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Advances in Bioresearch

ORIGINAL ARTICLE

Estimation of Favipiravir in Bulk Drug and Pharmaceutical Dosage form by Using RP-HPLC Method Development and Validation

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ABSTRACT
To create and test a new, simple, quick, precise, and accurate method. Development and Validation of the Eco-friendly RP-HPLC for Estimation of the Favipiravir in Bulk drug and Pharmaceutical Dosage Form RP-HPLC method development using Phenomenex ODS-3 and variables for estimating the Favipiravir in a bulk and formulations were optimised. The RP-HPLC method were developed on Phenomenex ODS-3 (250mm X 4.6mm i.d) 5µm column using buffer pH 3.5 a Methanol : Water [50:50] as mobile phase at flow rate is 1.0 ml/min and the PDA detector findings at 286 nm The maximum absorbance (Λmax) was found at 286 nm. The wavelength of 286 nm was chosen for further analysis of Favipiravir. The calibration curve should be determined using drug concentrations ranging from 1-15 µg/ml. The accuracy % recovery rate ranged from 99.07% to 99.37%. The method was to be precise with a % RSD value 0.731-0.742% and 0.837 – 0.845% for the intraday and Interday respectively. The limit of detection (LOD) and limit of quantification (LOQ) has been found to be 0.12 µg/ml and 0.36 µg/ml respectively by RP-HPLC method. We concluded that developed RP-HPLC methods for quantifying Favipiravir bulk and formulation are well precise, accurate, sensitive, and reproducible. The suggested approach can be employed by pharmaceutical enterprises for routine analysis of Favipiravir. The proposed method is economical and eco-friendly, with the retention time roughly 5.0 minutes.
Keywords: *RP-HPLC, Favipiravir, Method Development, Caliberation, Economical, Ecofriendly, Retenation time.*

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INTRODUCTION

Toyama Chemical located in Japan has been developed favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide), purine nucleic acid analogue used to treat viral illnesses such as influenza. This was recently studied and discovered to be a good choice for COVID-19 management. It works by inhibiting the RNA dependent enzyme RNA polymerase (Rd-Rp), a key enzyme that prevents the replication of RNA viruses[1]. Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazine carboxamide) is a antiviral drug which inhibits RNA-dependent RNA polymerase (RdRp) of RNA viruses selectively and potently. Toyama Chemical Co. Ltd. discovered Favipiravir by examining a chemical library for antiviral activity which is against the influenza virus. Recognized as substrate with RdRp and inhibits RNA polymerase activity[1,2] Because RdRp's catalytic domain is conserved among the different types of RNA viruses, this method of action provides a larger variety of antiviral actions than Favipiravir. Favipiravir is highly effective against a wide variety of influenza virus types and their subtypes, which includes strains resistant to

conventional influenza medication.. Of note, Favipiravir exhibits antiviral activity against the other RNA viruses which includes arenavirus, bunyavirus, and filovirus, all of these are known to causes fatal hemorrhagic fever. These exclusive antiviral profiles should be make the Favipiravir potentially a good promising drug for specially intractable viral RNA infections[1,3] Favipiravir has effective for treating patients which infected by COVID-19. However, research examines that the efficacy and the safety of the Favipiravir for the COVID19 patients is limited. Favipiravir induces viral spread after the 7 days and it should be contributes to clinically progress in 14 days. These results shows that Favipiravir has a strong ability to treat COVID-19 infection, mainly in patients which suffered from mild to moderate disease. Additional a well-designed studies, which examines the clinical trials of the doses and time duration of treatment, which are critical to getting firm conclusions[3,4] Favipiravir is a pro-drug that should be metabolized to the active form, favipiravirribofuranosyl-5'-triphosphate (favipiravir-RTP), are available in both oral and intravenous formulations[5] In 2014, favipiravir is approved in Japan for treatment against the influenza infection and recently in Covid-19 it's given as first line drug for reducing the infection[6]

FIG.1: STRUCTURE OF FAVIPIRAVIR

MATERIAL AND METHODS
MATERIALS AND INSTRUMENTS
Materials
Drugs
Note: Materials and intruments are in table form which is given in TABLE NO.1, TABLE NO.2, TABLE NO.3 in separate sheet
CHROMATOGRAPHIC CONDITIONS
Optimization of HPLC method
Developed a method until we get good chromatography
An acceptance criterion for good chromatography is as follows:
Retention time: Optimum R.T.
Asymmetry (Tailing factor): 0.8 to 2.0
Theoretical plates: NLT 2000
Following trials are taken for estimation of Favipiravir.
Principle: Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.
Trial 1:
Chromatographic Conditions:
Standard solution: Favipiravir100 PPM
Detector: U.V. Detector
Column: Phenomenex ODS-3,
Column Dimension: (250 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 35°C
Injection Volume: 20 µl
Wavelength: 286 nm
Mobile phase: Methanol : Water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 1 results are shown in results.
Trial 2:
Chromatographic Conditions:

Standard solution: Favipiravir100 PPM
Detector: U.V. Detector
Column: Phenomenex ODS-3,
Column Dimension: (250 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 35°C
Injection Volume: 20 µl
Wavelength: 286 nm
Mobile phase: Methanol : Water (50:50)
Flow Rate: 1.0 ml/min
Observation: The trial 2 results are shown in results.
Optimized Chromatographic condition: Trial no. 2 considered as optimized chromatography which is as follows
Standard solution: Favipiravir100 PPM
Detector: U.V. Detector
Column: Phenomenex ODS-3,
Column Dimension: (250 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 35°C
Injection Volume: 20 µl
Wavelength: 286 nm
Mobile phase: Methanol : Water (50:50)
Flow Rate: 1.0 ml/min

SELECTION OF THE ANALYTICAL WAVELENGTH

Selection of solvent

DMSO was selected as the solvent for dissolving Favipiravir.

Preparation of standard stock solutions for UV scan

In order to prepare stock solution, weighed accurately 25 mg Favipiravir and transferred into 50 ml volumetric flask, added 5 ml of DMSO and then sonicated for dissolve the standard completely and then diluted up to mark with a methanol (500 PPM).
Further diluted 0.4 ml to the 10 ml with a methanol. (20 PPM)[7]

Preparation of blank solution of UV scan: (Solution 1)

Added 5 ml of DMSO in 50 ml of a volumetric flask and then volume make up to mark with a methanol. Further diluted 0.4 ml to 10 ml with methanol.[7]

Selection of the analytical wavelength

Solution 1 as a blank and Favipiravir standard solution (20 PPM) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug. Favipiravir showed maximum absorbance at 286 nm shown in results.

Method Development by RP – HPLC

Preparation of the standard stock solution for the Chromatographic development:

Favipiravir Standard stock solution has been prepared by the dissolving 10 mg Favipiravir into a 20 ml clean and dried volumetric flask, added about 2 ml of DMSO to dissolve completely and make the volume up to mark with a methanol (500 PPM).
Added diluted 4 ml of the stock solution to 20 ml with a mobile phase (100 PPM).[8,9]

Selection of the analytical wavelength for HPLC method development:

Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 286 nm**.**

Preparation of System suitability test (Favipiravir standard solution):

Weighed about 10 mg of the Favipiravir and then transferred in 20 ml volumetric flask, added 2 ml of DMSO, sonicated to dissolve it, made the volume up to mark with a methanol. Pipette out 0.5 ml from standard stock solution and transferred into 25 ml of volumetric flask and make the volume up to mark with a mobile phase **(10 µ/ml working concentration),** chromatograms has been recorded.
System appropriateness is a pharmacopoeial condition that is used to determine whether the chromatographic system is sufficient to analysis which is to be performed. The tests was performed by the collecting data from the Five replicate injection of standard drug solution and the results are recorded.

Acceptance criteria

1. RSD should be less than 2.0 % for a five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency which is determined for Plate Count must be more than the 2000.

Analysis of the marketed Test sample:
Marketed test sample which Having Name Fluguard 400 mg tablets which are selected for the analysis and for performing the validation.

Average weight of test sample (Fluguard 400 mg Tablet):
Weighed the 20 tablets once at a time and then computed the average weight of the tablets using the following formula:

Average weight (mg) = Weight of 20 tablets (mg) / 20

Sample preparation of the Marketed test sample:
Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents uniformly with butter paper. Weighed the powder material equivalent to 100 mg of the Favipiravir and then transferred to the clean and dried 100 ml of the volumetric flask. Then added 10 ml of DMSO, sonicated for 15 minutes with alternating shaking. After 15 minutes solution allows to cool at room temperature and then made the volume up to mark with a methanol. Then filtered the solution through the suitable 0.45µ syringe filter then discard 3-5 ml of the initial filtrate. Added diluted 0.1 ml of the filtered stock solution to the 10 ml with mobile phase. (10 mcg of Favipiravir), injected the resulting solution and then chromatograms were recorded and results are recorded.

Note:- Data given in TABLE NO.4 in separate sheet

Formula for % Assay calculation:

%Assay =

$$\frac{\text{Fvipiravir spl area}}{\text{Favipiravir std avg area}} \times \frac{\text{Favipiravir STD wt(mg)}}{50} \times \frac{0.5}{\text{Weight(mg)}} \times \frac{100}{20} \times \text{Tablet Sample}$$

$$\frac{50}{0.5} \times \frac{\text{Avg wt of tablet(mg)}}{\text{Label claim of Favipiravir (mg)}} \times 100$$

VALIDATION OF RP-HPLC METHOD [10,11,12,13]
The developed method for estimation of Favipiravir were validated as per ICH guidelines for following parameters.[14]

FILTRATION STUDY
An analytical procedure's filtration research examines the interference of irrelevant components from the filter, deposition on the filter bed, and filter compatibility within the sample.
The study was conducted with Favipiravir Test sample (Tablet solution).
Filtration study should be carried out with the unfiltered and filtered test solution. During the filtration movement 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 ml of aliquot sample.

STABILITY STUDY OF THE ANALYTICAL SOLUTION
The stability study has been conducted for standard and the test sample solution. Stability study were performed at a normal laboratory conditions.
The solution should be stored at normal illuminate laboratory conditions then analyzed after 12-24 hours.
Standard and Test solution stability study was performed by calculating the difference between results of test solution at each stability time point to that of initial.

SPECIFICITY
Specificity is the ability to access unambiguously the analyte in the presence of the components which may be likely to be present.
Following solution shall be prepared and injected to prove the specificity nature of the method. (Checked peak purity for standard and test sample solution)

- Blank (Mobile phase as a diluent)
- Placebo
- Favipiravir Standard solution

- Tablet test sample solution

Analyzing marketed test sample contains the excipients (additives) which are completely unknown. So Placebo prepared at lab level by using the formula as follows:

Note:- Data given in TABLE NO.5 in separate sheet

Total 10 gm of placebo prepared:

LINEARITY AND RANGE

Preparation of the linearity solution

The capacity of an analytical process (within a specific range) to produce test results that should be directly proportional to concentration (quantity) of analyte in the sample is referred to as linearity.

5 levels of Linearity was performed from 10% to 150% of working concentration

Linearity Favipiravir stock solution:

Weighed 12.5 mg of Favipiravir and transferred in 25 ml volumetric flask, added 2 ml of DMSO, sonicated for dissolving it completely,then make up the volume up to mark with a methanol. Further diluted 2.5 ml of the stock solution to 25 ml with methanol (50 µg/ml)

Note:- Data given in TABLE NO.6 in separate sheet

Determination

Each level injected in triplicate and mean area calculated. Calibration curve was plotted graphically as a purpose of the analyte concentration in µg/ml on X-axis Vs mean area on y-axis as given in results.

Acceptance criteria

Correlation Coefficient: NLT 0.98
Intercept: To be report
Slope: To be report

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):

Detection limit:

The detection limit of an individual analytical process is the smallest quantity of analyte in the sample that has been detected but it does not essentially quantitated as an accurate number..

Quantitation limit

The limit of quantitation (LOQ) of an individual analytical process is the smallest amount of analyte in a sample that can be quantitatively quantified with sufficient precision and accuracy.

As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach on the basis of Calibration Curve in which residual standard deviation of regression line was calculated and determined in a LOD and LOQ by using following formula:

$LOD = 3.3\ \sigma / S$
$LOQ = 10\ \sigma / S$

Where,

σ = residual standard deviation of the regression line
S = Slope of the regression line

ACCURACY (% RECOVERY)

The analytical procedure's accuracy expresses the degree of agreement between the value which is acknowledged as either it is a conventional true value or acceptable reference value and value of the value which is found,

Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level has been prepared in a triplicate. Calculated % Recovery for both the sample, Mean % recovery for both level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Note:- Data given in TABLE NO.7 in separate sheet

Procedure for preparation of Accuracy sample solution:

Take clean and dried 9 volumetric flask of 100 ml. Weighed aprox 38.0 mg of placebo and transferred in each 100 ml of the volumetric flask. Weighed Favipiravir API as per accuracy level and transferred in same 100 ml of the volumetric flask. Added 10 ml of DMSO sonicated it for 15 minutes with alternating shaking. Make up the volume up to mark with a methanol. Then Filter the solution through the suitable 0.45 µ syringe filter after that discards 3-5 ml of filtrate. Add diluted 0.1 ml of filtrate in a 10 ml with mobile phase.

Acceptance criteria

1. % Recovery for each of one sample and Mean recovery and overall recovery should be in the range of 98-102%.
2. Relative Standard Deviation (RSD) value should be less than 2.0%.

PRECISION

The precision of analytical technique expresses the degree of agreement between a set of measurements acquired from multiple samplings of the same homogenous test under specified conditions. There are two types of precision, Repeatability and Intermediate precision. It is performed on tablet test sample.

Repeatibility:

Preparation of sample solution (6 Samples prepared):

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 100 mg of Favipiravir and then transferred in to the clean and dried 100 ml of a volumetric flask. Then added 10 ml of DMSO, sonicated for 15 min. with alternating shaking. After 15 min. allowed solution to cool at room temperature and make up the volume up to mark with a methanol. Then filtered the solution throughout the suitable 0.45 µ syringe filter and then discard 3-5 ml of the initial filtrate. Add diluted 0.1 ml of the filtered stock solution in to the 10 ml with a mobile phase. (10 mcg of Favipiravir), inject the resultant solution and then chromatograms has been recorded and results are recorded.

Six samples prepared.

Note:- Data given in TABLE NO.8 in separate sheet

Acceptance criteria:

% Assay: 90-110% for every sample and mean assay value

% RSD for % assay value of 6 samples: NMT 2%

Intermediate precision

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared).

Note:- Data given in TABLE NO.9 in separate sheet

Acceptance criteria:

% Assay: 90-110% for every sample and mean assay value

% RSD for % assay of 6 samples of Intermediate precision**:** NMT 2

% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

ROBUSTNESS

The robustness of analytical procedure is measure on its ability to remain unchanged by the minor but deliberate adjustments in the methods parameters and provides an indication to its dependability under normal conditions..

Determination: Standard solution injected under different chromatographic conditions as shown below to check system suitability of standard solution.

a). Changes in flow rate by ±10%. (± 0.1ml/min)

b). Change in column oven temperature. (± 2ºC)

c) Change in wavelength (± 3 nm)

RESULT

VALIDATION DATA OF RP-HPLC METHOD:

Optimization of HPLC method

Trial 1:

Chromatogram:

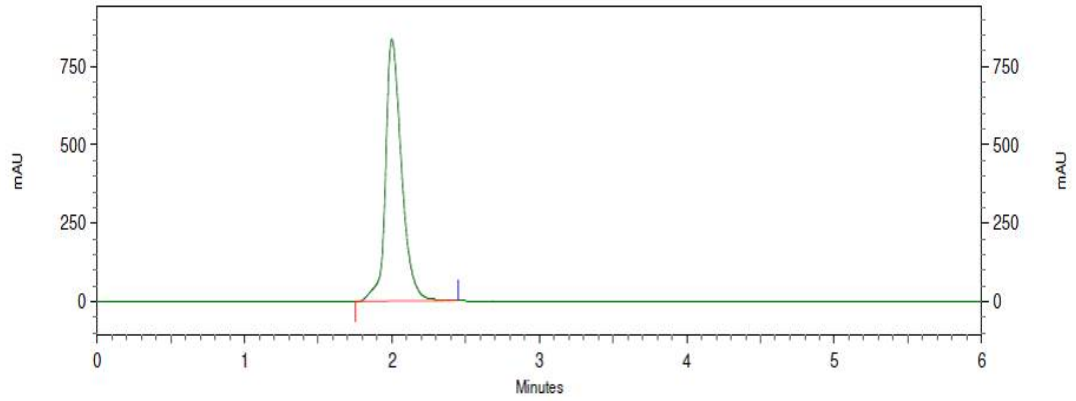


FIG. NO.2 TYPICAL CHROMATOGRAM OF TRIAL 1

Observation: Favipiravir eluted but Chromatography not acceptable. Fronting observed (Asymmetry = 0.82).
Conclusion: Method rejected
Trial 2:
Chromatogram:

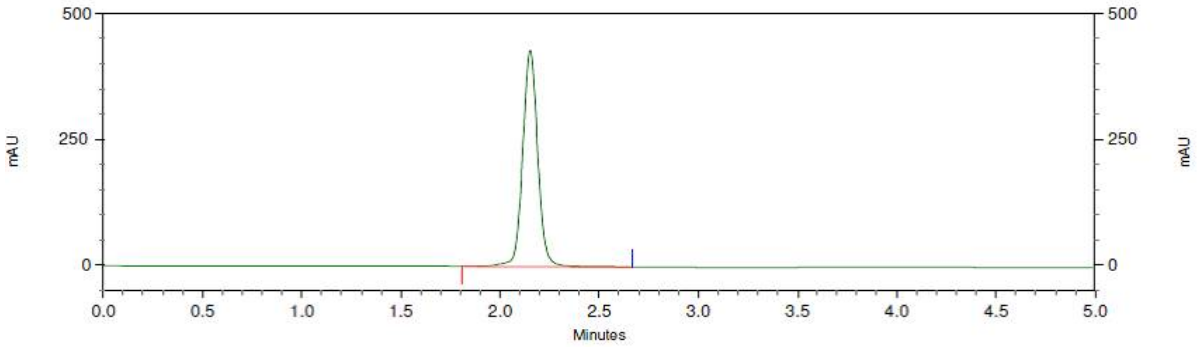


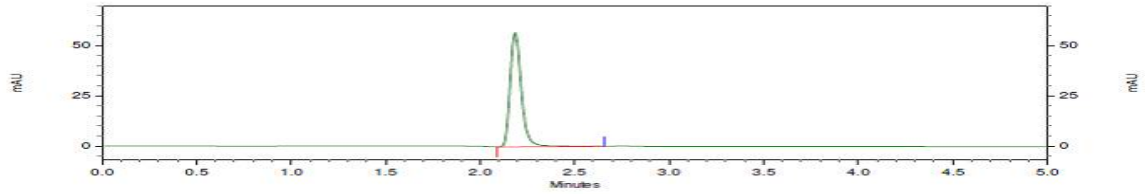
FIG. NO.3 TYPICAL CHROMATOGRAM OF TRIAL 2

Observation: Favipiravir eluted with Good Chromatography.
Conclusion: Method Accepted.
Conclusion: From the observations of trials first to two, it was observed that chromatographic conditions in trial two gives better peak, good retention time and tailing factor therefore chromatographic conditions in trial two was used for method validation.
Note:- Data given in TABLE NO.10 in separate sheet

FILTRATION STUDY:
Filtration analysis of an analytical technique examines the interference of unsuitable components from filter, deposition on filter bed, and filter compatibility with the sample. Performed on tablet test sample.
Note:- Data given in TABLE NO.11 in separate sheet

Chromatograms:

Sample Name: SAMPLE SOLUTION_UNFILTERED

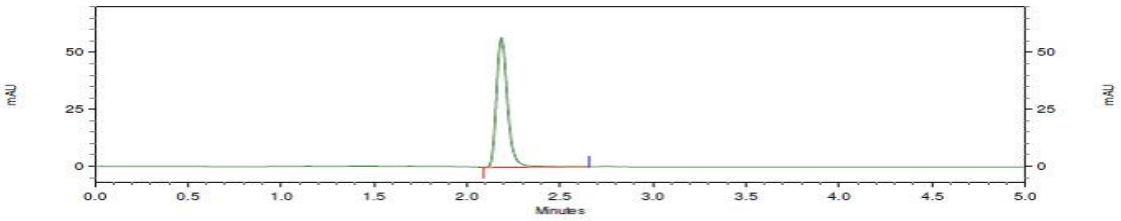


VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4546914	1.31	4556
Totals		4546914		

FIG. NO.4: CHROMATOGRAM OF THE UNFILTERED SAMPLE.

Sample Name: SAMPLE SOLUTION_0.45 µ PVDF FILTER

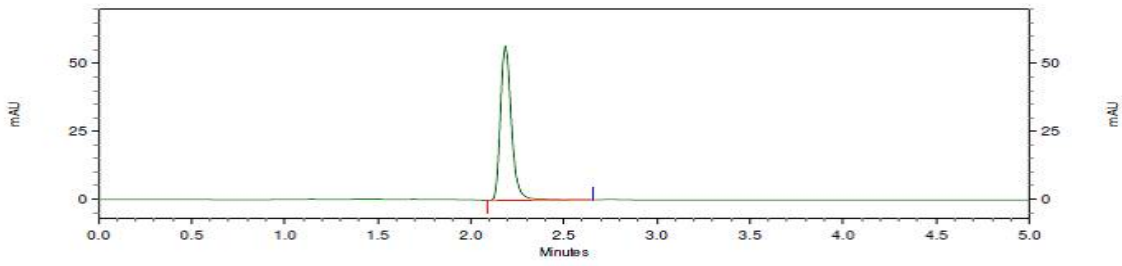


VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4530461	1.30	4523
Totals		4530461		

FIG. NO. 5: CHROMATOGRAM OF THE SAMPLE FILTERED THROUGH 0.45µ PVDF FILTER.

Sample Name: SAMPLE SOLUTION_0.45 µ NYLON FILTER



VWD: Signal A, 286 nm Results

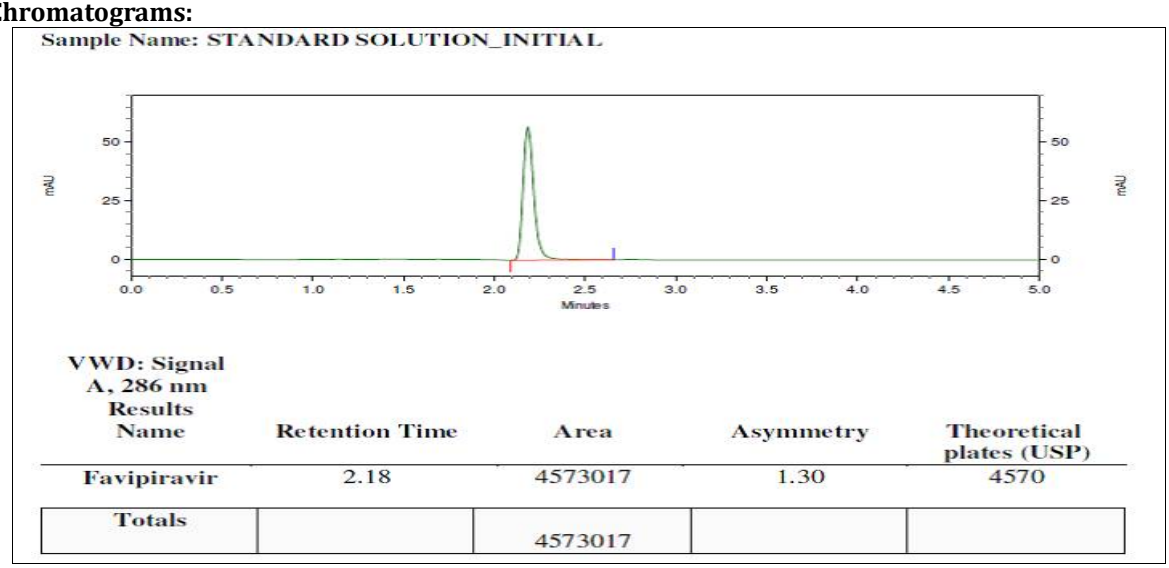
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4529645	1.30	4536
Totals		4529645		

FIG. NO. 6: CHROMATOGRAM OF THE SAMPLE FILTERED THROUGH 0.45µ NYLON FILTER.

Acceptance criteria: % Absolute difference of filtered samples NMT 2.0 w.r.t. Unfiltered sample.
Data interpretation: Both the filters PVDF and Nylon passes the criteria of the filter study, hence both the filters should be used. We used PVDF filter because it showed less absolute difference as compare to Nylon filter.

SOLUTION STABILITY:
A stability analysis was carried out for both the Standard and the Test Samples. The stability test was carried out under standard laboratory settings. The solution were stored in a standard lit laboratory environment and analysed at the start 12 hours and 24 hours.
Note:- Data given in TABLE NO.12 in separate sheet

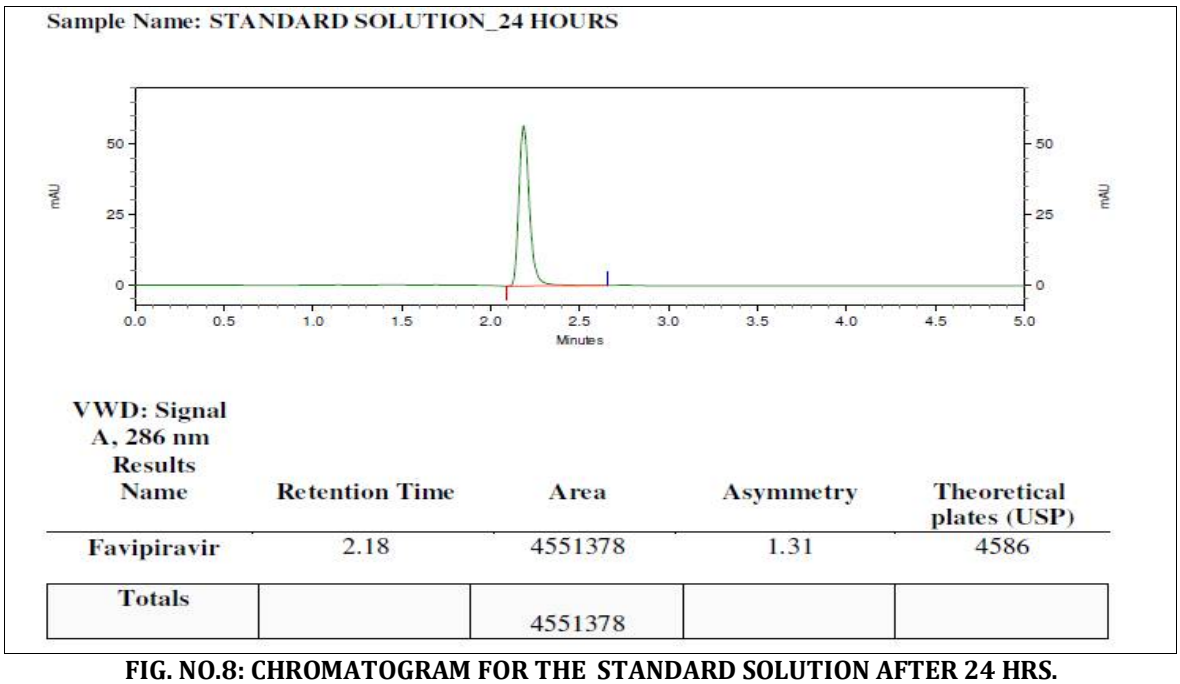
Chromatograms:



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4573017	1.30	4570
Totals		4573017		

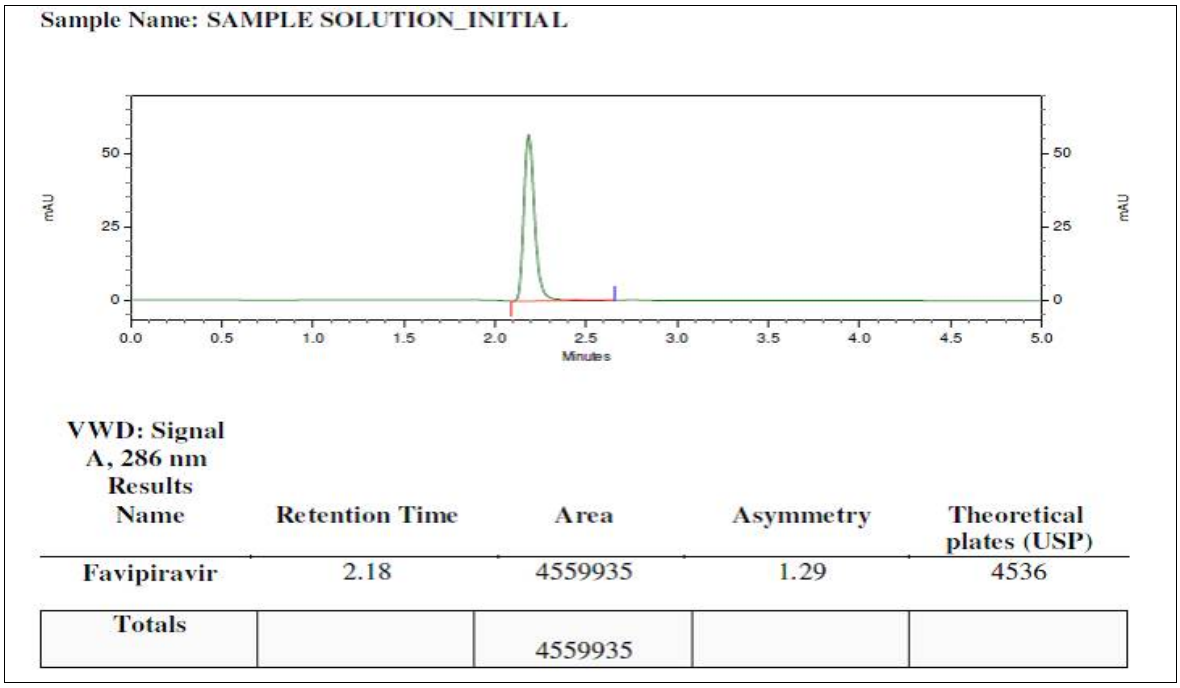
FIG. NO.7: CHROMATOGRAM FOR THE STANDARD SOLUTION INITIAL.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4551378	1.31	4586
Totals		4551378		

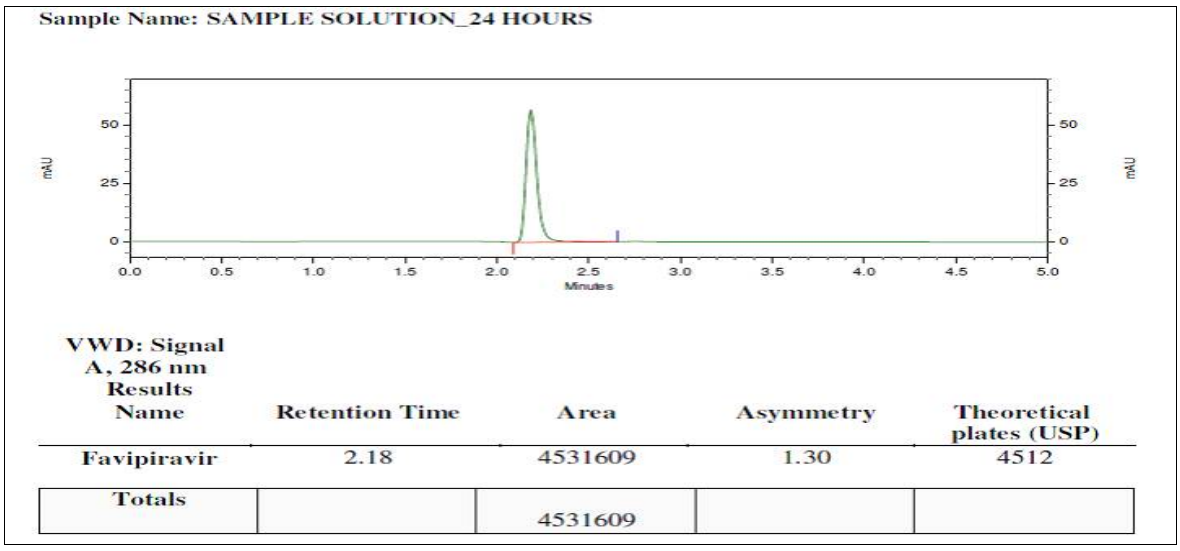
FIG. NO.8: CHROMATOGRAM FOR THE STANDARD SOLUTION AFTER 24 HRS.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4559935	1.29	4536
Totals		4559935		

FIG. NO.9 : CHROMATOGRAM OF THE TEST SOLUTION INITIAL.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4531609	1.30	4512
Totals		4531609		

FIG. NO. 10 : CHROMATOGRAM OF THE TEST SOLUTION AFTER 24 HRS.

Acceptance criteria: % Absolute difference of Stability solution: NMT 2.0 w.r.t. Initial solution.
Data interpretation: Standard solution and Test solution was found stable up to 24 Hrs. Hence both solutions should be used up to 24 Hrs.

SPECIFICITY:
Specificity is defined as ability to access the analyte unequivocally presence of components which are expected to be present.
Blank, standard solution prepared and then injected to check the peak purity.

Note:- Data given in TABLE NO.13 in separate sheet

Chromatograms:

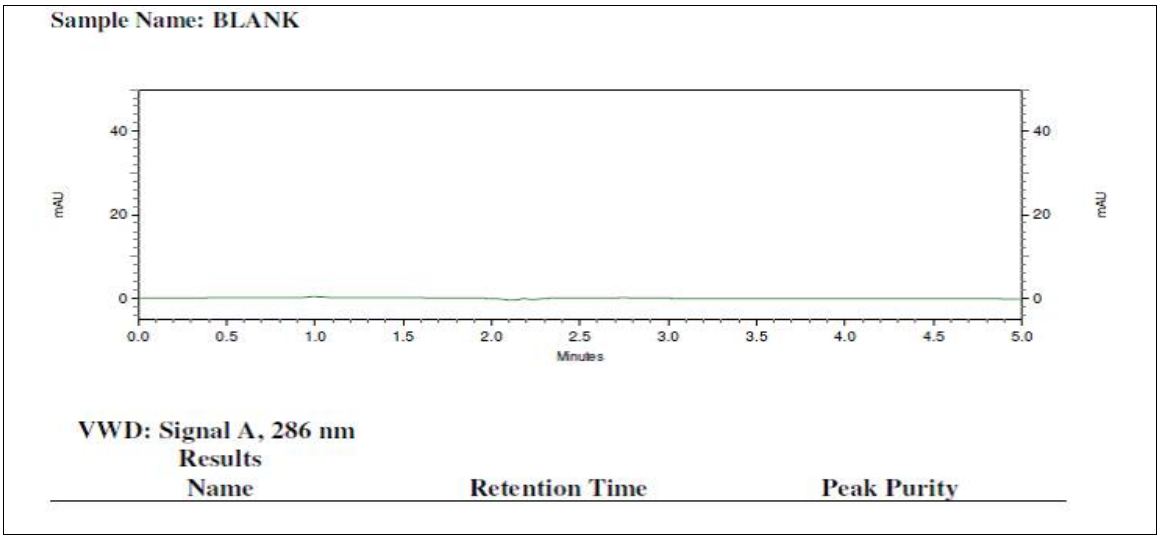


FIG. NO.11 : TYPICAL CHROMATOGRAM OF BLANK SOLUTION.

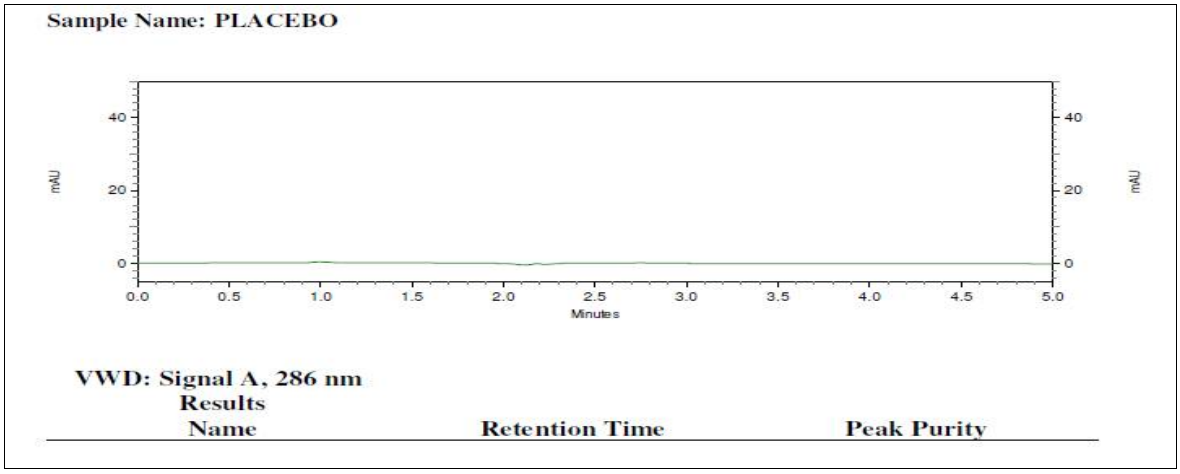


FIG. NO.12 : TYPICAL CHROMATOGRAM OF PLACEBO SOLUTION.

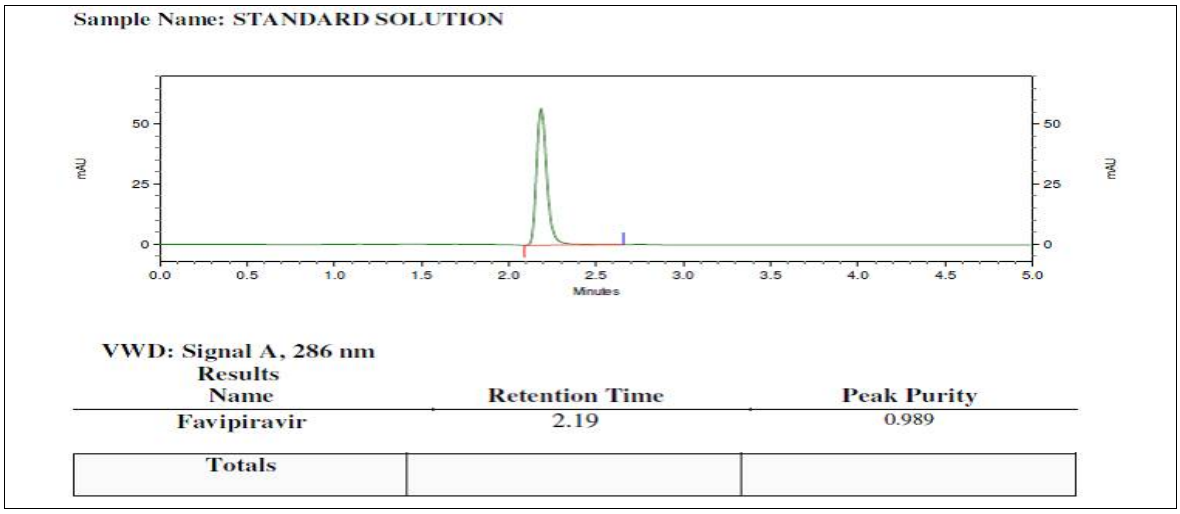


FIG. NO.13 : TYPICAL CHROMATOGRAM OF PEAK PURITY OF STANDARD SOLUTION

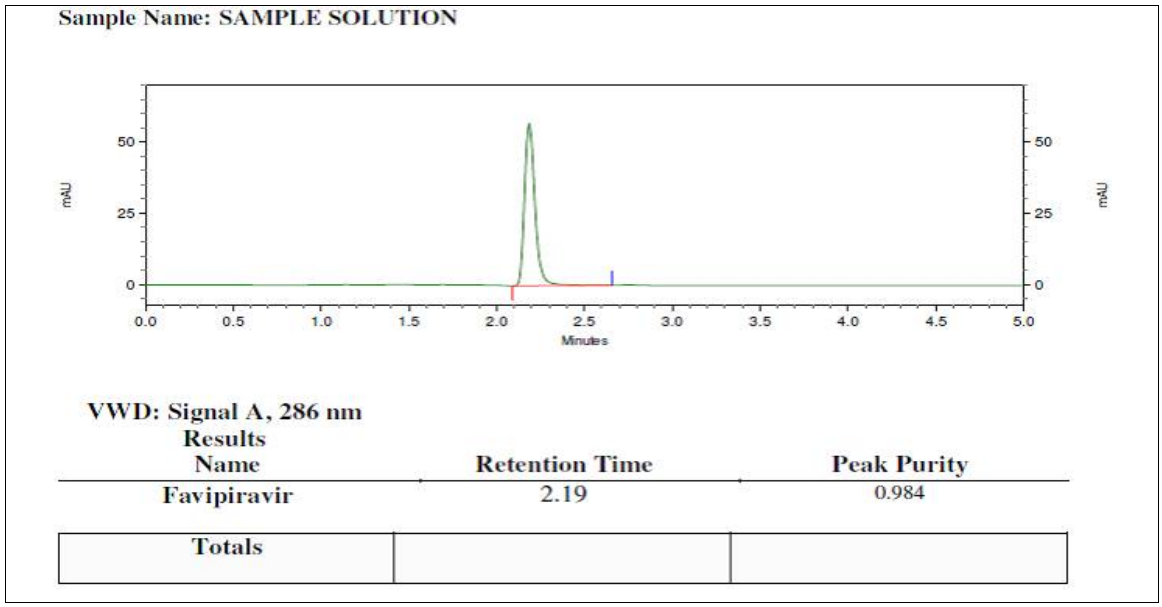


FIG. NO.14 : TYPICAL CHROMATOGRAM OF PEAK PURITY OF TEST SAMPLE SOLUTION.

Acceptance criteria:

Blank: There should be no Interference on R.T. of Favipiravir

Placebo: There should be no Interference on R.T. of Favipiravir

Standard and Test sample solution: Peak purity: NLT 0.95

Data interpretation: Blank and placebo does not having a interference on R.T. of the Favipiravir. Peak purity for Standard with test solution was well within limits. Hence the developed chromatographic method has been pass the criteria for the specificity.

LINEARITY AND RANGE

The ability of an analytical method to produce test findings that are proportional to concentration of analyte into a samples within certain range is referred to as linearity.

Note:- Data given in TABLE NO.14 in separate sheet

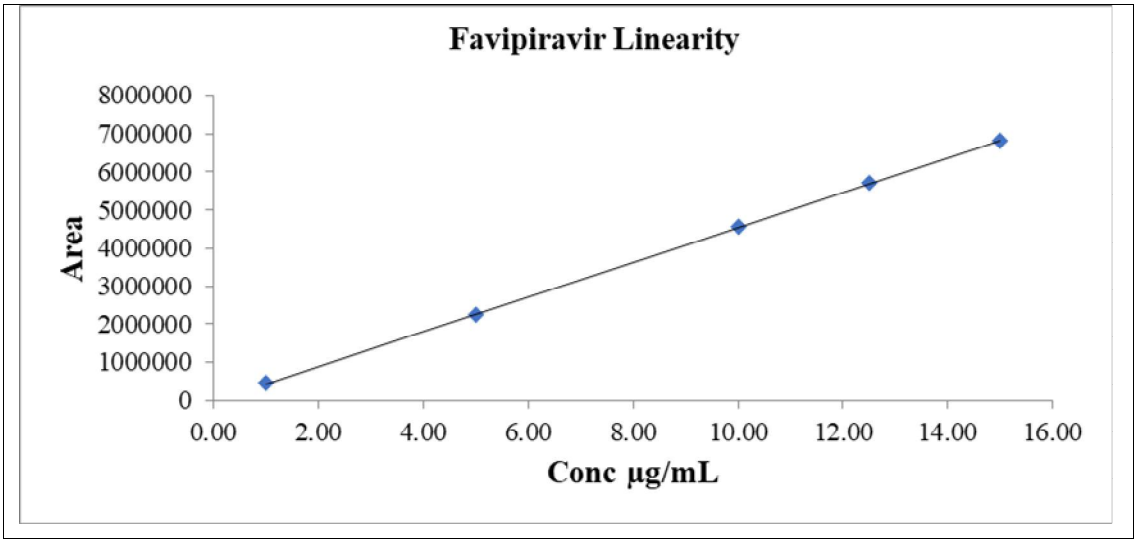


FIG. NO.15 : CALIBRATION FOR CURVE OF FAVIPIRAVIR

Note:- Data given in TABLE NO.15 in separate sheet

The respective linear equation for Favipiravir was

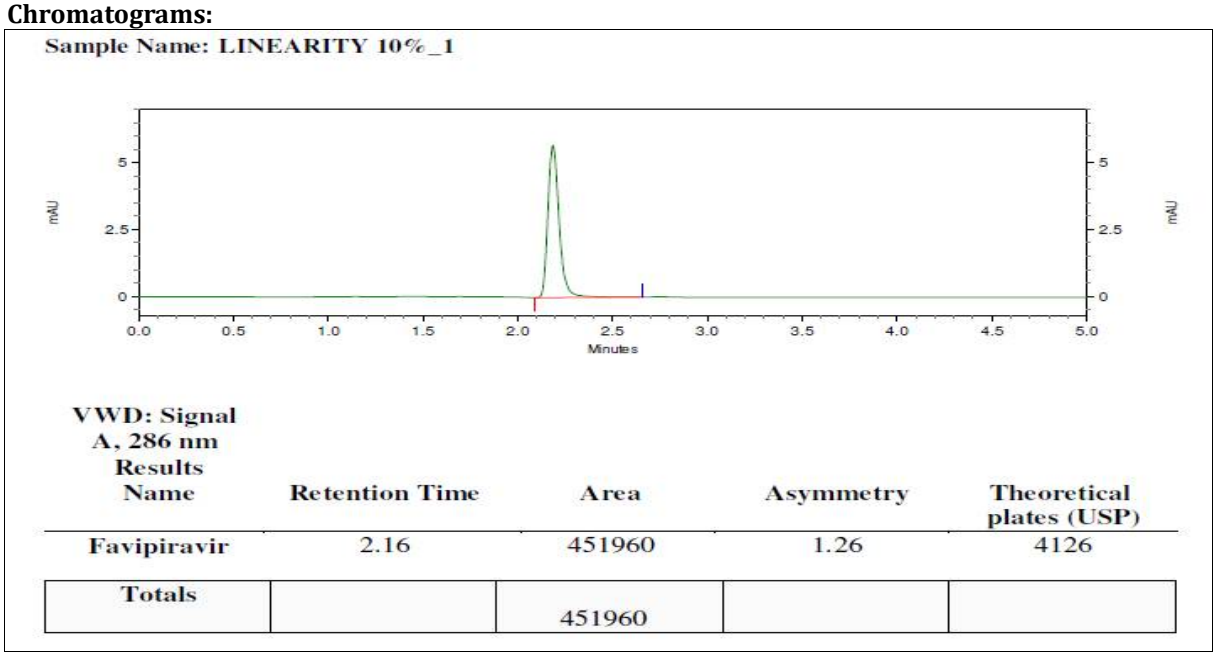
Y = M X + C

Y = 456883.4798 x + -20171.47438

where, x = concentration of analyte in µg/ml

y = is area of peak.
M = Slope
C= Intercept

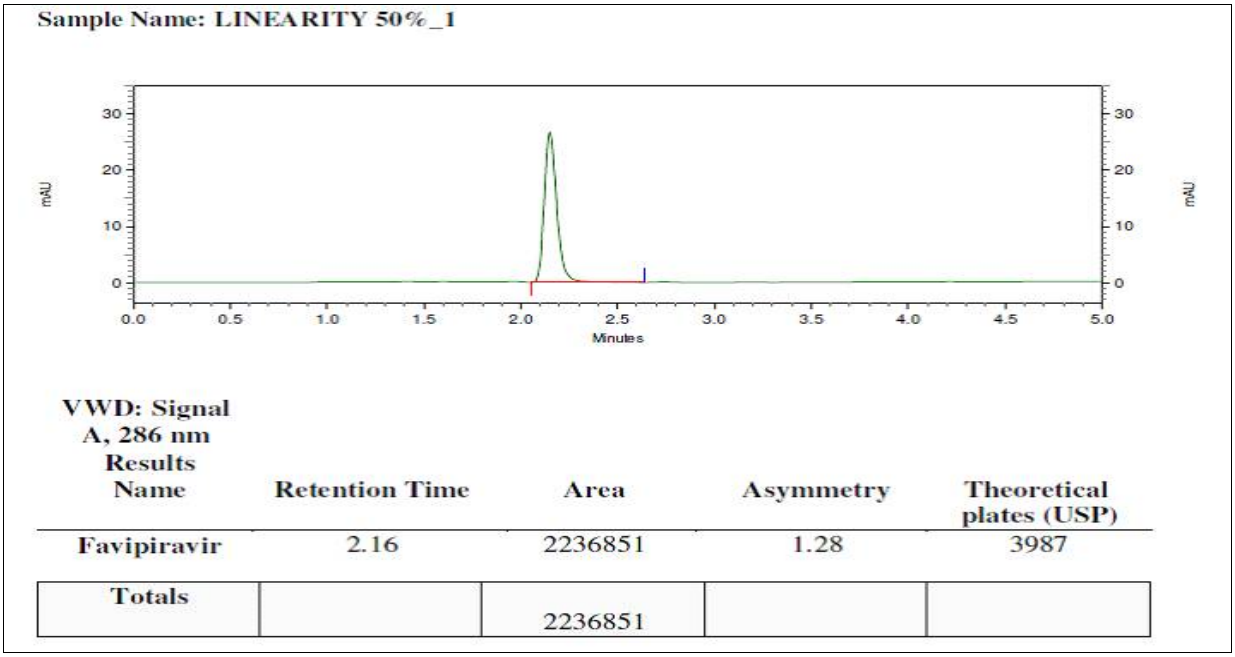
Chromatograms:



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.16	451960	1.26	4126
Totals		451960		

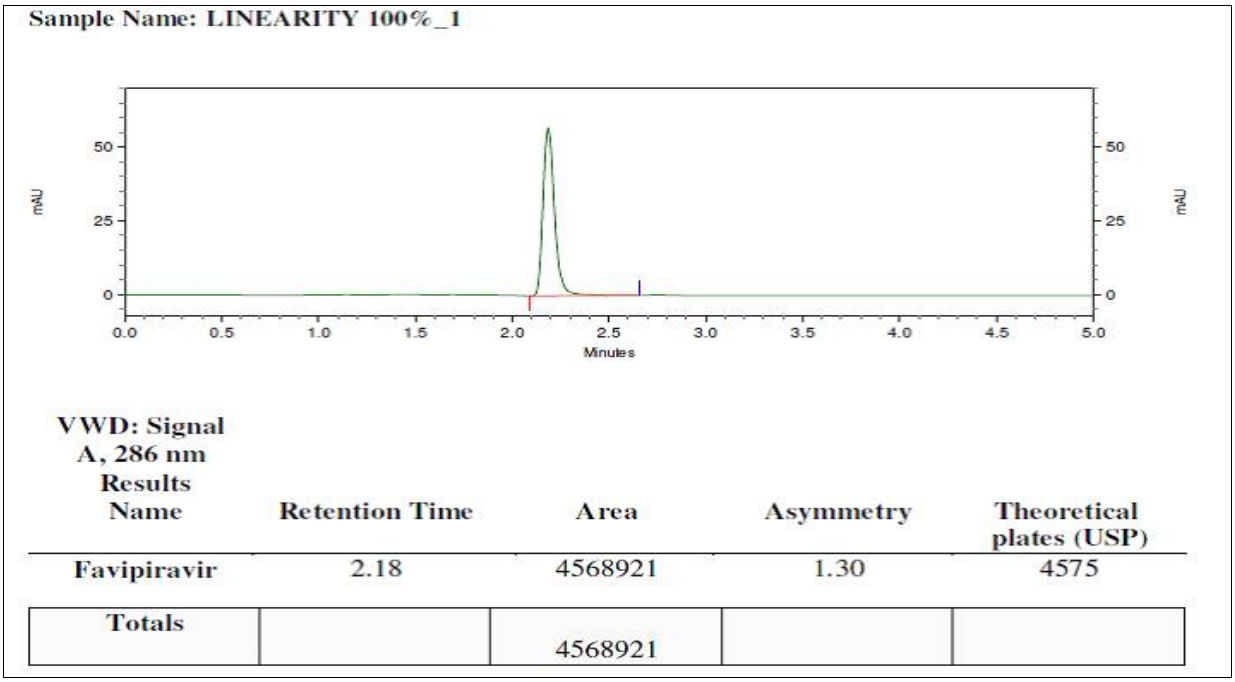
FIG. NO.16 : TYPICAL CHROMATOGRAM OF LINEARITY 10%.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.16	2236851	1.28	3987
Totals		2236851		

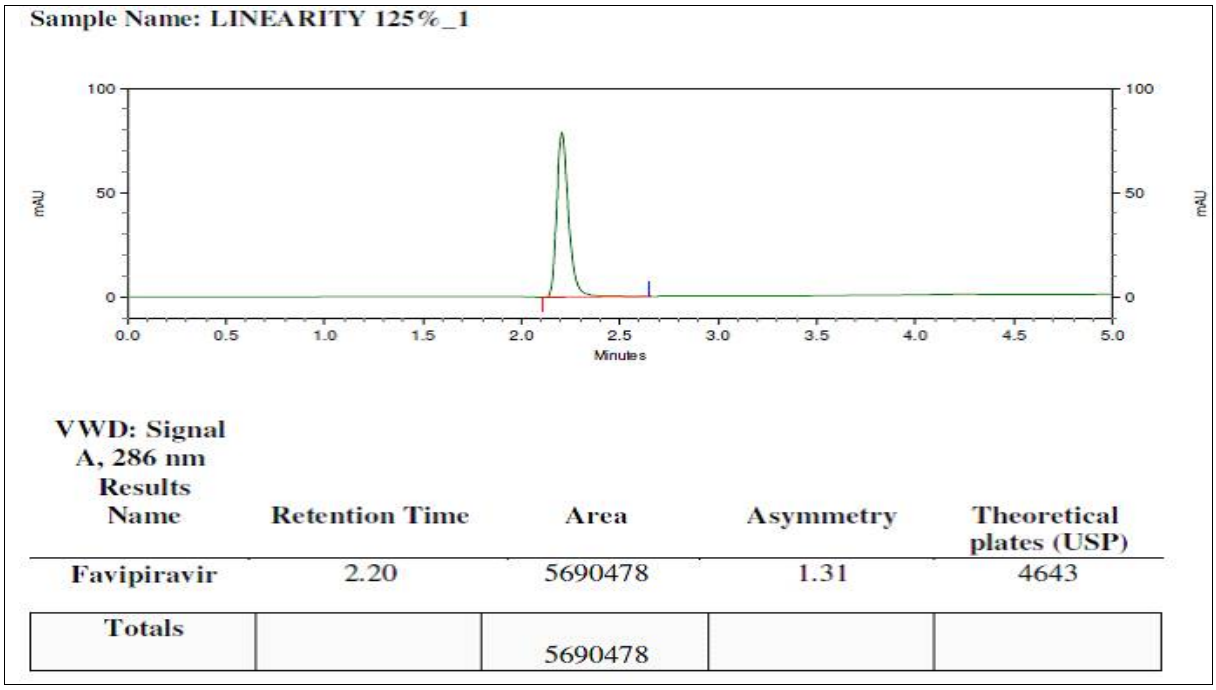
FIG. NO.17 : TYPICAL CHROMATOGRAM OF LINEARITY 50%.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4568921	1.30	4575
Totals		4568921		

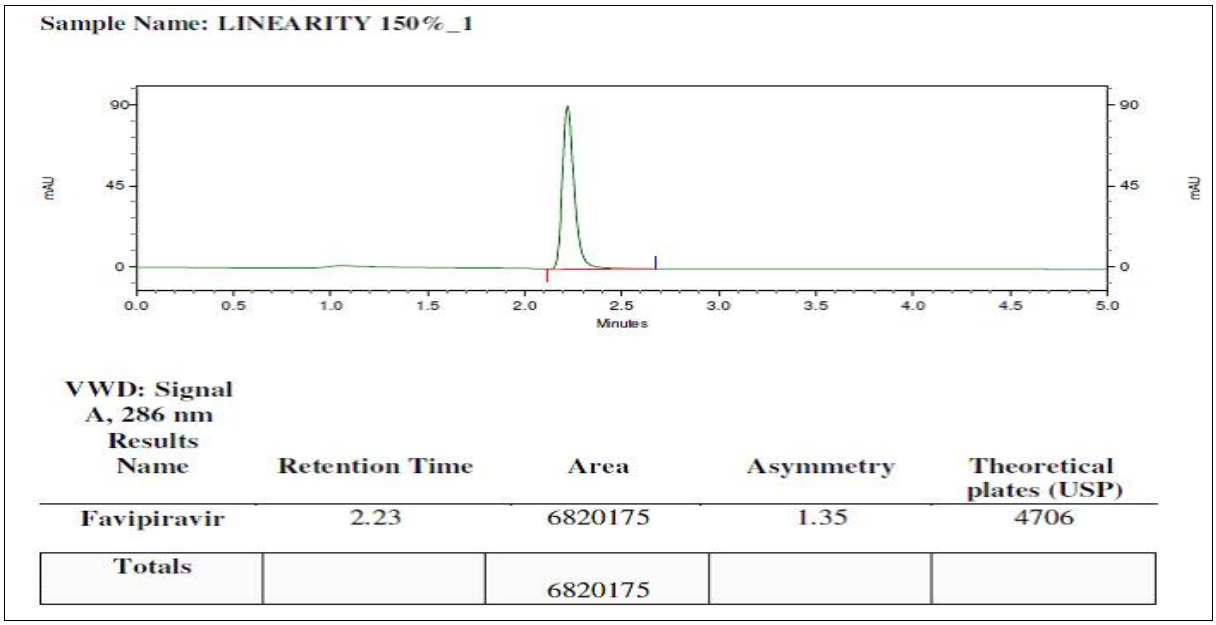
FIG. NO.18 : TYPICAL CHROMATOGRAM OF LINEARITY 100%.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.20	5690478	1.31	4643
Totals		5690478		

FIG. NO.19 : TYPICAL CHROMATOGRAM OF LINEARITY 125%.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.23	6820175	1.35	4706
Totals		6820175		

FIG. NO.20: TYPICAL CHROMATOGRAM OF LINEARITY 150%.

Conclusion:

From the calibration curve it was observed that the Favipiravir shows linear response in the range of 1.0-15.0 µg/ml. The Regression value were found well within the limit.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):

σ = 16377.584 (Residual standard deviation of regression line)

s = 456883.4798 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 16377.584 / 456883.4798

LOD = 0.12 µg/ml

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 16377.584 / 456883.4798

LOQ = 0.36 µg/ml

ACCURACY (RECOVERY):

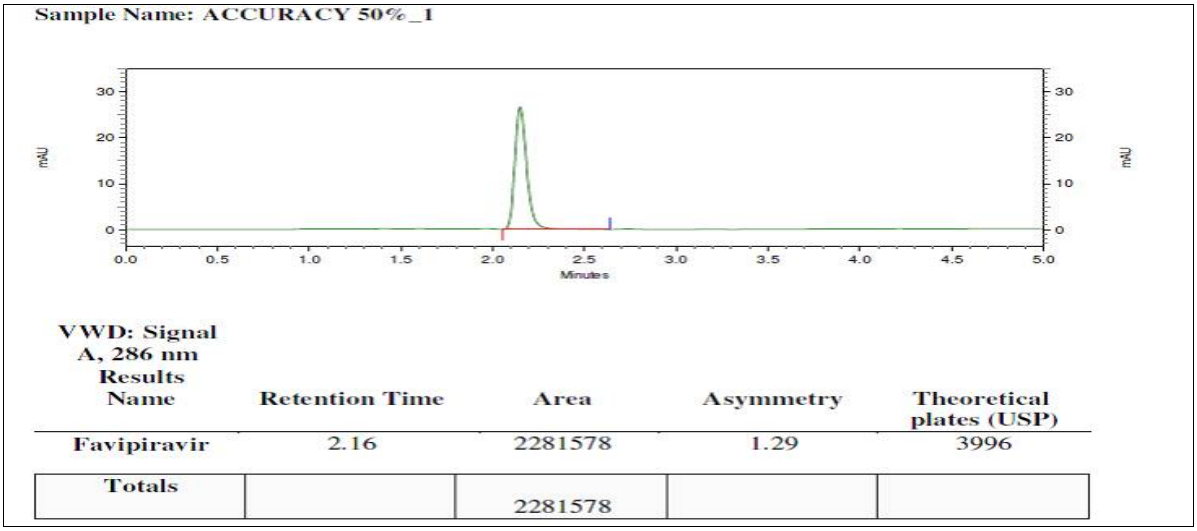
The accuracy of analytical method is closeness of the test results achieved by that method to the true value. Analytical method accuracy has been determined by the applying the procedure to analysed samples containing known concentrations of analyte.

Note:- Data given in TABLE NO.16 in separate sheet

Overall Recovery: 99.23 %

% RSD for Overall Recovery: 0.623

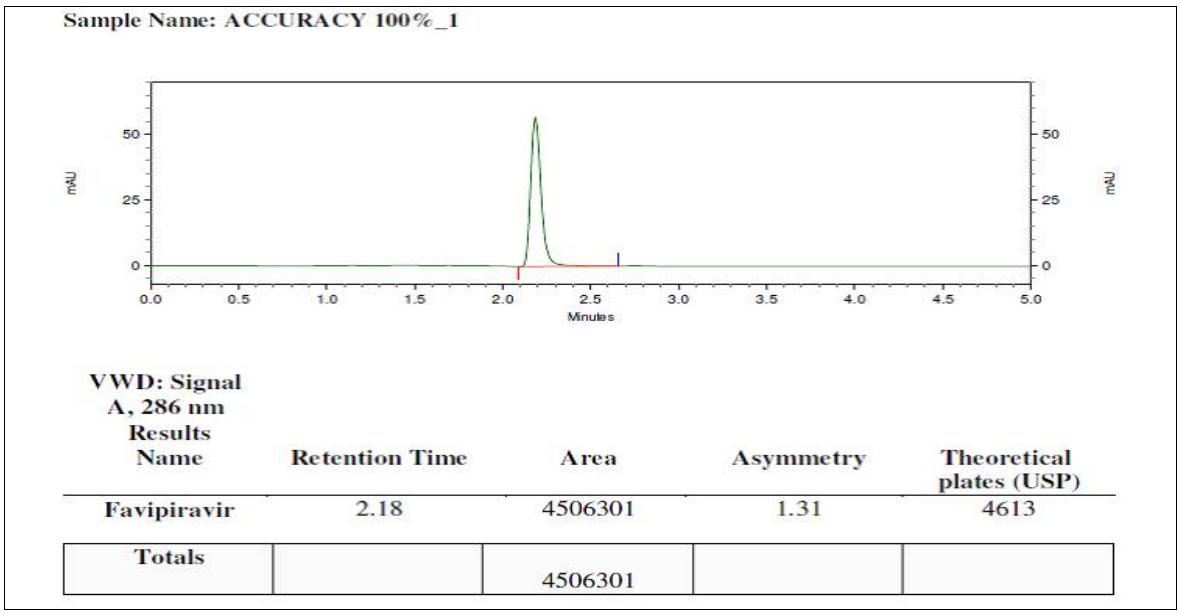
Chromatograms:



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.16	2281578	1.29	3996
Totals		2281578		

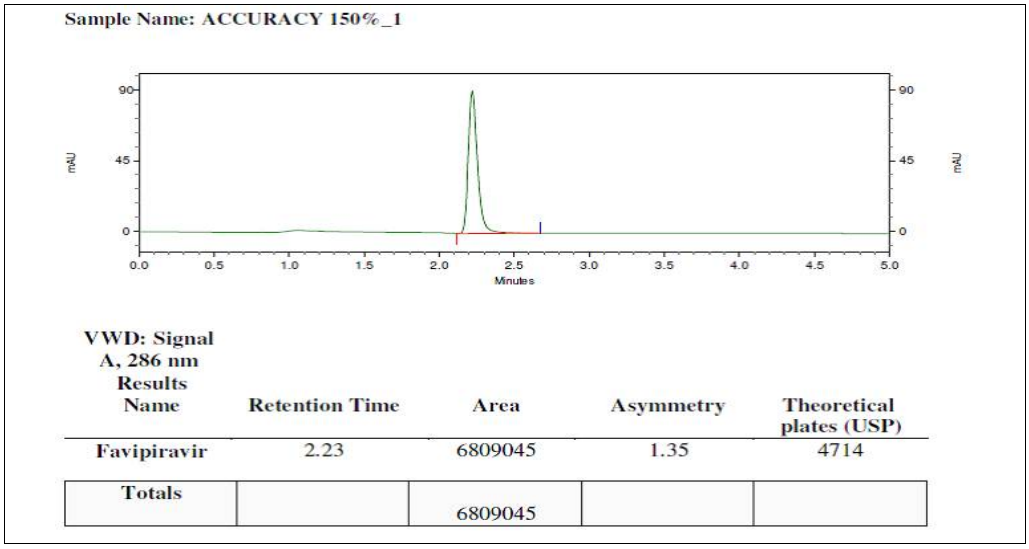
FIG. NO.21 : TYPICAL CHROMATOGRAM OF ACCURACY 50%.



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4506301	1.31	4613
Totals		4506301		

FIG. NO.22 : TYPICAL CHROMATOGRAM OF ACCURACY 100%.



VWD: Signal A, 286 nm Results

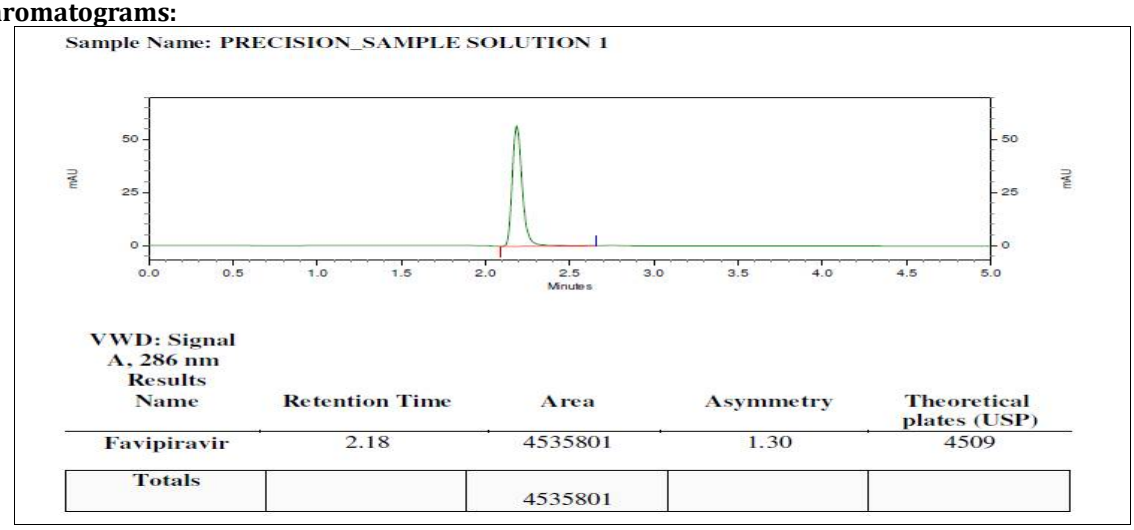
Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.23	6809045	1.35	4714
Totals		6809045		

FIG. NO.23 : TYPICAL CHROMATOGRAM OF ACCURACY 150%.

Acceptance criteria:
% Recovery for every level and overall recovery: 98.0 to 102.0%
% RSD for each level and overall recovery: NMT 2.0
Data interpretation: Recovery of analytical procedure were found well within a acceptance criteria at all 3 levels. % Recovery not get hampered by changed in analyte concentration.

PRECISION
The precision of analytical method is defined as degree of agreement amongst individual test findings when the method is conducted repeatedly to many samplings of a uniform sample. A method precision is typically stated as standard deviation or relative standard deviation. Precision testing should be carried out on a test sample.
Note:- Data given in TABLE NO.17 in separate sheet

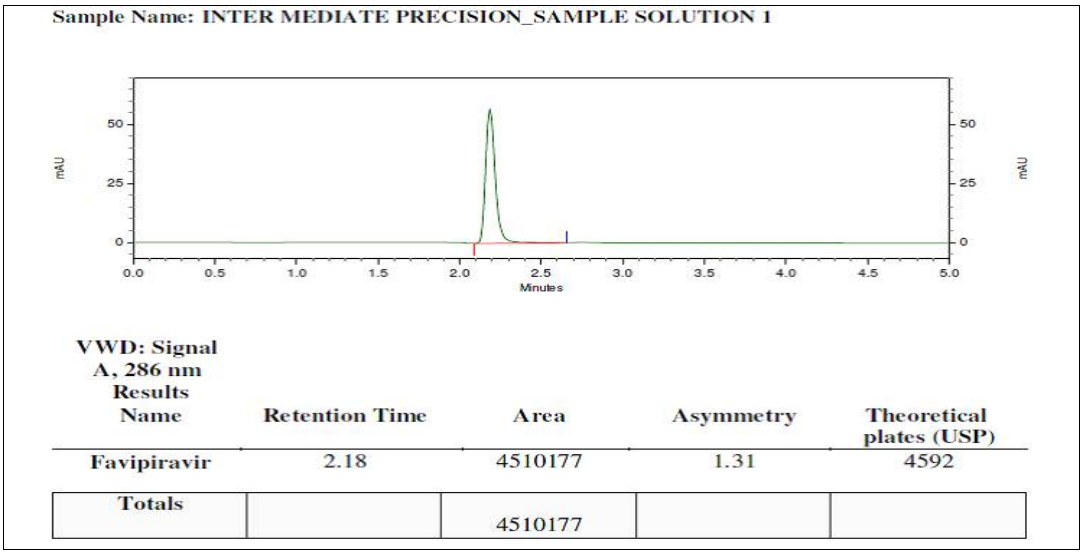
Chromatograms:



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4535801	1.30	4509
Totals		4535801		

FIG. NO.24 : TYPICAL CHROMATOGRAM OF REPEATABILITY PRECISION (SAMPLE 1).



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4510177	1.31	4592
Totals		4510177		

FIG.NO.25 : TYPICAL CHROMATOGRAM OF INTER-DAY PRECISION (SAMPLE 1).

Acceptance criteria:
% Assay: % Assay value for each sample (Individual sample) and mean assay value for precision (6 sample), mean assay value intermediate precision (6 sample), and mean assay value for precision plus intermediate precision sample (12 sample): 90-110%
% RSD: % RSD for precision study samples(6 sample), Intermediate precision study samples (6 sample) and precision plus intermediate precision sample (12 sample): NMT 2.0

Data interpretation: % Assay and % RSD were found within a acceptance limit and hence method is precise (Reproducible).

ROBUSTNESS:

The robustness is an analytical technique which is used for a measuring of its ability to remain unchanged by minor but calculated variations in a method parameters, and it indicates its dependability under normal conditions.

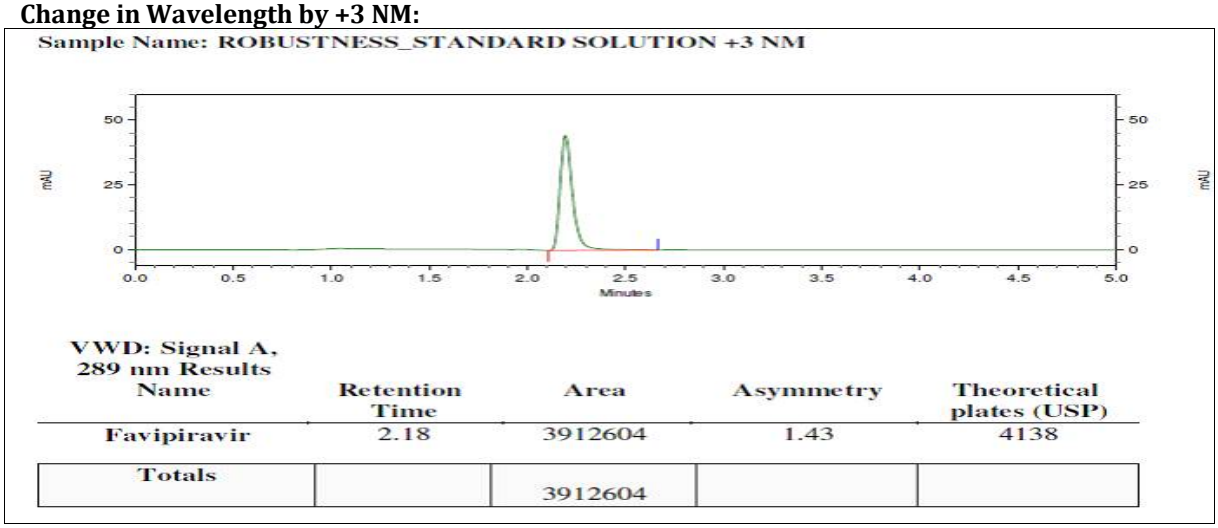
Following changes made under Robustness:

Change in Wavelength
Change in flow rate
Change in the column in oven temperature

Note:- Data given in TABLE NO.18 in separate sheet

Chromatograms:

Change in Wavelength by +3 NM:

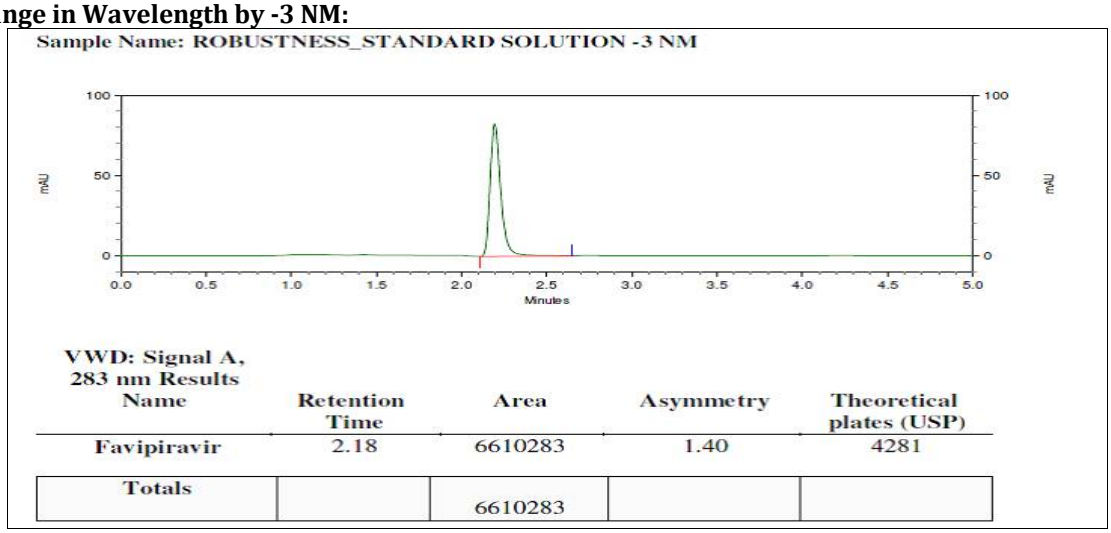


VWD: Signal A, 289 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	3912604	1.43	4138
Totals		3912604		

FIG. NO.26 : CHROMATOGRAM FOR THE STANDARD +3 NM.

Change in Wavelength by -3 NM:

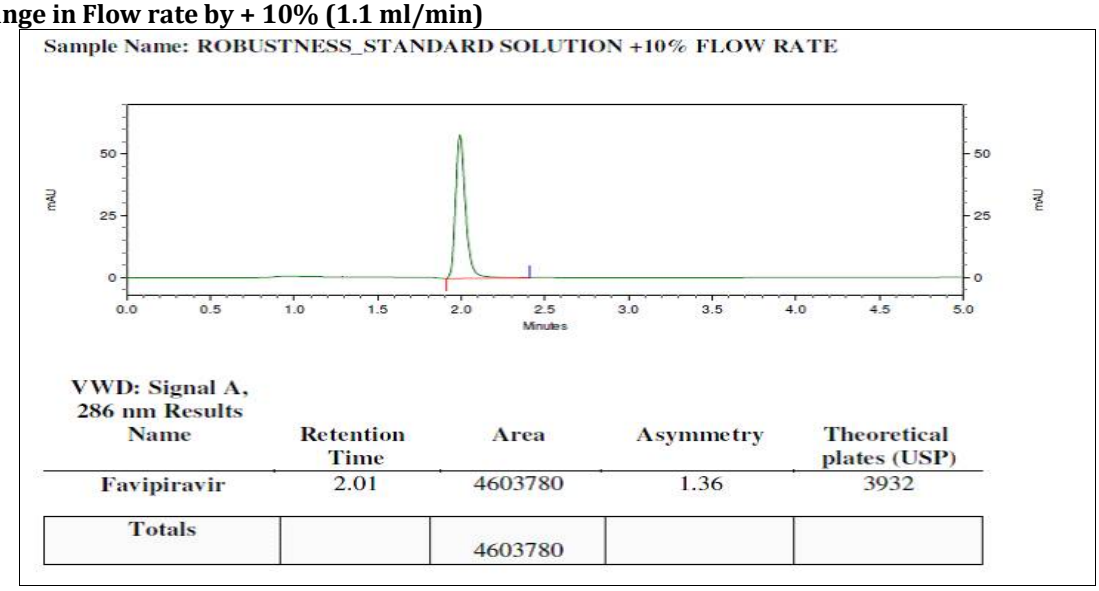


VWD: Signal A, 283 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	6610283	1.40	4281
Totals		6610283		

FIG. NO.27 : CHROMATOGRAM FOR THE STANDARD -3 NM.

Change in Flow rate by + 10% (1.1 ml/min)

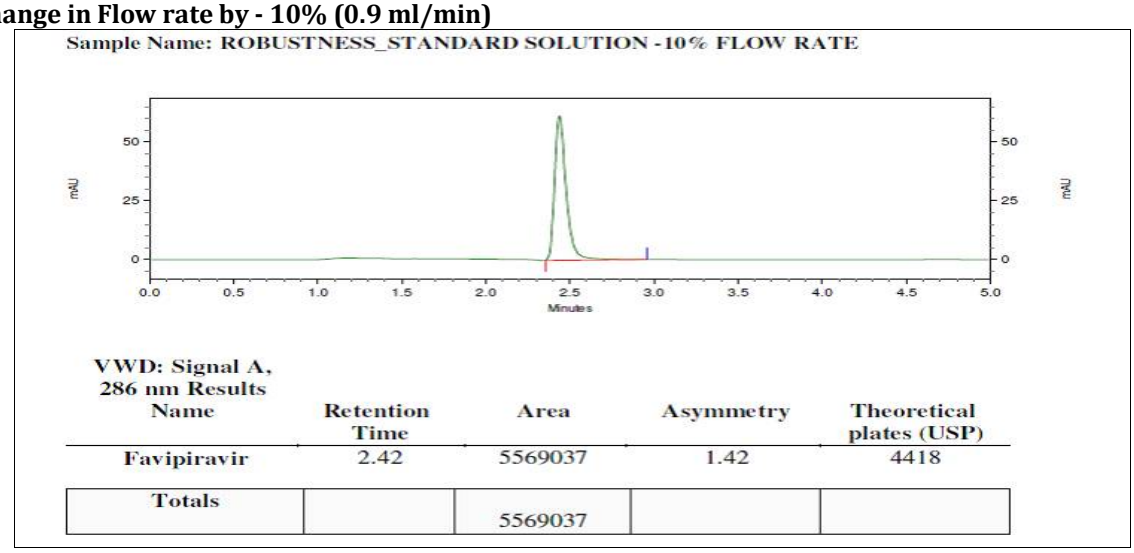


VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.01	4603780	1.36	3932
Totals		4603780		

FIG. NO.28: CHROMATOGRAM FOR THE STANDARD +10 F.R.%.

Change in Flow rate by - 10% (0.9 ml/min)

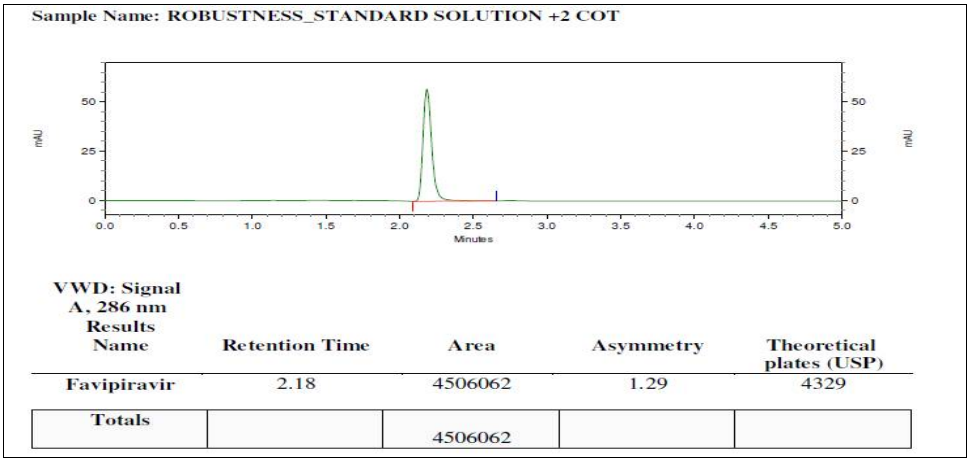


VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.42	5569037	1.42	4418
Totals		5569037		

FIG. NO.29 : CHROMATOGRAM FOR THE STANDARD -10 F.R.%.

Change in a Column in Oven temperature by +2°C:

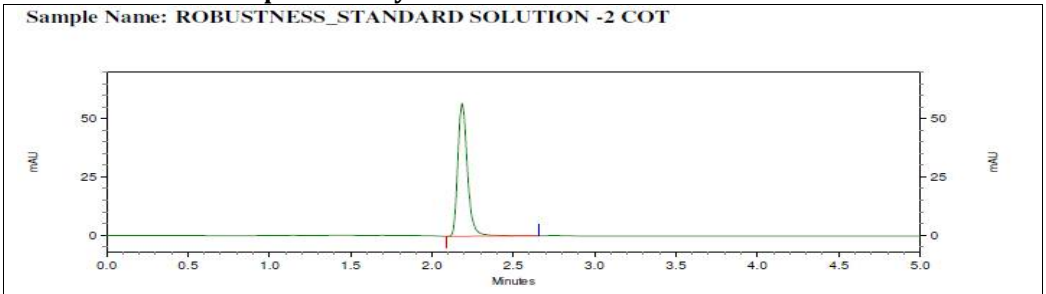


VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.18	4506062	1.29	4329
Totals		4506062		

FIG. NO.30 : CHROMATOGRAM FOR THE STANDARD +2°C C.O.T.

Change in Column Oven temperature by -2°C:



VWD: Signal A, 286 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Favipiravir	2.17	4483791	1.33	4503
Totals		4483791		

FIG. NO.31 : CHROMATOGRAM FOR THE STANDARD -2ºC C.O.T.

Acceptance criteria:
Chromatography (System suitability) acceptance criteria should not get failed.
Data interpretation: From the above results, it was observed that system suitability test result were found within the limits and analytical method was robust.

DISCUSSION

- The present work involved the development of simple, accurate, precise and suitable RP-HPLC method.
- Literature survey discovered that the several methods have been reported for determination of the Favipiravir in a bulk drug or in pharmaceutical dosage forms. Hence, in present study, a new, susceptible and suitable reversed-phase high performance liquid chromatography (RP-HPLC) method were newly developed and validated for the estimation of the Favipiravir in a bulk drug and pharmaceutical dosage form.
- In developed RP-HPLC method, the analyte should be resolved by using isocratic program and mobile phase was used Methanol : Water (50:50) with flow rate of 1.0 ml/min, on HPLC system containing UV- visible detector DEACX16446 and Phenomenex ODS-3 (250 mm X 4.6 mm i.d.). The detection should be carried out at 286 nm.
- The results founded in the analysis of newly developed method has been validated in conditions of parameter such as linearity, accuracy, precision, robustness, limit of detection and limit of quantification.

CONCLUSION

The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in a tablet dosage form. %RSD was also less than 2% showing high degree of precision of the proposed method.
Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for bulk drug of Favipiravir and also in pharmaceutical dosage form.

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