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Analytical Quality-by-Design Approach to Stability Indicating RP-HPLC Method Development and Validation for Estimation of Favipiravir in Bulk and Formulation

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ABSTRACT

Method: A simple, specific, accurate, precise and selective stability-indicating reverse phase high performance liquid chromatography method was developed for estimation of favipiravir in bulk and formulation by analytical quality-by-design paradigm. Cosmosil C-18 column (250 x 4.6 mm x 5µ) at ambient temperature and UV detector at 225 nm wavelength was employed. The mobile phase was methanol:water (60:40 % v/v) adjusted to pH 3.0 and flowrate 0.8 ml/min. The method involved varying three key parameters (composition of mobile phase, flow rate and wavelength) and evaluating their effects on the responses. Box-Behnken design was employed for method development and optimized using statistical software. ICH guidelines were followed for method validation as well as forced degradation study. The stability of drug in stress conditions of acid/base hydrolysis, oxidation, thermal and photolytic degradation was evaluated.

Results: A linear response was observed over the concentration range of 10 – 50 µg/mL with r^2=0.9993. Limit of detection (LOD) and limit of quantitation (LOQ) for favipiravir were 0.29 µg/mL and 0.88 µg/mL respectively. The developed method was found to be highly robust and efficient. Stability studies showed some degradation in alkali, acid and peroxide, with only minor degradation in heat and photolytic conditions.

Conclusion: The proposed stability indicating analytical QbD method can be used for routine analysis of favipiravir active pharmaceutical ingredient (API) and formulation in quality control laboratories. The method developed by analytical quality-by-design approach is a highly robust, efficient and offers the added advantages of QbD with enhanced quality.

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Keywords: Quality-by-Design (QbD), Reverse Phase High Performance Liquid Chromatography (RP-HPLC), Favipiravir, Box-Behnken Design (BBD), Forced degradation.

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INTRODUCTION

Quality-by-design is a holistic approach where product specifications, critical process parameters (CPP) and critical quality attributes (CQA) are included along with risk assesment and creation of a robust design space to build quality into the analytical process and drug product. This serves to ease the final approval and ongoing quality control of the drug [1]. In relation to analytical systems, it is termed Analytical Quality-by-Design or AQbD [2-5].

Since QbD technique implements the lifecycle concept for analytical methods and offers the advantage of regulatory flexibility for operation within the design space, it has become an area of interest for many pharmaceutical companies and research institutes [6-9].

The pandemic of Corona was witnessed globally in the year 2020 and 2021. Lakhs of people lost their lives due to the devastating effects of covid-19 virus on human health. Doctors were in dire need of antiviral drugs for treatment of the deadly virus [10]. Favipiravir (FAV), a newly discovered antiviral drug, was used extensively for the treatment of Covid-19 virus infection. It is 6-fluoro-3-hydroxy pyrazine-2-carboxamide with molecular formula $C_5H_4FN_3O_2$ and molecular weight 157.104. Favipiravir functions as a prodrug. The active drug binds to and inhibits RNA dependent RNA polymerase, which ultimately prevents viral transcription and replication [11]. Its chemical structure is given in figure-1.

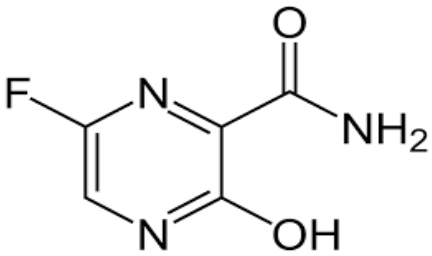


Fig. 1: Structure of Favipiravir

High Performance Liquid Chromatography (HPLC), especially, reverse phase HPLC (RP-HPLC) is the most popular and widely used analytical technique in the pharmaceutical industry. Its quality has gained huge importance with a QbD approach.

Literature reports few bioanalytical LC-MS/MS methods for estimation of Favipiravir alone or in combination with other drugs, analytical UPLC-MS/MS method, UV spectroscopic method and spectrofluorimetry design of experiment (DoE) method. There is also a bioanalytical HPLC method utilizing DoE with fluorescence detector [12-17].

It is evident from literature that no analytical HPLC methods are available for routine analysis of Favipiravir in pharmaceutical formulations developed through the AQbD approach. The mobile phase systems used in reported methods are also quite complex, expensive and not eco-friendly.

Over the past few years, literature reports have successfully demonstrated the immense applicability of DoE approach for developing effective and cost-efficient LC methods for estimation of drugs. [18].

Forced degradation study (FDS) is a necessary and integral part of drug development. It typically indicates stability of the drug/drug product by subjecting it to various stress conditions as outlined in the ICH Q1A (R2) guidelines. A stability-indicating analytical method should be capable of detecting degradation products and the assay method should be capable of detecting any decrease in drug content, during the product's shelf life [19].

Although, there are a few stability indicating analytical HPLC methods for estimation of Favipiravir in bulk and dosage form, not all parameters of stability studies are evaluated [20-23]. The reported stability-indicating methods are also not focused on any risk assessment, DoE and robustness of the method. Hence, there was a need to develop a simple, rapid, robust, precise, selective and cost-effective stability-indicating RP-HPLC method for estimation of Favipiravir in bulk and formulation through the AQbD approach.

MATERIALS AND METHODS

Chemicals and Reagents:

Favipiravir was procured as a gift sample from Glenmark Pharmaceuticals Pvt. Ltd. Chemicals and reagents used were of analytical grade and solvents used were of HPLC grade.

Instruments:

HPLC system of Analytical Technologies Ltd. with UV-3000-M detector and HPLC Workstation software was used.

Chromatographic conditions:

The Column employed was Cosmosil C18 (250 x 4.6 mm id., particle size: 5µ) at ambient temperature and 225 nm wavelength. The mobile phase was methanol:water (60:40) adjusted to pH 3.0 with orthophosphoric acid (OPA), flow rate was 0.8 ml/min. Injection volume was 20 µl.

Preparation of standard solutions:

Buffer (0.1% OPA)

About 1 ml of orthophosphoric acid solution was added in a 1000 ml of volumetric flask, about 100 ml of milli-Q water was added to it and final volume was made up to 1000 ml with milli-Q water. This was used to adjust the pH of the mobile phase to 3.0.

Standard preparation

Accurately weighed and transferred 25 mg of favipiravir standard into a 25 ml clean dry volumetric flask, three-fourth volume of diluent was added to it. This was then sonicated for 5 min, and made up to the final volume with diluent. The concentration of the solution was 1000 µg/ml. From this stock solution, working standard of 100 µg/ml was prepared.

HPLC method development by analytical QbD approach

The method employed is as follows.

Defining the analytical target profile (ATP)

This includes the goals or targets set according to the intended use of the analytical method. For example, quantitative analysis of API, degradation products, etc. [24]. Thus, ATP of present research was estimation of Favipiravir drug, formulation and forced degradation/stress study for assessing its stability.

Risk assessment

Risk assessment was performed for identification of critical quality attributes (CQAs, dependent variables) and critical process parameters (CPPs, independent variables). The CQAs like resolution, peak asymmetry, theoretical plates, area, retention time etc. and CPPs like flow rate, wavelength, column, ratio of mobile phase, pH etc. are crucial method parameters for quality on which the method performance rests [25].

The CPPs for the present method were identified to be ratio of mobile phase, flow rate and wavelength for a 3-variable Box-Behnken design (BBD), whereas the CQAs were identified to be peak asymmetry, theoretical plates, area, retention time and noted as quadratic responses of BBD.

Optimization of chromatographic conditions and design of experiment

Favipiravir maximum absorbance wavelength was selected at 225 nm by scanning the UV range of 200–400 nm using 30 µg/ml standard solution of the drug. The elution was carried on Cosmosil C18 column at ambient temperature. This column gave a peak shape with good system suitability parameters. The UV detector was used to detect favipiravir. The QbD method was optimized for three different parameters i.e. composition of mobile phase, wavelength and flow rate, using the design provided by the software. The Box-Behnken design was selected and 17 chromatographic runs were conducted as per DoE design. The responses were recorded under 4 headings of area, retention time, peak asymmetry and theoretical plates. The optimized chromatographic conditions were selected from the desirability indicated by the software.

Design software

Design Expert 10 software (free trial version) was used to plan the experiment's design.

Method validation

The method was ensured to be fit for its intended purpose by validating it as per ICH Q2 (R1) guideline. The parameters tested were linearity, accuracy, percentage recovery, precision, limit of detection (LOD), limit of quantitation (LOQ), assay and robustness [26].

Linearity

Pipette 1, 2, 3, 4, 5 ml of working standard solution into 10 ml volumetric flask and make volume upto the mark with diluent. The concentration of favipiravir in the prepared solution

was 10, 20, 30, 40 and 50 µg/ml. A calibration curve was plotted between concentrations versus peak area.

Precision

A solution of concentration 30 µg/ml was tested for intraday and interday precision. For intraday precision, the solutions were injected in the morning and evening in triplicate. Interday precision was calculated by analysing the solution on two different days in triplicate. The corresponding areas and the % relative standard deviation (RSD) was found.

Accuracy

As per ICH guidelines, accuracy is determined using a minimum of nine determinations over a minimum of three concentration levels covering the specified range of concentration (e.g., three concentrations/three replicates each). Three standards were defined as 10, 30 and 50 µg/ml, from the calibration range. Accuracy should be reported in terms of percent recovery by assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value.

Percentage Recovery

The percentage recovery was determined by calculating recovery of the drug by standard addition method. Known amount of sample (20 µg/ml) was added to standard solutions at 50, 100, 150 % recovery levels (10, 20, 30 µg/ml respectively) in triplicate and the mean area for each reading was calculated. The percentage recovery at every level was found.

Robustness

The robustness of the method was assessed by introducing small intentional variations in the method parameters of wavelength and pH of the mobile phase. The reliability of the method was ensured by evaluating its robustness.

LOD and LOQ

The limit of detection of an analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified and when quantitated, it is termed as limit of quantitation.

Assay

A total of 20 tablets of Favipiravir were taken and ground finely. Assay is reported as percent purity. Solutions of concentration 30 ppm were prepared from formulation as well as standard and injected to record the corresponding areas.

Standard preparation for degradation studies

A standard solution of 50 µg/ml concentration was treated with acid, base, hydrogen peroxide, heat and UV light individually. After degradation, the solutions were injected into LC system.

Forced degradation

Stress degradation studies of favipiravir were carried out under hydrolysis (acid and base), oxidation, thermal and photolytic conditions. The drug was treated with 0.1N HCl for 30 min at 60°C to perform acid degradation, 0.1N NaOH for 30 min at 60°C for base degradation and 3 % H_2O_2 for 12 hours at room temperature (R.T.) for peroxide degradation. The standard drug solution was treated thermally at a temperature of 60°C for 24 hours to study degradation by heat and also treated photolytically at R.T. for 24 hours.

RESULTS AND DISCUSSION

Optimization of HPLC method by analytical QbD approach

Trials were performed for optimizing the mobile phase composition by varying the mobile phase ratio of methanol:water at 40:60, 60:40 and 70:30. The initial chromatographic conditions employed are listed in table 1. The levels used for Box Behnken experimental design and its layout for 17 QbD trials are given in table 2 and 3 respectively.

Table 1. Initial chromatographic method development conditions

Trial No.	Wavelength (nm)	Mobile phase composition (% Methanol: Water)	pH of mobile phase	Sample volume (µl)	Flow rate (ml/min)	Pressure (MPa)	Run time (min)
1	225	70:30	3.0	20	0.8	9-10	8.21
2	225	40:60	3.0	20	0.8	9-10	9.17
3	225	60:40	3.0	20	0.8	9-10	8.25

Table 2. Box Behnken experimental design

Chromatographic Condition	Levels Used		
	Low (-)	Center (0)	High (+)
% Composition	40	50	60
Flow rate (ml/min)	0.8	0.9	1
Wave length (nm)	223	225	227

Table 3. Design of experiment showing factors and responses for 17 QbD trials using BBD

Run No.	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
	A:Composition of mobile phase	B:Flow rate	C:Wavelength	Retention Time (Rt)	Area	Theoretical Plates (N)	Asymmetry Factor
	(%)	(ml/min)	(nm)	(min)	(Area Unit)	(Units)	(Units)
1	50	1	227	3.974	1351450	7821	1.35
2	50	0.9	225	4.413	152656	7944	1.39

					4		
3	60	0.9	223	4.066	1522700	8351	1.24
4	50	0.9	225	4.413	1526564	7944	1.39
5	50	0.8	227	4.945	1732577	8087	1.26
6	40	0.9	227	5.083	1563301	5103	1.23
7	60	1	225	3.662	1290661	7594	1.12
8	50	0.8	223	4.945	1720900	6770	1.43
9	60	0.9	227	4.077	1528354	7625	1.33
10	50	0.9	225	4.413	1526564	7944	1.39
11	40	1	225	4.554	1585610	8267	1.18
12	50	0.9	225	4.413	1526564	7944	1.39
13	50	1	223	3.981	1343610	8016	1.26
14	40	0.9	223	5.106	1731998	8161	1.24
15	60	0.8	225	4.55	1701025	8047	1.25
16	40	0.8	225	5.698	1940398	4653	1.34
17	50	0.9	225	4.413	1526564	7944	1.39

Statistical analysis of method responses for peak asymmetry

Box-Behnken multifactor response surface quadratic model was adopted for the USP tailing factor of peak. The data was statistically analysed by analysis of variance (ANOVA) to evaluate the significance of the variables and interaction effects on the peak asymmetry response. The independent variables of the BBD selected are % composition of mobile phase, flowrate and detection wavelength. The statistical values provided by the software generated report are given in table 4.

The model F-value of 5.1819 and p-value less than 0.05 indicated that model terms were significant for optimization. The significant factors found were flowrate (p=0.031), interaction of flowrate x wavelength (p=0.0317) and % composition x % composition (p=0.0017). A positive relationship of these factors and their interaction effects on tailing of peaks could be predicted. “Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.011 indicated an adequate signal. This model fit well for

optimization. The effect of factors on responses and their interaction effects could be further studied from the 3-dimensional response surface and contour plots. The 3D response surface and contour plots could be utilized for navigation of design space (method operable design region) in development of a robust AQbD method.

Table 4. ANOVA table (partial sum of squares) for peak asymmetry response

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value**	
Model	0.11	9	0.0122	5.1819	0.0206	Significant
A-Composition	0.0003	1	0.0003	0.1323	0.7267	
B-Flowrate	0.017	1	0.0171	7.2488	0.031	Significant
C-Wavelength	1.39E-17	1	1.39E-17	5.88E-15	1	
AB	0.0002	1	0.0002	0.0953	0.7665	
AC	0.0025	1	0.0025	1.0590	0.3377	
BC	0.0169	1	0.0169	7.1585	0.0317	Significant
A^2	0.0569	1	0.0569	24.1034	0.0017	Significant
B^2	0.0110	1	0.0110	4.6846	0.0672	
C^2	0.0007	1	0.0007	0.3372	0.5797	
Residual	0.0165	7	0.0023			
Lack of Fit	0.0165	3	0.0055			
Pure Error	0	4	0			
Cor Total	0.1266	16				
ANOVA Summary						
Std. Dev.	0.0486	PRESS	0.2644	Adeq Precision	7.011	
R^2	0.8695	Adj R^2	0.7017	Pred R^2	-1.088	

The desirability value was high for the software suggested chromatographic conditions as shown in table 5. Therefore, conditions given in table 5 were selected as optimum chromatographic conditions for method development by AQbD approach to be reliably used for routine analysis of Favipiravir and the chromatogram for the optimized condition is given in Figure 2.

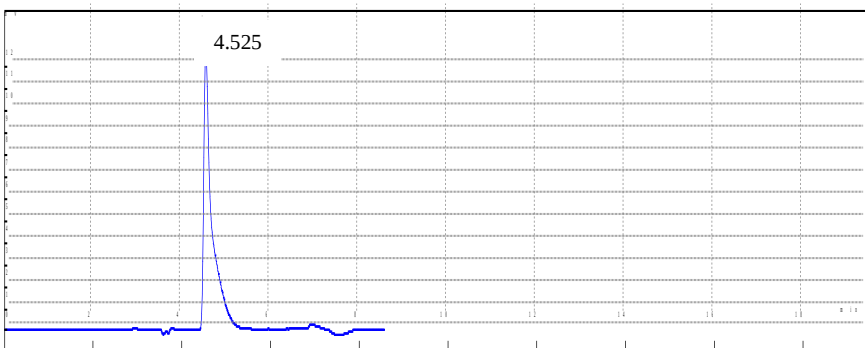


Fig. 2: Chromatogram for optimized condition of Favipiravir

Table 5. Suggested optimized chromatographic condition by AQbD approach

Flow rate (ml/min)	% Composition (methanol: water)	Wave length (nm)
0.8	60:40	225

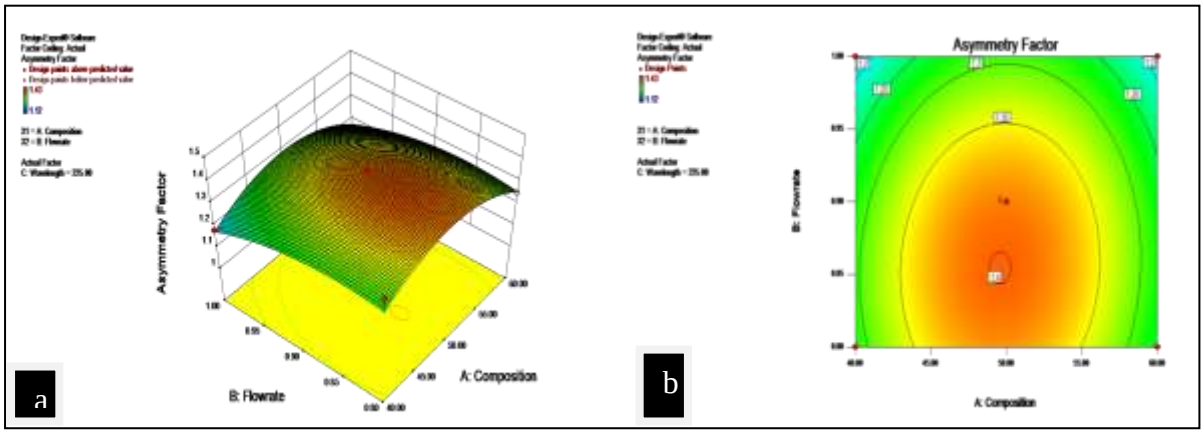


Fig. 3(a) The 3D Response surface and (b) Contour plot for peak asymmetry as a function of composition and flowrate (constant wavelength 225 nm).

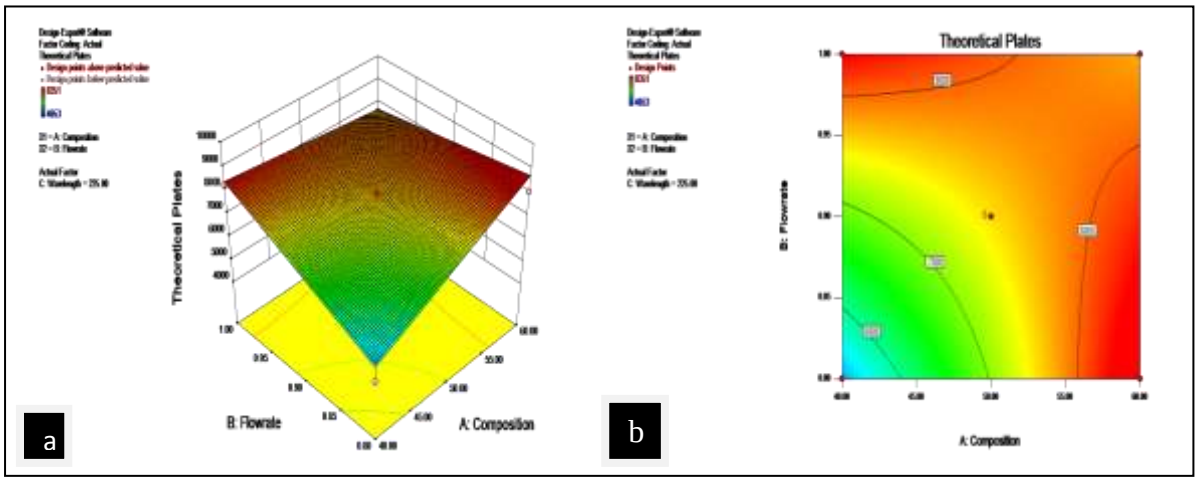


Fig. 4(a) The 3D Response surface and (b) Contour plot for theoretical plates as a function of composition and flowrate (constant wavelength 225 nm).

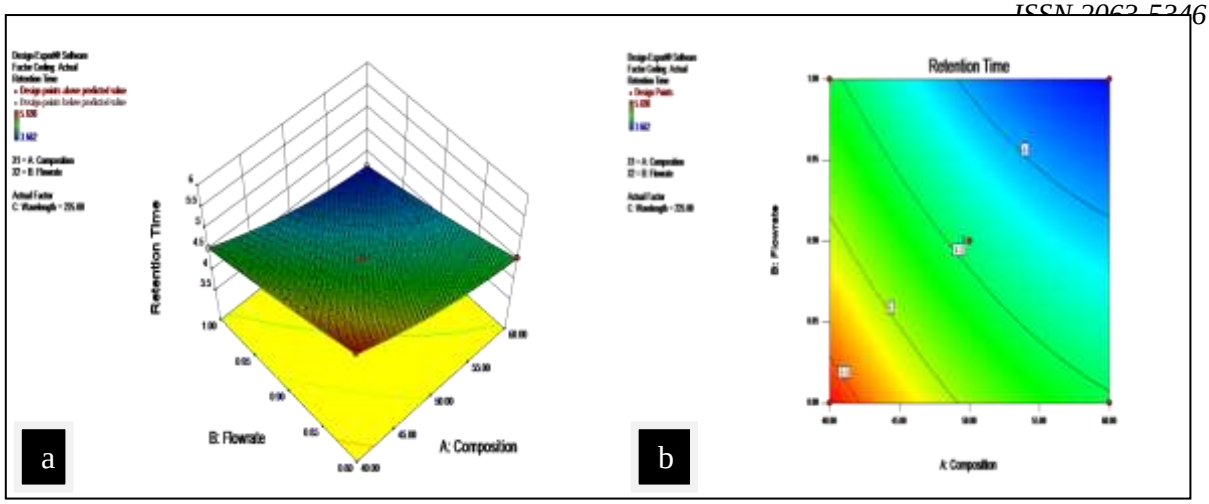


Fig. 5(a) The 3D Response surface and (b) Contour plot for retention time as a function of composition and flowrate (constant wavelength 225 nm).

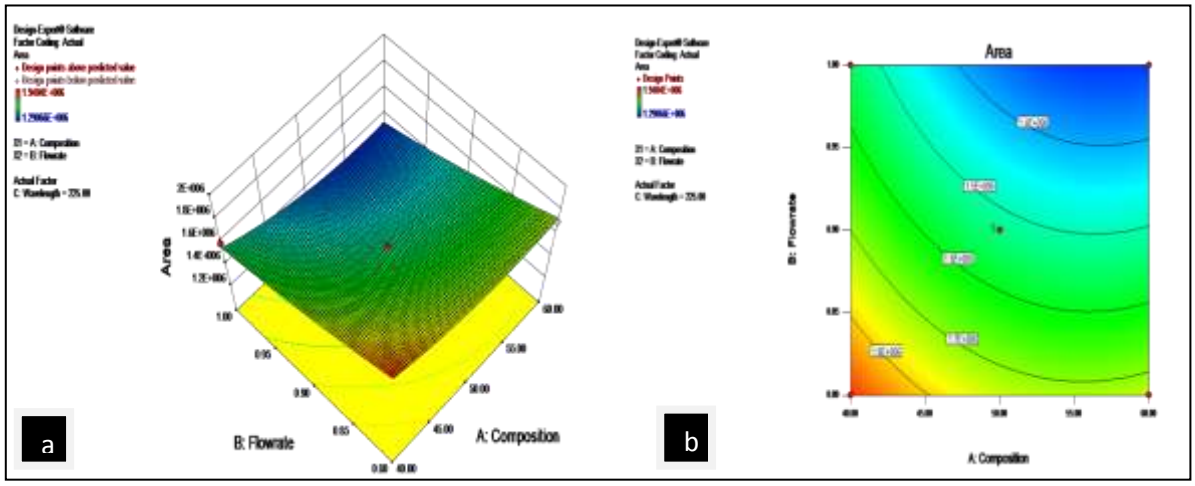


Fig. 6(a) The 3D Response surface and (b) Contour plot for area as a function of composition and flowrate (constant wavelength 225 nm).

Method Validation

Linearity

From the observations of linearity studies as shown in table 6, a calibration curve was plotted between drug concentration versus area, refer figure 7. A straight line was obtained with a correlation coefficient $r^2 = 0.9993$. The equation of line showing slope m and y-intercept c was obtained in the form of y = mx + c. A linear correlation between drug concentration and area was found within the range of 10-50 µg/ml. Therefore, this method could be used for estimating the concentration of Favipiravir quantitatively.

Table. 6 Data obtained from linearity study by HPLC

Concentration (ppm)	Area
10	205795
20	339210
30	449388
40	571062

50	688591

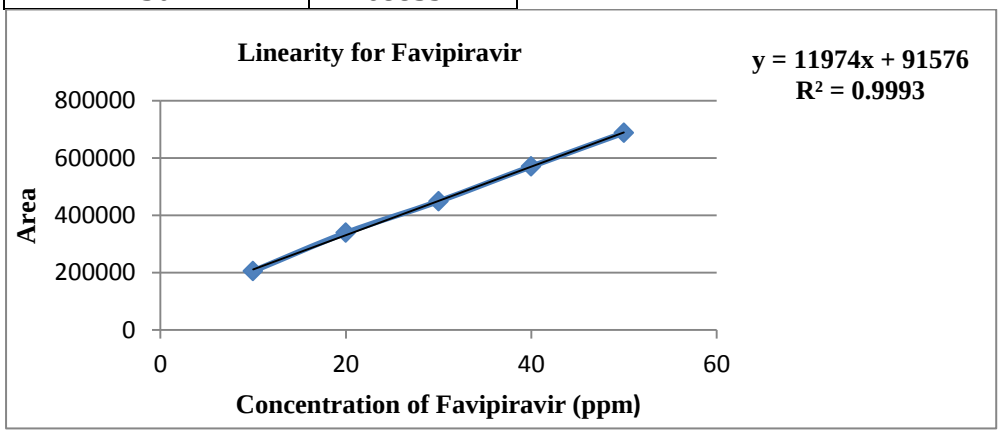


Fig. 7 Linearity for Favipiravir

Precision

Table 7 shows the results of intraday and interday precision. The mean area of the readings, standard deviation (SD) and relative standard deviation (% RSD) was calculated. The results complied with the limits for precision (% RSD < 2), proving the method is precise.

Table 7. Intraday and interday precision

Intraday Precision			**Interday Precision**		
Concentration (µg/ml)	Mean Area ± SD	% RSD	Concentration (µg/ml)	Mean Area ± SD	% RSD
30	445276.5	0.74	30	445251	0.75

Accuracy

Table 8 shows results of accuracy studies. Test was passed with specification RSD < 2 %.

Table 8. Accuracy

Sr. No.	**Concentration (µg/ml)**	**Area**	**Mean**	**SD**	**% RSD**
1	10	205795	204920.33	1062.45	0.5184
	10	203738			
	10	205228			
2	30	449388	447936.33	2366.96	0.5284
	30	449216			
	30	445205			
3	50	688591	687936.33	4015.22	0.5836
	50	683634			
	50	691584			

% Recovery

The results of recovery studies are given in table 9. The recovery of Favipiravir was within the compendial limits.

Table 9. Data obtained from recovery studies

Recovery level	Concentration of test (µg/ml)	Concentration of standard added (µg/ml)	Amount found (µg/ml)	% recovery
50% Recovery	20	10	29.74	99.16
100% Recovery	20	20	39.73	99.34
150% Recovery	20	30	49.62	99.25

Robustness

Table 10 shows the results of robustness studies. Despite small variations in the experimental parameters, the limit of % RSD < 2 indicated that the method was not affected significantly.

Table 10 Results of robustness studies

Parameter varied	Concentration (µg/ml)	Area	Mean	SD	% RSD
Wavelength (nm)					
223	20	341168	339870	1124.44	0.33
225	20	339210			
227	20	339231			
pH of mobile phase					
2.8	20	340178	339975	686.80	0.20
3.0	20	339210			
3.2	20	340538			

LOD and LOQ

The formula for calculation of LOD and LOQ according to ICH guidelines is,

$LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$

where σ = Standard deviation of the peak area response and
S = the slope of the calibration curve obtained from linearity.

From the above formula, the LOD was found to be 0.29 and LOQ was 0.88 µg/ml.

Assay

Assay results are shown in table 11. The results comply with the compendial standards.

Table 11. Assay of Favipiravir

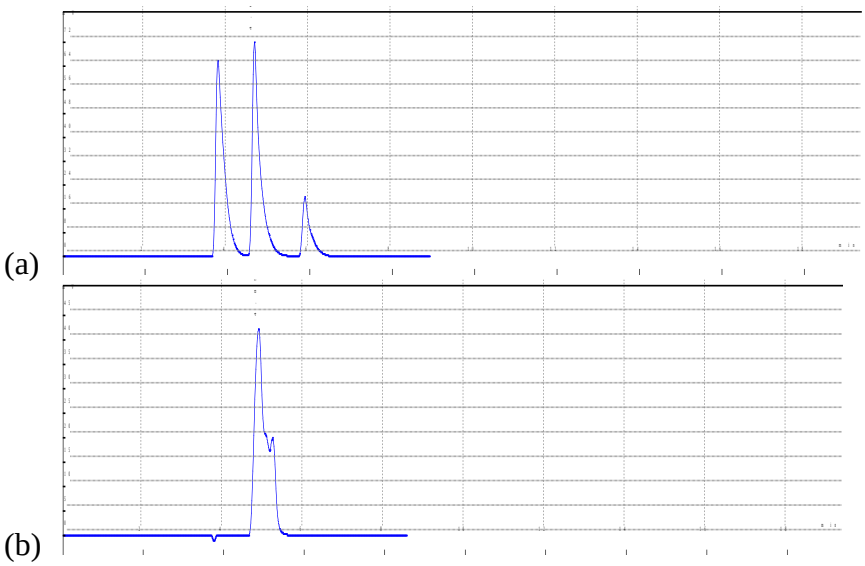
Test	Area of Standard	Area of Sample	% Assay
% Assay	449388	448512	99.80507

Forced degradation

The chromatograms of forced degradation studies of favipiravir are shown in figure 8. The favipiravir drug was stable under thermal and photolytic stress conditions. The drug was subjected to acid and base hydrolysis, and the chromatogram showed a degradant peak in the alkaline condition. In 3% hydrogen peroxide, the peroxide shows two degradant peaks which coeluted with the drug peak. It is evident from the values that the alkaline and acid hydrolysis stress conditions had a significant effect on the stability of drug than any other stress conditions.

Table 12. Degradation percentage of Favipiravir under various stress conditions

Degradation parameter	% Assay after degradation	% Degradation
Acid	87.19	12.80
Base	83.00	16.99
Peroxide	93.21	6.78
Photolytic (UV)	98.68	1.31
Thermal	98.51	1.48



(a)

(b)

Fig. 8 Forced degradation chromatogram of Favipiravir (a) Peroxide degradation: In 3 % H_2O_2 at RT for 12 hr, (b) Base degradation: In 0.1N NaOH at 60°C for 30 min.

CONCLUSION

The AQbD approach employing a Box-Behnken multifactor quadratic response surface model was successfully developed for routine analysis of Favipiravir in bulk and formulation.

Statistical analysis of results was provided by the software to aid method development in a novel way. The resultant method was not only efficient but also robust. The forced degradation studies further led to generation of a stability indicating RP-HPLC method. The scientific approach of QbD to analytical method development with strategies of risk assessment, establishing CQAs, CPPs and design of experiment leads to creation of a design space offering regulatory flexibility for changes in the approved design space. The novel analytical QbD approach thus gives an edge to analysis of drugs over the traditional method development approach and insulates against method failures during method transfer.

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REFERENCES

1. Guideline, I. H. T. (2009) Pharmaceutical development Q8 (R2). *Current step*, *4*. https://database.ich.org/sites/default/files/Q8%2 8R2%29%20Guideline.pdf
2. Ramalingam, P., & Jahnavi, B. (2019). QbD considerations for analytical development. In *Pharmaceutical Quality by Design* (pp. 77-108). Academic Press. https://doi.org/10.1016/B978-0-12-815799-2.00005-8
3. Urmi, KF, Nawaz M, Islam SM (2022) Analytical quality by design approach to RP-HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified-release dosage form. *Future Journal of Pharmaceutical Sciences*, 8(1), 1-16. https://doi.org/10.1186/s43094-021-00396-z
4. Kumar, L. (2023). Quality-by-design driven analytical method (AQbD) development and validation of HPLC-UV technique to quantify rivastigmine hydrogen tartrate in lipidic nanocarriers: Forced degradation, and assessment of drug content and in vitro release studies. *Microchemical Journal*, 108944. https://doi.org/10.1016/j.microc.2023.108944
5. Nagar, P., Garg, M., Chauhan, C., Kumar, R., & Chaudhary, A. K. (2022). Analytical Quality by Design (AQBD) Approach for HPLC Method Development, Method Optimization and Validation. *Quality Assurance*, *13*(2), 103-110. DOI: 10.25258/ijpqa.13.2.2
6. Volta e Sousa, L., Gonçalves, R., Menezes, J. C., & Ramos, A. (2021). Analytical method lifecycle management in pharmaceutical industry: A review. *Aaps Pharmscitech*, *22*, 1-14. https://doi.org/10.1208/s12249-021-01960-9
7. Alhakeem, M. A., Ghica, M. V., Pîrvu, C. D., Anuța, V., & Popa, L. (2019). Analytical quality by design with the lifecycle approach: A modern epitome for analytical method development. *Acta Marisiensis-Seria Medica*, *65*(2), 37-44. https://doi.org/10.2478/amma-2019-0010
8. Varnekar DS., & Sanjay, N. T. (2023) Quality by Design Approach-To Analytical Method Validation. *International Journal For Multidisciplinary Research*, *5*(2). https://doi.org/10.36948/ijfmr.2023.v05i02.1903

9. Mahapatra, A., & Meyyanathan, S. N. (2022). Approach of analytical quality by design and regulatory need. International Journal of Health Sciences, 6(S5), 2572–2592. https://doi.org/10.53730/ijhs.v6nS5.9208
10. Sanders, J. M., Monogue, M. L., Jodlowski, T. Z., & Cutrell, J. B. (2020). Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. *Jama*, *323*(18), 1824-1836. doi:10.1001/jama.2020.6019
11. Addendum 2021 to Indian Pharmacopoeia 2018, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, Ghaziabad. Website: www.ipc.gov.in.
12. Curley, P., Neary, M., Arshad, U., Tatham, L., Pertinez, H., Box, H., ... & Owen, A. (2021). Development of a highly sensitive bioanalytical assay for the quantification of favipiravir. *bioRxiv*. doi: 10.1101/2021.02.03.429628
13. Morsy, M. I., Nouman, E. G., Abdallah, Y. M., Zainelabdeen, M. A., Darwish, M. M., Hassan, A. Y., ... & Marzouk, H. M. (2021). A novel LC-MS/MS method for determination of the potential antiviral candidate favipiravir for the emergency treatment of SARS-CoV-2 virus in human plasma: Application to a bioequivalence study in Egyptian human volunteers. *Journal of pharmaceutical and biomedical analysis*, *199*, 114057. https://doi.org/10.1016/j.jpba.2021.114057
14. Hailat, M., Al-Ani, I., Hamad, M., Zakareia, Z., & Abu Dayyih, W. (2021). Development and validation of a method for quantification of favipiravir as COVID-19 management in spiked human plasma. *Molecules*, *26*(13), 3789. https://doi.org/10.3390/molecules26133789
15. Megahed, S., Habib, A., Hammad, S., & Kamal, A. (2021). Chemometric approach based on factorial and box-Behnken designs for determination of Anti coronavirus drug; Favipiravir in bulk and spiked human plasma by green HPLC method. *Turkish Journal of Analytical Chemistry*, *3*(2), 70-78. https://doi.org/10.51435/turkjac.963652
16. Ramadoss, K., Meerasa, S. S., Satyapal, A. K. S., Vijayakumar, A., & Venugopal, V. Quantitative analysis of favipiravir in bulk and pharmaceutical dosage forms using UV visible spectrophotometer. *Journal of medical pharmaceutical and allied sciences*, 12(2), 5680 – 5683. DOI: 10.55522/jmpas.V12I2.4410
17. Megahed, S. M., Habib, A. A., Hammad, S. F., & Kamal, A. H. (2021). Experimental design approach for development of spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: Application to spiked human plasma. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *249*, 119241. https://doi.org/10.1016/j.saa.2020.119241
18. Stojanovic, J., Krmar, J., Protić, A., Svrkota, B., Djajić, N., & Otašević, B. (2021). DoE Experimental design in HPLC separation of pharmaceuticals; a review. *Archives of Pharmacy*, *71*(Notebook 4), 279-301. https://doi.org/10.5937/arhfarm71-32480
19. Guideline, I. H. T. (2003) Guideline, Stability testing of new drug substances and products. *Q1A (R2), current step*, 4(1-24). https://database.ich.org/sites/default/files/Q1A%28R2%29%20Guideline.pdf
20. Lingabathula, S., & Jain, N. (2021). STABILITY Indicative And Cost Effective Analytical Method Development And Validation Of Favipiravir And Peramivir In Bulk

And Pharmaceutical Dosage Form By UsiNG RP-Hplc. *Int J Appl Pharm*, *13*(4), 265-271. DOI: https://dx.doi.org/10.22159/ijap.2021v13i4.40530

21. Varma, S., & Yadav, P. (2021) Stability Indicating HPLC Method Development and Validation for Estimation of Favipiravir in Pharmaceutical Dosage Form, *World Journal of Pharmacy and Pharmaceutical Sciences*, 10(5), 1749-1760. DOI: 10.20959/wjpps20215-19004
22. Ali, S. N. S., Mobina, L., Mehfuza, M., Seema, P., Ahmed, A., & Khan, G. J. (2021). Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form. *Journal of Pharmaceutical Research International*, 33(48B), 254-271. DOI: https://doi.org/10.9734/jpri/2021/v33i48B33283
23. Marzouk, H. M., Rezk, M. R., Gouda, A. S., & Abdel-Megied, A. M. (2022). A novel stability-indicating HPLC-DAD method for determination of favipiravir, a potential antiviral drug for COVID-19 treatment; application to degradation kinetic studies and in-vitro dissolution profiling. *Microchemical Journal*, 172, 106917. https://doi.org/10.1016/j.microc.2021.106917
24. Sawarkar, K. T., Asnani, A. J., Chaple, D. R., & Ikhar, J. S. (2022). Quality by Design (QbD): Building and Enhancing Quality of Pharmaceuticals. *International Journal of Pharmaceutical Research and Applications*, 7(3), 1738-1748. DOI: 10.35629/7781-070317381748
25. Dewi, M. K., Pratama, R., Arifka, M., & Chaerunisaa, A. Y. (2022). Quality By Design: Approach to Analytical Method Validation. *Sciences of Pharmacy*, *1*(1), 33-40. https://doi.org/10.58920/sciphar01010033
26. Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. *Q2(R1), 1(20), 05*. https://databa se.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf