DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF TOFACITINIB CITRATE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Tofacitinib Citrate in tablet dosage form.

Methods: The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer' s law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Tofacitinib in tablet dosage form and also validated as per ICH guidelines.

Results: The drug is freely soluble in analytical grade methanol, slightly soluble in ethanol and practically insoluble in water. So, the analytical grade methanol is used as a diluent in method. The melting point of Tofacitinib was found to be 201-203°C (uncorrected). It showed absorption maxima 287 nm in analytical grade

methanol. On the basis of absorption spectrum the working concentration was set on 30µg/ml (PPM). The linearity was observed between 10-50 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 99.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 1.44% and for Ruggedness is 0.97%.

Conclusion: A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Tofacitinib in tablet dosage form. The method could be considered for the determination of Tofacitinib in quality control laboratories.

Keywords: Tofacitinib, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Tofacitinib (3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d] pyrimidin-4-yl)amino) piperidin-1-yl)-3 oxopropane nitrile) is a new class of drug called Janus kinase inhibitor [1, 2]. Tofacitinib, a first oral non-biologic disease-modifying anti-rheumatic drug (DMARD) can be used as monotherapy or in combination with methotrexate or other non-biologic DMARD's, for treating adults with moderate or severe rheumatoid arthritis. It is contraindicated for use with biologic DMARDs or with immunosuppressive agents, such as azathioprine and cyclosporine [2]. Rheumatoid arthritis (RA) is a complicated, long-lasting autoimmune condition that causes synovitis and gradually destroys joints. In both the early and severe stages of the disease, synthetic and biological disease-modifying antirheumatic medications have been the backbone of care for individuals with RA [3]. It is crucial to have disease-modifying antirheumatic medicines with distinct modes of action that can enhance the present assemblage of therapies because RA is a complicated condition with multiple molecular subgroups. A new, selective Janus kinase (JAK) inhibitor called tofacitinib is used to treat RA as well as transplant rejection, psoriasis, and other immune-mediated diseases [4].
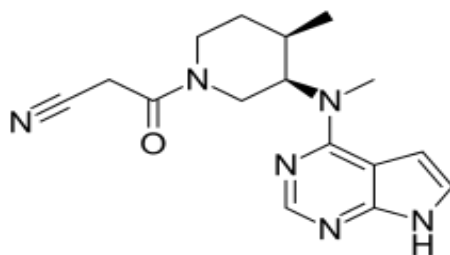

Fig. 1: Chemical Structure of Tofacitinib

A survey of literature revealed that RP- HPLC [5,6,7], LC-MS-MS [8,9], In-vivo marker study [10] and Pharmacokinetic study on volunteer [11] these methods have been reported for determination of Tofacitinib in rat plasma, bulk and its dosage forms. There are also found some UV method for Tolfacitinib [12, 13]. But, some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Tolfacitinib in tablet dosage form. So, the aim of work was to develop and validate an analytical method by UV-Visible Spectrophotometer for the estimation of Tofacitinib. In present study, simple, economical, accurate, reproducible analytical method with better detection range for estimation of Tofacitinib Citrate were developed and validated as per the ICH guideline Q2(R1).

MATERIALS AND METHODS

- **Instruments :**

  Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair

  Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

  Melting point apparatus of Equiptronics were used.

- **Materials:**

  Tofacitinib was obtained as a gift sample. Tofacitinib tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

  Method development:

A. Determination of λ max (10 PPM) [15, 16, 17]

Fig. 2: Calibration Curve

50 mg weighed amount of Tofacitinib was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 1 ml and added in 50 ml of volumetric flask dissolved and diluted up to the

mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of Working concentration

Preparation of Standard stock solution:

Standard stock was prepared by dissolving 50 mg of Tofacitinib in 100 ml of analytical grade methanol to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution:

Pipette out 3 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get concentration of 30 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution:

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution:

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations [15,16,17]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Tofactinib was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 3 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 287 nm. The absorbance was recorded:

MSN Laboratories Pvt. Ltd. (100 mg)		
Sr. no.	Sample	Absorbance
1	Blank	0.0000
2	Standard	0.3374
3	Sample	0.3321

Table 1: Absorbance of Dosage Form

No.	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	TOFADOZ - 10	05/2022	04/2025	B011415-	0.2501	98.43

MSN Laboratories Pvt LTD (10mg)			00		

Table 2: Dosage Form Specifications

E. Method of validation [14,16]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity:

The linearity of the proposed assay was studied in the concentration range 10 - 50 PPM at 287 nm. The calibration data showed a linear relationship between concentrations.

Sr. no.	Sample Concentration	Absorbance
1	10 PPM	0.1159
2	20 PPM	0.2278
3	30 PPM	0.3318
4	40 PPM	0.4417
5	50 PPM	0.5416
Correlation coefficient		0.9997 ~ 0.999

Table 3: Linearity Studies

Accuracy:

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 287nm.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	Qty weighed (mg)	Qty found (mg)	Recovery (98-102%)
80	0.8	0.78	98.75
100	1	0.99	99.00
120	1.2	1.19	99.17

Table 4: Accuracy Studies

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Sr. No.	Sample Solution	Absorbance

1	Sample Solution 1	0.3304
2	Sample Solution 2	0.3254
3	Sample Solution 3	0.3311
4	Sample Solution 4	0.3326
5	Sample Solution 5	0.3212
MEAN		0.3281
SD		0.0047
% RSD		1.4410

Table 5: Precision studies

Ruggedness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.3319	0.3323	98.47	0.9744
		0.3326			
2	Analyst 2	0.3345	0.3365	99.84	
		0.3384			

Table 6: Results for Ruggedness Studies

RESULTS

1. Solubility of Tofacitinib

Solubility test was passed as per criteria.

Sr. no.	Title	Result
1	Dimethylacetaamide	Freely Soluble
2	Methanol	Soluble
3	Water, Ethanol	Very Slightly Soluble

Table 7: Results for solubility studies

2. Melting point of Tofacitinib

The melting point of Tofacitinib was found to be 201-203˚C (uncorrected).

3. Results for linearity for assay method of Tofacitinib

The linearity of method was determined at concentration level ranging from 10 to 50   µg/ml (PPM).

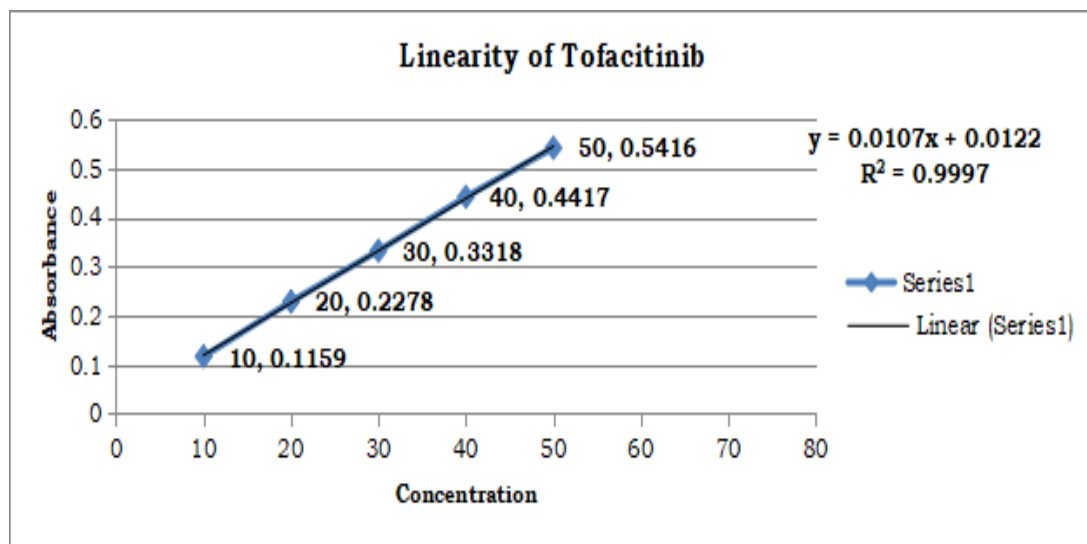The correlation coefficient value was found to be **(R^2) 0.9997 ~ 0.999**

Fig. 3: Tofacitinib Standard Curve

4. **Results for accuracy for assay method of Tofacitinib**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. **Results for precision for assay method of Tofacitinib**

The % RSD for different sample of precision was found to be 1.4410 ~ 1.44 and it is within acceptance criteria represented in Table - 5.

6. **Results for ruggedness for assay method of Tofacitinib**

The % RSD for different sample of ruggedness was found to be 0.9744 ~ 0.97 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Tofacitinib in tablet form has been developed. From the spectrum of Tofacitinib, it was found that the maximum absorbance was 287 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 10-50 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Tofacitinib in solid dosage forms. Hence, the method could be considered for the determination of Tofacitinib in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million

2. nm - Nanometer

3. HPLC - High Performance Liquid Chromatography

4. UV - Ultra violet

5. MS – Mass Spectroscopy

6. LC - Liquid Chromatography

7. ICH - International Council for Harmonization

8. RSD - Relative Standard Deviation

9. SD - Standard Deviation

10. Qty - Quantity

11. °C - Degree Celsius

12. M.D. - Manufacturing Date

13. E.D. - Expiry Date

14. µg/ml - Microgram per milliliter

15. Avg - Average

16. Wt - Weight

17. g - gm

18. DMARD - Disease-modifying anti-rheumatic drug

19. RA - Rheumatoid arthritis

20. JAK - Janus kinase

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