FORM-2

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COMPLETE SPECIFICATION

(SECTION 10, RULE 13)

TITLE OF THE INVENTION

RP-UPLC METHOD FOR ESTIMATION OF FAVIPIRAVIR IN PHARMACEUTICAL DOSAGE FORM AND USES THEREOF

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The following specification particularly describes the invention and the manner in which it is to be performed.

Technical Field of the Invention

The present invention relates to RP-UPLC method for estimation of favipiravir. The present invention more specifically relates to RP-UPLC method for estimation of favipiravir in pharmaceutical dosage form. The present invention provides a simple, precise and accurate RP-UPLC analytical method for estimation of favipiravir.

Background of the Invention

Favipiravir is chemically 6-fluoro-3-hydroxy-2-pyrazinecarboxamide. Favipiravir has antiviral activity against influenza virus and coronavirus. Liquid chromatography determination of Favipiravir can provide quality control method for routine analytical laboratory use. Patent CN112763622A discloses method of measuring liquid chromatography, with steps of preparing favipiravir standard and sample solution, and blank, standard and sample of 20 µl are injected into HPLC system, at column temperature 35 DEG C and flow rate is 1.0 mL/min and finally recording peak area and calculating favipiravir content by external standard method. CN113252800A relates to a detecting favipiravir and related substances by HPLC, using octadecylsilane column, column temperature 25-35 DEG C, detection wavelength is 220-250 nm, mobile phase A of aqueous solution with pH value of 3.5-6.0, mobile phase B as an organic solvent. The method had good resolution, and has usefulness as quality control method for analysis of favipiravir. Ultra-performance liquid chromatography (UPLC) has advantages of turnaround time, process dependability, method sensitivity, and drug specificity. There is still a need to develop a fast, simple, accurate and precise method for the estimation of favipiravir in pharmaceutical dosage form by reverse phase ultra-performance liquid chromatography (RP-UPLC).

Objects of the Invention

The main object of the present invention is to provide a RP-UPLC method for estimation of favipiravir in pharmaceutical dosage form.

Another object of the present invention is to provide simple, precise and accurate analytical method for estimation of favipiravir.

Summary of the Invention

The present invention relates to a RP-UPLC method for estimation of favipiravir in pharmaceutical dosage form.

In an embodiment of the present invention, the RP-UPLC method for estimation of favipiravir, comprising of dissolving Favipiravir using acetonitrile and 0.1% Ortho phosphoric acid as mobile phase in the ratio of 40:60 %v/v, running chromatogram through column C18, 100mm x 2.1 mm, 1.8m using mobile phase, optimizing conditions of column at flow rate 0.3ml/min, detecting wavelength at 226nm, injecting volume 0.50µL; and column temperature at 30ºC; running the sample and recording chromatogram from the chromatograph for estimation of Favipiravir.

In an embodiment of the present invention, the method for simultaneous estimation of Favipiravir, wherein the linearity ranges between 25% to150% levels, R^2 value 0.999, precision 1.0 for repeatability and 0.3 for intermediate precision, LOD 0.12µg/ml and LOQ 0.35µg/ml. The developed method is specific for the estimation of favipiravir in the bulk and pharmaceutical dosage forms. The present RP-UPLC method has excellent sensitivity, precision and reproducibility.

Brief Description of drawings

In the drawings accompanying the specification, Figure 1 shows linearity concentration and response of Favipiravir.

In the drawings accompanying the specification, Figure 2 shows the optimized chromatogram of Favipiravir.

Detailed description of the Invention

The present invention provides a RP-UPLC (Reversed Phase Ultra-Performance Liquid Chromatography) method for estimation of favipiravir in pharmaceutical dosage form. The RP-UPLC method for simultaneous estimation of favipiravir, comprising dissolving Favipiravir using acetonitrile and 0.1% Ortho phosphoric acid as mobile phase in the ratio of 40:60 %v/v, running chromatogram through column C_{18}, 100mm x 2.1 mm, 1.8m using mobile phase, optimizing conditions of column at flow rate 0.3ml/min, detecting wavelength at 226nm, injecting volume 0.50µL; and column temperature at 30ºC; running the sample and recording chromatogram from the chromatograph for estimation of Favipiravir.

In an embodiment of the present invention, the method for simultaneous estimation of Favipiravir, wherein the linearity ranges between 25% to150% levels, R^2 value 0.999, precision 1.0 for repeatability and 0.3 for intermediate precision, LOD 0.12µg/ml and LOQ 0.35µg/ml. The developed method is specific for the estimation of favipiravir in the bulk and pharmaceutical dosage forms. The present RP-UPLC method has excellent sensitivity, precision and reproducibility.

MATERIALS AND METHODS

Favipiravir pure drugs (API), Combination Favipiravir tablets (Fabiflu), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem™.

Equipment and Apparatus used:

UPLC instrument used with Auto Injector and PDA Detector. Software is used for detection. UV-VIS spectrophotometer with special bandwidth of 2mm and 10mm and matched quartz used for measuring absorbance for Favipiravir solutions. Based on drug solubility and Pka value following conditions are used to develop the method estimation of Favipiravir [Ramakrishna, B., Mondal, S. and Chakraborty, S., Development and Validation of Novel Method for the Determination of Favipiravir and Peramivir Using Reverse Phase Ultra Performance Liquid Chromatography; Balu, P.A. and Paresh, M.S., 2021. Stability-indicating RP HPLC method development for estimation of favipiravir in bulk and pharmaceutical dosage form. World Journal of Pharmaceutical Research, 10(14), pp.1444-1465; Yamani, N.S. and Annapurna, M.M., 2022. Stability Indicating RP-HPLC Method for the estimation of Favipiravir in API and Pharmaceutical Dosage Forms (Tablets). Research Journal of Pharmacy and Technology, 15(12), pp.5700-5706; Bulduk İ. (2021). Comparison of HPLC and UV Spectrophotometric Methods for Quantification of Favipiravir in Pharmaceutical Formulations. Iranian journal of pharmaceutical research: IJPR, 20(3), 57–65; Bulduk, İ., 2021. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatographica, 33(3), pp.209-215].

Optimized Method

Optimized Chromatographic Conditions

Column	:	STD SB C18 100mm x 2.1 mm, 1.8m.
Mobile phase	:	Acetonitrile:OPA (40:60)
Flow rate	:	0.3 ml/min
Detector	:	Acquity TUV 226nm
Temperature	:	30°C
Injection Volume	:	0.50µL

The optimized chromatogram of Favipiravir is provided in figure 2.

Observation: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and buffer taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 50mg of Favipiravir transferred 50ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of Favipiravir).

Preparation of Standard working solutions (100% solution): 1ml of Favipiravir from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Favipiravir).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/ml of Favipiravir).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (150µg/ml of Favipiravir)

Preparation of buffer

0.1%OPA Buffer: 1ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

0.01N KH2PO4 Buffer: Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of Milli-Q® water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.5 with dil. orthophosphoric acid solution.

SYSTEM SUITABILITY

A standard solution of favipiravir working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from six replicate injections are within the range and Results were shown in table 1.

Table 1: System Suitability Parameters

Peak Name: Favipiravir

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Favipiravir	1.064	904975	3053	1.32
2	Favipiravir	1.069	906470	2520	1.19
3	Favipiravir	1.081	912222	3127	1.19
4	Favipiravir	1.083	919282	2921	1.18
5	Favipiravir	1.084	929224	2865	1.18
6	Favipiravir	1.084	913447	2843	1.19
Mean			914270		
Std. Dev.			8953.8		
% RSD			1.0		

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Favipiravir (150ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (1000 µg/ml of Favipiravir).

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (150µg/ml of Favipiravir).

Six working sample solutions of 100ppm are injected and the % Amount found was calculated and %RSD was found to be 0.3 and chromatogram was shown in table 2.

Table 2. Repeatability data

Sr. No	**Peak Area**
1	916173
2	912235
3	919364
4	915180
5	917451
6	911346
AVG	915292
STDEV	3064.2
%RSD	0.3

Intermediate precision: Five working sample solutions of 100ppm are injected on the next day of the preparation of samples and the % amount found was calculated and %RSD was found to be 0.4. Table 3 shows intermediate precision data.

Table 3. Intermediate precision data

Sr. No	Peak Area
1	882996
2	884641

3	880651
4	889903
5	886486
6	884433
AVG	884852
STDEV	3146.3
%RSD	0.4

Linearity:

Preparation of Standard stock solutions: Accurately weighed 50mg of Favipiravir transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of Favipiravir).

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Favipiravir)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Favipiravir)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Favipiravir)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (100µg/ml of Favipiravir)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (125µg/ml of Favipiravir)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (150µg/ml of Favipiravir)

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 25 ppm to 150 ppm of Favipiravir. Plot a graph to concentration versus peak area. Slope obtained was 9096 Y-intercept was 716.8 and correlation co-efficient was found to be 0.999. Table 4 and Figure 1 shows linearity concentration and response.

Table 4. Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	25	229884
50	50	450335
75	75	682860
100	100	916240
125	125	1142473
150	150	1358768

Accuracy:

Preparation of Standard stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (1000µg/ml of Favipiravir).

Preparation of 50% Spiked Solution: 0.25ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 0.75ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The %recovery for each level should be between 98.0 to 102.

Three Concentrations of 50%, 100%, 150% are injected in a triplicate manner and %recovery was calculated as 100.04. Table 5 shows accuracy data.

Table 5. Accuracy data

% Level	Amount Spiked	Amount recovered	% Recovery	Mean %Recovery

	(µg/mL)	(µg/mL)		
50%	50	49.98	99.97	
	50	50.43	100.86	
	50	50.14	100.29	
100%	100	99.48	99.48	
	100	101.50	101.50	100.04%
	100	99.92	99.92	
150%	150	149.19	99.46	
	150	148.91	99.28	
	150	149.38	99.59	

Robustness: Small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guidelines.

Robustness conditions like Flow minus (0.7ml/min), Flow plus (0.9ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit. Table 6 shows Robustness Data.

Table 6. Robustness Data

Parameter	%RSD
Flow Minus	0.3
Flow Plus	1.4
Mobile phase Minus	0.3
Mobile phase Plus	0.3
Temperature minus	0.2
Temperature plus	0.8

LOD sample Preparation:

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml

Favipiravir, were transferred to 10ml volumetric flasks and made up with the same diluents

Detection limit of the Favipiravir in this method is found to be 0.12/ml.

LOQ sample Preparation

0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml Favipiravir, were transferred to 10ml volumetric flasks and made up with the same diluents.

Quantification limit of the Favipiravir in this method was found to be 0.35µg/ml.

Assay Methodology

Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae.

Calculate the percentage purity of Favipiravir present in tablet using the formula:

Calculation:

$$\text{Assay} = \frac{\text{Spl area}}{\text{Std area}} \times \frac{\text{Std. Dil. Fac}}{\text{Spl. Dil. Fac}} \times \frac{\text{Avg. Wt of Tab}}{\text{L.C}} \times \text{Potency of Std}$$

Spl area – Sample Peak area

Std area – Standard Peak area

Std. Dil. Fac- standard dilution factor

Spl. Dil. Fac- sample dilution factor

Avg. Wt of Tab- average weight of tablet

L.C – lable claim

Potency of Std

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula. Table 7 shows assay of formulation.

Table 7. Assay of Formulation

Sample No	%Assay

1	100.01
2	99.58
3.	100.36
4.	99.90
5.	100.15
6.	99.48
AVG	99.91
STDEV	0.33
%RSD	0.3

Table 8 shows summary table of parameters.

Table 8. Summary Table of parameters

Parameters		**Favipiravir**	**LIMIT**
Linearity Range (µg/ml)		25-150 µg/ml	R< 1
Regression coefficient		0.999	
Slope(m)		9096.3	
Intercept(c)		716.89	
Regression equation (Y=mx+c)		Y= 9096.3x + 716.89	
Assay (% mean assay)		99.91%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		1.0	NMT 2.0%
Method precision %RSD		0.3	NMT 2.0%
Accuracy %recovery		100.04%	98-102%
LOD		0.12	NMT 3
LOQ		0.35	NMT 10
Robustness	**FM**	0.3	%RSD NMT 2.0
	FP	1.4	
	MM	0.3	
	MP	0.3	

	TM	0.2	
	TP	0.8	

We claim:

1. A method for estimation of Favipiravir in pharmaceutical dosage form, comprising:

a) dissolving Favipiravir using acetonitrile and 0.1% Ortho phosphoric acid as mobile phase in the ratio of 40:60 %v/v;

b) running chromatogram through column C_{18}, 100mm x 2.1 mm, 1.8m using mobile phase of step a);

c) optimizing conditions of column of step b) at flow rate 0.3ml/min, detecting wavelength at 226nm, injecting volume 0.50µL; and column temperature at 30ºC;

d) running the sample of step c) and recording chromatogram from the chromatograph for estimation of Favipiravir.

2. The method for simultaneous estimation of Favipiravir as claimed in claim 1, wherein the linearity ranges between 25% to150% levels, R^2 value 0.999, precision 1.0 for repeatability and 0.3 for intermediate precision, LOD 0.12µg/ml and LOQ 0.35µg/ml.

Dated this 22nd day of February, 2023

To be signed digitally by
(Sanchita Tewari)
Agent for the Applicant
Patent Agent (IN/PA 2711)

Abstract

RP-UPLC METHOD FOR ESTIMATION OF FAVIPIRAVIR IN PHARMACEUTICAL DOSAGE FORM AND USES THEREOF

The present invention provides a simple, accurate and precise method for the estimation of favipiravir in pharmaceutical dosage form. The present invention relates a method for the estimation of favipiravir by RP-UPLC in bulk and tablet dosage forms. The method for simultaneous estimation of favipiravir in pharmaceutical dosage form, comprising of dissolving Favipiravir using acetonitrile and 0.1% Ortho phosphoric acid as mobile phase in the ratio of 40:60 %v/v, running chromatogram through column C_{18}, 100mm x 2.1 mm, 1.8m using mobile phase, optimizing conditions of column at flow rate 0.3ml/min, detecting wavelength at 226nm, injecting volume 0.50µL; and column temperature at 30ºC; running the sample and recording chromatogram from the chromatograph for estimation of Favipiravir. The method for simultaneous estimation of Favipiravir, wherein the linearity ranges between 25% to150% levels, R^2 value 0.999, precision 1.0 for repeatability and 0.3 for intermediate precision, LOD 0.12µg/ml and LOQ 0.35µg/ml. The developed method is specific for the estimation of favipiravir in the bulk and pharmaceutical dosage forms. The present RP-UPLC method has excellent sensitivity, precision and reproducibility.