

Analytical Method Development and Validation for Estimation of Tofacitinib Citrate in Pharmaceutical Dosage Form by Using UV Spectrophotometric Method

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ABSTRACT:

A simple, rapid, accurate, and cost-effective UV-visible spectrophotometric technique was developed and validated for quantitative determination of Tofacitinib Citrate in pharmaceutical dosage form. The procedure was carried out utilizing a LAB-INDIA UV-3000 double beam UV-visible spectrophotometer and dimethyl sulfoxide (DMSO) as the solvent. Tofacitinib Citrate has a maximum absorbance (λ_{max}) at 293 nm. The method demonstrated excellent linearity by following Beer-Lambert's law in the concentration range of 1-5 $\mu\text{g/ml}$, with a correlation coefficient (R^2) of 0.9996. The proposed method was validated according to ICH Q2 (R1) guidelines for parameters such as accuracy, precision, linearity, robustness, specificity, limit of detection (LOD), and limit of quantification (LOQ). The percentage recovery ranged between 98-100%, demonstrating good accuracy. The %RSD values for intra-day and inter-day precision were found to be less than 2%, confirming the precision of the method. The LOD and LOQ were found to be 0.120 $\mu\text{g/ml}$ and 0.211 $\mu\text{g/ml}$, respectively. The assay of Tofacitinib Citrate tablets showed a drug content of 99.25% of the labeled claim. The validated method is suitable for routine quality control analysis of Tofacitinib Citrate in bulk and pharmaceutical dosage forms.

KEYWORDS: Tofacitinib Citrate, UV-visible spectroscopy, Method development, Method validation, ICH guidelines, Pharmaceutical analysis.

I. INTRODUCTION:

Ultraviolet (UV)-visible spectroscopy is a type of absorption spectroscopy in which UV-visible light is absorbed by the molecule. Absorption of the UV-visible radiations results in

the excitation of the electrons from lower to higher energy levels. In organic molecules only certain functional groups (chromophores) that contain valence electrons of low excitation energy can absorb ultraviolet and visible radiation. UV visible spectroscopy is one of the most commonly used analytical techniques for qualitative and quantitative examination of pharmaceutical substances. UV-visible spectroscopy works on the concept that molecules absorb ultraviolet or visible radiation, causing electronic transitions between chemical orbitals. These electronic transitions yield essential information about the analyte's molecular structure, functional groups, and concentration.

Analytical technique development and validation play critical roles in drug discovery, development, and manufacture. Method creation, based on analytical chemistry, involves discovering, isolating, and quantifying the chemical components of therapeutic substances. Analytical method development aims to confirm the identification, purity, potency, and physical qualities of pharmaceuticals, including bioavailability and stability. This technique assures analytical methodologies are appropriate for evaluating pharmaceuticals, namely the active pharmaceutical ingredient (API).

Validation is the recorded process of ensuring that a procedure, process, equipment, material, activity, or system consistently achieves the desired outcome. ISO defines validation as objectively confirming that particular requirements for an intended usage are met. The FDA defines validation as providing recorded confirmation that a procedure regularly produces a product that fulfills established criteria and quality requirements.

The International Council for Harmonisation (ICH) has developed standards for the validation of analytical techniques, focusing on factors such as linearity, accuracy, precision,

specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). These validation parameters verify that the analytical method performs consistently under a variety of settings and may be used confidently for routine quality control testing. Validation is especially crucial in the pharmaceutical industry, since analytical data directly affect product release, stability studies, and regulatory compliance.

Tofacitinib Citrate is an orally active Janus kinase (JAK) inhibitor widely used in the treatment of autoimmune and inflammatory disorders such as rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis. It exerts its pharmacological action by selectively inhibiting JAK1 and JAK3 enzymes, thereby suppressing the JAK-STAT signaling pathway involved in inflammatory and immune responses. Due to its potent therapeutic activity and increasing clinical use, accurate estimation of Tofacitinib Citrate in pharmaceutical formulations is of significant importance.

Several advanced analytical techniques, including high-performance liquid chromatography (HPLC), have been described for estimating Tofacitinib Citrate. Although these approaches have excellent sensitivity and specificity, they frequently

necessitate costly instrumentation, extended analytical times, and sophisticated sample preparation. In contrast, UV-visible spectrophotometric methods are a simpler and more cost-effective option, particularly for routine analysis in quality control laboratories.

The selection of an adequate solvent is critical in UV spectrophotometric analysis. Dimethyl sulfoxide (DMSO) is a popular solvent because of its high solubilizing capacity for a wide range of medicinal molecules, including some that are weakly water soluble. The use of DMSO improves drug solubility and ensures precise and repeatable absorbance readings.

Therefore, the present study aims to develop and validate a simple, rapid, and precise UV-visible spectrophotometric method for the estimation of Tofacitinib Citrate in pharmaceutical dosage forms using dimethyl sulfoxide as a solvent. The developed method was validated in accordance with ICH Q2 (R1) guidelines to evaluate its linearity, accuracy, precision, robustness, specificity, LOD, and LOQ. The validated method is intended to be suitable for routine quality control analysis of Tofacitinib Citrate in bulk drug and tablet formulations.

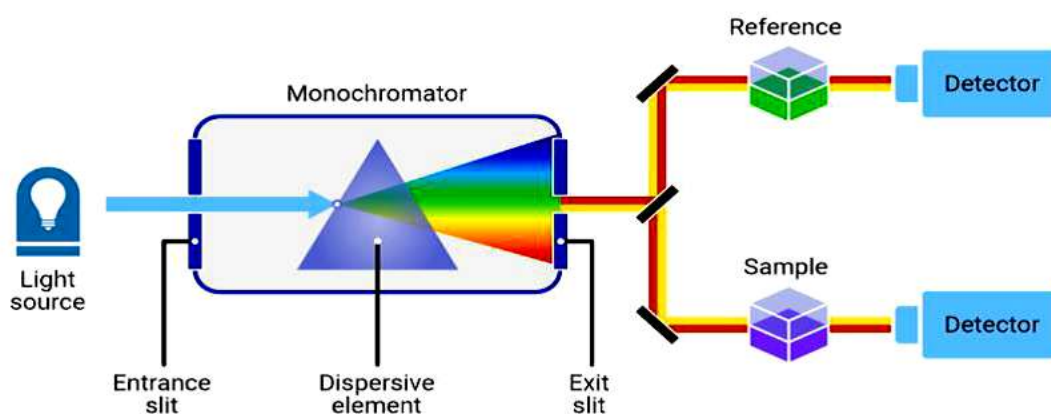


Figure.1. UV spectroscopy

II. DRUG PROFILE:

- **DRUG NAME:** Tofacitinib citrate
- **CHEMICAL NAME:** 3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d] pyrimidin-4-yl) amino) piperidin-1-yl)-3-oxopropanenitrile
- **MOLECULAR FORMULA:** C₂₂H₂₈N₆O₈
- **MOLECULAR WEIGHT:** 504.5 g/mol
- **STRENGTH:** 10 mg
- **APPEARANCE:** white to beige in colour.
- **SOLUBILITY:** It is freely soluble in Dimethyl sulfoxide (DMSO) and it is sparingly

soluble in ethanol (99.5% ethanol), it's slightly soluble in water.

- **SELECTION OF SOLVENT:** Dimethylsulfoxide (DMSO) is used as a solvent
- **MELTING POINT:** 212°C (413.6°F)
- **BOILING POINT:** 585.8 °C
- **BIOAVAILABILITY:** 74%
- **HALF-LIFE:** 3 hours
- **BRAND NAME:** XELJANZ

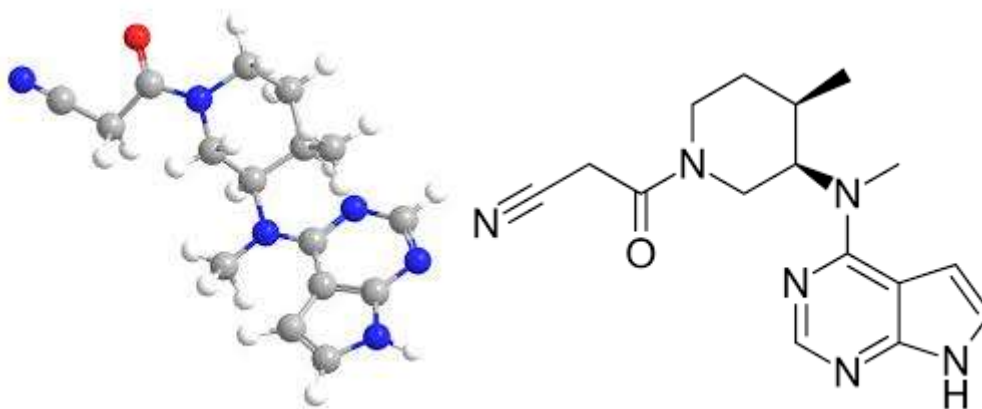


Figure.2. Chemical structure of Tofacitinib

DESCRIPTION: Tofacitinib inhibits Janus kinases, a class of intracellular enzymes involved in signalling pathways that influence hematopoiesis and immune cell activity. 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. Along with more severe immunologic and hematological side effects, known side effects include headache and nausea.

MECHANISM OF ACTION: Tofacitinib is an inhibitor of Janus kinase (JAK), tofacitinib functions by preventing the intracellular enzymes known as Janus kinases from participating in the signaling cascades that cause inflammation. By inhibiting these enzymes, tofacitinib reduces the inflammatory response associated with conditions like rheumatoid arthritis and psoriatic arthritis. Specifically, it inhibits JAK1 and JAK3, which are crucial for lymphocyte activation, function, and proliferation. This, in turn, affects the JAK-STAT signaling pathway, which plays a role in hematopoiesis and immune cell function.

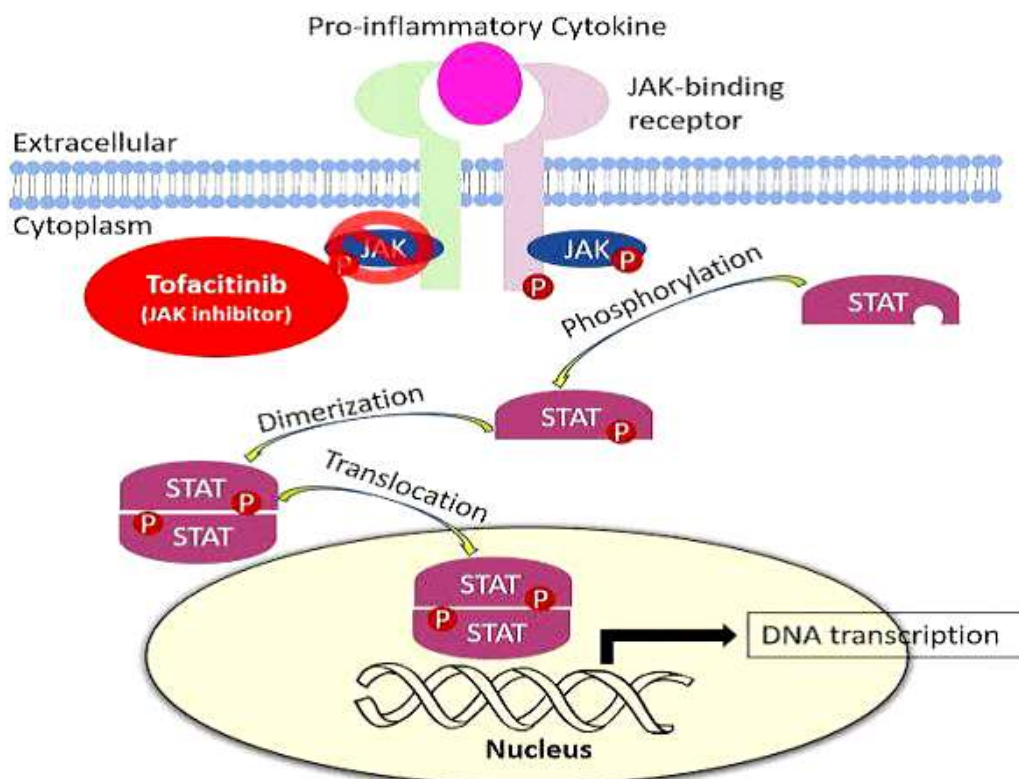


Figure.3. Mechanism of action

III. MATERIALS AND METHODS:

1.1 Instrumentation:

The absorbance was measured using a LAB-INDIA UV-3000 Double Beam UV-visible spectrometer with a 1.0 cm matching quartz cell for

the analytical wavelength selection. The UV spectra were captured between 200 and 400 nm in wavelength. Every medication and substance was weighed using an electronic digital laboratory balance.



Figure.4. LAB-INDIA UV-3000 Double Beam UV-visible spectrometer

3.2 Procedure:

3.2.1 Preparation of standard stock solution:

A precisely measured amount of 50 mg of Tofacitinib Citrate was separately transferred into 50 ml volumetric flask 10 ml dimethyl sulfoxide was added to the flask, shake it to dissolve the drugs, and then diluted with dimethyl sulfoxide to the mark, resulting in a stock solution with a concentration of 1000 µg/ml.

3.2.2 Preparation of working standard stock solution:

A 100 ml volumetric flask was filled with a 10 ml sample of the standard stock solution. To obtain the working standard solution, volume is adjusted for diluents. Tofacitinib Citrate, 1000µg/ml.

3.2.3 Procedure for determination of wavelength for measurement λ max:

Tofacitinib citrate was scanned in the spectrum mode between 200 and 400 nm in order to choose the analytical wavelength range for procedure 100µg/ml. As a blank, dimethyl sulfoxide DMSO was utilised. It was observed at the highest absorbance of 293 nm.

3.2.4 Preparation of calibration curve of Tofacitinib Citrate:

The several dilutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, and 5µg/ml were made from the normal stock solution. Absorbance was measured when the solutions were scanned at 293 nm. Plotting the graph of Tofacitinib Citrate revealed linearity, and the correlation was determined to be 0.9996.

3.2.5 Parameters of analytical method validation:

Analytical method validation: World journal of pharmaceutical research to ICH guidelines, the developed method was validate to assure the reliability of results of the analysis for different parameters like linearity, precision, accuracy, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ), specificity.

3.3 Linearity:

By analysing the absorbance of the Tofacitinib Citrate standard concentration (1–5µg/ml) at 293 nm, the linearity was ascertained. Measurements of absorbance are made. For the standard concentration of tofacitinib citrate, a regression equation and correlation coefficient were found. Table displays the linearity results. It was

discovered that the correlation coefficient was 0.9996.

3.4 Accuracy:

Accuracy was prepared by 3 sample of the solution 80, 100 and 120 % of working standard and added concentration of tofacitinib citrate in each sample solution and dissolved in 10ml of volumetric flask. Accuracy was assessed using a minimum of 9 determinations over a minimum 3 concentrations levels for each sample. The results of accuracy are shown in the table.2

3.5 Precision:

The precision of this method was estimated by two method variation. These two method variation are interday and intraday. Thus the above method at different time interval (morning ,afternoon, evening) on the same day (intraday precision) and on three consecutive day (interdayprecision). Interday precision was determined by checking absorbance is 10µg/ml on three different days. Calculate the mean of absorbance and %RSD. The results of precision are shown in the table 3&4.

3.6 Robustness:

Robustness was carryout by doing variation in method of parameter was done (i.e change in wavelength). Robustness of tofacitinib citrate was determined by variance if wavelength analysis by person to person. No significant difference was found in the absorbance and hence the proposed method was considered as robust which is shown in the table 5.

3.7 Limit Of Detection (LOD) And Limit Of Quantification (LOQ):

The limit of detection is the minimum amount of sample that can be detected. The limit of quantification is calculated using the smallest amount of analyte in the sample under the experimental conditions. Tofacitinib citrate has LOD and LOO values of 0.120µg/ml and 0.211µg/ml, respectively.

3.8 Specificity:

A solution containing mixture of tablet excipients were using the sample preparation procedure to evaluate the possible interference of the excipients. From the absorbance result no interference was observed from the excipients present in the formulation indicated that the method is specific.

3.9 Assay of tofacitinib citrate tablet formulation:

For the analysis 10 tablet for Tofacitinib citrate were weighed and finely powdered an accurately weighed quantity of powder is equivalent to 312.5 mg of Tofacitinib Citrate was taken in 100ml volumetric flask. Dimethyl sulfoxide is used as a solvent. The solution was

sonicated for 15mins and then filtered through by whatmann filter paper (No:41) and volume adjusted the solvent. Pipette out 10ml of the solution and makeup 100ml of solvent. From this further dilution was made to get the final concentration of 100µg/ml. The result of assay was shown in table.6.

IV. RESULT AND DISCUSSION:

4.1 Determination Of Wavelength For Measurement (λ max):

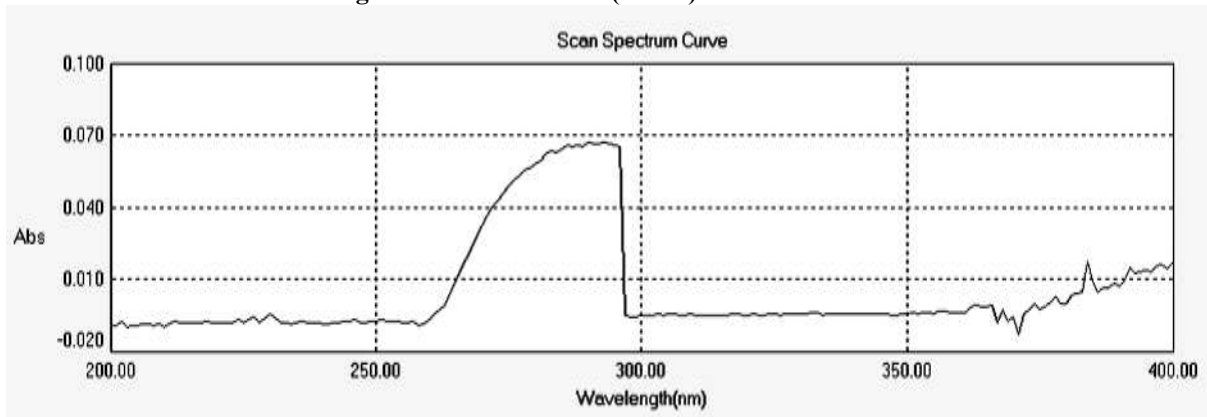


Figure.5. Determination of wavelength for measurement (λ max)

4.2 Linearity:

S.NO	CONCENTRATION (μ /ml)	ABSORBANCE
1.	1	0.092
2.	2	0.192
3.	3	0.277
4.	4	0.387
5.	5	0.463

Table.1. Linearity

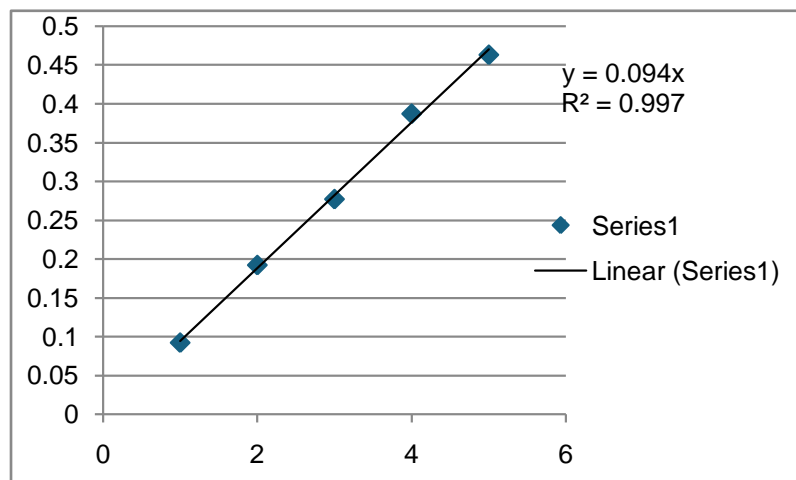


Figure.6. Linearity

4.3 Accuracy:

S.NO	CONCENTRATION	%RECOVERRY	%RSD
1.	80%	98.52	1.61
2.	100%	98.31	0.92
3.	120%	99.25	1.06

Table.2. Accuracy

4.4 Precision:

Table.3. Interday Precision data

S.NO	CONCENTRATION (µ/ml)	ABSORBANCE			MEAN	Std, DEVIATION	%RSD
		1	2	3			
1.	3 µ/ml	0.277	0.239	0.250	0.255	0.0196	0.14%
2.	4 µ/ml	0.387	0.362	0.341	0.363	0.0230	0.15%
3.	5 µ/ml	0.463	0.439	0.448	0.45	0.0121	0.11%

S.NO	CONCENTRATION (µ/ml)	ABSORBANCE			MEAN	Std, DEVIATION	%RSD
		1	2	3			
1.	3 µ/ml	0.275	0.235	0.249	0.245	0.02029	0.14%
2.	4 µ/ml	0.385	0.369	0.335	0.363	0.02553	0.15%
3.	5 µ/ml	0.460	0.415	0.442	0.445	0.01715	0.13%

Table.4. Intraday Precision data

4.5 Robustness:

PARAMETER	PARAMETE SEQUENCE	ABSORBANCE	MEAN	Std. DEVIATION	%RSD
WAVELENGTH	293	0.087	0.08	0.00655	0.08%
	284	0.074			
	287	0.079			

Table.5. Robustness

4.6 Assay Of Tofacitinib Tablet Formulation:

DRUG	LABEL CLAIM	AMOUNT FOUND	%LABEL CLAIM ASSAY (n=3) ± SD
TOFACITINIB	10 mg	312.5 mg	99.25±0.21

Table.6. Assay results of tablets using proposed methods

4.7 Summary:

S.NO	PARAMETER	NORMAL RANGE	RESULT
1.	LINEARITY	0.999	0.9996
2.	PRECISION	Intraday	NMT 2%
		Interday	NMT 2%
3.	ACCURACY	80%	98.45
		100%	98.71
		120%	99.72
4.	ROBUSTNESS	%RSD≤2	0.08%
5.	LIMIT OF DETECTION	-	0.120
6.	LIMIT OF QUANTIFICATION	-	0.211
7.	ASSAY	-	99.25±0.21

Table.8. Summary of validation parameter

V. DISCUSSION:

The approach described in this study provides a convenient and accurate method for analysing Tofacitinib Citrate in its pharmaceutical dosage form. Dimethyl sulfoxide is employed as a solvent. The investigation focused on the absorbance maxima of Tofacitinib citrate at 293nm. Linearity detector response was reported at concentrations of 1-5µg/ml. Tofacitinib citrate tablets tested at 99% of the label claim. The accuracy of the proposed approaches was investigated, and the findings were expressed as a percentage recovery. Tofacitinib citrate had a 100% recovery rate, showing closure accuracy in all procedures.

VI. CONCLUSION:

The proposed development of the UV-visible spectroscopy method is notably straight forward, fast, accurate, precise, and sensitive for the formulation of Tofacitinib citrate tablets. Upon validating this UV-visible spectroscopy method, it

is evident that the spectroscopic approach has been confirmed, using dimethyl sulfoxide as the solvent. All validation parameters, such as linearity, precision, accuracy, and robustness, were observed to be below 2% RSD in accordance with ICH guidelines, indicating the sensitivity of the proposed method. The correlation coefficient was determined to be 0.9996. The intra-day and inter-day % RSD values were found to be within the acceptable limits, and accuracy was also confirmed to be within the normal range. The limits of detection and quantification for the proposed method were established at 0.120 µg/ml and 0.211 µg/ml, respectively, making it a simple and rapid technique for quantifying Tofacitinib citrate tablet formulation. In conclusion, the developed method is straightforward, quick, nearly accurate, precise, and dependable. In line with ICH guidelines, the method is validated and suitable for estimating Tofacitinib citrate, exhibiting excellent linearity, precision, accuracy, robustness, and ruggedness.

This method is applicable for the routine analysis of Tofacitinib citrate in bulk formulations.

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