METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TOFACITINIB IN TABLET DOSAGE FORM BY USING RP-UHPLC

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Abstract: The new analytical method for the UHPLC method was established for Tofacitinib then optimized and then applied on pharmaceutical dosage forms. Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of Buffer, Acetonitrile and methanol in the ratio 40:40:20 gave a better resolution and sensitivity. The detection was carried out by using UV detector at 290nm using Thermo scientific (4.6mmX 3mm,5µm). Among these several flow rates tested, the flow rate of 0.7ml was found to be the best for Tofacitinib with respect to retention times and theoretical plates. The retention time is 2.129 for Tofacitinib. The asymmetry factor or the tailing factor was found to be 1.4 for tofacitinib, which indicates symmetrical nature of the peak. System suitability parameters such as retention time, tailing factor, capacity factor and number of theoretical plates were calculated. The number of theoretical plates was found to be around 2210 for Tofacitinib, which indicates efficient performance of the column.These parameters represent the specificity of the method. Linearity range was evaluated by the visual inspection of plot of peak area as a function of analyte concentration and the corresponding calibration graphs were shown in figure and results are shown in table. From the linearity studies, the specified concentration range was determined. It was observed that Tofacitinib was linear in the range of 50% to 150% for the target concentrations. The validation of the proposed method was verified by system precision and method precision. The %RSD for system precision and method precision of Tofacitinib was tabulated. Placebo interference studies were made by injecting placebo alone, then the standard and the placebo along with the standard. They did not show any interference of placebo at the RT of the analyte peak. Robustness studies were made by varying the flow rate and also by performing filter validation studies on to types of filters. The analytical data and results for filter validation were tabulated, hence the developed method was found to be robust. Study of ruggedness was made by conducting the study on different system and by two analysts. The results were found to be in limits and were tabulated and hence the developed method is found to be rugged.

Keywords: Method Development, Validation, Tofacitinib, Tablet Dosage, RP-UHPLC

INTRODUCTION:

Tofacitinib is an inhibitor of Janus kinases, a group of intracellular enzymes involved in signalling pathways that affect hematopoiesis and immune cell function. It is approved by the FDA for treatment of moderate to severe rheumatoid arthritis that responds inadequately to methotrexate or in those who are intolerant to methotrexate. Besides rheumatoid arthritis, Tofacitinib has also been studied in clinical trials for the prevention of organ transplant rejection, and is currently under investigation for the treatment of psoriasis. Known adverse effects include nausea and headache as well as more serious immunologic and hematological adverse effects. Tofacitinib is marketed under the brand name Xeljanz by Pfizer.[1-4] IUPAC name is 3-[(3R,4R)-4-methyl-3-[methyl({7H-pyrrolo[2,3-d]pyrimidin-4--yl})amino]piperidin-1-yl]-3-oxopropanenitrile. Molecular formula $C_{16}H_{20}N_6O$. Molecular Weight is 312.6.

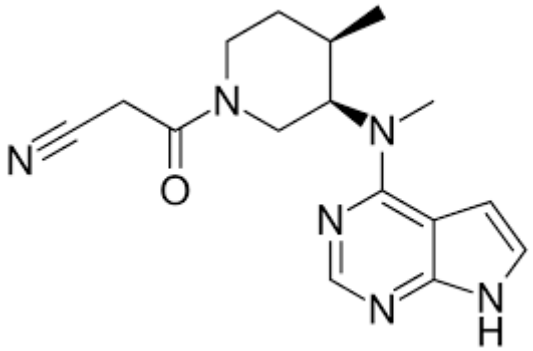

Figure 1: Structure of Tofacitinib

The 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, theoreticalplates 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

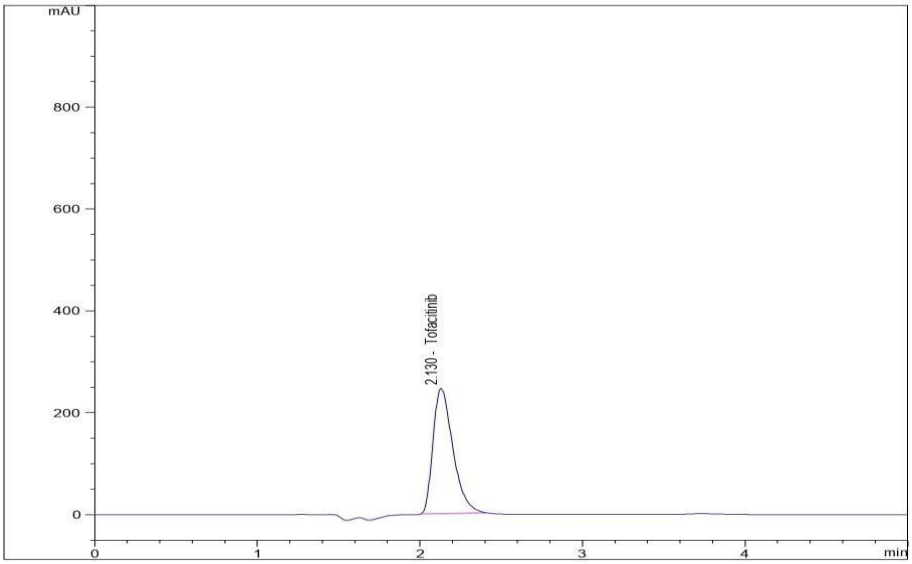

Figure 2: Standard chromatogram

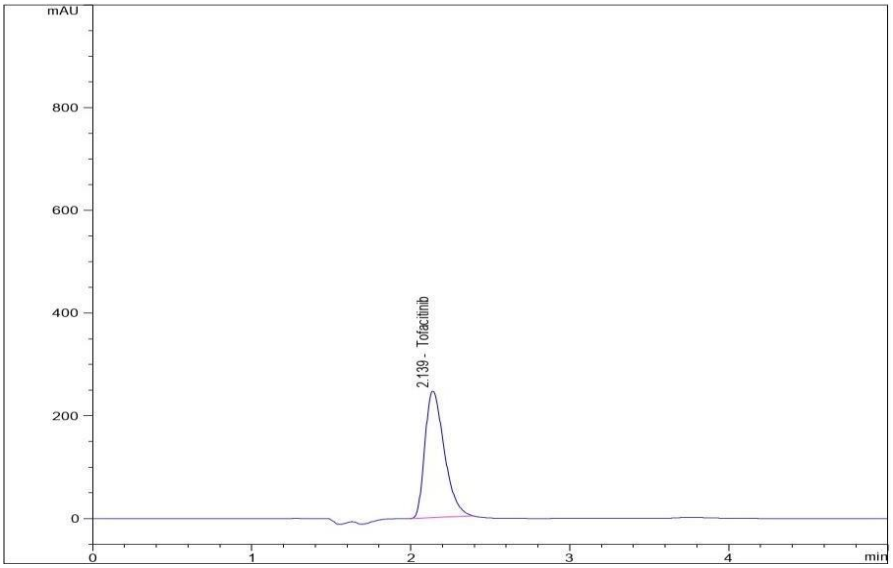

Figure 3: Sample chromatogram

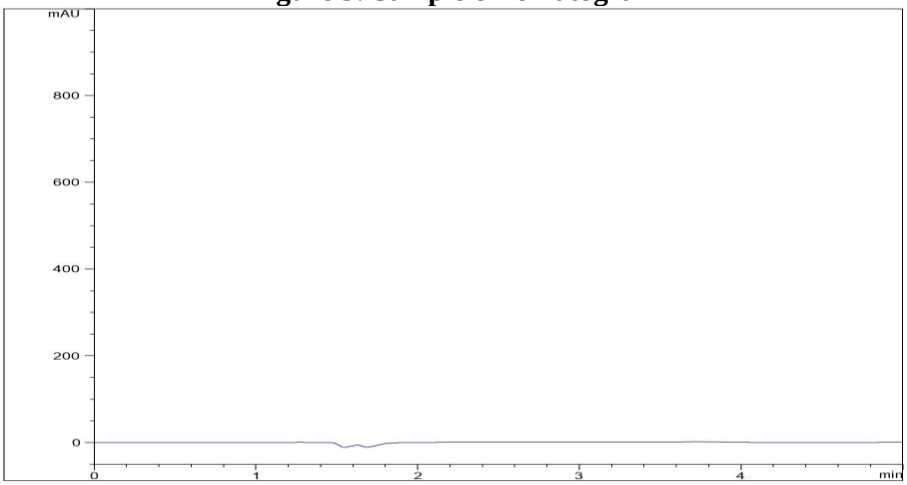

Figure 4: Blank chromatogram

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

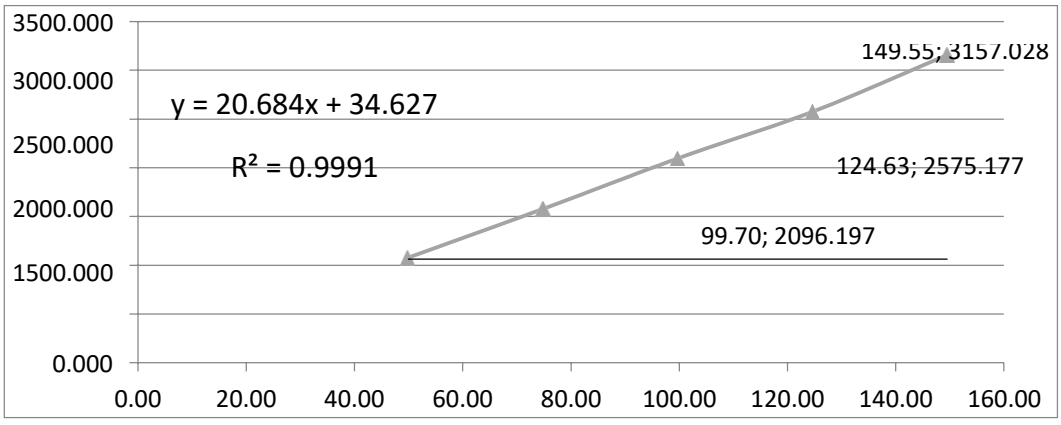

Figure 5: Linearity graph for Tofacitinib

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.

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