STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FAVIPIRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM.

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Abstract:

In this work, a new sensitive and accurate high-performance liquid chromatographic method for simultaneous determination of Favipiravir used for the treatment of corona disease was developed and validated. Inertsil ODS-3V C18 (4.6 mm X 100mm, 3.0 µm) column used for the chromatographic separation. Isocratic elution of mobile phase consisting 10 mM potassium dihydrogen phosphate (pH 4.0): Acetonitrile (90:10% v/v) was used for the separation. UV detector was used and detection wavelength was performed at 229 nm. The method was linear over the concentration range of 10 to 100 µg/mL for Favipiravir. The proposed method was validated and successfully applied for commercial dosage form. Method performed was highly accurate, precise and robust. Stability study was performed on different stress condition. Maximum degradation was achieved in alkali degradation.

Key words- Favipiravir, RP-HPLC, Stability indicating assay method

Introduction:

In early 2020 new virus began generating headlines all over the world because of unprecedented speed of its transmission. The virus SARS-coV-2 has been responsible for millions of infections globally. The disease caused by an infection with SARS-coV-2 is called as COVID-19 which stands for coronavirus disease. Chinese-born coronavirus disease (COVID-19) spread rapidly and became an epidemic, affecting almost all countries and regions around the world. COVID-19 case death rate ranges from 1% to 7% according to the reports of World Health Organization (WHO).It caused all people in the world to change their lifestyle. It still threatens the entire World. Since the outbreak of the COVID-19 began to affect the world, countries have implemented different treatment methods

Various antiviral drugs have been used for the treatment of COVID -19 like Favipiravir, Remdasvir, Lopinavir, Ritonavir etc.

Favipiravir initially marketed as an ant influenza agent in Japan in 2014. Favipiravir was first used against SARS-Co-V-2 in Wuhan where the pandemic started. Then as pandemic spread to Europe, this drug received approval for emergency treatment in Italy and currently it has been used in Japan, Russia, Ukraine, Saudi Arabia and UAE. In June 2020 Favipiravir received DCGI approval in India. It has a wide therapeutic safety margin for high dose and is available as an first oral formulation for covid, since 80% of the patients infected with COVID-19 have mild to moderate severity, oral formulation is more convenient. Favipiravir is a drug of choice for treatment of SARS-Co-V-2 in mild to moderate patient due to rapid viral clearance, greater improvement in chest CT and Better clinical recovery rate as compared to other anti-viral drugs.

Favipiravir is incorporated into the cells and converted to the favipiravir ibofuranosyl-5-triphosphate (Favipiravir-RTP) by host cells. The triphosphate form inhibits the activity of RNA dependent RNA polymerase (RdRp) of RNA viruses. The active favipiravir-RTP selectively inhibits RNA polymerase and prevents replication of the viral genome

2. Material methods.

2.1 Instrumentation and chromatographic conditions

The analysis utilized a high-performance liquid chromatography setup featuring a Analytical HPLC system coupled with a UV detector. This configuration included a quaternary solvent delivery pump, automatic sample injector. Chromatographic separation employed a Inertsil ODS-3V C18 (4.6 mm X 100mm, 3.0 µm), with a flow rate of 0.8 mL/min and a column temperature of 25°C. The mobile phase consisted of 10 mM potassium dihydrogen phosphate (pH 4.0): Acetonitrile (90:10% v/v), yielding satisfactory retention time. Optimization occurred at 292 nm. The entire process, including data acquisition and processing, was managed using Lab solution software. Each run lasted 8 minutes, conducted at ambient temperature.

2.2. Materials

Favipiravir, active pharmaceutical ingredients, were generously provided as gift samples by BDR Pharmaceuticals, Baroda, Gujarat. The pharmaceutical dosage form (BDFAVI – BDR pharmaceuticals, Baroda, Gujarat) was procured from a nearby pharmacy. High-quality solvents required for this study, meeting HPLC (reverse phase high-performance liquid chromatography) standards, were sourced from Merck Specialties Private Limited in Mumbai.

2.3 Preparation of Mobile phase.

Combine 10mM Potassium dihydrogen phosphate buffer: Acetonitrile (90:10%v/v)

2.4 Buffer Preparation

Preparation of 10mM potassium dihydrogen phosphate buffer pH 4.0: Weigh 1.36 gm of potassium dihydrogen phosphate and dissolve into 1000 mL of water, adjust pH to 4.0 with Orthophophoric acid.

2.5 Preparation of Diluent:

10mM Potassium dihydrogen phosphate pH 4.0: Acetonitrile (40:60%v/v)

2.6 Preparation of standard solution. (50 µg/mL)

An accurately weighed amount of Favipiravir (5 mg) working standards were transferred into 25 mL clean dry volumetric flasks, dissolved by sonication and made up to the final volume with the same diluent. 5 ml of above stock solution was pipette out and transferred into 20 ml volumetric flaks and made up with same diluent.

2.7 Preparation of sample solution. (50 µg/mL)

Weigh accurately 10 tablets to find the average weight of favipiravir. Crush all the tablets to fine powder. Accurately weigh and transfer the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. Add about 70% diluent and sonicate to dissolve the content with intermittent shaking for 30 minutes. After sonication dilute the volume of flask with diluent (250 µg/mL). Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)

3. Method Validation

The method was validated according to ICH guidelines in terms of: Specificity, Linearity, Accuracy, Precision, Robustness and Stability indicating capability.

3.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

3.2 System suitability Parameters

The system suitability parameters were determined by preparing standard solutions of Favipiravir And the solutions were injected five times and the parameters like peak tailing, retention time, peak area and USP plate count were determined.

3.3 Linearity

The linearity of the method is determined by preparing series of solutions in the range of (10–100 µg/ml). The obtained peak areas are plotted against concentration.

3.3.1 Preparation of linearity solutions

Preparation of Standard stock solutions: Accurately weighed and transferred 5 mg of Favipiravir, working Standards into a 25 ml clean dry volumetric flask, add 15 ml of diluent, sonicate to dissolve. Flasks labeled as Standard stock solution. From the stock solutions prepare the solution

to get 20%, 40%, 80%, 100%, 150% and 200% of standard solutions. Preparation of Linearity solution is mentioned in Table-1

3.4 Precision

3.4.1 Method precision (repeatability)

The method precision/ repeatability can be determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and % Relative standard deviation, % assay was calculated.

3.4.2 Intermediate precision

The intermediate precision can be determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments.Intermediate precision studied at 100% in triplicate on Different Days. The areas of all the injections were taken and % Relative standard deviation, % assay were calculated. The results obtained were within the acceptance criteria.

3.5 Accuracy

Accuracy is tested by the standard addition method at three different levels 50, 100 and 150%. The percentage recoveries of Favipiravir present in the pharmaceutical dosage form were calculated.

3.6 Method robustness

The Robustness of the developed method was determined by making small deliberate changes in flow rate (± 0.08 ml/min), change in mobile phase pH (± 2%), organic mobile phase ratio (± 2%), and change in detection wavelength (± 2 nm) along with the optimized method.

3.7 Forced degradation studies.

The stability studies were conducted by exposing the drug product to different stress conditions like acid, alkali, peroxide, thermal, photolytic and humidity conditions.

3.7.1 Control sample (As such) preparation:

Accurately weighed and transferred the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. About 70% diluent added and sonicate to dissolve the content with intermittent shaking for 30 minutes. After sonication dilute the volume of flask with diluent (250 µg/mL)

Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)

3.7.2 Acid stress sample preparation:

Accurately weighed and transferred the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. About 35 mL diluent added and sonicate to dissolve the content with intermittent shaking for 30 minutes. After sonication 2 mL of 0.05N HCl added and kept at room temperature for 2 Hour. After 2 Hour neutralize solution with 0.05N NaOH and dilute the volume of flask with diluent (250 µg/mL)
Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)
Note: Similarly blank sample also prepared for acid stress.

3.7.3 Alkali stress sample preparation:

Accurately weighed and transferred the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. About 35 mL diluent added and sonicates to dissolve the content with intermittent shaking for 30 minutes. After sonication 5 mL of 1N NaOH added and kept at 60°C for 4 Hour. After 4 Hour neutralize solution with 1N HCL and dilute the volume of flask with diluent (250 µg/mL)
Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)
Note: Similarly blank sample also prepared for alkali stress.

3.7.4 Peroxide stress sample preparation:

Accurately weighed and transferred the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. About 35 mL diluent added and sonicate to dissolve the content with intermittent shaking for 30 minutes. After sonication 5 mL of 30% H2O2 added and kept at 60° for 4 Hour. After 4 hour dilute the volume of flask with diluent (250 µg/mL)
Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)
Note: Similarly blank sample also prepared for peroxide stress.

3.7.5 Thermal stress sample preparation: (Sample exposed to 80°C for 7 Days)

Accurately weighed and transferred the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. About 70% diluent added and sonicate to dissolve the content with intermittent shaking for 30 minutes. After sonication dilute the volume of flask with diluent (250 µg/mL)
Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)

3.7.6 Humidity stress sample preparation: (Sample exposed to 40°C/75%RH for 7 days.)

Accurately weighed and transferred the fine powder equivalent to 12.5 mg of favipiravir to the 50 mL of volumetric flask. About 70% diluent added and sonicate to dissolve the content with intermittent shaking for 30 minutes. After sonication dilute the volume of flask with diluent (250 µg/mL)
Further dilute 2 mL of the above solution to 10 mL with diluent (50 µg/mL)

4. Results and discussions

4.1. Development and optimization of HPLC

The present work was focused to develop a stability indicating RP-HPLC [reverse phase high performance liquid chromatography] method for the simultaneous estimation of Favipiravir in pharmaceutical dosage form. The solubility of the active pharmaceutical ingredient was checked in different solvents like methanol, water, acetonitrile and in different ratios but finally the standard is soluble in 10 mM Potassium dihydrogen phosphate pH 4.0: Acetonitrile (40:60%v/v) so it was chosen as a diluent. The different mobile phases like acetonitrile and Ammonium acetate buffer, Methanol and OPA buffer and acetonitrile and OPA buffer were used in compositions with a flow rate of 1 ml/min but the theoretical plate, retention time and tailing factor were not satisfactory, so at last Potassium dihydrogen phosphate buffer pH-4.0 and acetonitrile was selected as a buffer at flow rate of 0.8 ml/min. Initially shimadzu, kromasil different columns with different temperatures like 30, 35, 40, 45°C were used but the retention time, run time and good peak shape were not exact and the problem was get rid by using Inertsil ODS 3V C18 (4.6 mm X 100mm, 3.0 µm) Column at ambient temperature with a run time of 8 min. Finally the method was optimized by altering the various mobile phase composition ratio and the optimized wavelength of Favipiravir was found to be at 229nm. Optimized chromatographic conditions mentioned in table-2. Chromatogram of optimized method was mentioned in figure-2

4.2 Specificity

There is no interference is observed at the retention time of favipiravir peak from blank and placebo. The chromatogram was shown in figure-3

4.3. System suitability parameters

The system suitability tests were conducted before performing the validation and the parameters were within the acceptable criteria like retention times were 3.89 min for Favipiravir, plate count was >2000, peak tailing was < 2 and the %RSD of peak areas of six injections were ≤ 2%. Hence the proposed method was successfully applied to routine analysis without any problems. Results of system suitability parameter is mentioned in table-3

4.4. Linearity range

The linearity range was in the interval of Favipiravir (10–100 µg/ml). These were represented by a linear regression equation as follows:
y = 10409x+2978.1. (r2 = 0.9991), Regression line was established by least squares method and correlation coefficient (r2) for Favipiravir was found to be greater than 0.999. Hence the curves established were linear. Results of Linearity are mentioned in table-4. Overlay chromatogram of linearity results and Linearity graph is mentioned in figure-4 and figure-5 respectively.

4.5. Precision

Six replicate injections at the same concentration were analyzed on the same day and also in two different days for verifying the variation in the precision and the %RSD for Favipiravir was within acceptable limit of ≤ 2. Hence the method is reproducible on different days with different analyst and column. This indicates that the method is precise. Results of precision parameter is mentioned in table-5

4.6. Accuracy

The percentage recoveries for Favipiravir were found to be 99.89%, 100.13% and 100.38% respectively Results of Accuracy parameter is mentioned in table-6. The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

4.7 Robustness

The method robustness studied under four different conditions, namely by changing the flow rate, pH, composition of mobile phase and wavelength proved that small but deliberate changes in the conditions does not affect the performance of the method significantly as evident from the precision values in the measurement of peak area of the analyte. Results of robustness is mentioned in table-7

4.8. Forced degradation studies

The forced degradation studies were conducted on Favipiravir drug products and all the parameters were within the acceptable limits. Favipiravir have shown significant sensitivity towards the treatment of acidic and basic solutions. The drugs gradually undergone degradation with time and prominent degradation was observed. Degradation peaks in acidic media at RT 14.24;19.08 min, in basic media at RT 1.565 were observed (Figures 6 and 7). Forced peroxide, thermal degradation, photolytic degradation and heat - humidity studies showed that the drug molecules did not undergo any degradation and was stable under these conditions. There was no change observed in retention times of all the drugs. From the chromatograms of the degradation products, the peak of the degradation products was not interfering with the peak of drug products and these degradation studies showed that the developed stability indicating method is specific. Force degradation study results mentioned in table-8

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5. Conclusion

In this paper a novel, simple, efficient, rapid and precise stability indicating Reverse Phase High Performance Liquid Chromatographic method was effectively developed and validated for the simultaneous estimation of Favipiravir in pharmaceutical drug product. The current method was validated according to ICH guidelines in terms of Linearity, Accuracy, Precision, Robustness and Stability indicating capability. We have conducted the stability studies by exposing the drug product to different stress conditions like acid, alkali, peroxide, thermal, photolytic, heat-humidity conditions and observed the degradation of drug product in acidic medium and basic medium. Based on the chromatograms of the degradation studies, we have detected that the peak of the degradation products was not interfering with the peaks of drugs products and so we conclude that the current developed Reverse Phase High Performance Liquid Chromatographic method is specific with respect to the Pharmaceutical drug product Favipiravir. Hence the current developed method can be fruitfully applied for the estimation of Favipiravir in drug testing laboratories and pharmaceutical industries.

List of tables

Table-1: Preparation of linearity solution.

Linearity Stock preparations: 5 mg of favipiravir → upto 25 mL With Diluent (200 µg/mL)			
Linearity Dilutions			
Level	Linearity stock taken (mL)	Diluted upto(mL)	Target concentration (µg/mL)
Level-1 (20%)	1	20	10
Level-2 (50%)	3	25	25
Level-3 (80%)	2	10	40
Level-4 (100%)	**5**	**20**	**50**
Level-5 (150%)	4	10	80
Level-6 (200%)	5	10	100

Table-2: Optimized chromatographic conditions

Parameters	**Description**
Column	Inertsil ODS-3V C18 (4.6 mm X 100mm, 3.0 µm)

Flow rate	0.8 mL/min
Column Temp (°C)	Ambient
Injection volume	5 µL
Detection wavelength	229 nm
Diluent	10mM potassium dihydrogen phosphate (pH 4.0): Acetonitrile (40:60% v/v)
Run time	8 minutes (Retention time- 4.2 minute)
Mobile phase	10 mM potassium dihydrogen phosphate (pH 4.0): Acetonitrile (90:10% v/v)

Table-3: Result of system suitability parameter.

Injection	Retention time	Area	Tailing factor	Theoretical plates
Injection-1	3.89	504781	1.23	4295
Injection-2	3.90	505323	1.23	4311
Injection-3	3.89	505539	1.22	4255
Injection-4	3.89	505456	1.22	4281
Injection-5	3.89	505648	1.22	4263
Mean	**3.89**	**505349**	**1.22**	**4281**
%RSD	**0.1**	**0.1**	**0.4**	**0.5**

Table-4: Results of Linearity parameter.

Level	Concentration (µg/mL)	Area
Level-1 (20%)	10.000	109318
Level-2 (50%)	25.000	253479
Level-3 (80%)	40.000	406086
Level-4 (100%)	50.000	499536
Level-5 (150%)	80.000	824603
Level-6 (200%)	100.000	1047050

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Correlation co-efficient (r)	**0.9995**
Y-intercept	**2978.1**
Slope	**10409**
Plot (Visual)	**Linear**

Table-5(a) Results of Method Precision

Sample ID	**Obtained mg**	**% Assay**	**%Mean**	**% RSD**
Set-1	201.3	100.6	100.1	0.7
Set-2	198.8	99.4		
Set-3	198.3	99.1		
Set-4	201.4	100.7		
Set-5	201.1	100.6		
Set-6	200.7	100.3		

Table-5(b) Results of intermediate precision

Interday				Intraday		
Sample ID	**% Assay**	**%Mean**	**% RSD**	**% Assay**	**%Mean**	**% RSD**
Set-1	100.6	99.9	1.3	101.1	100.6	1,0
Set-2	99.4			101.3		
Set-3	99.1			99.3		

Table-6: Results of accuracy

Level	**Sample ID**	**Amount Added(µg)**	**Amount Recovered(µg)**	**% Recovery**	**Mean**	**% RSD**
50%	Set-1	37.600	37.798	100.5	100.3	0.7
	Set-2	37.400	37.694	100.8		
	Set-3	38.200	37.994	99.5		
100%	Set-1	79.600	78.008	98.0	98.9	0.9
	Set-2	79.080	78.247	98.9		

	Set-3	79.400	79.261	99.8		
150%	Set-1	122.800	122.237	99.5	100.1	0.5
	Set-2	124.800	125.535	100.6		
	Set-3	123.800	123.830	100.0		

Table-7: Results of robustness parameters.

Sr. No.	Parameters	Condition	% RSD	% Assay
1	**As such**	**NA**	**0.1**	**101.0**
2	Flow Rate	0.72 mL/min	0.82	100.6
		0.88 mL/min	0.90	101.6
3	Mobile phase proportion	Buffer: Acetonitrile (88:12%v/v)	0.65	99.9
		Buffer: Acetonitrile (92:8%v/v)	0.42	102.1
4	pH of mobile phase buffer	3.8	0.70	101.5
		4.2	0.65	101.9
5	wavelength	227 nm	0.85	100.3
		231 nm	0.77	99.9

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Table-8: Force degradation study results.

Sr. no	Condition	Assay	% Area	% Difference with respect to control sample
1	As such (Control sample)	101.0	Favipiravir-100.0%	NA
2	Acid Degradation	92.3	Favipiravir- 90.8% Peak-1: 5.90% Peak-2: 3.35%	8.7
3	Alkali Degradation	98.3	Favipiravir- 98.9% Peak-1: 1.03%	2.7
4	Peroxide Degradation	100.2	Favipiravir- 100.0%	0.8
5	Thermal Degradation	100.6	Favipiravir- 100.0%	0.4
6	Humidity Degradation	99.7	Favipiravir- 100.0%	1.3
7	Photo Degradation	100.0	Favipiravir- 100.0%	1.0

List of figures.

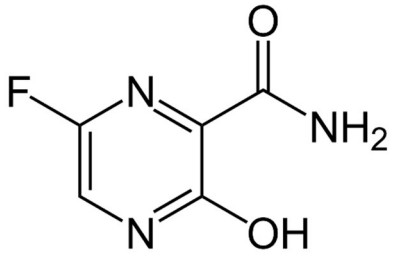

Figure-1 Structure of Favipiravir

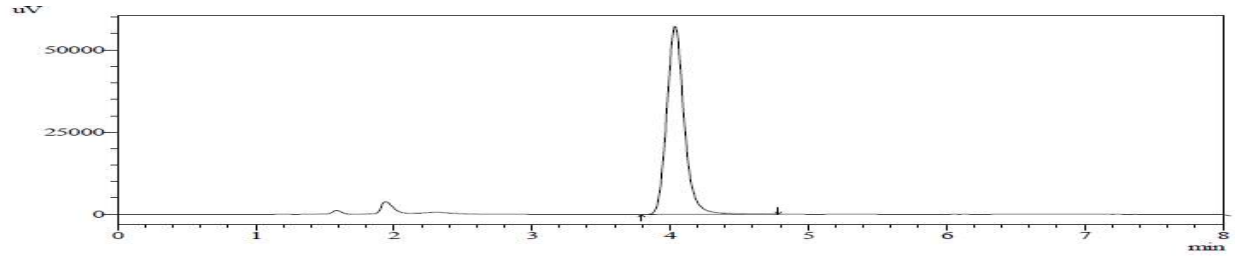

Figure-2: Chromatogram of standard Favipiravir. (50 µg/mL)

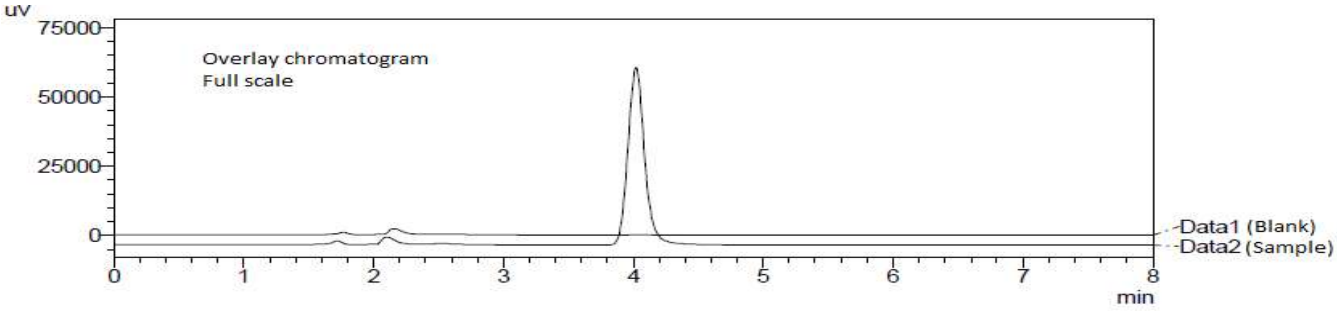

Figure-3 Chromatogram of Specificity

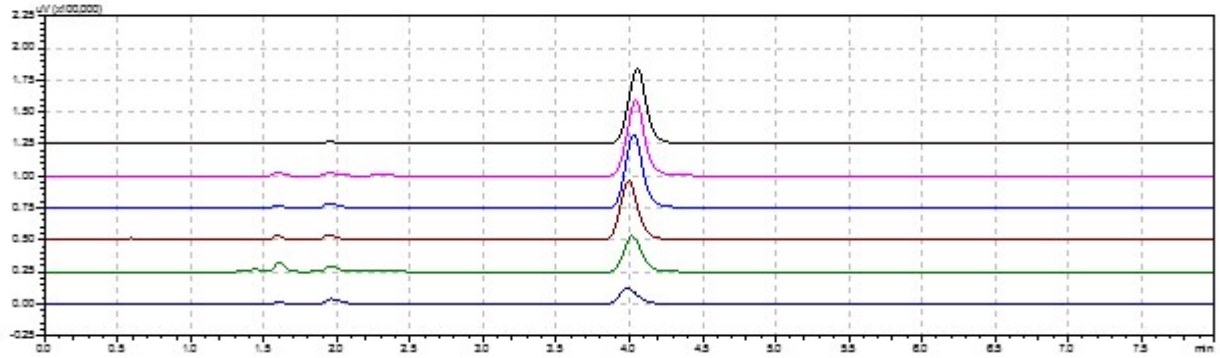

Figure-4 Linearity Overlay of Favipiravir

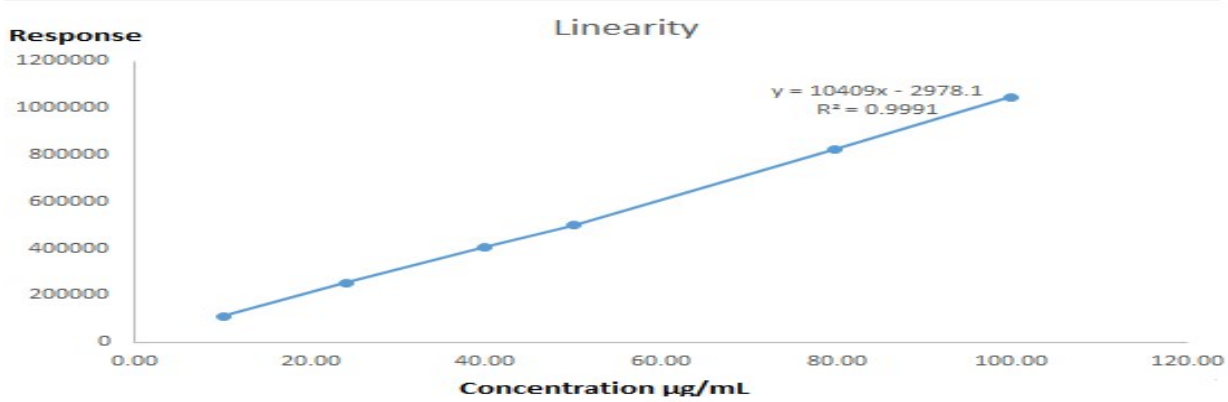

Figure-5: Linearity graph

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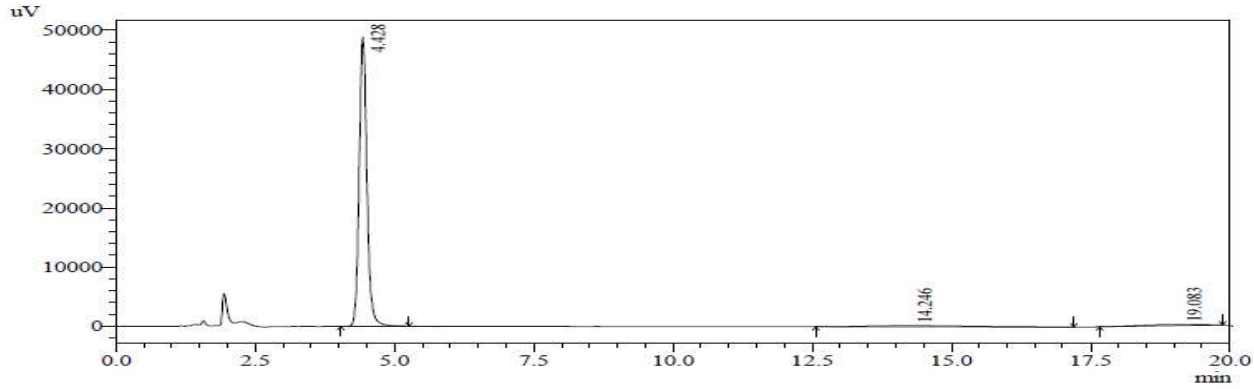

Figure-6: Acid stress chromatogram

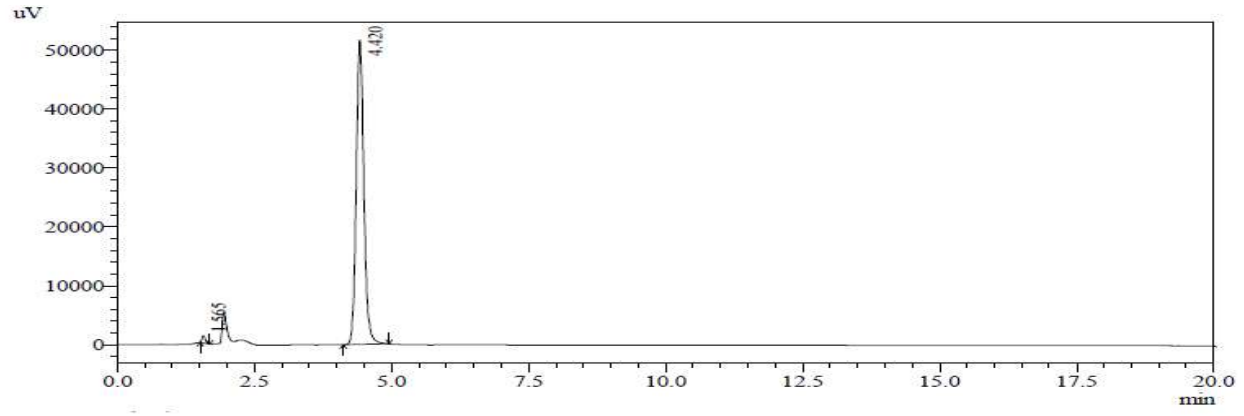

Figure-7: Alkali stress condition.

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