ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

FAVIPIRAVIR-ANALYTICAL METHODS

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Favipiravir-Analytical methods

High performance liquid chromatography (HPLC) and spectrofluorimetric methods have been developed for quality control of favipiravir in pharmaceutical formulations.

Abstract:

Favipiravir a pyrazine analogue its shows antiviral activity of viruses. The separation was carried out by C18 column. The mixture of potassium dihydrogen phosphate (mobile phase A) and acetonitrile (mobile phase B) for flowing phase. In HPLC column temperature was 30° and ultraviolet detection was 323nm. The column run time was 15min under chromatographic conditions. Excellent linear relationship between peak area and favipiravir concentrations has been observed. Developed HPLC method found to be sensitive, precise, accurate, specific and robust. Proposed method has successfully applied for quantification of favipiravir in pharmaceutical formulations.

Spectrofluorimetric method for determination of favipiravir. Different factors effecting fluorescence were carefully studied. The proposed method is based on measuring native fluorescence of favipiravir IN 0.2 M borate buffer at 432 nm after at 361nm. Relationship between favipiravir concentration and relative fluorescence intensity. The method was successfully implemented for determination of favipiravir in its pharmaceutical formulation.

Key words:

Favipiravir, antiviral, HPLC method, spectrofluorimetric method.

Introduction:

Favipiravir is an antiviral drug that used treat all the viral infections and that was initially developed for influenza by Toyama chemical. Chinese-borne corona virus disease spread rapidly so the active therapeutic alternatives are urgently needed for the treatment of corona virus. Many medications such as hydroxychloroquine, remdesivir and favipiravir are currently undergoes for clinical trials.

Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide) selectively inhibits the RNA polymerase of RNA viruses, thus preventing viral replication. It shows antiviral activity against alpha, filo, arena and noroviruses.

According to the literature search there are two methods for methods for determining Favipiravir (FVP) assay and impurities in active pharmaceutical ingredients. In both these methods a gradient HPLC mode was used for chromatographic separation and run time was 60 min.
Favipiravir functions as purine analogue and it's incorporated instead of adenine and guanine. The incorporation of single molecule of favipiravir termination of viral RNA. Inside the cell, favipiravir is converted into its active phosphorylated form and is then recognized as substrate by viral RDRP. It shows a broad spectrum of activity against different RNA viruses including influenza virus. In influenza the beneficial effect has attributed to decline in pulmonary viral load and TNF-alpha levels in airways. Favipiravir used for treatment of prophylaxis and Ebola virus infections.

HPLC - Method:

Chemicals:

Potassium dihydrogen phosphate, ortho-phosphoric acid and HPLC grade acetonitrile are analytical grade chemicals without purification were used. Deionised water was purified by Millipore without conductivity.

Sample solution:

Ten favipiravir tablets were accurately weighed and transferred to dry and clean mortar then make into fine powder. Then the powder transferred to 250ml volumetric flask and about 100ml deionised water was added to this flask was

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

attached to rotary shaker for 10min for complete disperse of ingredients. This mixture was sonicated for 30 min dilute volume with deionised water to give a solution containing 1000 µg/ml and filtered through 0.45µm filter.

Stock Solution:

One hundred milligram pure drug was accurately weighed, dissolved in about 30ml of deionised water and transferred to a 100ml volumetric flask for the 1 mg/ml of stock solution. Then this solution was sonicated for 30min and filtered through a 0.45µm filter and diluted with deionised water to obtain required concentration of standard solution before injected into system for analysis.

Determination of λ max:

Standard solution was subjected to scanning between 200 and 800nm on a ultraviolet spectrophotometer. λmax was obtained from the ultraviolet spectrum of standard solution.

Chromatographic conditions:

Chromatographic analysis was performed on a column of C18. Potassium dihydrogen phosphate (mobile phase A) and acetonitrile (mobile phase B).The mobile phase was filtered through 0.45 µm membrane filter before use and then pumped at flow rate of 1ml/min. The column should be thermo stated at 30°C. The run time was 15 min under these conditions.

SPECTROFLOUROMETRIC METHOD:

Apparatus:

FP-6300 spectrofluorometer was used for the scanning all the fluorescence spectra. Fluorometer was equipped with xenon lamp and holographic grating monochromaters. pH measurement were made with HANNA pH 211 microprocessor pH meter with double junction glass electrode.

Material and reagents:

Analytical grade chemicals and reagents are used. Favipiravir powder, acetyl trimethyl ammonium bromide, brij-35, sodium deodecyl sulphate, tween-80, carboxymethyl cellulose, boric acid, potassium chloride and sodium acetate, sodium hydroxide hydrochloric acid, acetonitrile, methanol, ethanol and 0.2M acetate buffer.

Stock solution:

Stock solution of favipiravir was prepared in distilled water at a concentration of 400µg/ml by dissolving accurately weighed 10 mg of favipiravir in 25 ml volumetric flask and diluted with water. Stock solution was stored in refrigerator for 3weeks at 4°C.

Standard solution:

Standard solution was prepared by taking 50ml of stock solution and diluting it into 25ml of distilled water in a calibrated 25 ml volumetric flask. The concentration of this solution was 800 mg/ml.

For the construction of calibration curve into 10 ml volumetric flask standard solution and 2.5 ml of 0.2M borate buffer pH 8.0. The solution were diluted to 10ml with water and mixed to yield final concentration in the range of 40-290mg/ml. the solution was measured at 432 nm after excitation at 361 nm relative fluorescence intensity of each solution was plotted against final drug concentration in mg/ml.

Analysis:

The tablet was prepared by mixing 200mg of favipiravir with recipients of povidone, colloidal, silicon dioxide hydroxypropyl cellulose, cross povidone, sodium steryl fumarate, talc, titanium dioxide. Accurate weighed amounts of laboratory synthetic tablet containing 10 mg of favipiravir was transferred into a 25 ml volumetric flask and add distilled water 10ml, and sonicate for 10 min and make the volume with distilled water. The solution was filtered to mesh nylon membrane filter, first portion of filtrate was discarded, and then 50 microliters of filtrate was transferred

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to a volumetric flask make with distilled water. And then take 1.5ml to a 10 ml volumetric flask add 2.5ml of borate buffer and dilute with distilled water to obtain 120ng/ml. Fluorescence intensity was determined at 432nm after excitation at 361 nm. Blank titration was also done and favipiravir concentration was calculated by regression equation.

Applications for HPLC:

The developed method and validated method has been applied successfully for determination of favipiravir in pharmaceutical formulations. The result obtained very closely related to amount indicated on labels of the tablets. This shows the method for content evaluation is useful.

Application of Spectrofluorometer:

This method can also be done by taking the biological fluids (human plasma). One ml of plasma was taken in one ml volumetric flask and spiked with favipiravir solution so that the concentration in plasma lies within the range of 6-24 microgram per millilitres. Then, the volume was completed to the mark with methanol to precipitate protein.

The mixture was mixed in vortex direction for two minutes and then centrifuged at 4000rpm for 10min. From this solution 400 microliters was transferred in 10 ml volumetric flask, 2.5 ml of borate buffer added and then distilled water was added to final volume and can be measured at previous nm. The human plasma used in study was approved by institution ethics committee.

Results and discussion:

Determination of λ max:

The wavelength corresponding to maximum absorbance was determined as 323nmfrom ultraviolet spectrum of standard solution.

Method development:

Mobile consists of several buffer systems were tried at the beginning of the study; they could not meet required system parameters. Then only potassium dihydrogen phosphate buffer was tested without using organic modifiers, long analysis times were obtained. Different acetonitrile solution ratio was investigated to obtain optimum conditions. Acetonitrile ratio was determined as 10% against mm potassium dihydrogen phosphate solution due to favipiravir peak being well shaped and asymmetrical using the system. Eventually, potassium dihydrogen phosphate and acetonitrile provides stronger theoretical plates and peak tailing factor and containing mixture of solvents and phosphate buffer, with ionic strength and pH ranges were tested. Best chromatographic conditions were achieved using isocratic mobile phase at flow rate in the C18 column that was kept at 30°C. The analysis was conducted at 30°C, which offers a lot of advantages of good chromatographic peak. However, continuing the analysis after 15 min ill increase both analysis time and cost. Over lapping peaks are not observed.

Effect of experimental parameters:

Favipiravir was found to be exhibit native fluorescence of high intensity at 432nm after excitation at 361nm different parameter affecting native fluorescence of favipiravir such as: diluting solvent, buffer system, effect of surfactants, pH, concentration of buffer, and volume of buffer were studied.

Effect of diluting solvent:

Different diluting solvents as water, ethanol, methanol, and acetone were studied. Water resulted in high fluorescence intensity after considering the blank reading. Water also an advantage regarding greenness of the method. Thus, water was selected as diluting solvent.

Effect of different buffer systems:

Different buffers such as acetate buffer and borate buffer in addition to 0.1N HCL and 0.1N NaOH were studied covering different pH ranges fluorescence intensity of favipiravir was to increase in alkali media.

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Effect of organized media:

Anionic, cationic and non ionic surfactants were investigated separately by adding 2% of 2ml aqueous solution of each surfactant to favipiravir solution. It was found that above surfactants increase the fluorescence intensity.

Conclusion:

The current study was carried out to investigate spectrofluorimetric was carried out to investigate method for rapid quantization of the new antiviral drug; favipiravir that potential therapeutic effect against corona virus. The present work is the spectrofluorimetric approach for assay favipiravir in pharmaceutical formulations. The adopted method is simple, fast, robust and green and can replace the only reported HPLC method for determination of favipiravir in routine analysis. Moreover, the high sensitivity of the proposed method allowed determination of favipiravir spiked human plasma.

A very quick, cost effective precise and accurate HPLC method for determination of favipiravir has been developed and validated in compliance with ICH guidelines. Besides the retention time, column run time and flow rate of mobile phase made method attractive because these features save analysis time and cost. Potassium dihydrogen phosphate, used as general purpose buffer, has many interesting properties. The most important of these features are good buffering capacity in selected pH range, easy availability, low toxicity and cost, greatly improved separation ability without colon degradation. In short this method is sensitive, selective, reproducible and rapid for favipiravir in bulk and tablets. The accuracy and precision are within reasonable limits, the maximum of quantification is as small as 3.60 microgram per millilitres and finally analytical method is reliable and robust.

Acknowledgements:

The author wants to thank Atabay pharmaceuticals and Fine chemicals for providing information about the high performance liquid chromatographic study of favipiravir and supporting this work. I would like thank Safa M. Megahed, Ahmed A. Habib and Amira H. Kamal for providing the information about spectrofourimertic study of favipiravir.

Declaration and competing interest:

This declares that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.

Reference:

1. Sohrabi, C.,Alsafi,Z;O'Nelli, N.;khan M; kerwan, A.;Al-jabir,A.;et al world health organization declares global emergency: A review of the 2019 novel corona virus , Int. J.Surg.2020, 76,71-76.

2. Saber- Ayed, M; Saleh, M. A; Abu-Gharbieh, E. The rationale for potential pharmacotherapy of corona virus Pharmaceuticals 2020, 13, 96-105.

3. Zhu, R.F.; Gao, R.I.; Robert, S.H.; Gao, J.P.; Yang, S.G.; zhu, C.Systematic review of the registered clinical trials of the corona virus disease 2019, J.Transl.Med.2020, 18, 274-279.

4. Prajapat,M ; Sharma, P; Shekhar, N; Avti, P; Sinha, S.; kaur, H.; et al. Drug targets for corona virus: A systematic review. Indian J. Pharm. 2020, 52(1), 56-63.

5. Dong, L.; Hu, S.; GAO, J. Discovering drugs to treat corona virus disease 2019. Drug discovery. Ther.2020, 14(1), 58-60

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

6. De Clercq, E. New nucleoside analogues for the treatment of hemorrhagic fever virus infections. Chem.- Asian J. 2019, 14(22),3962-3968

7. Delang, L.; Abdelnabi, R.; Neyts, J. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. Antiviral Res. 2018, 153, 85-94

8. Chen, C.; Huang, J.; Cheng, Z.; Wu, J.; Chen, S.; Zhang, Y.; et al. Favipiravir versus arbidol for corona virus: A randomized clinical trial, medRxiv 2020, 3,17-20

9. China patent. A kind of favipiravir has HPLC assay method of related substances. 21.09.2016.

10. Centre for drug evaluation and research. Reviewer Guidance: validation of chromatographic methods CMC, 3.

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

12. Tanaka T., Kamiyama T. T-705 (Favipiravir) suppressed tumour necrosis factor a production in response to influenza virus infection: a beneficial feature of T-705 as an anti-influenza drug. Acta viral. 2017; 61: 48. Doi: 10.4149/av_2017_01_48.

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

14. Elfiky A.A. SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: An in silicon perspective. J. Bio mol. Struct. Dyn.2020:1-9. Doi: 10.1080/07391102.2020.1761882.

15. Zhu N., Zhang D. A novel corona virus from patients with pneumonia in chaina, 2019. New Eng. J. Med. 2020. doi: 10.1056

16. A kind of favipiravir has the HPLC assay method of related substance, CN104914185B

17. Bulduk I. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta chromatogr.2020 doi: 10.1556/1326.2020.00828.

18. Little T.A. Design to the development of analytical methods development and validation. Biopharm Int.2014.

19. Orlandini s., Pinzautin S. Application of quality by design to the development of analytical separation methods. Anal.Bioanal.Chem. 2013;405:443-450. doi: 10.1007/s00216-012-6302-2.

20. Habib A.A., Mabrouk M.M. Implementation of factorial design for optimization of forced degradation conditions and development of validated stability indicating RP-HPLC method for lidocaine hydrochloride. Der pharma chemica. 1981; 7:198-211.

21. Habib A.A., Hammad S.F. Innovative quality by design approch for development of green micellar HPLC methods for simultaneous determination of atorvastatin and amlodipine. Chromatographia.2020;83:1221-1231. doi:10.1007/s10337-020-03937-5.

22. Perrin D. Springer science& Business media; 2012. Buffer for pH and metal ion control.

23. The united states pharmacopiea, USP 40, Online version,US pharmacopieal convention,Rockville,2017.

24. Pharmaceuticals medical devices agency: Avigan review report.

25. Rowe R.C., Sheskey P.Handbook of pharmaceutical excipients.Libros digitalis-pharmaceutical press.2009