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Development and Validation of Ultraviolet Spectroscopic Method for The Estimation of Tofacitinib Citrate

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ABSTRACT:

Background: Tofacitinib Citrate (TFC) is a Janus kinase (JAK) inhibitor known for its immunomodulatory properties. Recent research has brought attention to the potential efficacy of utilizing this therapeutic modality for specifically targeting nanoparticles in a diverse range of medical conditions. Scholars have been diligently endeavoring to establish a dependable method of analysis for assessing the quality of these compounds. However, the currently accessible methodologies are deemed too expensive and time-intensive. In this research, we proposed the validated design of a rapid, accurate, cost-effective, and unobtrusive ultraviolet spectroscopic method for quantifying TFC in manufacturing nanoparticulate formulations.

Objective: The main objective of this study is to find an innovative UV visible spectroscopic methodology that is both easy and robust, while also demonstrating its accuracy and precision in determining the concentration of TFC in nano-formulations.

Methodologies: The highest absorption 288.60 was detected using a Shimadzu UV-1800ENG240V, double-beam UV visible spectrophotometer, which subsequently facilitated the creation of a UV spectrophotometric method. The characterization of Tofacitinib was conducted using the DSC and FTIR techniques.

Results: The results indicate that the validated UV Spectroscopic method was developed as per the guidelines mentioned by the International Conference of Harmonization (ICH). The use of the Beer-Lambert rule involved a stepwise dilution process ranging from 10 to 50 g/ml, resulting in a highly favorable correlation coefficient value of R2 = 0.999. The experiment has successfully proved the methods show a great linearity throughout the whole concentration range. The present methodology's percentage recovery was determined to be below the criterion for maintaining secrecy, specifically, less than 2% stated as the relative standard deviation (% RSD). Additionally, the methodology's accuracy was demonstrated for inter- and intraday variations, as indicated by the % RSD values. The Limits of detection (LOD) and Limits of quantification (LOQ) were determined to be 0.05048 µg/ml & 0.1529 µg/ml, respectively, using accurate calculations. During the specificity testing, no instances were observed for interfering peaks.

Conclusion: In conclusion, the quantification of tofacitinib within the polymeric nanoparticle formulation has been effectively accomplished utilizing the UV method presented in this study. This analytical approach holds the potential for assessing diverse pharmacological dosage forms.

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Introduction

RA majorly affects the synovial joint lining and is responsible for progressive impairment, premature death, and a burden on society.[1-2] Disease-modifying antirheumatic drugs with several mechanisms of action are needed to better treat RA, a complex illness with many distinct molecular subgroups. TFC is a selective Janus Kinase (JAK) inhibitor that is effective against and other immune-mediated diseases such as transplant rejection and psoriasis.[3-4]

Method estimation is a crucial part of developing any drug delivery system, and it calls for a rigorous, accurate, and highly sensitive analytical approach. Adopting a strategy that places a priority on speed, simplicity, correctness, precision, and consistency in product displays helps speed up and improve the product development process. Analytical characterization of drug compounds is required before developing a drug delivery system. To validate and estimate tofacitinib before developing a polymeric nanoformulation loaded with TFC, none of the spectroscopic approaches were found effective. The powdered form of TFC is a white crystalline powder and to examine the melting point range and functional group present in tofacitinib citrate, differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) were used. This method validation approach employed ultraviolet (UV) spectroscopy to create and validate an evaluation method for TFC in both its pure bulk form and its TFC-loaded polymeric nano drug formulation , following guidelines established by the International Conference on Harmonization.[5-6]

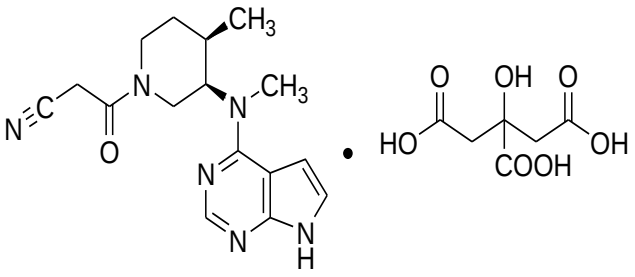

Fig. 1. Chemical structure of Tofacitinib Citrate

Material and Methods

Huirui Chemical Technology Co., ltd. in China was the source for our tofacitinib. The standard plot (Figure 2) was plotted with the help of a Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (UV-1800). Only Analytical-grade chemicals were purchased from Central Drug House, Delhi.

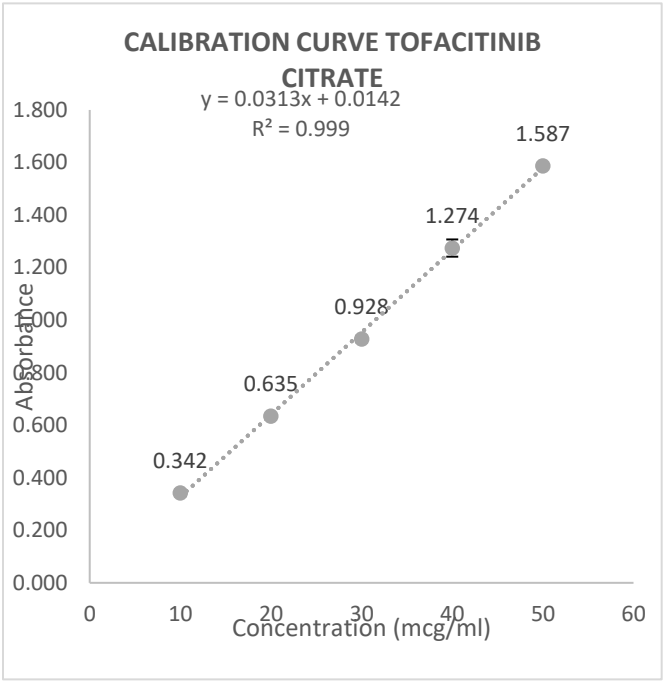

Fig. 2. Calibration curve of Pure drug TFC at wavelength 288.60nm.

I. Drug Characterization

The capillary fusion technique, also known as differential scanning calorimetry (DSC), is employed for drug characterization by the measurement of its melting point. The FTIR study spectra of TFC exhibit distinct features that indicate the presence of several functional groups.

Melting Point (MP) Assessment

The MP of TFC was calculated by the capillary fusion technique, which involved fusing a capillary tube made up of glass, measuring 90 mm, by applying heat on one side. The drug was then introduced into the tube from the opposite end until the liquid level reached a height of around 3 mm.

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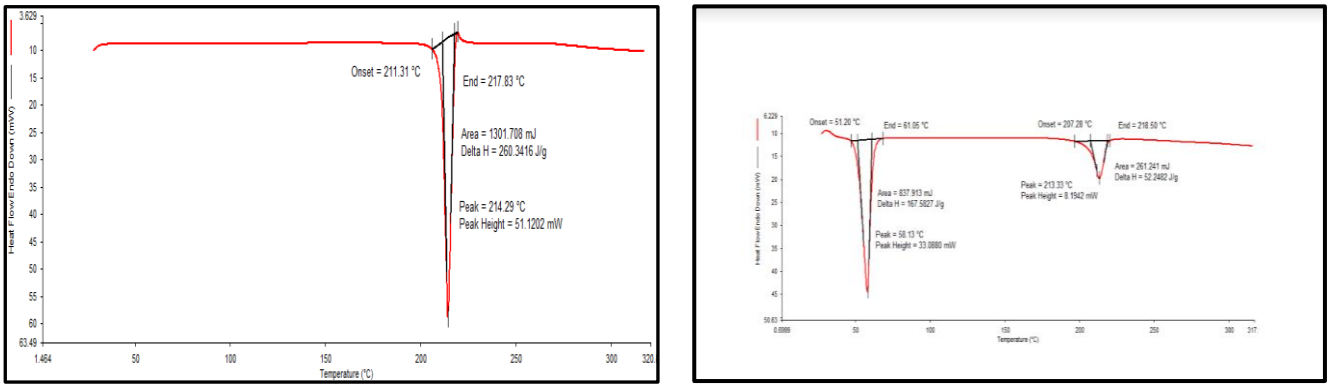
Subsequently, this was placed into the proper place on the melting point apparatus, and the melting temperature was measured and documented. The temperature at which the solid medicine undergoes a phase transition to a liquid state was observed. The technique of differential scanning calorimetry was initially performed in triplicate, as part of the computational process.

DSC and FTIR

DSC curve was obtained through the use of the Perkin-Elmer DSC 8000 instrument. The DSC thermogram curve of TFC and its physical mixture is clearly illustrated in Figure 3. A 5 mg sample was placed in an aluminum pan that was tightly sealed. The average amount of heat was applied with a rate of 10 ◦C/min while pure nitrogen was purged in the presence of the sample. 2-300º C was the set temperature range for this process.[7-8]



(a) (b)

Fig. 3. DSC thermogram of pure drug tofacitinib citrate (a) and Physical mixture (b)

Measurement of functional groups and stretching vibrations were measured by FTIR analysis present in the drug sample. The FTIR spectrum graph of pure TFC and its physical mixture as shown in Figure 4 (a and b) was obtained using an IR affinity 1S instrument manufactured by Shimadzu, Japan. The combination of Potassium Bromide and the pharmaceutical compound was made by use of an agate mortar. A disc was produced by subjecting a hydraulic pressure of 600 kg, resulting in the incorporation of roughly 100 mg of the combined pharmaceutical compound.

Subsequently, the FTIR spectra within the wavenumber range of 4000 to 500 cm-1 were taken. The resolution is commonly regarded as 4 cm-1. The techniques of DSC and FTIR are commonly employed to elucidate the structural characteristics and molecular conformation of drugs, as well as to investigate the interactions occurring between diverse materials. Hence, the utilization of the melting point and FTIR techniques is deemed suitable for identifying and characterizing the pharmaceutical compound, with the ultimate objective of formulating it into a nanostructure.[9-11]

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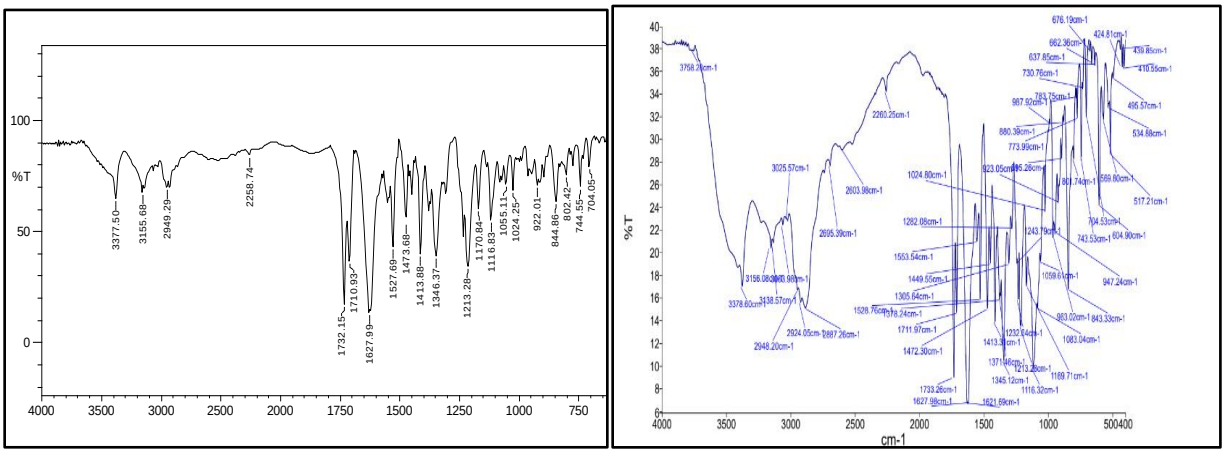

(a) (b)

Fig. 4. FTIR of pure drug TFC (a) and its physical mixture (b)

Stock Solution Preparation

To prepare a standard stock solution, a precise measurement of 10 mg of the pharmaceutical compound was done and transferred in a 100 ml volumetric flask (VF), followed by its dissolution in 1 ml dimethyl sulfoxide (DMSO). Subsequently, a specific volume was adjusted to 100 ml with the addition of freshly made hydrochloric acid with a concentration of 0.1N. To prepare a standardized solution of 100 mcg/ml, the resultant solution was subjected to a sonication process lasting for 5 minutes.

Detection Method Selection

The term "wavelength" refers to the distance between two consecutive points in a wave. The absorption spectra of a solution containing a medication concentration of 10 mcg/ml were examined within the 200–400 nm wavelength range depicted in Figure 5. The detection wavelength was determined to be 288 nm, which corresponded to the maximum wavelength seen.

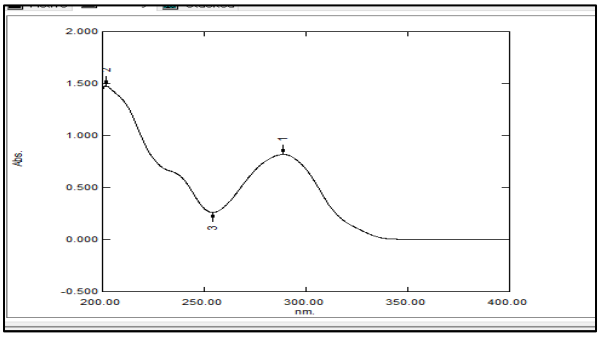

Fig. 5. UV Absorption spectrum of pure drug TFC.

Development of a Nanoparticle Formulation of TFC for Assay Purpose

The experiment involved subjecting polymeric nanoparticles prepared as per Table 1, containing 10 mg of the pure drug to centrifugation at a speed of 12,000 rpm for 30 minutes. The supernatant fluid obtained was mixed with 0.1 N hydrochloric acid and then it was transferred into a 10 ml VF. The total volume was adjusted up to the mark before passing it from the good-grade filter paper i.e. Whatman. To achieve the desired concentration i.e. 10 µg/ml, a specific dilution of the solution was made using 0.1 N hydrochloric acid (HCl) in the required quantity. Subsequently, an aliquot was subjected to scan from 200nm -400nm by UV visible spectrophotometer. The exact concentration of TFC entrapped in the polymeric

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nanoparticles was determined. The experimental protocol was conducted in a triplicate manner.[12]

TABLE I. List of ingredients of physical mixture of Polymeric Nanoparticle of TFC

S.No.	Ingredients	Quantity
1	Tofacitinib Citrate	10mg
2	Gelatin	27.5mg
3	Pluronic F68	15mg
4	Acetone	5ml
5	Glutaraldehyde	0.1ml
6	Distilled water	25ml

Validation of Methods

The validity of this approach has been confirmed in keeping all the considerations of the principles set forth by the ICH.

The concept of specificity

The evaluation of specificity was conducted by determining the TFC in the existence of all polymeric excipients. The spectrums were evaluated properly to assess the possibility of interference caused by excipients.

The Concept of Linearity and Range

Multiple aliquots were extracted from the TFC reference standard solution, with concentrations ranging from 10 to 50 mcg/ml. These aliquots were then assessed in triplicate. The absorbance was determined to be at a maximum of 288.60 nm. The determination of the R2 coefficient for the standard calibration curve equation was achieved by the utilization of UV spectroscopy, resulting in a value of 0.999. The calibration curve depicted in Figure 2 was generated by plotting the absorbance of the medication against the corresponding concentrations.

Accuracy and Precision

The concepts of accuracy and precision are important in various academic disciplines. Accuracy refers to the closeness of a measurement or results to the true or accepted value. To ascertain the consistency of the intended analytical procedure. The repeatability of this research method was evaluated in a triplicate manner of three distinct standard reference concentrations of TFC solution on the same day, at three different intervals of time. The Interday and Intraday preciseness were utilized to measure the repeatability of the approach. To express the results in terms of relative standard deviation (%RSD), an analogous experimental approach was implemented across three separate days. The reported results in this study were proved using UV spectroscopy and precision examinations, as outlined in Table 2 and Table 3. These findings specifically pertain to the fluctuations observed inside a single day (intraday) and across multiple days (interday).

The Concept of Robustness

To assess the robustness, a working reference standard concentration of 10 µg/ml was selected. The predictive capacity of the existing methodology was assessed by altering the wavelength by 2 nm for a sample with a concentration of 10 µg/ml.[13]

Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ)

The term "LOD" refers to the minimum number of analytes that can be detected in a sample following analysis. The LOQ can be explained as the minimum concentration of the analyte/drug in the sample under investigation that can be accurately and precisely detected or measured. The LOD and LOQ were determined using the calibration curve mathematical equation employed in the linearity study. The standard deviation of the intercept Y response and slope of the calibration curve, denoted as S, were utilized in the calculations. Specifically, LOD was found to be 3.3 σ/S, while LOQ was determined to be 10 σ/S.[14]

Result and Discussions

In this study, the characterization of TFC was conducted by the utilization of techniques such as melting point measurement, differential scanning calorimetry (DSC), and interpretation of Fourier-transform infrared (FTIR) spectra.

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Melting point

By utilizing the capillary fusion method, the melting point was evaluated yielding a range of 210 degree to 216 degree.

Table II. Presents the results obtained from research examining the intraday fluctuation of tofacitinib citrate.

Replicates	**Concentration**		
-	**5 µg/ml**	**10 µg/ml**	**15 µg/ml**
Absorbance			
1	0.199	0.336	0.479
2	0.199	0.338	0.481
3	0.201	0.335	0.479
Average	0.1996	0.3363	0.4796
SD	±0.0011547	±0.001527	±0.0011547
%RSD	0.5783	0.4541	0.2407

DSC and FTIR

The crystalline structure of TFC is evident from the exothermic peak observed at 214.29°C on the differential scanning calorimetry curve of pure tofacitinib citrate, as depicted in Figure 3. The temperature range of the peak observed in the experiment spans from 211°C to 217°C, exhibiting a close resemblance to the melting point of the material under investigation. Figure 4 is showing all the functional groups, as indicated by the analysis of the Fourier-transform infrared (FTIR) spectra of the pure pharmaceutical compound tofacitinib citrate. The Fourier Transform Infrared (FTIR) spectra of the Thin Film Composite (TFC) exhibited corresponding peaks at 1346 cm-1 and 1732 cm-1, signifying the saturated bending vibration of the -C=O group and the bending vibration of the -CH3 group, respectively. The stretching vibration peaks of the N-C group were seen at a wavelength of 1116 cm-1. The stretching vibration of the aromatic ring was quantified at a wavenumber of 1627 cm-1. The stretching vibration of the CN group.

The stretching vibration of the -CN group was seen at a wavenumber of 844.86 cm-1, among other findings. The structure and molecular functional groups of tofacitinib were confirmed using DSC and FTIR techniques. To ascertain the identity of pharmaceuticals, various parameters such as melting point, DSC, and FTIR were employed.

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Table III. Interday variation studies data for tofacitinib citrate.

Replicates	**Concentration**		
-	**5 µg/ml**	**10 µg/ml**	**15 µg/ml**
Absorbance			
1	0.198	0.332	0.458
2	0.196	0.334	0.459
3	0.197	0.335	0.461
Average	0.197	0.334	0.459
SD	±0.001	±0.00153	±0.00153
%RSD	0.5076	0.4577	0.3325

Validation of the Method

The non-interfering nature of widely used inactive formulation components has been demonstrated during the estimation process. This validates the unique technique for method validation, confirming its comparable level of specificity and selectivity to those ingredients. The proposed analytical method demonstrated a strong linear relationship throughout a selected concentration range i.e., 10-50 mcg/ml for TFC. Polymeric nanoparticles were employed for the purpose of extracting TFC. The extraction process yielded a discovery rate of 96.621%-97.21% (n=3). The accuracy of the measurements was assessed by calculating the percentage recovery. The precision of TFC was determined by calculating the mean and percent relative standard deviation (RSD) for the differential day fluctuation. The relative standard deviation (RSD) was found to be significantly below 2%. It was found that the limits of detection (LOD) and limits of quantification (LOQ) were 0.05048 and 0.1529 g/ml. As a result of the validation investigation, the methodology is shown to be interference-free and in compliance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) standards. In Table 5, the standard validation parameters are stated.

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Table V. Validation Parameters

S. No.	Parameters Investigated	Results obtained
1.	UV wavelength	288.6 nm
2.	Regression equation	Y=0.0313x + 0.0142
3.	Linearity range (µg/ml)	10-50 µg/ml
4.	Precision	(% RSD, n=5)
	Inter-Day (5,10,15 µg/ml)	0.5076,0.4577,0.3325
	Intra-Day (5,10,15 µg/ml)	0.5783, 0.4541, 0.2407
5.	Accuracy	(% Recovery ± SD)
	50%	96.621±0.012
	100%	95.756±0.023
	150%	97.210±0.008
6.	Correlation coefficient (R^2)	0.9990
7.	Limit of Detection (LOD) Limit of Quantification (LOQ)	0.05048 µg/ml 0.1529 µg/ml
8.	Specificity	Specific
9	Robustness	Robust

Conclusion

In conclusion, it can be inferred that the presented evidence supports the stated hypothesis. The analytical research technique proposed in this study offers a rapid, definite, sensitive, exact, accurate, simple, and cost-effective method for the determination of both pure TFCand its nanoparticulate formulation. The proposed task will attain a superior level of quality if validation methodologies are used in accordance with the recommendations outlined by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The efficacy of the methodology necessitates enhancement through prospective experimental endeavors. The statistical analysis of method validation data provides evidence for the acceptance of established processes in quality control laboratories. The unique strategy can be effectively utilized as an alternative approach for the study of TFC in large quantities and with modified dosages.

TFC is a pharmaceutical agent employed for the management of rheumatoid arthritis in people who exhibit intolerance to alternative therapeutic interventions. Nevertheless, individuals who possess suboptimal metabolic attributes may encounter adverse and incongruous clinical consequences due to heightened susceptibility to potential

drug interactions when concurrently supplied with other medications. The test results were determined to be in coherent agreement with the stated declaration, indicating that the excipients did not cause any interference. From an economic standpoint, the new approach demonstrates accuracy and simplicity in its utilization as an alternative method for conducting a comprehensive examination of TFC both in bulk and dosage forms.

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