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RESEARCH ARTICLE

Stress Degradation Studies and Development of Validated Spectrometric-Assay-Method For Determination of Tofacitinib In Pure and Physical Admixtures

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ABSTRACT:
Aim: The aim is to develop simple validated analytical method for analysis of Tofacitinib by UV Spectroscopy and to study the forced degradation and stress conditions have been used to detect the stability of Tofacitinib. **Method:** Tofacitinib was estimated at 285.9nm. Linearity range was found to be 10-50 mcg/ml. The correlation coefficient was found to be 0.9996. The molar absorptivity was found to be 12468.77mol/cm. The proposed method Sandell's sensitivity was found to be 0.040410 µg cm^2/0.001AU. The limit of detection and quantification were found to be 0.8169 and 2.4755 µg/ ml respectively. The degradation behavior of Tofacitinib was carried out as per the standard procedures and guidelines. Forced acid hydrolytic degradation, alkali degradation and oxidative degradation of was performed in bulk Tofacitinib and laboratory prepared admixtures using 1M Hydrochloric acid up to 48 hrs, in 10 % Hydrogen peroxide up to 48 hrs and for 1.0 M Sodium hydroxide up to 10 min at room temperature. The resulting solutions were analyzed for content by UV spectrophotometry at the maximum absorption of 285.9 n. The assay value of Tofacitinib in bulk and physical admixture was calculated at different time intervals for intraday and interday experiments. **Results and Conclusions:** The proposed method was successfully applied for the determination of tofacitinib in pure and laboratory prepared physical mixtures. The % RSD value of Tofacitinib in bulk and physical admixture was calculated at different time intervals for recovery , precision (Iintraday and Interday experiments) and quantification studies were found to be less than 2 %.

KEYWORDS: Tofacitinib, UV-Spectroscopy, Validation, ICH guidelines, isocratic

INTRODUCTION:
Tofacitinib(3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile) is a new class of drug called Janus kinase inhibitor.Tofacitnib an inhibitor of janus kinase(JAK)which is available orally with anti inflammatory and also with immunomodulatory.They binds with JAK inorder to protect activation of JAK signal transducers and the activators of STAT[(1).]

Janus kinases is inhibited by tofacitnib,it is a group of intracellular enzymes which takes place in signaling of the pathways which leads to the affect of immune cell function and hematopoiesis.These are approved by FDA which is used for the treatment of moderate to severe rheumatoid arthritis which leads to methotrexate or in some of them who are intolerant to methotrexate[(2).]

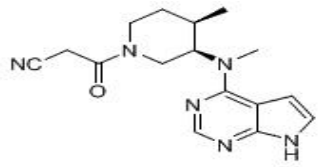

Figure 1: Structure of Tofacitinib

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Only limited analytical methods were reported in the literature for Tofacitinib. The aim of this work was to develop and validate a simple, fast, and reliable ultraviolet (UV) spectroscopic method for the determination of in Tofacitinib bulk and laboratory prepared physical mixtures. stress degradation study like alkali, acid and oxidative degradation were performed. Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH) to determine theTofacitinib in bulk and laboratory prepared physical mixtures. The objective of this study was to develop and validate an assay for the estimation of Tofacitinib using UV-Vis spectrophotometer.

MATERIALS AND METHODS:

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm), is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states (delta E = hδ). Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

1.Sources(UVandvisible)
2.Filterormonochromator
3.Samplecontainersorsamplecells
4. Detector

Preparation of Standard Stock Solution:

100 mg of Tofacitinib raw material was weighed and transferred in to 100 ml volumetric flask, then dissolved in water and made up to the volume with the same solvent. This solution contains 1000 µg/ ml concentration. The standard stock solution was further diluted with water to get the concentration of 10 µg/ ml and the solution was scanned between 200 and 400 nm using the same solvent as blank. The spectrum was observed in that range and the λ max was found to be 285.90nm and was selected as analytical wavelength.

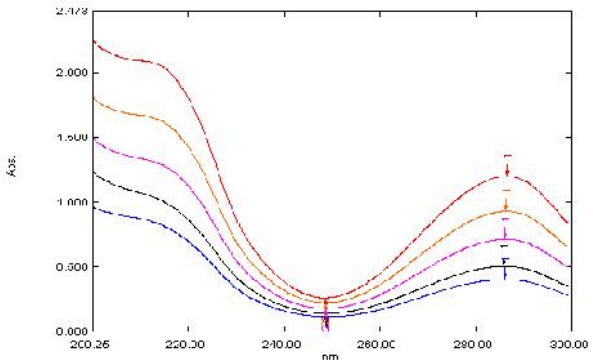

Fig.1 Linearity curve of Tofacitinib

Preparation of Solutions for Linearity Studies:

The standard stock solution of Tofacitinib was transferred into series of 10 ml volumetric flasks and made up to the volume with water. The absorbance of 10, 20, 30, 40, 50 µg/ ml solutions were measured at 285.90 nm. The calibration curve was plotted between concentration (vs) absorbance.Tofacitinib was linear within the concentration range of 10 – 50 µg / ml at 285.90 nm**.** (Fig-1)

Validation of Developed Method:

Linearity:

A calibration curve was plotted between concentration and absorbance (fig.2). Tofacitinib was linear in the concentration range of 10-50 µg / ml at 285.90 nm. The linearity was repeated for five times and LOD and LOQ values were calculated**.** The linearity is shown in fig.1

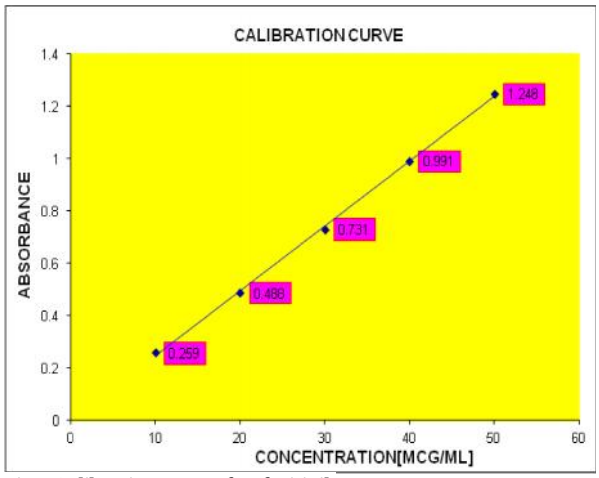

Fig.1 Calibration curve of Tofacitinib

Table.1: concentration and absorbance values:

S.no.	Concentration (µg /ml)	Absorbance
1.	10	0.259
2.	20	0.488
3.	30	0.731
4.	40	0.991
5.	50	1.248

Quantification of Physical Admixture:

1g of laboratory prepared physical admixture containing 100 mg of tofacitinib was made into powder form. The powder equivalent to 5 mg of tofacitinib was weighed and transferred into 100 ml volumetric flask. Add a minimum quantity of water to dissolve the substance and made up to the volume with the same (100 µg / ml). The solution was filtered with whatmann filter paper. From the clear solution, further dilutions were made in volumetric flask with water to get 25 µg/ml solution theoretically. The absorbance of six replicates was measured and the amount was calculated by using regression equation obtained in the linearity section. This procedure is repeated for six times.

PRECISION (Intraday-Interday):
The repeatability of the developed method was confirmed by the precision analysis. The intermediate precision of the method was confirmed by intraday and interday analysis i.e. the analysis of physical admixture was repeated three time in the same day and on three successive days. The amount of drugs present was determined and the percentage RSD also calculated.

ACCURACY:
Accuracy of the method was confirmed by the recovery studies. To the pre-analyzed physical admixture a known quantity of raw material of Tofacitinib was added in three levels of concentration and recovery process are followed as per the quantification process. The amount of recovery was calculated. This procedure is repeated for 6 times and the %RSD was calculated.

RESULTS AND DISCUSSION:

The new, simple and cost effective UV-Spectrophotometric method was developed for the estimation of Tofacitinib in bulk and physical admixtures and study of acid, alkali and oxidative degradation were studied. Tofacitinib was estimated at 285.90nm by using water as solvent. The drug was soluble in aqueous solution. Linearity range was found to be 10−50 µg/ml. The correlation coefficient was found to be 0.9996 and the molar absorptivity was found to be 12468.77L mol^{-1} cm^{-3} in water and in 0.1NHCl. The proposed method Sandell's sensitivity was found to be about 0.040306 µg cm^{-2}/0.001AU. The limit of detection and the limit of quantification were determined by the linearity studies, the process was repeated for six times and the limit of detection (0.8169) and the limit of quantification (2.4755) were calculated. It has been shown in table no.2.

Table no:2 Optical characteristics of tofacitinib

PARAMETERS	VALUES*
λ max (nm)	285.90nm
Beer's law limit (µg/ ml)	10-50
Sandell's sensitivity (g/cm^2/0.001 A.U)	0.0403
Molar absorptivity (L mol^{-1} cm^{-1})	12513.92
Correlation coefficient (r)	0.9996
Regression equation (y=mx+c)	Y = 0.0248x + -0.0009
Slope(m)	0.02481
Intercept(c)	-0.0009
LOD (µµg/ ml)	0.8169
LOQ (µg/ ml)	0.08185

From the linearity curve, the mean concentration of 25µg/ml was selected and quantification in physical admixture was performed. The 5 mg laboratory prepared physical admixtures was used for analysis. The amount present was determined by average of six replicate analysis and the amount present were found to be 24.596, 24.717, 24.959, 24.919, 25.161 respectively. The results were shown in Table no.3

Table no.3: Quantification of laboratory prepared physical admixture

	Sample no.	Amount added (µg/ ml)	Amount present (µg/ ml)	Percentage obtained	Average%	S.D	%RSD	S.E
	1.	25	24.596	98.38				
	2.	25	24.717	98.8				
Tofacitinib	3.	25	24.959	99.8	99.57	1.0872	1.0919	0.011791
	4.	25	24.919	99.67				
	5.	25	25.161	101.2				

CONCLUSION:

In this study a simple, precise, accurate and sensitive UV-spectroscopy methods were developed for the simultaneous estimation of Tofacitinib in bulk and in physical admixture. The Correlation coefficient (γ) value of the proposed method was close to 1.0, which indicates that the concentration used for plotting calibration curve obeys Beer's law strictly. Additives commonly present in the physical admixture but did not show any interference in the proposed method. Statistical validation was done and it shows that the method was reproducible and accurate. Also the various parameters were calculated such as standard deviation and percentage relative standard deviation. The values complies the entire limit as per ICH guidelines. The forced acid, alkali and oxidative degradation study of tofacitinib was studied by UV spectroscopy at various time interval (24hrs, 36hrs, 48hrs) it is observed that the drug Tofacitinib is degrading. Therefore the drug Tofacitinib has to be stored under such condition where the possibility of acid, alkali and oxidative hydrolysis does not arise.

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