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Analytical method development and validation for the estimation of favipiravir by using rp-uplc method

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ABSTRACT

Chromatographic separation was achieved on mobile phase consisting of a mixture of Methanol: Acetonitrile: Water in ratio 50: 30: 20 v/v/v with detection of 229 nm. Linearity was observed in the range 50-150µg/ml for Favipiravir (r^2 =0.9974) for drug estimated by the proposed methods was in good agreement with the label claim. It was concluded that, this newly developed method for the simultaneous estimation of Favipiravir was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Keywords: Favipiravir, water, methanol, pH, Method development

INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent. For many years, researchers have looked at "fast LC" to speed up analyses. The need for speed, the availability of affordable and easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other parameters) have been used.[1-5] Elevated temperature, having the dual advantages of lowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated. HPLC technology simply doesn't have the capability to take full advantages of sub-2µm particles. UPLC can be regarded as new invention for liquid chromatography. From the literature survey, it was revealed that few methods were developed but were not economical. Favipiravir is an antiviral used to manage influenza, and that has the potential to target other viral infections.[6-8]

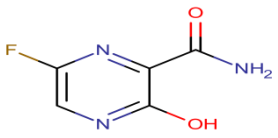

Fig 1: Structure of Favipiravir

The aim was to develop new RP-UPLC method for the simultaneous estimation of Favipiravir in bulk and pharmaceutical dosage form. Initially solubility determination of Favipiravir in various solvents and buffers was done, then determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for UPLC method development. Optimize the mobile phase and flow rates for proper resolution and retention times, validate the developed method as per ICH guidelines.[9-11]

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore, to develop newer analytical methods for such drugs.

MATERIALS AND METHODS

INSTRUMENTS

UV-Visible Spectrophotometer	Thermo Electron corporation
UV-Visible Spectrophotometer software	Vision Pro
UPLC software	OpenLab EZ Chrome
UPLC	Agilent technology Infinity 1290
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Thermo scientific
Electronic balance	Mettler Toledo
Column	Waters Acquity C18 (150x2.2mm ID 4.6µm)

REAGENTS

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Di hydrogen orthophosphate	AR Grade
Acetonitrile	HPLC Grade
Disodium hydrogen phosphate	AR Grade

DRUGS

Favipiravir	Gift Samples obtained from Chandra labs, Hyd.

Solubility Studies

These studies are carried out at 25 ^{0}C

Solvent Name	**Favipiravir**
Water	Soluble
Methanol	Soluble
Acetonitrile	Soluble
Acetone	Sparingly Soluble
Triethylamine	Soluble
Ethanol	Soluble
Phosphate buffer	Sparingly Soluble

Determination of Working Wavelength (λ_{max})

Preparation of Standard solution

Standard stock solution prepared by dissolving 10 mg of Favipiravir dissolved in sufficient methanol. Further dilution is prepared by adding 0.1 ml of stock solution to 10 ml of methanol.

The wavelength of maximum absorption (λ_{max}) of the solution of the drug in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows at 229 nm for Favipiravir was selected as detector wavelength for the UPLC chromatographic method.

METHOD DEVELOPMENT

Preparation of Phosphate buffer pH 6.8

2.72 gm of Potassium Di hydrogen orthophosphate was weighed and dissolved in 1000 mL of water. Adjust the pH to 4.0 ± 0.02 using diluted orthophosphoric acid. Buffer was filtered through 0.45µm filters to remove all fine particles and gases.

Preparation of standard solution

Standard stock solution prepared by dissolving 10 mg of Favipiravir dissolved in sufficient mobile phase. Further dilution is prepared by adding 0.1 ml of stock solution to 10 ml of mobile phase.

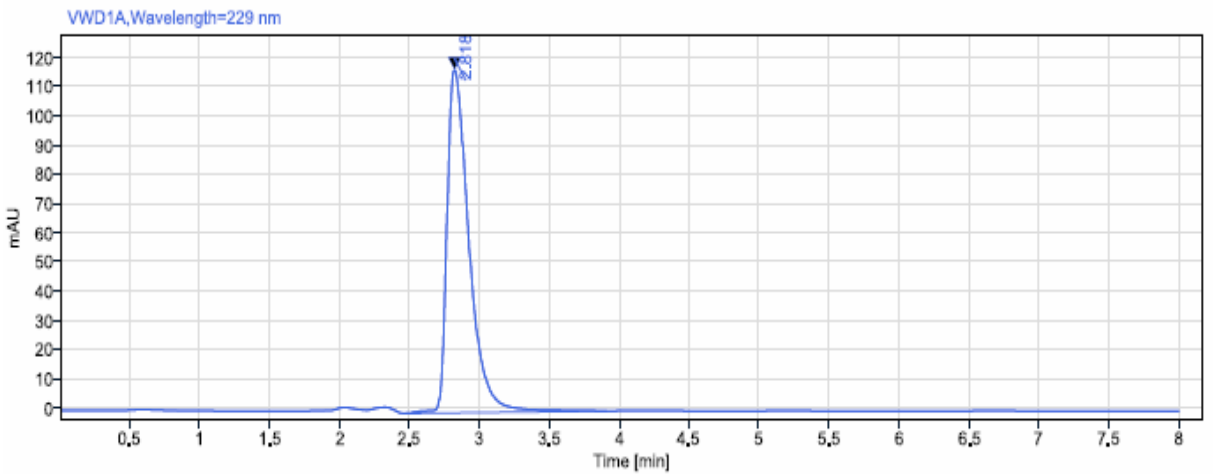

Fig 2: Optimized Chromatogram of Favipiravir

Results for Optimized chromatogram

S.NO	Name	RT	Area	TP	TF	RS
1	Favipiravir	2.818	1286.51	11206	1.81	-

The Favipiravir peak was observed at 2.818 mins with good efficiency (>2000) and peak shape and good resolution and tailing factor (< 2). So this trail was considered and validated according to ICH guidelines. Hence, this method was finalized for the simultaneous estimation of Favipiravir.

System Suitability& System precision

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 100µg/ml of FAVIPIRAVIR was injected six times and the chromatograms were recorded for the same. The plate count and tailing factor results were found to be within the limits and the % RSD was found to be 0.266% so system is suitable and giving precise results.

Method precision

Method precision was determined by injecting sample solutions of concentration FAVIPIRAVIR (100µg/mL) for six timesare prepared separately. The %RSD of Area and Retention times for 6 Sample determinations of FAVIPIRAVIR found to be within the acceptance criteria (less than 2.0%). Hence method is precise.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of FAVIPIRAVIR were prepared by dissolving 10 mg of FAVIPIRAVIR in 10 mL of mobile phase. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparation was found to be 0.98.

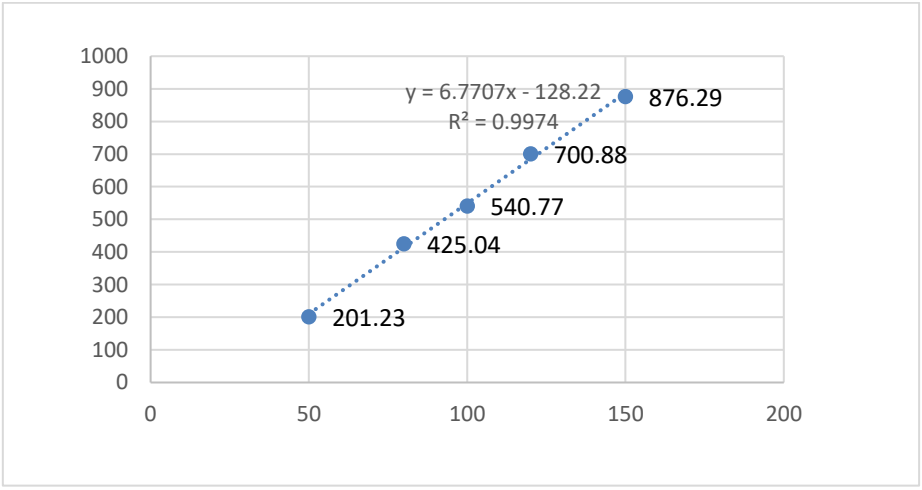

Fig 3: Graph for Linearity data of favipiravir

Specificity

Blank solution was injected, and the chromatogram was recorded for the same as given in figure below. Placebo solution was prepared, and it was injected, and the chromatogram was recorded. It was observed that diluent or excipient peaks do not interfere with the favipiravir peak.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs (50µg/ml, 150µg/ml and 250µg/ml) were added at the level of 50%, 150%, 250%. The recovery studies were carried out three times

and the percentage recovery and percentage mean recovery were calculated for drug. The percentage mean recovery of favipiravir was found between 98% and 102%.

Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters. The tailing factor and theoretical plates was found to be within the limits on small variation of flow rate and temperature.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. From the results % Assay and %RSD obtained acceptance criteria 2% so method is rugged

DISCUSSION

A simple and selective LC method is described for the determination of Favipiravir in bulk and tablet dosage forms. Chromatographic separation was achieved on mobile phase consisting of a mixture of Methanol : Acetonitrile : Water in ratio 50: 30: 20 v/v/v with detection of 229 nm. Linearity was observed in the range 50-150µg/ml for Favipiravir (r^2 =0.9974) for drug estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Favipiravir was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

REFERENCES

1. Skoog DA, West DM, Holler FJ. Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers, 1996, 1-5.
2. Sharma BK. Instrumental method of chemical analysis Meerut; 1999. p. 175-203.
3. Breaux J, Jones K. Understanding and implementing efficient analytical method development and validation. Pharm Technol. 2003;5:110-4.
4. Willard H, et al. Instrumental methods of analysis. 7th ed CBS publisher and distributors. New Delhi; 1991. p. 436-9.
5. Chatwal RG, Anand KS. High-performance liquid chromatography. Instrumental methods of chemical analysis. 5th ed. Mumbai: Himalaya publishers; 2010; 2.570-629.
6. Sharma BK. High-performance liquid chromatography. Instrumental methods of chemical analysis. 24th ed. Meerut: Goel publishers; 2005. p. 295-300.
7. Dong WM. HPLC instrumentation and trends. Modern HPLC for practicing scientists. USA; 2006. p. 5-10, 78-110.
8. Typical diagram of HPLC. Available from: http://www.comsol.com/stories/waters_corp_hplc_systems/full/Hplcdiagram.
9. HPLC solvent properties. Available from: http://www.sanderkok.com/techniques/hplc/eluotropic_series_extended.htm.
10. Swartz ME, Ira Krull S. Analytical method development. Analytical method development and validation. 1st ed. New York: Marcel Dekker, Inc; 2009. p. 17-80.
11. Satinder A, Dong MW. Method development and validation. Pharmaceutical analysis by HPLC. 15th ed. New York; 2005. p. 16-70.