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A REVIEW OF THE ANALYTICAL METHODS FOR THE DETERMINATION OF FAVIPIRAVIR

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Favipiravir, UV-spectroscopy, HPLC, HPTLC, Spectrofluorometric method

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ABSTRACT: Favipiravir is, an antiviral agent, used to treat diseases caused by RNA viruses like Ebola virus, SARS-CoV-2, *Influenza virus*, Polio measles *etc*. The prodrug Favipiravir enters the infected cells through endocytosis and undergo metabolism to become an active drug. An active form of Favipiravir selectively targets the catalytic domain of RNA-dependent RNA polymerase and then interrupts the nucleotide incorporation process during viral RNA replication. This dysregulation of viral RNA replication results in mutations where the replacement of guanine by adenine and cytosine by thymine occurs. This ultimately induces destructive mutagenesis in RNA viruses. Currently, Favipiravir is available in tablet and intravenous dosage forms. The following analytical methods have been carried over for Favipiravir: UV, HPLC, LC-MS/MS, Spectrofluorometric method, and HPTLC-densitometric techniques are reported as per literature. The present paper illustrates the review of analytical methods which involve the estimation of Favipiravir in bulk or dosage form. The review also describes the scope and limitations of many published analytical methods for the analysis of Favipiravir. This detailed review article will be of great help to the researcher who is working on Favipiravir.

INTRODUCTION: Favipiravir is an antiviral drug used to treat influenza infection. Fujifilm Toyama Chemicals first developed Favipiravir in the year 2014 in Japan to treat Influenza infection. Favipiravir is the top choice for COVID-19 treatment and was generally sold under the brand name Avigan, Avifavir and Areplivir. It is a guanine analogue with pyrazine carboxamide structure.

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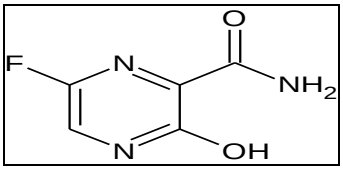
Favipiravir being a prodrug undergo phosphor-ribosylation and phosphorylation to produce an active form *i.e.*, Favipiravir ribofuranosyl -5'-triphosphate (Favipiravir-RTP), which inhibits viral replication by binding to RNA dependent RNA polymerase [1]. Favipiravir-RTP being a guanine analogue mimics both guanosine and adenosine for viral RNA-dependent RNA polymerase (RdRP) which inhibits primer extension.

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% [2].

Physical Characteristics: Favipiravir is a pale yellow color powder available in crystalline solid form. It was slightly soluble in water and completely soluble in acetonitrile and methanol. Melting point of Favipiravir is between 187 - 193°C. Other names for Favipiravir are Fapilavir and Favilavir [3].

Chemical Taxonomy: Favipiravir belongs to pyrazinecarboxamide class of organic compound which possess pyrazine nucleus and an amide group substituted on pyrazine nucleus.



Nomenclature: 6-fluoro-3-hydroxypyrazine-2-carboxamide

Molecular Formula: $C_5H_4FN_3O_2$

Molecular Weight: 157.104 g/mol

Pharmacokinetics Studies: The prodrug Favipiravir enters the infected cells through endocytosis and undergoes metabolism to become active drug. Favipiravir undergo phosphor-ribosylation and phosphorylation to produce an active form, *i.e.,* Favipiravir ribofuranosyl -5'-triphosphate.Favipiravir's bioavailability is almost near to 100%, *i.e.,* 97.6%.

Volume of Distribution: Approximately 54% of Favipiravir is plasma protein bound. Out of this 54%, 65% is serum albumin-bound, and 6.5% is ά1-acid glycoprotein bound.

Metabolism: Favipiravir is extensively metabolized in kidneys and excreted through urine. Favipiravir primarily undergoes hydroxylation by aldehyde oxidase and in minor concentration by xanthine oxidase. The inactive metabolite of Favipiravir produced by metabolism includes T705M1 and M2 (Favipiravir glucuronide conjugate).

Route of Elimination: Inactive metabolites of Favipiravir are excreted through renal route.

Half-life: Favipiravir possesses a short half-life, *i.e.,* 2 to 5.5 hours.

Clearance: 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, 600mg twice daily [4].

Analytical Methods for the Determination of Favipiravir in Pure Form or Dosage Form: Analytical methods are used to identify drug's physical and chemical properties in terms of qualitative and quantitative methods.[5]In this article, detailed information about instrumental analytical methods of Favipiravir has been discussed.

UV-Visible Spectroscopy: UV Visible spectroscopy is one of the instrumental analytical methods in which UV-Visible radiation are 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.

Jyothi B. *et al.,* developed a new ultraviolet spectrophotometric method for the estimation of Favipiravir by using Shimadzu UV–Visible spectrophotometer (UV JAPAN 1801). Favipiravir has poor solubility in the aqueous phase, so the drug is dissolved in the non-aqueous phase *i.e.,* ethanol, and then volume was made up with water. UV spectrum of Favipiravir in ethanol and water has a maximum absorption of 234 nm. Thus, obeyed Beer– Lambert's law in the concentration range of 1–10µg/ml, where linearity was observed between 2 to 10µg/ml with a coefficient of correlation (R^2) found to be 0.9995.

The developed method was statistically validated, and precise as %RSD values for intraday and interday precision were found to be 0.408% and 0.348–0.693%, respectively. 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 [6].

Zaranappa *et al.,* developed a simultaneous estimation of Aspirin and Favipiravir in bulk and tablet dosage form by UV-spectroscopic method. UV method was developed using acetonitrile solvent, and absorption maxima were observed in 225 nm and 321nm for Aspirin and Favipiravir, respectively. Beers range for Aspirin, and Favipiravir was found to be 4-20 µg/ml and 3-15 µg/ml, respectively. The developed method was validated, and linearity was obtained with correlation coefficient of 0.9995 and 0.9998 for Aspirin and Favipiravir, respectively.

Accuracy was performed in triplicates, and percentage recovery was found in the range of 93.93 to 106.17%. Robustness was performed by a slight variation in wavelength and %RSD was found to be within the limit, *i.e.*, 0.42-0.8 % for Aspirin and 0.35-0.62% for Favipiravir. Two instruments and two different analysts performed ruggedness, and percentage assay was found to be 98-102% and 98-106% for Aspirin, 98-101%, and 90-110% for Favipiravir. Precision was performed as method precision, system precision, intra-day precision, and inter-day precision in six replicates and %RSD was found to be within 2%. The developed method can be used for routine analysis of Favipiravir in pure or bulk dosage form [7].

Sandip *et al.,* developed a novel, simple and accurate UV spectroscopic method for the estimation of the potent antiviral drug Favipiravir in bulk and tablet formulation. Favipiravir is a modified pyrazine analogue *i.e.,* 6-Fluoro-3-hydroxypyrazine-2-carboxamide. By the UV method, absorption maxima were observed at 323nm, and zero-order derivative values quantitatively determined Favipiravir. ICH guidelines validated the developed method, and linearity was observed in the concentration range of 4-20µg/ml. Sensitivity of the method was expressed as the limit of detection and limit of quantification, which was found to be 0.08 µg/ml and 0.26 µg/ml respectively. Accuracy and precision were performed in replicates, and % RSD was found to be within the limit. The developed method was simple, sensitive, and accurate and can be used for routine drug analysis in marketed formulations [8].

Spectrofluorometric Method: Fluorescence emits visible light exhibited by certain substances when exposed to a beam of light. Substances showing this phenomenon are called fluorescent substances. The spectro-fluorometric method has advantages such as high specificity, high sensitivity and can be used to determine the fluorescence intensity

Safa M. Megahed developed a rapid, robust, sensitive, and green experimental design approach to develop a spectrofluorometric method for determining Favipiravir in spiked human plasma. Factors affecting the spectrofluorometric method were considered, and experimental parameters were optimized using Box Behnken Design. The method was developed in JASCO model FP 6300 spectrofluorometric with Spectra Manager software V1.53.0120622062by measuring the fluorescence of Favipiravir using 0.2 M borate buffer as solvent at 432 nm as emission wavelength and 361 nm excitation wavelength. Various solvents such as water, acetonitrile, methanol, ethanol, and acetone were used to check their effect on fluorescence, and it was found that water showed the highest fluorescence intensity.

The developed method was validated as per ICH guidelines and was found to be linear in the concentration range of 40–280 ng/ml. Sensitivity was performed as LOD and LOQ were found to be 9.44 ng/ml and 28.60 ng/ml, respectively. The concentration of Favipiravir in spiked plasma was found to be 48–192 ng/ml. Box Behnken Design was used for optimization as it aids in developing optimum conditions in three factors with fewer experimental runs. Optimization results were obtained in a contour plot, 3D response surface diagram, and interaction plot, and the result shows the optimum conditions by using 2.5 mL borate buffer at pH 8.0. The method's accuracy was performed in three replicates, and % recovery values were found to be 99.26 ± 0.87. The proposed method was eco-friendly according to the analytical eco-scale and can be used for routine analysis of Favipiravir in its pharmaceutical formulation.[9]

Colorimetric Method: An analytical method used to determine the concentration of analyte in the mixture using coloring agent.

Relerajan *et al.* determined Favipiravir from the pharmaceutical dosage form by the Extractive ion

pair complex Colorimetric method. The developed method was simple, sensitive, and accurate based on the formation of colored ion complexes of drugs with thiocyanate ions. The complex was then extracted using chloroform as solvent. Absorbance was measured at 618 nm in Shimadzu -160 double beam UV-Visible recording spectrophotometer with pair of 10mm matched quartz cells. The developed method was validated statistically. Accuracy was performed in replicates, and percentage recovery was calculated by the standard addition method. Linearity was observed in the concentration range of 1-12 µg/ml with a correlation coefficient of 0.9999. The reproducibility, repeatability, and precision of the method were within the limit, evidenced by low standard deviation values and % RSD. The developed method can be used for routine analysis of marketed dosage formulations [10].

Yukiko Moriiwa *et al.,* developed colorimetric assay for the quantification of Favipiravir in Human serum using Ferrihydrite. Ferrihydrite, color imparting agent, forms a complex with Favipiravir by substitution reaction. Favipiravir standard solution was prepared by using water as a solvent, and then further dilutions were made using MES/NaOH solution, pH was adjusted to 5.5. Iron (lll) hydroxyl of Ferrihydrite reacts with Favipiravir to form a yellow colored complex. Favipiravir adsorption on each Ferrihydritemicrobead is important for visual recognition of the yellow-color change.

The optimum packing length to recognize the yellow color was 1 mm. If it is longer than 1mm, then it results in lower intensity because of increased surface area, and vice versa resulting in easy judging of yellow color. Using Image-J software 1.8, yellow colored complex was transformed into red-green-blue pixels. Green and blue signal increases with the increase in the concentration of Favipiravir. The developed method was validated, and linearity was observed in the concentration range of 25–200 µg/ml with a correlation coefficient of 0.9913. Limit of detection and relative standard deviation was found to be 21.45 µg/ml and 4.3–15.4%, respectively. The developed method can be adopted for routine analysis of Favipiravir formulation as the method doesn't requires expensive equipment [11].

High-Performance Liquid Chromatography: HPLC is a physical separation technique conducted in the liquid phase. A sample is separated into its components by distributing the sample between mobile phase and a stationary phase under pressure applied using a pump.

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 thermostated to 30°C temperature and potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 90:10 as mobile phase. The flow rate was maintained at 1ml/min and the retention time was 15 min using 323 nm as the detection wavelength.

The developed method was validated as per ICH guidelines, and linearity was observed in the concentration range of 10-100 µg/ml with a correlation coefficient of 0.9999. 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 interday and intraday precision were less than 2%, i.e., 0.4 and 0.2%, respectively. Selectivity was performed by comparing the chromatograms of Favipiravir standard, tablet, and blank solutions where retention time, theoretical plate number, and peak tailing factor values observed were 7.696, 13,798, and 0.920, respectively. The method was also accurately indicated by % recoveries ranging 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. [12]

Abdallah I *et al.,* developed gadolinium-based magnetic ionic liquid for supramolecular dispersive liquid-liquid micro-extraction and HPLC/UV method for determining Favipiravir in human plasma. 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 10^3 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 [13].

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 observed in the concentration ranging from 2 to 10µg/ml with a correlation coefficient 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 has 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 method has been effectively adopted for routine analysis of Favipiravir in bulk and commercial formulations [14].

Sharaf A *et al.,* developed two green micellar HPLC and UV spectroscopic methods for the simultaneous determination of Molnupiravir and Favipiravir. The method was considered as green analytical method as the method was developed using no organic solvent, without extraction or derivatization steps and can also be applied for the simultaneous dissolution profile for Favipiravir tablets and Molnupiravir capsules. Multivariate chemometric model methods such as Classical least square, Principal component regression, Partial least squares and Genetic algorithm–partial least square methods were developed and validated using Matlab 8.2.0.701 (R2013b) software. Validation of chemometric model was performed using a set of nine binary mixture of Favipiravir and Molnupiravir. The developed method was found to be linear with correlation co-efficient of 0.9997.

Chromatographic method was developed using Kinetix® RP-C18 column (5 µm, 150 × 4.6 mm) and an isocratic mobile phase composed of 0.1 M SDS, 0.01 M Brij-35 and 0.02 M monobasic potassium phosphate mixture at the flow rate of 1 ml/min. Mobile phase pH was adjusted to 3.1 and analytes were detected at 230 nm using photodiode array detector. The developed method was validated according to FDA guidelines. Specificity was found to have good resolution between Favipiravir and Molnupiravir with the absence interference of excipients. The plot was found to be linear between the concentration range 0.5–50.0 µg/ml with R^2 value of 0.9999 to 1. Limit of detection and quantification was found to be 0.04 µg/ml and 0.02 µg/ml for Favipiravir, 0.12 µg/ml and 0.05 µg/ml for Molnupiravir. Accuracy was done for high, medium and low concentrations in triplicates according to QC standards and percentage recovery was found to be 99.99 ± 0.82 for Favipiravir and 99.99 ± 1.23 for Molnupiravir. The developed method can be applied for routine analysis of simultaneous estimation of drugs [15].

Aqeel Zeshan *et al.,* developed HPLC column screening for Favipiravir on six different columns i.e., Luna® C18(2) - 00D-4251-E0, Kinetex® C18 - 00D-4462-E0, Luna Omega PS C18 - 00D-4758-E0, Luna Omega Polar C18 - 00D-4760-E0, Kinetex 2.6 µm Biphenyl - 00D-4622-E0 and Kinetex F5 - 00D-4723-E0 to compare the retention time. 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 lack of significant differences in retention.

Hence, isocratic elution was followed to highlight the differences in retention between six different columns. 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. Retention time and peak tailing of Favipiravir in Luna C18(2), Kinetex® C18, Luna Omega PS C18, Luna Omega Polar C18, Kinetex 2.6 µm Biphenyl and Kinetex F5 - 00D-4723-E0 was found to be 5.099, 3.114, 4.838, 2.747, 3.523, 3.557 and 1.08, 1.29, 1.23, 1.40, 1.43, 1.46 respectively. The method has been developed using 500 µg/ml concentration of sample with best peak shapes in Luna C18(2), Kinetex C18 and Luna Omega PS C18 columns [16].

Nadendla Ramarao *et al.*, performed a validated High Performance Liquid Chromatographic method and UV spectroscopic method for the quantification of Favipiravir using PDA detector. The method was developed using SHIMADZU Prominence-i, LC-2030 system equipped with column i.e., Shim-Pack GIST C18 (250X 4.6 mm, 5µm) maintained at a temperature of 30°C. Mobile phase composed of potassium dihydrogenortho 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 calibration curve was found to be linear in the concentration range of 10-60 µg/ml. Sensitivity was calculated as LOD and LOQ which was 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, solvent with the drug. Solution stability was performed by storing the sample in solvent at ambient temperature for 24 hours, no changes were observed in sample solution and % RSD was within the limit. The developed method was found to be economic, eco-friendly with less retention time of 4.622 min and hence can be applied for routine analysis for marketed formulations of Favipiravir tablets [17].

Srinivaslingabathula *et al.*, developed stability indicative and cost effective analytical method 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) was 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, resolution and % RSD, found to be 38,417, 1.07, 0, 0.71 for Favipiravir and 3264, 1.04, 8.64, 0.89 for Peramivir respectively.

Degradation studies were carried out for Favipiravir and Peramivir to determine the conditions during which the drug is unstable so that measures are taken during formulation to avoid potential instabilities. Degradation studies were performed by acid, alkali, peroxide, reduction, thermal and hydrolysis method, percentage of drug degradation and drug recovered were calculated. The developed method was found to be fast and simple. Hence can be used for routine analysis of samples and to check its quality during stability studies [18].

Hebaelmansi *et al.*, developed green micellar solvent-free HPLC and spectrofluorometric determination of Favipiravir. To perform spectrofluorometric studies, factors affecting the method such as solvent type, buffer, pH, and surfactants were considered. The spectro-fluorometric method was performed using a Cary Eclipse fluorescence spectrophotometer equipped with Xenon flash lamp and the method was found to be sensitive by using Britton-Robinson buffer (pH 4) at 436 nm (emission wavelength) and 323 nm (excitation wavelength) with a concentration range of 20–350 ng/ml. Favipiravir has a remarkable native fluorescence and water was used as diluting solvent for more ecological safety. Micellar liquid chromatography uses micellar mobile phases, which has enhanced the column's separation efficiency, decreased retention time, and eliminated the environmentally hazardous organic solvents for elution. HPLC method was developed using Agilent HPLC1200 system with C18-RP (5 µm, 250 × 4.6 mm) column and a mobile phase consisting of 0.02 M Brij-35, 0.15 M SDS and 0.02 M disodium hydrogen phosphate, pH 5.0 at 1ml/min flow rate. The analytical method was validated as per FDA guidelines, where linearity was observed in the concentration range of 0.02 to

0.35 µg/ml and 10 to 100 µg/ml in Fluorescence spectroscopy and HPLC method with a correlation coefficient of 0.999. LOD and LOQ were found to be 0.004 µg/ml and 0.011 µg/ml, 0.985 µg/ml, and 2.986 µg/ml in fluorescence spectroscopy and HPLC method, respectively. The developed method can be used for routine analysis of marketed dosage forms and in the spiked human plasma sample. Besides, the method was found to be eco-friendly as the mobile phase used are biodegradable reagents. The eco-friendly properties were proven by their greenness metrics assessment i.e., GAPI and AGREE [19].

Mohammad Hailat *et al.,* developed and validated a method for quantifying Favipiravir in Human spiked 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 US-FDA 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 performed by calculating percentage recoveries at low, medium, and high concentration levels, and percentage recovery was found 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 carry-over 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 [20]. Inas A. Abdallah *et al.,* developed menthol-assisted homogenous liquid-liquid microextraction for HPLC/UV determination of Favipiravir as an antiviral for COVID-19 in human plasma. Microextraction was affected by factors such as extractant type, extractant volume, menthol amount, and vortex time. Optimum extraction was possible using 300 µL of tetrahydrofuran, 30 mg of menthol and subjecting the sample for 1 min vortexing before centrifuging it for 5 min. The method was developed using DionexUltiMate 3000 HPLC with Chromeleon 7 software on a Thermo® Hypersil ODS C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisting of 50 mM phosphate buffer and acetonitrile in a ratio of 60: 40 at a flow rate of 1ml/min and detection wavelength of 323nm was used for method development.

The developed method was validated according to the FDA bioanalytical method guidelines, and a calibration curve was obtained in the concentration range of 0.1 to 100 µg/ml with a coefficient of determination of 0.9992. Accuracy was performed in six replicates, and percentage recovery was found to be 97.1–103.9%. A stability study was done at different storage conditions, such as benchtop and freeze-thaw stability study of Favipiravir in human plasma. The sample was found to be stable as % RSD was within the limit, *i.e.,* 0.75 and 85 µg/ml, respectively. The validated HPLC method was used for the determination of Favipiravir in plasma samples, where $C_{max}$was found to be 6.40 µg/ml and 5.83 µg/ml for Avigan® 200 mg and Flupirava® 200 mg formulations, respectively. The developed method was simple, eco-friendly and hence can be used for biomedical applications [21].

Katharina Habler et al., developed simultaneous quantification of seven repurposed COVID-19 drugs Remdesivir, Chloroquine, Hydroxy-chloroquine, Lopinavir, Ritonavir, Favipiravir and Azithromycin by a two-dimensional isotope dilution LC-MS/MS method in human serum. A 2D Acquity UHPLC system along with an Oasis HLB Direct Connect HP column (30 mm x 2.1 mm, 20 M2066 for solid-phase extraction and MassTox® TDM MasterColumn® for analytical separation of analytes were used. A mobile phase consisting of water and acetonitrile with formic acid in the ratio 99.9:0.01 was used. The separated analytes from Master Column were detected with electrospray ionization in a positive mode in multiple reaction monitoring to record the mass transitions (m/z). The developed method was validated using the

European Medicines Agency bioanalytical method validation protocol. The linearity of the method was found to be ± 15 % of the nominal value and $R^2 \geq 0.993$ for all analytes in this study. Accuracy and precision were tested by replicating the analysis for five days, where accuracy was ≤ 9.59 %, and precision was ≤ 11.1 %. Stability studies were performed as benchtop stability, autosampler stability, freeze-thaw testing and long term stability which gave maximum deviations of 12.1 %, 9.3 %, 12.9 and 14.4 %, respectively. The proposed method was found to be an efficient tool for therapeutic drug monitoring to increase treatment efficacy and safety in COVID-19 patients [22].

Mosaad I. Morsy *et al.,* developed a novel LC-MS/MS method for determination of the potential antiviral candidate Favipiravir in human plasma: Application to a bioequivalence study in Egyptian human volunteers. Plasma sample was obtained by simple protein precipitation and separation of analyte was carried out in Exion LCTM chromatographic system using Eclipse plus C18 column (50 × 4.6 mm, 3.5 m). A mobile phase consisting of methanol and 0.2 % acetic acid in the ratio 20:80 with 0.6 ml/min flow rate was used. API4500 triple quadrupole tandem mass spectrometer with multiple-reaction monitoring (MRM) in negative electrospray ionization interface was used for Favipiravir.

The developed method was validated according to US-FDA guidelines. Selectivity was performed by analyzing six sets of blank human plasma from different sources, and found that there was no chromatographic interference from endogenous plasma constituents and concomitant medications. Linearity was observed in the concentration range of 100.0–20000.0 ng/mL by using a weighted linear regression strategy. Accuracy was performed in six replicates, and % Recovery was found to be 95.55 to 108.15% and 99.57–106.02% for intra-day and inter-day accuracy, respectively. Precision was carried out as intra-day and inter-day precision, and results were calculated as CV%, which was found to be 2.06 to 7.11% for both inter-day and intraday precision. The proposed method was applied to study the pharmacokinetic parameters of Favipiravir and for routine analysis of drugs [23]. Mamdouh R. Rezk developed a novel, rapid and simple UPLC–MS/MS method for quantifying Favipiravir in human plasma. Chromatography was carried out in AcquityUPLC® HSS C18 (100 × 2.1 mm, 1.8 μm) column and a mobile phase consisting of ammonium formate and methanol in gradient mode. Mass spectrometry was carried out in Xevo TQD LC–MS/MS with multiple-reaction monitoring mode using electrospray ionization. The developed method was validated as per the bioanalytical method validation protocol of the US FDA. For Favipiravir, linearity was observed in the concentration range of 0.25–16 μg/ml.

Precision and accuracy of the proposed method was performed in six replicates and the results were found to be in the range of 1.49 to 10.26% and 85.11–113.64% respectively. Stability studies were performed as short-term stability, post-preparative stability, long term stability, and freeze-thaw stability studies and % RSD was found to be 3.63, 4.84, 3.88, 4.96 respectively. Sensitivity was calculated as limit of quantification i.e., 0.25 μg/ml. The developed method involves simple sample preparation and also involves the determination of Favipiravir in human plasma at low concentration level [24].

İbrahim Bulduk developed a comparison of HPLC and UV Spectrophotometric method for quantification of Favipiravir in pharmaceutical formulations. UV spectrum was scanned from 200 nm to 800 nm using deionized water as a solvent and absorption maxima were found at 227 nm. The chromatographic method was developed in Agilent 1260 series liquid chromatograph on Inertsil ODS3 C18 (4.6 mm × 250 mm, 5.0 μm particle size) column maintained at 30°C and mobile phase consisting of sodium acetate solution: acetonitrile in the ratio of 85: 15 at 1ml/min flow rate. Both spectrophotometric and HPLC methods were validated in accordance with ICH guidelines. The method was found to be specific in spectrophotometric and liquid chromatographic methods, as there were no spectral and chromatographic interferences from the tablet excipients and solvent. In HPLC, robustness was analyzed under different conditions, such as changes in the mobile phase, flow rate, and in acetonitrile content in the mobile phase. For the spectrophotometric method, robustness was performed under different circumstances like change in solvent and detection wavelength. %

RSD was found to be within 2% for both the methods. Both the analytical methods were found to be linear in a concentration range of 10–60 mg/mlwith correlation coefficients greater than 0.999. The accuracy of the methods was performed in triplicates, and percentage recovery was found to be 99.57-100.10% for LC and 99.83–100.45% for UV. Intraday and interday precision was carried out, and %RSD was found to be 0.198 and 0.204, respectively. Both the developed and validated analytical methods can be used for routine analysis of Favipiravir in pharmaceutical formulations [25].

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 for structural elucidation using Mass-spectrometry operated in electrospray ionization mode.

Favipiravir was separated from its degradation products on Zorbax C18 column using isocratic elution mode and the column was maintained at 30°C. A mobile phase consisting of 25 mM phosphate buffer, methanol, acetonitrile in the ratio 62:28:10 delivered at flow rate of 1 ml/min was used. In HPLC, diode array detector was used for the detection of Favipiravir at 321 nm in the concentration range of 6.25-250 µg/ml. MS1 fragmentation pattern of Favipiravir was used to predict the possible mechanism of fragmentation of Favipiravir to form degradation products. 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 marketed dosage form [26]. Sonu A. Varma *et al.,* developed stability indicating HPLC method development and validation for estimation of Favipiravir in the pharmaceutical dosage form. The chromatographic method was developed on a stainless-steel inertsil column, packed with octadecylsilane (25 cm x 4.6 mm, 5 µm) maintained at 30°C, and a mobile phase consisting of 0.1% orthophosphoric acid and acetonitrile at 1 ml/min was used. The developed analytical method was validated as per ICH guidelines, and linearity was found in the concentration range of 10-30 µg/ml with a correlation coefficient R^2 of 0.9989. Accuracy was performed using 50%, 100%, and 150% standard solution, and percentage recovery was found to be within the limit.

Robustness was performed by deliberate modification in the mobile phase composition, flow rate, and pH values of the mobile phase. It was found that there was not much change in retention time, area, and peak symmetry. Forced degradation study was performed using methods such as acid degradation, basic degradation, oxidation degradation, thermal degradation, and photolytic degradation, and percentage recovery was calculated. The developed method can be used for quality control, routine analysis, and stability study of Favipiravir in marketed formulations [27].

Shyamala *et al.,* developed forced degradation studies for the determination of Favipiravir by Shimadzu LC-2010 HT RP-HPLC using a C18 column (250 X 4.6mm X 4µm) and a mobile phase consisting of orthophosphoric acid and acetonitrile in the ratio 60:40 at a flow rate of 1ml/min with a detection wavelength at 324nm. The developed method was validated according to ICH guidelines. System suitability parameters were determined using the number of theoretical plates and tailing factor, which were 82651 and 1.265, respectively. Linearity was observed in the concentration range of 4µg/ml - 20µg/ml, and the correlation coefficient was found to be 0.999. Precision was determined as intermediate precision, method precision, and results were found to be within 2% RSD, *i.e.,* 0.489 and 0.858, respectively. Accuracy was performed at three different ranges, *i.e.,* 50%, 100%, and 150%, and percentage recovery was found to be within the limit. Robustness was evaluated by deliberate changes in flow rate, *i.e.,* ± 0.2ml/min, and wavelength, *i.e.,* ± 5nm, and % RSD was found to be 0.89 and 1.6. Forced degradation studies were performed per ICH Q1A (R2) guidelines. A degradation study was performed using acid degradation, basic degradation, peroxide degradation, thermal degradation, and photolytic

degradation, and based on the results, it was found that Favipiravir was less stable in peroxide degradation. Mass spectral studies characterized the degradants of Favipiravir. The developed method can be used for routine analysis of Favipiravir in marketed formulations [28].

M. S. Kalshetti performed the development and validation of the HPLC method for quantification of Favipiravir in tablet in Young Lin Autochro-3000 HPLC instrument equipped with control panel Young Lin Autochro Software on Luna Phenomenex C8 (150x4.6 mm,5µm) column and a mobile phase consisting of water and methanol in the ratio of 95:5 at a flow rate of 1ml/min. 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 co-efficient 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. Precision was performed in six interday, intraday precision replicates, and the calculated % 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 method's sensitivity was performed as LOD and LOQ, which were found to be 1.15µg/ml and 3.49µg/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 formulation [29].

R. Suzuki *et al.,* developed a Quantitative Analysis of Favipiravir Spiked in Plasma Using HPLC. Nexera^{TM}XR 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 was 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 [30].

Pallavi *et al.,* performed bioanalytical method development and validation for the determination of Favipiravir in spiked human plasma by using RP-HPLC with carbamazepine as an internal standard. Chromatographic method was developed on Cromasil C18 column (250mm x 4.6 mm, 5 µ) with a mobile phase comprising of methanol and water in the ratio 35:65 maintained at pH 3 with 0.8ml/min flow rate. Run time and retention time were found to be 9.39 min and 6.2 min, respectively. Ethyl acetate was used as an extracting solvent. The developed method was validated from spiked human plasma as per ICH guidelines, and the method was found to be linear in the concentration range of 0.2-3.2 µg/ml. Accuracy was performed in replicates, and percentage recovery was found to be 97.6 to 100.2%. The detection and quantitation limit was System suitability parameters such as resolution factor, theoretical plates, and asymmetry factor was found to be 4.0, 4000, and 1.2, respectively. Long-term stability of was determined at -4°C for 60 days, and % RSD was found to be 1.56, 2.80, and 1.94 for three QC samples. The developed method was highly accurate, simple, precise and reproducible and hence can be applied for pharmacokinetic studies of Favipiravir [31].

Patil Aishwarya Balu *et al.,* developed stability indicating RP-HPLC method development for estimation of Favipiravir in the bulk and pharmaceutical dosage form. 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 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.

Precision was performed as intraday and interday precision using six replicates and %RSD was found to be 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, accurate and hence can be used for the routine determination of Favipiravir in bulk and pharmaceutical dosage form [32].

Yukiko moriiwa *et al.,* developed an optimization of analytical procedure for in-hospital rapid quantification of serum level of Favipiravir In the pharmacological treatment of COVID-19. Solid phase extraction (SPE) and RP-HPLC were used for quantification of Favipiravir. SPE was carried out using monolithic C18-silica disk built-in centrifugal spin-cartridge and then Subjected the sample for RP-HPLC. Chromaster HPLC system with Chromolith High-Resolution RP-18 column (100 × 4.6mm) equipped with a guard column (5 × 4.6 mm) and mobile-phase consisting of 0.1% phosphoric acid and CH3CN in the ratio 95:5 at a flow rate of 2ml/min. Favipiravir was detected by UV detector at 325nm.

The developed method was validated, and sensitivity was performed as the limit of detection and quantification, which was found to be 0.773 and 2.58 µg/ml, respectively. Accuracy was performed in replicates, and percentage recovery was found to be 97.9 to 105.0 %. The quantification method was well-validated and applied for measuring serum FPV level in clinical practice at a general hospital that acceptsCOVID-19 patients [33]. Süleyman Gökce *et al.,* performed the development and validation of the UPLC-MS / MS method for obtaining the Favipiravir tablet dosage form, and UPLC MS/MS was used for the recovery and stability study of Favipiravir. The chromatographic method was developed using 120 EC-C18 (4.6 mm × 50 mm, 2.7 µm) columns and water: methanol in the ratio 80:20 as mobile phase. Stability studies were done under forced conditions such as acidic, basic, oxidative, UV light, and thermal conditions in accordance with ICH guidelines. Based on the results, Favipiravir was sensitive to acidic, basic and oxidative conditions. The degraded products of the drug were characterized using electrospray jet stream ionization of mass spectrometry in negative ion mode. Molecular and daughter ion peaks were observed in 155.9 and 112.6, respectively. The calibration curve was found to be linear in the concentration range of 1-10 µg/ml with a correlation coefficient of 0.9999. Accuracy was performed at 80%, 100%, and 120% and percentage recovery was found to be 99.73-99.96%.

Robustness was performed by varying mobile composition, flow rate and column temperature, and %RSD was found to be 0.71, 0.6 and 0.33, respectively. The proposed method was found to be fast, reliable and hence can be used for routine medical and chemical analysis [34]. Nihal Saraner *et al.,* performed determination of Favipiravir in human plasma by using Liquid Chromatography-Tandem mass spectrometry: Application to Pharmacokinetic studies. Chromatographic method was developed using Shiseido Capcell PAK C18 column (250 × 4.6 mm, 5 µm) and mobile phase consisting of water and acetonitrile in the ratio 15:85. plasma was obtained from the protein precipitation method.

The separated analytes were characterized in mass spectrometry with electrospray ionization in negative ion mode and multiple reaction monitoring (MRM) mode. MRM transitions for Favipiravir and internal standard ($^{13}C^{15}N$) were found to be m/z 156.1 > 113.1 and m/z 158.1 > 113, respectively. Linearity was observed in the concentration range of 80- 30000 ng/ml and a correlation coefficient of 0.9977. The method's sensitivity was calculated as the limit of quantification, which was found to be 80 ng/ml. Stability studies were performed as bench top study and freeze-thaw stability studies and the co-efficient of variation was found to be within 3%. The validated method was found to be precise and sensitive and can be applied to pharmacokinetic studies of Favipiravir-marketed formulations [35]. Paul Curley *et al.* developed a highly sensitive

bioanalytical assay to quantify Favipiravir using liquid chromatography-tandem mass spectrometry. Emtricitabine was used as a standard internal drug. The chromatographic method was developed using Kinetex® F5 column (2.1x100mm 2.6µm) and mobile phases consisting of water and methanol in the ratio 95:5 with 0.1% acetic acid at a flow rate of 600 µl/min.

The separated analyte was detected on a SCIEX 6500+ QTRAP operating in negative mode. The developed method was validated as per FDA guidelines using mouse plasma, and linearity was observed in the concentration range of 0.78ng/ml to 200ng/ml. Recovery was determined at three QC concentrations, and percentage recovery was 76.5%, 98.5% and 95.0% for mouse plasma, human plasma and PBS, respectively. Accuracy was prepared in three different concentrations, i.e., 2ng/ml, 75ng/ml, 150ng/ml and percentage recovery was found to be 89%, 110%, and 106%, respectively. The reproducibility and robustness of the assay was determined based on accuracy and precision values. The developed method can be applied for both pre-clinical and clinical research and hence can be used for routine analysis of drug in marketed formulations [36].

Fathy Ibrahim Abd Allah *et al.*, developed a fully validated UPLC-MS/MS method for quantifying Favipiravir in human plasma boosted Lean Six Sigma: An application for a bioequivalence study. Valproic acid was used as an internal standard and the ACQUITY UPLCr BEH HILIC column was used to develop the chromatographic method. The developed method was quantified using triple quadrupole mass spectrometer. A bioequivalence study was performed using Favibrivix and Avigan; based on the results obtained, Favibrivix 200mg/tablet and Avigan® 200mg/tablet were statistically bioequivalent. Human plasma was separated using the protein precipitation method, and Lean Six Sigma verified the capacity and performance of the process. A mobile phase consisting of acetonitrile and 0.005% ammonia in water in the ratio 75:25 at a flow rate of 0.25ml/min was used, and the temperature was maintained at 10°C. The developed method was validated per FDA guidelines, and linearity was observed in the concentration range of 50–10,000 ng/ml with a coefficient of 0.9980. Accuracy was performed in replicates, and percentage recovery was within the limit. The developed method was found to be simple, accurate, economic and can be used for routine analysis of Favipiravir [37].

Nishanth V *et al.,* developed Multivariate optimization for determining Favipiravir, a SARS-CoV-2 Molecule, by the Reverse-Phase Liquid Chromatographic Method Using a QbD Approach. The chromatographic method was developed method utilized C18 column (5µm, 100 × 4.6 mm) and maintained at a temperature of 40°C. A mobile phase consisting of acetonitrile and ammonium acetate buffer (pH 4) in the ratio of 20:80 with a flow rate of 0.5 ml/min was used, and the analytes were detected at 323 nm in the UV-Vis detector. 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. The calibration curve was obtained in the concentration range of 0.062 – 4 µg/ml with a correlation coefficient of 0.9979. System suitability parameters such as resolution, peak asymmetry, and theoretical plates were performed, and the results were within the limit. Accuracy was performed in replicates, and percentage recovery was found to be in the range of 98.84-100%. The developed method was found to be simple and robust and thus can be used for routine analysis.[38]

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.

Roshdy E. Saraya *et al.,* performed highly sensitive high-performance thin-layer chromatography method for the simultaneous determination of Molnupiravir, Favipiravir and Ritonavir. 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 semi-automatic sample injection system, nitrogen stream, HamiltonR 100 µl, sampling syringe, and Camag densitometer scanner.2072 Retention factors of Favipiravir, Molnupiravir and Ritonavir were found to be at 0.22, 0.42 and 0.63 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 3.75–100.00 µg/ml for Favipiravir and Molnupiravir, 2.75– 100.00 µg/ml for Ritonavir. Sensitivity was performed by calculating LOD and LOQ where LOQ in µg/ml was found to be 3.38, 3.66, and 2.68 for Favipiravir, Molnupiravir, and Ritonavir, 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 [39].

Deena M *et al.,* developed a novel environment-friendly TLC-Densitometric method for the determination of anti-covid drugs "Remdesivir and Favipiravir". TLC method was developed using Camag® TLC scanner with linomat 5 equipped with WinCATS® programme on 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 co-efficient of 0.9999. The method's sensitivity was calculated as the limit of detection and quantification, where LOD was found to be 0.04 and 0.02 µg/band, and LOQ was 0.12 and 0.07 ng/band for Remdesivir and Favipiravir 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 eco-scale [40].

Other Methods Performed for Favipiravir Determination is as Follows: Ali Shokuhi Rad A *et al.,* developed Density functional theory (DFT) calculations towards the geometry optimization, electronic structure, Infrared spectroscopy, and UV–Vis analyses of Favipiravir absorption on the first-row transition metals doped fullerenes. This research provides detailed information about potential metalofullerene drug carriers using UV analysis and IR methods. The surface interaction of the drug with organometallic compounds such as titanium, chromium, iron, nickel, and zinc was studied by measuring adsorption properties. Maximum adsorption energy was found to be −148.2, −149.6, and −146.6 kJ/mol for Cr-, Fe-, and Ni-doped fullerenes, respectively.

Infrared spectroscopy (IR) study was performed to study the stretching vibration of bonds, *i.e.,* C—O bond in drug, C—M in metallofullereneand M—O in metallofullerene – drug complex. UV-Visible spectroscopy was performed using time-dependent functional theory, and features such as electronic transitions, excitation energy, absorbance, and oscillator strength of solvent were optimized through TD-ωb97xd/6-31++G method. UV studies was performed using water as solvent and λ_{max} was found to be 542, 456, 530, 481, and 459 nm for Ti-, Cr-, Fe-, Ni-, and Zn-doped fullerenes, respectively. The $\lambda_{max}$of Favipiravir molecule on metallofullerene was shifted to about 448 (Δ = −94 nm), 522 (Δ = +66 nm), 537 (Δ = +7 nm), 501 (Δ = +20 nm), 458 (Δ = −1 nm) nm for Ti-, Cr-, Fe-, Ni, and Zn-doped fullerenes, respectively. This shift can be concluded that blueshift occurs for Ti- and Zn-doped fullerenes and redshift for Cr-, Fe-, and Ni-doped fullerenes [41].

Soliman A., developed theoretical investigation of Favipiravir antiviral drug based on fullerene and boron nitride nanocages using UV method. C_{24} and

$B_{12}N_{12}$ functionalized nanocages are used to supplement the antiviral activity of Favipiravir. Density functional theory (DFT) was used to study the interaction energies of drug with perfect C_{24} and $B_{12}N_{12}$ functionalized nanocages and doped BC_{23} and $CB_{11}N_{12}$ nanocage. Based on DFT results, it was found that the interaction of –CO- group in Favipiravir with BC_{23} and $CB_{11}N_{12}$ is more favorable than with C_{24} and $B_{12}N_{12}$nanocages. The absorption spectrum of Favipiravir before and after the interaction with C_{24}, $B_{12}N_{12}$, BC_{23} and $CB_{11}N_{12}$ sites at maximum wavelength has been studied utilizing the time-dependent density functional theory. Analytical methods such as UV–Vis spectroscopy and Infrared spectroscopy are used for the investigation of the molecular orbitals of drugs and nanocages.

The adsorption energies of Favipiravir over C_{24}, BC_{23}, $B_{12}N_{12}$ and $CB_{11}N_{12}$ cages are − 0.085, − 1.434, − 1.120, and − 1.128 eV with corresponding binding distances of 3.355, 1.539, 1.557 and 1.555 Å, respectively. The functionalized $CB_{11}N_{11}$ has higher adsorption energy than the perfect $B_{12}N_{12}$ nanocage with Favipiravir. Binding energy was found to be higher in gas phase than in aqueous phase for C_{24} and BC_{23}. Bond length of B–N in $B_{12}N_{12}$ and $CB_{11}N_{12}$ cages clusters were found to be elongated due to the adsorption of Favipiravir at the nanocages i.e., 1.576 and 1.554 Å respectively. UV spectra provides detailed information about geometry optimization, molecular structure of drug with carriers and strong peaks were observed at 287.37, 208.63, 181.49, and 163.23 nm with energies of 4.314, 5.943, 6.831 and 7.596 eV for the main electron transitions using water as solvent. Based on the results, BC_{23}, $B_{12}N_{12}$ and $CB_{11}N_{12}$nanocages can be used as promising drug delivery vehicles for Favipiravir [42].

Mohammad Mehmandoust *et al.,* developed voltammetric sensor based on bimetallic nanocomposite for the determination of Favipiravir by using gold/silver Spectrofluorometric method core–shell nanoparticles (CSNP), conductive polymer poly (3,4-ethylene dioxythiophene) polystyrene sulfonate (PEDOT) and functionalized multi carbon nanotubes (F-MWCNT) on a glassy carbon electrode. Electrochemical sensors are widely used to determine trace amounts of drugs and other analytes in the sample because electrochemical sensors offer high catalytic portability, high chemical stability, and high sensitivity. A synergic effect was observed between F-MWCNTs, PEDOT: PSS and metal oxides; hence, there was a remarkable increase in in electrocatalytic activity towards Favipiravir. Limit of detection and quantification of Au@Ag CSNPs/PEDOT: PSS/F-MWCNTs/GCE to Favipiravir was found to be 0.005 to 0.009 and 0.009 to 2.0 µM, respectively.

The method was linear in the range of 0.005 to 0.009 and 0.009 to 1.95 µM. In order to determine the interference effect of volumetric method, electrochemical behavior was performed both individually and simultaneously using 1.0 µM of Favipiravir in the presence of 100-fold of other antiviral drugs like Tenofovir, Abacavir, Ribavirin and 300-fold of biological compounds like ascorbic acid, glucose, dopamine, uric acid, amino acids as interfering materials. The generated responses were not significantly affected by interfering agents, and it was found thatAu@Ag CSNPs/PEDOT: PSS/F-MWCNTs/GCE has an outstanding selectivity to determine Favipiravir in matrix samples. The developed electrochemical sensing platform was used for routine analysis of Favipiravir in human plasma, urine and tablet samples [43].

Shabnam Allahverdiyeva *et al.,* developed the first electrochemical evaluation of Favipiravir in the treatment of COVID-19: A study of its enhanced voltammetric determination in cationic surfactant media using a boron-doped diamond electrode. The study also focuses on the effect of cationic surfactant on the enhanced accumulation of Favipiravir at the surface of the boron-doped diamond electrode. The electrochemical properties of Favipiravir were determined without surfactant using cyclic voltammetry and then with surfactant using square-wave adsorptive stripping voltammetry. The electrochemical properties of Favipiravir were determined in the concentration range of 0.01-0.1 mg/ml and 0.1-20.0 mg/ml. LOD obtained from the proposed method can be used for sensing Favipiravir in routine quality control analysis, and the value was found to be 0.0028 mg/ml. The developed approach was applicable for routine quality control of commercial tablet formulations of Favipiravir and for the analysis of

urine samples without using expensive or sophisticated sample preparation [44].

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