Available online on 15.03.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Fluorimetric Determination of Antiviral (COVID-19) Drug Favipiravir in Bulk and Pharmaceutical Dosage Forms

K. Bhavya Sri*[1], Md. Saba Fatima[2], Shaheen Banu[3], M. Sumakanth[4]

1 Department of Pharmaceutical Analysis, HOD of Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India

2 Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India

3 Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India

4 Department of Pharmaceutical Chemistry, Principal at RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India

Article Info:

Article History:

Received 11 Jan 2023
Reviewed 26 Feb 2023
Accepted 07 March 2023
Published 15 March 2023

Cite this article as:

Bhavya Sri K, Fatima MS, Banu S, Sumakanth M, Fluorimetric Determination of Antiviral (COVID-19) Drug Favipiravir in Bulk and Pharmaceutical Dosage Forms, Journal of Drug Delivery and Therapeutics. 2023; 13(3):110-112

DOI: http://dx.doi.org/10.22270/jddt.v13i3.5770

*Address for Correspondence:

K. Bhavya Sri, Department of Pharmaceutical Analysis, HOD of Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Hyderabad, Telangana, India

Abstract

Favipiravir is a synthetic prodrug, which was first discovered while assessing the antiviral activity of chemical agents active against the influenza virus in the chemical library of Toyoma chemicals. It works by inhibiting RNA dependant RNA polymerase (RdRP), an enzyme required for RNA viral replication inside human cells. A simple, rapid, and economic method was developed for the quantitative determination of Favipiravirusing spectrofluorometer. The Favipiravir standard drug solution and sample tablet solution was prepared using double distilled water as a diluent. The different concentrations ofpure drugin the range 2-10 µg/ml and one sample solution were measured for the intensity at 432nm in the spectrofluorometer. The calibration curve was plotted and the sample's unknown concentration was calculated from the plot. The calibration curve was found to be linear with r^2 value obtained as 0.99.There are various other methods available for the quantification of Favipiravir which include RP-HPLC, UV-spectroscopic methods, FTIR, LC-MS with different extraction methods spiked in human plasma but not by using spectrofluorometer. Favipiravir shows fluorescence when dissolved in appropriate solvent hencethis method was developed to quantify Favipiravir which is a simple and efficient method. This method developed is easy and can be used for routine quality control test for Favipiravir pharmaceutical formulations.

Keywords: Favipiravir, spectrofluorometer, Calibration curve.

INTRODUCTION:

Favipiravir (FAV) is an antiviral drug which was approved in Japan in 2014 for use in the outbreak of influenza viral infections, where use of other antiviral drugswasinsufficiently effective in the treatment of influenza[1,2]. FAV was also used in the treatment ofEbola virus infections and for the post exposure prophylaxis[3]. When the pandemic hit the world inDecember 2019, there was no proper and approved specific drug to treat SARS-Cov-2. Few of the already approved antiviral drugs which have the safety data were tested for the activity against the covid-19 infection. The class of drugs which were preferably selected was the RNA dependent RNA polymerase (RdRP) inhibitors. One of the drugs belonging to that class is Favipiravir, which was then started to use to treat the Coronavirus infected patients.

Favipiravir chemically known as 6-fluoro-3-oxo-3,4-dihydro pyrazine 2-carboxamide as shown in Figure 1,acts by selectively inhibiting RNA dependant RNA polymerase. It is an enzyme which is required for the replication of RNA virus in the cells of humans[4]. It actsas a substrate for the RNA-dependent RNA-polymerase (RdRp) enzyme, which is mistaken by the enzyme as a purine nucleotide, thus inhibiting its activity leading to termination of viral protein synthesis[5]. Single molecule of FAV is enough to terminatethe further extension of viral RNA[6].

The reported methods for analysis of FAV include HPLC method with UV detection and UV-visible spectroscopic method[7,8]. There are no reported spectrofluorometric methods has been developed for quantitation of FAV neither in pure form nor in pharmaceutical preparation with the use of simple solvent as a diluent.Favipiravir shows fluorescence activity when dissolved in appropriate solvent. The solutions were measured at 432 nm after excitation at 361 nm.

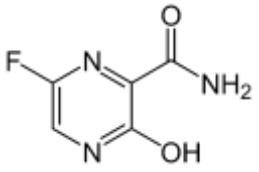

Figure 1: Chemical structure of Favipiravir.

MATERIALS AND METHODS:

Apparatus: Volumetric flasks, Pipette, Digital weighing balance.

Instrument: Spectrofluorometer.

Reagents: Favipiravir pure drug was a gift from pharmaceutical industry, Fabiflu (Favipiravir tablet-200mg).

Standard stock solution: Accurately weighed 10mg of Favipiravir pure drug was transferred into 10mL Volumetric flask and dissolved in Double distilled water. Thevolumetric flask was filled with the same up to the mark. This standard stock has aconcentration of 1000µg/mL.

Working Standard solution: From the standard stock solution,1mL solution was pipetted out and transferred in 10mL volumetric flaskand filled up to the mark with Double distilled water. This working standard has a concentration of 100µg/mL.

Standard Dilutions: From the working standard solution, standard dilutions were made from concentrations 2µg/mL to 10µg/mL using 10mL volumetric flasks. The absorbance of these standard dilutionswas checked in the fluorimeter. The readings were noted and a calibration curve was plotted.

Tablet analysis: 10 Fabiflutablets were accurately weighed and average weight was calculated. The equivalent weight of 10 mg was accurately weighed and dissolved in double distilled water and filled with the same up to the mark. From this solution a sample solutionwas prepared and checked for the absorbance value. From the calibration curve the actual concentration was calculated.

RESULTSAND DISCUSSION:

Figure 2 represents the calibration curve data of the Fluorescence activity of Favipiravir pure drug using double distilled water as solvent. The regression coefficient r^2 value was calculated to be 0.99 which is within limits. Table 1 shows the concentration and intensity data of the Favipiravir pure drug and that of sample. From the table, the concentration of Favipiravir in the Fabiflu tablet (sample) solution was found to be 4.89 µg/mL for a sample made with theoretical concemtration of 5 µg/mL.

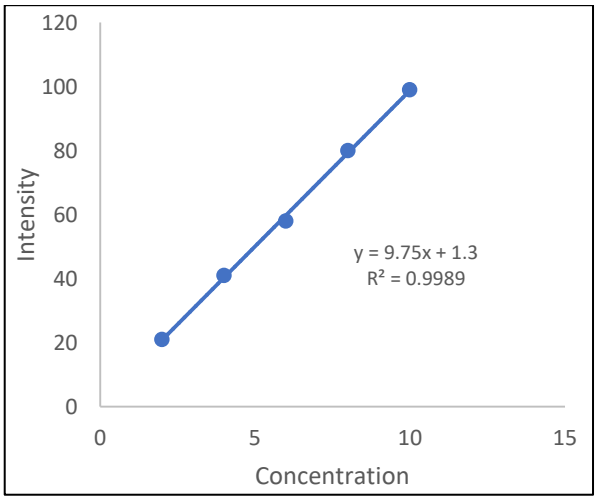

Figure 2: Calibration curve of Favipiravir.

Table 1: Calibration curve data of Favipiravir

Concentration	Intensity
2	21
4	41
6	58
8	80
10	99
Unknown sample	49
Concentration of unknown sample	4.893558895

The other methods available for the analysis and quantification of Favipiravir include HPLC using UV detection[7], HPLC using DAD detection[9],HPLC using bioanalytical samples (drug spiked in human plasma) using different extraction methods[10-12]. Addition to these, analysis of favipiravir was done by developing method and validating it using FTIR[13], NMR/LC-MS and invitro safety evaluation[14]. Comparative studies of method developed for analyzing Favipiravir in Pharmaceutical preparations using HPLC and UV were also found[15]. Concurrent Fluorescence based analysis of remdesivir and favipiravir[16], Spectrofluorimetric analysis of Favipiravir, remdesivir and hydroxychloroquine spiked in human plasma[17]are the two methods found for analysis of Favipiravir along with other drugs using Fluorescence. Since there were no reported methods found for individual analysis of Favipiravir using fluorescence, the present method was developed.

CONCLUSION:

The present method developed for the estimation of Favipiravir using spectrofluorometer is easy, rapid, simple without use of complex process or instrumentation. The method developed is according to ICH guidelines and results obtained fall under limits of the parameters set by the guidelines. This method developed can be used as a routine quality control test for Favipiravir pharmaceutical preparations.

ACKNOWLEDGEMENT:

We are thankful to our Principal, Prof. M. Sumakanth of RBVRR Women's College of Pharmacy for giving us this opportunity to perform this research work.

REFERENCES:

1. Furuta Y, Komeno T, et al., Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase, P. Jpn. Acad. B-Phys. 2017; 93:449-463, https://doi.org/10.2183/pjab.93.027

2. Tanaka T, Kamiyama T, et al., T-705 (Favipiravir) suppresses tumor necrosis factor a production in response to influenza virus infection: a beneficial feature of T-705 as an anti-influenza drug, Acta Virol. 2017; 61:48, https://doi.org/10.4149/av_2017_01_48

3. Bai C-Q, Mu J-S, et al., Clinical and virological characteristics of Ebola virus disease patients treated with favipiravir (T-705)-Sierra Leone, Clin. Infect. Dis. 2014; 63(2016):1288-1294, https://doi.org/10.1093/cid/ciw571

4. Megahed SM, Habib AA, Hammad SF et al, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2021; 249. https://doi.org/10.1016/j.saa.2020.119241

5. Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antivir Res. 2013 Nov; 100:446-454. https://doi.org/10.1016/j.antiviral.2013.09.015

6. Jin Z, Smith LK, Rajwanshi VK, Kim B, Deval J. The ambiguous base-pairing and high substrate efficiency of T-705 (favipiravir) ribofuranosyl 50 -triphosphate towards influenza A virus polymerase. PloS One. 2013; 8:e68347. https://doi.org/10.1371/journal.pone.0068347

7. Megahed S, Habib A, Hammad S Kamal A, 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. 2020; 249:119241. https://doi.org/10.1016/j.saa.2020.119241

8. 冯光玲丁文娟邓玉晓赵仁永龚艳艳段崇刚孙晋瑞, 2015, A kind of Favipiravir has the HPLC assay method of related substance, CN104914185B.

9. Taşkın D. Development and Validation of a Rapid HPLC-DAD Method for Determination of Favipiravir in Pharmaceutical Formulation. Clinical and Experimental Health Sciences. 2022; 12(3):648-652. https://doi.org/10.33808/clinexphealthsci.992869

10. Hailat M, Al-Ani I, Hamad M, Zakareia Z, Abu Dayyih W. Development and Validation of a Method for Quantification of Favipiravir as COVID-19 Management in Spiked Human Plasma. Molecules 2021; 26:3789. https://doi.org/10.3390/molecules26133789

11. Abdallah IA, Hammad SF, Bedair A, Elshafeey AH, Mansour FR. Determination of favipiravir in human plasma using homogeneous liquid-liquid microextraction followed by HPLC/UV. Bioanalysis. 2022; 14(4):205-216. Epub 2022 Jan 10. PMID: 35001648 https://doi.org/10.4155/bio-2021-0219

12. Onmaz DE, Abusoglu S, Onmaz M, Yerlikaya FH, Unlu A. Development and validation of a sensitive, fast and simple LC-MS/MS method for the quantitation of favipiravir in human serum. Journal of Chromatography B. 2021 Jun 30; 1176:122768. https://doi.org/10.1016/j.jchromb.2021.122768

13. RAGHAVENDRABABU PNN, PADMAVATHI Y, NEENA G, SUSHMA K, POOJITHA A. "NEW FTIR METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ANALYSIS OF FAVIPIRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS". International Journal of Current Pharmaceutical Research, 2022; 14(5):25-29, doi:10.22159/ijcpr.2022v14i5.2022.

https://doi.org/10.22159/ijcpr.2022v14i5.2022

14. Abdallah, I.A., El-Behairy, M.F., Ahmed, R.M. et al. The anti-COVID-19 drug Favipiravir: Degradation, Method development, Validation, NMR/LC-MS characterization, and In-vitro safety evaluation. Chem. Pap. 2022; 76:6415-6426. https://doi.org/10.1007/s11696-022-02327-5

15. Bulduk İ. Comparison of HPLC and UV Spectrophotometric Methods for Quantification of Favipiravir in Pharmaceutical Formulations. Iran J Pharm Res. 2021; 20(3):57-65. doi: 10.22037/ijpr.2020.114199.14725. PMID: 34903969; PMCID: PMC8653657.

16. El-Awady, M., Elmansi, H., Belal, F. et al. Insights on the Quantitative Concurrent Fluorescence-Based Analysis of Anti-COVID-19 Drugs Remdesivir and Favipiravir. J Fluoresc 2022; 32:1941-1948. https://doi.org/10.1007/s10895-022-02998-z

17. Ramzy S, Abdelazim AH, Osman AOE, Hasan MA, Spectrofluorimetric quantitative analysis of favipiravir, remdesivir and hydroxychloroquine in spiked human plasma, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Volume 2022; 281:121625, ISSN 1386-1425, https://doi.org/10.1016/j.saa.2022.121625