate a simple, precise, accurate, and robust RP-HPLC method for the estimation of Tofacitinib in bulk form. The developed method is expected to provide reliable quantitative analysis and serve as a useful tool for routine quality control and pharmaceutical research applications.

Systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". Recently, increased attention has been paid to Quality by design within the pharmaceutical industry to actively seek out quality using its underlying principles. As analytical techniques and methods are used for the quality control of pharmaceutical compounds and thereby assure patient safety and efficacy, they have become an essential part of pharmaceutical Quality by design. The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. This approach ensures a very high likelihood of method success during the product lifecycle. Thus, the validation which is usually performed after method development will serve the purpose of confirming method performance as opposed to identifying potential problem areas1.Pharmaceutical analysis plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulations and on finished products. It also plays an important role in building up the quality products through in process quality control. It also plays a major role in isolation and characterization of impurities

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine**.** It seeks ever improved means of measuring the chemical composition of natural and artificial materials. This branch of chemistry, which is both theoretical, and a practical science, is practiced in a large number of laboratories in many diverse ways while analytical method, is a specific application of a technique to solve an analytical problem. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces are based on the size of the particles (e.g. Size exclusion chromatography).
Different modes of chromatography are as follows –

- Normal Phase Chromatography
- Reverse Phase Chromatography
- Reverse Phase – ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography
- Size Exclusion Chromatography

Reverse Phase Chromatography : Methods can be chosen based on solubility and molecular mass**.** In most of the cases for non-ionic small molecules **(**$\mu < 2000$**)**, reversed phase methods are suitable
In 1960's chromatographers started modifying the polar nature of silanol group by chemically reacting silica with organic silanes. The objective was to make less polar or non-polar so that polar solvents can be used to separate water-soluble polar compounds. A large number of chemically bonded stationary phases based on silica are available commercially. Silica based stationary phases are still most popular in reversed phase chromatography however other absorbents based on polymer (styrene-divinyl benzene copolymer) are slowly gaining ground.
Simple compounds are better retained by the reversed phase surface, the less water- soluble (i.e. the more non-polar) they are. The retention decreases in the following order: aliphatic > induced dipoles (i.e. CCl4) > permanent dipoles (e.g.CHCl3) > weak lewis bases (ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong lewis acids (carboxylic acids). Also the retention increases as the number of carbon atoms increases. In reverse phase systems the strong attractive forces between water molecules arising from the 3-dimentional inter molecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Non- polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phase.
Chemically bonded octadecyl silane (ODS) an alkaline with 18 carbon atoms, it is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations use ODS-HPLC columns. The solvent strength in reverse phase chromatography is reversed from that of adsorption chromatography (silica gel) as stated earlier. Water interacts strongly highly with silanol groups, so that, adsorption of sample molecules become highly restricted and they are rapidly eluted as a result. Exactly opposite applies in reverse phase system; water cannot wet the non-polar (hydrophobic) alkyl groups such as C18 of ODS phase and therefore does not interact with the bonded moiety. Hence water is the weakest solvent of all and gives slowest elution rate. The elution time (retention time) in reverse phase chromatography increases with increasing amount of water in the mobile phase

Adsorption Chromatography /Normal Phase Chromatography : In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents.
The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary

phase. This forms a weak type of bond with any molecule in the vicinity when any of the following interactions are present.

- Dipole-induced dipole,
- Dipole-dipole,
- Hydrogen bonding,
- Complex bonding

These situations arise when the molecule has one or several atoms with lone pair electron or a double bond. The absorption strengths and hence k' values (elution series) increase in the following order. Saturated hydrocarbon < olefins < aromatics < organic halogen compounds < sulphides < ethers< esters < aldehydes and ketones < amines < sulphones < amides < carboxylic acids. The strength of interactions depends not only on the functional groups in the sample molecule but also on steric factors. If a molecule has several functional groups, then the most polar one determines the reaction properties. Chemically modified silica, such as the amino propyl, cyan propyl and diol phases is useful alternatives to silica gel as stationary phase in normal phase chromatography.
The amino propyl and cyan propyl phases provide opportunities for specific interactions between analyse and the stationary phases and thus offer additional options for the optimisations of separations. Other advantages of bonded phases lie in their increased homogeneity of the phase surface.
Resolution with water in weak mobile phase may be most conveniently achieved by drying the solvents and then adding a constant concentration of water or some very polar modifier such as acetic acid or triethylamine (TEA) to the mobile phase. The addition of such polar modifiers serves to deactivate the more polar shape as well as the reproducibility of the retention times and followed many more techniques for the analytical processes which are discussed in the materials and methods

In view of the analytical requirements for the quality assessment of Tofacitinib, the present study was undertaken to develop and validate a simple, accurate, precise, and robust RP-HPLC method for its estimation in bulk form. The developed method was validated in accordance with ICH guidelines to establish its suitability for routine pharmaceutical analysis. The materials employed and the experimental procedures adopted for method development and validation are described in the following section.

MATERIALS AND METHODS :

Chemicals used : In method development and validation of preservatives following chemicals and reagents were used.

Ingredients	Grade	Suppliers
Tofacitinib	API	R.S.I.T.C Jalgaon.
Orthophopsphoric acid(OPA)	HPLC	Avantor Performance material India Ltd. Thane, Maharashtra
Formic acid	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Es-tate 'A' Worli, Mumbai
methanol	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Es-tate 'A' Worli, Mumbai
Water	HPLC	Merck Specialities Pvt. Ltd.Shiv Sager Es-tate 'A' Worli, Mumbai

List of Marketed formulations:

List of brand names of combined formulations of Tofacitinib

Sr. No	**Brand name**	Formulation	**Available strength**	**Address of manufacturer**
1.	TFCT-NIB	Tablet	Tofacitinib=5 mg	IPCa

RP-HPLC :

Selection of Analytical Technique :

RP-HPLC was selected as analytical technique for estimation of Tofacitinib.

Instruments:
The analysis of the drug was carried out on Agilent (S.K.) Gradient System DAD Detector. Equipped with Reverse Phase (Agilent) C18 column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and running chemstation software.

List of instruments Name of Instrument		**Company Name**
1	RP-HPLC Instrument	Agilent 1100 with Auto sampler (Chemstation)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C18)	AgilentC18 (250mmX 4.6mm,5µm)
4	pH meter	VSI pH meter(VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonicator	Ultrasonics electronic instrument

Chromatographic conditions :
The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

chromatographic conditions (RP-HPLC) details used during method Development

1.	RP-HPLC	Agilent (S.K)Gradient System UV Detector
2.	Software	Chemstation
3.	Column	(Agilent) C18 column (4.6mm x 250mm)
4.	Particle size packing	5 um
5.	Stationary phase	C-18 (Agilent)
6.	Mobile Phase	MEOH : 0.5% formic acid 55:45
7.	Detection Wavelength	284 nm

8.	Flow rate	0.9 ml/min
9.	Temperature	Ambient
10.	Sample size	20 ul
11.	pH	2.7
12.	Run Time	10 min
13.	Filter paper	0.45 um

METHOD DEVELOPMENT OF RP-HPLC:

List of Mobile Phase **:**

Sr.No.	Mobile Phase
1.	90% MEOH +10% Water (0.5 % Formic acid) Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
2.	70% MEOH +30% Water (0.5 % Formic acid) Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
3	70 % MEOH + 30 % Water (0.5 % Formic acid) Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
4	MEOH : 0. 5 % formic acid 60%:40 % Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
5	MEOH : 0. 5% formic acid 65:35% Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
6	MEOH : 0. 5% formic acid 65:35 % Flow 1 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
7	MEOH : 0. 5% formic acid 68:32 % Flow 1 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
8	MEOH : 0. 5% formic acid 58:32 % Flow 1 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)
9	MEOH : 0. 5% formic acid 55:45 % Flow 0.9 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)

Analysis of standard drugs was done by following parameters:

- Melting point
- Solubility
- UV spectra and λmax
- RP-HPLC chromatogram and retention time.

Preparation of standard stock solution:-

1) **Tofacitinib standard stock solution : (Stock II)**

An accurately weighed quantity, 5 mg of Tofacitinib (TFT) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 500 µg/ml.

2) **Preparation of Stock Standard Solution :(Stock III) [TFT]**

Accurately weight and transfer 5 mg Tofacitinib working standard into 10 ml volumetric flask as about diluent methanol completely and make volume up to the mark with the same solvent to get

500 µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of Tofacitinib stock solution in ratio of 55:45% were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with meth-anol : Water (0.5 % Formic acid (PH 2.7), prepared in (5.5ml methanol: 4.5ml Water (0.5 % formic acid (PH 2.7)solvent.

RP-HPLC used for chromatographic condition apply on the Preparation of standard solution:-

1) Preparation of std**. Tofacitinib solution: (Stock II)** From the freshly prepared standard stock solution **(**500 µg/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml.

Selection of mobile phase:

Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter**.** The mobile phase was allowed to equilibrate until steady baseline was obtained**.** The standard solution containing mixture of Tofacitinib in was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak**.** From the various mobile phases tried, mobile phase containing methanol and Water **(**0.5 **%** formic acid**)** with pH adjust **(**2.7**)** was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Tofacitinib**.**

Optimization of Chromatographic condition:

The following chromatographic conditions were established by trial and error and were kept constant throughout the analysis**.**
Column **:** C18 **(**250mm× 4**.**6mm**)**
Particle size packing **:** 5µm
Detection wavelength **:** 284 nm
Flow rate **:** 0**.**9 ml/min
Temperature **:** Ambient
Sample size **:** 20 µl
Mobile phase **:** Methanol: water (0.5 % formic acid (ph 2.7)

Procedure for calibration curve of Tofacitinib:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained**.**

From the freshly prepared standard stock solution, pipette 5 mg Tofacitinib in 10ml of volumetric flask and diluted with mobile phase**.** From it 0**.**1, 0**.**2, 0.3, 0.4 and 0.5 ml of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 5,10,15,20 and 25µg/ml of Tofacitinib**.** Samples were injected and peaks were recorded at 284 nm as the graph plotted as concentration of drug verses peak area is depicted in respectively.

Study of system suitability parameters:

The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of standard solution.

Validation of method for analysis of Tofacitinib:

The developed method was validated as per ICH guidelines.

Linearity:
Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range
Determination:

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration; Percentage curve fittings are calculated**.**

Acceptance Criteria:

The plot should be linear passing through the origin**.**
Correlation Coefficient should not be less than 0.999. The Result are shown in;
Preparation of standard stock solution for Linearity:

5 mg of Tofacitinib were weighed and transferred to 10 mL volumetric flask & diluents was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted up to 10 ml volumetric flask with diluents was added to make up the volume.
Preparation of linearity solution:

A series of standard preparations of working standard of were prepared.

Tofacitinib
5
10
15
20
25

Accuracy (recovery):
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analysed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay,

Acceptance Criteria:

Mean recovery should be in the range of 98-102%.
The Relative Standard Deviation should not be more than 2.0%.

Preparation of standard stock solution:

5 mg of Tofacitinib working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume 0.1 ml of this solution diluted up to 10 ml with diluent.

Application of proposed method for analysis of tablet formulation:

Accuracy

The accuracy was determined by Tofacitinib (5 mg of Tofacitinib (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder mixture containing 5 mg of Tofacitinib were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy**.**

Accuracy for RP-RP-HPLC Method :

Sample	**Tofacitinib**	
Accuracy 80%	**Taken** **Added**	
Accuracy 100%	10	8
Accuracy 120%	10	10
	10	12

Repeatability:
Precision of the system was determined with the sample of RP-HPLC Method for. Six replicates of sample solution containing 10 µg/ml of Tofacitinib were injected and peak areas were measured and %RSD was calculated was repeated for Two times

Application of proposed method for analysis of Repeatability:

Weight of sample 5 mg of Tofacitinib was weighed and transferred to 10mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter 0.1 ml of this solution diluted upto 10 ml with diluent.

Precision:
Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an ana-lytical method is usually expressed as standard deviation or relative standard deviation.

Result of Intraday and Inter day Precision studies on RP-RP-HPLC method for Tofacitinib.
Intra-day precision:

Sample solutions containing 5 mg of Tofacitinib three different concentration 5 µg/ml, 15µg/ml, 25µg/ml Tofacitinib were analyzed three times on the same day and %R.S.D was calculated.

Inter-day precision:
Sample solutions containing 5 mg of Tofacitinib three different concentration 5µg/ml, 15µg/ml, 25µg/ml Tofacitinib different days and % R.S.D was calculated. It is usually expressed as standard deviation or relative standard deviation

Acceptance criteria:

The Relative Standard Deviation should not be more than 2% for test

Preparation of standard stock solution:

5 mg of Tofacitinib working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. 0.1 ml of this solution diluted up to 10 ml with diluent.

Robustness:

The mobile phase composition was changed in (±1 ml/ min-1**)** proportion of Methanol: 0.5% formic acid (PH 2.7) and the change in detection wavelength (±1 ml/ min-1**)** and the effect of the results were examine edit was performed using 20µg/ml of Tofacitinib in duplicate

Analysis of marketed formulation

To determine the content of Tofacitinib in marketed tablets (label claim 5 mg of Tofacitinib), 20 tablets powder weighed in 370 mg and average weight of powder was calculated in 18.5 mg. Tablets were triturated and powder equivalent to weigh in 18.5 mg, The drug was extracted from the tablet powder with 10 mL methanol. To ensure complete extraction it was sonicated for 15 min. 0.4 mL of supernatant was then diluted up to 10 mL with mobile phase.

Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Tofacitinib in the sample was calculated. The amount of Tofacitinib per tablet was obtained from the regression equation of the calibration curve

RESULT AND DISCUSSION:

Preliminary studies on Tofacitinib.

Melting point

The procured reference standard of Tofacitinib was found to melt in the range of 198.8 oC respectively.

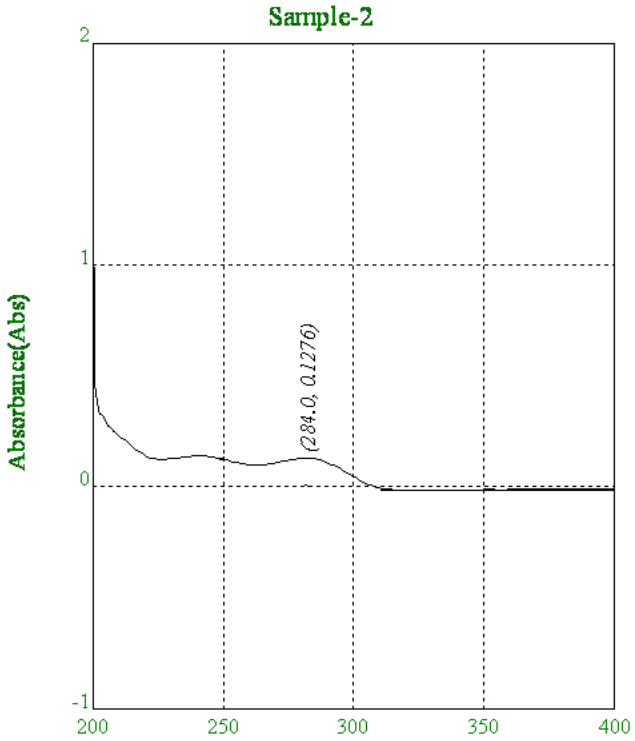
Solubility

The drug was found to be

Tofacitinib slightly soluble in water, soluble in methanol DMSO, ethanol. Insoluble in ether.

UV Spectroscopy

UV absorption of 20mcg solution of Tofacitinib in MEOH was generated and absorbance was taken in the range of 200-400 nm .λ max of Tofacitinib was found to be 284 nm respectively.



UV Spectrum of Tofacitinib

Chromatographic behavior of Tofacitinib mobile phase of various compositions.

Sr No.	**Mobile Phase**	**Retention time**	Remark
1.	90**%** MEOH **+**10**%** Water **(**0.5**%** Formic acid**)** Flow 0.7 ml/min abs at 284 nm **(**column 250mm X 4.6, 5 µm**)**	4.011	Broad peak
2	80% MEOH +20% Water (0.5% Formic acid) Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	4.754	broad peak
3	70 % MEOH + 30 % Water (0.5% Formic acid) Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	3.83	Peak Fronting obtained

4	MEOH : 0. 5% formic acid 60:40% Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	3.85	Larger RT
5	MEOH : 0. 5% formic acid 65:35% Flow 0.7 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	9.358	Sharpe peak were not obtained
6	MEOH : 0. 5% formic acid 65:35 % Flow 1 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	6.478	Sharpe peak were not obtained
7	MEOH : 0. 5% formic acid 68:32 % Flow 1 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	5.448	Sharpe peak were not obtained
8	MEOH : 0. 5% formic acid 58:32 % Flow 1 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	2.753	Sharpe peak were not obtained
9	MEOH : 0. 5% formic acid 55:45% Flow 0.9 ml/min abs at 284 nm (column 250mm X 4.6, 5 µm)	3.445	Sharpe peak were obtained

RP-**HPLC Method :** The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 5-25 µg/mL for Tofacitinib depict the calibration data of Tofacitinib The respective linear equation for RP-HPLC Tofacitinib was y **=** 37.69X**+**25.94 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Tofacitinib is depicted in

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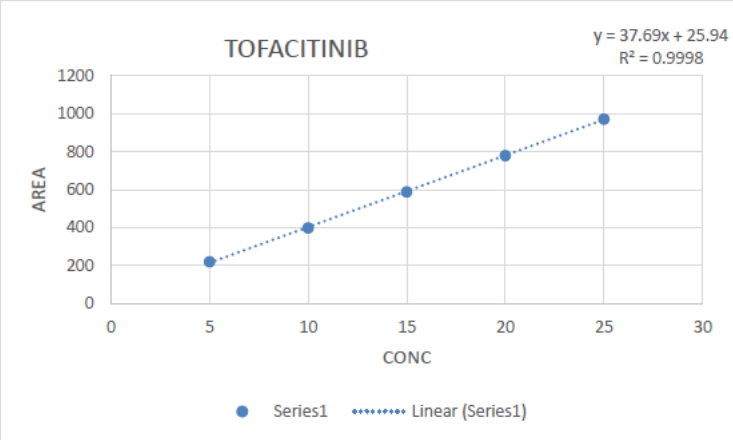

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	219.9235	219.9315	219.93	0.01	0.00
	10	396.8843	398.5861	397.74	1.20	0.30
	15	586.5391	589.9305	588.23	2.40	0.41
	20	777.9601	779.9569	778.96	1.41	0.18
	25	972.3008	970.8193	971.56	1.05	0.11
	Equation		y = 37.69x – 25.94			
	R^2		0.999			

The RP-HPLC Method for respective linear equation for Tofacitinib was y = 37.69x – 25.94 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Tofacitinib is depicted in

Attempts were made to develop RP-HPLC method for estimation of Tofacitinib from tablet. For the RP - HPLC method, Agilent Gradient System UV Detector and C18 column with 250mm x4.6 mm i.d and 5 µm particle size Methanol: **0.5%** formic acid pH 2.7 (55:45v/v) was used as the mobile phase for the method. The detection wavelength was 284 nm and flow rate was 0.9 ml/min. In the developed method, the retention time of Tofacitinib were found to be 3.44 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible.
So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Tofacitinib in bulk drug as well as in formulations.

Linearity: From Tofacitinib standard stock solution, different working standard solution (5-25µg/ml) were pre-pared in mobile phase 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatogram was recorded.

Linearity of Tofacitinib was observed in both methods the range of 5-25 ug/ml, Detection wavelength used was 284 nm.
The plot should be linear passing through the origin, Correlation Coefficient should not be less than 1 that concluded

Accuracy:- Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-101%

System suitability parameters : To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Tofacitinib system suitability parameters were studied. Repeatability studies on RP-HPLC method for Tofacitinib found to be 100.70% and %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded

Precision:- The method was established by analyzing various replicates standards of Tofacitinib. All the solution was analysed thrice in order to record any intra-day & inter-day variation in the result that concluded. Intraday and Inter day Precision studies on RP-HPLC for Tofacitinib which shows the high precision **%**amount in between 99**%** to 101**%** indicates to analytical method that concluded**.**

Robustness: The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.
The mobile phase composition was changed in (±1 ml/min-1**)** proportion, and wavelength change (±1 ml/min-1**)** of optimized chromatographic condition.

Limit Detection : The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:
LOD **= 3.3 (SD)/S**
Where, SD = Standard deviation of Y intercept
S = Slope

CONCLUSION : Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Tofacitinib in API and tablet dosage forms. HPLC method is suitable for the determination of Tofacitinib in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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