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Stability Indicating HPTLC Method for Determination of Tofacitinib Citrate

Thakariya NV*, Ezhava SB

Department of Quality Assurance, L.M. College of Pharmacy, Navrangpura, Ahmedabad-380009, Gujarat, India

ABSTRACT

Tofacitinib is a drug of the Janus kinase (JAK) inhibitor class. It is currently approved for the treatment of Rheumatoid Arthritis (RA) in the United States and other countries. A sensitive, selective, precise and stability-indicating High Performance Thin Layer Chromatography (HPTLC) method for quantitative analysis of tofacitinib citrate drug has been established and validated. Chromatographic separation was performed on aluminum plates precoated with silica gel and the mobile phase Chloroform: Methanol (9.5:0.5, v/v). Analysis of tofacitinib citrate was performed in absorbance mode at 287 nm. This system gave compact bands for tofacitinib citrate chromatogram of standard tofacitinib citrate (Rf=0.47 ± 0.03). Tofacitinib citrate was subjected to acid hydrolysis, base hydrolysis, neutral hydrolysis, oxidation, photolysis, dry heat induced degradation: Thermal degradation. The degradation products were well resolved from the pure drug. The linearity was obtained in the range 500-900 ng/spot with correlation coefficients (r^2=0.9974) for tofacitinib citrate. The developed technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of tofacitinib citrate as bulk drug without any interference from the degradation products. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use.

Keywords: Tofacitinib citrate, HPTLC, Validation, Stability studies

INTRODUCTION

Chemically, tofacitinib citrate is 2-hydroxypropane-1,2,3-tricarboxylic acid, 3-[(3R,4R)-4-methyl-3- [methyl({7H-pyrrolo[2,3-d]pyrimidin-4 l})amino]piperidin-1-yl]-3-oxopropanenitrile [1]. It is a drug of the Janus kinase (JAK) inhibitor class. It is currently approved for the treatment of Rheumatoid Arthritis (RA) in the United States and other countries. 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 (Figure 1) [1,2].

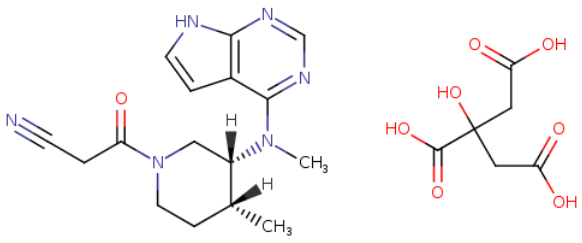

Figure 1: Tofacitinib citrate

A survey of literature revealed that Reverse Phase High Performance Liquid Chromatography (RP-HPLC) (RP-HPLC) and LC-MS-MS [3-9], methods have been reported for determination of tofacitinib in rat plasma, bulk and its dosage forms. However, there was no stability indicating analytical method reported for tofacitinib citrate by HPTLC method till date. So, the aim of work was to develop stability indicating HPTLC method for tofacitinib citrate. To validate the proposed methods as per International Conference on Harmonisation (ICH) guidelines [10]. To apply the above method for estimation of tofacitinib citrate in its synthetic mixture.

MATERIALS AND METHODS

Chemicals and reagents

Tofacitinib citrate was obtained from Pharm ACE Research Laboratory, Ahmedabad, India. Methanol (LR grade Finar, Ahmedabad, India), Toluene (LR grade RFCL Ltd., New Delhi, India). Ethyl acetate ($C_4H_8O_2$) (LR grade CDHL, New Delhi, India), Acetone (C_3H_6O) (LR grade Finar, Ahmedabad, India), Hydrochloric acid (HCl) (LR grade Finar Chemicals, Ahmedabad, India), Ammonia 0.91d min. 25% (Finar Chemicals, Ahmedabad, India), Hydrogen Peroxide (H_2O_2) 6% (Finar Chemicals, Ahmedabad, India), distilled water, pH strip, litmus paper, Sodium Hydroxide (NaOH) (LR grade Finar Chemicals, Ahmedabad, India).

INSTRUMENTS

A Camag Linomat IV (Semiautomatic spotting device), Camag Twin trough Chamber (10 ×10 cm), Camag TLC Scanner, Camag CATS4 Software, Hamilton Syringe, Shimadzu libror AEG-220 Balance, Constant temperature water bath, Veego melting point Apparatus VMP-D, Sonicator Frontline FS-4.

Chromatographic conditions

Stationary phase: Precoated silica gel G60 F_{254} aluminium sheets 20 × 20 cm^2, layer thickness 0.2 mm. Activation: TLC plates prewashed with methanol and activated in oven at 60°C for 5 min. Mobile phase: Chloroform: Methanol=9.5: 0.5 v/v. Chamber saturation time: 10 min. Temperature: Room temperature (25 ± 2). Migration distance: 60 mm

Preparation of standard stock solution

Stock Solution of tofacitinib citrate (S1)

Tofacitinib citrate (25 mg) was weighed accurately and transferred into 25 ml volumetric flask and diluted with methanol up to the mark (1000 µg/ml of tofacitinib citrate) (S1=1000 µg/ml of tofacitinib citrate).

Preparation of synthetic mixture

5 mg of the drug was accurately weighed and mixed with the excipients as croscarmellose sodium 10 mg, Hydroxypropyl Methylcellulose (HPMC) (20 mg), lactose monohydrate (150 mg), Polyethylene Glycol (PEG) 3350 (25 mg), magnesium stearate (10 mg), microcrystalline cellulose (20 mg) and titanium dioxide (5 mg) and the final synthetic mixture was mixed uniformly.

Preparation of solution

The whole synthetic mixture was taken in 10 ml volumetric flask and diluted with methanol. Than it was sonicated for 10 min and volume was make up to mark with methanol. This solution was filtered and filtrate (1.4 ml) is pipetted out and transferred in to 10 ml volumetric flask diluted with methanol up to the mark. An appropriate volume (10 µl) of standard solution (700 ng/spot) of tofacitinib citrate synthetic mixture was spotted on pre-coated TLC plate. The plate was developed, dried and photometrically analyzed as described previously.

RESULTS AND DISCUSSION

Mobile phase optimization

Different proportions of several solvents were tried while mobile phase selection. Finally Chloroform: Methanol (9.5:0.5 v/v) was finalized as mobile phase. The spots developed were dense, compact and typical peak of tofacitinib citrate was obtained as shown in Figure 2. Peaks were symmetrical in nature and no tailing was observed when plates were scanned at 287 nm.

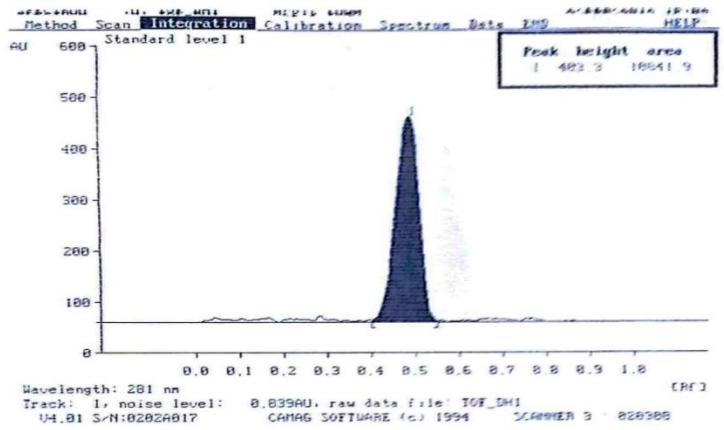

Figure 2: chromatogram of standard tofacitinib citrate (Rf=0.47 ± 0.03)

Acid degradation

Tofacitinib citrate was found to be unstable and degraded into three degradation products when refluxed with 0.1 N HCl for 3 h at 80°C temperatures (Figure 3).

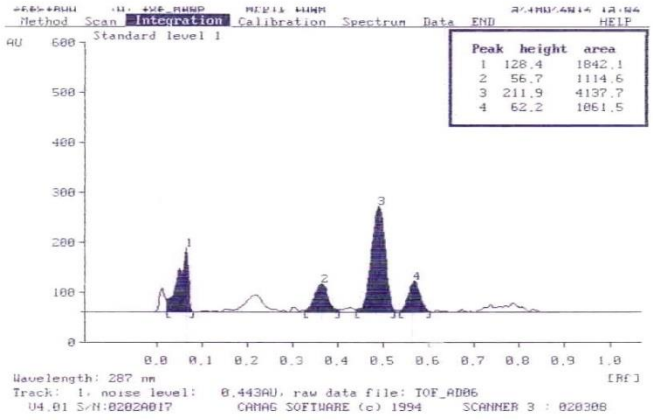

Figure 3: Chromatogram for acid degradation

Base degradation

Tofacitinib citrate was found to be unstable and degraded into four degradation products when treated with 0.1 N NaOH for 3 h at room temperature (Figure 4).

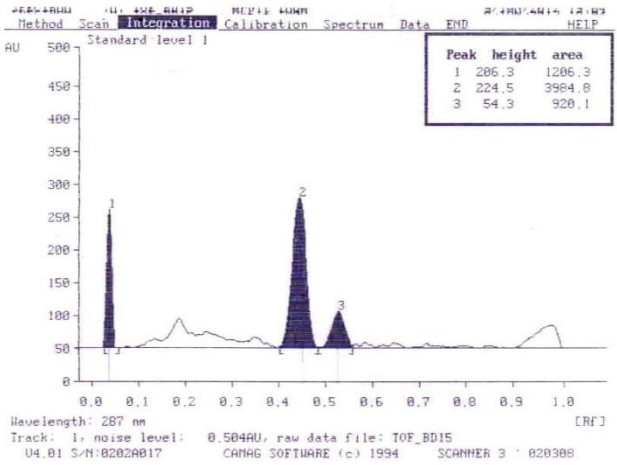

Figure 4: Chromatogram for base degradation

Thermal degradation

Tofacitinib citrate was found to be stable and no degradation product found when exposed to 80°C in Hot air oven for 24 h (Figure 5).

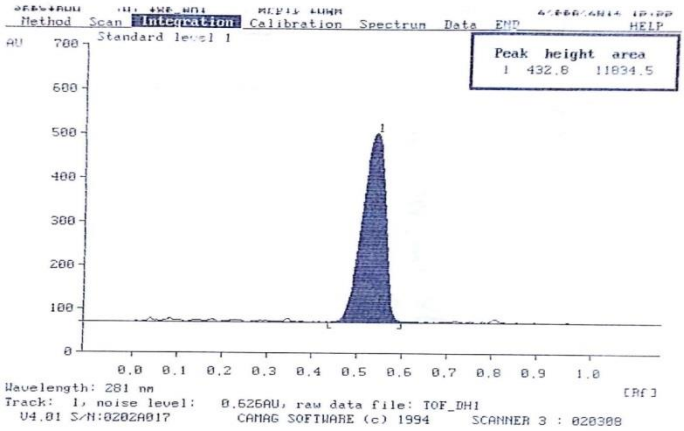

Figure 5: Chromatogram for thermal degradation

Photolytic degradation

Tofacitinib citrate was found to be stable and no degradation products were found when placed under UV light for 24 h at room temperature (Figure 6).

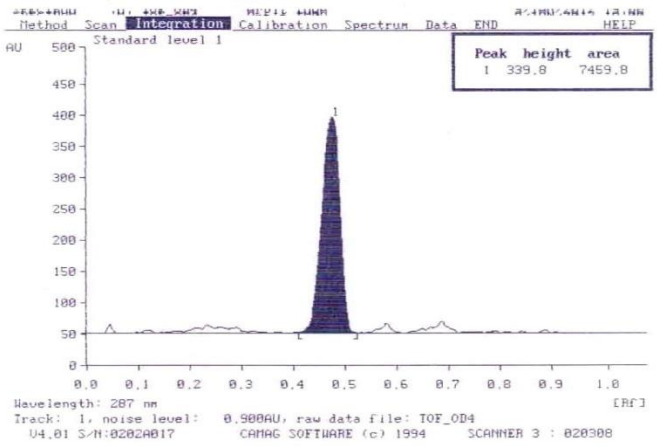

Figure 6: Chromatogram for photolytic degradation

Oxidative degradation

Tofacitinib citrate was found to be stable and no degradation products were observed when treated with 6% H_2O_2 for 48 h at room temperature (Figure 7).

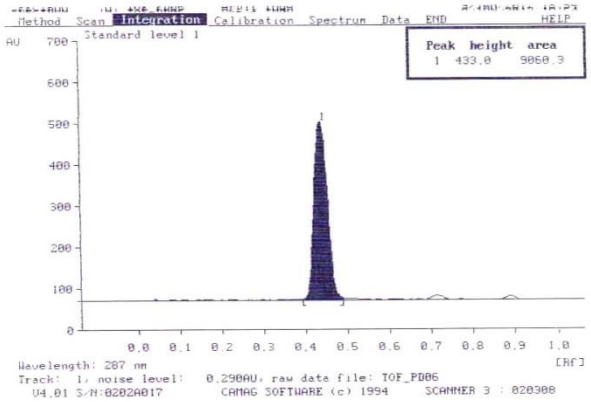

Figure 7: Chromatogram for oxidative degradation

Neutral hydrolysis

Tofacitinib citrate was found to be stable and no degradation products were found when refluxed with water for 24 h (Figure 8 and Table 1).

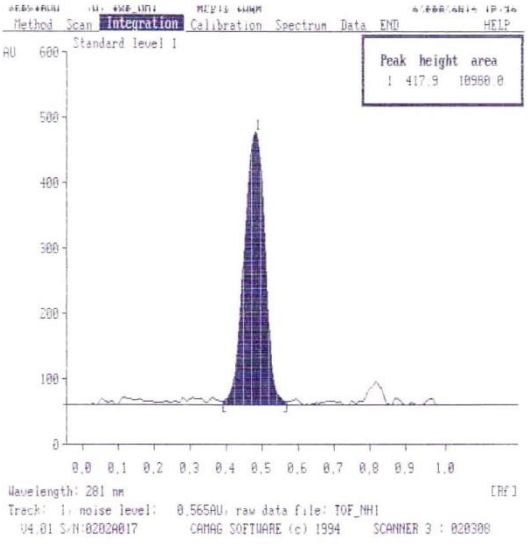

Figure 8: Chromatogram for neutral hydrolysis

Table 1: Results of forced degradation studies

S. No.	Condition	No. of degradation products	Rf of degradation products
1	Acid hydrolysis refluxed with 0.1 N HCl for 3 h	3	1. Rf=0.07 2. Rf=0.36 3. Rf=0.57
2	Base hydrolysis kept at room temperature for 30 min in 0.1 NaOH	2	1. Rf=0.04 2. Rf=0.52
3	Oxidative degradation kept at room temperature for 48 h in 6% H_2O_2	0	-
4	Photolytic degradation 24 h exposure to UV rays	0	-
5	Thermal degradation 24 h exposure to 80°C in hot air oven	0	-
6	Neutral hydrolysis refluxed with water for 24 h	0	-

Validation of HPTLC method

Due to its versatility and speed of analysis, HPTLC technique was found suitable for analysis of tofacitinib citrate in presence of its degradation product and impurities. A mixture of (Chloroform: Methanol=9.5: 0.5 v/v) could provide sharp peaks of tofacitinib citrate Rf=0.47 ± 0.03 (Figure 2) and was found to be specific separation method, which can separate tofacitinib citrate and its degradation product. It was observed that activation of TLC plates (pre-washing with methanol followed by drying at 50°C for 5 min) and pre-saturation of TLC chamber with mobile phase for 10 min ensures good reproducibility and peak shape. Photometric evaluation was performed at 287 nm. Using optimized conditions, developed HPTLC method was validated in terms of linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), precision.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity should be determined by minimum 5 different concentrations. The linearity of response for tofacitinib citrate was assessed by analysis of five independent levels of calibration curve in range of 50-90 µg/ml in terms of slope, intercept and correlation coefficient values. Coefficient of correlation was found to be 0.9974 (Figure 9 and Table 2).

Table 2: Linear regression data for calibration curves

Parameters	Tofacitinib citrate
Detection wavelength (nm)	287
Beer's Law Limit (ng/band)	500-900
Regression equation	5.3217x + 2489.7
Correlation coefficient (r)	0.9974
Intercept (c) ± SD	2489.7
Slope (m) ± SD	5.3217

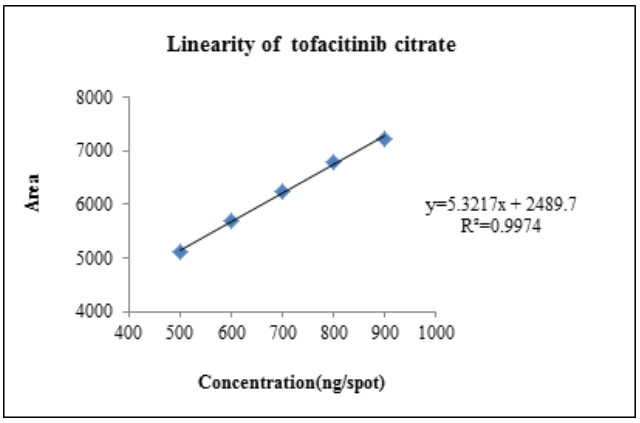

Figure 9: Calibration curve of tofacitinib citrate

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Repeatability, Intermediate precision, Reproducibility. The precision of the method was evaluated for its repeatability (sample application) and intermediate precision (Inter-day and Intraday).

Repeatability/Replication

An appropriate volume (10 µl) of standard solution (700 ng/spot) of tofacitinib citrate was spotted 6 times on pre-coated TLC plate. The plate was developed, dried and photometrically analyzed as described previously (Table 3).

Table 3: Repeatability of scanner peak area and peak height measurement

Concentration (ng/spot)	Repeatability of scanner peak area		Repeatability of sample application peak area	
	Peak area (n=3) MEAN ± SD	%RSD	Peak area (n=3) MEAN ± SD	%RSD
7000	6245.05 ± 9.91	0.16	6251.75 ± 12.35	0.19

Reproducibility

Variations of results within same day and amongst days are called as reproducibility. It includes following parameters,

Intraday precision and inter-day precision

A variation of results within same day is called intraday variation. It was determined by taking 3 determinations of 3 concentrations from linear range. The data for intraday precision of method are summarized in Table 4. The % Relative Standard Deviation (RSD) for intraday precision was found to be 0.06-0.17% for tofacitinib citrate. Variation of results amongst day is called interday variation. It was determined by taking 3 determinations of 3 concentrations from linear range. The data for inter day precision of method are summarized in Table 4. The %RSD for inter-day precision was found to be 0.13-0.16% for tofacitinib citrate.

Table 4: Intraday and Inter-day precision

Concentration (ng/spot)	Intraday precision		Inter day precision	
	Peak area (n=3) Mean ± SD	%RSD	Peak area (n=3) Mean ± SD	%RSD
500	5096.10 ± 7.69	0.15	5097.97 ±6.51	0.13
700	6253.33 ± 4.30	0.06	6248.53 ± 9.97	0.16
900	7225.8 ± 0.50	0.17	7218.90 ± 17.48	0.13

LOD and LOQ

It is the lowest amount of analyte in a sample that can be detected, but not necessary quantify under the stated experimental conditions. LOD is commonly used to substantiate that an analyte concentration is above or below a certain level. LOQ was calculated by using formula mentioned in ICH guidelines as follows:

$$LOD=(3.3\times\sigma)/S$$

σ=Standard deviation of the Y intercept, S=Slope of the calibration curve equation. LOD was calculated by using formula mentioned in ICH guidelines and were found to be 17.26 ng/spot. It is the minimum amount of analyte in a sample that can be quantitated with suitable precision and accuracy.

$$LOQ=(10\times\sigma)/S$$

LOQ was calculated by using formula mentioned in ICH guidelines and were found to be 52.29 ng/spot (Tables 5-7).

Table 5: Data for limit of detection and limit of quantitation for tofacitinib citrate

Validation parameter	Result (ng/spot)
LOD	17.26
LOQ	52.29

Specificity

The purity of tofacitinib citrate was ascertained by peak purity spectrum at three levels peak start, peak apex and peak end. It shows correlation coefficient of 0.9995 (M, E).

Accuracy

Table 6: Recovery for tofacitinib citrate

Level (%)	Amount spiked in placebo solution (µg/ml)	Target concentration (ng/spot)	Amount of tofacitinib citrate recovered (ng/spot) Mean ± SD (n=3)	%Recovery mean (n=3)
80	56	560	5457.83 ± 34.87	99.60
100	70	700	6248.43 ± 32.75	100.90
120	84	840	6960.47 ± 16.64	100.02

Assay

Table 7: Assay result

Formulation	Amount claimed (mg)	Amount obtained(mg) Mean ± SD (n=3)	%Assay(n=3)
Tofacitinib citrate synthetic mixture	5 mg/mixture	4.99 ± 0.048	98.80

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The standard deviation, %RSD and standard error calculated for the method are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The results of the stress studies indicated the specificity of the method. Hence, it can be concluded that the developed HPTLC method is accurate, precise, selective and can be employed successfully for the estimation of tofacitinib citrate.

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