Development and Validation of RP-HPLC Method for the Quantitative Estimation of Favipiravir in Bulk and Pharmaceutical Dosage Form

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Abstract:

Favipiravir is an antiviral drug, used in the treatment of SARS-COV-2, novel corona virus. It is a pyrazine carboxamide derivative with activity against RNA viruses A sensitive new RP-HPLC method was developed for the quantitative estimation of Favipiravir in bulk and pharmaceutical dosage form using Rivaroxaban as internal standard (IS). The separation of drug was achieved by Enable C18 column using acetonitrile and water as mobile phase at a ratio of 70:30 %v/v with a flow rate of 1mL/min and wavelength at 296nm respectively. The retention time of Favipiravir and Rivaroxaban were found to be 3.110 and 4.287 mins. The developed method was validated according to ICH Q2 (R1) guidelines over the concentration range of 5-25 µg /mL. The LOD and LOQ were found to be 2.17 µg /mL and 6.58 µg /mL. The method was found to be linear, specific, accurate, precise and robust. The developed method was used for determination of Favipiravir in pharmaceutical dosage forms.

Keywords- *Favipiravir, Antiviral, Rivaroxaban, ICH guidelines and Validation.*

1. Introduction

High performance liquid chromatography is now one of the foremost powerful tools in analytical chemistry. HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase so as to get a satisfactory flow rate, liquid must be pressurized to some thousands of pounds per square inch. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation is achieved. It has the flexibility to separate, identify and quantitate the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified.

Internal Standard Method: In this method a calibration plot is produced by

preparing and analyzing calibration solution containing different concentration of compound of interest with a fixedconcentration of internal standard added. Internal standard is known as concentration of a substance that is present in every sample that is analyzed.

Although each method is effective, the internal standard method tends to yield the most accurate and precise results. In this method an equal amount of an internal standard, a component that is not present in the sample, is added to both sample and standard solutions. The internal standard selected should be chemically similar. Additionally, it is important to ensure that the internal standard is stable and does not interfere with any of the sample components. The internal standard should be added before any preparation of sample so that extraction efficiency can be evaluated.Quantification is achieved by using ratios of peak height or area of the component to the internal standard.

FAVIPIRAVIR: Favipiravir is a broad spectrum anti-viral agent that selectively and potently inhibits the RNA-dependent RNA polymerase (RdRp) of RNA viruses. Favipiravir exerts its anti-viral activity as a pro-drug, since favipiravir is intra-cellularly phosphor ribosylation to be a vigorous form, favipiravir-RTP. It was approved by USFDA in 2014 for the treatment of influenza virus. It's chemically 6-fluoro-3-hydroxy-2-pyrazinecarboxamide with a chemical formula and molecular mass $C_5H_4FN_3O_2$ and 157.1g/mol respectively. Favipiravir is a selective and potent inhibitor of influenza viral RNA polymerase. It is effective against all subtypes and strains of influenza viruses including ones sensitive or immune to marketed neuraminidase and M2 inhibitors. It is an odorless, non-hygroscopic and white to yellowish powder which is soluble in organic solvents and is slightly soluble in water.

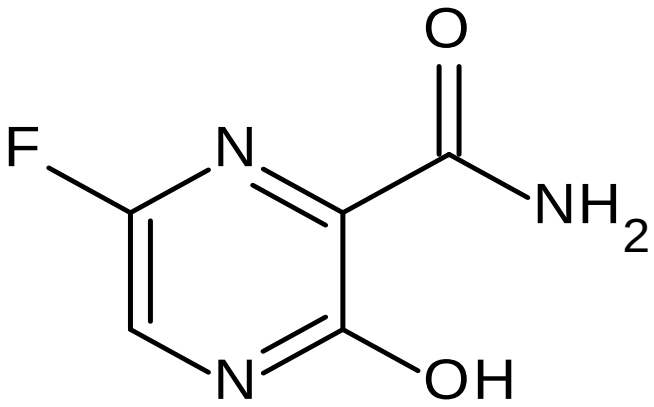

Fig 1 Structure of Favipiravir

RIVAROXABAN (Internal Standard): Rivaroxaban is an anticoagulant and first orally active direct inhibitor of factor Xa approved by FDA in the year 2011. It's accustomed treat and forestall blood clots. It is specifically used to treat deep vein thrombosis and pulmonary emboli. It is also used to prevent blood clots in atrial fibrillation and following hip or knee surgery. It is administered orally. It is

chemically 5-Chloro-N-({(5S)-2-oxo-3- [4-(3-oxo-4- morpholinyl) phenyl]-1, 3-oxazolidin-5yl}-methyl)-2-thiophenecarboxamide with an empirical formula and molecular weight of $C_{19}H_{18}ClN_3O_5S$ and 435.89g/mol respectively.

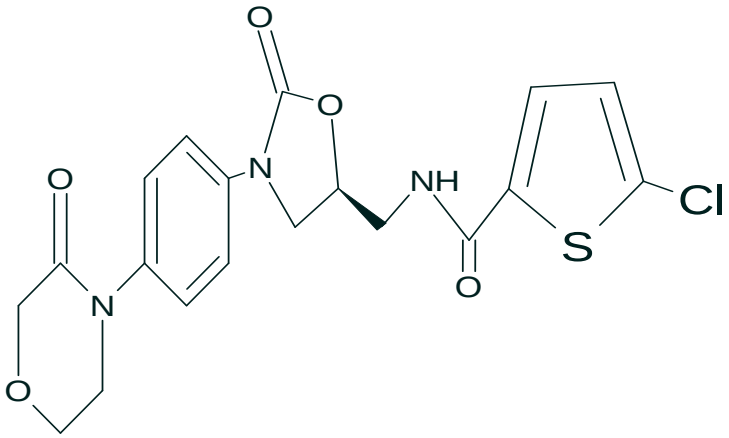

Fig 2: Structure of Rivaroxaban

Review of literature reveals that there are HPLC, spectrophotometric, LC-MS/MS methods for the estimation of Favipiravir. As there is **no internal standard** method for the estimation of favipiravir which is considered as the most accurate method, the present work is an attempt to develop a sensitive method for the quantitative estimation of favipiravir in bulk and pharmaceutical dosage form by RP-HPLC.

2.Materials and Methods:

Instrumentation:

A gradient high performance liquid chromatograph (SHIMADZU, HPLC) equipped with HPLC pump LC-20AT, UV-visible detector SPD-20A, LC solutions software and Enable-C18 column (250×4.6mm, 5µm) was used.

Chemicals and reagents:

Hubert Drugs Pvt. Ltd and Nifty labs provided a free sample of Favipiravir (standard) and Rivaroxaban (Internal standard) respectively. Methanol and water were purchased from Rankem Laboratories Pvt Ltd. Acetonitrile was purchased from Merck laboratories Pvt Ltd.

Preparation of Standard Stock Solution

Appropriately 10.0 mg of favipiravir was weighed into a 10 mL graduated tube and volume was made up to the mark with acetonitrile of HPLC grade. This gives a solution of 1000µg/mL. 1mL of 1000µg/mL solution was transferred into 10mL graduated tube and the volume was made up to the mark with HPLC grade acetonitrile. This gives 100µg/mL solution. Further dilutions were done by serial dilution method for obtaining respective concentrations with acetonitrile. Mobile phases of varying compositions of solvents like acetonitrile, water, methanol, pH 6.8 buffer, pH 3 buffer were used for the method development.

Preparation of Internal Standard Stock Solution

Appropriately 10.0 mg of rivaroxaban was weighed into a 10mL graduated tube; volume was made up to the mark with acetonitrile of HPLC grade. This gives 1000µg/mL solution. 1mL of 1000µg/mL solution was transferred into 10mL graduated tube and the volume was made up to the mark with acetonitrile. This gives 100µg/mL solution. 1 mL of 100µg/mL was transferred into 10 mL, graduated tube and the volume was made up to the mark with acetonitrile. This gives 10µg/mL solution.

Preparation of Standard Solution for Internal Standard Method

From both the standard solutions i.e., standard solution of favipiravir (5-25µg/mL)and standard solution of IS (10µg/mL) equal amounts of solutions were taken (1mL each), mixed and injected into the column of HPLC for the response.
The method was developed by selecting C18 column as stationary phase, acetonitrile and water as mobile phase solvents with a flow rate of 1mL/min at a wavelength of 296nm usingUV detector.

Preparation Of Mobile Phase

Mobile phase consists of acetonitrile and water at a ratio of 70:30%v/v. The mobile phase was filtered through 0.2µm cellulose acetate filters & degassed in sonicator prior to use.

Selection of Solvent

Analyte solubility is checked in different solvents like HPLC grade water, methanol, pH 3 buffer, pH 6.8 buffer and acetonitrile.

Selection of Wavelength

Favipiravir and rivaroxaban standard solutions were prepared in acetonitrile individually and were scanned in UV region from 200 to 400nm. The point at which maximum absorbance of both the drugs was observed, is selected as detection wavelength.

Selection of Mobile Phase
Most of the chromatographic separations can be achieved by choosing the optimum mobile phase composition. Most widely used solvents in RP-HPLC are methanol and acetonitrile. A mobile phase which separates all the impurities and degradants from each other and from analyte will be selected.

Selection of Column
Selection of the column is the first and the most important step in method development. Generally longer columns provide better separation due to higher theoretical plate numbers. As the particle size decreases the surface area available for coating increases. Columns with 5-µm particle size give the best compromise of efficiency, reproducibility and reliability.

Selection of Flow Rate
Time taken for the elution of drug (retention time) is greatly influenced by flow rate. Therefore, several trials were performed to select appropriate flow rate depending upon the elution of the drug from column. Flow rate between 0.5 mL/min to 1 mL/min is the acceptable limit.

Selection of Mode of Separation

Selection of separation of mode greatly depends on nature of analyte. Favipiravir being a polar drug it can be separated by RP-HPLC method.

Analyte Solution Stability Studies

Solution Stability Study of Favipiravir:

The solution stability of favipiravir was checked using UV-Visible Spectrophotometer. The solution was scanned in the range of 200-400nm in the duration of 0^{th}-6^{th} hour cycle. The stability of solution was observed for 6hrs.

Solution Stability Study of Rivaroxaban:

The solution stability of rivaroxaban was checked using UV-Visible Spectrophotometer. The solution was scanned in the range of 200-400nm in the duration of 0^{th}-6^{th} hour cycle. The stability of solution was observed for 6hrs.

Analytical Method Validation

All analytical procedures were validated according to ICH Q2(R1) guidelines. Following parameters were performed for method validation:

System Suitability

The system suitability tests were conducted by injecting a standard solution of favipiravir and rivaroxaban (internal standard) to ensure the readiness of system before analysis. Under optimum conditions various parameters such as column efficiency (theoretical plates), tailing factor, resolution etc. were checked.

Selectivity

Selectivity of a method refers to the extent to which it can determine particular analytes in a solution without interference from other components in the mixture. Selectivity of the method was evaluated by preparing blank and standard solution of drug and internal standard followed by injecting them into the HPLC system.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain results/response which are directly proportional to the concentration. Linearity of method is demonstrated with five different concentration levels. Standard solutions of favipiravir were prepared at five different concentration levels. 10mg of favipiravir was weighed accurately and dissolved in 10mL of acetonitrile which gives 1000µg/mL solution. From the above solution 1m was diluted to 10mL to get 100µg/mL solution. From 100µg/mL solution 0.5, 1, 1.5, 2 and 2.5mL were taken and made up to 10mL using acetonitrile individually in order to get 5µg/mL, 10µg/mL, 15µg/mL, 20µg/mL and 25µg/mL solutions respectively. To the above solutions equal amount of 10µg/mL solution of internal standard was added. All the solutions were filtered followed by degassing using ultrasonic bath sonicator. These solutions were checked for response (peak area ratio) using high performance liquid chromatograph. Calibration curve was drawn by plotting concentration against the peakarea ratios. Correlation coefficient, slope of the curve is reported to show the developed method was linear.

Sensitivity (LOD and LOQ)

The detection limit and quantitation limit can be calculated based on the standard deviation of the response and the slope.

$$LOD = 3.3\ \sigma/S \quad (1)$$

$$LOQ = 10\ \sigma/S \quad (2)$$

where, σ = standard deviation of response,
S = slope of the calibration curve

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the values which is accepted either as conventional true value or an accepted reference value and the value found. A standard solution of favipiravir containing internal standard will be prepared at three concentration levels corresponding to 80%, 100% and 120%. Suitable dilutions were made to get 12µg/mL, 15µg/mL and 18µg/mL solutions respectively. To the above solutions equal amount of 10µg/mL solution of internal standard was added. All the solutions were filtered followed by degassing using ultrasonic bath sonicator. These solutions were checked for response (peak area ratio) using high performance liquid chromatograph. The percentage RSD of three levels will be calculated. It should be within the limits.

Precision

The precision of an analytical procedure defines the degree of closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of a homogenous sample under prescribed conditions. Precision may be considered at three levels- repeatability, intermediate precision, reproducibility.
Precision of the method shall be reported by injecting six replicates of standard solution (100%) consecutively under the same analytical conditions (repeatability). Percentage relative standard deviation for six replicates should be calculated.
Precision of the method is also determined for both intra-day and inter-day variations. Three different concentrations (80%, 100% and 120%) of standard favipiravir solutions within the linear range will be analyzed on three consecutive days for inter-day precision and three times (morning, afternoon and evening) within the same day for intra-day precision. The percentage RSD of three levels was calculated. It should be within the limits.

Recovery Studies

Test solutions of favipiravir shall be prepared at three concentration levels corresponding to 80%, 100% and 120%. Tablet powder(225mg) equivalent to 1 tablet weight of favipiravir was weighed and dissolved in methanol and centrifuged at 5000rpm for 10mins and the supernatant was collected and evaporated. The drug was collected and 10mg was dissolved in 10mL of acetonitrile which gives 1000µg/mL solution. From this solution 100µg/mL solution was prepared by taking 1mL of above solution and making up the volume to 10mL with acetonitrile. From 100µg/mL solution 1.2mL, 1.5mL and 1.8mL was taken into volumetric flasks individually and the volume was made up to 10mL using acetonitrile in order to get 12µg/mL, 15µg/mL and 18µg/mL solutions respectively. To the above solutions constant amounts of standard favipiravir solution of 15µg/mL concentration and internal standard solutions were added. The percentage recovery of three levels will be reported against standard concentrations. Calculation of percentage recovery is based on the amount of the test solution added vs. standard concentration. Percentage recovery value

should be not less than 80% and not more than 120%. The formula used to calculate percentage recovery is given below

$$\text{Amount found} = \frac{\text{peak area ratio of sample}}{\text{Peak area ratio of standard}} * \text{concentrate ion of standard added} \quad (3)$$

$$\text{Percentage recovery} = \frac{Amount\ found}{Amount\ added} * 100 \quad (4)$$

Robustness

The robustness of the method is determined to check the reliability of an analysis with respect to deliberate variations in method parameters. In this method the robustness was calculated for two parameters- flow rate and pump B concentration.
Standard solution of Favipiravir of 15µg/mL concentration was prepared by serial dilution method. The response of the solution was noted at 0.9mL/min, 1mL/min and 1.1mL/min flow rate and 69%, 70% and 71% pump B concentration. Percentage RSD was calculated. It should be within the limits.

3.Results and Discussion

A new sensitive RP-HPLC method was developed for estimation of Favipiravir in bulk and pharmaceutical dosage form. The Application of the method for analysis in marketed formulation was performed using a gradient high performance liquid chromatograph (SCHIMADZU, HPLC) equipped with HPLC pump LC-20AT, LC solutions software, UV-Visible detector SPD-20A and Schimadzu-C18 column (250×4.6mm, 5µm). The developed chromatographic conditionswere demonstrated in table 1.

Selection Of Wavelength (λmax):

Absorption maximum of favipiravir was observed at 322nm and absorption maximum of internal standard rivaroxaban was observed at 248nm. The isobestic point was found to be at 296nm, thus the wavelength of 296nm was selected as λmax based on various trials. The individual spectrums and overlay UV spectrum of favipiravir and rivaroxaban.

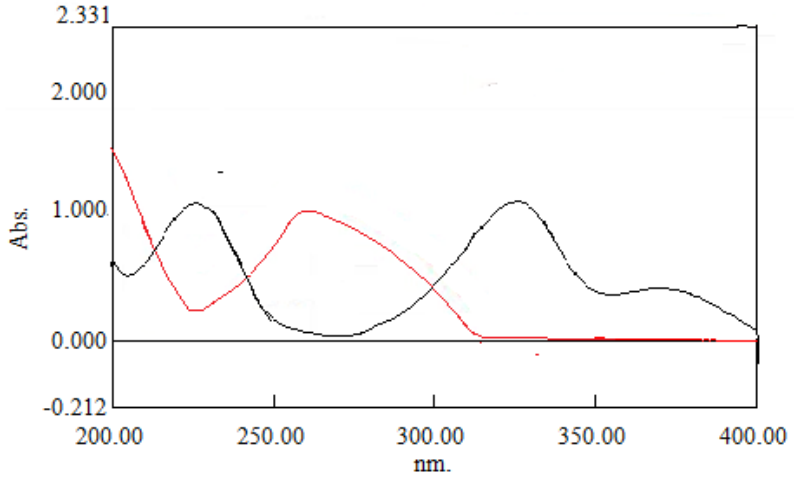

Fig 3: Overlay UV- Spectrum of favipiravir and rivaroxaban

Table 1: Optimized RP-HPLC conditions

Parameters	Conditions
Stationary phase (column)	C18 column (250×4.6mm, 5µm)
Mobile phase	Acetonitrile: Water (70:30)
Flow rate	1mL/min
Run time	10mins
Column temperature	Ambient
Volume of injection loop	20µl
Detection wavelength	296nm
Drug retention time	3.154mins
Internal standard retention time	4.471mins

Suitability Parameters:

System suitability parameters were found to be satisfactory. All the parameters (theoretical plate count, resolution etc.) were found to be within acceptable limits. System suitability parameters are given in table 2.

Table 2: System suitability parameters

S. No	Parameters	Found	Acceptable limits
1.	Retention time	3.154 mins	-
2.	Resolution (n=6)	6.308	Rs >2
3.	Theoretical plate count (n=6)	5549.7 20	N>2000

Selectivity And Sensitivity (LOD & LOQ)

There were no interference peaks obtained in blank, at the retention times of analyte peak and internal standard peak. The chromatograms of blank, drug and internal standard are shown in fig.4,5,6 respectively. The chromatograms of blank, drug and internal standard demonstrate the selectivity results.

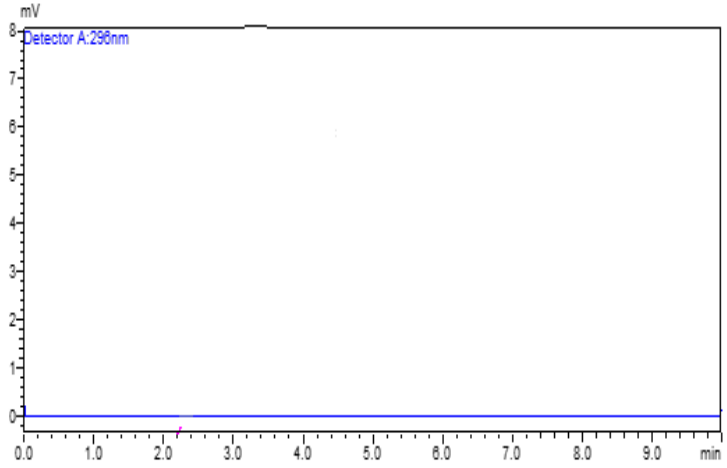

Fig4: Chromatogram of blank solution

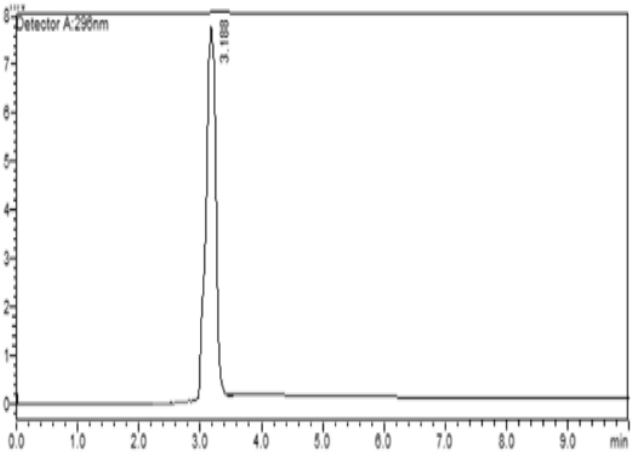

Fig5: Chromatogram of Favipiravir

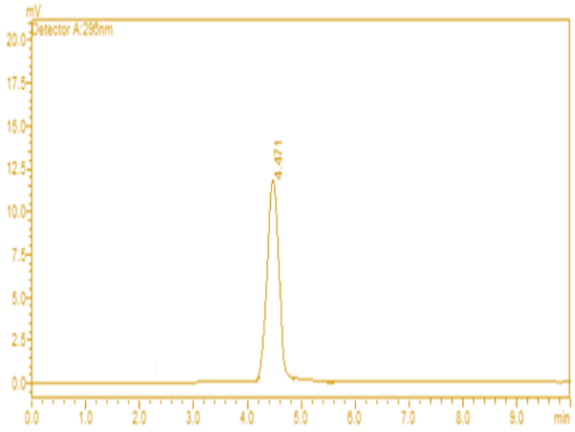

Fig 6: Chromatogram of rivaroxaban

Sensitivity (LOD and LOQ)

The concentration of favipiravir for determination of LOD was 2.173µg/mL, which indicates the sensitivity of the method. Similarly, LOQ was found to be 6.586µg/mL, which indicates favipiravir can be estimated at low concentrations.

Linearity

The response of the drug was found to be linear in the concentration range 5-25µg/mL. The linear regression equation for favipiravir was y = 0.1036x -0.0444 and r^2 value is 0.9991. The regression analysis details of calibration graph for the developed RP- HPLC method was shown in table 3.

Table 3: Calibration curve data

S. No	Concentration (µg/mL)	Peak area ratio*
1	5	0.493
2	10	0.95
3	15	1.53
4	20	2.03
5	25	2.542

***Average of three determination (n=3)

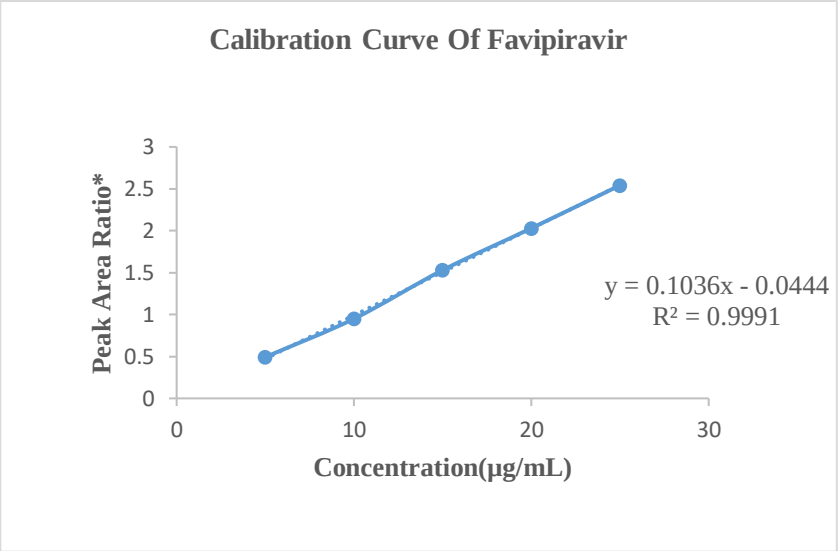

Fig 7: Calibration curve of favipiravir

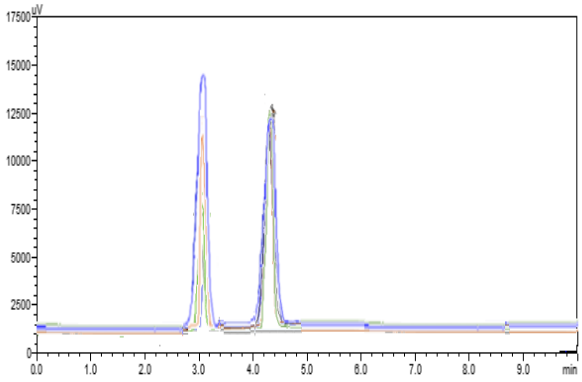

Fig 8: Linearity-overlay chromatogram of favipiravir and rivaroxaban(Internal standard)

The response of the drug was found to be linear in the concentration range 5-25µg/mL. The linear regression equation for favipiravir was y = 0.1036x -0.0444 and r^2 value is 0.9991. The regression analysis details of calibration graph for the developed RP- HPLC method was shown in table 4.

Table 4: Regression analysis of calibration graph for the developed RP-HPLCmethod

Parameters	
Concentration range	5-25µg/mL
Slope	0.1036
Standard error of Intercept	0.030515
Standard deviation of intercept	0.068234
Correlation	0.9991
Regression equation	0.1036x -0.0444

Acceptance criteria: Coefficient of determination: < 0.999

Precision

The precision of the developed method was determined in terms of repeatability, inter and intra-day precisions. The %RSD was calculated for the mentioned parameters and they were found to be within the acceptable limits. The data of precision studyobtained is shown in table5.
The chromatograms of precision studies are shown in the overlay spectrum in fig 9.

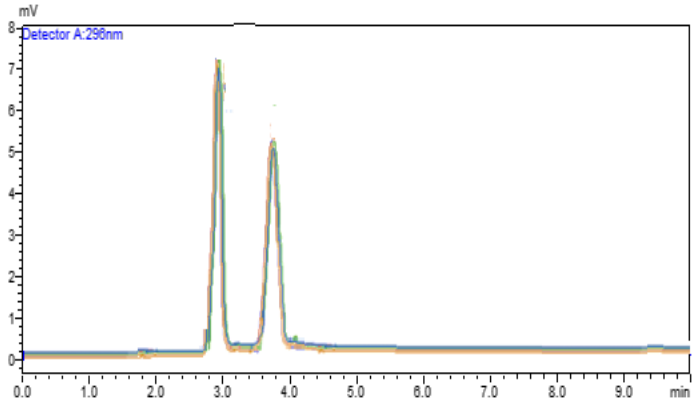

Fig 9: Overlay Chromatogram of Precision (Repeatability)

Table 5: Repeatability data

S. No	Concentration (µg/mL)	Peak area ratio	Mean*± Standard deviation	%RSD
1	15	1.5595	1.55425 ± 0.01775	1.14 %
2	15	1.5316		
3	15	1.5684		
4	15	1.5318		
5	15	1.5668		
6	15	1.5674		

*- Average of six determinations.

Table 6: Intra-day precision data

S. No	Concentration (µg/mL)	Mean peak area ratio*		Mean*± Standard deviation	% RSD
1	12	Morning	1.4447	1.4575± 0.01115	0.76%
		Afternoon	1.4627		
		Evening	1.4651		
2	15	Morning	1.1604	1.2066± 0.00465	0.38%
		Afternoon	1.2060		
		Evening	1.2534		
3	18	Morning	1.5175	1.5235 ± 0.005724	0.37%
		Afternoon	1.5289		
		Evening	1.5241		

*- Average of three determinations

Table 7: Inter-day precision data

S. No	Concentration (µg/mL)	Mean peak area ratio*		Mean*± Standard deviation	% RSD
1	12	Day-1	0.8686	0.8527 ± 0.01404	1.64%
		Day-2	0.8418		
		Day-3	0.8479		
2	15	Day-1	1.1768	1.1914 ± 0.01507	1.25%
		Day-2	1.1905		
		Day-3	1.2069		
3	18	Day-1	0.9273	0.9163 ± 0.012914	1.40%
		Day-2	0.9021		
		Day-3	0.9196		

*- Average of three determinations

The calculated %RSD values are very low indicating that the method was precise.
Acceptance criteria: The % RSD for peak area ratio of analyte solution should not be more than 2%.

Accuracy (Drug substance)

Accuracy of the method was determined at three different concentration levels (80%,100% and 120%). Mean and %RSD values were calculated and are shown in Table 8. The chromatograms of accuracy study are show in fig 10.

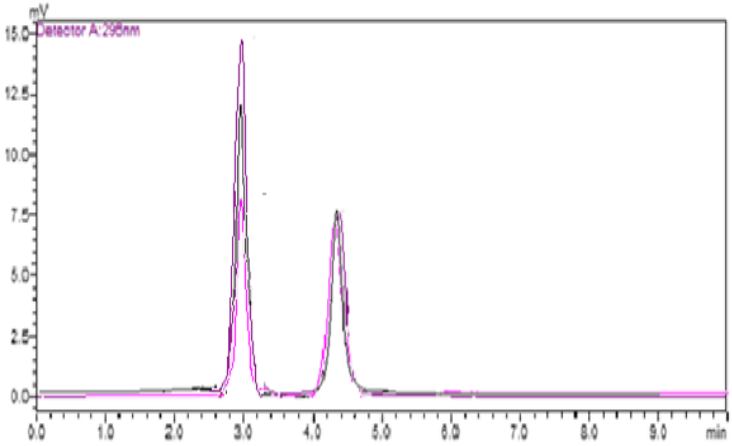

Fig 10: Accuracy Overlay chromatogram of favipiravir

Table8: Accuracy (Drug substance) data

S. No	Concentration (µg/mL)	Peak area ratio	Mean*± Standard deviation	% RSD
1	12	1.4547 1.4427 1.4561	1.451167± 0.007366	0.50%
2	15	1.1684 1.212 1.2443	1.208233± 0.003809	0.31%
3	18	1.5297 1.5219 1.5273	1.5243± 0.004686	0.30%

*-Average of three determinations

As the %RSD values were found to be within the acceptable limits the results indicate that the method was accurate.
Acceptance criteria: The % RSD for peak area ratio of analyte solutions should not be more than 2%.

Recovery Studies (Drug Product)

Recovery of Favipiravir from pharmaceutical dosage form ranged from 97.4-99.8 %.
The results are given in table-9 and Chromatograms are shown in fig 11.

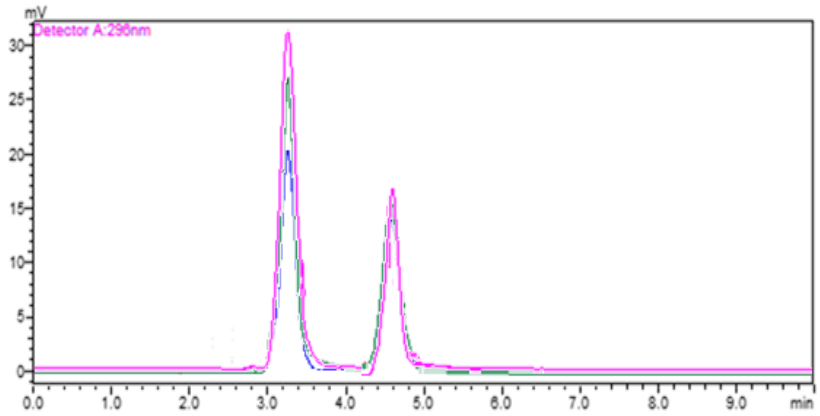

Fig 11: Recovery studies- Overlay chromatogram of favipiravir

Table 9: Recovery Studies Data

S. No	Conc of tablet solution taken (µg /mL)	Conc of standard solution added (µg/ mL)	Peak area ratio	Mean*	Conc found (µg/mL)	Percentage recovery
1	12	15	1.1400 1.1501 1.1402	1.1433	26.30	97.4%
2	15	15	2.0010 1.9002 2.0996	2.0002	29.41	98.0%
3	18	15	2.9732 3.0026 3.0010	2.9922	32.96	99.8%

The observed results indicate that the analyte was consistent and reproducible.
Acceptance criteria: % Recovery should be between, 80% to 120%

Robustness:

In different deliberate varied chromatographic conditions (flow rate and pump B concentration) all analytes were adequately resolved and elution orders remained the same. Results are given in table and chromatograms are shown in fig 10.

Table 10: Robustness data (Flow rate)

S. No	Flow rate	Concentration	Mean peak area ratio (n=3)	Mean*± Standard deviation	% RSD
1	0.9mL/ min	15µg/mL	1.6625	1.658967 ± 0.010841	0.65 %
2	1.0mL/ min	15µg/mL	1.6676		
3	1.1mL/ min	15g/mL	1.6468		

*-Average of three determinations

Table 11: Robustness data (Pump B concentration)

S. No	Pump B Concentration	Concentration	Mean peak area ratio (n=3)	Mean*± Standard deviation	% RSD
1	69%	15µg/mL	1.6264	1.6371 ± 0.011173	0.67 %
2	70%	15µg/mL	1.6487		
3	71%	15µg/mL	1.6363		

*- Average of three determinations

As the %RSD values were found to be within the acceptable limits the results indicate that the method was accurate.
Acceptance criteria: The % RSD for peak area ratio of analyte solutions should not be more than 2%.

Analyte Solution Stability Studies:

There was no much variation in the absorbance values and thus the solution of favipiravir in acetonitrile (HPLC grade) was found to be stable for 6-hour cycle. The solution stability data is shown in table 4.1 and the overlay spectrum is shown in fig.12.

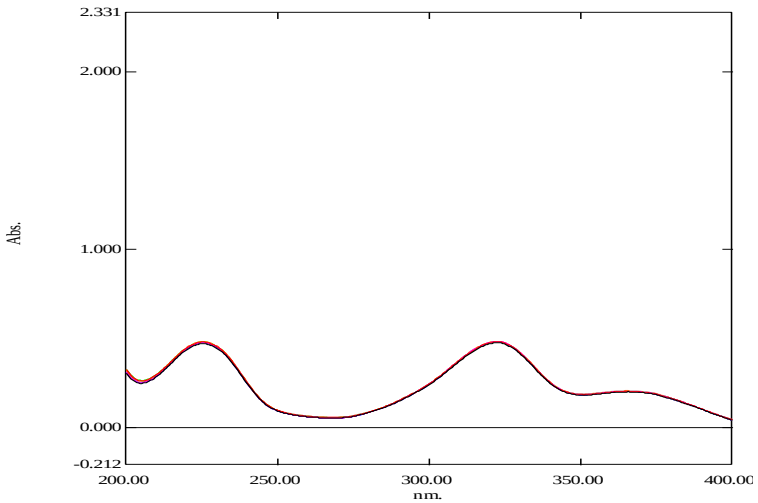

Fig.12: Overlay UV-spectrum of solution stability of favipiravir

Table 12: Solution stability of favipiravir (0-6 hour)

Time Point (hours)	Absorbance
0	0.474
0.5	0.479
1	0.480
2	0.480
4	0.481
6	0.480

There was no much variation in the absorbance values and thus the solution of rivaroxaban in acetonitrile (HPLC grade) was found to be stable for 6-hours cycle.The solution stability data is shown in table 4.2 and the overlay spectrum is shown in fig.13.

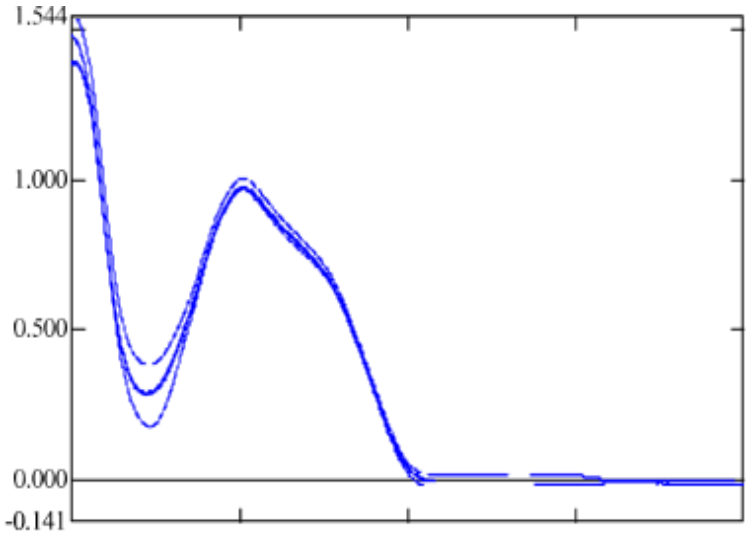

Fig.13: Overlay UV-spectrum of solution stability of rivaroxaban

Table 13: Solution stability of rivaroxaban (0-6 hour)

Time Point (hours)	Absorbance
0	0.995
0.5	0.995
1	0.990
2	0.992
4	0.994
6	0.996

As the analyte solutions were found to be stable in acetonitrile (HPLC grade) solvent, analysis can be carried on.

Assay

The validated HPLC method was used to determine the favipiravir in commercially available tablets (200 mg). 20 tablets were weighed and average weight was calculated, from the tablet powder equivalent to one tablet weight was taken and it was dissolved in methanol and it was centrifuged at 5000rpm for 10mins. The supernatant was collected and allowed to dry and the drug was collected and dissolved in acetonitrile volume was made up to the mark. This solution was further diluted to get a solution having concentration of 15µg/mL and analyzed. Assay results are shown in table 12.

Table 12: Assay data

Brand Name of Tablets	Label Claim of drug	Mean peakarea ratio* ± Standard deviation	Percentage purity	Amount of Drug present
Fabiflu	200mg	1.2534 ± 0.000208	107.76%	215.5mg

*Average of three determinations

Acceptance criteria: Percentage purity of the analyte solution should be in the range of 80% to 120%

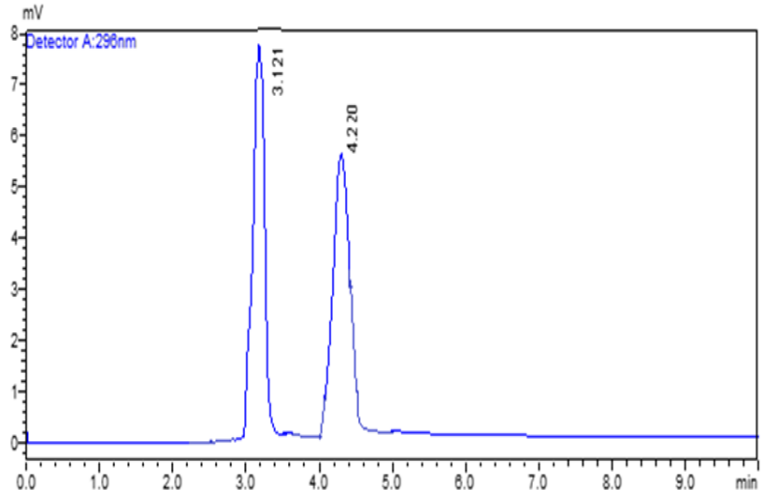

Fig:12: Assay chromatogram of favipiravir (15µg/mL)

Conclusion

A sensitive RP-HPLC method was developed for the quantitative estimation of Favipiravir in bulk and pharmaceutical dosage form. The developed method was optimized prior to validation studies in terms of stationary phase, mobile phase composition and flow rate. The chromatographic peak of FVP was eluted at a retention time of 3.110 mins and that of

IS Rivaroxaban was at 4.287 mins. The linear regression equation for favipiravir was $y = 0.1036x - 0.0444$ and r^2 value is 0. 9991.The developed method was subjected to method validation as recommended by ICH Q2 (R1) guidelines. A mobile phase without preparation of any buffer solution or adding ion-pairing agents and a short retention time are advantageous. From the results obtained it is concluded that the developed method was found to be selective, sensitive, linear, accurate, reproducible, robust and applicability of the method can be studied in biological samples.

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