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Determination of Antiviral Drug, Favipiravir by a Stability-indicating Chromatographic Method

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ABSTRACT

To determine favipiravir in bulk and marketed formulation, a stability-indicating reversed-phase high performance liquid chromatographic approach has been established that was proved to be sensitive and accurate. SPOLAR C18 column with 250 mm x 4.6 mm, 5 μ dimensions, 0.1% ortho-phosphoric acid buffer: acetonitrile in 50:50 as a mobile phase at 30 °C were used to achieve the chromatographic separation. The retention time of 2.613 min was recorded, when favipiravir measured at 323 nm using 1 mL/min flow rate. The suitable chromatographic conditions were identified through optimization studies. The method showed appreciable linearity (R^2 = 0.999) over 10-60 μg/mL concentration range. The calculated values for detection and quantification of favipiravir were 0.12 and 0.37 μg/mL, respectively. The methodology was verified, and the validation parameters results fell within the acceptable range outlined by ICH protocols. Satisfactory result was obtained on adopting optimized protocol in marketed formulation. Hence, this chromatographic methodology is suitable for the regular analysis of favipiravir in various marketed formulations.

Keywords: Favipiravir, High-performance liquid chromatography, Optimization, Validation, Forced-degradation.

INTRODUCTION

Favipiravir, chemically known as 6-fluoro-3-hydroxypyrazine-2-carboxamide, is an antiviral agent which specifically suppresses influenza's RNA-dependent RNA polymerase (RdRP) and various other RNA viruses[1]. The chemical structure of favipiravir is given in Fig. 1. It has $C_5H_4FN_3O_2$ as molecular formula and 157.1 g/mol as molecular weight. It slightly dissolves in water and it occurs as white amorphous powder with a melting range of 187 to 193 °C[2]. Being a prodrug, it undergoes ribosylation and phosphorylation within the cells to convert in to the active favipiravir-RTP. Upon binding of the later to RdRp, inhibits and thus prevents further viral transcription and replication[3]. The

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potential of favipiravir against SARS-Co V-2 was also investigated recently[4].

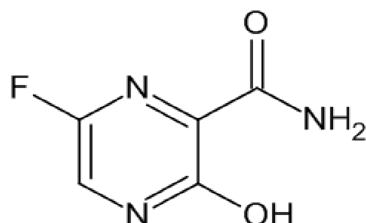

Fig. 1. Chemical structure of favipiravir

Literature review enabled to identify a number of quantification strategies involving either single or multi-drug formulations containing favipiravir. Ultra-violet spectroscopic methods using solvents, such as distilled water[5], ethanol[6], ethanol-water[7] and 0.1 N hydrochloric acid[8], spectrofluorimetry,[9,10] HPLC in both normal and reversed-phase[5,11-16], ultra performance liquid chromatography[17] were reported in the literature. The HPLC techniques described in the literature varied in linearity range, retention time (RT) and sensitivity. Table 1 had the corresponding details.

High performance liquid chromatographic (HPLC) separations are in high demand due to wide choice of stationary and mobile phases, high resolution, accuracy and precision. Favipiravir exhibits relative polarity because of its hydroxyl and amide groups. The same is reflected in its distinctive solubility characteristics, when tested at micro concentration range during the analysis. Due to the use of polar mobile and non-polar stationary phases, RP-HPLC (reversed-phase) technology is a potential tool for the analysis of polar substances[18]. Though several RP-HPLC methods are mentioned in the literature (Table 1), high sensitivity, early RT and stability were given more attention in the present work. Further, the chromatographic separation conditions meeting with the above features were determined through optimization. ICH Q2R1 guidelines[19] were utilized to validate the optimized method and the details were provided in subsequent sections.

Table 1: Comparison among current and reported HPLC methods

S. No	Stationary and mobile phase; detection wavelength	Linearity, sensitivity and RT	Reference
1	C18 column (Inertsil ODS-3V, 250 mm x 4.6 mm x 5 µ), Potassium dihydrogen phosphate 50 mM (pH 2.3) and acetonitrile (90:10, v/v); 323 nm	Linearity: 10-100 µg/mL LOD: 1.20 µg/mLLOQ: 3.60 µg/mLRT: 7.696 min	(13)
2	C18 column (250 mm x 4.6 mm x 4 µ), ortho-phosphoric acid and acetonitrile (60:40); 324 nm	Linearity: 4-20 µg/mL LOD: 1.26 µg/mL LOQ: 3.83 µg/mLRT: 4.4 min	(14)
3	C18 column (Inertsil ODS-3V), Potassium dihydrogen phosphate 50 mM (pH 3.5) and acetonitrile (90:10, v/v); 358 nm	Linearity: 15-250 µg/mL LOD: 2.186LOQ: 6.626RT: 4.622 min	(17)
4	C18 column (Zorbax, 250 mm x 4.6 mm x 5 µ), 5.0 mM phosphate buffer (pH 3.5 ± 0.05) containing 0.1% (w/v) heptane sulphonic acid sodium salt-methanol–acetonitrile (62:28:10, by volume); 321 nm	Linearity: 6.25–250.00 µg/mL LOD: 1.02 µg/mL LOQ: 3.10 µg/mL RT: 3.5 min	(12)
5	C18 (Sunfire) column (250 mm x 4.6 mm x 5 µ), ammonium acetate buffer pH 6.5 and methanol; 323 nm.	Linearity: 10–50 µg/mL LOD: 1.0 µg/mL LOQ: 3.5 µg/mL RT: 2.65 min	(6)
6	SPOLAR C18 column (250 mm x 4.6 mm x 5 µ), acetonitrile: 0.1% OPA (50 : 50 v/v); 320 nm	Linearity: 10-60 µg/mL LOD: 0.37 µg/mL LOQ: 1.12 µg/mL RT: 2.613 min	Current method

RT: Retention time; LOD: Limit of detection; LOQ: Limit of quantification.

MATERIALS AND METHODS

Instrumentations and reagents

A Shimadzu HPLC system (LC-20AD, LC solution software) containing SPOLAR C18 column (250 mm x 4.6 mm x 5 µ) for separation and UV detector for the measurement were used. The acquisition of analytically pure sample of favipiravir was achieved from Hetero Laboratories Ltd. (Hyderabad, India). Merck provided HPLC grade water, methanol and acetonitrile (Mumbai, India). Glassware used in the present investigation were treated overnight with chromic acid reagent (chromic acid-sulfuric acid mixture), washed liberally with double-distilled water and the same were used after drying in a hot air oven (Bio Technics India, Mumbai, India). A solution containing acetonitrile and water in 50:50 v/v was utilized as diluent throughout the investigation. Ortho-phosphoric acid (OPA; 0.1%, v/v) was prepared by transferring OPA (1 mL) in to a 1000 mL volumetric flask and the finally volume was done with water of HPLC. Upon dissolving 20 mg of

the analyte in 50 mL of a combination of water and acetonitrile in a volumetric flask, a standard stock solution of favipiravir (400 μg/mL) was produced.

Methods

System suitability test

The standard solution of favipiravir (400 μg/mL) was suitably diluted with diluent to obtain working stock solution containing 40 μg/mL. By examining six injections, system suitability characteristics like USP plate count, peak tailing and resolution were computed.

Method optimization

The chromatographic method was investigated for the appropriate selection of stationary (column) and mobile phases. Accordingly, different trials were conducted by keeping other chromatographic conditions (diluent, UV detector, wavelength of detection, column temperature, mobile phase flow rate, sample injection volume and run time) same. Details about the same are provided in the sections that follow.

Method validation

Linearity

Transferring aliquots of standard analyte solutions (400 μg/mL) into a set of 10 mL volumetric flasks and measuring the volume with diluent, analyte solutions with concentration ranges of 10–60 μg/mL were obtained. In order to establish linearity, each concentration was examined in triplicate under ideal chromatographic conditions.

Sensitivity

The chromatographic methodology's responsiveness was confirmed through the determination of the limit of detection (LOD) and limit of quantification (LOQ). They were derived from standard deviation (SD) method using below given formulae:

$LOD = 3.3 (\sigma/S)$ $LOQ = 10 (\sigma/ S)$

Where, σ = SD of intercepts of calibration curves and S is the slope of the linear curve.

Accuracy

The closeness in the measurements of favipiravir by chromatographic procedure was demonstrated by computing %recoveries using standard addition method. Discrete volumes of standard solutions of analyte (0.5, 1.0, 1.5 mL from 400 μg/mL stock solution) were spiked to the formulation of the same at a fixed concentration and accuracy was examined by computing mean %recovery and corresponding %RSD at each level.

Precision

The preciseness in the method was arbitrated by analyzing specific concentration of analyte (40 μg/mL) repeatedly within the day and three different days across the week. Peak areas were calculated for six working sample solutions of the analyte in each case and variations in the corresponding measurements were expressed as %RSD.

Specificity

The explicitness of the methodology was ascertained through careful observation of chromatograms of blank, placebo and the analyte. Verification was conducted to confirm the interference between the peaks in the placebo and blank at analyte retention time.

Robustness

The method robustness was ensured by adopting the methodology with slight alterations. The flow rate (±0.1 mL), solvent ratio in mobile phase (±5 mL), and column temperature (±5 °C) were executed and the %RSD for each variable was calculated.

Degradation studies

Acidic degradation

Stock solution of favipiravir (1 mL) was added to 2 N hydrochloric acid (1 mL). The resultant is refluxed at 60 °C for 30 minute.

Alkali degradation

A mixture containing favipiravir stock solution (1 mL) and 2 N sodium hydroxide (1 mL) was refluxed at 60 °C for 30 minute.

Oxidative degradation

The sample solution prepared by adding hydrogen peroxide (1 mL) to the stock solution (1 mL) of favipiravir was maintained undisturbed for about half an hour at 60 °C.

Dry heat degradation

The standard analyte solution underwent dry heat degradation by exposure to an oven set at 105 °C for duration of 6 hours.

Photo stability

A beaker containing standard analyte solution (400 μg/mL analyte) was placed in a UV chamber for week period.

The resultant solutions in all the above tests were diluted to get solutions of the required concentrations and they were injected into HPLC. Utilizing the corresponding chromatograms, the stability of the sample was evaluated and assessed.

Assay

Twenty Fabiflu tablets (each containing 200 mg of favipiravir) were ground to obtain fine powder. The powder analogous to 40 mg of favipiravir was shifted to a volumetric flask (100 mL) containing diluent (approximately 50 mL). The mixture was screened via HPLC filters after sonication for 15 minute. The optimized HPLC procedure was adopted for the resulting favipiravir solution (400 μg/mL) and the output chromatograms were analyzed. The details were elaborated in results section.

RESULTS

Method development and optimization

Primitively, favipiravir solubility profile was examined in a variety of solvents including acetonitrile, water, methanol, ethanol, chloroform, and acetic acid. It has been shown to be insoluble in ethanol and chloroform, soluble in acetonitrile and water, and sparingly soluble in methanol, at the concentration used in the study (1000 μg/mL). Based on these results, acetonitrile and water (50:50) was selected as diluent for the further investigations.

Through a series of studies, the ideal chromatographic conditions required for separation of favipiravir were found. Various stationary and mobile phase combinations were utilized, while other chromatographic conditions (flow rate: 1 mL/min, volume of injection: 10 μL, temperature of column: 30 °C, wavelength: 320 nm and UV detector) were maintained unchanged. The details of trials were portrayed in Table 2.

Table 2: Details of chromatographic conditions in various trials

Trial	Column	Mobile phase	Observation
1	Discovery C18 column (150 mm x 4.6 mm, 5 μ)	Water:Acetonitrile (50:50 v/v)	Peak was broad in shape.
2	SPOLAR C18 column (250 mm x 4.6 mm, 5 μ)	Acetonitrile:Methanol (50:50 v/v)	Peak extended the specification limit.
3	SPOLAR C18 column (250 mm x 4.6 mm, 5 μ)	Water:Methanol (40:60 v/v)	Peak splitting and baseline disturbances were detected.
4	SPOLAR C18 column (250 mm x 4.6 mm, 5 μ)	Acetonitrile:0.1% OPA (45:55 v/v)	Peak tailing and high retension time was observed.
5	SPOLAR C18 column (250 mm x 4.6 mm, 5 μ)	Acetonitrile:0.1% OPA (50:50 v/v)	Good resolution, no tailing, high theoretical plate count and reduced retention time was observed.

After carefully examining all trial runs from 1 to 5, the chromatographic conditions in trial 5 with good resolution, high theoretical plate count without peak tailing, and a shortened retention time were determined to be ideal for the method. Thus, SPOLAR C18 column (250 mm x 4.6 mm, 5 μ) and acetonitrile: 0.1% OPA (50:50, v/v) as mobile phase were selected for further analysis. In Fig. 2 the optimized chromatogram was displayed.

Method validation

The HPLC method was further justified through various validation parameters as per ICH Q2 (R1) protocols.

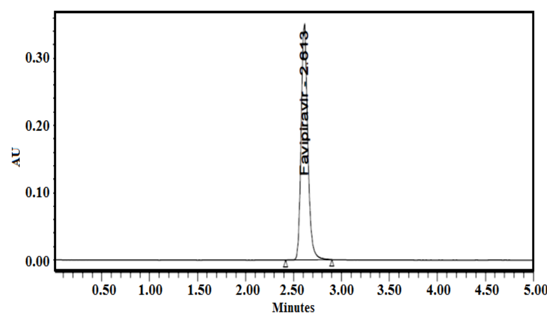

Fig. 2. Optimized chromatogram of favipiravir

System suitability

Table 3 presents the system suitability parameters that were acquired from chromatograms after injecting six samples of the analyte at a concentration of 40 μg/mL. In accordance with ICH criteria, the USP plate count, tailing factor, and resolution values %RSD were determined to be satisfactory (less than 2.0).

Table 3: System suitability parameters data for favipiravir

Injection*	RT	Peak area	USP plate count	USP tailing
1	2.613	1622499	7108	1.18
2	2.618	1631340	7282	1.21
3	2.628	1640862	7246	1.17
4	2.628	1635074	7281	1.17
5	2.63	1651974	7261	1.19
6	2.638	1648214	7205	1.19
Mean	2.626	1638327	7230.5	1.185
SD	0.009	10957.6	66.377	0.015
% RSD**	0.341	0.669	0.918	1.279

*Favipiravir 40 μg/mL; RT: Retention time; SD: Standard deviation; RSD: Relative standard deviation; **Acceptance limit: %RSD less than or equals to 2.0.

Linearity

Six different concentrations of favipiravir (10-60 μg/mL) in triplicate were injected and the corresponding chromatographic system responses were recorded in the form of calibration curve Fig. 3. The linearity study resulted in y = 40226x+5454.8 as linear regression equation. Further, correlation coefficient (R^2) value 0.999 indicated appreciable linear relationship of the chromatographic response over the measured concentration range of favipiravir.

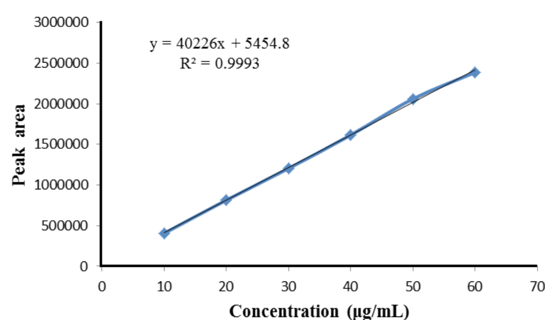

Fig. 3. Calibration curve for favipiravir

Sensitivity

As a result of rigorous evaluation, the method exhibited LOD & LOQ values of 0.37 and 1.12 μg/mL, respectively.

Accuracy

The correctness of the chromatographic methodology was demonstrated by assessing the %recovery of the analyte. The mean percentage recovery was noted as 99.21-101.61 among the three concentration levels of analyte (50%, 100%, and 150%). The %recoveries of the triplicate measurements, their mean and %RSD were computed and propound in Table 4. Since the percentage RSD readings were less than 2.0, the method's accuracy was confirmed.

Table 4: Accuracy data of favipiravir

%Level	Amount Spiked (μg/mL)	Amount recovered (μg/mL)	%Recovery	Mean	%RSD
50	20	19.98	99.88	99.88	0.12
	20	19.96	99.72		
	20	19.99	99.96		
100	40	39.68	99.21	100.27	1.22
	40	40.64	101.61		
	40	40.01	100.01		
150	60	59.84	99.73	100.11	0.54
	60	60.44	100.73		
	60	59.94	99.89		

RSD: Relative standard deviation

Precision

Multiple replications of the working sample solutions of 40 μg/mL were injected repeatedly in to the system in order to measure intra-day precision and multiple samples containing same concentration was tested on consecutive days over a week period to determine inter-day precision of the method. The discrepancies in the responses, such as RT and peak area were determined and mean values were computed Table 5. The %RSD values in both the studies were found to be below 2.0. The results evidenced the preciseness of the method.

Table 5: Precision data of favipiravir

Injection	Intra-day precision		Inter-day precision	
	RT	Peak Area	RT	Peak Area
1	2.605	1629908	2.646	1633498
2	2.625	1639581	2.652	1657172
3	2.668	1650782	2.641	1642032
4	2.634	1658234	2.645	1621777
5	2.702	1647201	2.641	1617728
6	2.714	1628245	2.656	1639487
Mean	2.658	1642325	2.647	1635282
SD	0.044	11902.52	0.006	14395.33
%RSD*	1.65	0.72	0.23	0.88

RT: Retention time; SD: Standard deviation; RSD: Relative standard deviation; *Acceptance limit: %RSD less than or equals to 2.0.

Specificity

Specificity is the capability of a chromatographic technique to exclude the analyte response when all known substances and contaminants are present at the same time. The

results indicated the absence of intruding peak in blank and placebo at favipiravir's RT (Fig. 4). Thus, the method was found to be specific.

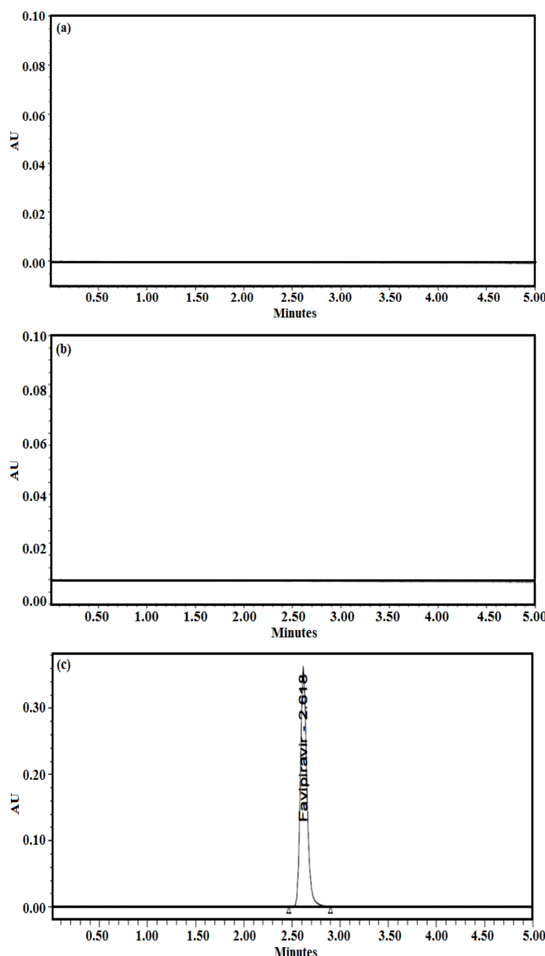

Fig. 4. Chromatograms of (a) Blank, (b) Placebo and (c) Favipiravir

Robustness

The outcomes of robustness study were provided in Table 6. Slight changes in the flow rate, solvent ratio and temperature didn't produce any significant change in the chromatographic response. The method's robustness is evidenced by %RSD values below 2.0, as outlined in Table 6.

Table 6: Robustness data of Favipiravir

Parameter	%RSD
Mobile phase flow rate (0.9 mL/min)	0.4
Mobile phase flow rate (1.1 mL/min)	0.8
Mobile phase (45A:55B)	0.8
Mobile phase (55A:45B)	0.2
Temperature (25 °C)	0.6
Temperature (35 °C)	1.1

A:Acetonitrile;B:0.1% ortho-phosphoric acid;RSD:Relative standard deviation

Degradation Studies

Upon subjecting the formulation to various stress conditions, the stability of favipiravir was scrutinized. The degraded samples of each test were loaded in to HPLC and the amount of drug degraded was calculated (Table 7). The %drug degraded was observed to be relatively more in acid (6.32%), while in all other degradation tests it was minimal (< 5%). Fig. 5 depicts the chromatogram of acidic degradation.

Table 7: Degradation studies data

S. No	Degradation Condition	% Drug Degraded
1	Acid	6.32
2	Alkali	4.65
3	Oxidation	4.33
4	Thermal	2.53
5	UV	1.39

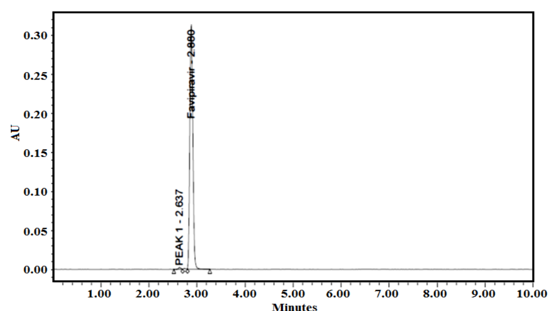

Fig. 5. Chromatogram in acidic degradation

Assay

The results obtained by determining the favipiravir content in the commercial formulation are shown in Table 8. The mean %assay and %RSD for favipiravir was observed to be 100.15 and 0.72, respectively. These measurements were observed to be accord with the protocols mentioned under ICH.

Table 8: Assay data of formulation

Injection	%Assay
1	99.39
2	99.98
3	100.66
4	101.11
5	100.44
6	99.29
Mean	100.15
SD	0.73
%RSD	0.72

SD: Standard deviation; %RSD: %Relative standard deviation.

DISCUSSION

The present investigation was aimed to establish a stability-indicating RP-HPLC

method, which can precisely measure favipiravir without interference of excipients and degradation products. Various stationary and mobile phases were used to optimize the separation efficiency of the chromatographic method. The optimum chromatographic resolution was achieved by using SPOLAR C18 column and 0.1% OPA:acetonitrile (50:50 v/v) as mobile phase. Furthermore, appropriate selection of chromatographic conditions for the method was accomplished by checking system suitability parameters and they found to be satisfactory.

The optimized methodology was validated and all tested variables met with guidelines of ICH. The method's ability to separate was shown to be linear over concentration range of 10-60 µg/mL, and its correlation coefficient value (R^2 reaching to unity) was noteworthy. The method's exactness was assessed at three levels by considering 40 µg/mL as 100% and multiple injections (n = 6) of the same were loaded in to the chromatographic system to demonstrate precision of the method. The %RSD values in both intra- and inter-day precision studies were noted justifiable (< 2.0). The perceptivity of the optimized method was manifested through low LOD (0.37 µg/mL) and LOQ (1.12 µg/mL) values.

The chromatograms for each the analyte, placebo and blank were recorded in order to assess the method's particularity. The results showed no interference from formulation excipients or degradation products. The approach was attempted by doing minor adjustments in the mobile phase solvent ratio, mobile phase flow rate, and column temperature. However, it was found that the chromatographic separation was unaffected by any of these modifications.

In a study on forced-degradation, more favipiravir degradation in acid was discovered (6.32%), than in alkali, oxidative, thermal and UV-degradation. This method was chosen specifically to ascertain the amount of favipiravir present in the commercial formulation. The results verified that this approach is appropriate even for the tested commercial formulation. In comparison to previous findings, the RT of favipiravir using the established method was discovered to be short (2.613 min) and with remarkably low LOD and LOQ values[6,12-14,17]. These findings provide more proof that the approach under investigation is preferred for the regular examination of favipiravir in the pharmaceutical industry.

CONCLUSION

The chromatographic method established for the quantification of favipiravir was found to be straightforward, quick, and sensitive. When compared to published HPLC techniques, the approach demonstrated a respectable linearity range (10-60 µg/mL) without sacrificing sensitivity (low LOD and LOQ values) or RT (2.613 minute). Stability testing and validation analysis helped to expand the method's scope. With these benefits, the contemplated method can be used to analyze the quality control of favipiravir in various dosage forms.

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Conflict of interest

The authors disclose no competing interests.

REFERENCES

1. Yousuke, F.; Brian B. G.; Kazumi, T.; Kimiyasu, S.; Donald, F. S.; Dale, L. B.; Favipiravir (T-705), a novel viral RNA polymerase inhibitor., *Antiviral Research*., **2013**, *100*(2), 446-454. Doi: https://doi.org/10.1016/j.antiviral.2013.09.015
2. Favipiravir. https://go.drugbank.com/drugs/DB12466. Accessed 24 April **2024**.
3. Furuta, Y.; Komeno, T.; Nakamura, T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proceedings of the Japan Academy., *Series B Physical and Biological Sciences*., **2017**, *93*(7), 449-463. Doi: 10.2183/pjab.93.027
4. Umang, A.; Reyma, R.; Zarir, F. U. Favipiravir: A new and emerging antiviral option in COVID-19., *Medical Journal, Armed Forces India*., **2020**, *76*(4), 370–376. Doi: https://doi.org/10.1016/j.mjafi.2020.08.004
5. Sayyed Nazifa, S. A.; Lajporiya, M.; Manjra, M.; Patel, S.; Aejaz, A.; Khan, G. J. Analytical method development and validation and forced degradation stability-indicating studies of Favipiravir by RP-HPLC and UV in bulk and pharmaceutical dosage form., *Journal of Pharmaceutical Research International*., **2021**, *33*(48B), 254-271. Doi: 10.9734/JPRI/2021/v33i48B332833

6. Nandeesha I.; Kailash, S. C.; Basappa, C. Y.; Manjunatha D. H. New analytical methods for the determination of new anti-viral drug Favipiravir: A potential therapeutic drug against Covid-19 virus, in bulk and dosage forms., *Pharmaceutical Chemistry Journal*., **2023**, *56*, 1419–1425. Doi: 10.1007/s11094-023-02807-2
7. Jeevana Jyothi, B.; Venkata Kavya, R. Ultraviolet spectrophotometric method development for estimation of new antiviral repurposing drug favipiravir., *Asian Journal of Pharmaceutical and Clinical Research*., **2021**, *14*(7), 67-69. Jeevana Jyothi, B., Venkata Kavya, R. Ultraviolet spectrophotometric method development for estimation of new antiviral repurposing drug favipiravir., *Asian Journal of Pharmaceutical and Clinical Research*., **2021**, *14*(7), 67-69. Doi: 10.22159/ajpcr.2021.v14i7.41966
8. Santosh V. G.; Hanumant A. B. Development and validation of UV-spectrophotometric mehod for estimation of favipiravir., *International Journal of Pharmacy and Pharmaceutical Research*., **2022**, *24*(4), 172-179.
9. Megahed, S. M.; Habib, A. A.; Hammad, S. F.; Kamal, A.H. Experimental design approach for development of spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: application to spiked human plasma., *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*., **2021**, *249*, 119241. Doi: https://doi.org/10.1016/j.saa.2020.119241
10. Mikhail, I.E.; Elmansi, H.; Belal, F.; Ehab Ibrahim, A. Green micellar solvent-free HPLC and spectrofluorimetric determination of favipiravir as one of COVID-19 antiviral regimens., *Microchemical Journal*., **2021**, *165*, 106189. Doi: 10.1016/j.microc.2021.106189
11. Inas, A. A.; Sherin, F. H.; Alaa, B.; Fotouh, R. M. Menthol-assisted homogenous liquid-liquid microextraction for HPLC/UV determination of favipiravir as an antiviral for COVID-19 in human plasma., *Journal of Chromatography B*., **2022**, *1189*, 123087.Doi:https://doi.org/10.1016/j.jchromb.2021.123087
12. Hoda, M. M.; Mamdouh, R. R.; Amira, S. G.; Ahmed, M. A. A novel stability-indicating HPLC-DAD method for determination of favipiravir, a potential antiviral drug for COVID-19 treatment: application to degradation kinetic studies and *in-vitro* dissolution profiling., *Microchemical Journal*., **2022**, *172*, 106917. Doi: 10.1016/j.microc.2021.106917
13. Ibrahim, B. HPLC-UV proposed Method for quantification of favipiravir in pharmaceutical formulations., *Acta Chraomatographica.,* **2020**, *33*(3), 209–215. Doi: https://doi.org/10.1556/1326.2020.00828
14. Shyamala.; Ashok.; D. Development of forced degradation studies of favipiravir by RP-HPLC., *Journal of Medical Pharmaceutical and Allied Sciences*., **2021**, *10*(6), 3823-3826. Doi: 10.22270/jmpas.2021.V10I6.1493
15. Aishwarya Balu, P.; Sonali Paresh.; M. Stability indicating RP HPLC method development for estimation of favipiravir in bulk and pharmaceutical dosage form., *World Journal of Pharmaceutical Research*., **2021**, *10*(14), 1444-1465.
16. Mohammad, H.; Israa, A.; Mohammed, H.; Zainab, Z.; Wael, A. D. Development and validation of a method for quantification of Favipiravir as COVID-19 management in spiked human plasma., *Molecules*., **2021**, *26*, 3789. Doi: https://doi.org/10.3390/molecules26133789.
17. Suleyman, G.; Ay en H.; Ibrahim, B. Development and validation of UPLC-MS/MS method for obtaining favipiravir tablet dosage form and evaluation of its behaviour under forced conditions., *Journal of Pharmaceutical Research International*., **2021**, *33*(56A), 130-140.
18. Sharma, B. K. Instrumental Methods of Chemical Analysis, 26th Edition, Goel Publishing House, Meerut., **2007**.
19. ICH Harmonized Tripartite Guideline. Validation of analytical procedures: text and methodology Q2 (R1). International Conference on Harmonization, Geneva., **2005.**