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Research Article

Optimized RP-HPLC Method Development For Precise Estimation Of Favipiravir: A Quality By Design Approach

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ABSTRACT

A robust and precise method for the estimation of Favipiravir in bulk and pharmaceutical dosage forms was developed using a Quality by Design (QbD) approach with RP-HPLC. The chromatographic analysis was conducted on a Waters Alliance 2695 HPLC system equipped with a C18 column using a mobile phase of Methanol:Water (60:40, v/v) at a flow rate of 0.9 mL/min. The optimized wavelength was set at 229 nm with a column temperature of 30°C, achieving a retention time of 4.60 minutes for Favipiravir. Method validation demonstrated %RSD of 0.07 and recovery of 99.18%, while LOD and LOQ values were determined as 0.16 and 0.48 respectively. The method exhibited linearity (R^2 = 0.9999) with a regression equation of y = 19106x + 96204. Optimization efforts reduced retention times and overall run time, ensuring simplicity and cost-effectiveness suitable for routine quality control in laboratories and industries. This validated RP-HPLC method is simple, linear, and precise meets ICH guidelines, making it reliable for the quantitative analysis of Favipiravir.

INTRODUCTION

The quality of pharmaceuticals plays prime role in ensuring the safety and efficacy of the medicines. The quality assurance and control of pharmaceutical products and chemical formulations is essential for ensuring the

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availability of safe and effective drug formulations to consumers. Hence, Analysis of pure drug substances and their pharmaceutical dosage forms hold a significant role in estimating the suitability to use in patients. The quality of the analytical data depends upon the quality of the methods employed in generation of the data (1). Hence, development of sturdy and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

Analytical chemistry is the science that seeks improved means of measuring the chemical composition of natural and artificial materials. Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why to utilize. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte as well as to the final needs or requirements of it. Quality by Design (QbD) has become an important concept for the pharmaceutical industry that is further defined in the International Conference on Harmonization (ICH) guidance on pharmaceutical development as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified.

MATERIALS & METHODS

Selection of Diluent:

The diluent consisted of Methanol and Water in a ratio of 60:50 with pH adjusted to 3.0 using Ortho-Phosphoric Acid, chosen based on the solubility of the drugs.

Preparation of Standard Stock Solutions:

Accurately weighed 10 mg of Favipiravir was transferred to individual 50 ml volumetric flasks. Three-fourths of the diluent was added, sonicated for 10 minutes, and then made up to volume with diluent to obtain Standard stock solution 1 (200 µg/ml of Favipiravir).

Preparation of Standard Working Solutions:

One millilitre of Standard stock solution 1 was pipette into a 10 ml volumetric flask and made up with diluents to obtain Standard working solution (20 µg/ml of Favipiravir).

Preparation of Sample Stock Solutions:

Twenty tablets were weighed, and the average weight was calculated. An amount equivalent to 10 mg (16.25 mg) of Favipiravir was transferred to a 10 ml volumetric flask, sonicated with 5 ml of diluent for 25 minutes, made up to volume with diluents, and filtered (1000 µg/ml of Favipiravir).

Preparation of Sample Working Solutions:

Two hundred microliters of filtered sample stock solution was transferred to a 10 ml volumetric flask and made up with diluent (20 µg/ml of Favipiravir).

Method Development through QbD Approach:

Optimal chromatographic conditions were determined using a Quality by Design (QbD) approach. Various parameters affecting chromatographic separation, including mobile phase composition, were systematically studied to achieve a well-resolved chromatogram with good peak shape and resolution between the drugs.

Selection of Wavelength:

A standard solution of Favipiravir was scanned using a UV spectrophotometer over a wavelength range of 200 nm to 400 nm, with diluent used as the blank. The maximum absorption wavelength (λ max) for Favipiravir was determined to be 229 nm.

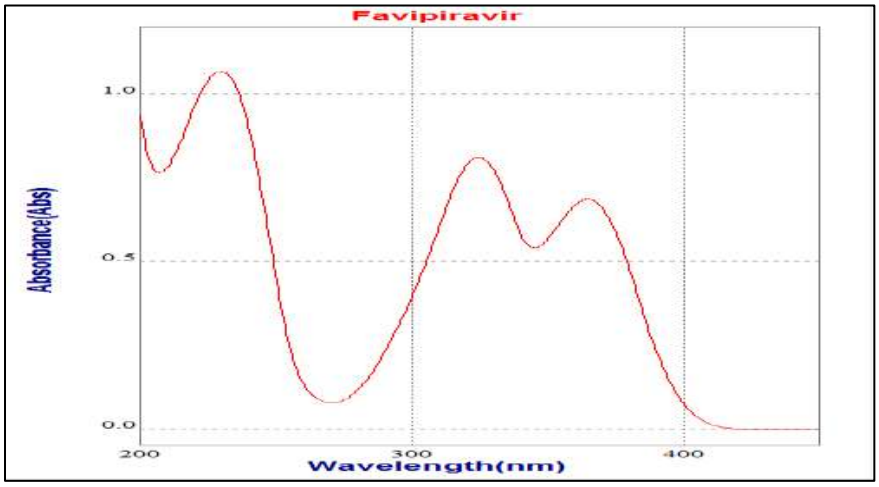

Figure 1 UV Spectrum of Favipiravir showing λmax at 227 nm

Development of HPLC Method:

The aim of this study was to optimize the assay method for the quantification of Favipiravir. Based on a comprehensive literature review, optimization was pursued through a series of experimental trials.

Validation of Developed Method:

Method validation is a crucial step ensuring the accuracy and reliability of an analytical method for its intended application. Through meticulously documented experimental investigations, validation confirms that the method meets necessary performance criteria. In the pharmaceutical industry, where precise and consistent results are essential, method validation holds significant importance.

The validation process encompasses the evaluation of various parameters to establish the method's robustness and accuracy. These parameters include specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Adherence to guidelines from the International Conference on Harmonization (ICH) ensures that the validated method meets regulatory standards.

RESULTS AND DISCUSSIONS:

Optimization of Chromatographic Conditions Using CCD

Central Composite Design (CCD) is employed to optimize High-Performance Liquid Chromatography (HPLC) separations by examining main effects and interactions of factors. In this study, a three-factor CCD with seventeen experimental runs and five center points was used. The independent variables were flow rate (A), mobile phase (B), and temperature (C), with responses measured for twenty experimental runs. Insignificant model terms were removed via backward elimination, simplifying the model. Statistical analysis, including ANOVA, indicated a P value <0.05, showing the model terms' significance. Polynomial terms had P values <0.5, indicating their influence on responses. The R^2 values exceeded 0.8, confirming the quadratic model's fit and reliability.

Adequate precision values, all above 4, assured reproducible optimization. Low percentage CV indicated high model reproducibility with minimal variation. ANOVA helped build a polynomial equation for response prediction at given factor levels. Perturbation graphs allowed simultaneous comparison of all factors against responses.

Table 1 Factor for QBD Design for method development of Favipiravir

Factor	Name	Units	Minimum	Maximum	Values	Mean	Std. Dev.
A	Composition	%	30	60	1.000=60.00	45	10.28992
B	Flow rate	ml/min	0.8	1	1.000=1.00	0.9	0.068599
C	Wavelength	nm	225	229	1.000=229.00	227	1.371989

Table 2 Effect of Factor on QBD Design

Name	Units	Model	Minimum	Maximum	Mean	Std. Dev.	Ratio
Retention Time	min	Quadratic	4.128	7.653	5.520	0.948	1.854
Area	Units	Quadratic	1358752	2242931	1607461	284204.5	1.651
Theoretical Plates	Units	Quadratic	6336	8134	7478.765	571.342	1.284

Table 3 Model of Central Composite Design (CCD)

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
Std	Run	A:Composition %	B:Flowrate ml/min	C:Wavelength nm	Retention Time min	Area Units	Theoretical Plates Units	Asymmetry Factor Units
10	1	45	1	225	4.809	1541769	7993	1.3
5	2	30	0.9	225	6.883	1467656	6764	1.34
16	3	45	0.9	227	5.334	1362561	7891	1.3
3	4	30	1	227	6.273	1358752	6828	1.29
15	5	45	0.9	227	5.334	1362561	7891	1.3
14	6	45	0.9	227	5.334	1362561	7891	1.3
17	7	45	0.9	227	5.334	1362561	7891	1.3
13	8	45	0.9	227	5.334	1362561	7891	1.3
6	9	60	0.9	225	4.527	2046084	8134	1.23
9	10	45	0.8	225	5.828	1460824	7480	1.28
7	11	30	0.9	229	6.878	1588787	6567	1.3
12	12	45	1	229	4.805	1590853	7113	1.28
8	13	60	0.9	229	4.533	2242931	7755	1.24
11	14	45	0.8	229	5.843	1535937	8026	1.3
4	15	60	1	227	4.128	1734750	7520	1.28
1	16	30	0.8	227	7.653	1966302	6336	1.33
2	17	60	0.8	227	5.006	1979394	7168	1.29

Software Aided Method Optimization

Design of Experiments (DoE) is a valuable tool for optimizing composition parameters, allowing for the evaluation of both principal effects and their interactions. Central Composite Design (CCD), a component of Response Surface Methodology (RSM), effectively displays quadratic response surfaces without necessitating a three-level factorial design. The critical factors and experimental levels investigated were based on univariate preliminary studies conducted for chromatographic method development. For the optimization of Favipiravir, twenty experiments were performed, including five center points, examining three factors.

Table 4 ANOVA table for Area using CCD

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	Squares	df	Square	Value	Prob > F	
Model	1.08E+12	9	1.2E+11	3.883141	0.0437	
A-Composition	3.29E+11	1	3.29E+11	10.66996	0.0137	
B-Flowrate	6.41E+10	1	6.41E+10	2.081958	0.1923	
C-Wavelength	2.44E+10	1	2.44E+10	0.793287	0.4027	
AB	3.29E+10	1	3.29E+10	1.068711	0.3356	
AC	1.43E+09	1	1.43E+09	0.046521	0.8354	
BC	1.69E+08	1	1.69E+08	0.005498	0.943	significant
A^2	5.18E+11	1	5.18E+11	16.80208	0.0046	
B^2	9.15E+09	1	9.15E+09	0.29691	0.6027	
C^2	6.39E+10	1	6.39E+10	2.073541	0.1931	
Residual	2.16E+11	7	3.08E+10			
Lack of Fit	2.16E+11	3	7.19E+10			
Pure Error	0	4	0			
Cor Total	1.29E+12	16				
Fit Statistics						
Std. Dev.	175523		R-Squared	0.833128		
Mean	1607461		Adj R-Squared	0.618578		
C.V. %	10.91927		Pred R-Squared	-1.66996		
PRESS	3.45E+12		Adeq Precision	5.576528		

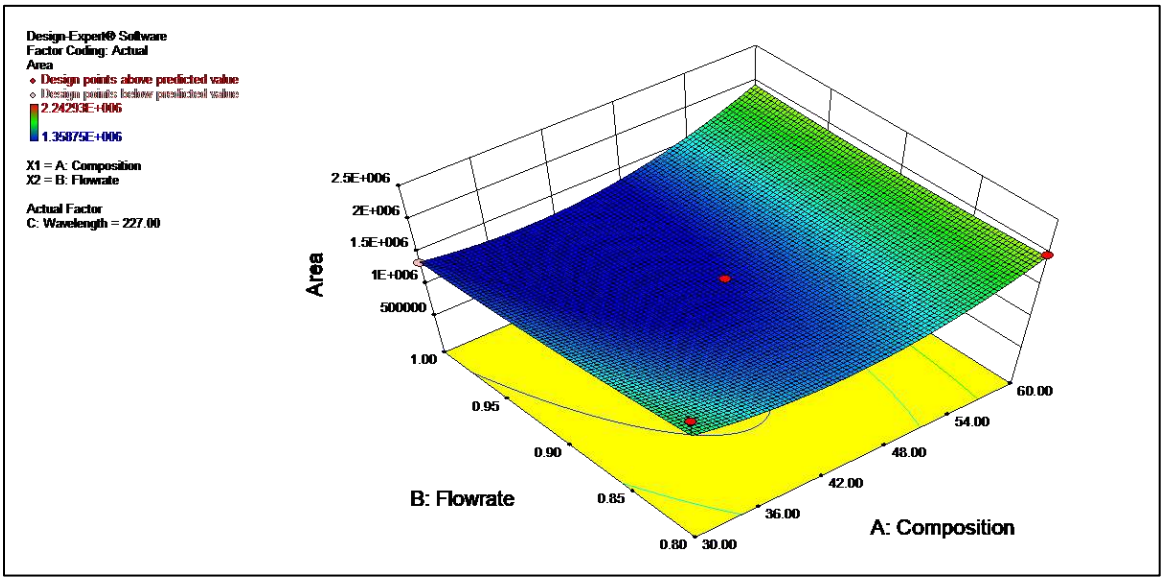

Figure 2 3D counter plot area as a function of organic ratio of Mobile Phase

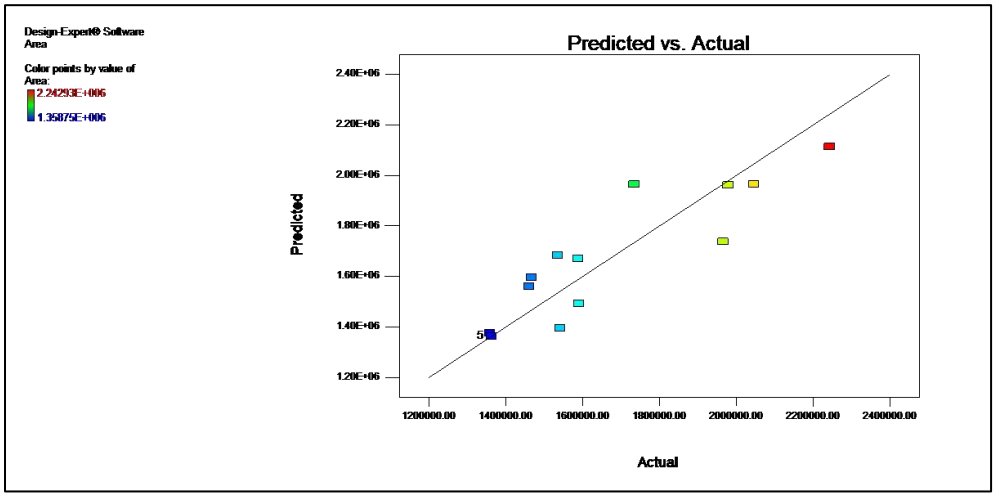

Figure 3 Graph for QBD Factor Area Predicted Vs Actual

Table 5 ANOVA table for retention time using CCD

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	14.36353	9	1.595948	1832.091	< 0.0001	
A-Composition	11.26463	1	11.26463	12931.4	< 0.0001	
B-Flowrate	2.327403	1	2.327403	2671.776	< 0.0001	
C-Wavelength	1.80E-05	1	1.80E-05	0.020663	0.8898	
AB	0.063001	1	0.063001	72.32291	< 0.0001	
AC	3.03E-05	1	3.03E-05	0.034726	0.8575	
BC	9.02E-05	1	9.02E-05	0.103604	0.7569	significant
A^2	0.699184	1	0.699184	802.6386	< 0.0001	
B^2	0.002325	1	0.002325	2.669319	0.1463	
C^2	0.005533	1	0.005533	6.351566	0.0398	
Residual	0.006098	7	0.000871			
Lack of Fit	0.006098	3	0.002033			
Pure Error	0	4	0			
Cor Total	14.36963	16				
Fit Statistics						
Std. Dev.	0.029515		R-Squared	0.999576		
Mean	5.519765		Adj R-Squared	0.99903		
C.V. %	0.534706		Pred R-Squared	0.99321		
PRESS	0.097564		Adeq Precision	152.4964		

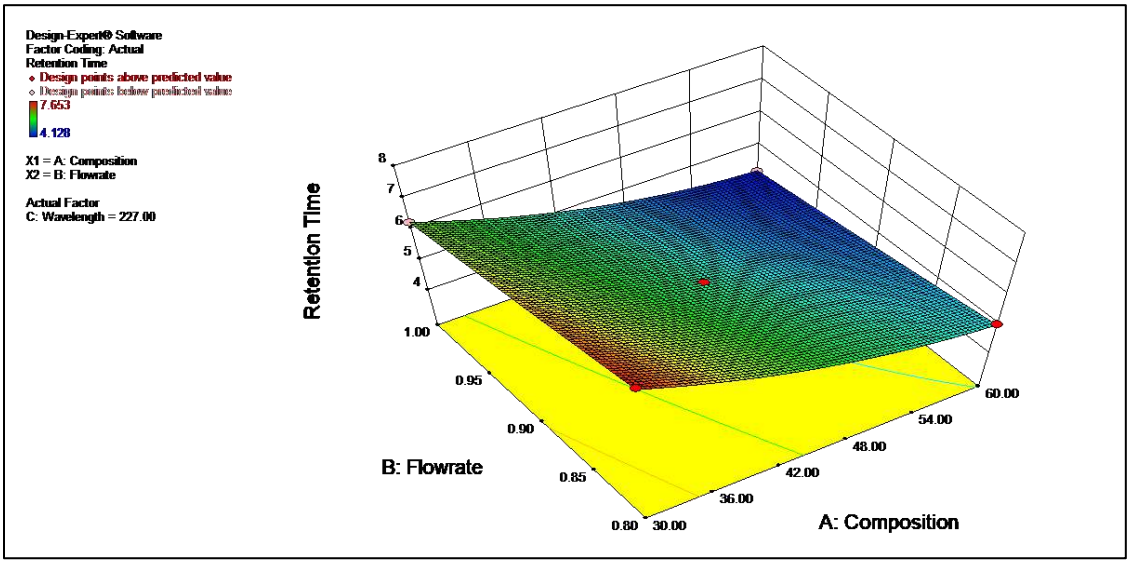

Figure 4 3D counter plot of Retention Time as a function of organic ratio.

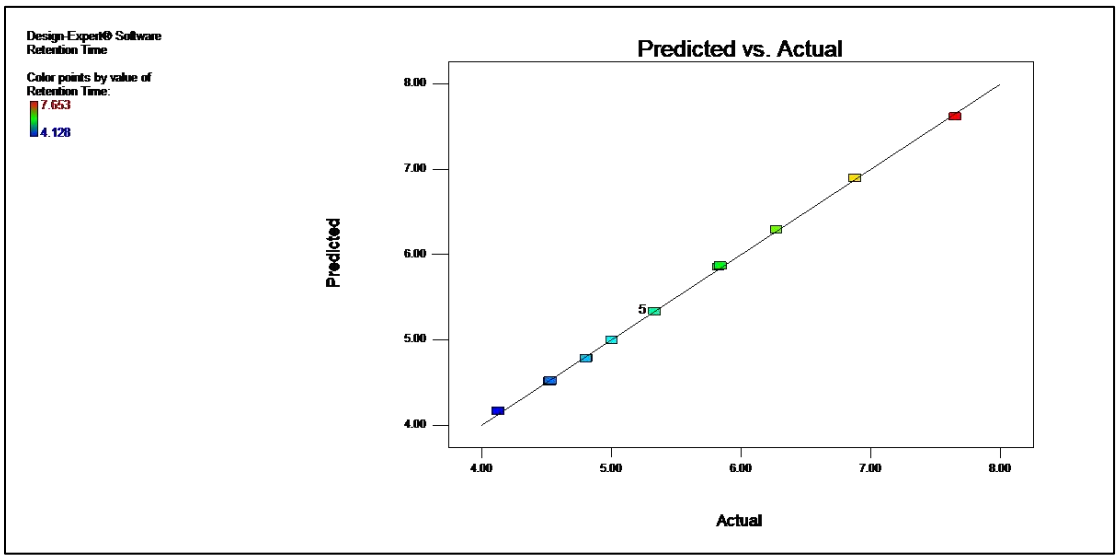

Figure 5 Graph for QBD Factor Retention Time Predicted Vs Actual

Table 6 ANOVA for theoretical plates using CCD

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	4888496	9	543166.2	11.36987	0.0021	significant
A-Composition	2082841	1	2082841	43.59922	0.0003	
B-Flowrate	24642	1	24642	0.515821	0.4959	
C-Wavelength	103512.5	1	103512.5	2.166783	0.1845	
AB	4900	1	4900	0.10257	0.7581	
AC	8281	1	8281	0.173343	0.6896	
BC	508369	1	508369	10.64147	0.0138	
A^2	1713869	1	1713869	35.8757	0.0005	
B^2	354105.3	1	354105.3	7.412335	0.0297	
C^2	11385.26	1	11385.26	0.238323	0.6403	

Residual	334407	7	47772.43			
Lack of Fit	334407	3	111469			
Pure Error	0	4	0			
Cor Total	5222903	16				
Fit Statistics						
Std. Dev.	218.569		R-Squared	0.935973		
Mean	7478.765		Adj R-Squared	0.853652		
C.V. %	2.922529		Pred R-Squared	-0.02443		
PRESS	5350512		Adeq Precision	10.83606		

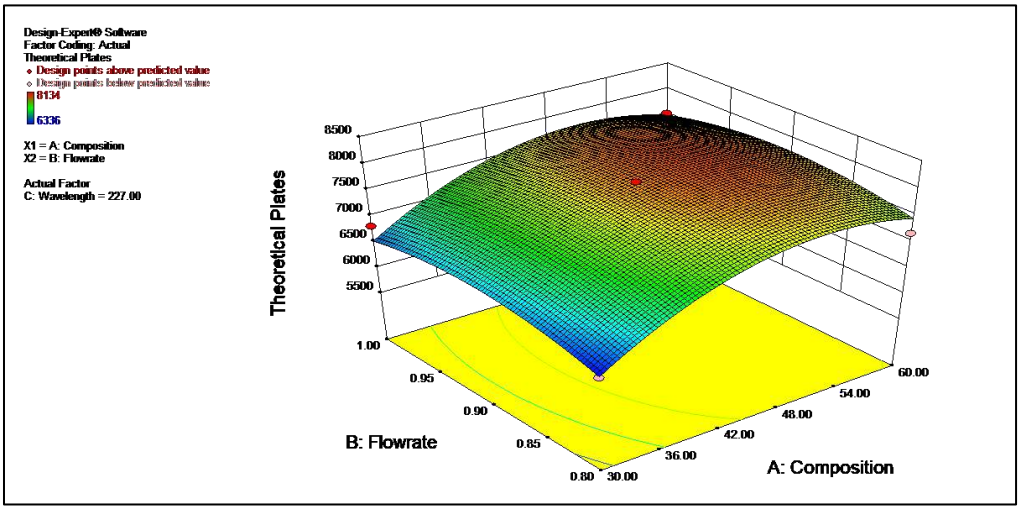

Figure 6 3D counter plot of Theoretical Plates as a function of organic ratio.

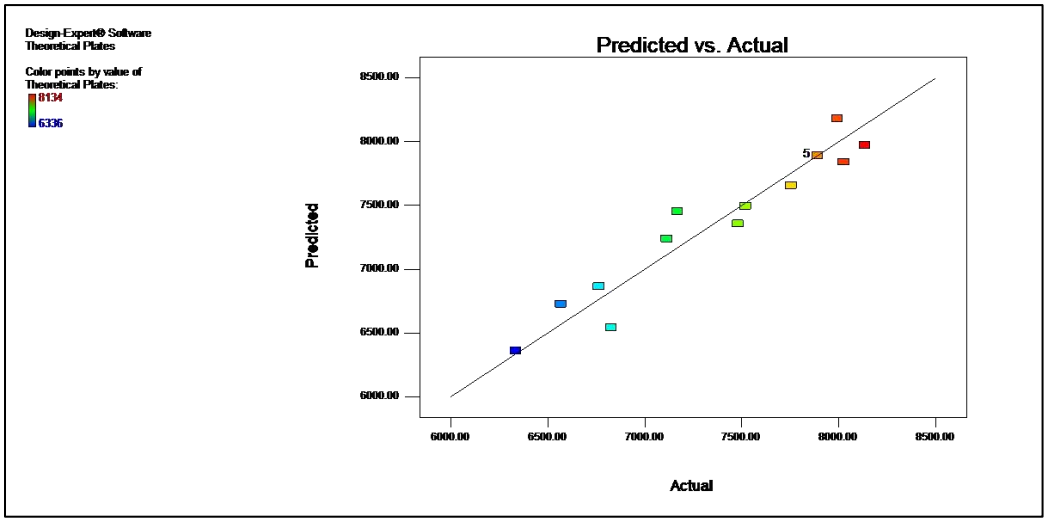

Figure 7 for QBD Factor Theoretical Plates Predicted Vs Actual

Table 7 ANOVA for asymmetrical factor using CCD

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	0.006475	3	0.002158	5.879712	0.0092	significant
A-Composition	0.00605	1	0.00605	16.48136	0.0014	
B-Flowrate	0.000312	1	0.000312	0.85131	0.373	

C-Wavelength	0.000112	1	0.000112	0.306471	0.5892	
Residual	0.004772	13	0.000367			
Lack of Fit	0.004772	9	0.00053			
Pure Error	0	4	0			
Cor Total	0.011247	16				
Fit Statistics						
Std. Dev.	0.019159		R-Squared	0.575706		
Mean	1.291765		Adj R-Squared	0.477792		
C.V. %	1.483193		Pred R-Squared	0.14092		
PRESS	0.009662		Adeq Precision	7.263016		

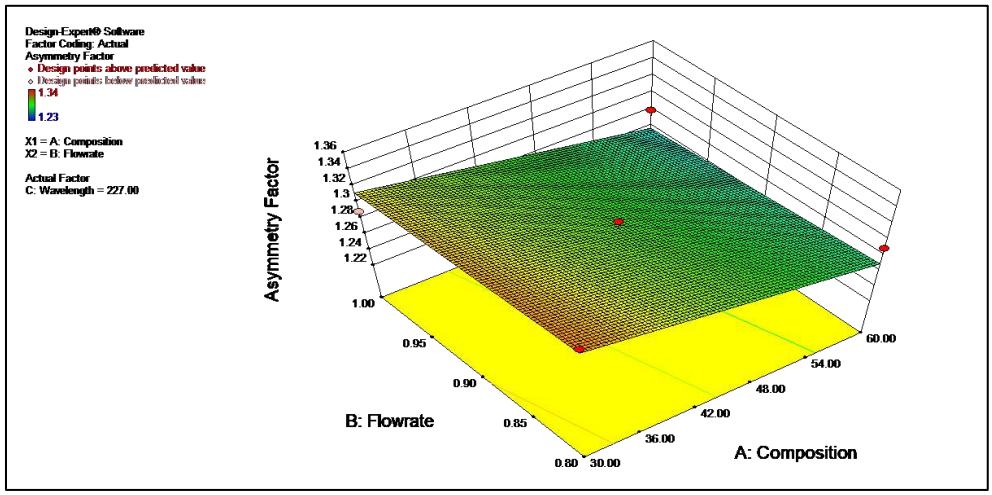

Figure 8 3D counter plot of Asymmetry Factor as a function of organic ratio.

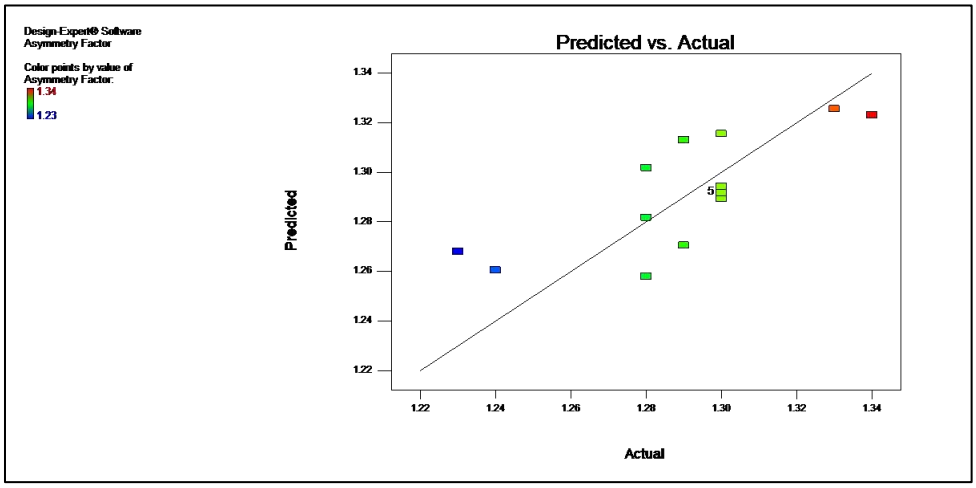

Figure 9 Graph for QBD Factor Asymmetry Factor Predicted Vs Actual

System Suitability:

All the system suitability parameters met the criteria specified by ICH guidelines. Specifically, the plate count exceeded 2000, the tailing factor was less than 2, and the resolution was greater than 2. Therefore, all system suitability parameters were within the acceptable limits and considered satisfactory according to ICH guidelines.

Mobile phase: Methanol: Water (60:40 v/v)

pH of Mobile Phase: 3 (pH is adjusted with o-phosphoric acid)
Flow rate: 0.9ml/min
Column: Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Detector wave length: 229nm
Column temperature: 30°C
Injection volume: 20 µL
Run time: 8.0 min
Result: Sharp Peak Observed

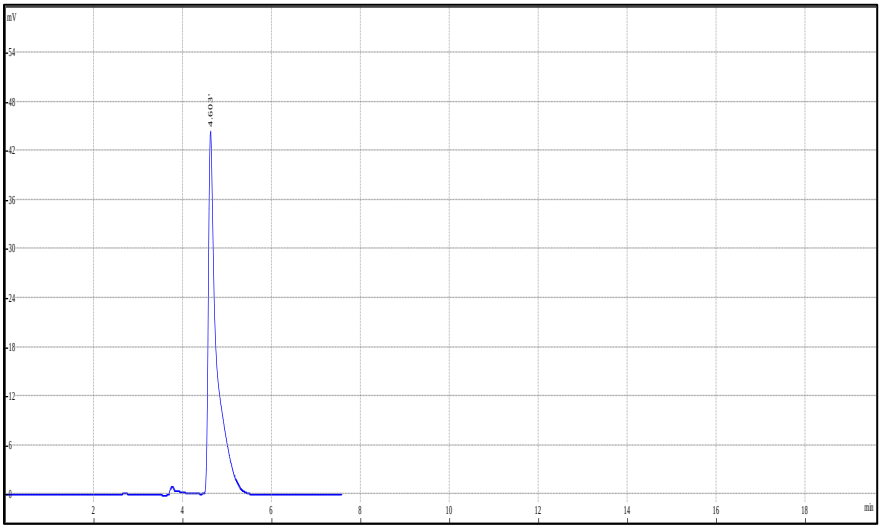

Figure 10 Chromatogram of Optimized Parameters

Method Validation:

Specificity: No interfering peaks were observed at the retention times of the drug, as demonstrated in Figure 10.

Precision:

Six injections were made from a single volumetric flask of the working standard solution. The areas obtained from these injections were used to calculate the average area, standard deviation, and %RSD for Favipiravir. The %RSD was found to be 0.34% for interday precision and 0.61% for intraday precision. Since the %RSD values were less than the precision limit of 2%, the system precision for this method was confirmed to be satisfactory.

Table 8 Interday Precision Table

Morning	Afternoon	Evening	Mean	% RSD
651006	64989	650079	646046	0.61%
644329	644342	643301		
646690	648894	640871		

Table 9 Intraday Precision Table

Day 1	Day 2	Mean	% RSD
651006	648218	647082	0.34%
644329	647012		
646690	647082		

Linearity:

Favipiravir was evaluated at concentrations ranging from 0 to 50 µg/ml. Six different concentrations within this range were analyzed, each in duplicate. The average peak areas were recorded. The linearity equation for Favipiravir was determined to be y = 20086x + 59238, with a correlation coefficient of 0.9996.

Table 10 Linearity Table for Favipiravir

Conc. In µg/ml	Area
10	260620
20	465563
30	651006
40	868313
50	1063524

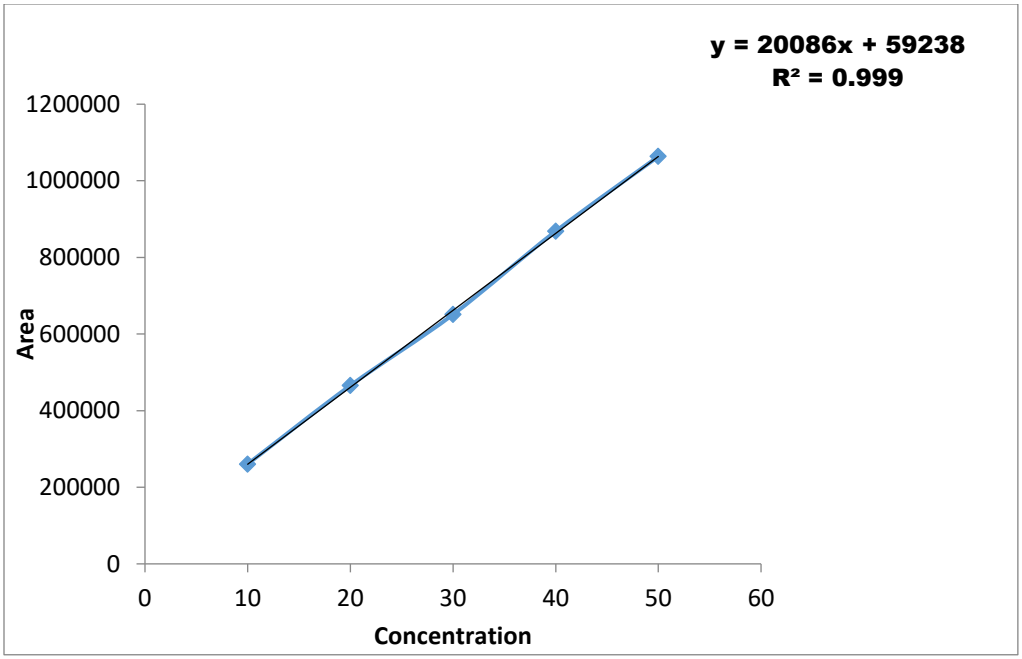

Figure 11 Standard Linearity Curve of Favipiravir

Accuracy:

The accuracy of the method was evaluated using spiked sample solutions at three levels (50%, 100%, and 150%). Three levels of accuracy samples were prepared using the standard addition method. Triplicate injections were performed for each level, and the mean %Recovery for Favipiravir was found to be 99.185%.

Table 11 Accuracy data table of Favipiravir

Sr. No.	% Composition Added	Average Area of Standard	Average Area of Sample	% Recovery
1	50%	651006	650424	99.911
2	100%	868313	862047	99.278
3	150%	1063524	1046142	98.366

Sensitivity:

Calculated from standard deviation and slope of calibration curve.

Table 12 Sensitivity table of Favipiravir

	LOD	LOQ
Favipiravir	0.1594 µg/ml	0.4830 µg/ml

Robustness:

The robustness of the method was tested by making small, deliberate changes in flow rate, mobile phase ratio, and temperature. Additionally, variations in wavelength and pH were evaluated with samples injected in duplicate. The system suitability parameters remained largely unaffected, and all criteria were met. The %RSD values were within acceptable limits, confirming the method's robustness.

Table 13 Robustness Data for Change in Wavelength

Sr. No.	Conc. In µg/ml	Area	Mean	Deviation	%RSD
1	20	465563	463020	3512.42	0.75859012
2	20	464484			
3	20	459012			

Table 14 Robustness Data for Change in pH

Sr. No.	Conc. In µg/ml	Area	Mean	Deviation	%RSD
1	20	465563	464447	2600.29	0.55986714
2	20	466303			
3	20	461475			

Assay:

The assay for Favipiravir 400 formulation was conducted as described. The average % assay for Favipiravir was found to be 99.740%.

Table 15 Assay Data for Favipiravir

Sr. No.	% Composition	Area of Standard	Area of Sample	% Assay
1	**% Assay**	651006	649315	99.740

CONCLUTION

A simple, accurate, and precise method was developed for estimating Favipiravir in bulk and pharmaceutical dosage forms using a Quality by Design (QbD) approach. The chromatographic analysis was carried out using a Waters HPLC Alliance 2695 model with a C18 column (50 x 2.1 mm, 1.7 µm). The mobile phase consisted of Methanol: Water (60:40 v/v) and was pumped through the column at a flow rate of 0.9 ml/min, with the temperature maintained at 30°C. The optimized wavelength was 229 nm, and the retention time for Favipiravir was 4.60 minutes. The %RSD for Favipiravir was 0.07, and the %Recovery was 99.18%. The LOD and LOQ values, obtained from regression equations, were 0.16 and 0.48, respectively. The regression equation for Favipiravir was y = 19106x + 96204 with an R^2 of 0.9999. The method showed reduced retention times and run times, making it simple and economical for routine quality control testing in industries. The QbD approach facilitated a comprehensive understanding of the method variables, reducing the likelihood of failure during validation. Optimization of chromatographic conditions, such as mobile phase composition, was achieved through multiple trials to ensure good resolution and symmetric peak shapes. All validated parameters met the acceptance criteria as per ICH guidelines.

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