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GREEN ASSESSMENT OF A SIMPLE COST-EFFECTIVE ULTRAVIOLET PHOTOMETRIC METHOD FOR QUANTITATIVE ASSESSMENT OF TOFACITINIB IN TABLET FORMULATION BY USING AGREE SOFTWARE

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ABSTRACT:

The basic objective of this study was to establish a green assessment straightforward method to use, economical UV spectroscopic method for the estimation of dosage formulations for tofacitinib tablets. The recommended approach employs a solvent mixture of ethanol and water (20:80), with the detection wavelength at 293 nm. It is highly specific, showing no interference with blank. The method demonstrates linearity within the spectrum of concentration of 2.00 to 14.01 mcg/mL, with a remarkable coefficient of correlation value is 0.999. Precision is confirmed with RSD below 2 % and % recovery of drug found to be 99.55 to 99.84, while the limits of quantification and detection are determined as 0.515 µg/mL and 1.562 µg/mL accordingly. Moreover, the solution remains stable for 24 hours under room temperature. Validation in accordance with ICH Q2(R1) requirements, were shown to be satisfactory with regard to the regular analysis of tofacitinib in tofacitinib tablets by UV spectroscopy. Moreover, the environmental friendliness of the developed methods—which rely on solvents like ethanol and water—was underlined by the greenness assessment carried out using the AGREE software. These results support replacing currently used procedures for the determination of tofacitinib in tablet formulation with our environmentally friendly and analyst-friendly approaches.

Keyword: Tofacitinib, Method development, UV- Spectroscopy, Validation, ICH, Tablet

INTRODUCTION:

Researchers' interest in developing green analytical methods has significantly increased in the past few years.[1-4]Tofacitinib Formally identified as 3-[(3R,4R)-4-methyl-3-[methyl({7H-pyrrolo[2,3-d]pyrimidin-4yl})amino] piperidin-1-yl]-3-hydroxypropanenitrile.[5] It is a Inhibitor of Janus Kinase Jak-1 and Jak-3 by interfering GAK-STAT signaling pathway taken orally to treat rheumatoid arthritis.[6,7] These are FDA-approved medications used for the management of mild rheumatoid arthritis that responds poorly to methotrexate or in certain patients who cannot tolerate the medication.[8] Tofacitinib structure was given below in fig.No.1

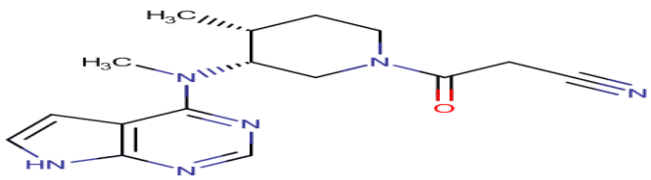

Fig No.1: Structure of Tofacitinib

The literature on tofacitinib only reported a limited number of analytical methods in UV spectroscopy. There aren't many published UV spectroscopic techniques that can be used to figure out tofacitinib in different matrices.[9] These methods are selective and sensitive but frequently involve using hazardous solvents. Therefore, ecologically friendly solutions must be developed to have a greater positive impact on both the environment and the workforce. These methods are selective and sensitive but frequently involve using hazardous solvents. Therefore, ecologically friendly solutions must be developed to have a greater positive impact on both the environment and the workforce. Green analytical chemistry's main objective is to create ecologically friendly pharmaceutical analysis techniques for the quality control industry. Examining a novel approach to achieving consistent growth in analytical chemistry is the aim. We assessed the whiteness and greenness using a range of tools, such as the advanced green analytical AGREE program. To determine tofacitinib in tablet formulations selectively while adhering to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) criteria was the aim of this study, which developed and validated environmentally friendly spectroscopic methods.

The main objective of this research was to create and verify a eco-friendly straight forward, quick, accurate, simple and cost effective ultraviolet Spectral analysis technique for the measurement of tofacitinib in its tablets formulation using Green chemistry Agree Software. Validation of the Compliance of the developed method for determining the dosage forms of tofacitinib in tablets and bulk, which was verified in accordance with the ICH Q2 (R1) guideline.

MATERIALS AND METHODS:

Tofacitinib was received from pharmaceutical industries as a sample gift with ethanol from Merck Specialties Pvt. Ltd., India. and distill water used. Tofacitinib tablets are purchased from the local market. UV spectrophotometer of shimadzu make is use for analysis.

When a molecule absorbs ultraviolet light within the range of 200 to 400 nanometers, its electrons become excited, transitioning from their usual ground state to a more energized state. The energy disparity between these states, denoted as delta E and calculated as hδ, corresponds to the energy absorbed from the ultraviolet radiation. [10-11]

Instrumentation:

A Shimadzu UV-VIS spectrophotometer Model UV-1700 with a computer interface and quartz cells measuring one centimeter was used. Data processing and interpretation were done using UV Probe software.

Greenness evaluation software:

Analytical GREEnness calculator (AGREE) version 0.5 used for evaluation.

METHOD DEVELOPMENT:

Solubility: Solubility of tofacitinib was studied using different solvents such as distilled water, ethanol, methanol, and acetonitrile,0.1N NaOH and 0.1N HCl were used to assess the solubility of tofacitinib. The above solvents were chosen for method development.[12]

Working Wave Length (λ max) Determination:

An instrument for measuring UV-VIS was used to scan a drug solution (10 µg/mL) in ethanol and water (80:20) in the UV between 200 and 400 nm. [13]

Preparation of Standard stock Solution:

Accurately weigh 100.0 mg of tofacitinib API and transfer into a flask with volumetric measurement with a capacity of 100 ml. To dissolve, add 50 millilitres of diluents and sonicate. Add diluent to make up the volume and stir. Use diluent to dilute 5 mL of this solution to 50 mL.(100 mcg/mL)

Preparation of standard solution:

Transferring 5 mL of the standard stock solution into a 50 mL volumetric flask and adding diluents to get the final volume was the process of creating the working standard solution.(10 mcg/mL)

Preparation of Sample Solution:

We bought tablets with 10 mg of tofacitinib from the local store. Take the mean weight and grind it. A precisely weighed amount of powder that is equivalent to 10 mg of tofacitinib, was poured into a 100 flask with volumetric capacity, diluted, and then sonicated to dissolve for 15 minutes.The final volume that was required with the addition of diluents. After passing through a 0.45µm nylon filter, further diluted 5 mL to 50 mL using diluents (10 µg/mL).

Evaluation of green Profile:

The created methods' environmental friendliness is assessed through the use of the AGREE Analytical Greenness Metric software (version 0.5 beta), which may be accessed at https://mostwiedzy.pl/wojciech-wojnowski,174235-1/AGREE.[13-14] To evaluate how environmentally friendly analytical techniques are, AGREE uses a set of fundamental criteria. The greenness score, which is rounded to two decimal places and represents a benchmark value weighted average, is shown in the center of the graphic. A score below 0.50 indicates an unpleasant method, a score between 0.50 and 0.75 recommends acceptability, and a score over 0.75 indicates a good level of greenness. The range of scores is 0.00 to 1.00.[15-17]

Validation: [18-20]

The goal of this structural process is to create written proof that a certain operation regularly yields results or products that adhere to predefined standards of quality and specifications. The following important parameters were used to further validate the suggested spectrophotometric technique in compliance with the recommendations provided by the International Council for Harmonization (ICH) in Q2 (R1). System appropriateness, linearity, accuracy, precision, detection and quantification limits, solution stability, and resilience are all included in this parameter.

System suitability:

To prove that the UV spectrophotometric system used for the analysis was suitable, system suitability tests were performed. Establishing Use of a UV spectrophotometer, the absorbance at 293 nm was measured for each of the six replicates of a single standard (10 mcg/mL) of tofacitinib that prepared from a working standard solution in diluents. Next, we computed the absorbance's relative standard deviation expressed percentage (% RSD).

Specificity:

The sample was made using blank as the diluents, and standard. The absorbance was determined at 293 nm and any interference were examined.

Precision:

Precision of a measurement is the degree to which findings from several measurements of the same homogenized sample agree. The level of precision of the approach was demonstrated by evaluating its reproducibility, intermediate precision, and intraday precision. Six samples of the 10 μg/mL concentration were analysed on the same day for repeatability, and six replicates of the same concentration were analysed on successive days for intermediate precision. In both cases, the assay findings' % relative standard deviation was computed.

Linearity:

The linearity of the analytical process confirms the direct correlation between test results and analyte concentration in the sample, in accordance with ICH Q2 (R1) criteria. Using a working standard solution of tofacitinib, seven standard preparation of various concentrations (2, 4, 6, 8,10,12 and 14 μg/mL) were produced in ethanol and water (20;80).A calibration curve for absorbance and concentration was plotted after each solution's measurement of absorbance was made at 293 nm. Regression analysis utilized to calculate the coefficient of correlation and percentage relative standard deviation.

Accuracy:

The in-house tablet formulation of tofacitinib was subjected to standard addition procedures, where by known amounts of the standards were implemented at three different levels (80, 100, and 120%) and the method was then used to assess accuracy.Tofacitinib standard was spiked at these levels in order to conduct recovery studies, and calculated the percentage recovery for each level.

Solution stability:

In beginning and over 24 hours after that, solutions, both standard and sample were analyzed, and the stability of the solution was reported.

Robustness:

This evaluation of the robustness parameter of the proposed method takes into account method's ability to be reported with minor changes to the technique's parameters, like wavelength (291 nm and 295 nm) or solvent composition (12:88) and (28:72), in different change in method parameter under different conditions, without unchanged differences in results.

Limits to quantification and detection:

A substance's threshold for detection is the minimum concentration in the given sample at which it can be found but not always accurately calculated

On the other hand, the limit of quantification refers to the material's lowest concentration in the sample that can be reliably and accurately measured. Applying the slope technique and residual standard deviation of respons, these tofacitinib criteria were determined in accordance with ICH guidelines. To do this, a calibration curve from the linearity plot was used. The formula utilized to compute the LOD was (3.3*σ)/S, where S is the slope of the calibration curve, and σ is the response standard deviation. Equation (10*σ)/S was also used to determine the LOQ.

RESULTS and DISCUSSION:

Solubility study was carried out by different solvents and the results are given in table No-1.

Table-1 Solubility Data of tofacitinib

Solvent	**Solubility**
Dist. Water	**Soluble**
Ethanol	**Soluble**
Methanol	**Soluble**
0.1(N) NaOH	**Soluble**
0.1N HCl	**Soluble**

Determination of Working Wave Length (λ_{max})

As evidenced by the UV-scan report in figure No. 2 and the tofacitinib lamdamax shown at 293 nm.

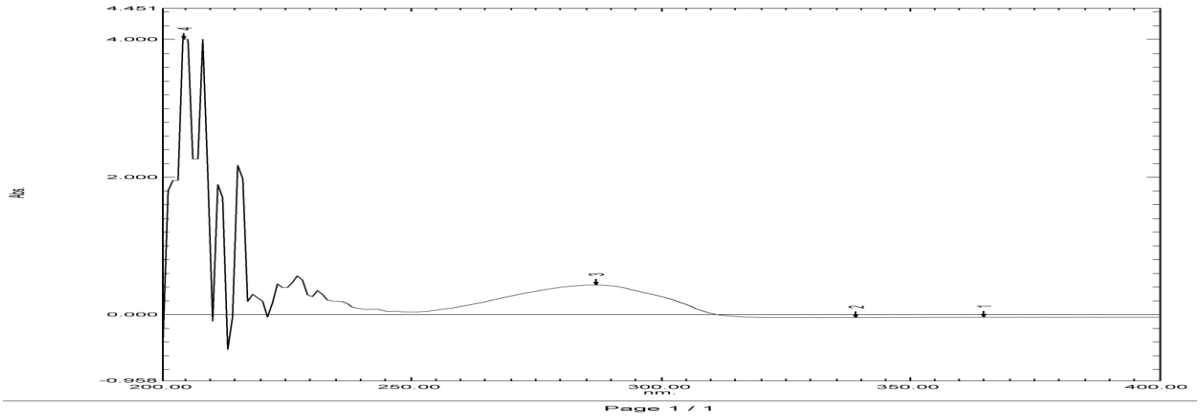

Fig.2 Lamda max spectra of Tofacitinib

System precision Data of Tofacitinib

Absorbance data of the tofacitinib standard solution at 10 µg/mL was recorded, and the system suitability results are provided in Table No. 2.

Table III : System precision Data of Tofacitinib

Sr. No.	Absorbance of Tofacitinib
1	**0.861**
2	**0.863**
3	**0.862**
4	**0.861**
5	**0.862**
6	**0.865**
Mean	**0.863**
SD	**0.002**
%RSD	**0.18**

Specificity:

At the lamda max of 293 nm, no interference was seen in the blank; the findings are shown in Table No. 2-2.

Table 2: Specificity data

Sample	Absorbance	% Interference
Blank	**0.000**	**NA**
Standard	**0.861**	**NA**
Test 5 mg	**0.865**	**NA**

Table IV: Method precision data of Tofacitinib

Six samples 10 µg/mL of same homogenius batch is prepared and analyzed and calculated assay value of tofacitinib given in table no-3

Sr. No	Absorbance	% Assay of Tofacitinib
1	0.865	100.21
2	0.862	99.75
3	0.867	100.39
4	0.861	99.50
5	0.871	100.85
6	0.863	99.69
Mean		100.07
SD		0.510
%RSD		0.51

Table V: Intermediate precision data of Tofacitinib

% Assay of Tofacitinib			
Sr. No.	Absorbance	Intermediate precision	MethodPrecision
1	0.861	99.72	100.21
2	0.864	99.99	99.75
3	0.863	99.72	100.39
4	0.871	100.82	99.50
5	0.865	99.93	100.85
6	0.863	99.88	99.69
Mean		100.01	100.07
SD		0.412	0.51
%RSD		0.41	0.51
Over all Mean		100.04	
Over all SD		0.44	
Over all %RSD		0.44	
Analyst		Analyst II	Analyst I
UV-Vspectrophotometer ID		UV-1800	UV-1700

Table VI: Solution stability of standard solution data of Tofacitinib

Time (In hours)	Tofacitinib	
	Absorbance	% Difference

Initial	**0.681**	**-**
24 hour	**0.678**	**0.4**

Table VII: Solution stability of Test solution data of Tofacitinib

Time (In hours)	**Tofacitinib**	
	%Assay	**% Difference**
Initial	**99.7**	**-**
24 hour	**99.3**	**0.46**

Table VIII: Linearity & Range data of Tofacitinib

Sr.No	**Concentration of Tofacitinib in µg/mL**	**Absorbance**
1	**2.00**	**0.151**
2	**4.00**	**0.326**
3	**6.00**	**0.519**
4	**8.00**	**0.691**
5	**10.01**	**0.864**
6	**12.01**	**1.037**
7	**14.01**	**1.221**
Slope	**0.089**	
Intercept	**-0.031**	
Correlation	**0.999**	

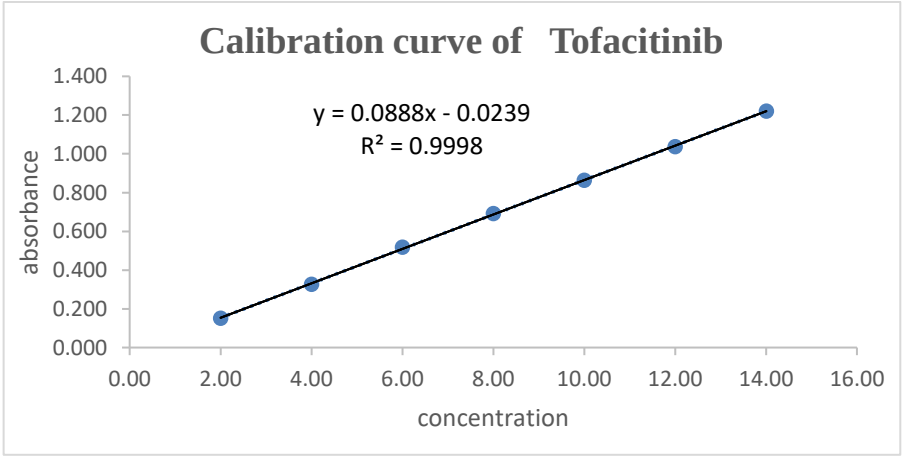

Fig. 3 Calibration plot of Tofacitinib

Linearity plot of Tofacitinib

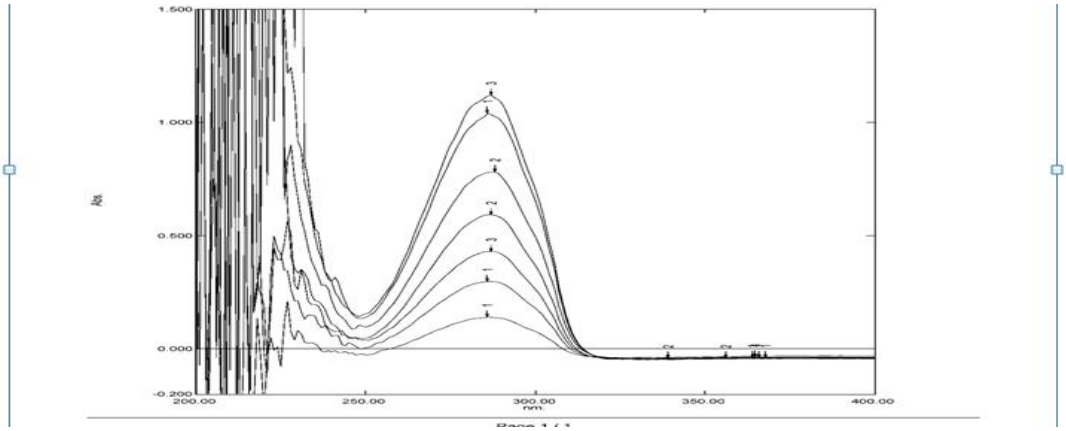

Fig-4 Linearity UV spectra of Tofacitinib

Table IX: Accuracy as recovery data

Level	Amount Added	Abs	Amount Recovered	% Recovery	Mean % Recovery	SD	% RSD
Level 1 (80%)	8.10	0.691	8.02	99.01	99.55	0.91	0.92
	8.21	0.701	8.13	99.03			
	8.31	0.721	8.36	100.60			
Level 2 (100%)	10.010	0.862	10.00	99.90	99.67	0.21	0.21
	10.100	0.867	10.06	99.60			
	10.060	0.863	10.01	99.50			
Level 3 (120%)	12.21	1.050	12.18	99.75	99.84	0.54	0.54
	12.32	1.055	12.24	99.35			
	12.12	1.049	12.17	100.41			
Over all Mean						99.68	
Over all SD						0.55	
Over all % RSD						0.56	

Table X: Robustness data

Parameter	%Assay	% RSD	Over All% RSD Of Assay
Plus Wavelength (295nm)	99.81	0.19	0.43

Minus Wavelength (291 nm)	**99.46**	**0.35**	**0.54**
Plus Organic Ethanol: Water (28:72)	**100.12**	**0.40**	**0.45**
Minus Organic Ethanol: Water (12:88)	**100.02**	**0.38**	**0.45**

An assessment of sustainability profiles or eco-friendly attributes.

AGREE graphical representations of the established strategies are shown in Figure 4. AGREE analytical grades of 0.80 and 0.90 were given to the developed spectrophotometric procedures. The AGREE greenness scale classifies scores as follows: less than 0.50 indicates insufficiency, 0.50 to 0.75 indicates reasonableness, and more than 0.75 indicates excellentness. The results of this study's AGREE analytical evaluations indicate that both of the recommended analytical methods for tofacitinib quantification are very environmentally friendly.

The Principles of Green Chemistry are demonstrated to be fulfilled by the AGREE tool. But energy use is an important factor to take into account, especially when utilizing spectrophotometric and chromatographic techniques, which allow the carbon footprint to be used to quantify environmental effects. The carbon footprint, expressed in kilograms of CO2 equivalent, evaluates the negative consequences of an approach by taking into account variables including power consumption of the equipment, operating time, and energy emissions. As per the description provided by Ballester-Caudet et al. (2019), this metric is calculated using the formula present in ESI S1 within the HEXAGON tool.[21-22]

The HEXAGON method indicates that the spectrophotometric approach produced a total carbon footprint of 0.00550 kg CO2 equivalent. Since both numbers are less than 0.1, the final score , as determined by the HEXAGON technique, is 0 out of 5.[23-24]. For a carbon footprint of less than 0.1, the total competency score is zero on a 5-point rating system. Our methods are more ecologically friendly because their 5-minute analysis time is associated with a lower carbon footprint score. [25]

A B

Figure No- A. Agree Pictogram scale and B. UV-Spectrometric method Agree pictograms

Discussion:

This work investigated the spectrophotometric characteristics of tofacitinib using solvents that are safe for the environment and operators. From sample preparation to detection, the novel approaches' environmental friendliness was assessed. Water and ethanol, which were chosen for their safety for analysts and the environment, were utilized in the processing of the sample; ethanol served as an organic modifier. Ethanol and ultrapure water were the solvents employed for spectrophotometric studies.

The new procedures were validated in accordance with ICH guidelines, and they performed remarkably well. In the selective analysis of tofacitinib, they showed outstanding linearity, precision, accuracy, specificity, and robustness. It was also discovered that the techniques had extremely poor quantification and detection limitations.

Furthermore, the system appropriateness parameters showed that the procedure performed satisfactorily. This study's main discovery is that the trash generated is non-toxic. Since all validation requirements were satisfied without sacrificing performance standards, the study's goals were accomplished. This study validates the

viability of performing spectrophotometric studies and shows how ethanol- and water-based mobile phases may be used successfully in pharmaceutical analyse

CONCLUSION:

A precise, cost-effective, and reliable UV-spectroscopic technique has been established for quantifying tofacitinib in tablet formulations This method stands out for its simplicity and speed, boasting precision with %RSD values below 2. To demonstrate its reproducibility and precision, the method has been rigorously tested. The recovery range of 99.55-99.84% attests to its accuracy and specificity. The high recovery percentage of the drug suggests that the presence of other ingredients in the tablet formulation doesn't interfere with tofacitinib determination, affirming the method's specificity and dependability.

The future of humanity depends more than ever on developing ecologically responsible solutions, as clean water sources are being depleted and air pollution is rising. It is crucial to manage trash, cut back on energy use, and address environmental contamination. Thus, in this setting, the creation of environmentally friendly approaches becomes greater significance.

The degree of greenness was confirmed using the AGREE assessment tool, confirming the suggested method's eco-friendliness. Notably, a review of the literature revealed that no method for detecting tofacitinib that uses a mobile phase composed of safer solvents is currently in use.

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