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ANALYTICAL QUALITY BY DESIGN (AQBD) APPROACH FOR RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF TOFACITINIB: A SYSTEMATIC OPTIMIZATION STRATEGY

Manisha Keshav Gavit[1]*, Dr. Aejaz Ahmed[2], Dr. G. J. Khan[3]

[1*]Ali-Allana College of Pharmacy, Akkalkuwa, Dist. Nandurbar (M.S.)-425 415, India. E-mail: gavitm7@gmail.com
[2]Department of Pharmaceutical Chemistry, Ali-Allana College of Pharmacy, Akkalkuwa, Dist. Nandurbar (M.S.)-425 415, India
[3]Department of Pharmacology, Ali-Allana College of Pharmacy, Akkalkuwa, Dist. Nandurbar (M.S.)-425 415, India.
***Corresponding Author**: Manisha Keshav Gavit,E-mail:gavitm7@gmail.com

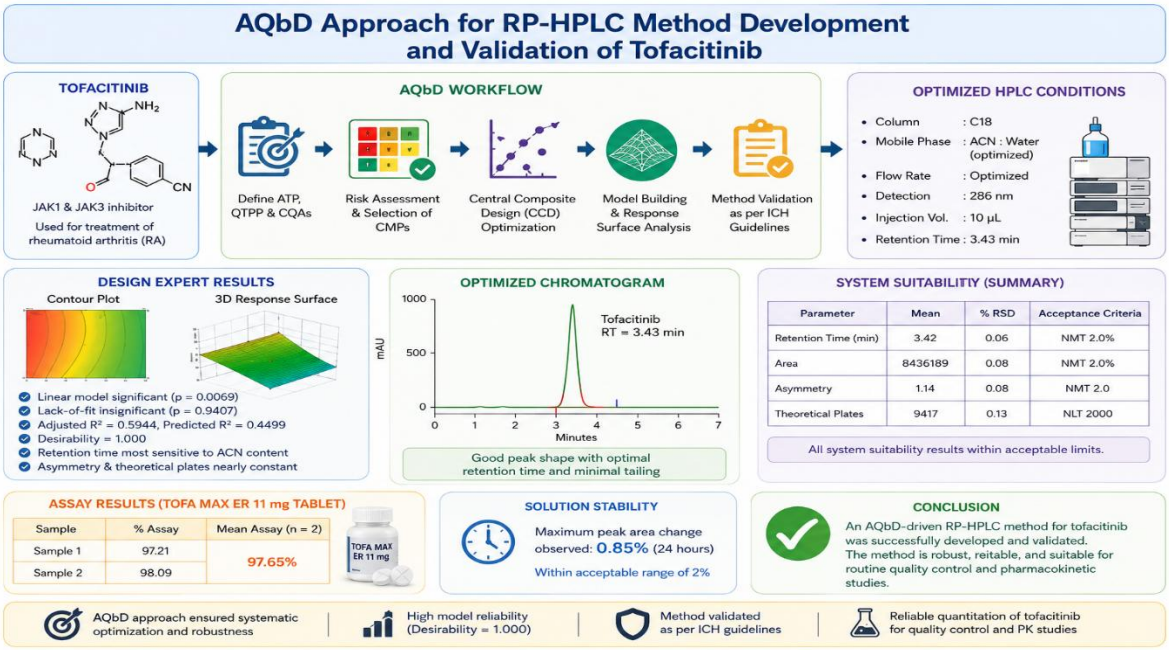


Figure: Graphical Abstract

ABSTRACT

Background: Analytical Quality by Design (AQbD) is a systematic, science-based methodology for building robust analytical techniques through identification and management of essential method parameters. A reliable RP-HPLC method is needed for routine quality control and pharmaceutical analysis of tofacitinib, a Janus kinase (JAK) inhibitor used in the treatment of rheumatoid arthritis.

Aim: To develop, improve and validate RP-HPLC technique based on AQbD for quantitative measurement of tofacitinib utilizing Design Expert® software and response surface methodology as per ICH requirements.

Method: The effect of three important technique parameters (acetonitrile concentration, flow rate and column oven temperature) was studied using a Box-Behnken experimental design with 14 trials. Retention duration, peak asymmetry and theoretical plate count were the analytical responses evaluated. The improved chromatographic conditions were verified according to ICH criteria for system applicability, specificity, linearity, accuracy, precision, robustness, solution stability, limit of detection (LOD) and limit of quantitation (LOQ).

Results and Discussion: The linear regression model was statistically significant ($F = 7.35$, $p = 0.0069$) with a non-significant lack-of-fit ($p = 0.9407$), supporting the applicability of the AQbD model. The desirability value of the optimized method was 1.000. The chromatographic peak was crisp with a retention period of around 3.43 min, an asymmetry of 1.14 and theoretical plate count of roughly 9417. The method displayed good linearity over the concentration range 5–15 µg/mL with LOD and LOQ values of 0.22 and 0.67 µg/mL, respectively. Accuracy was obtained with a total recovery of 99.81%. Precision and robustness were within ICH acceptance criteria. Adequate stability was confirmed through solution stability studies, showing a maximum peak area variation of just 0.85% after 24 h. The results indicated that AQbD was able to optimize the analytical method and improve its robustness and reliability.

Conclusion: An AQbD based simple, rapid, accurate and precise RP-HPLC technique has been developed and validated for the quantification of tofacitinib. The method showed good selectivity, precision, accuracy, robustness and stability and it is applicable for routine quality control, pharmaceutical analysis and pharmacokinetic studies.

KEYWORDS: Tofacitinib, Quality by Design approach, RP-HPLC method, ICH guideline

INTRODUCTION

Tofacitinib (tofacitinib citrate) is an orally active JAK inhibitor targeting mainly JAK1 and JAK3 and shows negligible inhibitory effect on JAK2 and TYK2. Tofacitinib is the first approved JAK inhibitor by the Food and Drug Administration for the treatment of rheumatoid arthritis (RA). The mechanism of action of tofacitinib involves the modulation of the JAK/STAT pathway resulting in the downregulation of cytokine-induced inflammatory response associated with the development of RA [1,2,3,4,5]. Accurate quantification of tofacitinib is crucial for quality control, bioequivalence, and pharmacokinetic studies. Highly sensitive HPLC–MS/MS assays with lower limits of quantification around 0.1 ng/mL have been developed, but many conventional RP-HPLC methods still rely on trial-and-error optimization, which can reduce robustness and increase sensitivity to experimental variation [6].

The Quality by Design (QbD) approach provides a well-planned and scientifically backed approach to drug development, stressing the importance of comprehensive knowledge about the product and process with defined objectives. This approach is described in ICH guidelines Q8(R2), Q9, and Q10 and is based on the definition of the quality target product profile (QTPP), CQAs, CMAs/CPPs, and the application of suitable control strategy [7,8].

Quality by Design for analysis (AQbD) applies quality by design (QbD) concepts to analytical methods through the incorporation of quality considerations from the start, as opposed to depending on testing of the final product alone. The first step in AQbD is the development of an Analytical Target Profile (ATP), followed by risk assessment and determination of critical method parameters (CMPs) [5]. Recent AQbD-based HPLC applications have shown superior performance compared with conventional approaches. For example, AQbD-driven methods for ponatinib in rat plasma and for simultaneous estimation of metformin, linagliptin, and empagliflozin have achieved wide linear ranges, improved sensitivity, and clearly defined operable design regions; AQbD-based green HPLC methods for thalassemia drugs have also demonstrated high efficiency with reduced environmental impact.

In view of the increasing clinical application of tofacitinib and the lack of fully documented QbD-based RP-HPLC methods specifically targeting tofacitinib, there is definitely a need for developing an AQbD-based analytical methodology that assures robust and reliable quantitation of the drug for pharmacokinetic studies and quality control purposes. In the current work, the aim is to develop and validate a robust RP-HPLC method for quantifying tofacitinib via AQbD approach. This will be accomplished through multi-variable optimization by the means of central composite design, risk analysis for selecting CMPs, and full validation of the method as per ICH guidelines [9,10,11,12,13].

MATERIAL AND METHODS:

2.1 Chemicals and Drugs:

The ACN used in this work was of HPLC grade and was obtained from Merck India Pvt. Ltd., Mumbai, India. Likewise, water of HPLC grade was obtained from the Siddhi Lab water purifier system. All other reagents were of analytical grade. The source of tofacitinib was V & S Laboratory, Maharashtra, India. For this analysis, Tofa Max XR/ER 11 mg extended-release tablet of Sunovion Pharmaceuticals (India) Private Ltd. containing the drug tofacitinib was used.

2.2 Equipment:

Double beam UV-visible spectrophotometry was performed using the instrument, Model V-550 of Jasco, Japan with Spectra Manager software. High Performance Liquid Chromatographic (HPLC) analysis was conducted using the Quaternary Gradient HPLC system model 1260 Infinity II from Agilent Technologies, USA with the help of Open Lab EZChrom software. Design Expert® software (version 13.0.0; State-Ease Inc., Minneapolis, MN, USA) was employed for experimental design and optimization.

2.3 Chromatographic conditions:

Table 1: Baseline RP-HPLC chromatographic conditions for Tofacitinib

Parameter	Description
Mode	Isocratic
Column Name	Phenomenex C18, 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	286 nm
Column Oven temp	40ºC
Mobile Phase	Acetonitrile: Water (70:30%V/V)
Flow Rate	1.0 ml/min
Run time	3.43 Minutes

2.4 Standard Stock Solution preparation for Chromatographic analysis:

Tofacitinib standard stock solution was prepared by dissolving 16.16 mg Tofacitinib Citrate (Equivalent to 10 mg of Tofacitinib) into a 20 mL clean and dried volumetric flask and added mixture of mobile phase, sonicated until all the standard is dissolved and finally diluted up to the mark with remaining acetonitrile and water mixture (500 PPM). Furthermore, 2 ml of the stock solution was diluted to 10 ml with the mobile phase for each trial. (100 PPM)

2.5 Application of Design Expert and Factorial Design:

The USFDA has given extensive guidelines for the application of QbD principles in immediate-release formulations, extended-release formulations, and biopharmaceuticals. Regulatory bodies keep emphasizing the need to comply with ICH guidelines, particularly Q8, Q9, Q10, and Q11.

In the present study, the authors utilized experimental design techniques in optimizing their HPLC method. They used a randomized response surface design, popularly called Box-Behnken design, consisting of 14 experimental trials.
The study focused on three factors that affect three important responses. The factors in the study were the mobile phase (X1) from 60 to 80, flow rate (X2) 0.8 to 1, and temperature of the column oven (X3) 30 to 40. For the dependent variables, the team focused on retention time (RT), peak asymmetry, and theoretical plates. Response surface methodology was used to figure out how those three factors affected the dependent variables. The independent variable R1, R2 and R3 ware Asymmetry, Retention time and Thermotical plates respectively and total 14 runs ware selected as depicted follows in Table 1.

Table 2: The independent variable R1, R2 and R3 ware Asymmetry, Retention time and Thermotical plates respectively and total 14 runs

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
1	60	1	35	1.1	6.4	4500
2	70	1	40	1	3.43	8500
3	50	1	30	1.17	4.5	5635
4	70	1.2	35	1.08	5.3	5350
5	60	1.2	30	1.12	6.5	7335
6	50	1.2	35	1.15	7.5	6700
7	70	0.8	35	1.09	5.2	6921
8	70	1	30	1.11	5.9	4950
9	60	1.2	40	1.13	6.3	7302
10	60	0.8	40	1.11	5.9	6700
11	50	1	40	1.14	7.3	5870
12	60	0.8	30	1.16	5.3	6780
13	60	1	35	1.18	4.9	7350
14	50	0.8	35	1.18	6.9	6890

The developed method using HPLC was rigorously tested for selectivity, precision, and robustness based on system suitability and other parameters as per general principles of chromatography. This allowed the development of a method suitable for quantification as suggested by (Bose et al., 2014) [14].

2.6 RP HPLC Method Validation:

In the case of RP-HPLC method for determination of Tofacitinib, all important validation parameters suggested by ICH have been evaluated such as system suitability with respect to retention time, peak area, asymmetry, theoretical plates and %RSD, specificity by checking blank and placebo injections to ensure no interference at the retention time of analyte, linearity with respect to the range of concentration from 5 to 15 µg/mL with regression analysis and %RSD of responses, accuracy by performing recovery study at various levels (50%, 100% and 150%), precision with respect to intra-day (repeatability) and inter-day (intermediate precision) on tablet samples, sensitivity by calculating LOD and LOQ, stability of solutions of standards and samples at 0, 12 and 24 hours, and robustness by deliberately changing the wavelength, flow rate and column oven temperature while ensuring that the system suitability is within the specified limits [15-22].

RESULTS:

3.1 Application of Design Expert Model:

Fit statistics show that the linear regression model is preferred because there is a significant improvement when additional terms are added to this model (p = 0.0069). The graphical optimization shows the high correlation between selected factors and the prediction of model (Figure 1).

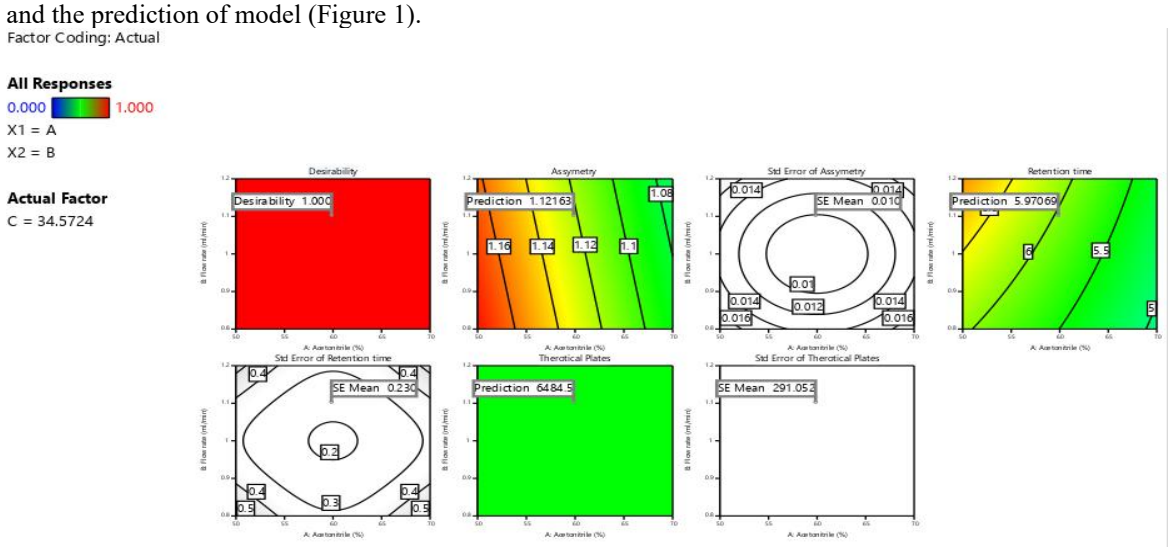


Figure 1: Contour surface response for all response 1, 2 and 3

This shows the reliability of the used model and its predictions as in Figure 2.

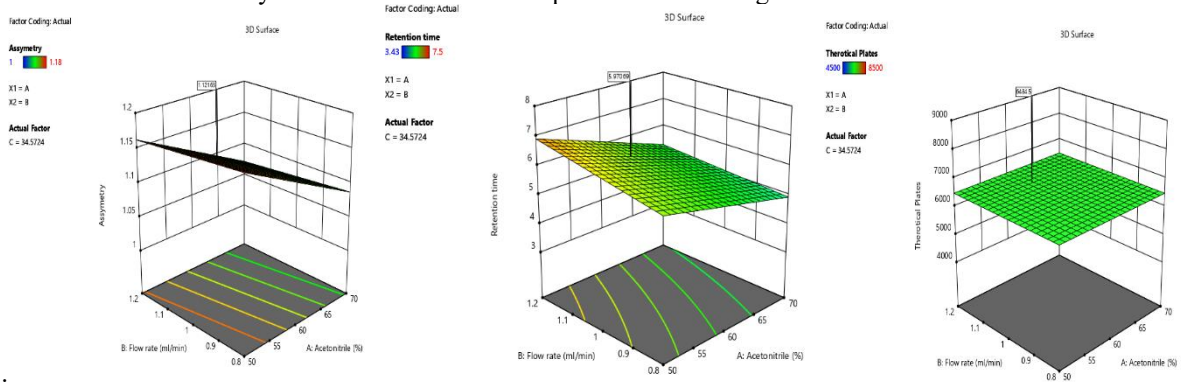


Figure 2: 3D response for all three independent variables like asymmetry, retention time and thermotical plats respectively

As seen from the analysis of the response surface, both concentration and flow rate have shown significant impact on retention time but limited effect was observed on asymmetry and plates.

3.2 HPLC method development and Validation:

Tofacitinib eluted at Retention Time of 3.43 and good chromatography observed.

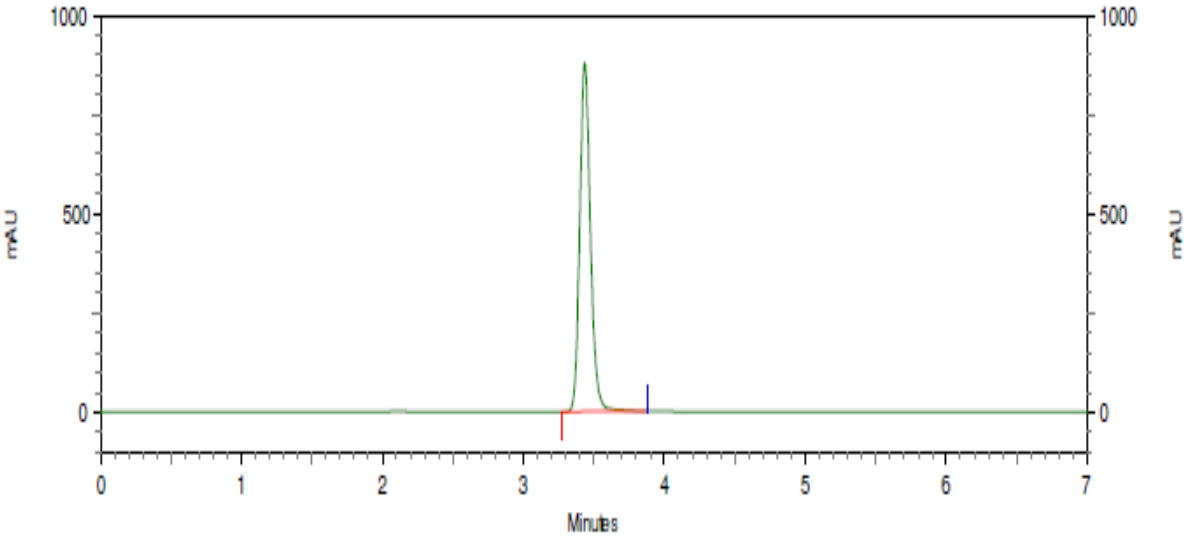


Figure 3: Optimize Chromatography Peak selection

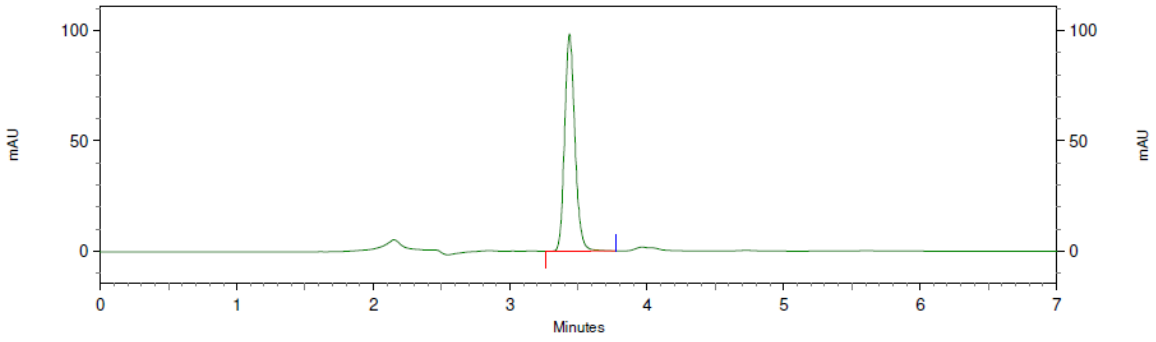
Chromatographic circumstances in trial five were submitted to method validation after it was determined that the conditions in optimize trial produced a better peak, good retention time, and tailing factor.

3.3 System suitability test:

Table 3: System Suitability Test of Tofacitinib

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Tofacitinib Std 1	8432651	1.14	9416
2	Tofacitinib Std 2	8431506	1.14	9421
3	Tofacitinib Std 3	8433105	1.15	9405
4	Tofacitinib Std 4	8435657	1.15	9428
5	Tofacitinib Std 5	8448025	1.14	9417
Mean		8436189	1.14	9417
STD Dev		6788.46280		
% RSD		0.08		

Sample Name: STANDARD SOLUTION_1



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Tofacitinib	3.42	8432651	1.14	9416
Totals		8432651		

Figure 4: Chromatogram Standard solution 1 of system suitability solution

The asymmetry factor is 1.14, an optimal value that assures peak shape consistency. The theoretical plates were about 9,417, indicating column efficiency and appropriate chromatographic behaviour.

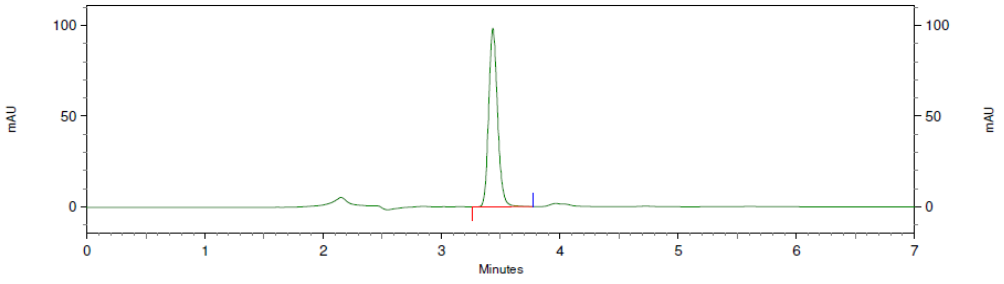
3.4 Analysis of Marketed Test samples (Assay):

Weight of 20 tablets 2.7280 gm and average weight 136.4 mg

Table 4: Assay results of Tofa max ER 11 mg Tablet

Sample	Area	% Assay	Mean Assay
Sample 1	8210539	97.21	97.65
Sample 2	8301429	98.09	

Sample Name: TEST SAMPLE SOLUTION_1



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Tofacitinib	3.42	8210539	1.15	9391
Totals		8210539		

Figure 5: Chromatogram sample solution-1 of Tablet sample.

Validation of RP-HPLC method:

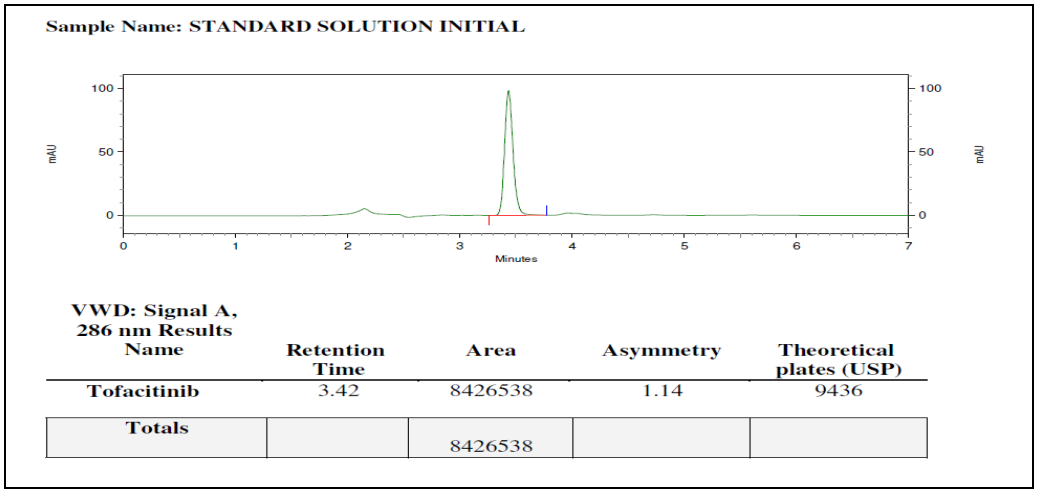
3.5.1 Solution Stability: The stability study was done on both the standard and test sample.

Table 5: Results of Solution stability.

Time point	Standard solution		Sample solution	
	Area	% Absolute difference	Area	% Absolute difference
Initial	8426538	NA	8335128	NA
12 Hours	8412305	0.17	8305217	0.36
24 Hours	8365429	0.73	8264100	0.85

As seen from the results above, there was negligible change in the peak area at various intervals of times.

3.5.2 Chromatograms:



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Tofacitinib	3.42	8426538	1.14	9436
Totals		8426538		

Figure 6: Chromatogram of Standard solution Initial

% Absolute difference of Stability solution: NMT 2.0 w.r.t. Initial solution. Standard and Test solution was found stable up to 24 Hrs Hence both solutions can be used up to 24 Hrs.

3.5.3 Specificity: Specificity refers to the capability to uniquely identify the analyte amidst the presence of components that could otherwise be expected. Blank and Placebo solution are injected to assess peak purity.

3.5.4 Chromatograms:

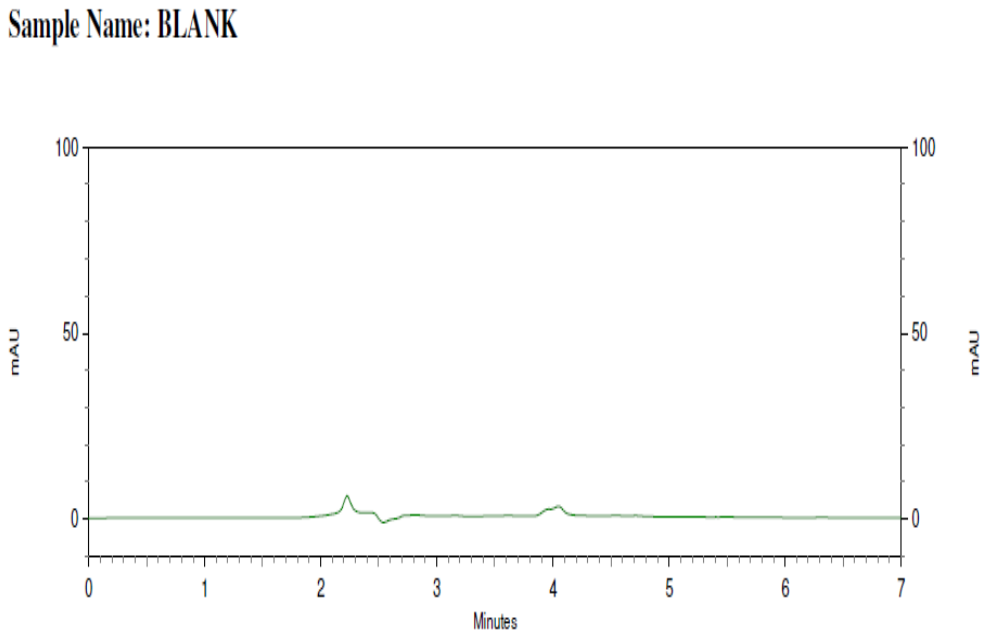


Figure 7: Chromatogram of Blank solution.

Linearity and Range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.

Table 6: Linearity Data for Tofacitinib

Level	Conc (µg/mL)	Area	Mean	% RSD
50%	5.00	4256748	4250296	0.298
		4258459		
		4235680		
75%	7.50	6468429	6472424	0.071
		6471425		
		6477419		
100%	10.00	8432106	8436938	0.060
		8436527		
		8442181		
125%	12.50	10513102	10522472	0.082
		10524186		
		10530128		
150%	15.00	12635204	12652106	0.143
		12671258		
		12649857		

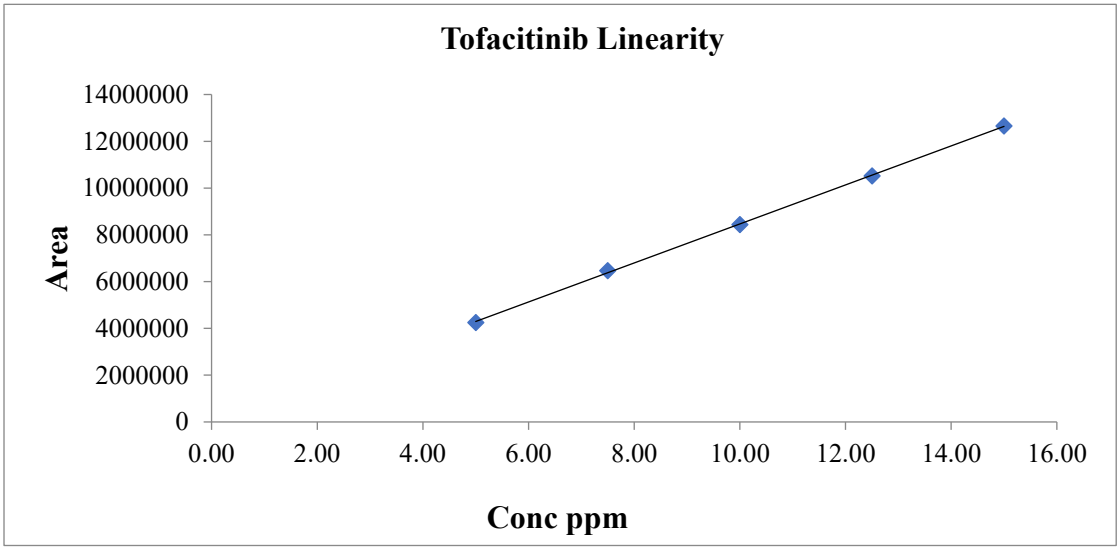


Figure 8: Tofacitinib linearity

The Regression value was found well within the limit significantly lower than the commonly used threshold of 2.0%.

3.7 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 55588.04

s = 834151.31

LOD = 0.22 µg/mL

LOQ = 0.67 µg/mL

Accuracy:

Table 7: Result and statistical data of Accuracy of Tofacitinib

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4265210	5.06	5.01	101.00	100.33	0.9753
	4321568	5.12	5.08	100.79		
	4221070	5.00	5.04	99.21		
100	8436527	10.00	10.03	99.70	99.66	0.6580

	8513269	10.09	10.06	100.30		
	8295346	9.83	9.93	98.99		
150	12635240	14.98	14.95	100.20	99.45	0.7695
	12595428	14.93	15.01	99.47		
	12542155	14.87	15.07	98.67		

Overall Recovery: 99.81 %
% RSD for Overall Recovery: 0.810
Precision:
The precision of an analytical procedure is the closeness of the agreement between individual measurement results obtained from repeated measurements ware as depicted in table 9.

Table 8: Intra- day and Inter- Day Precision for Tofacitinib test sample assay:

	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	248.3	8352149	98.88
	Sample 2	248.5	8265743	97.78
	Sample 3	247.8	8312429	98.61
	Sample 4	249.1	8467415	99.93
	Sample 5	248.5	8221530	97.26
	Sample 6	248.1	8341251	98.83
	Mean			98.55
	STD DEV			0.933
	% RSD			0.947
Intermediate precision (Inter-Day)	Sample 1	247.8	8332567	98.85
	Sample 2	248.6	8312567	98.30
	Sample 3	248.2	8352106	98.92
	Sample 4	249.2	8325671	98.21
	Sample 5	248.7	8210238	97.05
	Sample 6	248.0	8256741	97.87
	Mean			98.20
	STD DEV			0.6903
	% RSD			0.703
Repeatability Plus Inter-day	Mean			98.374
	STD DEV			0.8033
	% RSD			0.817

Robustness:
The robustness of an analytical method is a measure of its capacity to remain retention time 3.12 to 3.81.
3.10 Effect of Wavelength, Flow Rate and Oven Temperature Variation:
The effect of the slight change of the wavelength was measured for two different wavelengths: 288 nm, and 284 nm. It was found out that the R.T. for each case did not vary; it stayed equal to 3.43 min. From the robustness experiment, it was evident that there was some impact of the change in flow rate on retention time. When the flow rate increased by 10%, retention time decreased to 3.12 min was decreased by 10%, retention time became 3.81 min. Values of asymmetry were between 1.12 to 1.15, which means that the shape of peaks did not get changed. Influence of deviations in temperature during column chromatography was examined at +2°C and -2°C. There were no variations in retention time (3.41-3.42 min), signifying that the process is insensitive to small changes in temperature. The areas of standard peaks also revealed slight differences.

DISCUSSION:
Sequential Sum of Squares of the Model (Type I) was used based on the application of a linear model: linear terms significantly accounted for the variability in asymmetry ($F = 7.35$, $p = 0.0069$); while the terms of interaction (2FI), quadratic and higher orders had no influence on decreasing the residual variance, which means that curvature and interaction effects are insignificant within the considered range of acetonitrile concentration, flow rate, and column temperature. Small value of residual mean square together with non-significant lack-of-fit indicates the presence of random errors only. With regard to chromatography, variations in asymmetry are mostly dependent on first-order effects of the method parameters, and therefore, slight changes in acetonitrile concentration, flow rate, or oven temperature result in proportional changes in peak shape and asymmetry adjustment, however, due to relatively low there are some other sources of variability in the peak shape. Stability testing of the solution revealed that peak area decreased from 8,335,128 initially to 8,305,217 after 12 hours (0.36% difference) and to 8,264,100 after 24 hours (0.85% difference), which are lower than the generally accepted 2.0%, and therefore, no degradation was observed.

The specificity tests established that there was no interference from the blank and placebo at the retention time of the analyte. In addition, there was also precision at the concentration range of 5.00 to 15.00 µg/mL, which provided very low %RSD results indicating high reproducibility. The robustness test results indicated that small but controlled changes in the wavelength, flow rate, and column temperature had no significant effect on retention time, asymmetry, and column efficiency with the criteria for system suitability being within the acceptable range.

CONCLUSION:

The linear relationship model was found useful for asymmetric optimization by using QbD approach, which can be used for process optimization and prediction by response surfaces. There was the good separation of the analyte peak from other peaks, which indicates sufficient selectivity of the method and no interfering peaks at the retention time of the analyte for Tofacitinib quantification. Robustness experiments showed the accuracy of results obtained even with slight changes of conditions (wavelength, flow rate, and column temperature), with exception of retention time change with flow rate changing. Stability of asymmetry and sufficient theoretical plate counts show the reproducibility of system. The developed HPLC method is precise, efficient and reliable.

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