Development And Validation Of RP-HPLC Method For Estimation Of Favipiravir API And It's Tablet Dosage Form Using Quality By Design Approach

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Abstract

Favipiravir is a pyrazine carboxamide derivative discovered by Toyama chemical of Japan to act against many RNA viruses (potent broad spectrum inhibitor of Influenza & other RNA viruses). The present study gives the development of the RP HPLC method by using Quality by Design followed by validation of developed method for estimation co Favipiravir in bulk and tablet dosage form. The central composite method was applied on organic Phase concentration & buffer PH for initial Screening studies. The selection of optimum chromatographic condition was carried out by design space numerical, graphical optimization on retention time, Peak asymmetry & Theoretical Plates. Optimized analytical method consisted Acetonitrile: water (80:20%v/v) as mobile phase, pH 5, flow rate 1ml/min, a wavelength 323 nm. Favipiravir was eluted with retention time 2.7 min & peak area 51248, Favipiravir showed good linear relationship in range of 10-90 µg/ml with a correlation coefficient of 0.9985. The % RSD for intraday, inter day precision & Repeatability was found to be 0.88, 0.65 & 0.97 respectively. Limit of detection & quantification was found to be 104.44 µg/ml &. 316.5 µg/ml. The method validation parameters were in the prescribed limit as per ICH guidelines.

Keywords: RP- HPLC, COVID 19 , favipiravir, Quality by Design approach

BACKGROUND

The corona virus (COVID19) expanded quickly from China to the rest of the world and has now become an epidemic that has spread to practically every Country. The COVID19 death rate ranges from 1% to 7%, according to a study from the World Health Organization (WHO). Several drugs which include chloroquine, arbidol, remdesivir, and favipiravir are currently undergoing clinical studies to test their efficacy and safety in the treatment of corona virus disease 2019 (COVID-19) in China.[1] Favipiravir is an antiviral drug of pyrazine carboxamide derivative and chemically 5-fluoro-2-oxo-1H-pyrazine-3-carboxamide (Fig 1), The mechanism of its actions is thought to be related to the selective inhibition of viral RNA-dependent RNA polymerase.[2] Favipiravir is a pro-drug that is metabolized to its active form, favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP), available in both oral and intravenous formulations.[3] In 2014, favipiravir was approved in Japan for stockpiling against influenza pandemics and recently in Covid-19 it's given as first line drug for reducing the infection.[4]

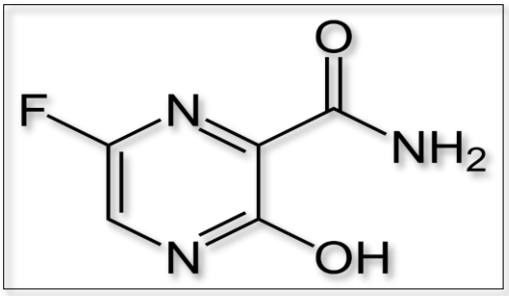


Figure 1: Structure of Favipiravir

In 2005, QbD was approved by the International Conference on Harmonization (ICH), whose ICH Q8(R2) guideline defines it as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management."[5] In order to identify and control the sources of variability in the analytical technique by designing the control strategy, it is essential to apply the AQbD approach to gather information.[6]

MATERIALS AND METHODS

Material

Favipiravir, pure drug substances was obtained from Yarrow chemical Products from Mumbai. Methanol, Acetonitrile, Triethylamine, water and o- Phosphoric acid used were of analytical grade and purchased from Oswal scientific, Pune (India). The FAPVIR™ tablet (Abbott Healthcare Pvt. Ltd) containing 200 mg of favipiravir was procured from local market.

Instruments

The HPLC Jasco with detector JASCO UV – 1575, Pump JASCO PU – 2080 PLUS and C18 column (150mm X 4.6 mm X 5µm particle size) was used for analysis at an ambient temperature.

Chromatographic Condition

The C18 column with mobile phase consisting Acetonitrile: water (80:20%v/v) was used at ambient temperature. The mobile phase pH 5 was adjusted with the help of o-Phosphoric acid. The flow rate kept 1 ml/min and wavelength was set 323 nm. The very sharp and the symmetric peak of FAP were obtained with the above chromatographic condition. The HPLC method was optimized for Mobile phase, pH of buffer as two variables at three different level using Central Composite Design with Design Expert Software.

Preparation of reference standard Solution

10 mg Favipiravir accurately weighed and was transferred to 10 ml volumetric flask containing little amount of methanol. The volume was made up to the mark using same methanol to make 1000ppm solution. From the stock solution, withdraw 1 ml and transfer it to 10 ml volumetric flask and dilute it with the methanol up to 10 ml (100 ppm). The resulting solution is sonicated for 25 min.

Selection of detection wavelength

100 µg/ml Solution of Favipiravir was scanned in the range 200-400nm with UV spectroscopy & λmax was found at 323 nm.

HPLC Method development by QbD

Steps of Analytical HPLC method development by using QbD approach was follows.

Selection of Quality Target method Parameter

Pharmaceutical Formulations are tested for assay and Drug release to determine of drug efficacy. In the same way, impurities are determined in pharmaceutical products for safety assessment. Hence the common targets are assay determination, impurities estimation, drug release in method development. The assay determination was selected as QTMP for analytical method development. [7,8,9]

Selection of Critical Quality Attributes

The CQA's plays a significant role for identification of variables, which affect on method performance and can influence the results. The retention time, Theoretical Plate and peak asymmetry were selected as CQAs for RP-HPLC method development.[10,11]

Factorial design

After finalized the QTMP & CQA, the risk assessment was carried out on the basis of prior Knowledge to shortlist CMP's for further evaluation. The central composite experimental design was applied for optimization of mobile phase and pH of buffer solution. The central composite method was used to study the interaction & quadratic effect of mobile phase & pH of buffer solution on retention time, peak area, Theoretical Plate and peak asymmetry.

The two factors, mobile phase & pH of buffer solution at three level was considered with Design Expert (Version 8.00 stat- ease Inc., MM), the suited response for second order polynomial exploring quadratic response surface.[12]

$Y = \beta_0+\beta_1A+\beta_2B +\beta_{12}AB + \beta_{11}A_2 +\beta_{22}B_2B+\beta_{22}B_2A+\beta_{11}A_2$

The mobile phase & pH of buffer solution were finalized as independent variables & coded as A & B respectively. It is shown in Table 1. Y is given result associated with each factor level combination. From the experimental runs of the observed experimental value of Y obtained β_1 to β_{22} as regression coefficients. β_0 is an intercept. The term AB, A_2 & B_2 represented the interaction between factors and quadratic terms.

Since there is a multivariable interaction of variables and process parameters have been studied, the factors have been selected on a preliminary analysis.[13] The variables were chosen based on preliminary study since multivariable interactions of variables and process parameters have been examined. The pH of buffer system and mobile phase composition were selected as independent variables and shown in Table 1. Retention time, Theoretical Plate and peak asymmetry served as the dependent variables for the independent factors that were suggested.[14]

Table1:- Coded values for independent variables

Factor	**Coded values given factor**	**Levels**		
		-1	**0**	**+1**
Acetonitrile: water	A	60	70	80
pH of buffer solution	B	5	6	7

Evaluation of experimental results and selection of final method condition

The CCD technique was used to evaluate these method conditions. The initial phase involved evaluating the peak asymmetries, theoretical plates, and retention time conditions. This led to distinctive chromatographic conditions for Favipiravir. The demonstrated acceptable ranges are from robust areas where purposeful changes to the procedure parameters have no impact on the quality. This assures that the method didn't fail later on while being validated. The variable must be tuned at various levels until the answers were within the acceptable ranges if the modeling studies do not provide the intended result.[15,16] Utilizing the Design Expert tools, the ideal chromatographic conditions must be optimized.

Risk Assessment

The developed method's attributes, such as its efficiency and ability to remain operational throughout the duration of the product's life, are taken into consideration while choosing the optimum final method.[17] The evaluation of the method to study the robustness and ruggedness was conducted using a risk-based approach based on the QbD principles outlined in the ICH Q8 and ICH Q9 standards.[18,19] For robustness evaluation, the parameters of the method or its performance under multiple conditions such as change of mobile phase, detection wavelength, flow rate, pH of buffer were assessed.[20]

Implement a Control strategy

The deployment of a control strategy must follow after the development of the method. For the development of the analytical control strategy, the analytical target profile was established. The proposed set of controls defined as the analytical control strategy was established from a study of the many parameters, including risk management, analytical method, and suitability for purpose.[21] All of these variables assure that the method's effectiveness and the outputs' quality fall within the specified analytical target profile. For sample preparation, measurement, and replicate standard operating procedures, an analytical control strategy was prepared.[22]

Continual improvement for managing analytical life cycle

The ideal method for managing the analytical lifecycle is to continuously improve, which can be done in the laboratory by monitoring the consistency of the quality and performing routine maintenance on HPLC equipment, computers, and software.[23]

Analytical Method Validation

Analytical method validation is a process of documenting/proving that an analytical method provides analytical data acceptable for the intended use. The parameters required for validation according to the ICH Q2(R1) are Specificity, Linearity, Accuracy, Precision, Limit of detection (LOD), Limit of quantification (LOQ), Range, and Robustness.[24]

Linearity

The linearity of Favipiravir was determined by taking 5 independent level of concentration range of 10-90 µg/ml. The calibration graph was plotted by taking concentration on x axis and mean peak area on y axis. The correlation coefficient and regression line equation were determined.

Precision

Precision was determined by analyzing six sample concentration of 50 µg/ml of Favipiravir. The intraday & inter day precision was determined by analyzing six sample of 50 µg/ml at three different time on same day and different day respectively. The % RSD was found in the acceptable limit.

Accuracy

In accuracy study, percentage recovery was calculated from marketed formulation by at three levels 80%, 100% & 120% of standard addition. The percentage recovery of Favipiravir was found in the acceptable limit.

LOD and LOQ

The LOD & LOQ were determined from the standard deviation of the responses & slope of calibration curve as per the formula enlisted in Equation 1.

LOD =3.3 X σ /SD

LOQ=10X σ/SD

Where σ is the standard deviation of the y-intercept of the regression line, and SD is the slope of the calibration curve.

Robustness

To evaluate the robustness of the RP-HPLC analytical method, the effect of mobile phase composition, detection wavelength, flow rate and pH of the buffer was employed to check the response of method whether remain unaffected by small changes in chromatographic conditions or not. Serial dilutions of 20µg/ml solution were injected and calculated the % relative standard deviation.

System Suitability studies

The system suitability study was carried out by injecting six replication of Favipiravir. The peak asymmetry, column efficiency theoretical plates were calculated for standard solution.

RESULT & DISCUSSION

Initially, mobile phase methanol: Buffer & Methanol: Water in was tried but peak was unable to fulfill acceptable criteria. In mobile phase, Acetonitrile: Water 50:50 v/v was tried. The improvement of peak shape, asymmetry & Theoretical plate was carried out by adjusting pH of Aqueous Phase. All system suitability parameters were fulfilled with optimized chromatographic condition.

So Acetonitrile: Water 80:20 v/v & pH of aqueous phase 5 were optimized as mobile phase by using Central composite design.

Development HPLC method by QbD Approach [25, 26]

Quality Target method Parameter

The assay determination was selected as QTMP for analytical method development. The retention time, peak area, Theoretical Plate and peak asymmetry were selected as CQAs for RP-HPLC method development.

Critical Method Parameters (CMP)

The mobile phase Acetonitrile: Water & pH of aqueous phase was adjusted by 0.01% triethylamine & O- phosphoric acid.

Factorial Design

The central composite experimental design was applied for optimization of mobile phase and pH of buffer solution. Design matrix as per Centric Composite Method for optimization of the HPLC method of Favipiravir showed in Table 2.

Table 2: Design Matrix as per Centric Composite Method of the HPLC for Favipiravir

Sr. No.	Coded Factor Levels	
	Factor 1	Factor 2
1.	-1	1
2.	1	-1
3.	0	0
4.	0	-1.41
5.	-1.41	0
6.	0	0
7.	-1	-1
8.	0	0
9.	0	1.41
10.	1.41	0
11.	0	0
12.	1	1
13.	0	0

Design Space

In response surface, Central Composite design with quadratic model was applied on Mobile phase composition & pH of Buffer system against retention time, theoretical plates and peak asymmetry. The proposed CCD was given 13 runs.

From Figure 2 and equation retention time (Actual Values) = 9.492 - 0.187 X A - 0.493 X B -1.013 X AB +0.00206 X A^2 +0.106 X B^2, it indicated that as β_1 negative coefficient (-0.187) suggests that as the amount of Acetonitrile in mobile phase (A) decrease and β_2 negative coefficient (-0.493) suggests that as pH of buffer (B) decrease, the value of retention time was increased.

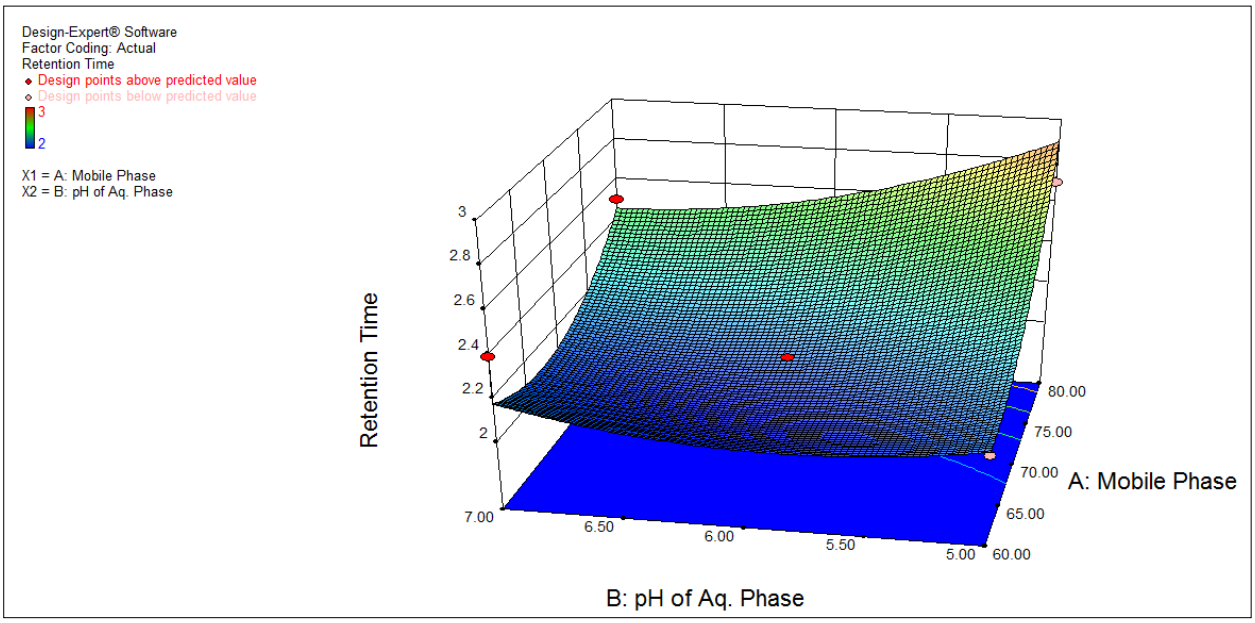


Figure 2: 3D surface plot for effect of combination of factors on retention time of Favipiravir by using Central Composite design

From Figure 3 and equation theoretic plates (Actual Values) = 14865.85 – 440.33 X A + 3.71.77 X B - 69.975 X AB + 6.507 X A^2 + 348.10 X B^2, it indicated that as β_1 negative coefficient (-440.33) suggests that as the amount of Acetonitrile in mobile phase (A) decrease and β_2 positive coefficient (371.77) suggests that as pH of buffer (B) increase, the value of theoretic plates was increased.

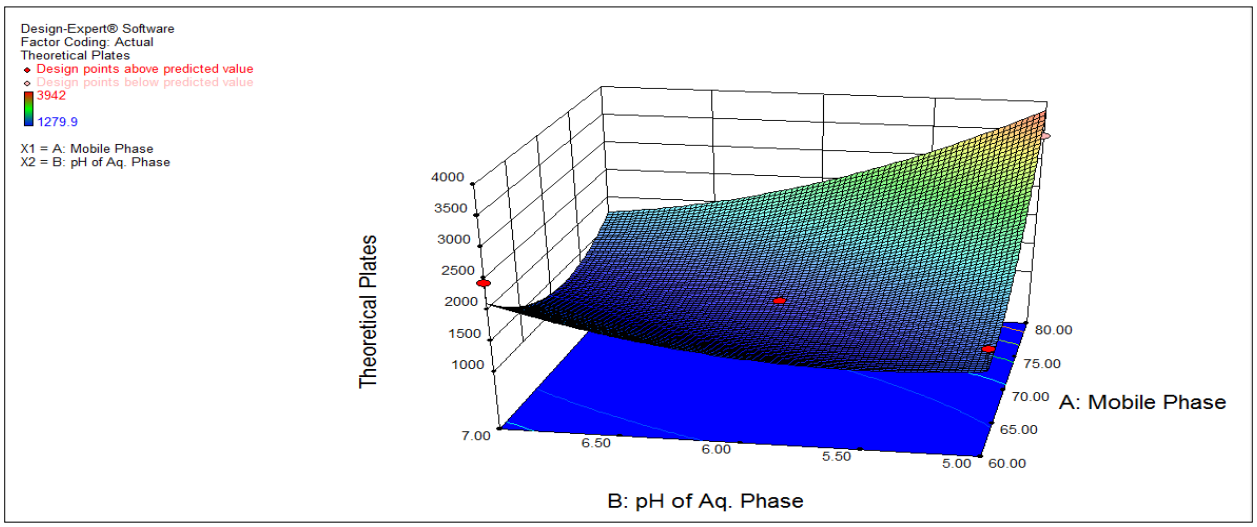


Figure 3: 3D surface plot for effect of combination of factors on Theoretical plates of Favipiravir by using Central Composite design

From Figure 4 and equation peak asymmetry (Actual Values) = 9.050 + 0.000821 X A – 2.974 X B + 0.0175 X AB – 0.000625 X A^2 + 0.162 X B^2, it indicated that as β_1 positive coefficient (0.000821) suggests that as the amount of Acetonitrile in mobile phase (A) increase and β_2 negative coefficient (-2.974) suggests that as pH of buffer (B) decrease, the value of Peak asymmetry was increased.

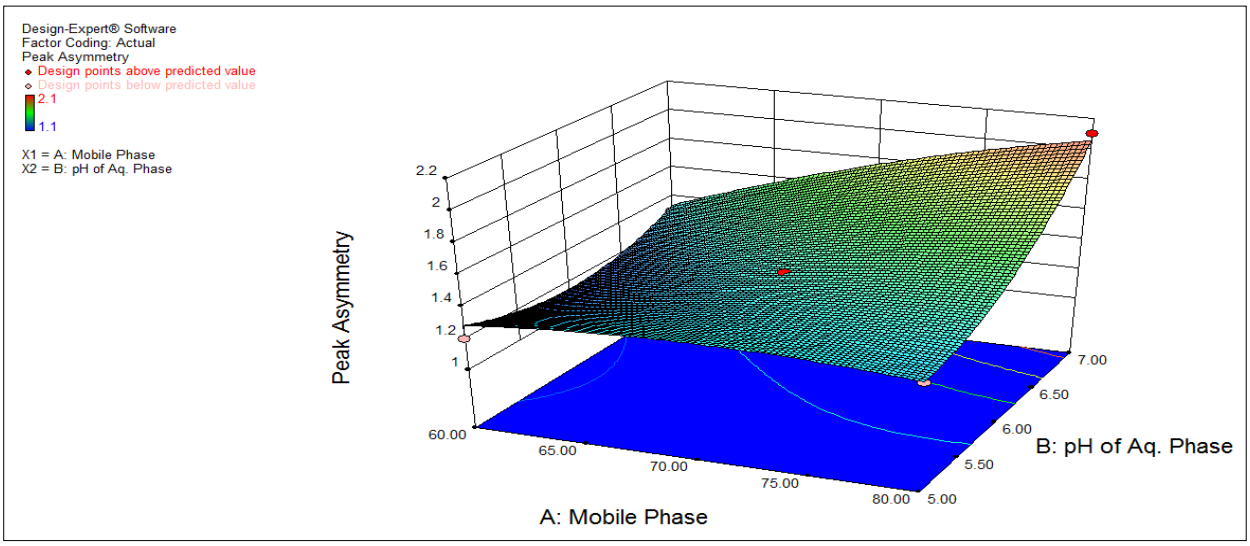


Figure 4: 3D surface plot for effect of combination of factors on Peak Asymmetry of Favipiravir by using Central Composite design

The R^2 value of retention time, theoretical plate, peak asymmetry was found to be 0.8532, 0.8862 and 0.9757 respectively. High value of R^2 values were given guarantee that the chosen quadratic model is fitting the data and may be used to interpolate. Adequate precision is a signal-to-noise ratio. The average prediction error is compared to the range of the predicted values at the design points. A model's discrimination is sufficient when the ratio is larger than 4. The Adequate precision value of retention time, theoretical plate, peak asymmetry was found to be 7.352, 8.720 and 26.434 respectively. After optimization, a model's repeatability is determined by its percentage CV. Low percentage CV is usually a bonus for generating consistent outcomes with less variance. All responses were shown low %CV. For the optimization, Derringer's desirability function was used. The solution with the highest level of desire among those offered by the software was chosen, and it was tested.

Optimized condition obtained

The optimized condition was obtained after doing complete study of all factors in varies experimental condition by using design expert software version 8 and predicted results shown in Table 3.

Table 3: Obtained solution for optimized solution

Acetonitrile: Water	pH of buffer Solution	Retention Time	Theoretical Plates	Peak Asymmetry	Desirability
80:20	5	2.895	3855.86	1.30	0.965

Analytical Method Validation

System Suitability

According to USP, system suitability studies are important part of liquid chromatographic method. The retention time was found to be 2.7, Peak area was 51576, Peak asymmetry was 1.35 and % RSD of six replicate injection was 0.87. For optimized formulation obtaining 3D surface plot of desirability is shown in figure 5.

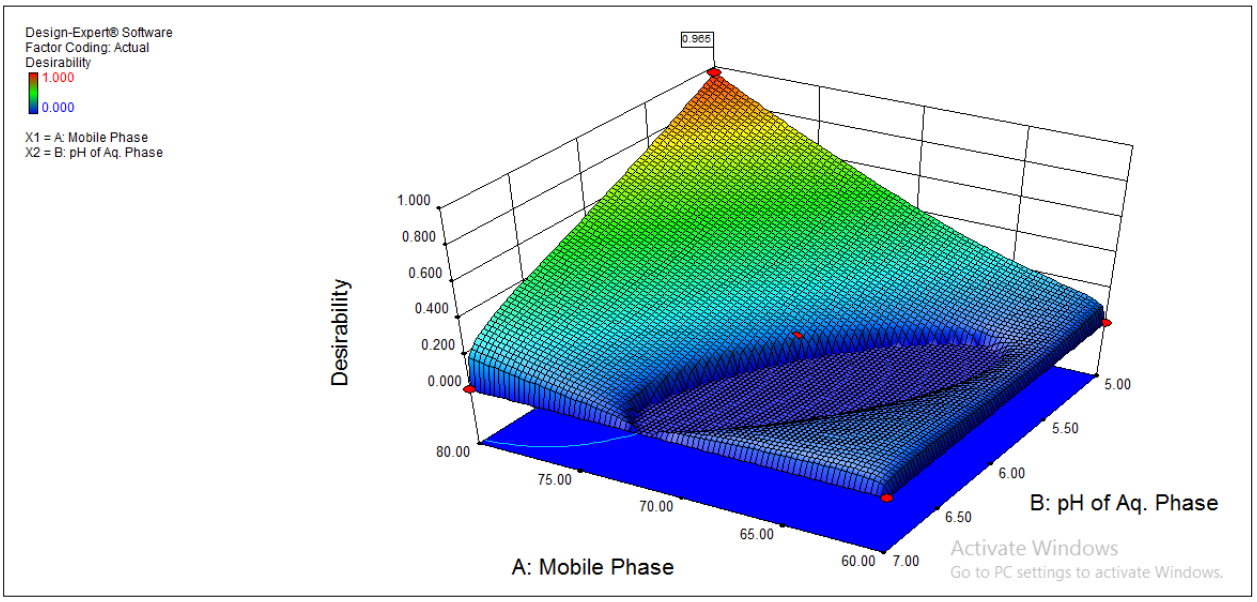


Figure 5: 3D surface plot desirability for obtaining optimized formulation

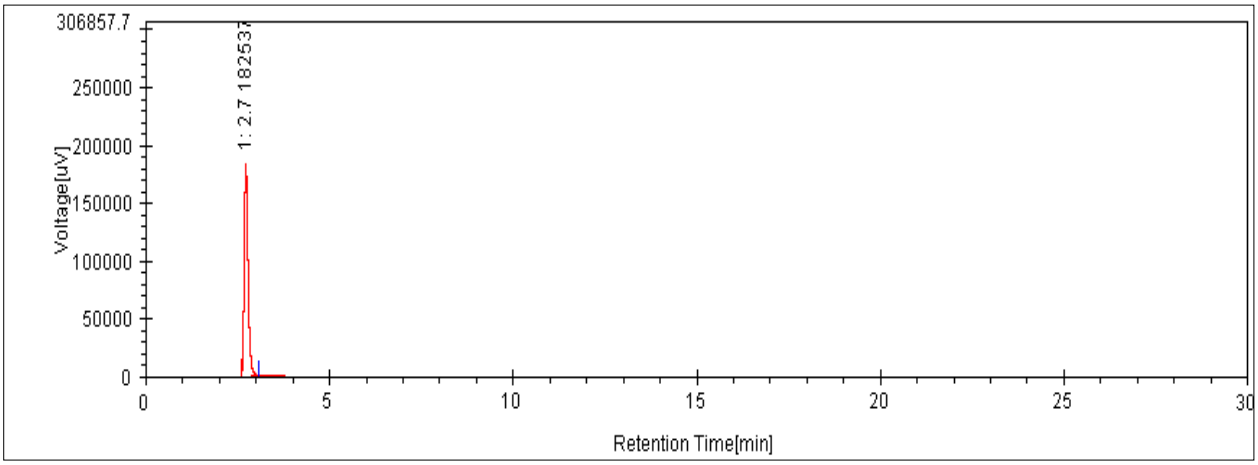
Linearity

The mobile phase containing combination of Acetonitrile: water (80:20%v/v), pH 5 given sharp peaks having Rt 2.7 min of FAP. The calibration curve for FAP was found linear over the range of 10-90 µg/ml. The regression line equation was found to be y = 978.15x + 769.48 with correlation coefficient 0.9986. The data of calibration curves is mentioned in Table 4.

TABLE 4: CALIBRATION CURVE DATA FOR FAP

Sr. No	**Concentration (µg/ml)**	**Peak Area (AU) (Mean ± SD : n=5)**
1	10	9912.10
2	30	30086.27
3	50	50171.25
4	70	70888.93
5	90	87325.37

Figure4: Chromatograph Graph for standard FAP



Precision

% RSD for intra-day, inter-day and repeatability precision for FAP were found 0.88, 0.65 & 0.97 respectively (Table 6)

TABLE 5: INTERMEDIATE PRECISION DATA FOR FAP

Sr. NO	Concentration (µg/ml)	Intraday Precision	Interday Precision	Repeatability
		Peak area	Peak area	Peak area
1	50	51565.9	51259.4	50715.3
2	50	51234.1	50841.3	50685.1
3	50	51311.2	50953.5	49989.8
4	50	50597.4	51454.2	50536.7
5	50	50427.3	50576.1	51120.1
6	50	51265.9	51311.4	51425.2
Average		51066.97	51065.98	50745.37
Standard Deviation		448.50	332.11	494.28
RSD%		0.88	0.65	0.97

LOD & LOQ

The LOD and LOQ for FAP were found to be 104.44 µg/ml and 316.45 µg/ml respectively.

Recovery

The Recovery for FAP were found to be 99.95-100.02%. (Table 7).

Table 7: Recovery Data for FAP

Level	Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount of drug Recovered (µg/ml)	% Recovery
50%	40	20	19.989	99.95

100%	40	40	39.99	99.98
150%	40	60	60.01	100.02

Robustness

The Robustness of Favipiravir was evaluated by slightly change in mobile phase composition, detection wavelength, flow rate and pH of the buffer. The % RSD of peak area was found to be less than 2. The results were shown in Table 8.

TABLE 8: Robustness data of FAP

Acetonitrile : Water			**Retention Time (min)**	**Detection Wavelength**	**Peak Area**
(%V/V)				**(nm)**	
1	79	21	2.73	322	50659
2	80	20	2.7	323	51248
3	81	19	2.67	324	49752
Average			2.700	Average	50553
Standard Deviation			0.030	Standard Deviation	753.61
RSD%			1.11	RSD%	1.49
Flow Rate			**Retention Time (min)**	**pH of Buffer**	**Peak Area**
(ml/min)				**(mmol/L)**	
1	0.9		2.76	4.7	51241
2	1		2.7	5	51877
3	1.1		2.686	5.3	50789
Average			2.715	Average	51302
Standard Deviation			0.0393	Standard Deviation	546.59
RSD%			1.45	RSD%	1.06

The results for validation are summarized in Table 9.

TABLE 9: SUMMARY OF VALIDATION PARAMETERS

Sr. NO	**Parameters**	**Result**
1	Linearity Range (µg/ml)	10-90 µg/ml
2	Regression Equation	y = 978.15x + 769.48
3	Correlation Coefficient	0.9986
4	LOD	104.4 µg/ml
5	LOQ	316.5 µg/ml
6	% Recovery	99.95-100.02%
7	Precision (%RSD)	
	Intraday Precision	0.88

	Interday Precision	0.65
	Intermediate Precision	0.97
8	Robustness (%RSD)	
	Acetonitrile : Water (Organic Phase ±1ml)	1.11
	Detection Wavelength (± 1 nm)	1.49
	Flow Rate (±0.1ml)	1.45
	pH of Buffer (± 0.3 mmol/L)	1.06
9	System Suitability (% RSD)	0.87

Assay of Marketed Formulation

The content of Favipiravir in marketed formulation (FAPVIR™ Tablet) was found to be 104.6 % effective (Table 12). The chromatogram showed peak area of FAP was shown 2331, with no additional excipients showing interference in FAP estimation.

TABLE 10: ANALYSIS OF MARKETED FORMULATION

Drugs	**Label Claim (mg)**	**Average Amount found(mg)**	**Assay (%)** **(n=3)**
FAP	200	199.03	99.52%

CONCLUSION:

The HPLC analytical QbD concept were extended for determination of best performing method & the final design space, a multivariate study of several important analytical method parameter namely mobile phase & pH of buffer at three different level was performed. The interrelation of these two factors with CQAs were studied & optimized at different level by using central composite method. The optimal selection of chromatographic condition was in the analytical design space using desirability function. The very sharp and the symmetric peak of FAP were found in mobile phase Acetonitrile: Water (80:20%v/v) & pH 5. The retention time of peak was found at 2.7 min. Validation of AQbD Method showed all parameters within acceptable criteria. This design space offers good practical knowledge that help in future. This method was given various advantages like high speed of analysis, more convenience & ecofriendly procedure of quantification of FAP in bulk drug as well as in pharmaceutical formulation. The developed method by using QbD approach has helped to understand method variables which affected on response hence leading to less chances of failure during method variable. As compare to conventional method, QbD approach analytical HPLC method development by using automated QbD design Expert Software has given more robust method in less time. The statistical analysis of data indicates that developed method is more accurate, precise, robust & reproducible.

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