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Original Article

STABILITY INDICATIVE AND COST EFFECTIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FAVIPIRAVIR AND PERAMIVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredients of Favipiravir and Peramivir and their related substances.

Methods: A simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Favipiravir and Peramivir. The chromatographic strategy utilized Inertsil ODS column of dimensions 250x4.6 mm, 5 micron, using isocratic elution with a mobile phase of acetonitrile and 0.1 percent orthophosphoric acid (70:30). A flow rate of 1 ml/min and a detector wavelength of 260 nm utilizing the PDA detector was given in the instrumental settings. Using the impurity-spiked solution, the chromatographic approach was streamlined.

Results: Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. LOD and LOQ for the two active ingredients and their impurities were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R^2>0.999, which means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness was determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in RS condition. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Favipiravir, Peramivir, RP-HPLC, Development, Validation

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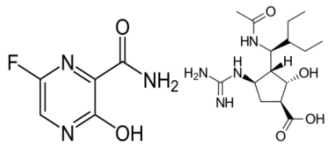
INTRODUCTION

Peramivir (trade name Rapivab) is an antiviral medication developed for the treatment of influenza by BioCryst pharmaceuticals [1]. In October 2009, the experimental antiviral drug peramivir was confirmed to be "life saving" effective in the intravenous treatment of 8 extreme cases of swine flu [2]. On October 23, the U. S. An emergency use authorization for peramivir was provided bt the Food and Drug Administration (FDA), authorizing the use of the drug in intravenous form for hospitalized parents only in cases where the other treatment options available are inadequate or unavailable [3]. Two new anti-influenza medications were launched clinically in Japan in 2010. Rapiacta (peramivir), which is provided by intravenous drip injection, was one of them [4-6]. Peramivir was the first intravenous neuraminidase inhibitor (NAI) approved by the US food and drug administration (FDA) for the treatment of influenza [7]. Data from randomized controlled trails have shown that peramivir is safe and well-tolerated in uncomplicated influenza, with similar or superior clinical efficacy to those of placebo and oseltamivir [8-10]. Signs of an allergic reaction hive, difficult breathing, swelling in face or throat, severe skin reactions [11]. Favipiravir, sold under the brand name Avigan among others, is an antiviral medication used to treat influenza in Japan [12]. It is also being studied to treat a number of other viral infections [13]. There is evidence that use of during pregnancy may result in harm to the baby [14, 15]. Research in 2014, suggested that Favipiravir may have efficacy in humans, it went unaddressed.



A **B**

Fig. 1: Structure of (A) Favipiravir and (B) Peramivir

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade orthophosphoric acid, water, were purchased from Merck India Ltd, Mumbai, India. APIs of Favipiravir, Peramivir standards were procured from Glen mark, Mumbai.

The instrumentation

Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of photodiode array (model 2998) was used for this study [16].

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode were tested. However, the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally, 0.1% OPA buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredient and their impurities. During the optimization of the method various stationary phases such as C_8, C_{18} phenyl and amino, inertsil ODS columns were tested. From these trials the peak shapes were relatively good with a inertsil ODS column of 250 x 4.6 mm, 5 µ with a PDA detector. The mobile phase flow rate has been done at 254 nm in order to obtain enough sensitivity. By using above conditions we get retention times of Favipiravir and Peramivir were about 3.003 and 7.271 min with a tailing factor of 1.063 and 1.401. The number of theoretical plates for Favipiravir and Peramivir were 2089456,1889412 which indicate the column's successful output the % RSD for six replicate injections was around 0.94% the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

There are some HPLC methods [17-19] reported in the literature, but these methods are developed only for routine analysis of ESC

and FLU in bulk and formulation studies. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method. Different extractions were tried using acetonitrile, methanol, and dimethylformamide [20].

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [21-29].

Preparation of buffer

1 ml of ortho phosphoric acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

Chromatographic conditions

The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% OPA and Inertsil ODS column (250x4.6 mm, 5 µ) column with a flow rate of 1 ml/min.

Diluent

Water and Acetonitrile in the ratio (50:50) is used as diluent.

Preparation of the standard stock solution

For standard stock solution preparation, add 70 ml of diluents to 100 mg of Favipiravir and 100 mg of Peramivir taken in a 100 ml volumetric flask and sonicate for 10 min to fully dissolve the contents and then make up to the mark with diluent.

Preparation of standard solution

1 ml of solution is drawn from the above normal stock solution into a 10 ml volumetric flask and diluted up to the level.

Preparation of sample solution

Take 130 mg of the sample drug Favipiravir and 100 mg of the sample drug Peramivir into a 100 ml volumetric flask and add 70 ml of diluents and sonicate for 10 min to fully dissolve the contents and then make up the mark with diluent. This solution is filtered into a device using a 0.45µ nylon syringe in a vial.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients from their impurities. In order to provide a good performance, the chromatographic conditions were optimized.

System suitability

In System, suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Specificity

In this test method placebo, sample and standard solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Favipiravir	Peramivir
USP Plate Count	NLT 2000	38417	3264
USP Tailing	NMT 2.0	1.07	1.04
USP Resolution	NLT 2.0	-	8.64
% RSD	NMT 2.0	0.71	0.89

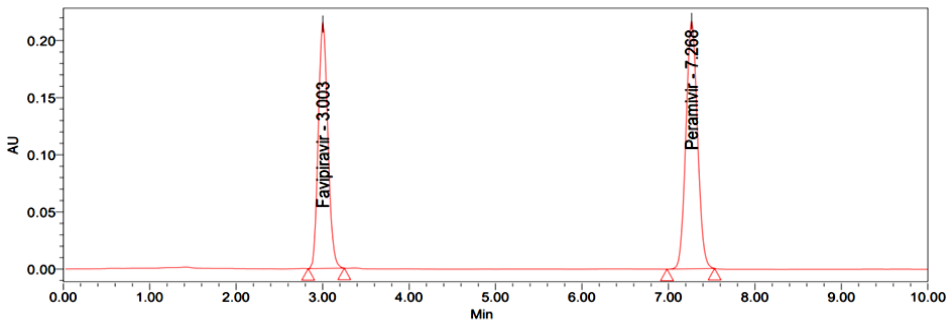


Fig. 2: Chromatogram of system suitability

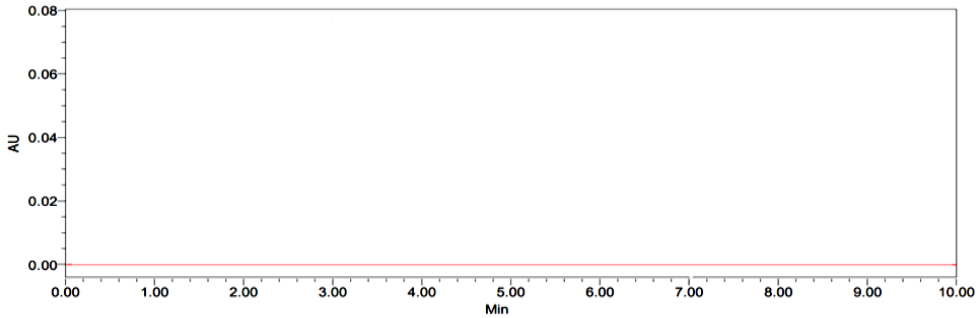
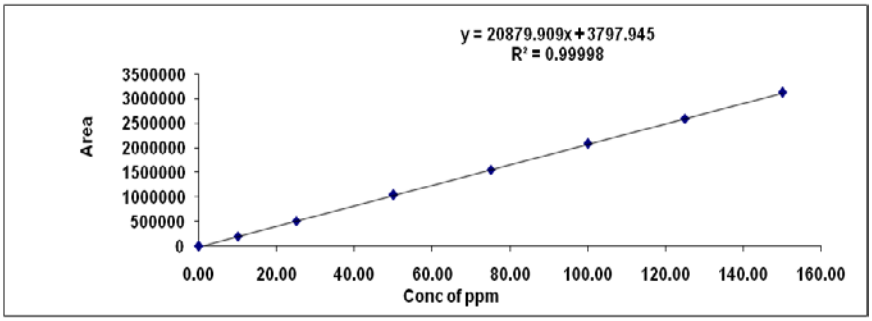


Fig. 3: Chromatogram of blank

Table 2: Linearity of favipiravir and peramivir

S. No.	Conc µg/ml	Favipiravir area count	Conc. µg/ml	Peramivir area count
1	10	208946	10	188941
2	25	509623	25	460832
3	50	1044728	50	944706
4	75	1554351	75	1405538
5	100	2089456	100	1889419
6	125	2599079	125	2350244
7	150	3134184	150	2834118
Correl coef		0.99998		0.9999
Slope		20879.909		18880.871
intercept		3797.945		3434.331



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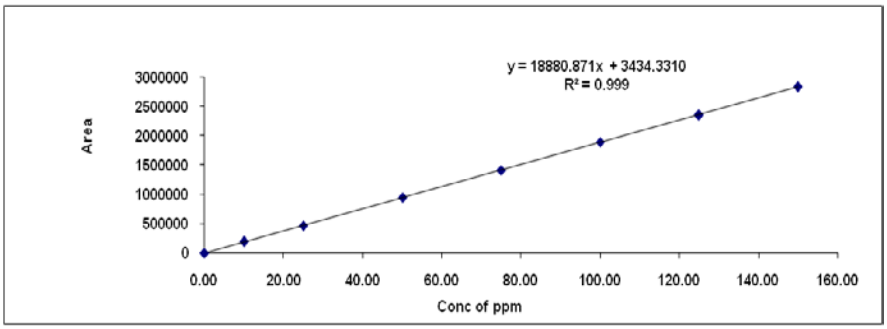

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Fig. 4: Calibration plots of (A) Favipiravir (B) Peramivir

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Favipiravir, Peramivir, as 10,25,50,75,100,125,150 percent respectively. Linearity was performed in the range of 10-150µg/ml of Favipiravir and 10-150µg/ml of Peramivir. The correlation coefficients achieved greater than 0.999 for all.

Accuracy

In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution spiked with known amounts of all the impurities at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. For all impurities, percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of 100.154-100.624% for Favipiravir and 99.512-99.918% for Peramivir. The results are given in table 3, 4 and 5.

Precision

In method precision study prepare six different samples in the concentration of Favipiravir (100 ppm) and Peramivir (100 ppm) are injected into HPLC system. Favipiravir %assay found to be in the range of 100.136-100.561 and Peramivir %assay found to be in range of 100.261-100.517. These results are given below table 4.

Table 3: Results of accuracy

S. No.	% Level	Favipiravir % recovery	Peramivir % recovery
1	50	100.154	99.512
2	100	100.624	99.861
3	150	100.417	99.981
mean		100.534	99.78467
SD		0.106108	0.24364

Mean+SD (n=3)

Table 4: Intraday precision results of favipiravir and peramivir

Favipiravir				Peramivir		
S. No.	**Conc.(µg/ml)**	**Area counts**	**% assay as is**	**Conc.(µg/ml)**	**Area counts**	**% assay as is**
1		2089456	100.561		1889419	100.264
2	100	2089451	100.478	100	1889426	100.517
3		2089444	100.362		1889435	100.284
4		2089441	100.287		1889441	100.361
5		2089420	100.514		1889422	100.485
6		2089450	100.136		1889434	100.234
% RSD	1.06			0.918		
mean	100.3897			100.3575		
SD	0.160168			0.119252		

Mean+SD (n=6)

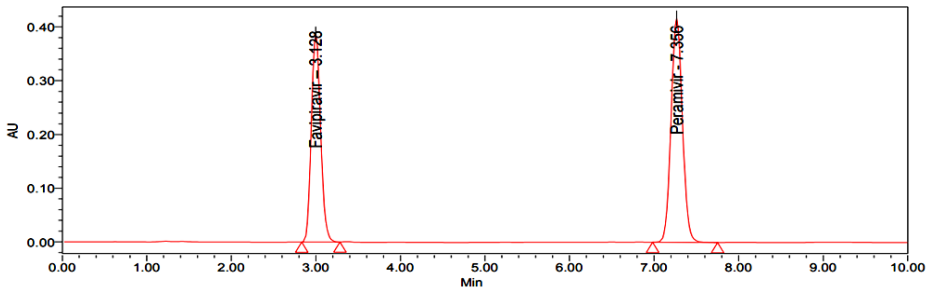


Fig. 5: Chromatogram of sample

Intermediate precision

Six replicates of the sample solution were studied by various researchers, and on separate days different instruments were tested. The peak regions used to determine mean percent RSD values have been calculated. The results are given in the following table.

Intraday precision

Six replicates of a sample solution containing Favipiravir (100µg/ml) and Peramivir (100µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Interday precision

Six replicates of a sample solution containing Favipiravir (100µg/ml) and Peramivir (100µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5 [16].

Table 5: Inter-day outcomes of accuracy of favipiravir and peramivir

Favipiravir				Peramivir		
S. No.	**Conc.(µg/ml)**	**Area counts**	**% assay as is**	**Conc.(µg/ml)**	**Area count**	**% assay as is**
1		2089451	100.241		1889414	100.482
2	100	2089461	100.054	100	1889406	100.612
3		2089312	100.354		1889396	100.536
4		2089366	100.687		1889354	100.417
5		2089414	100.471		1889341	100.532
6		2089457	100.289		1889411	100.417
%RSD	0.89			1.04		
Mean	2089410			100.4993		
SD	60.078			0.07611		

Mean+SD (n=6)

LOD and LOQ

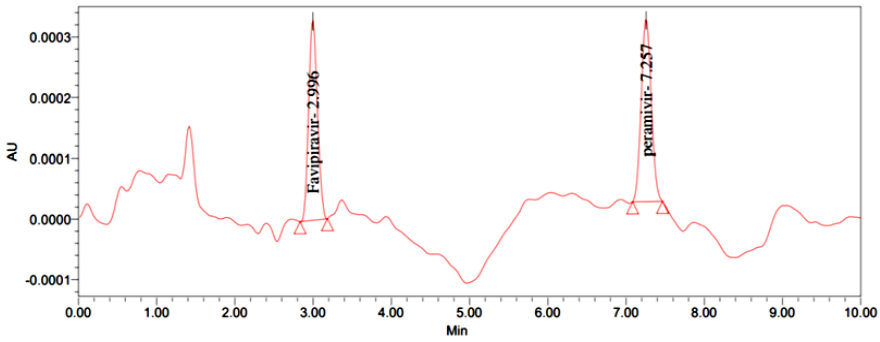
The LOD concentrations for Favipiravir are 1.818 µg/ml and s/n values is 8 and Peramivir 1.818 µg/ml and s/n value 6. The LOQ concentration for Favipiravir 6.248 µg/ml and their s/n values are 23 and Peramivir their 6.248 µg/ml and s/n value is 28. The method is validated as per the US FDA guidelines [30].

Robustness

The conditions of the experiment were designed to test the robustness of the established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for favipiravir and peramivir found to be within the limit and results are tabulated in table 7.

Table 6: LOD and LOQ for favipiravir and peramivir

Favipiravir				Peramivir			
LOD		**LOQ**		**LOD**		**LOQ**	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
1.818µg/ml	8	6.248µg/ml	23	1.818µg/ml	6	6.248µg/ml	28



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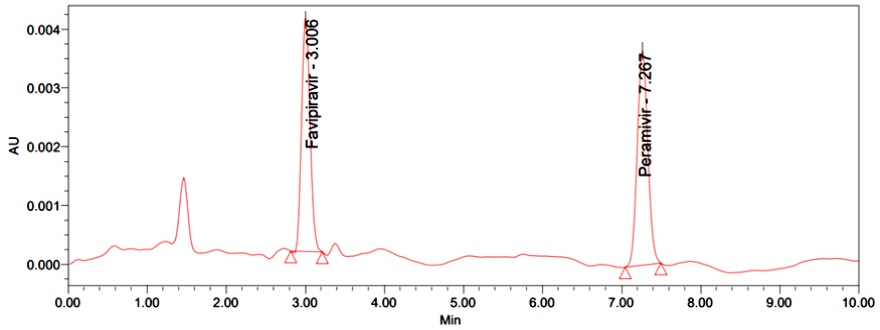

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Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Table 7: Robustness data of favipiravir and peramivir

Parameter name	% RSD	
	Favipiravir	Peramivir
Flow minus (0.8 ml/min	0.62	0.70
Flow plus (1.2 ml/min)	0.34	0.66
Organic minus (-10%)	0.56	0.26
Organic plus (+10%)	0.51	0.61

Stability

The standard and sample solution was kept at room temperature and at 2-8 °C up to 24 h. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 h [31]. There was no significant deviation observed and confirmed that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Favipiravir and Peramivir drugs. The results are given below table 8.

Table 8: Stability results of favipiravir and peramivir

Stability	Favipiravir		Peramivir	
	Purity	% of deviation	Purity	% of deviation
Initial	99.86	0.14	99.91	0.09
6 H	99.74	0.12	99.84	0.07
12 H	99.62	0.24	99.73	0.18
18 H	99.51	0.35	99.61	0.30
24 H	99.46	0.40	99.54	0.37

Degradation studies

The Peramivir and Favipiravir sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have been carried out to find out that the method is suitable for products of degradation. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities.

Acid degradation

In acid degradation was done at 1N HCl and degradation was formed 12.7% for Favipiravir and 13.5% for Peramivir.

Alkali degradation

In alkali degradation was done at 1N NaOH and degradation was formed 13.7% for Favipiravir and 14.7% for Peramivir.

Peroxide degradation

In peroxide degradation was peroformed at 20% hydrogen peroxide at 15.14% Favipiravir at 15.14% and 15.15% for Peramivir.

Reduction degradation

In reduction degradation, they formed 13.5% Favipiravir and Peramivir.

Table 9: Forced degradation results of favipiravir and peramivir

Degradation condition	Favipiravir		Peramivir	
	% Assay	% Deg	% Assay	% Deg
Acid degradation	87.246	12.754	86.421	13.579
Alkali degradation	86.271	13.729	85.284	14.716
Peroxide degradation	84.854	15.146	84.841	15.159
Reduction degradation	86.412	13.588	86.472	13.528
Thermal degradation	99.231	0.769	99.547	0.453
Hydrolysis degradation	99.457	0.543	99.145	0.855

Thermal degradation

In thermal degradation the sample was degradated 0.76% for Favipiravir and 0.45% for Peramivir.

Degradation of hydrolysis

In hydrolysis degradation the sample was degradated 0.54% for Favipiravir and 0.85% for Permaivir.

All degradation results are tabulated in table 9.

CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Favipiravir and Peramivir. All the products of degradation formed during the stress conditions and the related active pharma ingredients are well separated and peaks were well resolved from each other and separate with an appropriate retention time indicating that the proposed method to be fast, simple, feasible and affordable in RS condition. Therefore the developed method during stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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