ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FAVIPIRAVIR IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Golla Bazari[1], K. Venkataramana*[1], G.Somasekhar[1]

[1] Department of Pharmaceutical Analysis, SKU College of Pharmaceutical Sciences (SKUCOPS), Anantapuramu, Andhra Pradesh, India-515003

** Address for correspondence:*

K. Venkataramana,

Department of Pharmaceutical Analysis, SKU College of Pharmaceutical Sciences (SKUCOPS), Anantapuramu, Andhra Pradesh, India-515003. Email: venkat.n.pharma@gmail.com, 9398814224

Abstract

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation ofFavipiravir in bulk form and marketed formulation. Separation of Favipiravir was successfully achieved on a Develosil ODSHG-5RPC18,5µm,15cmx4.6mmi.d.column in anisocratic mode of separation utilizing Methanol : Phosphate buffer (0.02M, pH-3.6) in the ratio of 45:55%v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Favipiravir. The correlation coefficient was found to be 0.9995 for Favipiravir. The LOD and LOQ for Favipiravir were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Favipiravir, which indicates that the proposed method is highly accurate.Thespecificityofthemethodshowsgoodcorrelation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Key words: Accuracy, Favipiravir, Precision, Robustness, RP-HPLC, ICH Guidelines

Introduction

Forced degradation studies provide the approach to analyse the stability of drug samples inpharmaceuticalindustries.Drugproductsafetyandefficacyisaffectedbythechemicalstability of the molecule. Stability of molecule information provides the data for selecting proper formulation, package, proper storage conditions and shelf life. These data also play a significant role which is required in the regulatory documentation. Before filling registration dossier it is obligatory to execute stability studies of new drug molecules.[1]

International Conference on Harmonisation (ICH) guidelines, make it essential to organize the forced degradation studies and it is evidently mandated to perform forced degradation of new drug products. These studies offer the information to support detection of potential degradants. It also illustrates the degradation pathways of pharmaceutically active molecules. The drug molecule intrinsic stability can be estimated by forced degradation studies. Probable polymorphic or enantiomeric substances and variation between drug related degradation and excipients interferences can also be evaluated by forced degradation studies. ICH guidelines mandatory oblige the forced degradation studies under a range of conditions, like pH, light, oxidation, dry heat, acidic, basic, hydrolysis etc. Moreover, it provides the separation of drug from degradation products. The FDA and ICH guidance mandate the requirement of forced degradation to recognize how the quality of a drug substance and drug product varies with time and different environmental factors. [2]

The developed and validated analytical method permits the analysis of each degradation products. Unfortunately, there is less guidance available to establish true selective forced degradation methods. Appropriate experimental conditions for forced degradation studies(temperatures, duration, and extent of degradation, etc.) are not specified properly. The main aim of the present study is development of accurate, precise, sensitive, selective,reproducible and rapid analytical technique for cost effective estimation of Favipiravir in bulkformand marketed pharmaceutical dosageform.

Materials and methods

HPLC Method development:

Preparation of Standard Solution:

Accuratelyweighandtransfer10mgofFavipiravirworkingstandardintoa10mlofcleandry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Favipiravir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:
Twenty capsules were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantityofpowderofFavipiravirequivalentto10mgweretransferredtocleananddry10mlvolumetricflask and7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (0.02M, pH-3.6)in proportion 45:55%v/v.

Optimization of Column:
The method was performed with various C18 columns like, X- bridge column, Xterra, andC18 column. Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. was found to be ideal as it gave good peak shape and resolution at1.0ml/min flow.

Preparation of buffer and mobile phase:

Preparation of Potassium Dihydrogen Phosphate (KH_2PO_4) Buffer (0.02 M) (pH-3.6):
Dissolve 2.72172 g of potassium dihydrogen phosphate in 1000ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

Preparation of Mobile Phase:
Accurately measured 450 ml (45%) of Methanol and 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µfilter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

Results and discussion

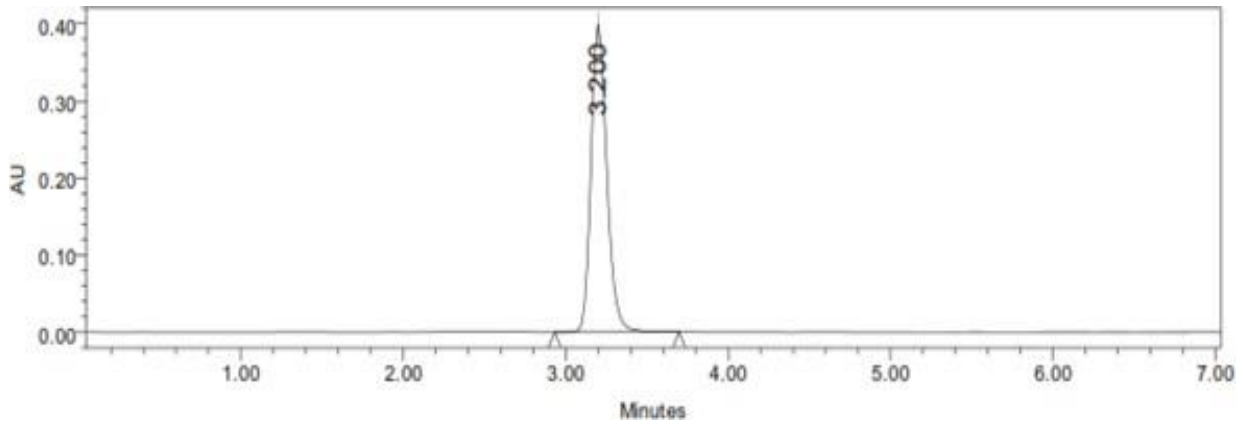

Fig-1: Chromatogram of Favipiravir in Sample

In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So it is an optimized chromatogram.

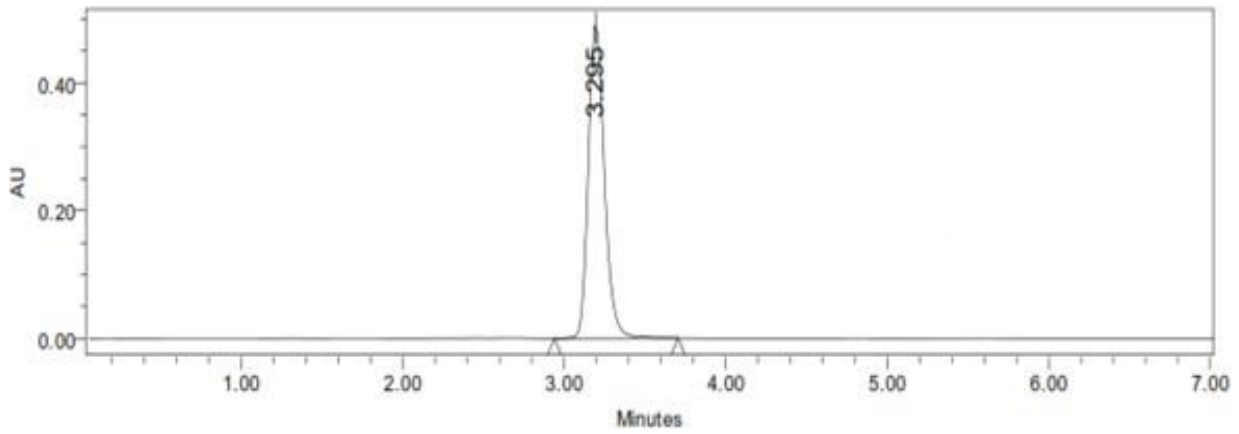

Fig-2: Chromatogram of Favipiravir in Optimized Chromatographic Condition

In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So it is an optimized chromatogram.

Method validation

System Suitability:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established.

Table-1: Data of System Suitability Test

S.No.	ectionNo.	RT	Area	USP PlateCount	USP Tailing
1	Injection1	3.253	284568	7368	1.26
2	Injection2	3.254	285684	7295	1.25
3	Injection3	3.215	283659	7346	1.27
4	Injection4	3.297	284754	7394	1.29
5	Injection5	3.253	283695	7425	1.25
6	Injection6	3.213	284578	7385	1.27
Mean			**284489.7**	**7368.833**	**1.265**
S.D			**752.5617**		
%RSD			**0.26453**		

Table-2: System suitability results for Favipiravir (Flowrate)

S.No.	Parameter	Limit	Result
1	Asymmetry	T□ 2	Favipiravir=0.12
2	Theoreticalplate	N□ 2000	Favipiravir=7258
3	TailingFactor	(Tf)<2	Favipiravir=1.25

Specificity: Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing three drugs was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method wass pecific.

Linearity: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Favipiravir. The prepared solutions were filtered through Whatman filter paper (No.41).From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area(Y-axis) against the concentration (X-axis).

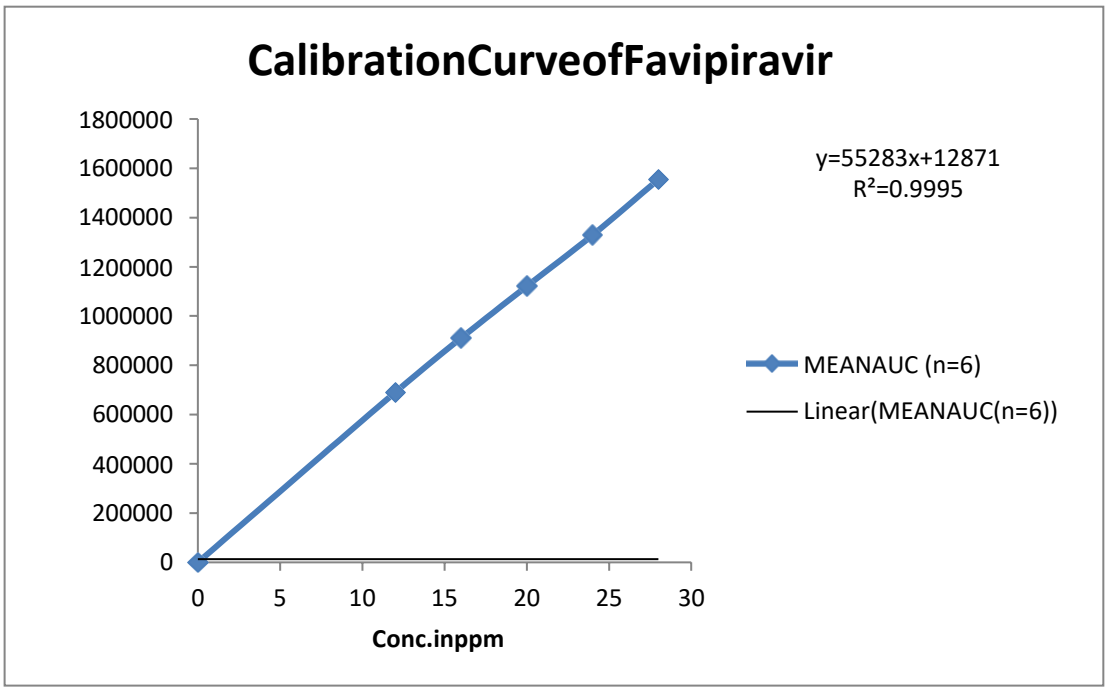

Fig-3: Calibration Curve of Favipiravir

Accuracy:

Inject the three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Favipiravir and calculate the individual recovery and mean recovery values. Accuracy at different concentrations (80%, 100%, and 120%) was prepared and the % recovery was calculated. The results obtained for recovery at 80%, 100%, 120% are within the limits. Hence method is accurate.

Precision: The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Favipiravir

Table-3: Repeatability Results of Favipiravir

HPLC Injection Replicates	**AUC for Favipiravir**
Replicate–1	285479
Replicate–2	284571
Replicate–3	286954
Replicate–4	283261
Replicate–5	285964
Replicate–6	284259
Average	**285081.3**
StandardDeviation	**1318.666**
%RSD	**0.462558**

.**Table-4: Results of Ruggedness for Favipiravir**

S.No.	Peak Name	RT	PeakArea	TheoreticalPlates	TailingFactor
1	Favipiravir	3.297	294754	7394	1.29
2	Favipiravir	3.253	293695	7425	1.25
3	Favipiravir	3.213	294578	7385	1.27
4	Favipiravir	3.297	296534	7584	1.23
5	Favipiravir	3.210	296571	7745	1.24
6	Favipiravir	3.254	298698	7658	1.25
Mean			**295805**		
Std.Dev.			**1819.334**		
%RSD			**0.615045**		

Robustness: Robustness is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Acid Degradation: An accurately weighed 10 mg of both the pure drug were transferred to two different clean & dry round bottom flasks. 30ml of 0.1 N HCl was added to it and it was refluxed in a water bath at 60^0C for 4 hours. Allowed to cool to room temperature. The sample was then neutralized using dilute 0.1 N NaOH solution & final concentration was prepared to50µg/ml for Favipiravir with mobile phase. It was injected into the HPLC system against a blank of mobile phase (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Favipiravir in0.1N HCl.

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Favipiravir, different chromatographic conditions were applied & the results observed are presented in previous chapters.
Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.
In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RPC18,5□m,15cmx4.6mmi.d.columnwaspreferredbecauseusingthiscolumnpeakshape,resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studyingthe solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1NNaOH,0.1NHCl). [3]

Thedrugwasfoundtobefreelysolubleinn,N-dimethylformamide,solubleindichloromethane, very slightly soluble in ethanol (96%). and practically insoluble in water.Solubility in water is increasing with lowering of pH within the physiological range. Using thesesolventswith appropriate composition newer methods can be developed and validated. [4]

Detection wavelength was selected after scanning the standard solution of drug over 200to 400nm. From the U.V spectrum of Favipiravir it is evident that most of the HPLC work can be a ccomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Favipiravir in different formulations. [5]

Conclusion

In the present investigation the selected drug combinations were analyzed in both bulk and pharmaceutical formulations by a simple, fast, precise and reliable Reverse Phase High Performance Liquid Chromatographic methods with the search for a suitable stationary and new mobile phase which were not been used until. Based on the results obtained in this study, it is concluded that the present validated method can be successfully applied for the estimation of Favipiravirin bulk form and Marketed Pharmaceutical Dosage form.

References

1. *Vibha G et al., Development and validation of HPLCmethod- a review. InternationalResearchJournal of Pharmaceutical and AppliedSciences. 2012, 2(4), 22-23.*
2. *Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.*
3. *Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11.(CPMP/ICH/281/95).*
4. *Development and validation of HPLC method - A Review, Vibha Gupta et al, InternationalResearchJournal of PharmaceuticalandAppliedSciences, 2012;2(4):17-25.*
5. *A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al.International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015*