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# Stability Indicating Analytical Method Development And Validation For The Determination Of Favipiravir By Rp-Hplc

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**ABSTRACT**
A simple, Precised, Accurate method was developed for the determination of Favipiravir by RP-HPLC technique. Chromatographic conditions used are stationary phase Agilent c18 150 x 4.6 mm, 5m. Mobile phase 0.1% OPA buffer: Acetonitrile in the ratio of 50:50 and flow rate was maintained at 1ml/min, detection wave length was 230 nm, column temperature was set to 30oC and diluent was Acetonitrile: Water (50:50), Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R2 value was found to be as 0.999. Precision was found to be 0.7% for repeatability and 1.2% for intermediate precision. LOD and LOQ are 1.140µg/ml and 4.424µg/ml respectively. By using above method assay of marketed formulation was carried out 100.37% was present. The above method can be used in routine quality control analysis.
**Keywords**: HPLC Favipiravir , Method development. ICH Guidelines.

## INTRODUCTION:

**Pharmaceutical Analysis** is that core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and qualitative measurements. In practice, quantifying an analyte in a complex sample becomes an exercise in problem solving. To be efficient and effective, an analytical chemist must know the tools that are available to tackle a wide variety of problems. Analytical chemistry is divided into two branches qualitative and quantitative. A qualitative method provides information about the identity of atomic or molecular species or functional groups in sample. A quantitative method provides numerical information as to the relative amount of one or more of the components[1,2]. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and biological samples. In pharmaceutical industry, spectrophotometric and chromatographic methods have gained the significance in recent years. Spectrophotometric methods[3,4] It is defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. The electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types,

**Qualitative And Quantitative:** Qualitative Inorganic Analysis seeks to establish the presence of a given element or inorganic compound in a sample. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample. Quantitative analysis seeks to establish the amount of a given element or compound in a sample. There are various techniques used for analysis of mixtures. Spectroscopy measures the interaction of the molecules with electromagnetic radiation. Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated. Analytical Chromatography is used to determine the

existence and possibly also the concentration of analyte(s) in a sample[6]. Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, forensic science and so on.

**1.SPECTROSCOPY**

**ABSORPTION SPECTROPHOTOMETRY:**

When a molecule is exposed to an electromagnetic radiation (EMR), certain amount of energy associated with the particular radiation is absorbed by molecule. As a molecule absorbs energy, an electron is promoted from an occupied orbital to an unoccupied orbital of greater potential energy. Generally, the most probable transition is from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The energy differences between electronic levels in most molecules vary from 125 to 650 KJ/mole7.

**ULTRA VIOLET – VISIBLE SPECTROSCOPY:**

The name Ultra violet, means "beyond violet" (from Latin ultra, "beyond"), violet being the color of the shortest wavelengths of visible light. The color violet has the shortest wavelength in the visible spectrum. UV light has a shorter wavelength than that of violet light. One among the various analytical techniques used for analysis of mixtures is spectrophotometry. It involves separation of drugs by physical and chemical methods and then to read them at their _max to obtain results. This technique involves two steps and has relatively low sensitivity. Derivative spectroscopy is slight advanced which do not require separation but the difference in _max of component of drugs need to be at least 208 nm. Amongst the instrumental methods Spectrophotometric occupies very important position, which utilizes the measurement of intensity of electromagnetic radiation, emitted or absorbed by the analyte. The careful manipulation of the experimental parameters in UVvisible spectrophotometer often enables the analyte to estimate simultaneously more than one component present in a mixture without their prior separation.

**1.2 INTRODUCTION TO HPLC**

- The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography. Both terms can be abbreviated as HPLC.[7]
- High-pressure liquid-solid chromatography (HPLC) is rapidly becoming the method of choice for separations and analysis in many fields. Almost anything that can be dissolved can be separated on some type of HPLC column.[8]

**Characteristics of HPLC method** [9,10]

- Efficient, highly selective, widely applicable
- Only small sample required.
- May be nondestructive of sample
- Readily adapted to quantitative analysis.
- High resolving power.

**Modes of HPLC:** [11]

**1) Normal phase chromatography:** In normal phase mode, the nature of stationary phase is polar and the mobile phase is non-polar. In this technique, non-polar compounds travel faster and are eluted first because of the lower affinity between the non-polar compounds and stationary phase. Polar compounds are retained for longer time and take more time to elute because if their higher affinity with the stationary phase. Normal phase mode of separation is, therefore, not generally used pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

**2) Reversed phase chromatography:** Reversed phase mode is the most popular mode for analytical and preparative separations of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with octyl and octadecyl functional group bonded to silica gel and the mobile phase is a polar solvent, often a partially or fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds is reversed (thus the name reverse-phase chromatography).

**Method Devlopment on HPLC**[12]

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians (researchers) and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution.

Alternate analytical methods are developed for the drug product to reduce the cost and time. When alternative analytical methods are intented to replace the existing procedure, analyst should collect the literature for all types of information related to analyte and define the separation goal. Then estimate the best separation condition from trial runs. After optimizing the separation condition, validate the method for release to routine laboratory.

**Getting Started on Method Development** [13]

"Best column, best mobile phase, best detection wavelength, efforts in separation can
make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results – a validated method of separation."

**a) The Mobile Phase:** In reverse-phase chromatography, the mobile phase is more polar than the stationary phase. Mobile phase in these systems is usually mixtures of two or more individual solvents with or without additives or organic solvent modifiers. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solutes. The simple alteration of composition of the mobile phase or of the flow rate

allows the rate of the elution of the solutes to be adjusted to an optimum value and permits the separation of a wide range of the chemical types. First isocratic run followed by gradient run is preferred.

**b) The Detector:** The next consideration should be the choice of detector. UV-visible detectors are the most popular as they can detect a broad range of compounds and have a fair degree of selectivity for some analytes.

**c) The Column Length:** Many chromatographers make the mistake of simply using what is available. Often this is a 250 cm × 4.6 mm $C_{18}$ column. These columns are able to resolve a wide variety of compounds. While many reverse phase separations can be carried out on such column. Method development can be streamlined by starting with shorter columns; 150, 100 or even 50 cm long.

**d) The Stationary Phase:** Selecting an appropriate stationary phase can also help to improve the efficiency of method development. For example, a $C_8$ phase (reversed phase) can provide a further time saving over a $C_{18}$, as it does not retain analytes as strongly as the $C_{18}$ phase. For normal phase applications, cyano (nitrile) phases are most versatile.

**e) The Internal Diameter:** By selecting a shorter column with an appropriate phase, run times can be minimized so that an elution order and an optimum mobile phase can be quickly determined.

**f) Gradient Programming:** The fastest and easiest way to develop a method is to use a mobile phase gradient. Always start with a weak solvent strength and move to a higher solvent strength. To begin, use a very fast gradient (e.g.10 minutes) and then modify the starting and finishing mobile phases to achieve a suitable separation.

**g) Retention:** Analytes may be too strongly retained (producing long run times). If this occurs, the solvent strength should be increased. In reverse phase analysis this means a higher % of organic solvent in the mobile phase.

**h) Poor Separation:** Analytes often co-elute with each other or impurities. To overcome this, the analysis should be run at both higher and lower solvent strengths so the best separation conditions may be determined.

**i) Peak Shape:** This is often a problem, especially for basic compounds analyzed by reversed phase HPLC. To minimize any potential problems always use a high purity silica phase such as Wakosil II. These modern phases are very highly deactivated so secondary interactions with the support are minimal. To maximize the reproducibility of a method, it is best to use a column heater to control the temperature of the separation. A temperature of 35 – 40°C is recommended.

**j) Buffer selection:** In reverse phase HPLC, the retention of analytes is related to their hydrophobicity. The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic and, therefore, its retention decreases. When separating mixtures containing acid and/or bases by reversed phase HPLC, it is necessary to control the pH of mobile phase using appropriate buffer in order to achieve reproducible results.

Buffers play an additional role in the reproducibility of a separation. The buffer salts reduce peak tailing for basic compounds by effectively masking silanols. They also reduce potential ion-exchange interactions with unprotonated silanols (Figure 1.2).

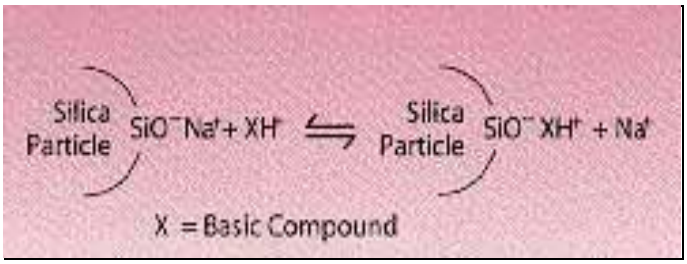



**Fig. 1.1: Peak Tailing Interaction**

**k) Selection of pH:** The pH range most often used for reversed-phase HPLC is 1 - 8 and can be divided into low pH (1 - 4) and intermediate pH (4 - 8) ranges. Each range has a number of advantages. Low pH has the advantage of creating an environment in which peak tailing is minimized and method ruggedness is, maximized. For this reason, operating at low pH is recommended. At a mobile phase pH greater than 7, dissolution of silica can severely shorten the lifetime of columns packed with silica-based stationary phases.

The pKa value (acid dissociation [ionization] constant) for a compound is the pH at which equal concentrations of the acidic and basic forms of the molecule are present in aqueous solutions. Analytes may sometimes appear as broad or tailing peaks when the mobile phase pH is at, or near, their pKa values. A more rugged mobile phase pH will be at least 1 pH unit different from the analyte pKa. This shifts the equilibrium so that 99% of the sample will be in one form. The result is consistent chromatography.

## INTRODUCTION TO METHOD VALIDATION

**"Doing thorough method validation can be tedious, but the consequences of not doing it right are wasted time, money, and resources."**

**Defination:** Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. The real goal of validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method.[20]

**Type of analytical procedures to be validated:** Validation of analytical procedures is directed to the four most common types of analytical procedures.

- Identification test.
- Quantitative test for impurities content.
- Limit test for the control of impurities.
- Quantitative test of the active moiety in samples of drug substance on drug product on other selected components in the drug product.

In our method of validation, we are following last type.

Assay procedures are intended to measure the analyst

present in given sample, assay represent a quantitative measurement of the major component(s) in the drug sample. Two steps are required to evaluate an analytical method.

1) First determine the classification of the method.

2) The second step is to consider the characteristics of the analytical method

For analytical method validation of pharmaceuticals, guidelines from the International Conference on Harmonization (ICH), United States Food and Drug Administration (US FDA), American Association of Official Analytical Chemists (AOAC), United States Pharmacopoeia (USP) and International Union of Pure and Applied Chemists (IUPAC) provide a framework for performing such validations in efficient and productive manner.

**Reasons for method validation**

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation.

**Performance characteristics examined when carrying out method validation** [21]

- Specificity
- Linearity
- Range
- Accuracy
- Precision ( Repeatability and Ruggdness )
- Detection and Quantitation limit
- Robustness.
- System suitability

The different parameters of analytical method development are discussed below as per ICH guideline:-

**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.

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

**2) Linearity:**

The linearity of an analytical procedure is its ability (within given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

**3) Range:**

The range of analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The specified range is normally derived from linearity studies and depends on the intended application of the procedure.

**4) Accuracy:**

Accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy should be established across the specified range of the analytical procedure.

**5) Precision:**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

**6) Detection Limit:**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated under stated experimental conditions.

**7) Quantitation Limit:**

The quantitation limit of an individual analytical procedure is defined as the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

**8) Robustness:**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

**9) System Suitability Testing:**

The system has to be tested for its suitability for the intended purpose. System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

Parameters such as plate count, tailing factors, resolution and reproducibility (% RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method.

**A. Retention Time (Rt):** Retention time is the time of elution of peak maximum after injection of compound.

**B. Theoretical Plates (N):** The number of theoretical plates in column is given by the following relationship,

$$N = 16 (t / w)^2$$

Where, t is retention time and w is width at the base of the peak.

HETP = L / N

Where L=length of column.

The theoretical plates should be more than 2000.

**C. Resolution(R):** Resolution of two components in mixture is determined by following equation,

$$Rs = \frac{2 (t_2 - t_1)}{\text{----------}}$$

$$W_1+W_2$$

Where, $t_2$ and $t_1$ is the retention time of second and first compound respectively, whereas $W_2$ and $W_1$ are the corresponding widths at the bases of peak obtained by extrapolating straight sides of the peaks to baselines.

R should be more than 2 between peaks of interest and the closest eluted potential interferences (impurities, excipients, degradation products or internal standard).

**D. Tailing Factor (T):** It is the measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing become more pronounced.

$$T = \frac{W_{0.05}}{2F}$$

Where, $W_{0.05}$ is the width of peak at 5% height and F is the distance from the peak maximum to the leading edge of the peak height from baseline.

Tailing factor should be less than 2.

**E. Capacity Factor (K'):** It is calculated by using the formula,

$$K' = \frac{t}{t_a - 1}$$

Where, t is the retention time of drug and $t_a$ is the retention time of non-retarded component, air with thermal conductivity detection.

The following table lists the terms generally used and their recommended limits obtained from the analysis of the system suitability sample.

System suitability parameters and Characteristics to be validated in HPLC method are given in **Table 1.1** and **1.2**, respectively.

**Table 1.1: System Suitability Parameters and their recommended limits.**

| Parameter | Recommendation |
|---|---|
| Capacity Factor (K') | The peak should be well-resolved from other peaks and the |
| Repeatability | RSD ≤ 1% |
| Relative | Not essential as the resolution is stated |
| Resolution($R_S$) | $R_S$ of > 2 between the peak of interest and the closest eluting |
| Tailing Factor(T) | T ≤ 2 |
| Theoretical | In general should be > 2000 |

**Table 1.2: Characteristics to be validated in HPLC.**

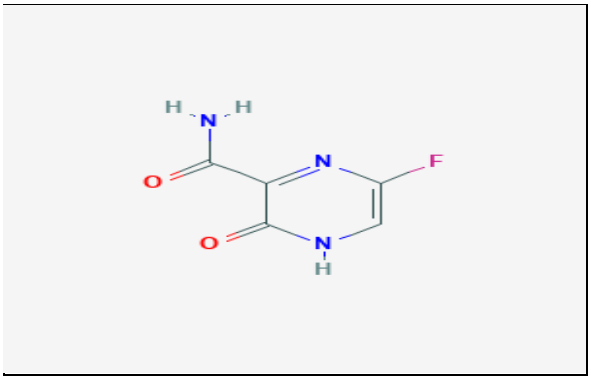
| Characteristics | Acceptance Criteria |
|---|---|
| Accuracy/trueness | Recovery 98-102% (individual) |
| Precision | RSD < 2% |
| Repeatability | RSD < 2% |
| Intermediate Precision | RSD < 2% |
| Specificity / | No interference |
| Detection Limit | S/N > 2 or 3 |
| Quantitation Limit | S/N > 10 |
| Linearity | Correlation coefficient $R^2$ > 0.999 |
| Range | 80 –120 % |

## 2. DRUG PROFILE[17]

**Favipiravir**

**Description**: Discovered by Toyama Chemical Co., Ltd. in Japan, favipiravir is a modified pyrazine analog that was initially approved for therapeutic use in resistant cases of influenza.The antiviral targets RNA-dependent RNA polymerase (RdRp) enzymes, which are necessary for the transcription and replication of viral genomes.Not only does favipiravir inhibit replication of influenza A and B, but the drug has shown promise in the treatment of avian influenza, and may be an alternative option for influenza strains that are resistant to neuramidase inhibitors. Favipiravir has been investigated for the treatment of life-threatening pathogens such as Ebola virus, Lassa virus, and now COVID-19.

**Structure:**




**CAS number:** 259793-96-9

**IUPAC Name:** 6-fluoro-3-hydroxypyrazine-2-carboxamide

**State:** Solid

**Melting point (°C)**: 187°C to 193°C

**Solubility** : slightly soluble in water

**pKa** :5.1

**Indication:** In 2014, favipiravir was approved in Japan to treat cases of influenza that were unresponsive to conventional treatment. Given its efficacy at targetting several strains of influenza, it has been investigated in other countries to treat novel viruses including Ebola and most recently, COVID-19.

**Pharmacodynamics:** Favipiravir functions as a prodrug and undergoes ribosylation and phosphorylation intracellularly to become the active favipiravir-RTP. Favipiravir-RTP binds to and inhibits RNA dependent RNA polymerase (RdRp), which ultimately prevents viral transcription and replication.

**Mechanism of action:** The mechanism of action of favipiravir is novel compared to existing influenza antivirals that primarily prevent entry and exit of the virus from cells. The active favipiravir-RTP selectively inhibits RNA polymerase and prevents replication of the viral genome. There are several hypotheses as to how favipiravir-RTP interacts with RNA dependent RNA polymerase (RdRp). Some studies have shown that when favipiravir-RTP is incorporated into a nascent RNA strand, it prevents RNA strand elongation and viral proliferation. Studies have also found that the presence of purine analogs can reduce favipiravir's antiviral activity, suggesting competition between favipiravir-RTP and purine nucleosides for RdRp binding.

**Volume of distribution:** The apparent volume of distribution of favipiravir is 15 - 20 L.

**Proteinbinding :** Favipiravir is 54% plasma protein-bound Of this fraction, 65% is bound to serum albumin and 6.5% is bound to α1-acid glycoprotein.

**Metabolism :** Favipiravir is extensively metabolized with metabolites excreted mainly in the urine.10 The antiviral undergoes hydroxylation primarily by aldehyde oxidase and to a lesser extent by xanthine oxidase to the inactive metabolite, T705M1.10

**Route of elimination:** Favipiravir's metabolites are predominantly renally cleared.9

Half-life : The elimination half-life of favipiravir is estimated to range from 2 to 5.5 hours.9

## 3. LITERATURE REVIEW[18-23]

1. **Ibrahim bulduk.** Favipiravir (FVP), a pyrazine analog, has shown antiviral activity against a wide variety of viruses. It is considered to be worth further investigation as a potential candidate drug for COVID-19. It is not officially available in any pharmacopoeia. A rapid, simple, precise, accurate, and isocratic high performance liquid chromatography (HPLC) method has been developed for routine quality control of favipiravir in pharmaceutical formulations. Separation was carried out by C18 column. The mobile phase was a mixture of 50 mM potassium dihydrogen phosphate (pH 2.3) and acetonitrile (90:10, v/v) at a flow rate of 1 mL $min^{-1}$. The ultraviolet (UV) detection and column temperature were 323 nm, and 30 °C, respectively. The run time was 15 min under these chromatographic conditions. Excellent linear relationship between peak area and favipiravir concentration in the range of 10–100 µg $mL^{-1}$ has been observed ($r^2$, 0.9999). Developed method has been found to be sensitive (limits of detection and quantification were 1.20 µg $mL^{-1}$and 3.60 µg $mL^{-1}$, respectively), precise (the interday and intraday relative standard deviation (RSD) values for peak area and retention time were less than 0.4 and 0.2%, respectively), accurate (recovery, 99.19–100.17%), specific and robust (% RSD were less than 1.00, for system suitability parameters). Proposed method has been successfully applied for quantification of favipiravir in pharmaceutical formulations.

2. **M.J.Navas & A.M.jimenez.** Analytical techniques employed in anti-influenza drugs analysis are summarized. The literature reviewed covers the papers of analytical interest that have appeared in approximately the last 10 years. Specific applications to Amantadine, Rimantadine, Oseltamivir, Zanamivir, and Peramivir are included. Possibilities and limitation of various analytical methods are evaluated.

3. **Abdul Rahaman Shaik and N.khaleel.** Specific stability-indicating reversed-phase high performance liquid chromatography (HPLC) method has been developed and validated for the quantification of tolvaptan in bulk drug and pharmaceutical dosage form. The optimized conditions for the developed HPLC method are; Inertil ODS-3V column (150 x 4.6 mm, 5.0 mm) maintained at 30°C with mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio 40:60%v/v on isocratic mode at flow rate of 1.0 mL/min and detection wavelength 254 nm. The retention time of tolvaptan was found to be 2.59 min with linearity in the concentration range from 37.5 – 225.0 µg/mL, respectively. The mean percentage recovery of tolvaptan was found to be 98.30 – 101.13 %, respectively. The percent relative standard values were less than 2.0 at all the levels and indicates a satisfactory accuracy and precision. The robustness of the method found to meet the acceptance criteria. The stress study against qualified working standard of Tolvaptan, indicated that the developed HPLC method was stability- indicating, conducted as per ICH requirements. The developed method can be handy in the quality control of bulk and pharmaceutical dosage forms.

4. **Brian B. Gowen, et al.,(2015):** Favipiravir (T-705) is a new anti-influenza drug approved for human use in Japan and progressing through Phase 3 clinical trials in the U.S. In addition to its potent inhibitory effects against influenza virus infection, the compound has been shown to be broadly active against RNA viruses from 9 different families, including the Arenaviridae. Several members of the Arenaviridae family of viruses are significant human pathogens that cause viral hemorrhagic fever, a severe systemic syndrome where vascular leak is a cardinal feature. Because arenaviral infections are unlikely to be diagnosed and treated until the illness has progressed to a more advanced state, it is important to understand the effects of the disease state on favipiravir pharmacokinetics (PK) and biodistribution to help guide therapeutic strategy. During acute arenavirus infection in hamsters, we found reduced plasma favipiravir concentrations and altered kinetics of absorption, elimination and time to maximum drug concentration. In addition, the amounts of the favipiravir M1 primary metabolite were higher in the infected animals, suggesting that favipiravir metabolism may favor the formation of this inactive metabolite during viral infection. We also discovered differences in favipiravir and M1 PK parameters associated with arenavirus infection in a number of hamster tissues. Finally, analysis at the individual animal level demonstrated a correlation between reduced plasma favipiravir concentration with increased disease burden as reflected by weight loss and viral load. Our study is the first to show the impact of active viral infection and disease on favipiravir PK and biodistribution, highlighting the need to consider alterations in these parameters when treating individuals with viral hemorrhagic fever of arenavirus or other etiology Hiep X. Nguyen et al,.(2015): This paper presents a sensitive, specific and reliablegradient reverse-phase high-performance liquidchromatography method for determination of favipiravir.Favipiravir was analyzed on a Phenomenex Luna C18analytical column (150x4.60 mm, 5 µm) usingacetonitrile – water (0.05 % v/v trifluoroacetic acid) asmobile phase at a flow rate of 1.0 mL/min. The method provided a good linearity (R 2 = 1.000) ver the range 0.1 – 50 µg/mL. The assay method was successfully applied tostudy favipiravir solubility in 10 mM phosphate bufferedsaline, propylene glycol, polyethylene glycol 400, 10 mM phosphate buffered saline:ethanol (75:25 v/v), 10 mM phosphate buffered saline:polyethylene glycol 400 (50:50v/v); and the drug stability in ethanol and these solventsunder different

storage conditions. This method allows forassessment of favipiravir concentrations for transdermalin vitro permeation studies

**5. Thi Huyen Tram Nguyen et al., (2017):** In 2014–2015, we assessed favipiravir tolerance and efficacy in patients with Ebola virus (EBOV) disease (EVD) in Guinea (JIKI trial). Because the drug had never been used before for this indication and that high concentrations of the drugs were needed to achieve antiviral efficacy against EBOV, a pharmacokinetic model had been used to propose relevant dosing regimen. Here we report the favipiravir plasma concentrations that were achieved in participants in the JIKI trial and put them in perspective with the model-based targeted concentrations.

## 4. OBJECTIVE AND AIM OF THE STUDY

**Aim:** For the Favipiravir drug, one HPLC-UV method have been written (12-14). The main aim of the present study is to develop an new accurate, precise, sensitive, selective, reproducible and rapid analytical technique for estimation of Favipiravir drug in bulk ant Pharmaceutical dosage form.

## OBJECTIVE OF WORK:

- To develop a new stability indicating HPLC method for determination of `Favipiravir and to develop the validated method according to ICH guidelines..
- Literature survey for selection of drug and to gain theoretical knowledge on analytical method development and validation.
- Procurement and standardization of drugs.
- Development and optimization of analytical method.
- Apply in the pharmaceutical formulation the validated procedure for estimating Favipiravir.

## 5. METERIALS AND METHODS

### *5. 1. EQUIPMENTS AND CHEMICALS*

**Equipment and Apparatus used:**

1. HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Favipiravir solution.
2. Sonicator (Ultrasonic sonicator)
3. $P^H$ meter (Thermo scientific)
4. Micro balance (Sartorius)
5. Vacuum filter pump

**Reagents used:**

1. Methanol HPLC Grade (RANKEM)
2. Acetonitrile HPLC Grade (RANKEM)
3. HPLC grade Water (RANKEM)
4. Glacial Acetic acid
5. APT received from spectrum lab
6. Test sample received from local market (DalsiClear), Abbott India Ltd.

**Methods:**

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

**Preparation of buffer:**

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

**Buffer: 0.01N Potassium dihyrogen ortho phosphate:** Accurately weighed 1.36gm of Potassium dihyrogen 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 added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution

**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, 5ml 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. (100µg/ml of Favipiravir)

**Validation:**

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solutions of Favipiravir (100ppm) 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 Standard stock solutions:** Accurately weighed 50mg of Favipiravir transferred to 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 stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.(100 µg/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, 5ml 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. (100µg/ml of Favipiravir )

**Linearity:**

**Preparation of Standard stock solutions:** Accurately weighed 50mg of Favipiravir transferred to two separately 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 pipettede out and made up to 10ml. (150µg/ml of Favipiravir)

**Accuracy:**

**Preparation of Standard stock solutions:** Accurately weighed 50mg of Favipiravir transferred to two separately 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 50% 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 100% Spiked Solution:** 1.0ml 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:** 1.5ml 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.

**Acceptance Criteria:**

The % Recovery for each level should be between 98.0 to 102

**Robustness:** Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/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.

**LOD sample Preparation:** 0.25ml Standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml Favipiravir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

**LOQ sample Preparation:** 0.25ml standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml Favipiravir of, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

**Degradation studies:**

**Oxidation:**

To 1 ml of stock solution of Favipiravir, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at $60^0$c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:**

To 1 ml of stock ssolution Favipiravir, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at $60^0$c .The resultant solution was diluted to obtain 1 0 0 µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:**

To 1 ml of stock solution Favipiravir, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at $60^0$c. The resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:**

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

**Photo Stability studies:**

The photochemical stability of the drug was also studied by exposing the 1000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies:**

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

### 5.2 METHOD DEVELOPMENT

Based on drug solubility and $P^{ka}$ Value following conditions has been used to develop the method estimation of Favipiravir.

**Trial 1**

**Chromatographic conditions:**

**Mobile phase:** Water: Acetonirile (50:50 v/v)

**Flow rate:**1 ml/min

**Column :** Discovery C18 (4.6 x 150mm, 5µm)

**Detector wave length:**230nm

**Column temperature** : 30°C

**Injection volume** : 10 µL

**Run time** : 6 min

**Diluent** : Water and Acetonitrile in the ratio 50:50

**Results** : In this trail favipiravir peak was eluted but broad peak shape observed. So, further trail was carried out.

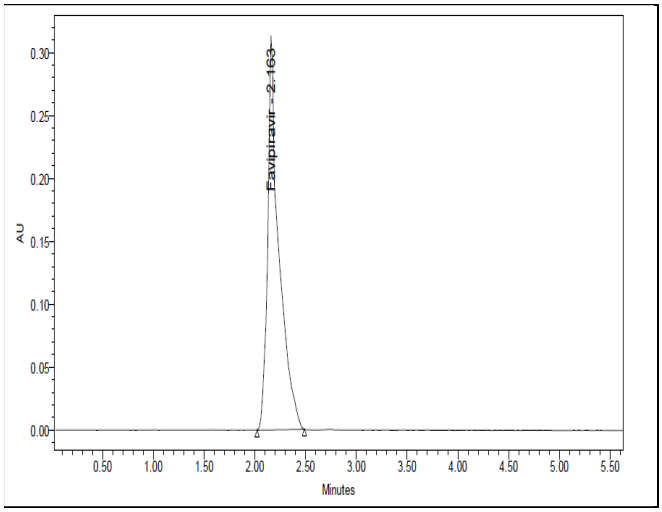



**Fig 6.1 Trial chromatogram 1**

**Trial 2**

**Chromatographic conditions:**

**Mobile phase** : Acetonitrile:Kh2po4(40:60 v/v)

**Flow rate** : 1ml/min

**Column** : Kromasil C18 (4.6 x 150mm, 5µm)

**Detector wave length** : 230nm

**Column temperature** : 30°C

**Injection volume** : 10 µL

**Run time** : 5 min

**Diluent** : Water and Acetonitrile in the ratio (50:50)

**Results** : In this trail favipiravir peak was elutede but peak splitting is observed. So, further trail was carried out.

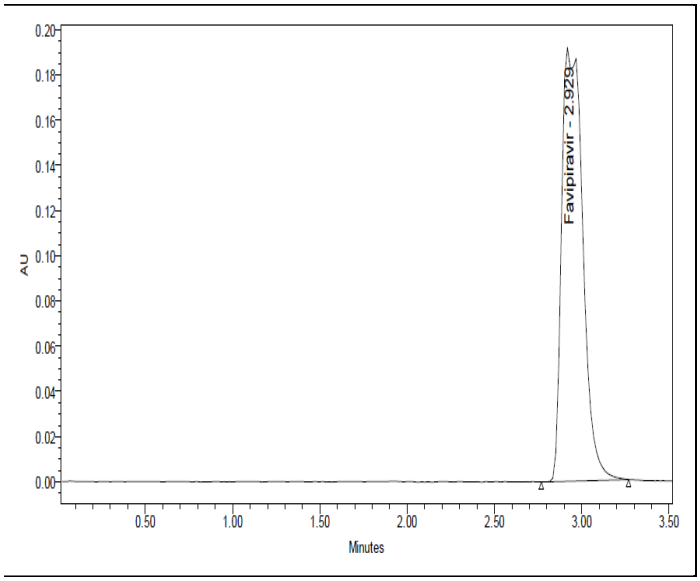



**Fig 6.2 Trial chromatogram 2**

**Trial 3**

**Chromatographic conditions:**

**Mobile phase** : Acetonitriile: kh2po4 (50:50 v/v)

**Flow rate** : 1 ml/min

**Column** : Zodiacsil C18 (4.6 x 150mm, 5µm)

**Detector wave length** : 230nm

**Column temperature** : 30°C

**Injection volume** : 10 µL

**Run time** : 5.0 min

**Diluent** : Water and Acetonitrile in the ratio 50:50

**Results** : In this trail by changing the column peak was eluted but broad peak shape and Baseline disturbance is observed. So, further trail was carried out.

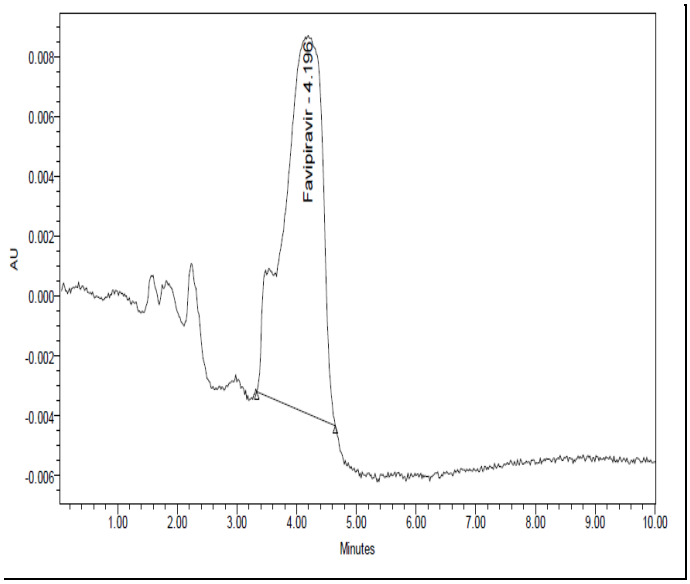



**Fig 6.3 Trial chromatogram 3**

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**Trail_4**

**Chromatographic conditions:**
**Mobile phase** : Acetonitriile: Water (50:50 v/v)
**Flow rate** : 1 ml/min
**Column** : Agilent C18 (4.6 x 150mm, 5µm)
**Detector wave length** : 230nm
**Column temperature** : 30°C
**Injection volume** : 10 µL
**Run time** : 10 min
**Diluent** : Water and Acetonitrile in the ratio 50:50
**Results** : In this trail the peak was eluted with good shape and all parameters were in the limit but the is to reduce the retention time. So, further trail was carried out.

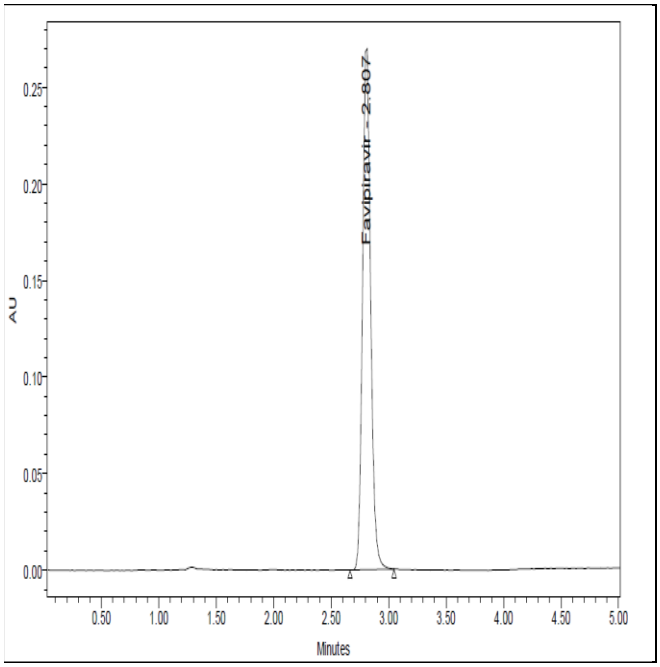



**Fig 6.4 Trial chromatogram 4**

**Optimized Chromatographic Conditions:**
**Mobile phase** : Acetonitriile: 0.1% OPA (50:50 v/v)
**Flow rate** : 1 ml/min
**Column** : Agilent C18 (4.6 x 150mm, 5µm)
**Detector wave length** : 230nm
**Column temperature** : 30°C
**Injection volume** : 10 µL
**Run time** : 10 min
**Diluent** : Water and Acetonitrile in the ratio 50:50
**Observation** : Favipiravir eluted with good peak shape and retention time and tailing was passed.

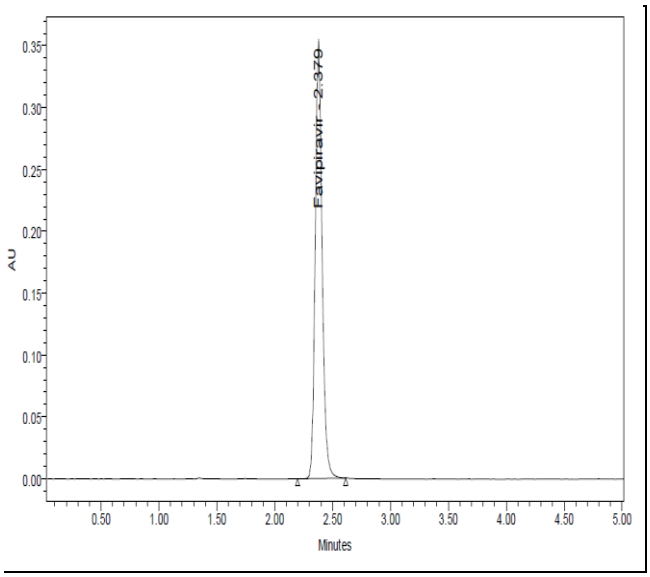



**Fig 6.5 optimized chromatogram**

## 6. RESULTS AND DISCUSSIONS

### 6.1 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 five replicate injections are within range and Results were shown in table 6.1.

Peak Name: Favipiravir

| | Peak Name | RT | Area | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|
| 1 | Favipiravir | 2.370 | 1794002 | 8107 | 1.14 |
| 2 | Favipiravir | 2.414 | 1769910 | 8105 | 1.14 |
| 3 | Favipiravir | 2.414 | 1772002 | 8034 | 1.14 |
| 4 | Favipiravir | 2.416 | 1753365 | 8343 | 1.13 |
| 5 | Favipiravir | 2.416 | 1771396 | 8251 | 1.14 |
| 6 | Favipiravir | 2.419 | 1750261 | 8310 | 1.14 |
| Mean | | | 1768489 | | |
| Std. Dev. | | | 15714.4 | | |
| % RSD | | | 0.9 | | |

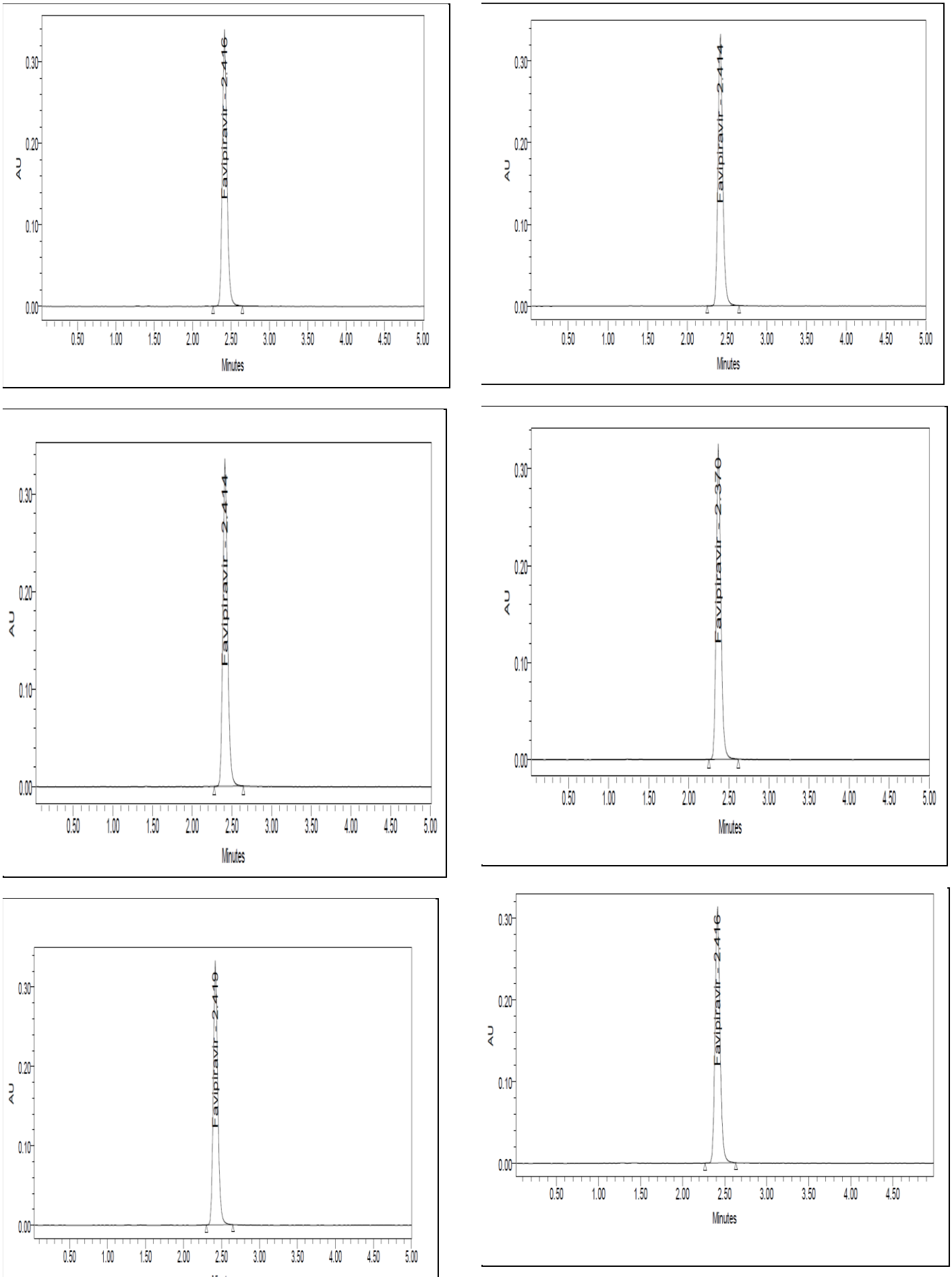



**Fig 6.9 System suitability Chromatogram**

**Discussion:** According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Validation: Specificity**

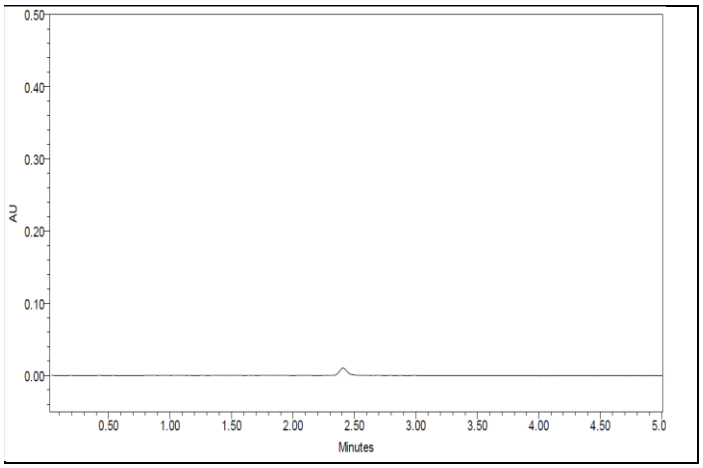



**Fig 6.8 blank Chromatogram**

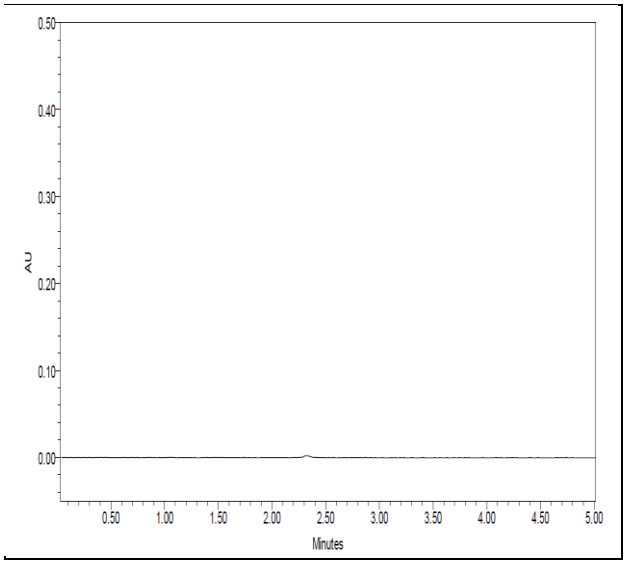


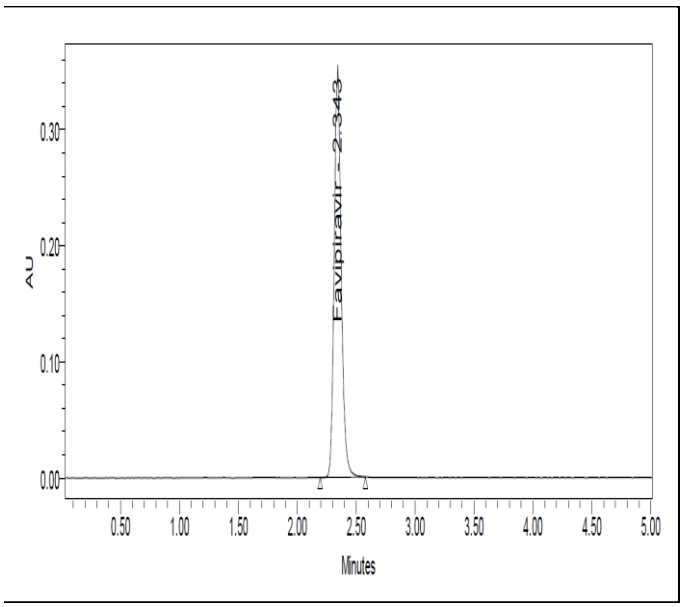

**Fig 6.9 Placebo Chromatogram**

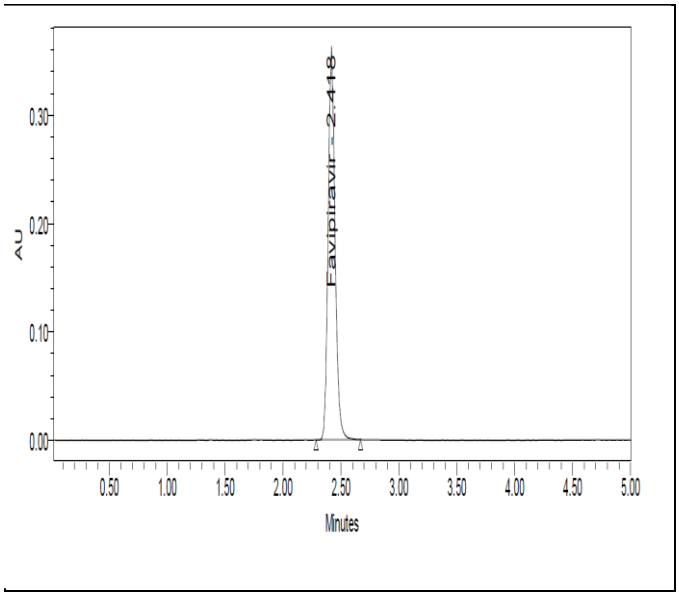
**6.2 Precision:**

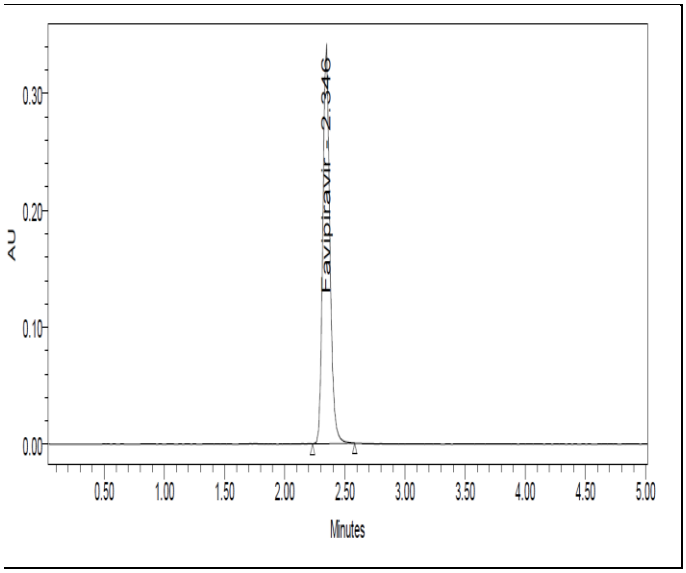
**Repeatability:** Six working sample solutions of 100ppm are injected and the % Amount found was calculated and %RSD was found to be 0.9 and chromatogram was shown in fig 6.2.

**Table 6.2 Repeatability data**

| S.No | Peak Area |
|---|---|
| 1 | 1767890 |
| 2 | 1795927 |
| 3 | 1782310 |
| 4 | 1771645 |
| 5 | 1787820 |
| 6 | 1799210 |
| AVG | 1784134 |
| STDEV | 12673.7 |
| %RSD | 0.7 |



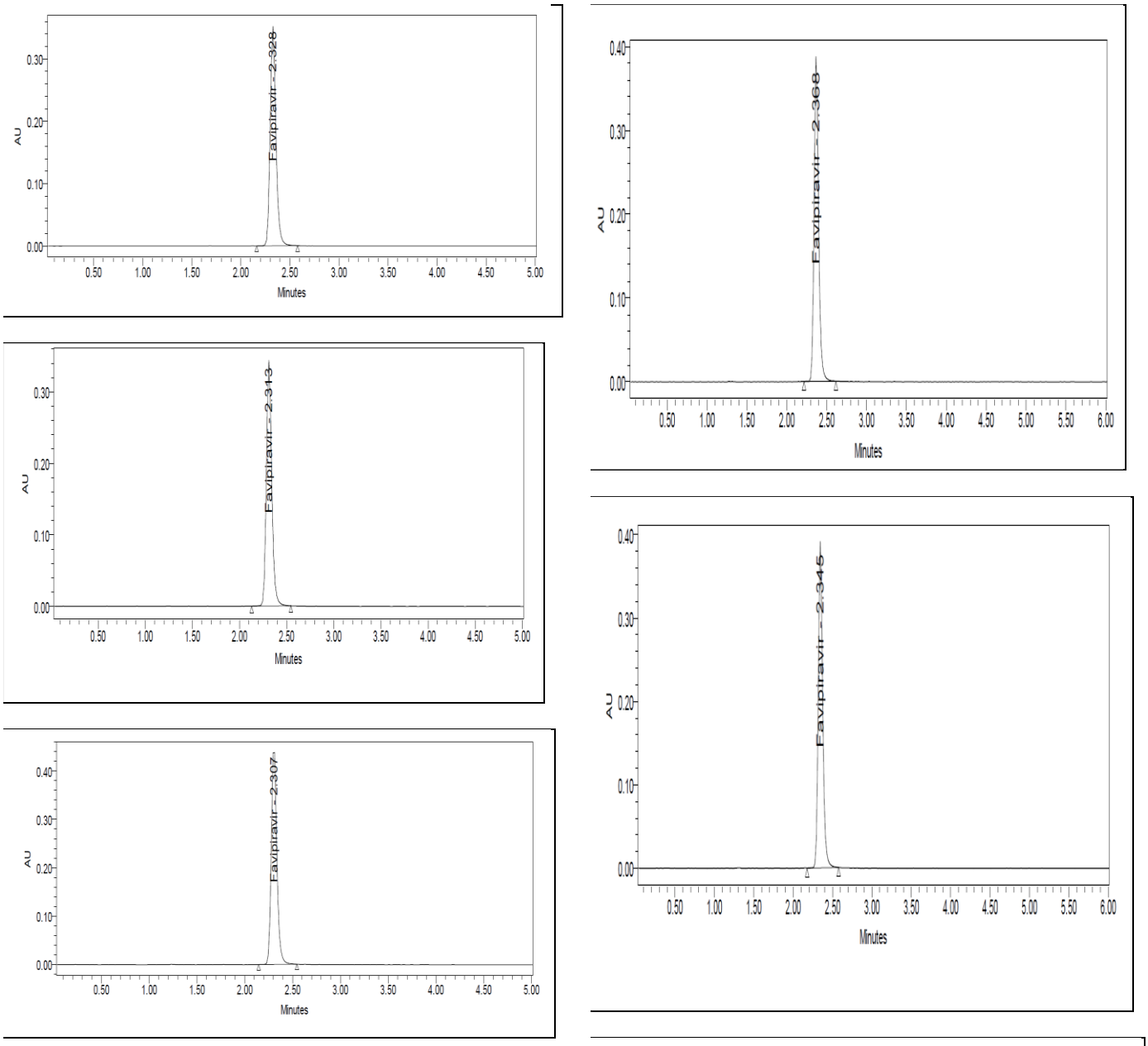
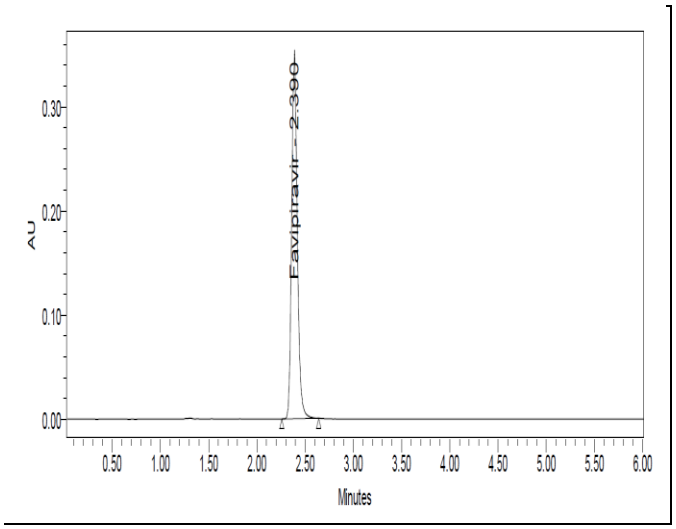


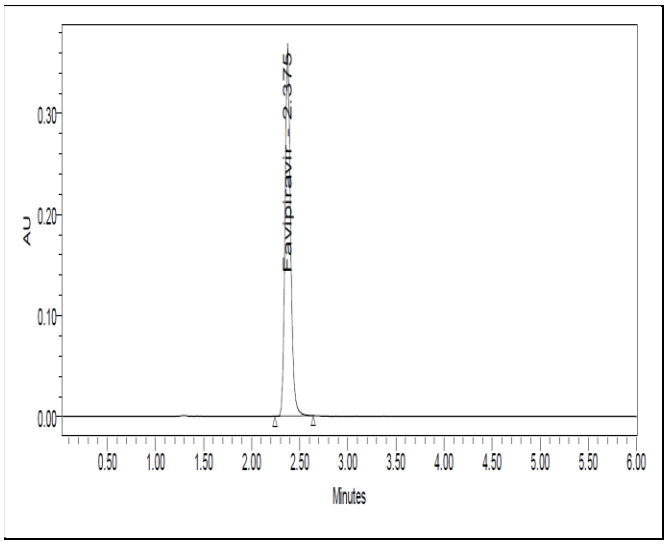

**Fig 6.10 Repeatability Chromatogram**

**Intermediate precision:** Six 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 0.7 and chromatogram was shown in fig 6.3.

**Table 6.3 Intermediate precision data**

| S.No | Peak Area |
|---|---|
| 1 | 1725503 |
| 2 | 1718547 |
| 3 | 1707234 |
| 4 | 1719121 |
| 5 | 1765381 |
| 6 | 1721596 |
| AVG | 1726230 |
| STDEV | 20125.7 |
| %RSD | 1.2 |



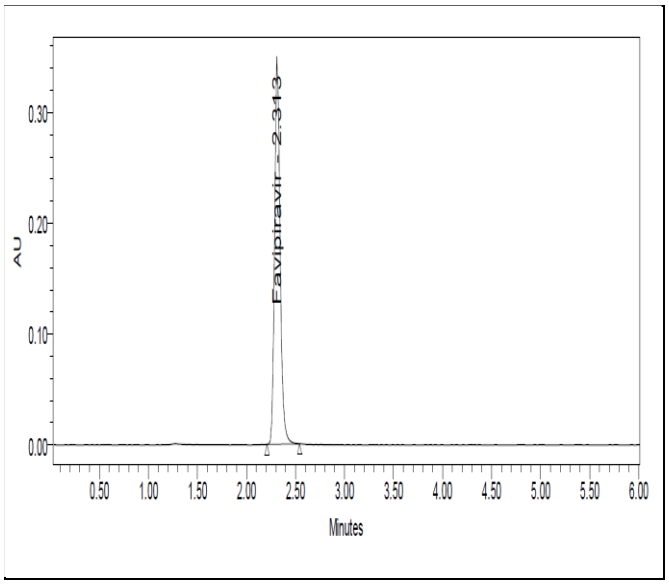




**Fig 6.11 Intermediate precision Chromatogram**

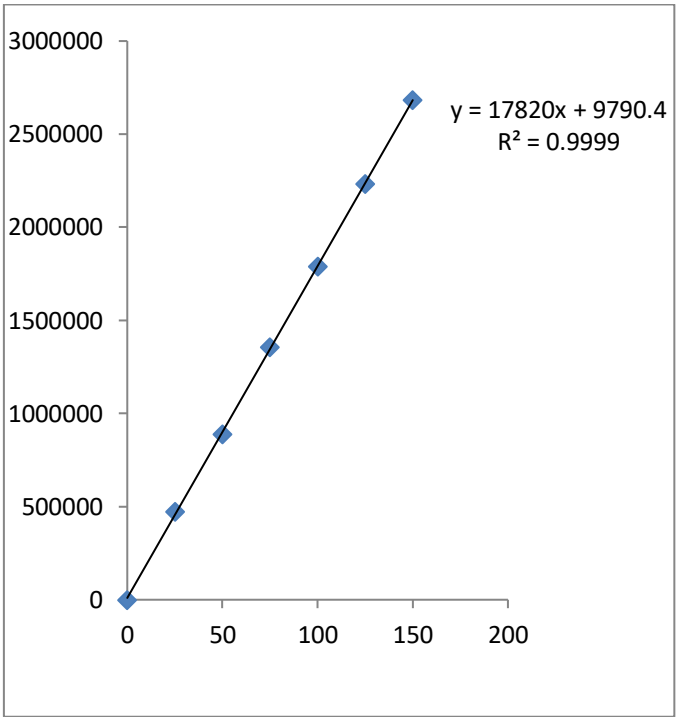
### 6.3 LINEARITY:

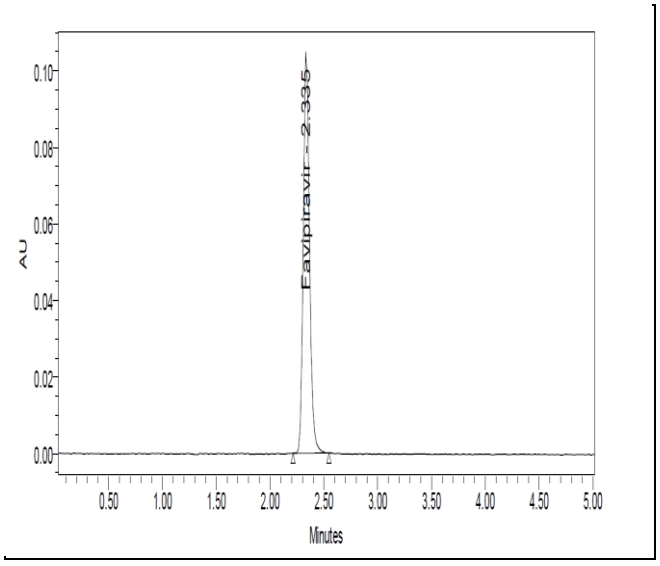
To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 25 ppm to 150 ppm of Favipiravir . Plot a graph to concentration versus peak area. Slope obtained was y = = 17820x + 9790 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig 6.10.

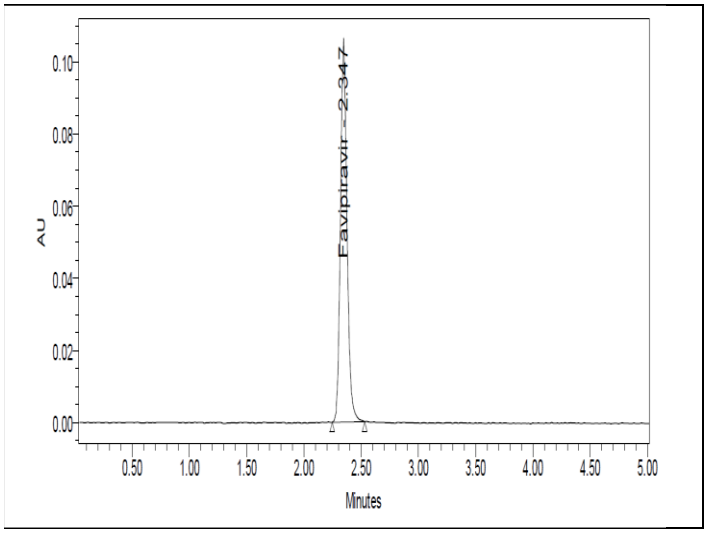
**Table 6.4 Linearity Concentration and Responce**

| Linearity Level (%) | Concentration (ppm) |
|---|---|
| 0 | 0 |
| 25 | 472839 |
| 50 | 888560 |
| 75 | 1355705 |
| 100 | 1789301 |
| 125 | 2233739 |
| 150 | 2683764 |

**Fig 6. Linearity Plot**





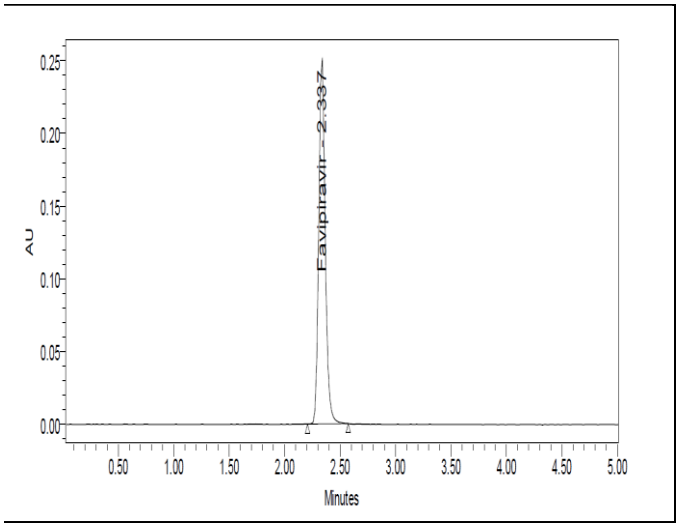
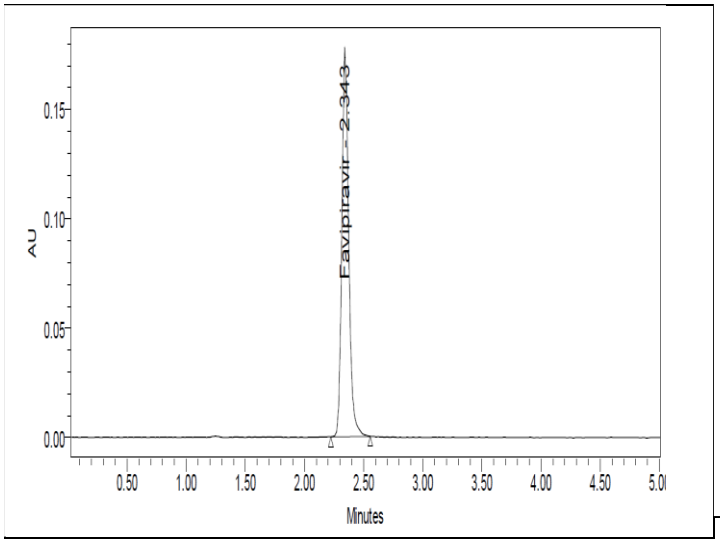




**Fig 6. Linearity 25% Chromatogram**



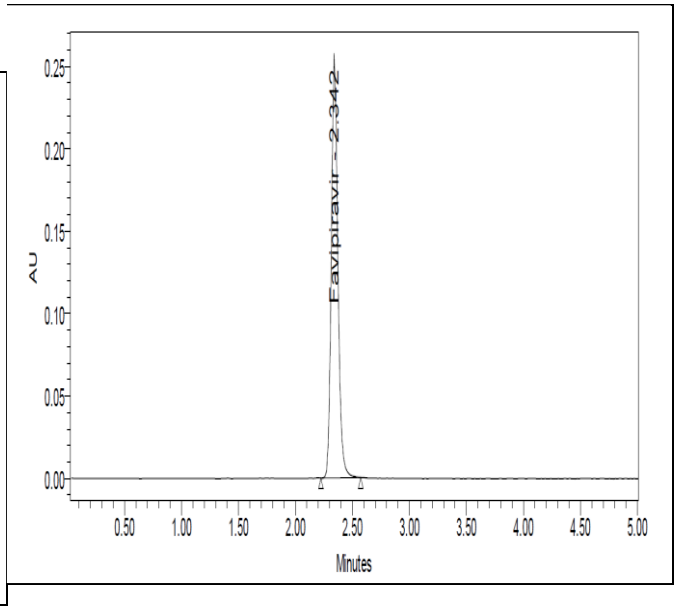
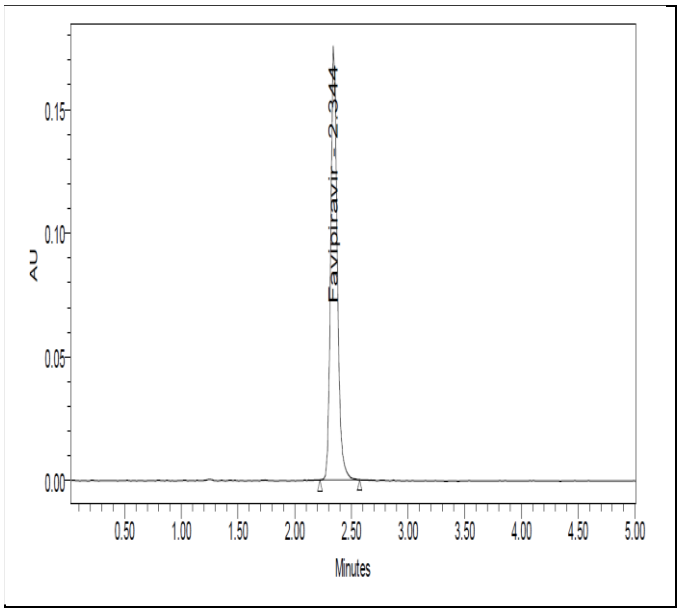




**Fig 6. Linearity 75% Chromatogram**



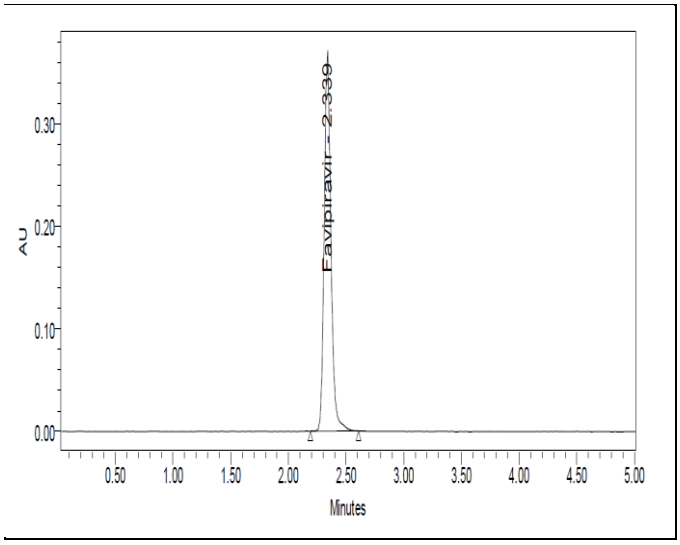




**Fig 6. Linearity 50% Chromatogram**

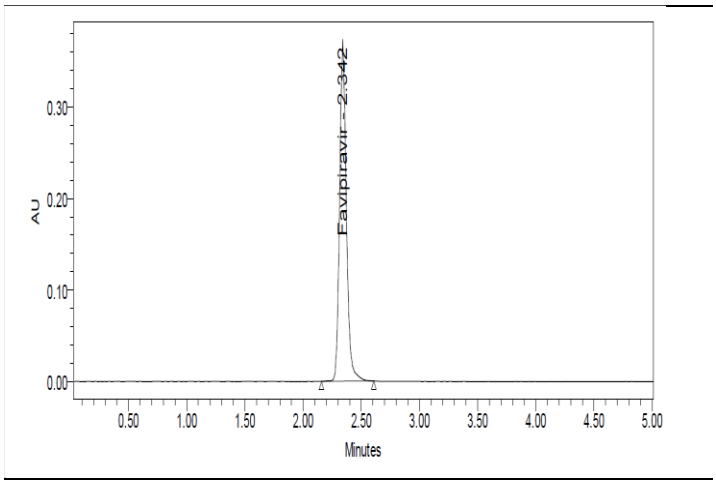
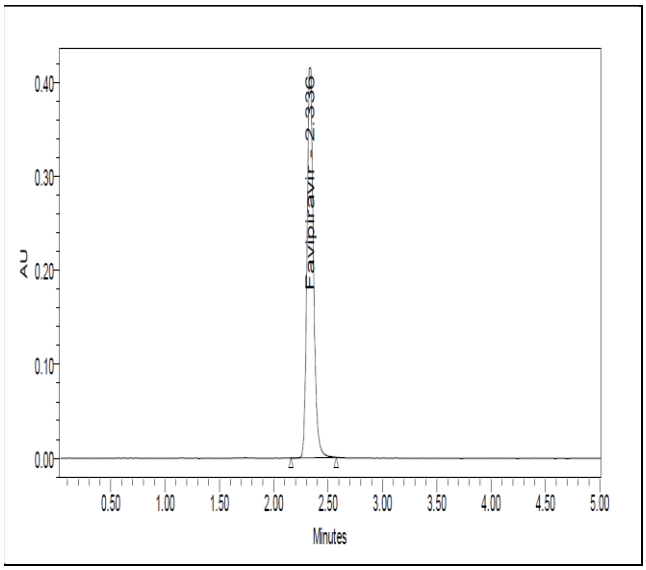



**Fig 6. Linearity 100% Chromatogram**



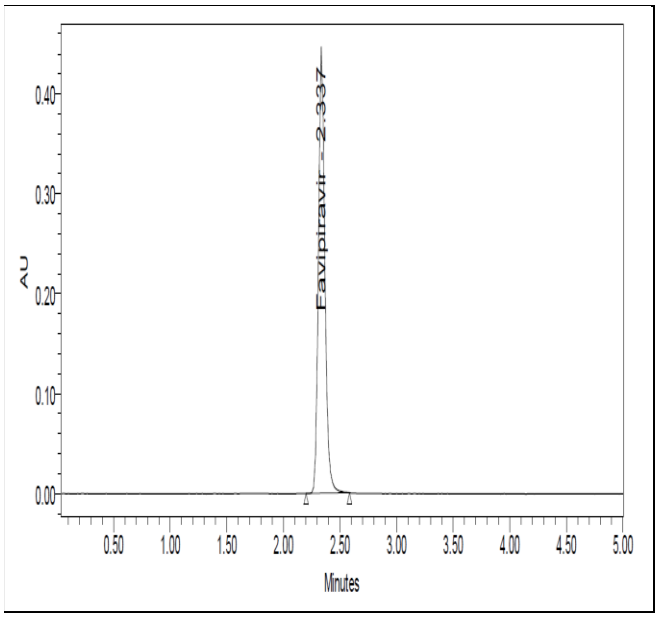
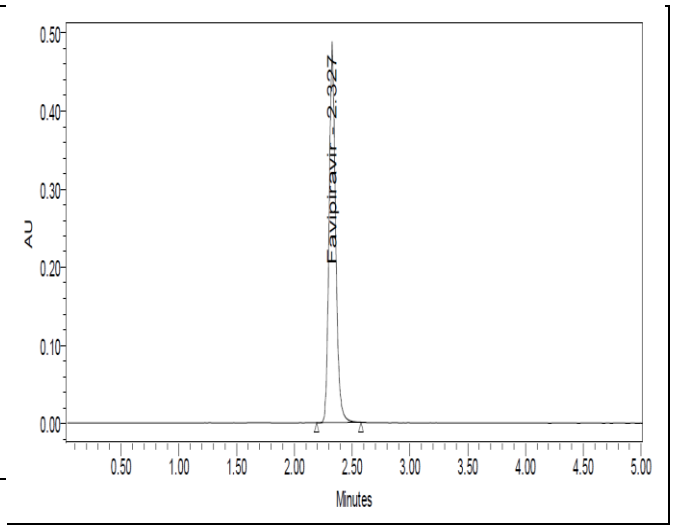




**Fig 6. Linearity 125% Chromatogram**



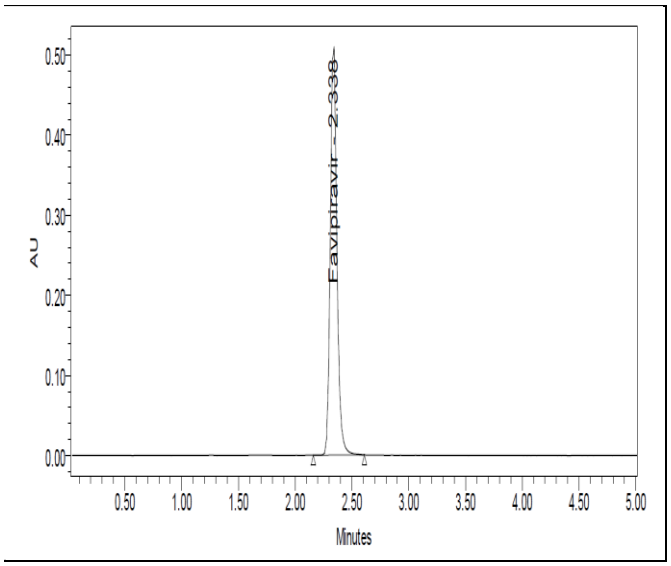
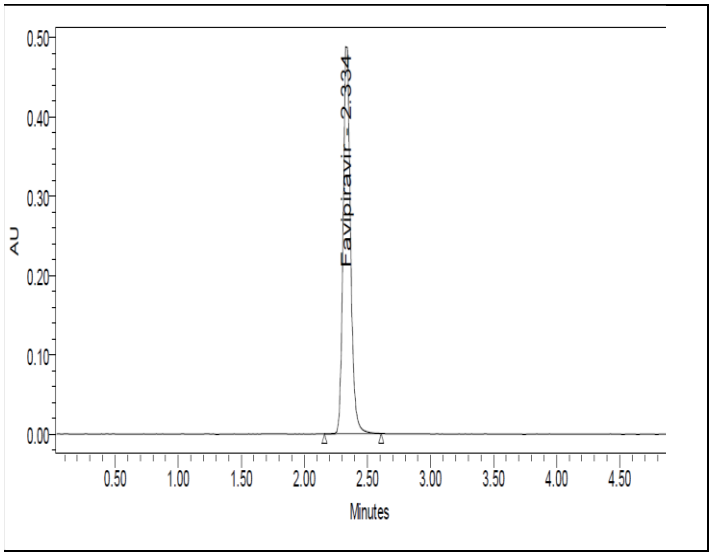



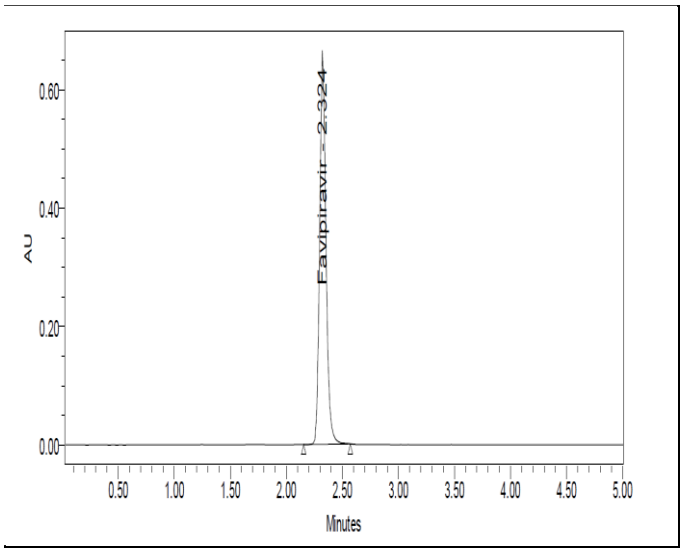

**Fig 6. Linearity 150% Chromatogram**

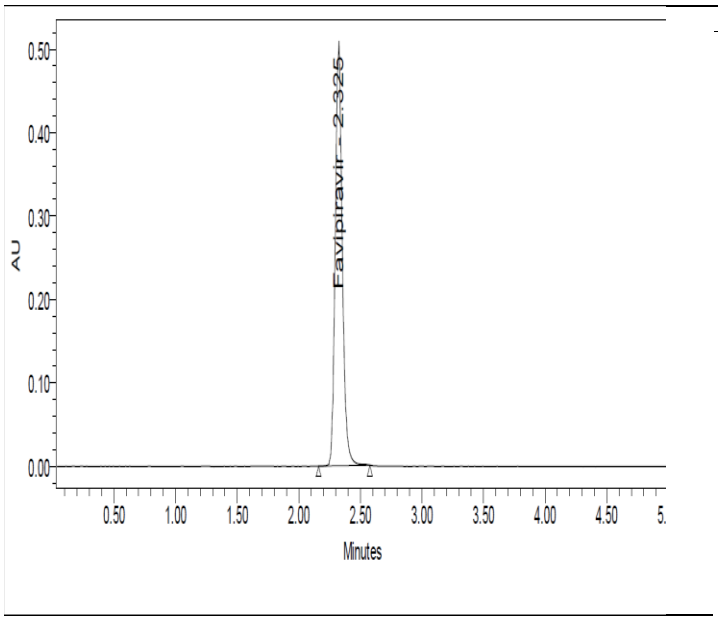
**6.4 Accuracy:** Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recover was calculated as 100.37 % and %RSD was found to be 0.68 and chromatograms were shown in fig 6.11-6.13.

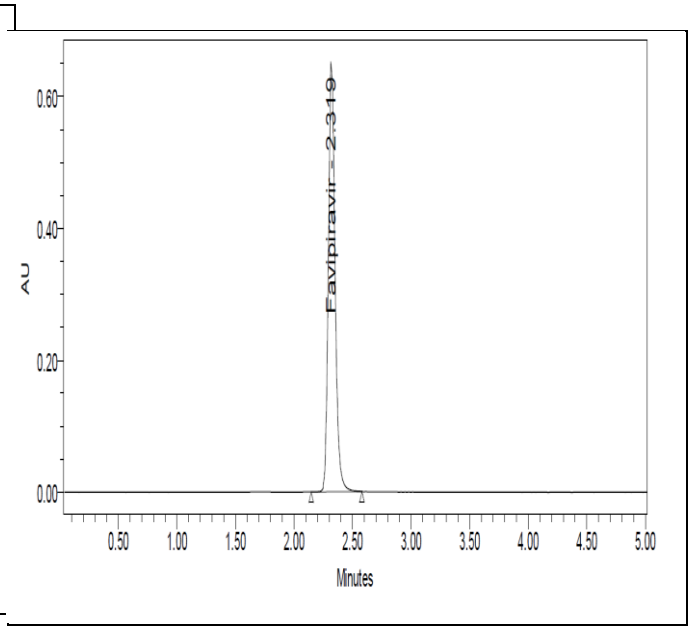
**Table 6.5 Accuracy data**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 50 | 50.55 | 101.10 | 100.37% |
| | 50 | 50.35 | 100.69 | |
| | 50 | 49.59 | 99.17 | |
| 100% | 100 | 101.04 | 101.04 | |
| | 100 | 100.30 | 100.30 | |
| | 100 | 99.80 | 99.80 | |
| 150% | 150 | 151.68 | 101.12 | |
| | 150 | 149.77 | 99.85 | |
| | 150 | 150.38 | 100.25 | |



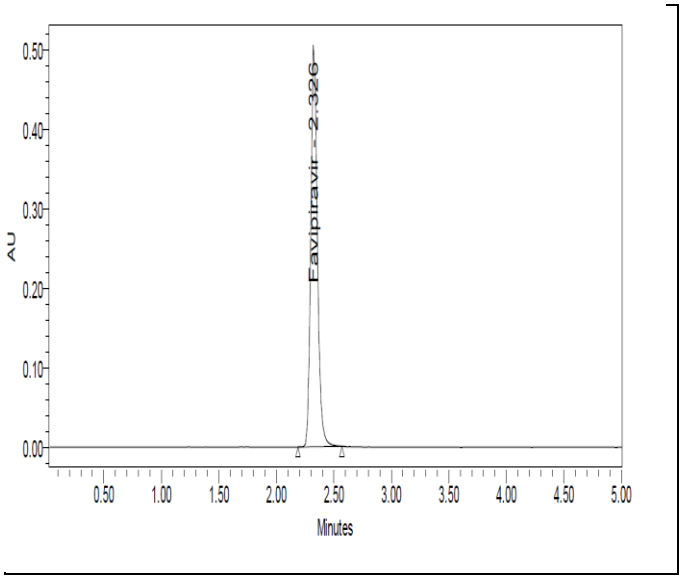




**Fig 6. Accuracy 50% Chromatogram**

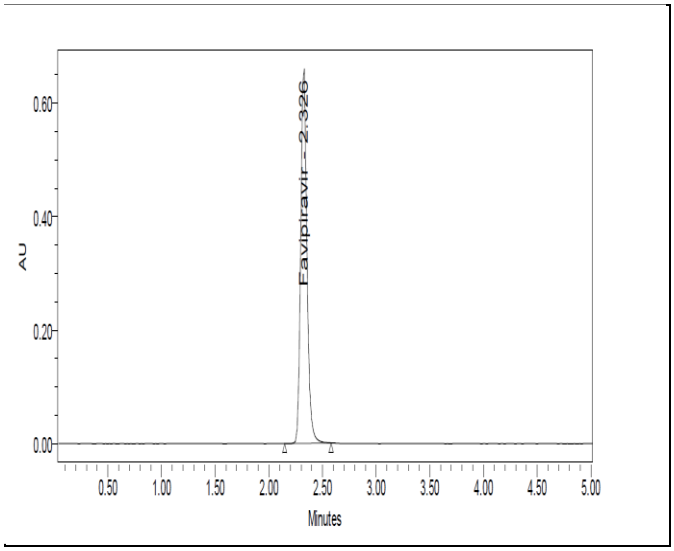
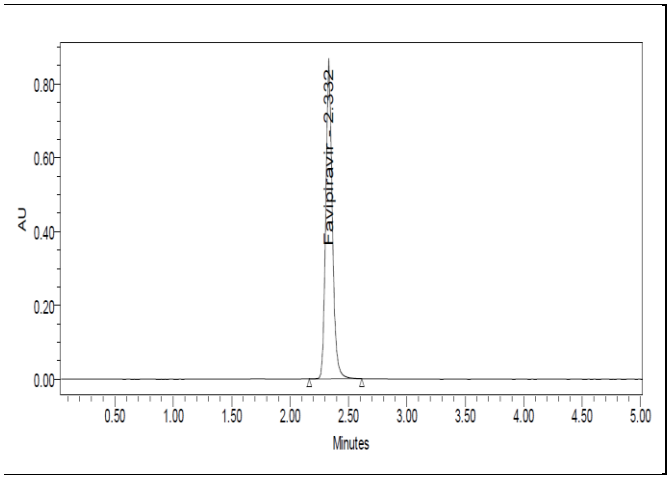
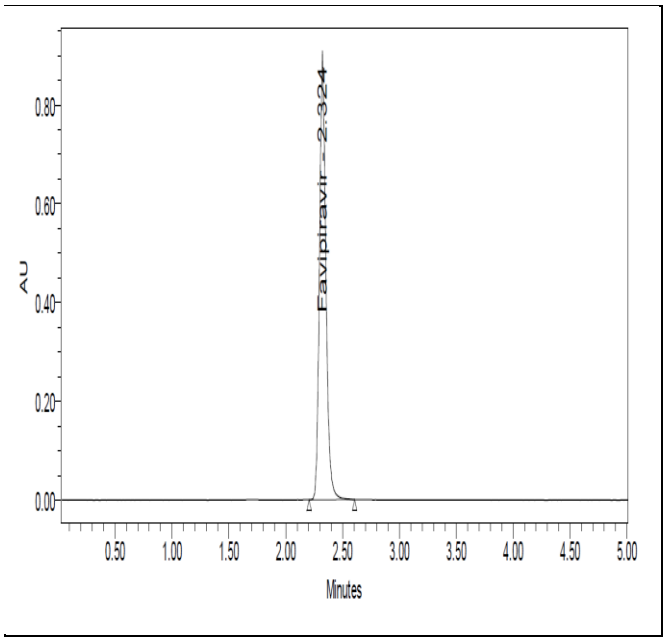



**Fig 6. Accuracy 100% Chromatogram**



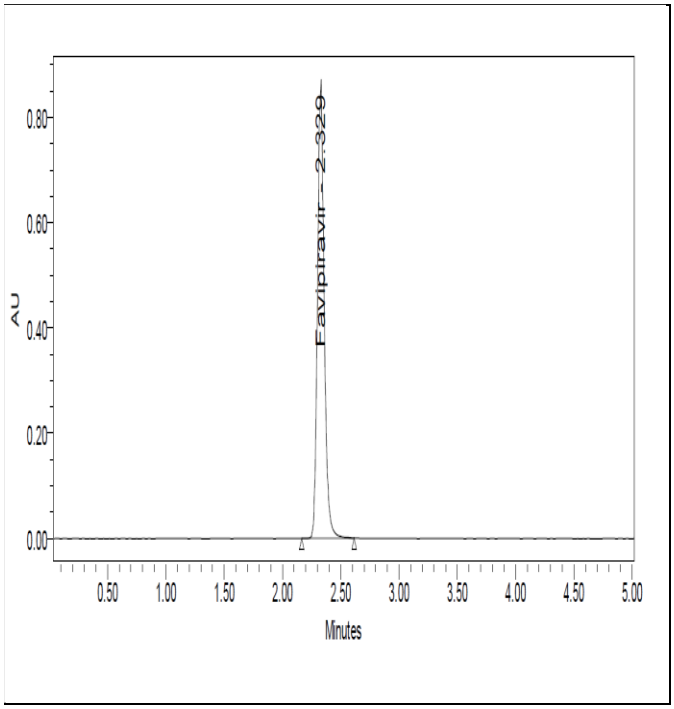




**Fig 6.Accuracy 150% Chromatogram**

**6.5 LOD:** Detection limit of the Favipiravir in this method was found to be 1.460µg/ml.

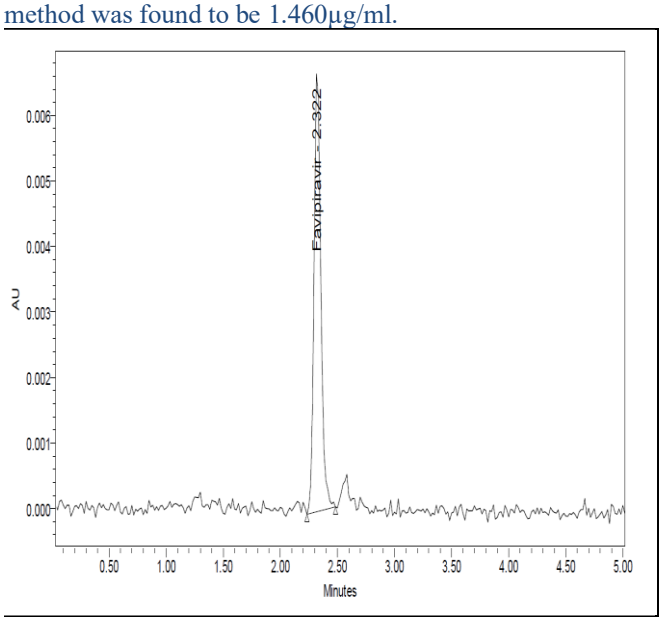



**Fig 6. LOD Chromatogram of Favipiravir**

**6.6 LOQ:** Quantification limit of the Favipiravir in this method was found to be 4.424µg/ml.

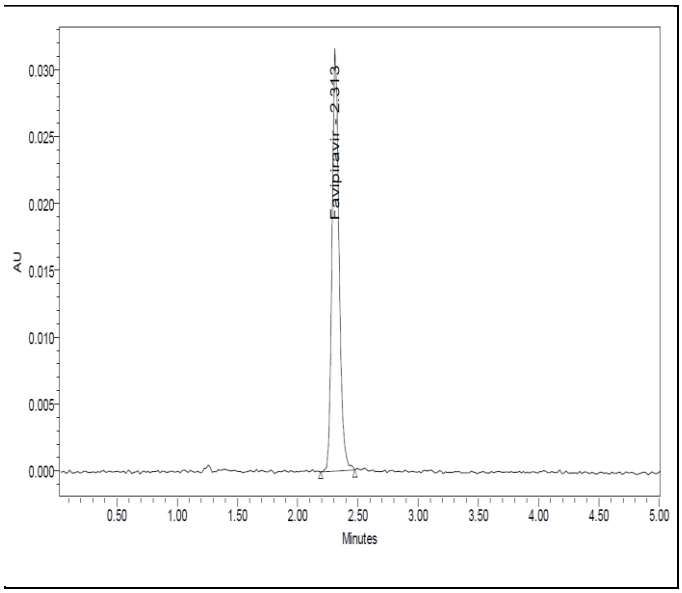
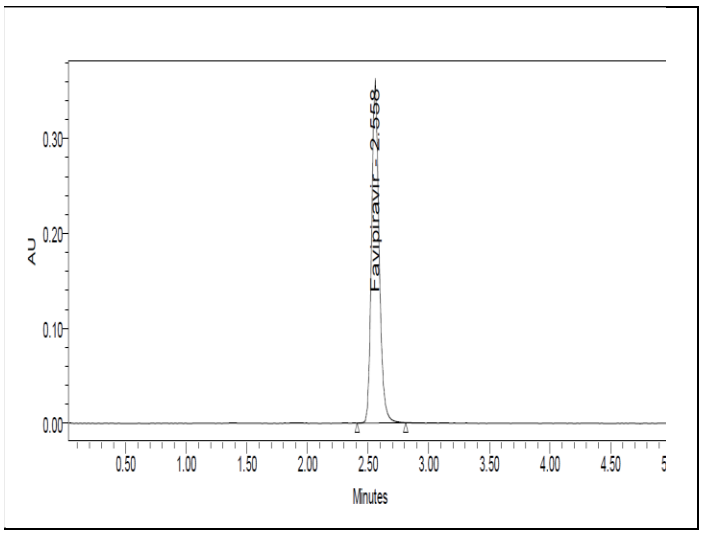


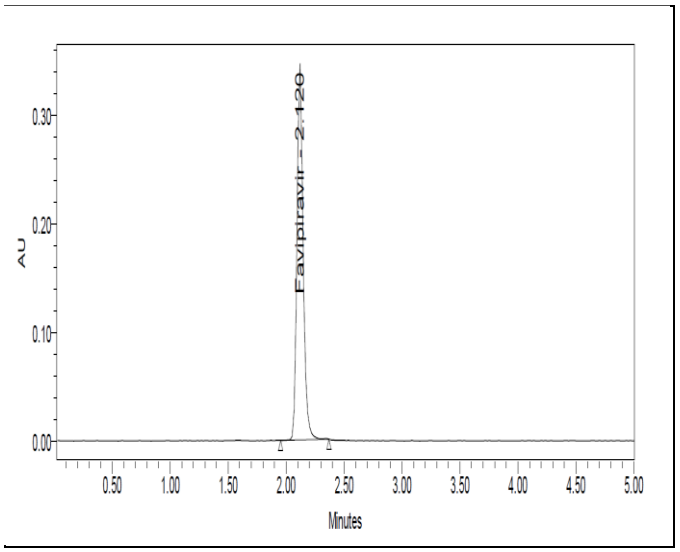

**Fig 6. LOQ Chromatogram of Favipiravir**

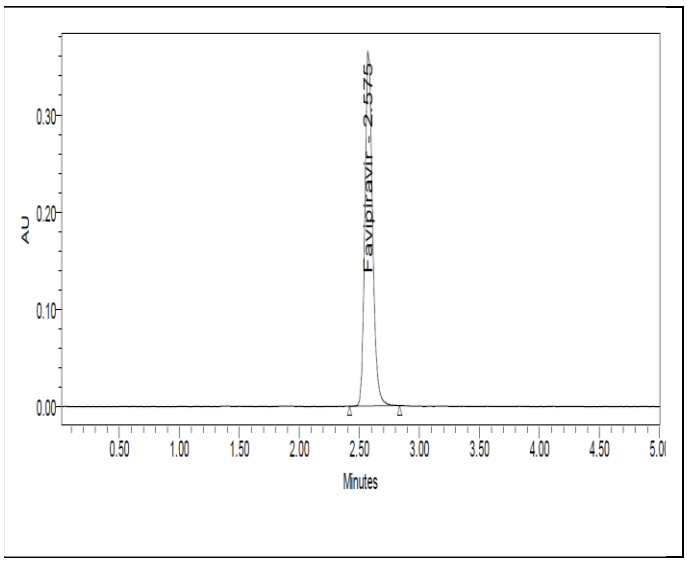
**6.7 Robustness:** Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated.

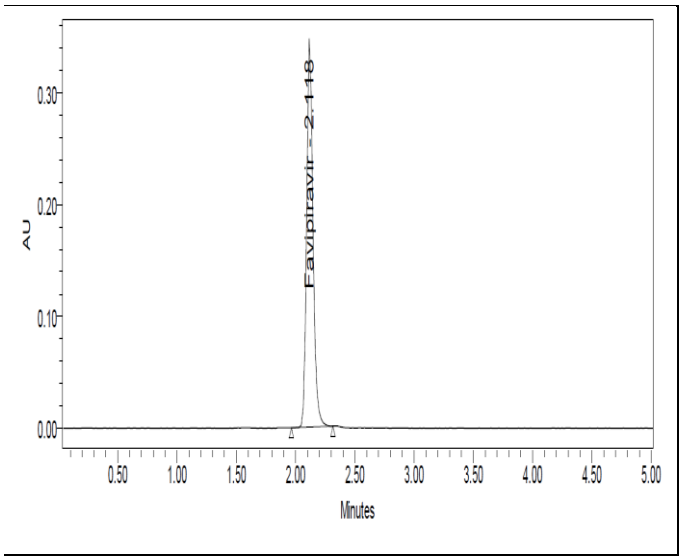
**Table 6.6 Robustness Data**

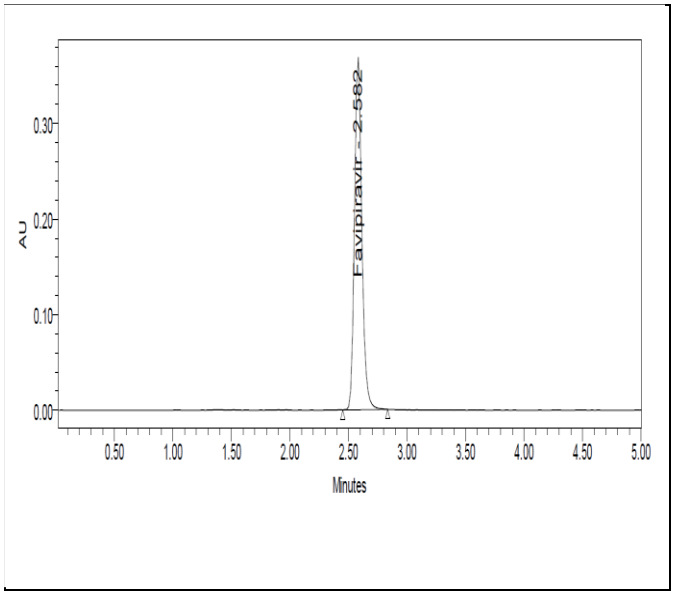
| Parameter | %RSD |
|---|---|
| Flow Minus | 1.6 |
| Flow Plus | 0.3 |
| Mobile phase Minus | 0.4 |
| Mobile phase Plus | 1.1 |
| Temperature minus | 1.5 |
| Temperature plus | 0.6 |



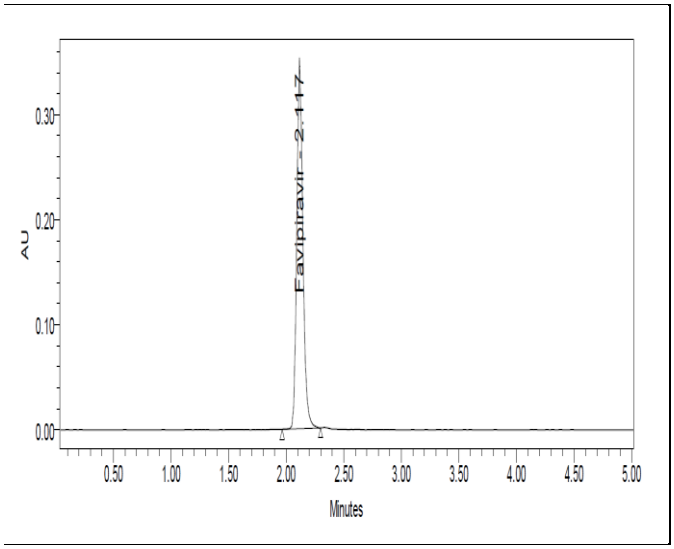
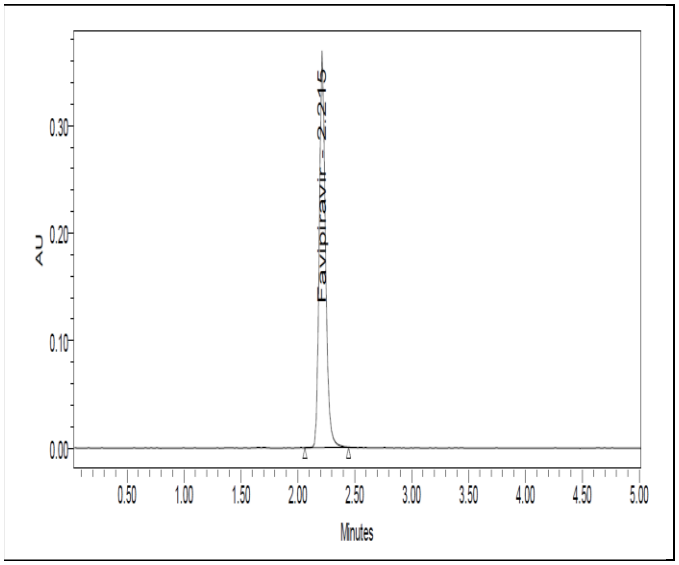
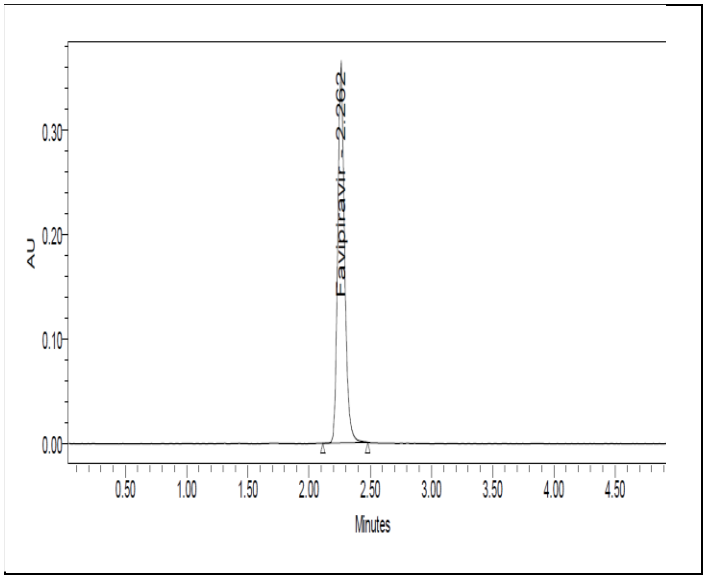




**Fig 6. Flow plus Chromatogram of Favipiravir**

**Fig 6. Flow minus Chromatogram of Favipiravir**



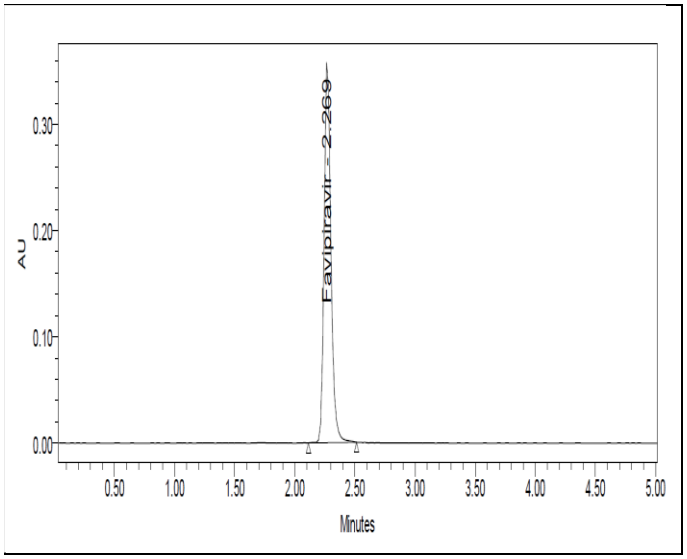
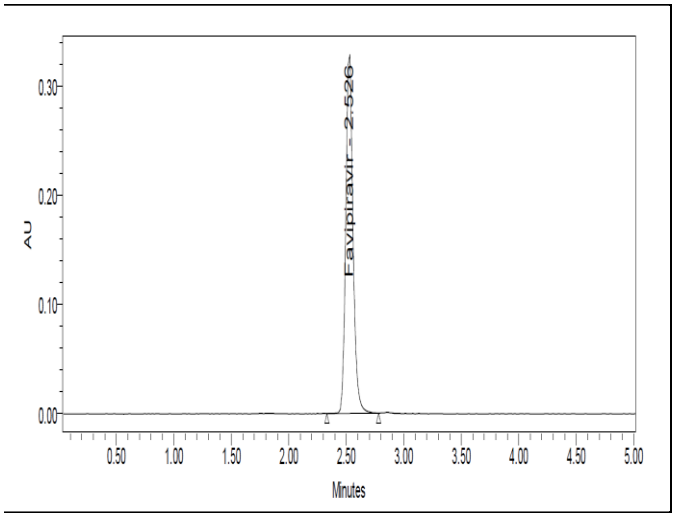
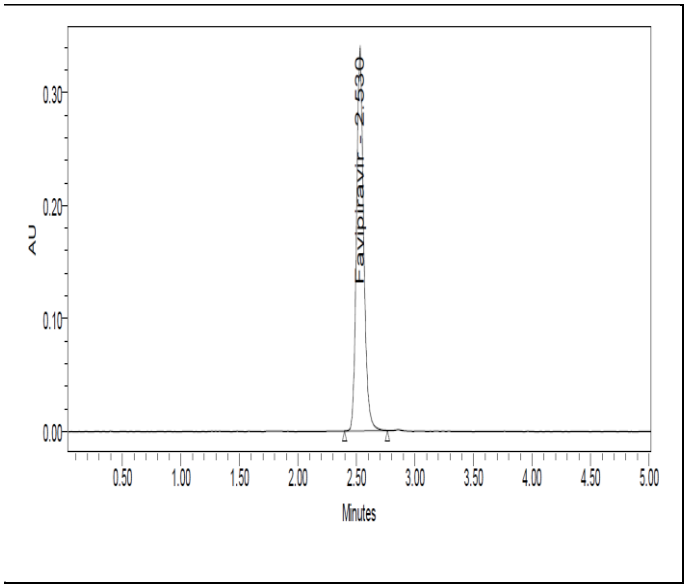




**Fig 6.Mobile phase minus Chromatogram of Favipiravir**



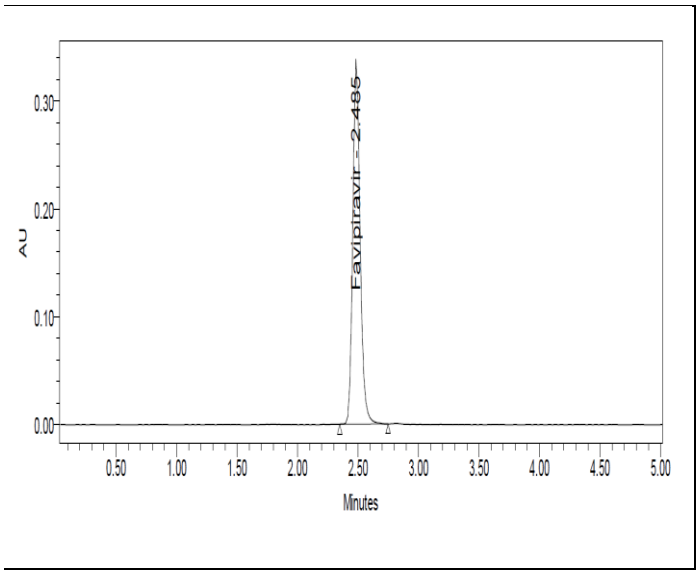
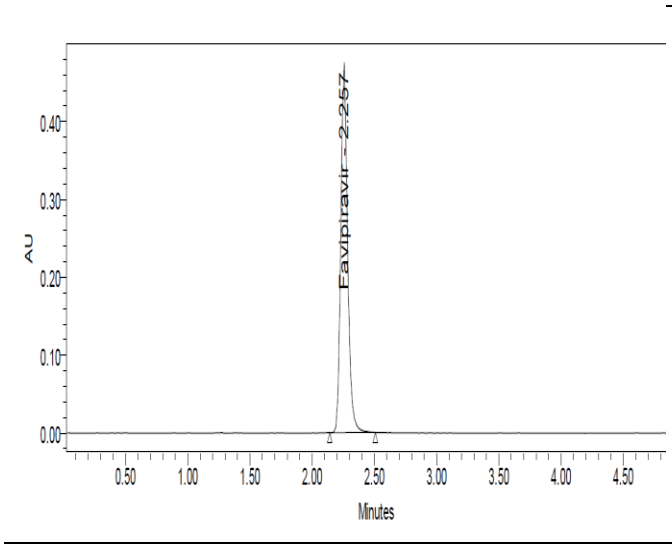
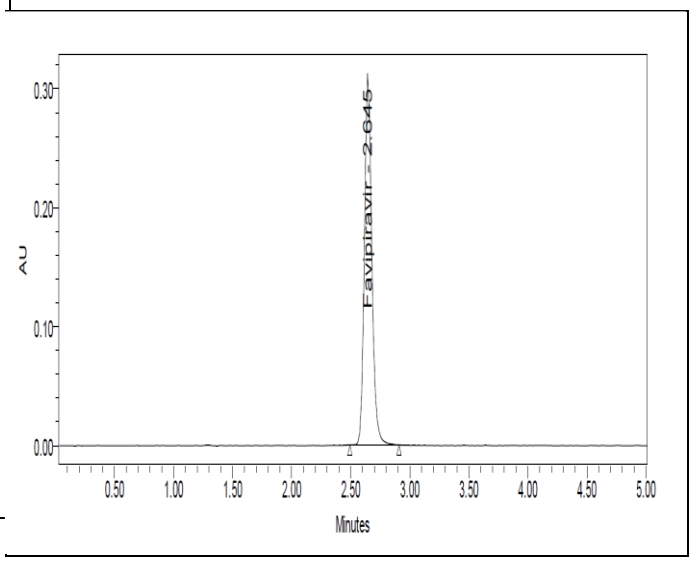
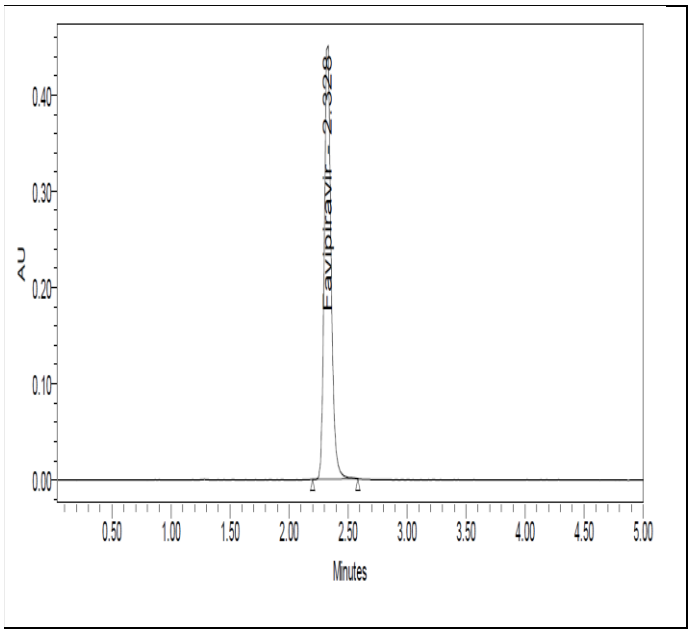
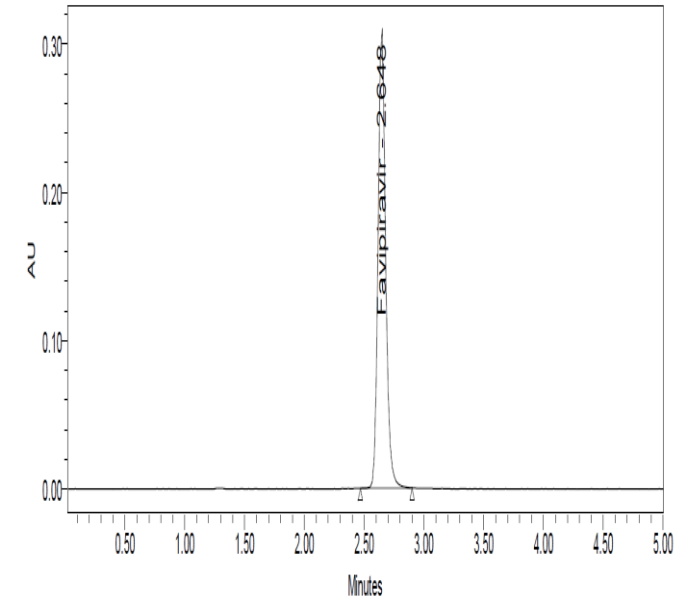



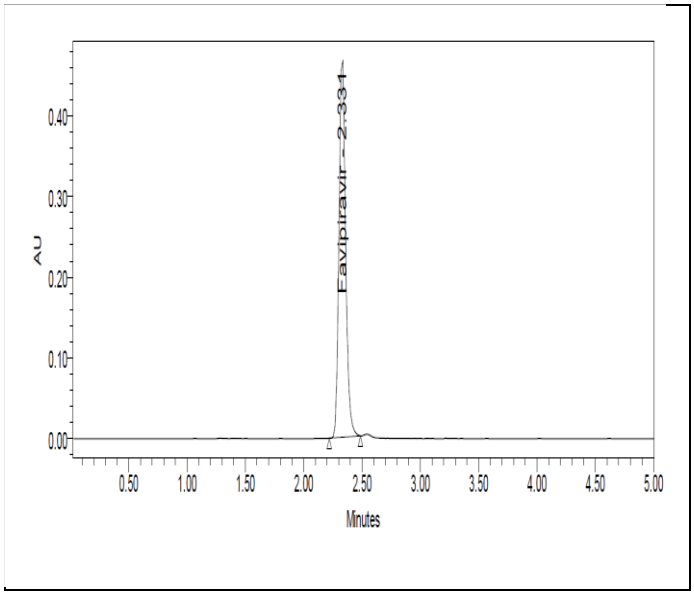

**Fig 6. Mobile phase plus Chromatogram of Favipiravir**



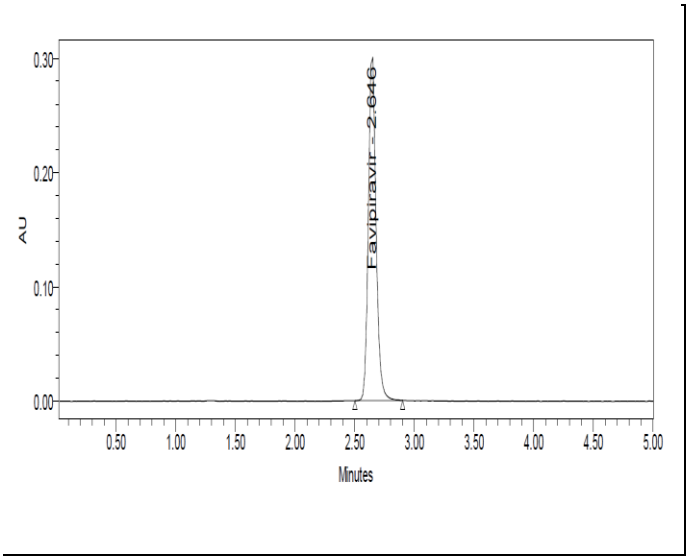




**Fig 6. Temperature minus Chromatogram of Favipiravir**

**Fig 6. Temperature plus Chromatogram of Favipiravir**

### 6.8 ASSAY OF MARKETED FORMULATION

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 6.7 Assay of Formulation**

| Sample No | %Assay |
|---|---|
| 1 | 99.87 |
| 2 | 101.45 |
| 3. | 100.68 |
| 4. | 100.08 |
| 5. | 100.99 |
| 6. | 101.64 |
| AVG | 100.78 |
| STDEV | 0.7159 |
| %RSD | 0.71 |

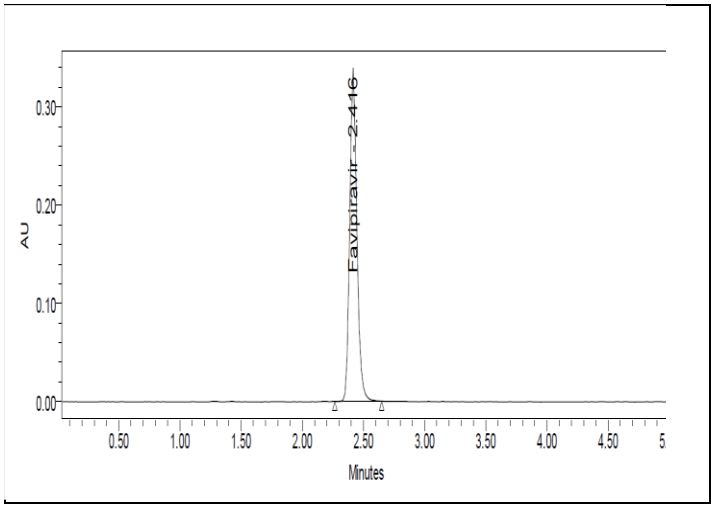



**Fig 6.28 Assay of Chromatogram**

### Degradation Studies:

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

**Degradation Data of Favipiravir**

| S.NO | Degradation Condition | % Drug Degraded |
|---|---|---|
| 1 | Acid | 5.70 |
| 2 | Alkali | 5.10 |
| 3 | Oxidation | 5.26 |
| 4 | Thermal | 3.05 |
| 5 | UV | 1.09 |
| 6 | Water | 0.78 |

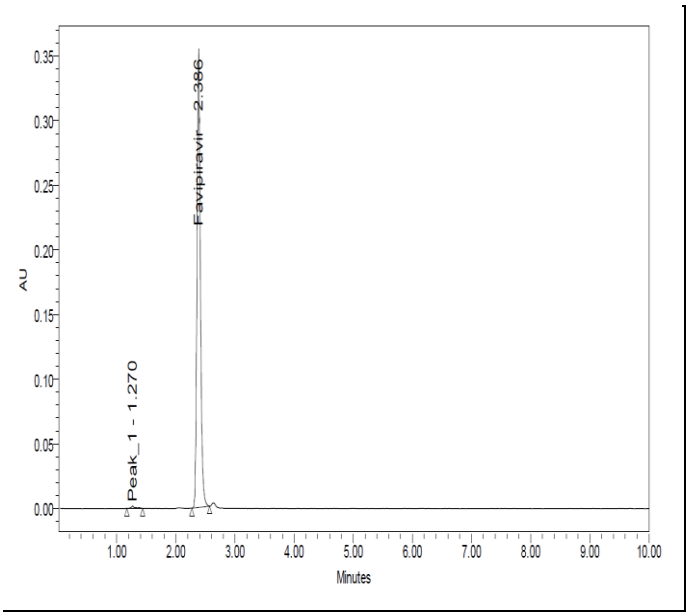



**Fig 28 Acid degradation chromatogram**

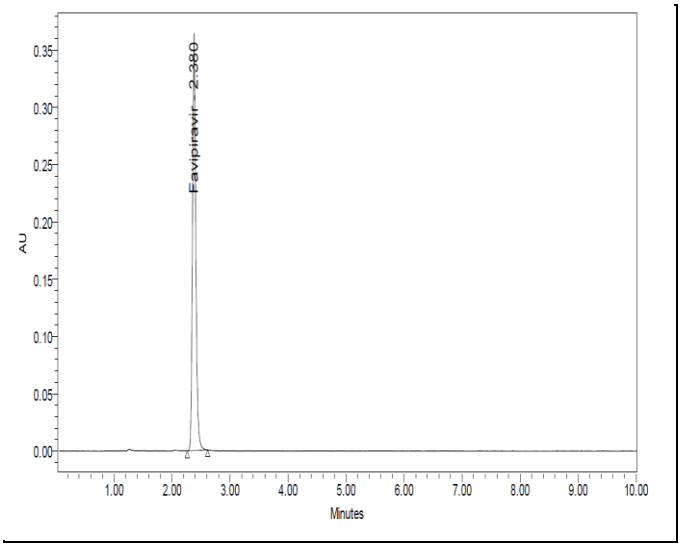



**Fig 29 Base degradation chromatogram**

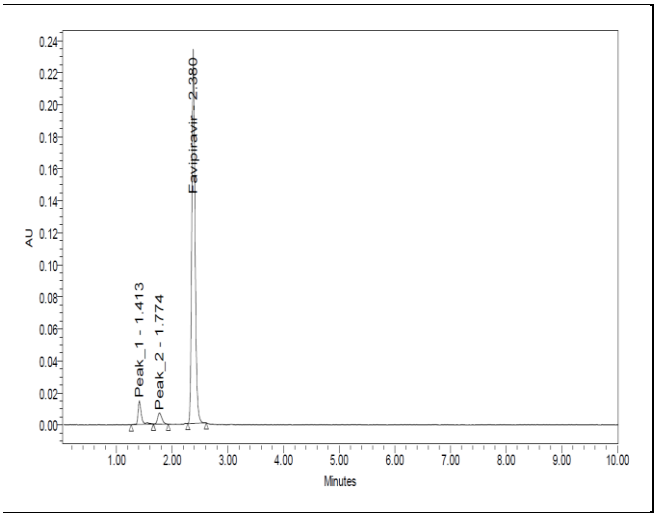



**Fig 30 Peroxide degradation chromatogram**

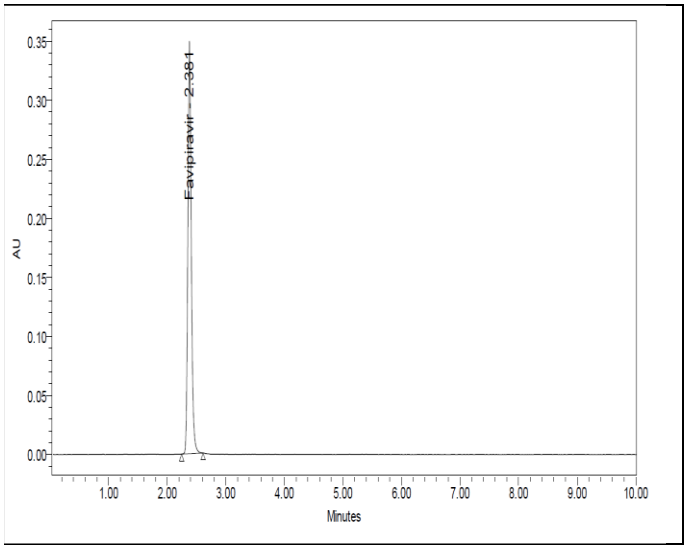



**Fig 31 Thermal degradation chromatogram**

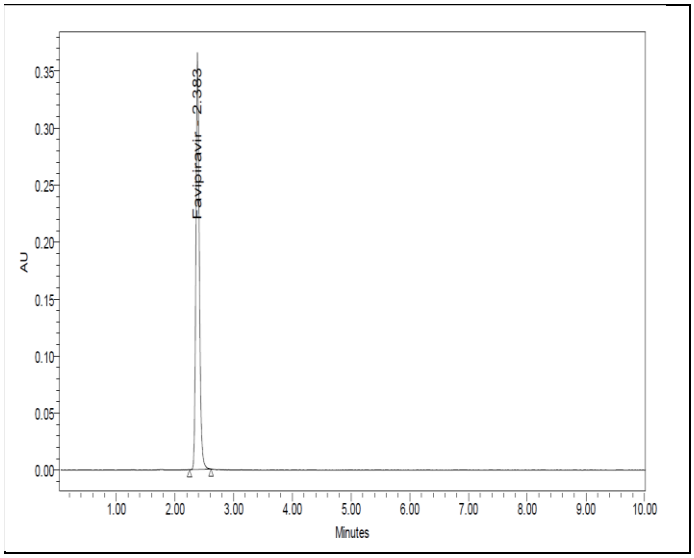



**Fig 32 UV degradation chromatogram**

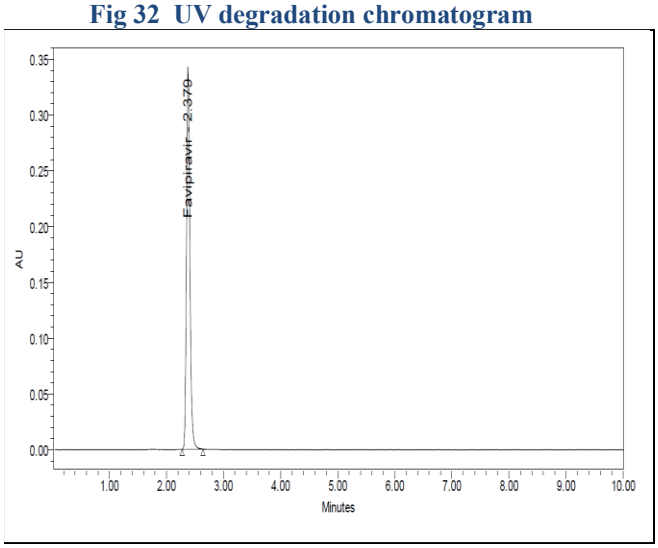



**Fig 33 Water degradation chromatogram**

## 7. SUMMARY AND CONCLUSION

### 7.1 Summary Table

| Parameters | | Favipiravir | LIMIT |
|---|---|---|---|
| Linearity:Range(µg/ml) | | 25 - 150 ppm | R< 1 |
| Regressioncoefficient | | 0.999 | |
| Slope(m) | | 17820 | |
| Intercept(c) | | 9790 | |
| Regression equation(Y=mx+c) | | y = 17820x + 9790 | |
| **Assay(% mean assay)** | | 100.78% | 90-110% |
| **Specificity** | | Specific | No interference of any peak |
| **System precision %RSD** | | 0.9 | NMT 2.0% |
| **Method precision %RSD** | | 0.7 | NMT 2.0% |
| **Accuracy %recovery** | | 100.37% | 98-102% |
| **LOD** | | 1.460 | NMT 3 |
| **LOQ** | | 4.424 | NMT 10 |
| **Robustness** | **FM** | 1.6 | %RSD NMT 2.0 |
| | **FP** | 0.3 | |
| | **MM** | 0.4 | |
| | **MP** | 1.1 | |
| | **TM** | 1.5 | |
| | **TP** | 0.6 | |

**CONCLUSION:**

Chromatographic conditions used are stationary phase Agilent c18 150 x 4.6 mm, 5µ. Mobile phase 0.1% OPA buffer: Acetonitrile in the ratio of 50:50 and flow rate was maintained at 1ml/min, detection wave length was 230 nm, column temperature was set to 30°C and diluent was Acetonitrial: Water (50:50), Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, $R^2$ value was found to be as 0.999. Precision was found to be 0.7 for repeatability and 1.2 for intermediate precision. LOD and LOQ are 1.140µg/ml and 4.424µg/ml respectively. By using above method assay of marketed formulation was carried out 100.37% was present. The above method can be used in routine quality control analysis.

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