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Research Article

BIOANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF FAVIPIRAVIR AND REMDESIVIR IN RAT PLASMA BY LCMS/MS AND ITS APPLICATION TO A PHARMACOKINETIC STUDY

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ABSTRACT

Objectives: Food and drug administration approved and antiviral drugs of favipiravir and remdesivir were used in the treatment of Covid. For the bioanalytical approach of favipiravir and remdesivir, quick and easy, exact, active, and repeatable liquid chromatography-mass spectrometry (MS) MS methodology were created, employing D_5-favipiravir and D_5-remdesivir as internal standards.

Methods: In this study, a symmetry C_{18} column (150 mm×4.6 mm, 3.5 µm) was used for separation, with an elution by isocracy using 0.1% formic acid in water and acetonitrile in a 50:50 v/v combination of mobile phase with 1 mL/min flow rate at room temperature.

Results: Favipiravir (r^2=0.9999) was tested across a reasonable concentration. According to USFDA requirements, we discovered that the medications remained stable throughout the stability trials, just because the validated approach has successfully conducting to the pharmacokinetic studies of two drugs.

Conclusion: It is concluded that if both drugs are used in combination, they may produce a beneficial effect in the treatment of COVID-19 patients.

Keywords: Liquid chromatography-mass spectrometry/mass spectrometry, Favipiravir, Remdesivir, Validation, Rat plasma.

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INTRODUCTION

Avigan is an antiviral drug that is used to treat influenza [1,2], such as influenza A and influenza B, in Japan. Researchers are studying a new treatment for severe acute respiratory syndrome coronavirus 2 [3,4] as well as middle-east respiratory syndrome coronavirus is one of several different viral illnesses. It is a pyrazine carboxamide derivative [5,6]. Simply said, recommended for new virus variants (those trigger more severe effects symptoms) instead of yearly flu strains [7] (which is relatively harmless in the majority of cases). Researchers have found that pregnant [8] women who use this have a higher chance of having a baby with birth defects. The four animal species had teratogenic [9] and embryotoxic effects demonstrated on them [10]. Also thought to work by blocking viral RNA-dependent RNA polymerase [11,12], these actions are driven by the decrease in viral RNA-dependent RNA polymerase selectively. The drug favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP) is formed by metabolizing favipiravir and is both oral and intravenous preparations are available [13,14]. As a result, it has not been established that favipiravir is efficacious in primary human airway cells, which leaves open the possibility that the drug will not help during the flu season [15]. Favipiravir-RTP is a nucleoside analogue. The sequence of G and A mimics the roles of guanosine and adenosine [16,17] are found in viral RdRP. When two bases are used in a row to begin primer extension, the process is halted, though the method remains unclear as of 2013.

Gilead Sciences [18] developed the antiviral medication remdesivir, marketed under the name Veklury. Injection is given into a vein [19]. COVID-19 was on the verge of a pandemic, remdesivir, an experimental antiviral drug was also used to treat COVID-19 in around 50 countries. Remdesivir was initially developed to treat hepatitis C [20] infections and was later studied as a treatment for the aforementioned infections, including that of Ebola virus [21,22], as well as Marburg virus [23] infections. A raised liver enzyme [24,25] level is the most commonly observed side effect in healthy volunteers (a sign of liver problems). Remdesivir is a prodrug that is intended to allow intracellular delivery of GS-441524 monophosphate and subsequent biotransformation into GS-441524 triphosphate [26], a ribonucleotide [27] analogue inhibitor of viral RNA polymerase. Remdesivir had the most commonly reported adverse effects, which included respiratory failure [28] and other indicators of organ function, such as blood biomarkers of organ function, such as insufficient albumin [29], potassium deficiency anemia cell [30] count, the absence of thrombocytes [31], and elevated bilirubin [32] (jaundice). Various other side effects have been reported, including gastrointestinal distress [33], liver enzyme levels [34] in the blood, and allergic reactions at the injection site and heart rhythm abnormalities [35]. Some side effects associated with remdesivir, such as low blood pressure [36], nausea, vomiting, sweating, or shivering can occur during infusion. Structures of the chemicals used to make favipiravir and remdesivir (Fig. 1). The aim of the study is to develop a new rapid and sensitive liquid chromatography-mass spectrometry (LC-MS/MS) method for the simultaneous estimation of favipiravir and remdesivir in rat plasma using D_5-favipiravir and D_5-remdesivir as internal standard.

Very few articles were reported in the last few decades for determining the favipiravir and remdesivir using high-performance liquid chromatography (HPLC) [37,38] and LCMS [39,40]. We encountered problems such as long runtime, preparations of samples, and mobile phases, which were very costly in previous methods. However, our developed method is validated as per USFDA guidelines and has a shorter run time. It is more precise, less costly, and possesses good linear calibration curves, optimized MRM transitions, and an excellent recovery rate. The bioanalytical assay was applied successfully to the

pharmacokinetic study of favipiravir and remdesivir. But to date, no novel method for the simultaneous determination of favipiravir and remdesivir was evolved.

MATERIALS AND METHODS

Materials

Drugs and chemicals

Zydus Cadila, Ahmedabad, provided favipiravir and remdesivir (Internal Standards D_5-Favipiravir and D_5-Remdesivir) with purity levels of 99.99%. Merck Ltd, Worli, Mumbai, India, provided acetonitrile (LCMS grade), water (Milli Q), and formic acid (HPLC grade). The remaining elements and components were all grade AR and widely available.

Instrumentation

For the creation of a bioanalytical test, mass spectrometer QTRAP 5500 triple quadrupole instrument linked to an HPLC system (Waters Alliance e2695 model) [41], to read results AB SCIEX software was used. The positive ion electrospray ionization interface of the QTRAP 5500 triple quadrupole mass spectrometer was used for the study. The mass ion pair following was tracked using MRM mode: m/z 560.28 to 158.63 and m/z 603.33 to 193.65 for favipiravir and remdesivir, m/z 365.21 to 163.48, m/z 608.45 to 513.29 for D_5-favipiravir and D_5-remdesivir (Internal standards). Following optimization, the operating mass spectrometry parameters are specifically: Ion spray voltage 5500V, temperature source 550°C, drying gas temperature 120–250°C, collision gas – Nitrogen, pressure 55psi and drying gas flow stream 5 mL/min, 40V was declustering potential, 45V entry potential, 15V exit potential, 5500V capillary voltage, and a dwell time 1 sec. The instrumentation specifications are detailed, as shown in below (Table 1).

Chromatographic conditions

To accomplish chromatographic separation, a symmetric C_{18} (150×4.6 mm, 3.5 µm) column was employed on isocratic model at ambient conditions. Acetonitrile (1.0 mL/min) with 0.1% formic acid in water was mixed 50:50 v/v in the mobile step. This experiment used an injection volume of 10 µL and run for a total of 15 min.

Diluent: Methanol + Water (50+50)

Favipiravir parent stock solution

To prepare favipiravir parent stock solution of concentration 5,000 ng/mL, weigh 5 mg of favipiravir and transferred into a 10 mL volumetric flask, add about 6 mL of diluent and sonicate for 15 min to dissolve and make up to the mark. Further dilute 0.1 mL of this solution to 10 mL.

Remdesivir parent stock solution

To prepare remdesivir parent stock solution of concentration 5,000 ng/mL, weigh 5 mg of remdesivir and transferred into a 10 mL volumetric flask, add about 6 mL of diluent and sonicate for 15 min to dissolve and make up to the mark. Further dilute 0.1 mL of this solution to 10 mL.

Favipiravir and remdesivir stock solution

Take 0.16 mL of favipiravir parent stock solution and 0.08 mL of remdesivir parent stock solution into another 10 mL volumetric flask and make up to the mark with diluents (Favipiravir concentration - 80 ng/mL and Remdesivir concentration - 40 ng/mL).

Prepare D_5-favipiravir and D_5-remdesivir stock solutions in the same way.

Standard solution preparation for plasma samples

In standard preparation, 200 µL of rat plasma was spiked with 500 µL of internal standard working solution, 500 µL of standard stock solution, 300 µL of acetonitrile, and 500 µL of diluent. The solution was centrifuged at 5,000 rpm for 15 min and then the supernatant was collected, filtered through a 0.45 µ nylon syringe filter, and subsequently fed into the LC-MS apparatus.

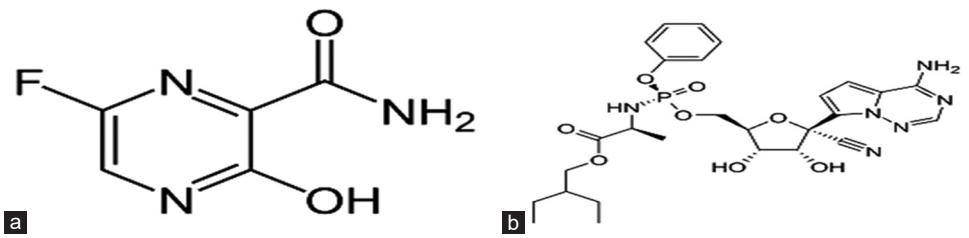

Fig. 1: (a) Structure of favipiravir; and (b) structure of remdesivir

Table 1: Liquid chromatography-MS/MS conditions

LC conditions		MS conditions	
HPLC	**Waters Alliance e2695**	**MS**	**A Sciex QTRAP 5500**
Mobile Isocrates	ACN: 0.1% formic acid in water 50:50 v/v	Ionization source	Nitrogen gas (N2), used for drying Flow that dries rate: 5 mL/min at 55 psi
	Flow level: 1 mL/min		Source temperature: 550°C
	Injection volume: 10 µL		Capillary voltage: 5500V
Symmetry C_{18}	150 mm length	Gases from colliding cells	Ultra-pure nitrogen
	4.6 mm ID	Mode	MRM[b]
	3.5 µm PS		
Analyte	Favipiravir	Favipiravir changes in MRM	m/z-560.28→m/z-158.63 CE[a]–15V
	Remdesivir	Remdesivir MRM transitins	m/z-603.33→m/z-193.65 CE[a]–15V
Internal standard	D_5-Favipiravir	D_5-Favipiravir changes in MRM	m/z-365.21→m/z-163.48 CE[a]–14V
	D_5-Remdesivir	D_5-Remdesivir changes in MRM	m/z-608.45→m/z-513.29 CE[a]–14V

CE: Collision energy, MRM: Multiple-response-monitoring shifts, ID: Internal diameter, PS: Particle size, MS: Mass spectrometry, HPLC: High-performance liquid chromatography

Animal characteristics

The six healthy rats used in this investigation (body weight range 250–350 g) were procured through Biological E Limited, Hyderabad.. The animal study Institutional Animal Care and Use Committee approved the procedure (Reg.No:1074/PO/Re/S/21/CPCSEA). These creatures are sheltered by a comparable laboratory setting, endive, carrots, and other fresh produce and fresh maize (in limited quantities). Animal feed needs to be stored at a level of 21–24°C and a relative humidity of 50–55%. Before the trial, the animals were fasted overnight and allowed unrestricted access to water. Favipiravir sample tablet and remdesivir solid injection powder were evaluated for pharmacokinetics. Each medication was given orally to all of the rats to a certain extent, 3.3 mg/kg of favipiravir and 0.08 mg/kg of remdesivir. A 1.5 mL of blood sample was taken from the rat body at 0.17, 0.33, 0.5, 0.67, 1, 2, 3, 4, 6, 8, and 12 h and then centrifuged the plasma for 30 min at 5,000 rpm. The supernatant solution was loaded onto a chromatographic column and plasma specimens were kept at temperatures ranging from 2 to 8° Celsius until the investigation was finished.

Statistical analysis

The mean, standard deviation, and coefficient of variation (% CV) were calculated to assess data variability and consistency. One-way analysis of variance was used to determine statistical significance. $p<0.05$ was considered statistical significance.

RESULTS AND DISCUSSION

Development of bioanalytical methods

The ESI has the strongest response in this stage compared to the chemical ionization by atmospheric pressure chemical Ionization method. Favipiravir, remdesivir, and their internal standards were quantified using the MRM method. Favipiravir, remdesivir, and their internal standards show a significant positive ion response mode as compared to ion-negative. The mass spectrum displayed in (Fig. 2).

For isocratic and gradient mode, we tested several buffers using acetonitrile as the mobility phase in various ratios to obtain the best chromatographic conditions. At each trial, the mobile step composition was tweaked to improve determination and accomplishment reasonable retention intervals. Finally, 0.1% formic acid and ACN at 50:50 v/v ratios in isocratic mode chosen as the mobile stage as it provides the best response of the medications. We used different stationary phases in the optimization process C18, C8, and CN-propyl which are examples. Utilizing a symmetry C18 column, 150 mm × 4.6 mm, and 3.5 µ connected to a PDA detector. We obtain strong peak shapes of favipiravir and remdesivir from various trials. Flow rates in the mobile process were set to 1 mL/min. Favipiravir and remdesivir had retention durations of 3.999 and 8.887 min, respectively. Six replicate injections yield % CV in acceptable limit,

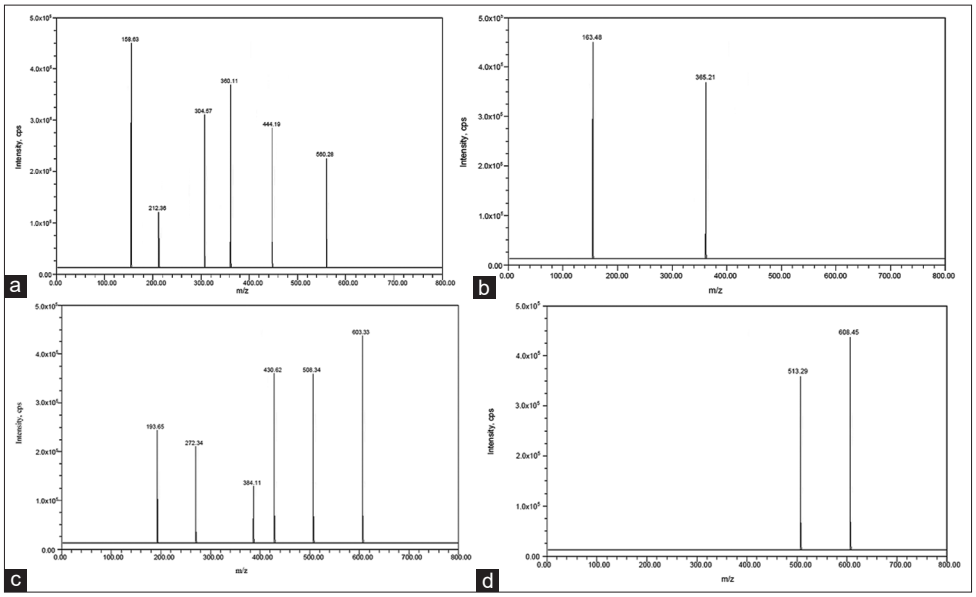

Fig. 2: (a) Mass spectra of favipiravir; (b) mass spectra of D_5-favipiravir; (c) mass spectra of remdesivir; and (d) mass spectra of D_5-remdesivir

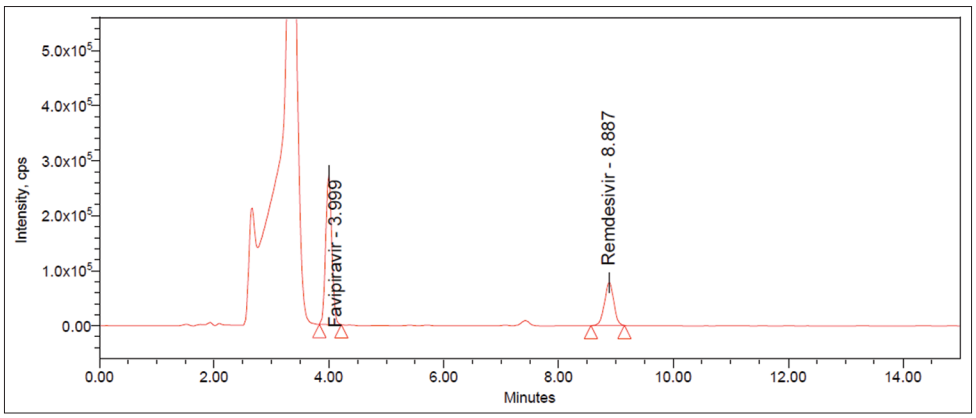

Fig. 3: Chromatogram of standard

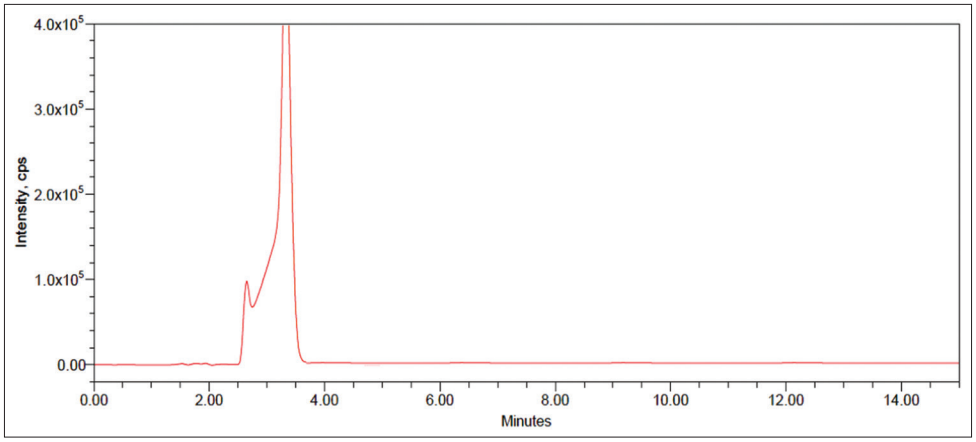

Fig. 4: Chromatogram of blank

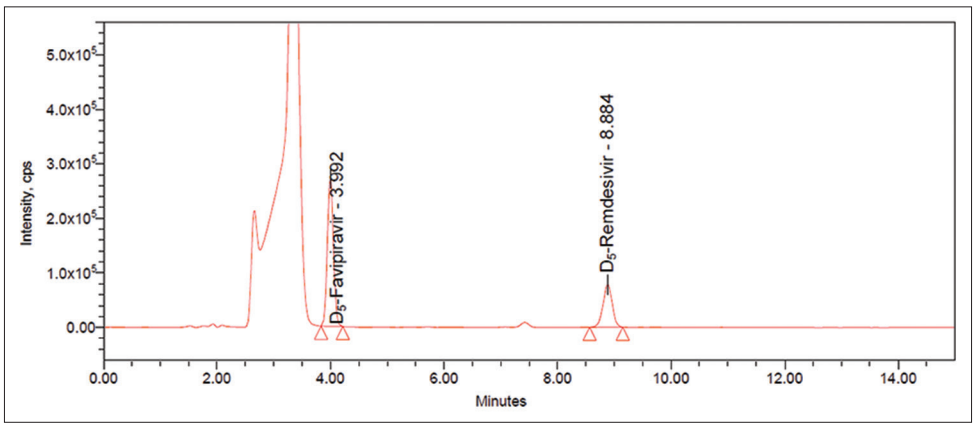

Fig. 5: Chromatogram of blank plasma spiked with internal standard

Table 2: Results of matrix variability and recovery percentage of favipiravir and remdesivir in rat plasma

Analyte	Matrix	Bias of the matrix factors (%)		Percentage RSD	
		LQC±SD	HQC±SD	LQC	HQC
Favipiravir	Plasma	98.22±0.0253	99.84±0.0362	1.56	0.63
Remdesivir	Plasma	97.73±0.0478	99.27±0.0189	0.63	1.42

Mean (n=18), RSD: Relative standard deviation, LQC: Low-quality control, HQC: High-quality control. Mean±SD (n=18). SD: Standard deviation

Table 3: Correlation effects of favipiravir and remdesivir

Indicator of validity	Favipiravir			Remdesivir		
Measures of quality control	Low	Medium	High	Low	Medium	High
QC concentration (ng/mL)	10	20	30	5	10	15
Linearity range	2–40 ng/mL			1–20 ng/mL		
Correlation (r^2)	0.9999±0.017			0.9992±0.009		

Mean±SD (n=3). SD: Standard deviation

indicating that the suggested technique is very specific. According to USFDA guidelines, the method in development has been validated. Standard, blank, and internal standard chromatograms are shown in following (Figs. 3-5).

Table 4: Linearity results of favipiravir and remdesivir

Linearity	Favipiravir Concentration (ng/mL)	Favipiravir peak area ratio	Remdesivir concentration (ng/mL)	Remdesivir peak area ratio
1	2.00	0.133	1.00	0.112
2	5.00	0.267	2.50	0.271
3	10.00	0.521	5.00	0.557
4	15.00	0.778	7.50	0.782
5	20.00	1.027	10.00	1.030
6	25.00	1.280	12.50	1.318
7	30.00	1.512	15.00	1.583
8	40.00	2.044	20.00	2.139
Slope	0.0493		0.1053	
Intercept	0.03281		0.00718	
CC	0.99991		0.99921	

Validation of bioanalytical process

Sensitivity

Six distinct plasma samples and plasma samples spiked with the IS were analyzed using optimized chromatographic and mass spectrometry conditions to assess specificity and selectivity of the method. The % CV for favipiravir and remdesivir was found to be 0.60% and 1.14%. % recovery was 99.3% and 99.4%. Hence, the sensitivity was passed.

Matrix effect

Favipiravir and remdesivir had matrix impact results of 98.22, 99.84% and 97.73, 99.27% at low-quality control (LQC) and high-quality control (HQC) stages, respectively. The medications' percent CV was determined to be 1.56, 0.63, and 0.64, 1.42 while comparing LQC and

Table 5: Accuracy and precision measurements for favipiravir and remdesivir plasma from rats

Matrix	Sample	Favipiravir			Remdesivir		
		Accuracy (% Bias)	Precision (%CV)		Accuracy (% Bias)	Precision (%CV)	
			Intra-day	Inter-day		Intra-day	Inter-day
Plasma	LLOQC	−1.18	2.57	2.30	−1.21	0.53	1.49
	LQC	0.54	1.32	0.77	0.66	0.64	0.72
	MQC	0.26	1.48	0.49	0.43	0.17	0.61
	HQC	0.13	0.67	0.16	0.57	1.65	0.84

LQC: Low-quality control, HQC: High-quality control

Table 6: Dilution integrity results

Analyte	ULOQC concentration (ng/mL)	Calculated concentration±SD	CV%
Favipiravir	40	39.98±0.0471	2.45
Remdesivir	20	19.91±0.0369	1.27

Mean±SD (n=6). SD: Standard deviation

Table 7: The outcomes of carry over

Concentration	Percentage of carry over	
	Favipiravir	Remdesivir
Blank	0	0
LLOQC	3.18	3.57
ULOQC	1.49	1.22

HQC standards. The findings show that the matrix effect on analyte ionization and internal specifications were both within reasonable limits (Table 2).

Linearity and precision

Under emphasis was the area at its height proportions in change norms. This method's linearity range for favipiravir was 2–40 ng/mL and for remdesivir 1–20 ng/mL. For favipiravir and remdesivir at various QC levels, calibration curves were seen over a linear concentration spectrum and correlation coefficients were found to be higher than 0.9999. Linearity results of Favipiravir and Remdesivir were shown in Tables 3 and 4 and the calibration plot is shown in Fig. 6 [42].

By aggregating all test data from diverse QC specimens, the accuracy and exactness were verified. The percent CV of favipiravir and remdesivir was <5% for all quality control samples at varied doses. All of the exactness and accuracy values were within the quantification range (Table 5).

Dilution integrity and carry over

Consistency in diluting may be demonstrated analyte tampering matrix concern with the ULOQC and diluting this specimen has a blank matrix. At ULOQC (40 molecules per milliliter of favipiravir and 20 ng/mL of remdesivir) dilution integrity was tested. Moreover, the percent CV for two components was found to be within suitable ranges at 2.45 and 1.27 (Table 6).

System mistake which could alter the measured sample value is known as transportation. The Waters Alliance analyzed samples collected using the following procedure for an LC/MS system. The system was built around a Z-spray three-fold four-fold mass detector with a blank injector capacity of 10 µL for a mixture of formic acid in water (0.1%) and acetonitrile (50/50). We may conclude that this process had no implications for the reliability and precision of suggested method (Table 7).

Reinjection reproducibility

The reproducibility of the reinjection was tested during actual subject sample analysis to validate the system after harsh product deactivation due to any instrumental disappointment. The difference on scales between LQC and HQC was <2.0. Due to an equipment failure, the group was re-infused, and the samples were produced which was repeated 24 h, the percent both the LQC and HQC had decreases of <2.0%. As a result, during genuine specimen analysis, if an instrument fails, a batch may be re-injected after 24 h.

Stability

To test the bench top stability of favipiravir and remdesivir, the prepared stock solution was maintained left out for 18 h. In an autosampler

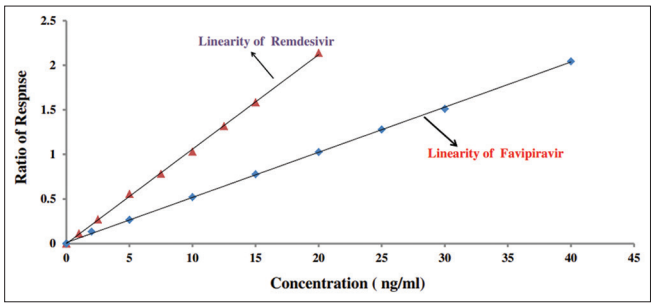

Fig. 6: Calibration plots of favipiravir and remdesivir

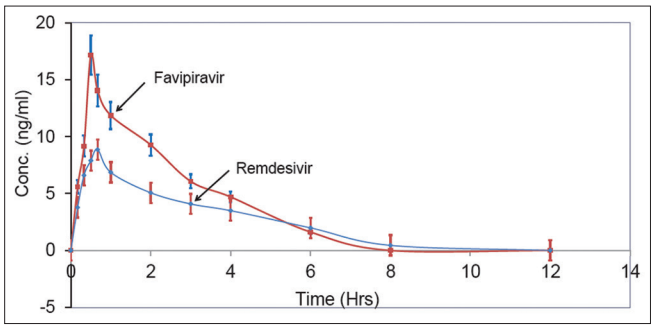

Fig. 7: Recovery plot of favipiravir and remdesivir

dependableness, the standard solution was held for 24 h at room temperature in the autosampler to provide dependable stability behavior under these conditions. The stock arrangement was kept for 24 h at (−28±5)°C for freeze thaw stability testing, 18 h at 2–8°C for wet recovery of consistency testing, and 18 h at (−20±3)°C for dry extract stability testing. Drugs were held for 7 days at (5±3)°C for short-term stability, and 28 days at (−20±3)°C for long-term stability before being injected into the LCMS. The stability of a freshly prepared stock solution was compared to a stock solution created before 24 h [43]. Favipiravir and remdesivir % change were determined to be 1.25%, 0.84%, and 0.43%, respectively, demonstrating that solutions are stable for up to 24 h.

Favipiravir and remdesivir were stable in plasma under various circumstances at room temperature. It was determined that the multiple incidents of subzero temperature and defrosting with plasma specimens enhanced by favipiravir and remdesivir had no effect on their stability. The durability of the freeze thaw was achieved by contrasting the durability samples that were frozen at -30°C and three times thawed with freshly

Table 8: Results for stability testing of favipiravir in rat plasma following various periods of storage

Stability	State of storage	Level of concentration	Concentration (ng/mL) (mean±SD, n=6)	RSD%	Recovery (%)	Accuracy (%Bias)
Bench top stability	18 h at room temperature	10	10.201±0.64	0.54	98.32	−1.68
		20	20.264±0.49	0.42	101.48	1.48
		30	30.157±0.83	0.28	100.27	0.27
Auto sampler stability	24 h in autosampler at room temperature	10	10.049±0.41	0.33	100.42	0.42
		20	20.043±0.52	1.17	100.55	0.55
		30	30.162±0.38	1.04	100.64	0.64
Long term stability	28 days at (−20±3)°C	10	10.968±0.95	2.31	90.99	−9.01
		20	19.712±0.85	1.46	92.36	−7.64
		30	30.241±0.34	0.85	91.23	−8.77
Freeze thaw stability	24 h at (−28±5)°C then exposed between three rounds of freezing and thawing	10	10.192±1.07	1.27	101.33	1.33
		20	20.117±0.81	1.15	99.14	−0.86
		30	30.896±0.59	0.93	98.27	−1.73
Stability of Wet Extract	18 h at 2–8°C	10	10.997±0.27	2.26	99.09	−0.91
		20	20.121±0.42	0.47	100.14	1.14
		30	30.036±1.28	0.36	100.23	0.23
Dry extract stability	18 h at (−20±3)°C	10	10.069±3.14	2.42	100.58	0.58
		20	20.223±1.08	1.07	101.73	1.73
		30	30.163±2.96	0.82	100.69	0.69
Short term stability	7 days at (5±3)°C	10	10.831±1.42	3.33	97.55	−2.45
		20	20.169±2.85	0.45	99.43	−0.57
		30	30.893±2.03	0.74	98.81	−1.19

Mean±SD (n=6). SD: Standard deviation

Table 9: Stability test results remdesivir plasma from rats kept in various freezers

Stability	State of storage	Level of concentration	Concentration (ng/mL) (mean±SD, n=6)	RSD%	Recovery (%)	Accuracy (%Bias)
Stability in the upper tier	18 h at room temperature	5	5.759±1.25	0.24	98.44	−1.56
		10	10.326±4.29	0.43	100.74	0.74
		15	15.625±0.49	0.26	98.55	−1.45
Automatically Sampled Stability	24 h in auto sampler neutral Temperature	5	5.623±0.68	1.03	99.38	−0.62
		10	10.321±0.63	0.87	100.28	0.28
		15	15.053±1.58	0.48	100.01	0.01
Long term stability (day 28)	28 days at (−20±3)°C	5	5.174±1.24	2.41	91.44	−8.56
		10	10.639±0.63	1.35	93.93	−6.07
		15	15.858±0.78	0.46	92.52	−7.48
Freeze thaw stability	24 h at (28±5)°C then exposed to a total of three freeze-thaw cycles	5	5.743±0.49	1.05	100.47	0.47
		10	10.632±0.47	0.89	99.36	−0.64
		15	15.632±0.33	1.68	101.48	1.48
Stability of Wet Extract	18 h at 2–8°C	5	5.852±0.68	3.43	100.06	0.06
		10	10.247±0.47	0.44	99.47	−0.53
		15	15.675±0.89	0.91	100.25	0.25
Dry extract stability	18 h at (−20±3)°C	5	5.284±0.54	2.25	101.49	1.49
		10	10.339±1.62	1.67	101.63	1.63
		15	15.264±3.47	0.46	100.74	0.74
Short term stability	7 days at (5±3)°C	5	4.893±1.22	0.69	96.66	−3.34
		10	10.124±1.54	0.53	97.45	−2.55
		15	14.457±2.38	0.94	98.62	−1.38

Mean±SD (n=6). SD: Standard deviation

Table 10: Clinical pharmacokinetic studies favipiravir and remdesivir

Variables in pharmacokinetics	Favipiravir	Remdesivir
AUC_{0-t} (ng h/mL)	190±0.0703	113±0.0529
C_{max} (ng/mL)	17.2±0.0261	8.859±0.0135
$AUC_{0-\infty}$ (ng h/mL)	190±0.0516	113±0.0487
$T_{1/2}$ (h)	6±0.04	8±0.07
T_{max} (h)	0.5±0.01	0.67±0.02

Mean±SD (n=6). SD: Standard deviation

spiked quality control samples. The freeze-thaw stability assessment used six aliquots each of the LQC and HQC concentration ranges. The concentration obtained after 24 hrs was compared with the initial concentration for long-term stability assessment. The stability values in the following table illustrate the overall stability test results, which indicate that the favipiravir and remdesivir samples remain within the permissible range of variation throughout the entire analysis procedure.

Pharmacokinetic studies

To investigate the pharmacokinetic properties of favipiravir market formulation, a dose of 3.3 mg/kg and remdesivir dose formulation market 0.08 mg/kg was administered into the rats' orally and superior normalized plasma concentration time profiles were obtained (Fig. 7). In pharmacokinetic investigations following oral administration, favipiravir and remdesivir show considerable variations. A 1.5 mL favipiravir blood sample was taken from the rat body at 0.17, 0.33, 0.5, 0.67, 1, 2, 3, 4, 6, 8, and 12 h after the drugs were administered into the rat body. Following that, a test substance sample was produced, into the chromatography apparatus, and the findings were recorded. This dose's accurate bioavailability was found to be within the acceptable limit of C_{max} after oral administration of favipiravir and remdesivir

(a combination of the two) (Table 10). The outcomes of this investigation yielded crucial pharmacokinetic parameters: C_{max} of favipiravir and remdesivir were 17.2±0.0261 and 8.859±0.0135, T_{max} of favipiravir and remdesivir were 0.5±0.01 and 0.67±0.02, $T_{1/2}$ of favipiravir and remdesivir were 6±0.04 and 8±0.07, AUC_{0-t} of favipiravir and remdesivir were 190±0.0703 and 113±0.0529 ng h/mL, and $AUC_{0-\infty}$ of favipiravir and remdesivir were 190±0.0516 and 113±0.0487 ng h/mL.

CONCLUSION

The established and verified LC-MS/MS methodology demonstrated robustness, reliability, and efficiency in quantifying favipiravir and remdesivir in rat plasma. The optimized chromatographic and mass spectrometric conditions achieved excellent separation, identification, and quantification of the analytes with high sensitivity and precision. The method demonstrated strong linearity, selectivity, specificity, and matrix independence, with recoveries and coefficients of variation well within acceptable limits. Stability studies confirmed the analyte's integrity under various processing and storage conditions, further validating the method's suitability for long-term application. After oral administration, favipiravir and remdesivir were rapidly absorbed from the rat body and showed pharmacokinetic behavior; here, the described method is fast, rugged, and reproducible. This confirms that the method is highly suitable for therapeutic drug monitoring and pharmacokinetic profiling, providing a valuable tool for clinical and research purposes involving favipiravir and remdesivir.

AUTHORS' CONTRIBUTIONS

Sunil Rayudu; Literature review, methodology, data curation, writing-original draft, and evaluation; and M. Manoranjani; Literature review, writing original draft, review and editing, supervision, evaluation, and visualization.

CONFLICTS OF INTEREST

The authors express no conflicts of interest.

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