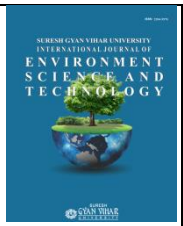

SGVU International Journal of Environment, Science and Technology

Journal homepage: https://www.gyanvihar.org/researchjournals/envirmental_science.php

E-ISSN: 2394-9570

Vol.10 Issue Page No 31- 45

Review article

A REVIEW OF ANALYTICAL DEVELOPMENT METHODS FOR THE DETERMINATION OF FAVIPIRAVIR

Suyog Subhash Hole[1]and Dr. Gaurav Gupta[2]

School of Pharmacy, Suresh Gyan Vihar University, Jaipur, India

Keywords

Favipiravir, development, method, HPLC

Abstract

Favipiravir is an antiviral drug that can be used to prevent COVID-19 and other influenza viral diseases. The prodrug Favipiravir enters into the infected cells through endocytosis and undergoes metabolism to become an active drug. An active form of Favipiravir targets the catalytic domain of RNA-dependent RNA polymerase and then disrupts the nucleotide incorporation process during viral RNA replication. This deregulation of viral RNA replication results in mutations where the replacement of guanine by adenine and cytosine by thymine occurs. This ultimately induces ruinous mutagenesis in RNA viruses. Nowadays, Favipiravir is available in intravenous and tablet dosage forms. The present paper accentuates the review of analytical methods including UV, HPLC, LC-MS/MS, Spectrofluorometric method, and HPTLC-densitometric techniques, etc., which involve the estimation of Favipiravir in bulk or dosage form. This review also describes the scope and limitations of developed analytical methods for the analysis of Favipiravir. This detailed review article will be of great help to the researcher who is working on the analytical development method of

	Favipiravir.

Introduction

Favipiravir was developed in the year 2014, by the Fujifilm Toyama Chemicals Company in Japan to treat Influenza infection. Favipiravir is an antiviral drug used to treat influenza infection. It is a guanine analog with a pyrazine carboxamide structure. Favipiravir being a prodrug undergoes phosphor ribosylation and phosphorylation to produce an active form i.e., Favipiravir ribofuranosyl -5'-triphosphate (Favipiravir-RTP), which selectively inhibits RNA dependant RNA polymerase (RdRP), an enzyme required for RNA viral replication inside the human cells. It functions as a purine analog and is incorporated instead of guanine and adenine. The elongation of viral RNA terminates due to the incorporation of a single molecule of Favipiravir. Inside the cell, Favipiravir is converted into its active phosphorylated form and is then recognized as a substrate by viral RdRP. It shows an ample spectrum of activity against different RNA viruses including influenza virus, etc.

In 2014, Favipiravir was approved in Japan for use in the outbreak of novel or recurrent influenza viral infections, where other antiviral drugs usually used in influenza are insufficiently effective. In Influenza, the beneficial effect has been attributed to the decline in pulmonary viral load and TNF-alpha levels in the airways. Favipiravir was also used for the post-exposure prophylaxis and treatment of patients with Ebola virus infections. In December 2019, the first cases infected with the COVID-19 virus (also known as SARS-Cov-2) were reported in Wuhan, China. Now this virus becomes a pandemic all over the world. SARS-Cov-2 is a beta coronavirus that is enveloped by positive-strand RNA viruses like MERS (Middle East respiratory syndrome)-Cov and SARS (severe acute respiratory syndrome)-Cov. For SARS-Cov-2, the viral genome codes for sixteen non-structural proteins (Nsps) required for virus replication and pathogenesis and four structural proteins. However, several already existing antiviral drugs which have been proven to be safe and effective against other viruses are tested for their activity against the SARS-Cov-2. Special concern is given to RNA-dependent RNA polymerase (RdRp) inhibitors. One of these drugs is favipiravir (FAV) which is known as T-705. Chemically, it is 6-fluoro-3-oxo-3,4- dihydro pyrazine 2-carboxamide.

Favipiravir is an excellent choice for the treatment of COVID-19 and it is available in the market under the brand name Avifavir, Avigan and Areplivir. Several methods have been reported related to the analysis of Favipiravir in both pharmaceutical preparations and

biological fluids. To date, many analytical methods have been developed for quantitative determination related to Favipiravir levels. (Itigimatha et al., 2023)

Favipiravir has the chemical name 6-fluoro-3-hydroxypyrazine-2- carboxamide with the chemical formula C5H4FN3O2 and a molecular weight of 157.104 g/mol. The structure of Favipiravir can be seen in Figure 1. The class of chemical compounds known as pyrazine carboxamides includes favipiravir, which has an amide group substituted on its pyrazine nucleus. Favipiravir is a pale-yellow color powder available in crystalline solid form, slightly soluble in water and completely soluble in acetonitrile and methanol. The melting point of Favipiravir is between 187 - 193°C. Other names for Favipiravir are Fapilavir and Favilavir.

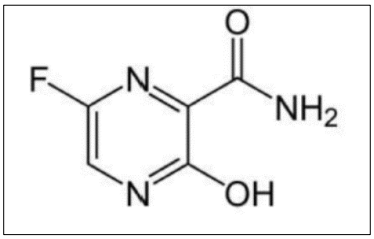

Figure 1: Structure of Favipiravir

Based on clinical evidence, it is not suitable to suggest Favipiravir during pregnancy because of its teratogenic effects. Upon oral administration, the maximum drug concentration occurs at 2 hours and then has a short half-life, i.e., 2 to 5.5 hours. The plasma protein binding capacity of Favipiravir was found to be 54%. (Furuta et al., 2017)

As a prodrug, favipiravir undergoes intracellular phosphorylation and ribosylation to transform into the active favipiravir-RTP.7,10 RNA-dependent RNA polymerase (RdRp) is a protein that favipiravir-RTP binds to and inhibits, preventing the transcription and replication of viral DNA. Favipiravir's bioavailability is almost near 100%, i.e., 97.6%. Favipiravir is extensively metabolized in the kidneys and excreted through urine. Aldehyde oxidase is primarily responsible for hydroxylating favipiravir, with xanthine oxidase playing a minor role. The inactive metabolite of Favipiravir produced by metabolism includes T705M1 and M2 (Favipiravir glucuronide conjugate). Inactive metabolites of Favipiravir are excreted through the renal route. (Pruthvishree, 2023)

The major mechanism of clearance of Favipiravir in humans is hydroxylation. Based on studies, Favipiravir is given for five days. The recommended dose for the first day through the oral route is 1600 mg twice a day, and then for the next four days, 600 mg twice daily. Analytical methods are used to identify a drug's physical and chemical properties in terms of

qualitative and quantitative methods. In this article, detailed information about the instrumental analytical methods of Favipiravir has been discussed. (Chakraborty & Mondal, 2023)

Analytical Methods for the Determination of Favipiravir in Pure Form or Dosage Form:

UV-Visible Spectroscopy:

UV Visible spectroscopy is one of the instrumental analytical methods in which UV-Visible radiation is used to analyze the sample. Molecules undergo electronic transitions and show absorption in this wavelength range which is accessible to UV-Visible spectrophotometer. Based on the type of beam used in UV instruments, there are two types of spectrometers, i.e., single beam spectrometer and double beam spectrometer. (Hassanipour et al., 2021)

A brand-new, user-friendly, and precise UV spectroscopic approach was created by Sandip et al. to estimate the amount of the highly effective antiviral medication Favipiravir in both tablet and bulk form. By using the UV technique, absorption maxima at 323 nm were seen, and Favipiravir's zero-order derivative values were quantified. ICH guidelines validated the developed method, and linearity was observed in the concentration range of 4-20μg/ml. The sensitivity of the method was expressed as the limit of quantification and limit of detection, which was found to be 0.26 μg/ml and 0.08 μg/ml respectively. Accuracy and precision were performed in replicates, and % RSD was found to be within the limit. The developed method was sensitive and accurate. It can be used for routine drug analysis in marketed formulations. (Sandip Firke et al., n.d.)

By utilizing a Shimadzu UV-Visible spectrophotometer (UV JAPAN 1801), Jyothi B. et al. created a novel ultraviolet spectrophotometric approach for the measurement of Favipiravir. Due to the drug's limited solubility in water, favipiravir is typically dissolved in ethanol before being reconstituted with water. The maximum UV spectrum absorption for favipiravir in ethanol and water is 234 nm. As a result, the concentration range of 1 to 10 g/ml conformed to Beer- Lambert's law, and linearity was noted between 2 and 10 g/ml with an R^2 coefficient of 0.9995. After the developed approach was statistically validated, it was discovered that the precise %RSD values for intraday and interday precision were, respectively, 0.408% and 0.348-0.693%. The method was also found to be Accurate as indicated by % recoveries ranging from 99.30–99.91%. The detection limit and quantitation

limit of the test was found to be 0.095µg/ml and 0.290µg/ml, respectively. The analysis results are validated as per ICH guidelines and this method can be employed for routine analysis. (Jyothi B & Kavya, 2021)

High-Performance Liquid Chromatography:

In order to evaluate drug products, high-performance liquid chromatography (HPLC) is a crucial analytical instrument. The different medicines and drug-related degradants should be able to be separated, detected, and quantified using HPLC procedures. A sample is separated into its components by distributing the sample between a mobile phase and a stationary phase under pressure applied using a pump. (Maheshwara Rao, 2014)

The gadolinium-based magnetic ionic liquid for supramolecular dispersive liquid-liquid micro-extraction and the HPLC/UV method for locating favipiravir in human plasma was developed by Abdallah I et al. The gadolinium-based magnetic ionic liquid is used as an extractant, and factors affecting microextraction include the extractant, amount of extractant, type of disperser, and disperser volume. Extraction efficiency was enhanced by using 50 mg of the Gd-magnetic ionic liquid and 150 µl of tetrahydrofuran. The developed bioanalytical method was validated according to ICH guidelines, and the coefficient of determination was 0.9999 with a linear concentration range of 25 to 1.0 x 103 ng/ml. Accuracy was found to be within the range i.e., 99.83% to 104.2% with the RSD values ranging from 4.07% to 11.84%. The duration of extraction time was about 12min and the HPLC analysis time was found to be 5 mins. The developed method was found to be simple and sensitive for the analysis of Favipiravir in human plasma. (Abdallah, Hammad, et al., 2022)

Bulduk I et al. developed the HPLC-UV method for quantifying Favipiravir in pharmaceutical formulations. The isocratic HPLC method was developed using Inertsil ODS-3V C18 (4.6 mm 3.250 mm, 5.0 mm) column, which is thermostatic to 30°C temperature and potassium dihydrogen phosphate buffer and acetonitrile as the mobile phase at a 90:10 ratio. The flow rate was maintained at 1ml/min and the retention time was 15 min using 323 nm as the detection wavelength. With a correlation coefficient of 0.9999, linearity was seen in the concentration range of 10-100 g/ml after the devised technique had been validated in accordance with ICH requirements. Linearity was evaluated by Least squares linear regression analysis using average peak area versus drug concentration data.

The developed method was precise as % RSD values for intraday and interday precision were less than 2%, i.e., 0.2 and0.2 and 0.2 and 0.4 %, respectively. Selectivity was performed by comparing the chromatograms of Favipiravir standard, tablet, and blank solutions where, theoretical plate number, retention time, and peak tailing factor values observed were 13.798, 7.696, and 0.920 respectively. Indicators of the method's accuracy included percent recoveries that ranged from 99.1 to 100.17%. The drug detection and quantification limits were 1.20 µg/ml and 3.60 µg/ml, respectively. This method has been applied for the estimation of Favipiravir in pharmaceutical dosage formulation for routine analysis. (BULDUK, 2021)

In order to compare the retention times, Aqeel Zeshan et al. developed HPLC column screening for favipiravir on six different columns, including Kinetex® C18, Luna® C18(2), Luna Omega PS C18, Luna Omega Polar C18, Kinetex 2.6 m Biphenyl, and Kinetex F5. The method was initially developed using gradient elution i.e., 5% B for 0.5 minutes, to 95% B over 10 minutes but there was a lack of significant differences in the retention. Hence, isocratic elution was followed to highlight the differences in retention between six different columns.

The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile in the ratio 95:5 with a flow rate of 1ml/min maintained at 30°C. Peak tailing and Retention time of Favipiravir in Luna C18(2), Kinetex F5 - 00D4723-E0, Kinetex® C18, Kinetex 2.6 µm Biphenyl, Luna Omega PS C18 and luna Omega Polar C18 was found to be 1.08, 1.46, 1.29, 1.43,1.23, 1.40 and5.099, 3.557, 3.114, 3.523, 4.838, 2.747 respectively. The method has been developed using a 500 µg/ml concentration of sample with the best peak shapes in Luna C18(2), Kinetex C18, and Luna Omega PS C18 columns. (Aqeel et al., 2020)

Nadendla Ramarao et al., performed a validated High-Performance Liquid Chromatographic method and UV spectroscopic method for the quantification of Favipiravir using a PDA detector. The method was developed using the SHIMADZU Prominence-LC2030 system equipped with a column i.e., Shim-Pack GIST C18 (250X 4.6 mm, 5µm) maintained at a temperature of 30°C. The mobile phase is composed of potassium dihydrogen ortho-dihydrogen ortho phosphate buffer (pH 4.0) and acetonitrile (90:10 v/v) at 1ml/min flow rate.

The developed method was validated as per ICH guidelines and a calibration curve was found to be linear in the concentration range of 10-60 µg/ml. Sensitivity was calculated as LOD and LOQ which were found to be 0.18 µg/ml and 0.53 µg/ml, respectively. Accuracy was performed by using recovery studies and was found to be 99.47- 100.80%. Specificity was

performed for blank, sample, and standard solution and it was found that there was no interference of excipients or solvent with the drug. Solution stability was performed by storing the sample in the solvent at ambient temperature for 24 hours, no changes were observed in the sample solution and % RSD was within the limit. The developed method was found to be economical, and eco-friendly with less retention time of 4.622 min and hence can be applied for routine analysis for marketed formulations of Favipiravir tablets. (Nadendla & Abhinandana, 2021)

Srinivas Lingabathula et al. developed stability-indicative and cost-effective analytical methods for developing and validating Favipiravir and Peramivir using RP-HPLC. The chromatographic method was developed using an Inertsil ODS column of (250x4.6 mm, 5 microns) with a mobile phase of acetonitrile and 0.1 percent orthophosphoric acid in the ratio 70:30 at 1 ml/min flow rate. Waters Alliance liquid chromatography, empower 2.0 data handling software, and a photodiode array detector (model 2998) were used for the method development. The proposed method was validated according to ICH guidelines, where the calibration charts plotted were linear with a concentration range of 10-150 µg/ml and a regression coefficient of 0.999. System suitability parameters include USP plate count, tailing factor, and % RSD, found to be 3264, 1.04, 0.89 for Peramivir and 3841, 1.07,0.71 for Favipiravir respectively.

Degradation studies were carried out for Favipiravir and Peramivir to discover the conditions during which the medicine is unstable so that precautions be made during formulation to avoid potential instabilities. Degradation studies were performed by acid, alkali, peroxide, reduction, thermal and hydrolysis methods, percentage of drug degradation and drug recovery were calculated. The developed method was found to be fast and simple. Hence can be used for routine analysis of samples and to check their quality during stability studies. (Lingabathula & Jain, 2021)

Nazifa S et al. developed a new analytical method for estimating Favipiravir in bulk and pharmaceutical dosage form using HPLC/UV method and to study forced degradation stability indicating studies on Favipiravir. The chromatographic method was developed using Inertsil ODS-3V C18 column, potassium dihydrogen phosphate 50 mM (pH 3.5), and acetonitrile (90:10, v/v) as mobile phase at a flow rate of 1ml/min with a retention time of 10 min. The developed method was validated as per ICH guidelines for precision (method precision, system precision, intraday, and interday precision), linearity, accuracy, and recovery. Linearity was reported in the concentration spanning from 2 to 10µg/ml with a

correlation value of 0.990. The limit of detection and quantification were found to be 0.0723µg/ml and 0.219 µg/ml, respectively.

The intraday and inter-day variation was carried out at three different concentrations i.e., 2, 8, and 12 g/ml, and % RSD was within the limit. Various attempts have been made for forced degradation to perform stability-indicating studies. Forced degradation studies were performed using acid, alkali, thermal, photolytic, and peroxide degradation methods. The proposed UV approach has been efficiently implemented for regular analysis of Favipiravir in commercial and bulk formulations. (Ahmed et al., 2021)

Mohammad Hailat et al. developed and validated a technique for measuring Favipiravir in Human spiking plasma. The method was developed on Symmetry® C18-(250 cm × 4.6 mm, 5 µm) with the mobile phase consisting of methanol: acetonitrile: 20 mM phosphate buffer in the ratio 30:10:60 at the flow rate of 1 ml/min and detector wavelength of 242 nm. Dichloromethane was used as an extracting solvent for the complete recovery of drugs from plasma and retention time for Favipiravir and Acyclovir was found at 7.40 min and 4.64 min, respectively.

The bioanalytical method was validated as per USFDA guidelines, and the developed method was found to be linear in the concentration range of 3.1–60.0 µg/ml with a regression coefficient of 0.9976. Accuracy was done by calculating percentage recoveries at low, medium, and high concentration levels, and percentage recovery was determined to be 89.99%, 89.09%, and 90.81%, respectively. Stability studies were performed using three methods, i.e., stability studies at room temperature, freeze-thaw stability study, and benchtop long-term stability studies where % nominal values were between 85–115%. A carryover study was performed using the blank solution, unextracted ULOQ, extracted blank plasma, and extracted ULOQ. It was found that no carry-over effect was seen in the developed method. This method can be used for routine drug analysis in marketed dosage formulations and spiked plasma samples. (Hailat et al., 2021)

M. S. Kalshetti developed and validated the HPLC method for quantification of Favipiravir in tablets using a Luna Phenomenex C8 (150x4.6 mm,5 m) column and a mobile phase made up of water and methanol in a 95:5:1 ratio at a flow rate of 1 ml/min in a Young Lin Autochro3000 HPLC instrument with a control panel and Young Lin Autochro Software. Favipiravir was detected using a UV-Visible detector at 229 nm, and retention time was found to be 4.3 min.

The developed method was validated using ICH guidelines, and linearity was observed in the concentration range of 10-50 µg/ml with a correlation coefficient of 0.9997. Specificity was performed using the blank solution, drug solution, and marketed product solution, and it was found that there was no interference in the developed chromatogram from solvent and excipients. Six interday and intraday precision replicates were used to accomplish the precision, and the computed% RSD was found to be 0.98 and 1.09, respectively. Accuracy was done using standard addition methods of 80%, 100%, and 120% and percentage RSD was found to be 0.62, 0.2, and 0.23, respectively. The LOD and LOQ used to determine the method's sensitivity were determined to be 1.15 g/ml and 3.49 g/ml, respectively. The developed method was found to be economical, sensitive, accurate, precise, and reproducible and can be used for routine analysis of drugs in marketed formulations. (Kalshetti & Adlinge, n.d.)

Marzouk Hoda M et al. developed a novel stability-indicating HPLC-DAD method for the determination of Favipiravir, a potential antiviral drug for COVID-19 treatment and its application to Degradation Kinetic Studies and In-Vitro Dissolution Profiling. Stability-indicating HPLC method was performed by exposing the drug to various stress conditions such as acid, base, oxidative, and hydrolysis degradation. The degradation products of Favipiravir were subjected to structural elucidation using Mass-spectrometry operated in electrospray ionization mode. Favipiravir was isolated from its degradation products on the Zorbax C18 column using the isocratic elution mode, and the column was maintained at 30°C. A mobile phase consisting of 25 mM phosphate buffer, methanol, and acetonitrile in the ratio 62:28:10 delivered at a flow rate of 1 ml/min was used.

Favipiravir was detected in HPLC using a diode array detector at 321 nm in the concentration range of 6.25-250 g/ml. The likely mechanism of Favipiravir's fragmentation to produce degradation products was predicted using the drug's MS1 fragmentation pattern. The developed method was also used to study the degradation kinetics of Favipiravir. Besides, different solvents were used to determine the dissolution profile of Favipiravir. The developed method was found to be accurate, reliable, time-saving, and cost-effective and hence can be used for routine analysis of Favipiravir in the marketed dosage form. (Marzouk et al., 2022)

Stability indicating RP-HPLC method development for determination of Favipiravir in the bulk and pharmaceutical dose form was created by Patil Aishwarya Balu et al. The chromatographic method was developed in Agilent 1120 compact LC HPLC System on C18

column with a mobile phase consisting of methanol: water (0.05% Triethylamine) in the ratio 70:30 at a flow rate of 0.8 ml/min using UV detection at 360 nm. The retention time of Favipiravir was 2.66 min.

The developed method was validated as per ICH guidelines, and linearity was found in the concentration range of 20-100 µg/ml with a correlation coefficient of 0.9997. Six replicates were used to perform intraday and interday precision, and the results showed that the %RSD was 0.99 and 1.05, respectively. Accuracy was done in three different concentrations LQC, MQC, and HQC and percentage recovery was found to be 99.7%. The limit of detection and limit of quantification was found to be 1.73 µg/ml and 5.26 µg/ml, respectively. Stability studies were performed as forced degradation studies, hydrolytic method, oxidative degradation, photolytic and thermal degradation. Based on the results, it was found that the drug was stable in basic and oxidative conditions and sensitive toward acidic conditions. The developed method was found to be simple, and accurate and hence can be used for the routine determination of Favipiravir in bulk and pharmaceutical dosage form. (Aishwarya Balu et al., 2021)

R. Suzuki et al. developed a Quantitative Analysis of Favipiravir Spiked in Plasma Using HPLC. Nexera TMXR HPLC was used for quantitative analysis in Shim-pack scepter C18 column (150mm x 4.6mm 5.0 µm) with guard column (10mm x 4mm 5. 0 µm) maintained at 30°C. A mobile phase consisting of phosphate buffer and methanol at a 1ml/min flow rate was used. The analytes were detected at 360 nm in the Fluorescence detector.

The calibration curve was prepared by spiking healthy human plasma with Favipiravir. The developed method was validated and linearity was observed in the concentration range of 1-100 µg/ml with a correlation coefficient of 0.999. Accuracy and precision were performed in replicates where percentage recovery in accuracy was found to be 92.1% – 106%, and % RSD for precision was found to be 0.21% – 0.31%. The developed method provides high-sensitivity quantitative analysis using a fluorescence detector and can be used for routine analysis. (Performance & Chromatography, n.d.)

Nishanth V et al., developed Multivariate optimization for determining Favipiravir, a SARSCoV-2 Molecule, by the Reverse-Phase Liquid Chromatographic Method Using a QbD Approach. The chromatographic method was developed method utilized a C18 column (5µm, 100 × 4.6 mm) and maintained at a temperature of 40°C.

The analytes were detected at 323 nm in the UV-Vis detector using a mobile phase that contained acetonitrile and ammonium acetate buffer (pH 4) in a 20:80 ratio with a flow rate of 0.5 ml/min. The box-Behnken design was used to optimize the analytical method by maintaining critical parameters such as the volume of acetonitrile, temperature, and flow rate.

The retention time of Favipiravir was observed at 3.4min. The developed method was validated following ICH guidelines. With a correlation coefficient of 0.9979, the calibration curve was achieved in the concentration range of 0.062 - 4 g/ml. System suitability parameters such as resolution, peak asymmetry, and theoretical plates were performed, and the results were within the limit. Replicated accuracy tests revealed a percentage recovery that ranged from 98.84 to 100%. The developed method was found to be simple and robust and thus can be used for routine analysis. (Nishanth G et al., 2022)

Inas A. Abdallah et al. developed an HPLC – UV method for the determination of Favipiravir, since these degradation products may have deadly effects on the human physiological system, it is crucial to define them in order to assess the safety of therapeutic medications. Our goal was to learn more about the toxicity, safety margins, and degradants of favipiravir (FVP), a breakthrough anti-Covid-19 medication that has recently gained widespread use and had a significant impact on humanity.

On a reversed-phase Eclipse XDB C18 column (4.6 150 mm, 5 mm), favipiravir and its oxidative and alkaline degradation products (FDP1 & FDP2) were separated using HPLC - UV in isocratic mode at a flow rate of 1 mL/min and detected at 332 nm.

The method was validated according to the ICH guidelines and was linear over the concentration range of 0.5 to 100 µg/mL With an IC50 of less than 100 g/ml and no cytotoxicity observed when treated NHSF cells were examined under an optical microscope, FVP, and its degradants were evaluated in vitro on normal skin fibroblast cell lines. (Abdallah, El-Behairy, et al., 2022)

High-Performance Thin Layer Chromatography:

HPTLC is a chromatographic technique that separates complicated components. The plates are prepared from optimized uniformly sized even particles and hence have more separation efficiency. HPTLC has advantages such as shorter analysis time, and detection is possible

with nanogram sample concentration. This chromatographic method is suitable for qualitative and quantitative separation of the sample.

Molnupiravir, Favipiravir, and Ritonavir were simultaneously determined using a highly sensitive, high-performance thin-layer chromatography approach by Roshdy E. Saraya et al. The method was developed using Silica gel 60F254 thin layer chromatography plates as the stationary phase and methylene chloride: ethyl acetate: methanol 25% ammonia in the ratio 6:3:4:1 as the mobile phase. HPTLC system was supplied with a semiautomatic sample injection system, nitrogen stream, Hamilton R 100 µl, sampling syringe, and Camag densitometer scanner. Retention factors of Favipiravir, Ritonavir, and Molnupiravir were found to be at 0.22, 0.63, and 0.42 min, respectively. Densitometric detection was performed at 289 nm and the developed method was validated as per ICH guidelines. The method was found to be linear in the concentration range of 2.75– 100.00 µg/ml for Ritonavir, 3.75– 100.00 µg/ml for Favipiravir and Molnupiravir,

Sensitivity was performed by calculating LOD and LOQ where LOQ in µg/ml was found to be 3.38, 2.68, and 3.66, for Favipiravir, Ritonavir, and Molnupiravir, respectively. Deliberate changes in mobile phase composition carried out robustness, and the recovery percentage was within the limit. The proposed method was found to be simple, eco-friendly, and cost-effective. It can be used for routine analysis of drugs when co-formulated shortly as single dose combinations. Besides, the method provides the highest throughput using recyclable reagents and simple economic tools.(Saraya et al., 2022)

Deena M et al. developed a novel environment-friendly TLC-Densitometric method for the determination of anti-covid drugs ―Remdesivir and Favipiravir‖. The TLC method was developed using a Camag® TLC scanner with lino mat 5 equipped with WinCATS® program on a normal phase TLC plate using ethyl acetate-methanol-ammonia as mobile phase in the ratio 8:2:0.2. The separated spots were detected in UV spectroscopy at 235nm and retardation factor was found to be 0.18 and 0.98 for Remdesivir and Favipiravir respectively. The factors affecting the TLC-densitometric method, such as eluent composition, saturation time, and scanning wavelength, were optimized.

The developed method was validated as per ICH guidelines, and the percentage recovery of Remdesivir and Favipiravir was found to be in the range of 97.21 to 101.31%. The calibration curve was found to be linear in the concentration range of 0.20 – 4.50 µg/band and 0.08 – 5.00 µg/band for Remdesivir and Favipiravir, respectively, with a correlation coefficient of 0.9999. The method's sensitivity was calculated as the limit of detection and quantification,

was the LOQ was found to be 0.07 and 0.12 ng/band and LOD was 0.02 and 0.04 µg/band for Favipiravir and Remdesivir respectively. Accuracy and precision were performed in replicates, and the results were within the limit. The greenness of the method was performed using the standard of greenness profile, and eco-scale, and the method passed the test an 80 score was achieved on the eco-scale. (Noureldeen et al., 2022)

Several analytical methods have been developed to estimate Favipiravir in bulk or dosage form, including UPLC-MS/MS method, LC-MS, Spectrofluorometric method, HPTLC-densitometric techniques, surfactant-assisted dispersive liquid liquid-liquid microextraction (SA-DLLME) combined with thin-layer chromatography–digital image colorimetry (TLC-DIC), electrochemical techniques. (Monicha et al., 2020)

Conclusion:

Overall, various analytical methods have been used to determine favipiravir levels. Spectrophotometry and thin-layer chromatography methods are simple and easy to apply. However, the HPLC analysis method is often used in research because it can detect samples with low concentrations. The HPLC method has specific advantages and sensitivity for favipiravir analysis in dosage form and biological matrices.

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