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RESEARCH ARTICLE
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"A REVIEW OF ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND ESTIMATION TECHNIQUES OF FAVIPIRAVIR"

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Abstract
Favipiravir, a Pyrazinecarboxamide derivative, is a broad-spectrum antiviral drug originally approved in Japan for influenza and subsequently investigated for emerging viral infections, including Ebola and COVID-19. Its mechanism of action involves conversion into the active ribofuranosyl-5'-triphosphate form that inhibits viral RNA-dependent RNA polymerase, thereby disrupting replication of RNA viruses. Due to its therapeutic importance, reliable analytical methods for its estimation in bulk drug, formulations, and biological matrices are essential. This review summarizes the development, validation, and application of various analytical techniques such as UV spectrophotometry, RP-HPLC, LC–MS/MS, and UPLC for the determination of Favipiravir. Key validation parameters—including specificity, linearity, accuracy, precision, LOD, LOQ, robustness, and system suitability—are discussed in accordance with ICH guidelines. The reported methods demonstrate high sensitivity, reproducibility, and robustness for quality control, pharmacokinetic studies, stability testing, and therapeutic drug monitoring. This comprehensive overview highlights the significance of analytical method development in ensuring the safety, efficacy, and regulatory compliance of Favipiravir formulations.

INTRODUCTION: -

A member of the pyrazine class, favipiravir is a pyrazine that has been modified at positions 2, 3, and 6 by aminocarbonyl, hydroxy, and fluoro groups, respectively [1]. It is an antiviral drug that is authorized for the treatment of influenza in Japan and inhibits the RNA-dependent RNA polymerase of a number of RNA viruses. It functions as a RNA-directed RNA polymeraseinhibitor, an antiviral medication, and an anticoronaviral agent [2]. It is an organofluorine chemical, a hydroxy pyrazine, and a primary carboxamide. Favipiravir was authorized in Japan in 2014 to treat influenza illnesses that did not improve with standard care [3]. It has been studied in various nations to treat new viruses including Ebola and, most recently, COVID-19 because of its effectiveness in combating many influenza strains [4].

As a prodrug, favipiravir is intracellularly phosphorylated and ribosylation to produce the active Favipiravir-RTP.In the end, favipiravir-RTP stops viral transcription and replication by binding to and inhibiting RNA dependent RNA polymerase (RdRp). Favipiravir is mode of action differs from those of other influenza antivirals, which mainly stop the virus from entering and leaving cells [5]. By specifically inhibiting RNA polymerase, the active Favipiravir-RTP stops the viral genome from replicating. The way that Favipiravir-RTP interacts with RNA dependent RNA polymerase (RdRp) is the subject of various theories [6].

According to certain research, Favipiravir-RTP inhibits viral growth and RNA strand elongation when it is integrated into a developing RNA strand. Studies have also found that the presence of purine analogues can reduce Favipiravir's antiviral activity, suggesting competition between Favipiravir-RTP and purine nucleosides for RdRp binding [7]. The IUPAC Name of Favipiravir is 5-fluoro-2-oxo-1H pyrazine-3-carboxamide.The identification of the degradation products, followed by isolation and characterization stages, can be used to forecast the degradation process of the drug molecule subjected to various environments, including hydrolysis, oxidation, and photolysis.

Despite the fact that there have been a few stability-indicating HPLC methods previously published, no thorough stability-indicating method that isolates and characterizes the degradation products has been released as of yet for the quantification of FVP. Additionally, the medicine under research underwent forced degradation studies under various circumstances, which produced distinct degradation pathways [8].In the work that is being described, an effort has been made to investigate the oxidative and alkaline breakdown products of FVP in accordance with ICH criteria. Flash chromatography and HPLC-MS have been used for their respective isolation and characterisation. Furthermore, chemical structures and the degradation mechanism have been anticipated based on the 1H-NMR and MS of the discovered degradation products [9].

DRUG PROFILE OF FAVIPIRAVIR

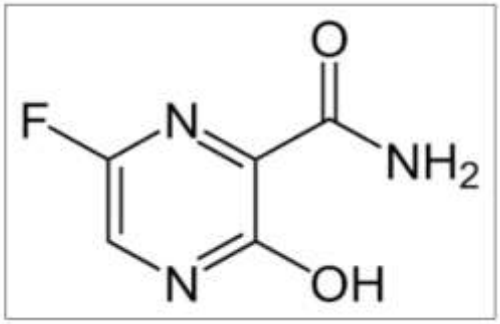

Fig 1: Chemical Structure of Favipiravir

IUPAC Name: 6-fluoro-3-hydroxypyrazine-2-carboxamide
Molecular Formula: $C_5H_4FN_3O_2$
Molecular Weight: 157.10 g/mol
Chemical Class: Pyrazinecarboxamide derivative; antiviral agent (RNA-dependent RNA polymerase inhibitor)
Appearance: White to light yellow crystalline solid powder
Melting Point: ~187–190 °C
pKa: ~5.1 (weakly acidic, due to hydroxypyrazine moiety)
Log P (Partition Coefficient): ~0.25 (low lipophilicity; favors aqueous solubility)
Solubility Profile:

- Freely soluble in water (~15–20 mg/mL at room temperature)
- Soluble in methanol and ethanol
- Slightly soluble in non-polar solvents (chloroform, hexane)

Stability:

- Stable under normal light and temperature conditions

- Susceptible to hydrolytic degradation under strong acidic/alkaline environments
- Sensitive to high moisture and prolonged heat exposure

Structural Characteristics:

- Contains a pyrazine ring with fluorine substitution (enhances metabolic stability)
- Carboxamide group contributes to hydrogen bonding and aqueous solubility
- Balanced hydrophilic–lipophilic profile supports oral bioavailability

PHARMACOKINETIC ACTION OF FAVIPIRAVIR

Absorption

High oral bioavailability (\~97%)
Rapid absorption after oral administration
Peak plasma concentration (Tmax): 1–2 hours

Distribution

Widely distributed in body tissues
Volume of distribution (Vd): \~15–20 liters

Metabolism

Hepatic metabolism
Metabolized mainly by aldehyde oxidase (AO) and xanthine oxidase (XO)
Converted to inactive metabolite (T-705 M1)

Elimination

Mostly excreted in urine as inactive metabolite
Elimination half-life: \~2–5 hours (can be prolonged at higher doses)
Non-linear kinetics due to saturable metabolism

MECHANISM OF ACTION

Activation in the body:

Favipiravir is a prodrug that requires metabolic activation after administration. Inside cells, it is converted by cellular enzymes (primarily hypoxanthine-guanine phosphoribosyltransferase, HGPRT) into its active ribofuranosyl-5'-triphosphate form (favipiravir-RTP)[10].

Targeting viral RNA polymerase:

The active favipiravir-RTP acts as a substrate mimic for purine nucleosides and selectively inhibits **viral RNA-dependent RNA polymerase (RdRp)**, an enzyme essential for replication of RNA viruses [11].

Inhibition of viral replication:

By incorporating into the viral RNA chain, favipiravir-RTP causes premature chain termination or induces lethal mutagenesis. This leads to the production of non-functional viral RNA strands, thereby blocking efficient replication of the virus [11].

Selectivity towards viruses:

Favipiravir shows a higher affinity for viral RdRp compared to human DNA/RNA polymerases, which explains its antiviral activity with relatively low toxicity to host cells [12].

Broad-spectrum antiviral effect:

Because RdRp is conserved among many RNA viruses, favipiravir demonstrates **broad-spectrum activity** against influenza viruses, flaviviruses, arenaviruses, filoviruses, and coronaviruses [12].

ANALYTICAL TECHNIQUES FOR FAVIPIRAVIR

Official methods for estimation of Favipiravir

Matrix	Method	Column / Principle	Mobile phase / Reagent (typical)	Ref.
Bulk drug (API)	HPLC Isocratic	C18, 4.6 × 150 mm, ~3 µm	Phosphate buffer (pH ~3.0) : Acetonitrile	13
Tablets	HPLC Isocratic	C18, 4.6 × 150 mm, 3 µm	Diluted phosphoric acid (pH ~3.0) : Acetonitrile	14

Tablets	Liquid Chromatography	C18, 250 × 4.6 mm, 5 µm).	Sodium acetate buffer : Acetonitrile (90:10), pH 3.0	15
Oral Suspension	HPLC	C18, 150 × 4.6 mm, 3 µm	Diluted phosphoric acid (pH ~3.0) : Acetonitrile	16

Reported methods for estimation of Favipiravir

Matrix	Method	Column / Principle	Mobile phase / Reagent (typical)	Ref.
Bulk Drug (API)	RP-HPLC	C18,150–250 × 4.6 mm ,5 µm	Methanol : Water (35:65 v/v) or other phosphate/organic mixes	17
Tablet	RP-HPLC, Isocratic	C18, 150 × 4.6 mm, 5 µm	Water : Methanol (30 : 70), pH ~3.0 with 0.1% orthophosphoric acid	18
Oral Suspension	RP-HPLC	C18, 150 × 4.6 mm,5 µm	Phosphate buffer (5 mM) pH 3.5 Methanol in ratio 75 : 25	19

ANALYTICAL METHOD VALIDATION PARAMETERS FOR FAVIPIRAVIR

Parameter	Description	Application for Favipiravir
Specificity	Ability of the method to measure Favipiravir accurately in presence of excipients, impurities, or degradation products [20].	Confirmed using placebo, spiked samples, and stressed samples (acid, base, oxidative, photolytic, thermal conditions).
Linearity	Ability of the method to obtain test results proportional to the concentration of analyte within a given range [20].	Linearity typically observed over **5–50 µg/mL** for UV/HPLC methods with correlation coefficient (R^2) ≥ 0.999.
Accuracy (Recovery)	Closeness of measured values to true value. Expressed as % recovery [20].	Standard addition method shows **98–102% recovery** in formulations.
Precision	Repeatability (intra-day) and intermediate precision (inter-day, analyst-to-analyst). Expressed as %RSD [20].	For Favipiravir, %RSD reported < 2%, confirming acceptable precision.
LOD (Limit of Detection)	Lowest concentration detected (not quantified) [20].	For Favipiravir in HPLC: ~0.02–0.05 µg/mL.
LOQ (Limit of Quantification)	Lowest concentration quantified with precision and accuracy [20].	For Favipiravir in HPLC: ~0.1–0.2 µg/mL.
Robustness	Ability to remain unaffected by small changes in method parameters (pH, mobile phase ratio, flow rate) [20].	Favipiravir method remains robust within slight changes (±0.1 pH, ±2% organic phase, ±0.1 mL/min flow).
System Suitability	Ensures system performance before analysis (parameters: retention time, peak area, tailing factor, theoretical plates) [20].	Typical acceptance criteria: Tailing factor < 2.0, %RSD of peak area < 2%, plate count > 2000.

APPLICATIONS OF DEVELOPED ANALYTICAL METHODS FOR FAVIPIRAVIR

Spectrophotometric Methods (Stability-Indicating)

Determination of favipiravir in bulk and pharmaceutical formulations.

Stability studies in presence of its acid degradation products.

LC–MS/MS in Biological Samples (Rat Plasma)

Pharmacokinetic studies in preclinical (animal) models.

Detection of favipiravir in biological fluids.

LC–MS/MS for Tablets and Pure Drug

Assay and quality control of favipiravir tablets.

Ensures correct drug dosage in marketed formulations.

RP–HPLC and UV Spectroscopy

Routine quality control testing in pharma labs.

Estimation of favipiravir in bulk and dosage forms.

Extractive Spectrophotometric Methods (Using Dyes)

Cost-effective estimation of favipiravir in formulations.

Ideal for resource-limited labs without advanced instruments**.**

HPLC–DAD from Volumetric Absorptive Microsampling (VAMS)

Therapeutic drug monitoring (TDM) and pharmacokinetics.

Suitable for minimal blood volumes, remote sample collection.

UPLC for Dissolution and Filter Compatibility

Dissolution testing during formulation development.

Ensures no interaction between drug and filters used in testing.

Simultaneous RP-HPLC for Multiple Antivirals

Simultaneous analysis of COVID-19 antiviral combinations.

Time-saving in multi-drug QC or research setups.

CONCLUSION

Favipiravir plays a vital role as an antiviral agent with proven activity against influenza and emerging viral diseases, necessitating reliable estimation methods for pharmaceutical and clinical applications. The reviewed analytical techniques, particularly RP-HPLC and LC–MS/MS, provide accurate, sensitive, and validated approaches for its quantification in bulk drug, formulations, and biological samples. Stability-indicating methods further support quality assurance by identifying degradation pathways under stress conditions. Overall, the development and validation of analytical methods for Favipiravir are crucial for routine quality control, pharmacokinetics, dissolution testing, and therapeutic monitoring. Future research should focus on advanced, eco-friendly, and cost-effective analytical tools to meet growing demands in pharmaceutical industries and clinical practice.

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