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Development and Validation of Stability Indicating Assay Method for Estimation of Tofacitinib in Tofacitinib Citrate Immediate Release Tablet Dosage Form

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ABSTRACT:

A simple, precise and accurate stability indicating RP-HPLC method for estimation of Tofacitinib form Tofacitinib citrate immediate release tablet dosage form was developed & subsequently validated. Tofacitinib was separated and estimated using Agilent RP-HPLC 1200 series, UV detector. Chromatographic separation was achieved in Isocratic mode with Inertsil ODS-3V (150mm * 4.6mm I.D, particle size 5 µm) column. The mobile phase used was phosphate buffer (pH 5.0): Acetonitrile in the ratio of 65:35 % v/v. The elution of analyte was achieved with the flow rate of 1.0 ml/min and run time of 7 min. Detection was carried out at the wavelength of 287 nm. The different HPLC parameters were optimized & method was validated according to standard ICH guideline. Forced degradation study was carried out by exposing Tofacitinib for acid, alkali, oxidative, thermal & photolytic stress conditions. Retention time of Tofacitinib was found to be 3.292 min. The detector response was linear in the concentration range of 19.85µg/ml – 158.831µg/ml. The results show that Tofacitinib & other degradation products were fully resolved & thus the proposed method is stability indicating.

KEYWORDS: Tofacitinib, RP-HPLC, Stability indicating, Validation

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INTRODUCTION:

Tofacitinib is chemically 3-[(3R,4R)-4-methyl-3-[methyl({7H pyrrolo[2,3-d]primidin-4-yl})amino]piperidin -1-yl]-3-oxoprpanenitrile used in the treatment of Rheumatoid Arthritis patients who have inadequate response with Methotraxate.

The molecular formula of Tofacitinib is $C_{16}H_{20}N_6O$ with the molecular weight of 312.3696 g/mol. Tofacitinib is reversible and partial janus kinase inhibitor that prevents the phosphorylation and activation of STATs pathway and decrease inflammatory response.[1-2]

Tofacitinib is not official in Indian United States and European Pharmacopoiea. Literature survey reveals that only few analytical methods are reported for analysis of drug. There is no stability indicating analytical method was reported for estimation of Tofacitinib from Tofacitinib citrate immediate release tablet dosage form.[3-6]

Therefore, it was thought of interest to develop precise, accurate, sensitive, selective chromatographic method for estimation of Tofacitinib form Tofacitinib citrate immediate release tablet dosage form which will provide valuable information that can be used to assess the inherent stability of the drug under various stressed conditions, eventually to improve formulation and manufacturing process. The aim of work was to carry out RP-HPLC method development and validation for estimation of Tofacitinib from Tofacitinib citrate immediate release tablet dosage form.[7-8]

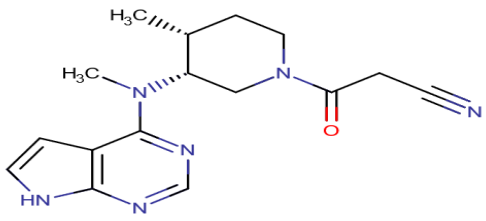

Figure 1: Chemical structure of Tofacitinib

MATERIALS AND METHODS:

In the present research work Acetonitrile and Potassium dihydrogen phosphate monohydrate were used of Merck Life science, Methanol and 1- Octane sulphonic acid sodium salt monohydrate were used of Spectrochem Pvt. Ltd. Potassium hydroxide was used of Sigma Aldrich. The sample of Tofacitinib API and Tablets were kindly gifted by ZYDUS CADILA HEALTHCARE, Moraiya, Ahmedabad.

Equipment

The analysis was performed on HPLC Agilent technologies 1200 series, Isocratic mode, Photodiode array detector, injector of 100 µL loop volume. Intertsil ODS-3V (150mm * 4.6mm, 5 µm) columnwhich is maintained at 40 ºC temperature. Chromeleon software was used for data collecting and processing.

Preparation of 5.0 pH phosphate buffer

Weigh 2.72 gm of Potassium dihydrogen phosphate and 1 gm of 1- Octane sulphonic acid sodium salt monohydrate and dissolve in 1000 ml Milli Q water, adjust pH 5.0 with diluted Potassium hydroxide solution.

Preparation of mobile phase

Prepare the mixture of Phosphate Buffer and Acetonitrile in the volume ratio of 65:35 % v/v and sonicate for degassing. Filter through 0.45 µ Millipore filter.

Diluent

Water and Acetonitrile is used in the ratio of 50:50 % v/v as diluent.

Preparation of standard stock solution (100ppm)

Transfer an accurately weighed quantity of 80.8 mg of Tofacitinib citrate (Eq. to 50 mg of Tofacitinib) API to a 50ml volumetric flask, add about 35ml of diluent and sonicate to dissolve. Make up the volume upto the mark with diluent and mix dilute 5.0ml of above solution to 50ml with diluent and mix.

As such sample solution (100ppm)

[Label claim: 5 mg]

Weigh accurately 20 tablets and calculate the average weight. Weight 10 intact tablets and transfer into 500 ml of volumetric flask. Add 300 ml of diluent and sonicate with occasional shaking for 45 mins. Make volume upto mark with diluent.

Filter the solution through 0.22 µm Millipore PVDF filter; collect the filtrate by discarding first 5 ml of filtrate.

Chromatographic conditions

Inertsil ODS- 3V (150mm * 4.6mm, 5 µm) column was used as stationary phase. Phosphate buffer (5.0 pH) and Acetonitrile were used in the ratio of 65:35 % v/v as Mobile phase. It was filtered through 0.45 µ(micron) membrane filter and degassed. The mobile phase was pumped at 1.0

ml/min. The eluent was monitored at 287 nm. The injection volumes of sample and standard were 10 µL (micro liter). Total run time is 7 min.

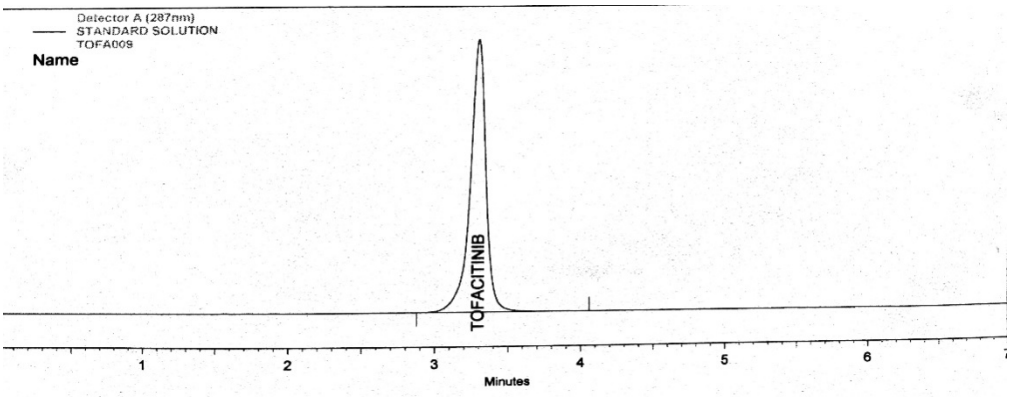

Figure 2: Chromatogram of Tofacitinib

The developed method was validated for linearity, precision, accuracy, robustness and is applied for forced degradation studies as per ICH guidelines.

RESULT AND DISCUSSION:

Method Development

ICH prescribed stress conditions such as acid, alkali, oxidative, thermal and photolytic stresses were carried out.

Acid Degradation

Sample preparation

20 ml reconstituted solution transfer into 100 ml volumetric flask, Add approx. 50 ml of diluent and sonicate for 10 mins, add 5 ml of 1 N HCl and heat for 2 hours on boiling water bath at 110□C for acid hydrolysis. Then the solution was neutralized with 1N NaOH and made volume upto mark with diluent. Then pipette out 5 ml of solution in 50 ml volumetric flask and make volume upto mark with diluent.

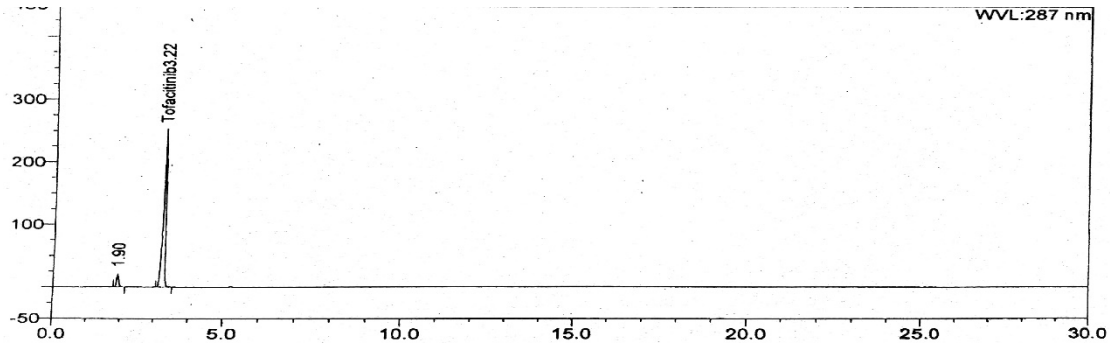

Figure 3: Acid degradation of Tofacitinib

Alkali Degradation

Sample preparation

20 ml reconstituted solution transfer into 100 ml volumetric flask, Add approx. 50 ml of diluent and sonicate for 10 mins, add 5 ml of 1N NaOH and heat for 5 mins on Room Temperature for Alkali hydrolysis. Then the solution was neutralized with 1N HCl and made volume upto mark with diluent. Then pipette out 5 ml of solution in 50 ml volumetric flask and make volume upto mark with diluent.

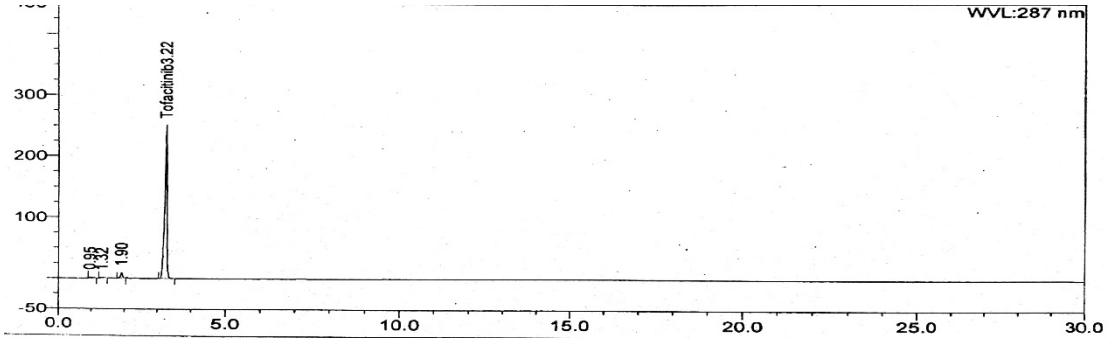

Figure 4: Alkali degradation of Tofacitinib

Oxidative Degradation

Sample preparation

20 ml reconstituted solution transfer into 100 ml volumetric flask, Add approx. 50 ml of diluent and sonicate for 10 mins, add 5 ml of 5% H_2O_2 and heat for 30 mins on Boiling water bath. Then pipette out 5 ml of solution in 50 ml volumetric flask and make volume upto mark with diluent.

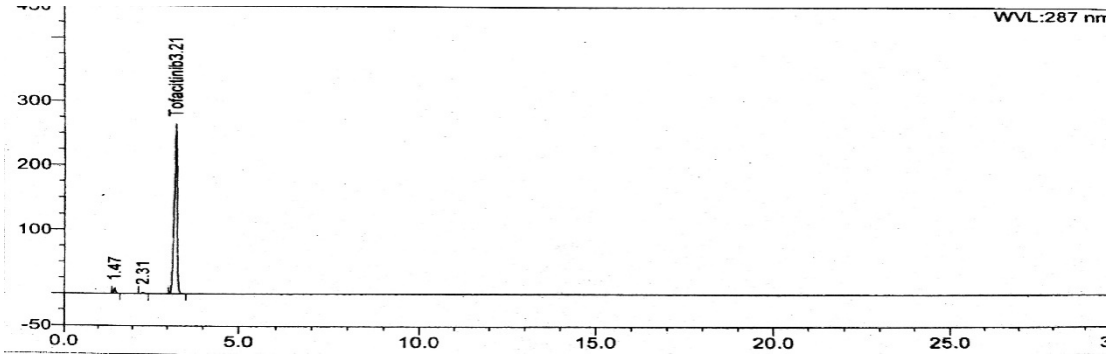

Figure 5: Oxidative degradation of Tofacitinib

Thermal Degradation

Sample preparation

1 vial kept at 100□C for 5 days, reconstitute vial with 19 ml water and transfer in 100 ml volumetric flask, make up the volume with diluent. Pipette out 5 ml of solution in 50 ml volumetric flask and make volume upto the mark with diluent.

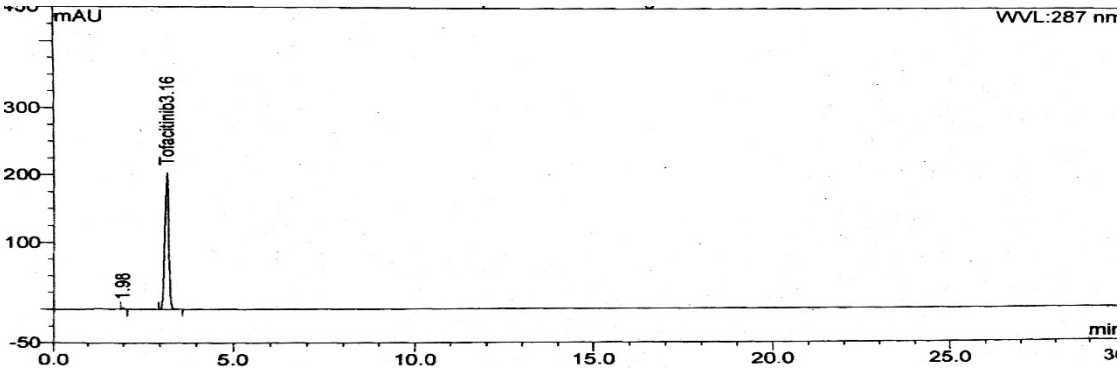

Figure 6: Thermal degradation of Tofacitinib

Photolytic Degradation

Sample preparation

1 vial kept into UV chamber for 22 hours, reconstitute vial with 19 ml water and transfer in 100 ml volumetric flask, make up the volume with diluent. Pipette out 5 ml of solution in 50 ml volumetric flask and make volume upto mark with diluent.

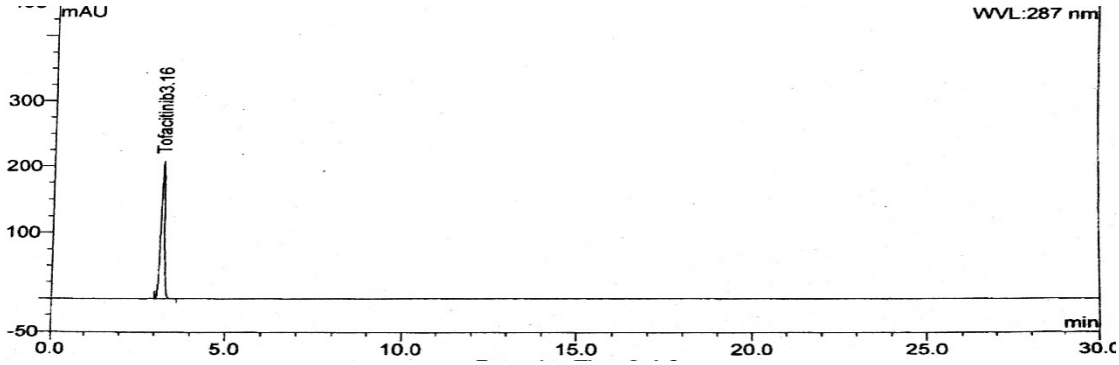

Figure 7: Photolytic degradation of Tofacitinib

Table 1: Degradation summary

Sr. No.	Stress Condition	Duration	Area after Degradation	% Degradation	% Mass Balance
1	Acid Hydrolysis (1N HCl)	110ºC for 2 hour	791930	8.4	97.2
2	Base Hydrolysis (1N NaOH)	R.T for 5 mins	783285	9.4	94.85
3	Peroxide Degradation (5 % H_2O_2)	110ºC for 30 mins	837751	3.1	98.68
4	Thermal Degradation	100ºC for 5 days	825649	4.5	96.46
5	Photolytic degradation	UV chamber for 22 hours	842940	2.5	98.0

Method Validation

System suitability/ System precision

System suitability and precision was demonstrated by making five replicate injections as per the test method. The peak area of analyte for replicate injection was recorded. The theoretical plates and tailing factor were evaluated for the analyte peak.

Standard Sample Preparation (100 ppm)

Transfer an accurately weighed quantity of 80.8 mg of Tofacitinib citrate (Eq. to 50 mg of Tofacitinib) API to a 50ml volumetric flask, add about 35ml of diluent and sonicate to dissolve. Make up the volume upto the mark with diluent and mix dilute 5.0ml of above solution to 50ml with diluent and mix.

Table 2: System suitability/ system precision parameter

Injection No.	Peak area of Tofacitinib
1	1525.909
2	1518.697
3	1519.979
4	1520.524
5	1521.528
Average	1521.327
% RSD	0.2

- Theoretical plates of Tofacitinib peak : 4026
- Tailing factor of the Tofacitinib peak : 1.2
- % RSD for five replicates standard injections is not more than 2 %.

Method Precision

Method precision was demonstrated by preparing six samples as per the test method representing a single batch. The assay of these samples was determined and the precision and the precision of method was evaluated by computing the percentage relative standard deviation of assay results.

Table 3: Method precision parameter

Method Precision	% Assay
1	99.9
2	99.7
3	99.0
4	99.5
5	98.4
6	97.9
Average	99.1
% RSD	0.8

Acceptance criteria

% RSD for assay of six preparations should not more than 2.0.

Sonication Time optimization

Three sample dilution were made as per the proposed test methods, sonicate the above three preparations for different time period namely 30 minutes and 45 minutes and 60 minutes after adding a suitable quantity of diluent. After completion of sonication they were suitably diluted and the assay of these samples were determined.

Table 4: Sonication time optimization parameter

Sonication time	% Assay
30 min	98.8
45 min	99.2
60 min	99.0

Linearity

The linearity of detector response for Tofacitinib was demonstrated by preparing solutions of Tofacitinib working standard over the range of 20- 160 % of standard concentrations. These solutions were injected into the HPLC system and the area of analyte peak was recorded. A graph of concentration vs. analyte peak response was plotted. The concentration co efficient between concentration & analyte peak response and Y- intercept of the correlation plot was evaluated.

Table 5: Linearity parameter

Linearity Level (%)	Conc. Of Tofacitinib (µg/ml)	Peak area of analyte
20	19.854	316.913
50	49.6351	791.376
80	79.416	1253.631
100	99.270	1567.910
120	119.123	1901.669
160	158.831	2500.778

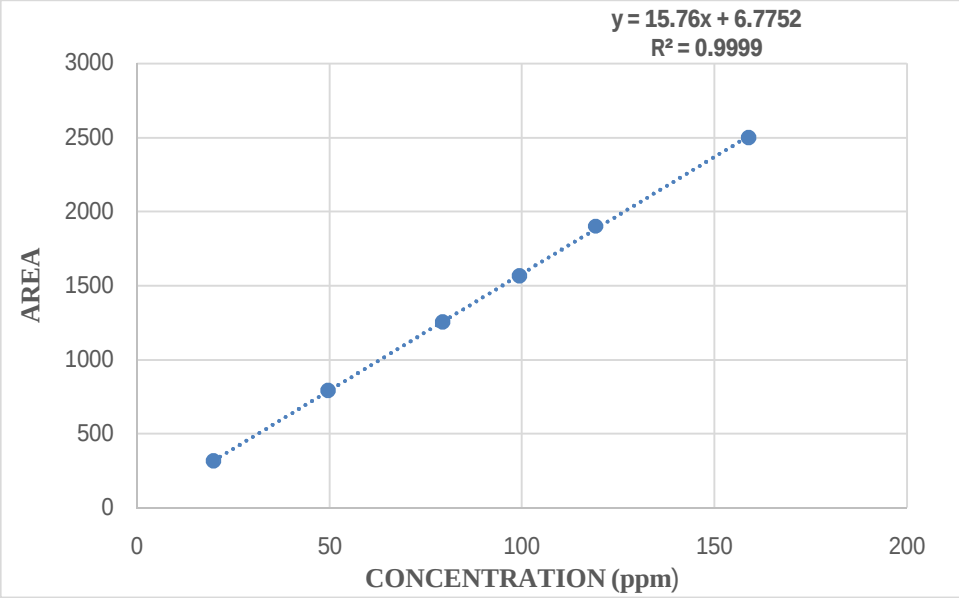

Figure 8: A graph of concentration vs. analyte peak response

The plot was found to be linear with a correlation co efficient of for Tofacitinib 0.9999 with respect to 100% linearity level response.

Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples (i.e. spiking of placebo with known quantities of API at the level of 50%, 100%,150% of target concentration. The recovery samples were prepared in duplicate. The above samples were chromatograph and the percentage recovery for amount added was estimated. The precision of the recovery was determined by computing the relative standard deviation of duplicate recovery results.

Table 6: Accuracy parameter

Recovery at 50 % level

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	25.19	25.37	100.7
2	25.38	25.46	100.3
Average			100.5
% RSD			0.3

Recovery at 100 % level

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	49.88	49.71	99.7
2	49.88	49.69	99.6
Average			99.7
% RSD			0.3

Recovery at 150 % level

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	73.40	73.16	99.7
2	73.50	73.25	99.7
Average			99.7
% RSD			0.0

Acceptance criteria

The recovery is 98- 102 % and the RSD is NMT 2 %.

Filter compatibility & saturation

The filter paper saturation was verified by preparing the assay samples with optimized samples preparation and analyzed the samples by discarding different volume of analyte. The assay of these samples was determined. Different filters are used. Here the filters are used are as follows, Millipore PVDF (0.22 µm), Millipore Nylon (0.22 µm), Whatman GMF PVDF (0.45 µm).

Table 7: Filter compatibility and saturation parameter

Millipore PVDF (0.22 µm)

Discarded volume	% of Tofacitinib	% Difference
Unfiltered	99.4	-
After 1 ml	97.6	1.8
After 2 ml	97.8	1.6
After 3 ml	98.0	1.4
After 5 ml	98.7	0.7
After 7 ml	97.9	1.5

Millipore Nylon (0.22 µm)

Discarded volume	% of Tofacitinib	% Difference
Unfiltered	99.4	-
After 1 ml	98.9	0.5
After 2 ml	98.3	1.0
After 3 ml	98.9	0.5
After 5 ml	99.1	0.2
After 7 ml	98.9	0.4

Whatman GMF PVDF (0.45 µm)

Discarded volume	% of Tofacitinib	% Difference
Unfiltered	99.4	-
After 1 ml	98.6	0.7
After 2 ml	98.4	0.9
After 3 ml	98.8	0.6
After 5 ml	98.9	0.4
After 7 ml	98.8	0.5

Acceptance criteria: The difference between the unfiltered and filtered samples is NMT 2.0 %.

Robustness

According to Robustness, there is minor but deliberate change made in chromatographic parameter with reference of Floe rate and column temperature. To observe robustness, 100% level solution is used.

Table 8: Robustness parameter

Parameter	Change	Area	Mean	SD	RSD
Flow Rate(ml/min)	0.8	843382	860837.6	9017.077	1.047477
	0.8	864013			
	0.8	857761			
	1.0	864553			
	1.0	854431			
	1.0	870001			
	1.2	869931			
	1.2	854553			
	1.2	868913			
Column Temperature	35ºC	859903	851714.6	13179.98	1.547464
	35ºC	862223			
	35ºC	839903			
	40ºC	864223			
	40ºC	849921			
	40ºC	850031			
	45ºC	824421			
	45ºC	864493			
	45ºC	850313			

CONCLUSION:

Stability Indicating RP-HPLC method has been developed and validated for the estimation of Tofacitinib from Tofacitinib citrate immediate release tablet dosage form. The methods are found to be specific as there was no interference of any co-eluting impurities after degradation study. The degraded products are well resolved, indicating the method can also be useful for determination of degraded products.

All the parameters and results are found within the acceptance limit as given in the validation protocol. So, we can conclude that developed RP-HPLC method is found to be specific, linear, accurate and robust. Therefore, method is to be specific with good resolution. Thus, the proposed method can be used in pharmaceutical analysis for forced degradation study and routine quality control samples for Tofacitinib citrate immediate release tablet dosage form.

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