IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2022 Vol.:26, Issue:1

Analytical Method Development and Validation of Favipiravir in Bulk and Tablet Formulation

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Pritee R. Atkar*, Mirza Shaihed Baig

Y. B. Chavan College of Pharmacy, Aurangabad. India. 431003

Submitted: 20 November 2022
Accepted: 26 November 2022
Published: 30 December 2022

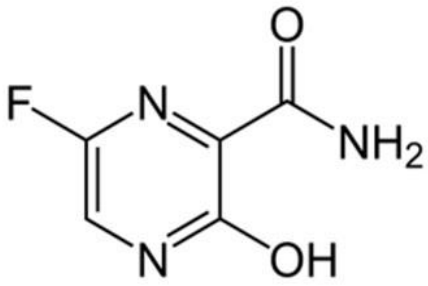
Keywords: Favipiravir, Accuracy, Precision, LOD, Validation.

ABSTRACT

Favipiravir is an antiviral that is active against many RNA viruses, & also used in COVID-19. A simple, fast & reliable UV-spectroscopic method was developed for the estimation of Favipiravir in a bulk and pharmaceutical dosage form. It is a pyrazine carboxamide derivative. The solution of standard & sample was prepared in ethanol. The λmax was found to be 323 nm. Pure drug concentration were prepared in the range of 100µg/ml and the linear regression analysis data showed a good linear relationship with an R2 value of 0.9994. The calibration graphs constructed at their wavelength of determination were found to be linear for UV and derivative spectrophotometric methods. The developed method was validated as per ICH guidelines by concerning accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), & ruggedness. The LOD, LOQ & % RSD indicate that the developed method is suitable for the analysis of commercial samples.

INTRODUCTION

Favipiravir, is one among potential drugs that may possibly be used in the management of COVID-19 infection. It is a pyrazine carboxamide derivative, that showed effective antiviral activity against a variety of RNA viruses including influenza A virus, adenovirus, and SARS Corona virus [1]. Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) is a pyrazine analog. The mechanism of action is linked to transcription inhibition and viral gene replication which finally prevents the synthesis of viral RNA inside infected cells. [2] Literature search reveals that there are fewer method reported for the determination and quantification of Favipiravir and there is less research work done on this antiviral drug. No official pharmacopoeial method found for this particular antiviral drug. In the view of this criterion, the main aim and objective of this study is to develop and validate a sensitive method for the estimation of Favipiravir with applicability of Favipiravir in routine analysis [3].



The chemical structure of Favipiravir [4]

The present work was carried out with the view of establishing a simple, rapid, accurate, economic, precise, and robust UV method for the estimation of favipiravir in bulk and capsule dosage form, using methanol as the solvent. [2]

MATERIALS AND METHODS

Chemicals and Equipment

The pharmaceutical grade of Favipiravir was obtained from Swapnroop Reaserch Private Limited, Aurangabad. The ethanol is used as solvent. The tablet formulation of Favipiravir was bought from local pharmacy. Shimadzu UV 1800 double beam UV-visible spectrophotometer was used for UV analysis and for weighing purpose digital weighing balance was used.

Preparation of Standard solution

Stock solution of the drug 100 µg/ml is prepared by dissolving 5 mg each drug in 50 ml volumetric flask and the volume is make up by ethanol.

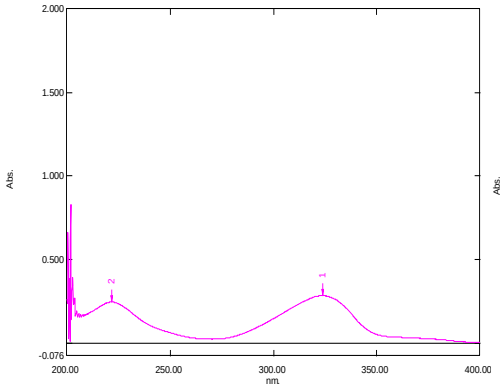
Selection of Analytical Wavelength

By appropriate dilution of standard solution of FVP 2µg/ml of each was prepared and scanned. Following peak were observed.

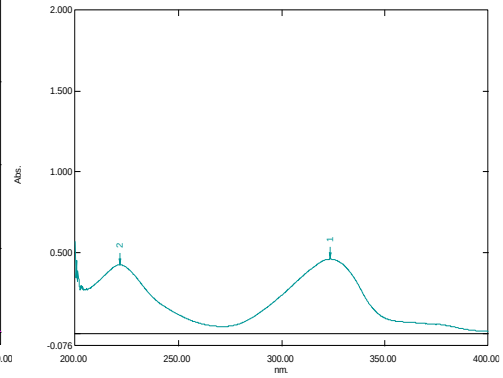
Drug	**Lamda Max At Zero Order**
FVP	323.08nm

Zero order derivative spectroscopy

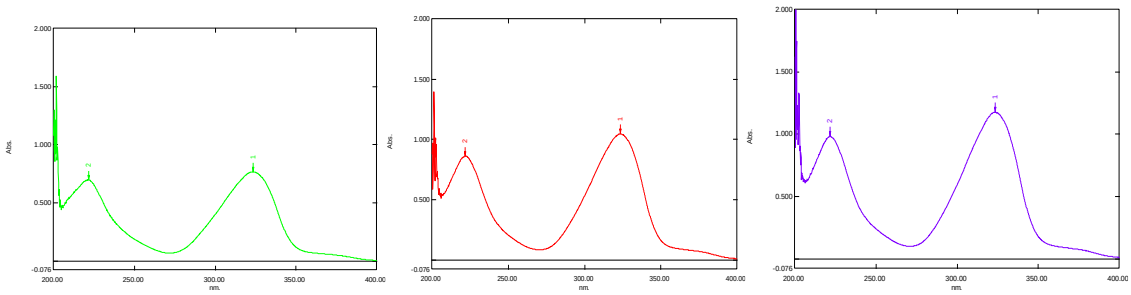
The derivative process provides two general advantages: first, an effective enhancement of resolution, which can be useful to separate two or more components with overlapping spectra; second, discrimination in favor of the sharpest features of a spectrum, used to eliminate interferences by broad band constituents. Five dilutions of FVP tab dilutions are prepared and there spectra are taken which are as follows:



0.5µg/ml

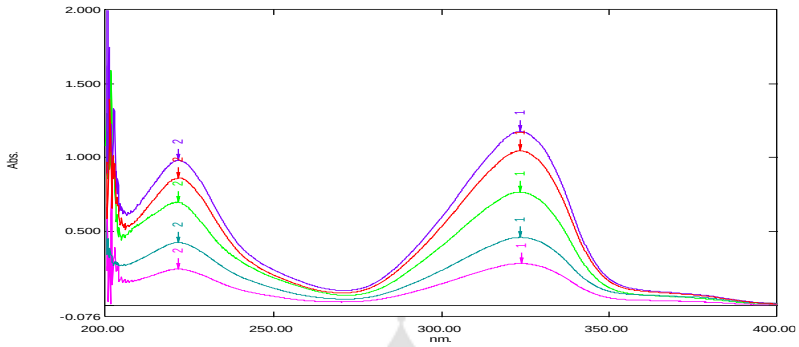


1µg/ml



1.5 µg/ml 2µg/ml 2.5µg/ml

Overlain of zero order spectroscopy spectra



First order derivative spectroscopy

The derivative spectrophotometric method is one of the advanced modern spectrophotometric techniques that offer a useful means for extracting both qualitative and quantitative information from the spectra composed of overlapped bands.

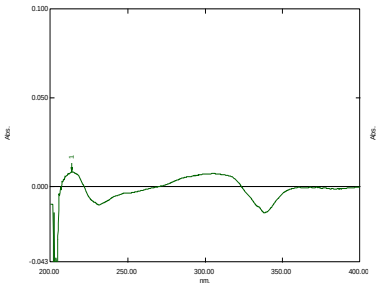
Solvent Used: Ethanol

Stock Solution:

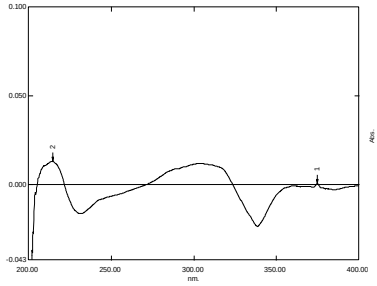
Stock solution of the drug 100 µg/ml is prepared by dissolving 5 mg each drug in 50 ml volumetric flask and the volume is make up by ethanol.

Selection of Analytical Wavelength

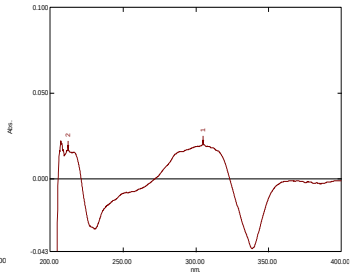
By appropriate dilution of standard solution of FVP 2µg/ml of each were prepared and scanned. The λmax was found at 323nm. Five diutions are prepared and there spectra are taken which are as follows-



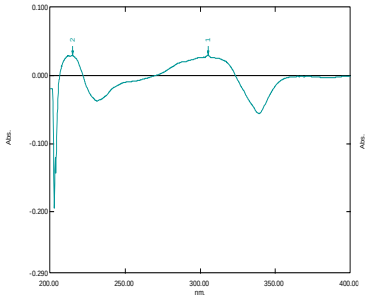
0.5µg/ml



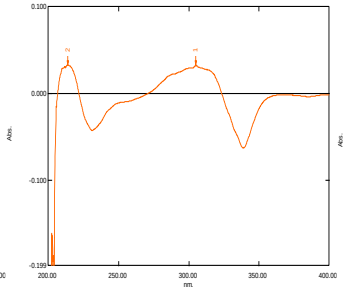
1µg/ml



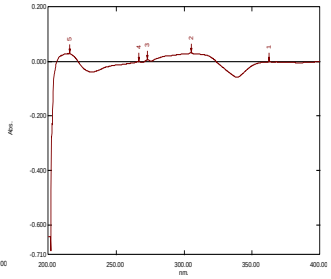
1.5µg/ml



2µg/ml

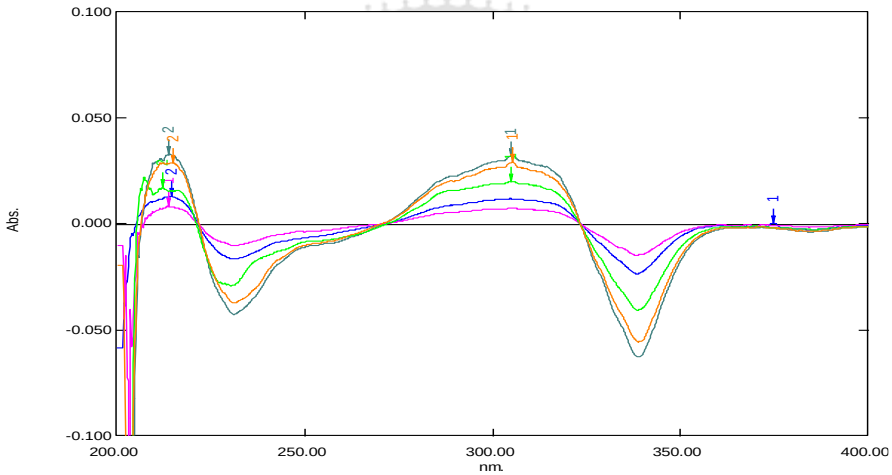


2.5µg/ml



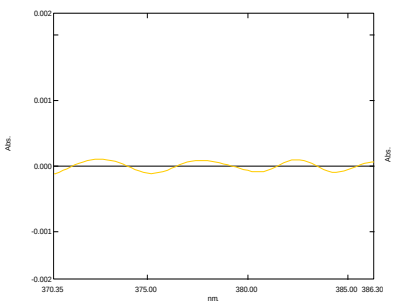
2µg/ml FVP tab

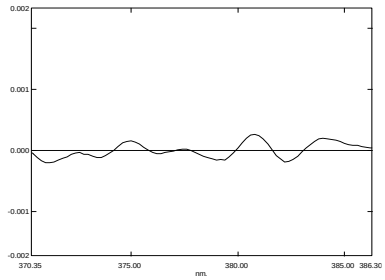
Overlain of first order spectroscopy spectra

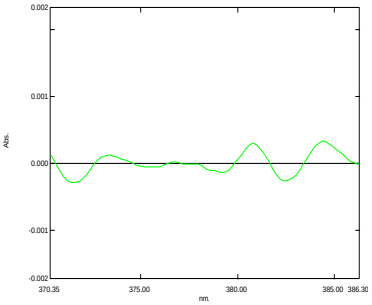


Second order derivative spectroscopy:

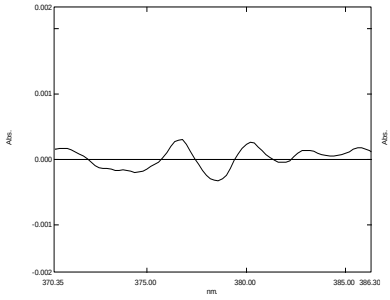
Second derivative spectroscopy is a technique which enhances the separation of overlapping peaks. The second derivative spectroscopy method requires the use of a UV/Visible scanning spectrophotometer. Five dilutions are prepared and there spectra are taken which are as follows-

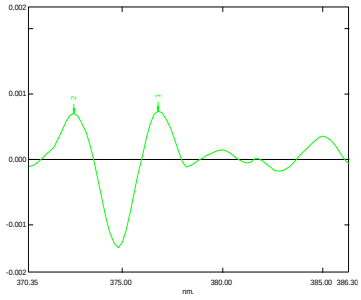






0.5µg/ml 1µg/ml 1.5µg/ml

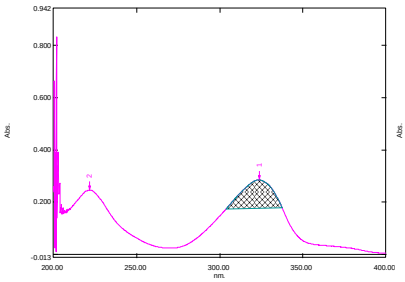


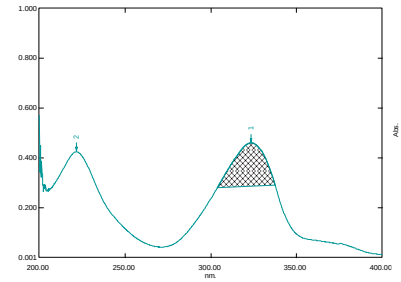


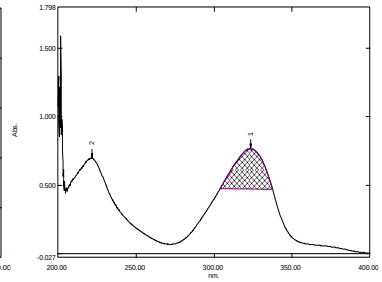
2µg/ml 2.5µg/ml

Area undercurve method

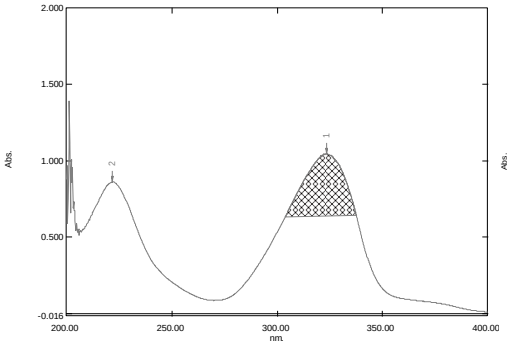
The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area should be calculated. Five dilutions & FVP tab dilutions are prepared and there spectra are taken which are as follows-



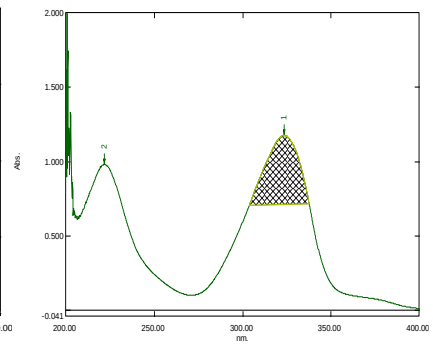




0.5µg/ml 1µg/ml 1.5µg/ml



2µg/ml



2.5µg/ml

Method validation

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. It is one of the most critical parameter in method validation. It confirms the suitability of method to the greatest extent and hence method developer must design suitable extraction procedure to assure accurate quantification of analyte in presence of sample matrix.

Precision

Precision measures of how close individual measurements are to each other. It is the term used to see how reliable and consistent the measurement of the experiment is. Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It is measured by injecting a series of standards or analyzing series of samples from multiple samplings from a homogeneous lot.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity is measuring the linear response of the method. Linearity studies are important because they define the range of the method within which the results are obtained accurately and precisely.

Limit of detection (LOD) and limit of quantification (LOQ)

Calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels.

Limit of detection (Lod) = 3.3*standard deviation/ slope

Limit of quantification (Loq) = 10* standard deviation/slope

Ruggedness

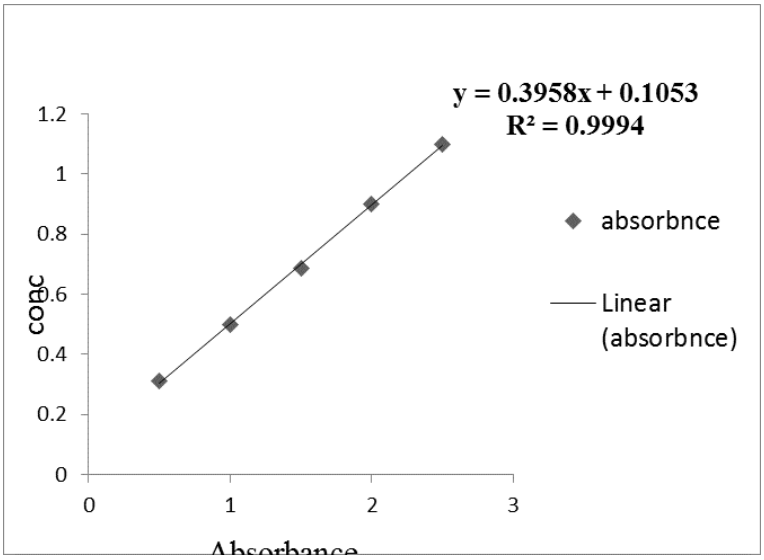
Ruggedness is normally expressed as the lack of influence on the test results of operational and environmental variables of the analytical method. It is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory & from analyst to analyst. The purpose of a ruggedness test is to find the factors that strongly influence measurement results, and to determine how closely one needs to control these factors.

RESULTS AND DISCUSSION

A calibration graph of concentration versus absorbance was plotted. The drug followed the Beers Lamberts law in the concentration range of 0.5-2.5µg/ml. Favipiravir gives λmax at 323nm. The linearity graphs for spectroscopic methods are as shown in following graphs, the sensitivity of the method was assessed by determining LOD & LOQ which is found to be 0.3625 and 1.099µg/ml. Accuracy is performed with level of percent recovery 80,100 & 120.

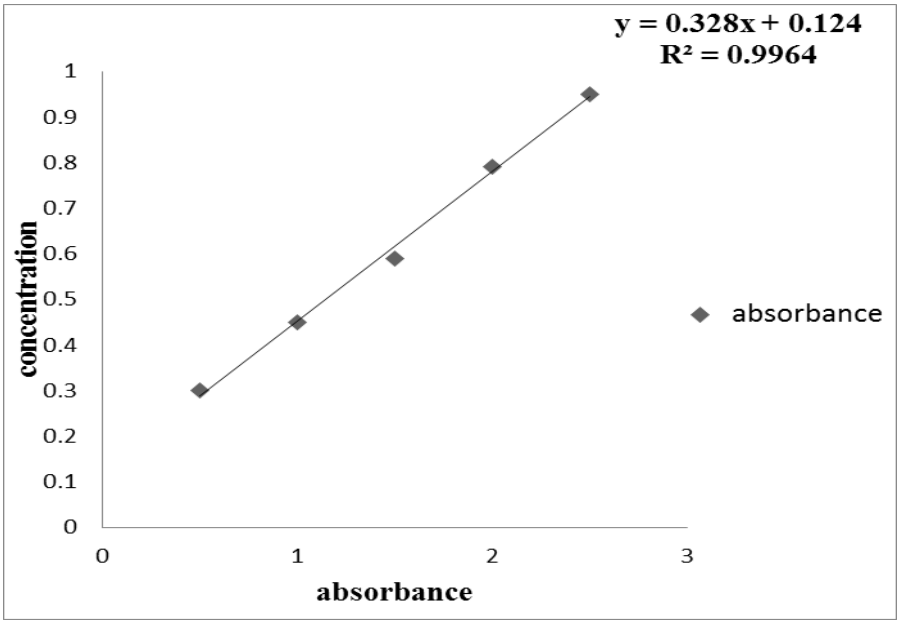
Table No. 1: Results of Linearity

Concentration	Absorbance
0.5	0.311
1	0.498
1.5	0.687
2	0.899
2.5	1.1
Regg. Equ.	y=0.395+0.105
R2	0.999



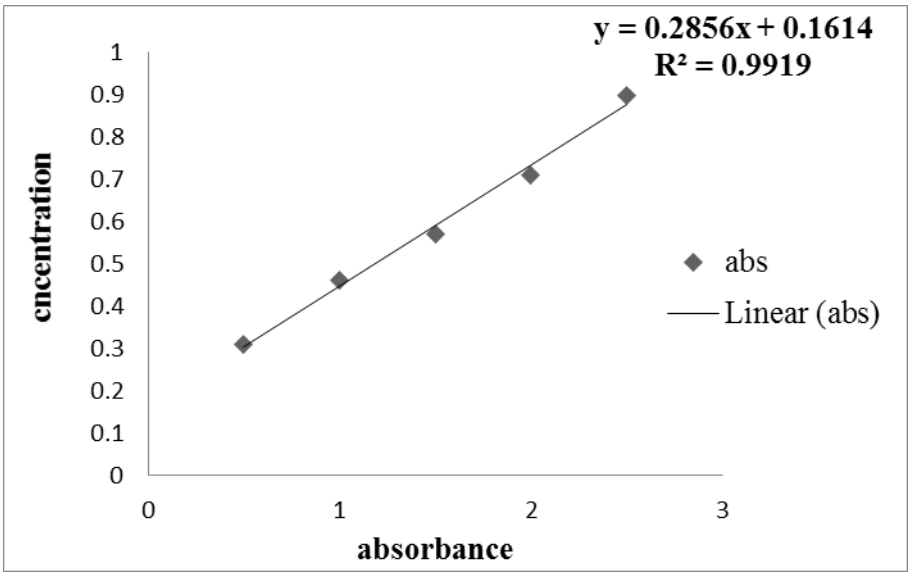
ZERO ORDER

Concentration	Absorbance
0.5	0.3
1	0.45
1.5	0.59
2	0.79
2.5	0.95
Regg. Equ.	y=0.328x+0.124
R2	0.996



FIRST ORDER

Concentration	Absorbance
0.5	0.31
1	0.46
1.5	0.57
2	0.71
2.5	0.899
Regg. Equ.	y=0285x+0.161
R2	0.991



SECOND ORDER

	Zero order			First order			Second order		
Level of percent recovery	**80**	**100**	**120**	**80**	**100**	**120**	**80**	**100**	**120**
Amount present (Label claim)	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**
Amount of standard added (mg)	**160**	**200**	**240**	**160**	**200**	**240**	**160**	**200**	**240**
Total amount recovered	**359.1**	**400.3**	**438.2**	**358.2**	**399.1**	**438.5**	**359.7**	**400.2**	**439.6**
% recovery	**99.43**	**100.15**	**99.25**	**98.87**	**99.55**	**99.37**	**99.81**	**100.1**	**99.83**
% mean	**99.61**			**99.26**			**99.91**		

Table No. 2: Result of Accuracy

	Intraday		
Sr no.	**Concentration (µg/ml)**	**Time**	**% recovery**
1	**2**	**Morning**	**99.43**
2	**2**	**Afternoon**	**98.87**
3	**2**	**Evening**	**99.25**

Table No. 3: Result of Precision

	Interday		
Sr no.	**Concentration (µg/ml)**	**Day**	**% recovery**
1	2	Day 1	99.10
2	2	Day 2	98.88
3	2	Day 3	98.59

Table No. 4: Result of Ruggedness

Sr no.	Concentration	Analyst 1 % recovery	Analyst 2 % recovery
1	2	99.87	99.44
2	2	99.25	99.84
3	2	99.89	99.51
4	2	99.79	99.20
5	2	99.58	99.37

CONCLUSION

No derivative spectroscopic methods have been described for the determination of Favipiravir. Therefore simple, fast, and reliable derivative spectroscopic methods were developed for the Favipiravir. The developed method can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulations.

REFERENCES

1. Wrushali A. Panchale 1, Sonam B. Bisen 2, Jagdish V. Manwar 2, *, Ravindra L. Bakal 1, Tejaswini V. Tidke 1, Mitali N. Paithankar 1 and Aparna G. Vakhare 1 Development of visible spectrophotometric methods for the analysis of favipiravir in pure drug and tablet formulation, 2022.

2. Santosh V. Gandhi*, Hanmant A. Bade Development and validation of UV-spectrscopic method for Estimation of Favipiravir, July 2022.

3. Prof. Ramarao Nadendla1* and Dr. Abhinandana Patchala, A Validated high Performance Liquid Chromatographic Method for the Quantification of Favipiravir by PDA Detector, March 2021.

4. Alla V. Yegorovaa*, Yulia V. Scrypynetsa, Georgy V. Maltsevb, Inna I. Leonenkoa, Valery P. Antonovicha, Sergey N. Kashutskyyb, Olga D. Voitiukb, Spectroscopic Studies on the Interaction Between Novel Antiviral Drug Favipiravir and Serum Albumins, 2020.

5. Yousuke FURUTA,*1,† Takashi KOMENO*2 and Takaaki NAKAMURA, Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase, 2017.

6. Monographs on Favipiravir and on Favipiravir tablets International Meeting of World Pharmacopoeias (IMWP).

7. Kiyan Kiyanmehr 1, Gholamreza Moussavi 1*, Samira Mohammadi 1, Kazem Naddafi 2, Stefanos Giannakis 3 "The efficacy of the VUV/O3 process run in a continuous-flow fluidized bed reactor for simultaneous elimination of Favipiravir and bacteria in aqueous matrices".

8. GuangyiWang,*, † Jinqiao Wan,‡ Yujian Hu,‡ Xiangyang Wu,‡ MarijaPrhavc,† Natalia Dyatkina, 'Synthesis and Anti-Influenza Activity of Pyridine, Pyridazine, and Pyrimidine C-Nucleosides as Favipiravir (T-705) Analogues ''.

9. Alla V. Yegorovaa*, Yulia V. Scrypynetsa, Georgy V. Maltsevb, Inna I. Leonenkoa, “Spectroscopic Studies on the Interaction Between Novel Antiviral Drug Favipiravir and Serum Albumins ” 2020.
10. Sonu A. Varma and 1 Priyanka Yadav “stability indicating hplc method development and validation for estimation of favipiravir in pharmaceutical dosage form” 2021.
11. Ibhahim Bulduk Department of Chemistry, School of Health, Us¸ ak University, 64200, Us¸ ak, Turkey Department of Chemistry, School of Health, Us¸ ak University, 64200, Us¸ ak, Turkey “HPLC-UV method for quantification of favipiravir in pharmaceutical formulations” August 31, 2020.
12. Duygu Eryavuz Onmaza,*, Sedat Abusoglu a, Mustafa Onmaz b, Fatma Humeyra Yerlikaya a,“Development and validation of a sensitive, fast and simple LC-MS / MS method for the quantitation of favipiravir in human serum” 2020.
13. Yasmine Ahmed Sharaf 1,†, Sami El Deeb 2,3,* , Adel Ehab Ibrahim 3,4,†, Ahmed Al-Harrasi 3 “Two Green Micellar HPLC and Mathematically Assisted UV Spectroscopic Methods for the Simultaneous Determination of Molnupiravir and Favipiravir as a Novel Combined COVID-19Antiviral Regimen” 2022.
14. Lieve Naesens, Luke W. Guddat, Dianne T. Keough, André B. P. van Kuilenburg, Role of Human Hypoxanthine Guanine Phosphoribosyl transferase in Activation of the Antiviral Agent T-705 (Favipiravir).
15. Selin Seda TİMUR 1, Meltem ATAŞOĞLU 2, Yalçın ÖNER 2, ‘Tutku Ceren KARABULUT 2, Hakan EROĞLU 1 *In vitro* studies for BCS classification of an antiviral agent, 26 August 2021.
16. Jeevana jyothi b*, venkata kavya r ultraviolet spectrophotometric method development for estimation of new antiviral repurposing drug favipiravir’ 28 may 2021.
17. Yasmine Ahmed Sharaf 1,†, Sami El Deeb 2,3,* , Adel Ehab Ibrahim 3,4,†, Ahmed Al-Harrasi 3 “Two Green Micellar HPLC and Mathematically Assisted UV Spectroscopic Methods for the Simultaneous Determination of Molnupiravir and Favipiravir as a Novel Combined COVID-19 Antiviral Regimen” 2022.
18. Ioannis Skarmoutsos 1, *, Guillaume Maurin 2, Elvira Guardia 3, Jannis Samios Hydration structure and dynamics of the favipiravir antiviral drug: A molecular modelling approach.