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DEVELOPMENT AND VALIDATION OF A RELIABLE SPECTROPHOTOMETRIC METHOD FOR FAVIPIRAVIR ANALYSIS IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

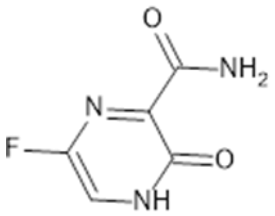
Simple, precise and accurate zero order derivative spectroscopic method has been developed and validated for the estimation of favipiravir in bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ max) at 361nm in 0.1N NaOH and obeys Beer's law in the concentration range of 2-12µg/ml. The linearity study was carried out and regression coefficient was found to be 0.9988 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 99.16 -101.8. The LOD and LOQ were found to be 0.137 and 0.416µg/ml. The % relative standard deviation were found to be less than 2. According to ICH guidelines the technique has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantification of favipiravir in bulk and pharmaceutical dosage form.

KEYWORDS: Favipiravir, zero order derivative spectroscopy, validation, pharmaceutical formulations.

INTRODUCTION

Favipiravir is a well-known medication used to treat influenza, and its potential to treat COVID-19 is also being investigated. It is the first antiviral medication that can be taken orally for mild to moderate COVID-19. Studies that have previously been finished in China, Japan, and Russia have demonstrated that favipiravir is a possible treatment for this illness. Japan's Toyama Chemical created Favipiravir, is a derivative of pyrazine carboxamide that inhibits a variety of RNA viruses. It was initially reported to be a minimally cytotoxic, selective inhibitor of influenza virus replication. Favipiravir is thought to specifically target the RdRp catalytic region, blocking viral replication in cells and reducing infection. As a prodrug, favipiravir is phosphorylated and ribosylated to yield an active form known as favipiravir ribofuranosyl-5'-triphosphate (Favipiravir-RTP), which binds to RNA dependent RNA polymerase and inhibits viral replication by mimicking guanosine and adenosine for viral RNA-dependent RNA polymerase (RdRP), which prevents primer extension. Clinical research indicates that Favipiravir's teratogenic properties make it inappropriate to recommend during pregnancy. When used orally, the medication reaches its maximal concentration after two hours and has a brief half-life of two to five hours. Favipiravir's plasma protein binding capability was discovered to be 54%.

Chemically favipiravir is 6-fluoro-3-hydroxypyrazine-2 carboxamide. Favipiravir is a pale-yellow powder available in crystalline solid form and it is soluble in organic solvents such as N, N-Dimethyl Formamide and methanol, DMSO, NaOH, and even soluble in deionized water. Its molecular formula is $C_5H_4FN_3O_2$ and its molecular weight is 157.1g/mol and having melting point in the range of 187 - 193˚C.



6-fluoro-3-hydroxypyrazine-2-carboxamide

Fig.1: Chemical structure of Favipiravir.

Literature survey revealed that there were few analytical methods have been reported for the determination of favipiravir in pure drug and pharmaceutical dosage forms by using UV[1-5], HPLC[6-13], RP-HPLC[14-24] and HPTLC[25] so far. The aim of present work is to develop and

validate a novel, rapid, simple, precise and specific Zero order derivative UV Spectrophotometric method for estimation of favipiravir in bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Favipiravir pure drug was obtained as a gift sample from Glenmark Pharmaceutical, Bengaluru and its pharmaceutical dosage favipiravir 20 tablets (feravir-200) labelled claim 200mg from local pharmacy manufactured by Mascot Health Series Pvt. Ltd.

Solvent: 0.1N NaOH is used as a solvent.

Selection of analytical wavelength: Appropriate dilutions of favipiravir were prepared from standard stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. The absorption spectra obtained and show maximum absorbance at 361nm, as the wavelength for detection.

Preparation of standard stock solution: 100mg of Favipiravir was weighed accurately and transferred into 100ml volumetric flask and diluted in 0.1N NaOH up to mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml into 10ml individual volumetric flask and diluted in 0.1N NaOH up to mark, this gives 2, 4, 6, 8, 10, and 12µg/ml concentration.

Preparation of sample solution: 20 tablets of Favipiravir marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Favipiravir was transferred into a 100ml of volumetric flask then it was diluted with 0.1N NaOH and made up to the mark.

METHOD AND VALIDATION

The method was validated according to the ICH guidelines.[26-28]

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity: The linearity of an analytical method is its capacity to show the test results that are directly proportional to the concentration of the analyte in the sample within the range.

The linearity was established in the range of 2-12µg/ml was measured at 361nm and absorbance values are shown in table-1. The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in Fig-3. Statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined and shown in table-2.

Precision: The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was established by intra-day and inter-day studies. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. shown in table-3.

Accuracy: The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. Shown in table-4.

Ruggedness: The ruggedness is defined as the reliability of results when the method is performed under the variation in conditions. This includes distinct analyst, laboratories, instruments, temperature etc. Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (table-5).

LOD and LOQ: The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is a discrete analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated by using following formula.

$$LOD = 3.3(SD)/S \text{ and } LOQ = 3(LOD)$$

LOD and LOQ value of Favipiravir were found be 0.137 and 0.416µg/ml.

Table 1: Results of calibration curve at 361nm by zero order spectroscopy.

SL.NO	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	2	0.137±0.0010
3	4	0.249±0.0011
4	6	0.388±0.0055
5	8	0.508±0.0043
6	10	0.643±0.0018
7	12	0.746± 0.0026

**Average of six determinations.*

Table 2: Regression parameter of Favipiravir by zero order spectroscopy.

Regression parameter	Results
Range(µg/ml)	2-12
Λ_{max} (nm)	361
Regression Equation	Y= 0.062X+0.0117
Slope(b)	0.0621
Intercept(a)	0.0105
Correlation coefficient(r^2)	0.9988
Sandell's equation	0.0154
Limit of detection(µg/ml)	0.137
Limit of quantitation(µg/ml)	0.416

Table 3: Determination of precision results for Favipiravir at 361nm by zero order spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
2	0.137±0.0010	0.729	0.137±0.00094	0.686
4	0.249±0.0011	0.846	0.244±0.0020	0.819
6	0.388±0.0055	0.441	0.362±0.0024	0.662
8	0.508±0.0043	0.279	0.519±0.0025	0.481
10	0.643±0.0018	1.417	0.623±0.0027	0.433
12	0.746± 0.0026	0.348	0.722± 0.0012	0.166

**Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Accuracy results for Favipiravir at 361nm by Zero order spectroscopy.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	6	3	9.17	101.8±0.20	0.196
100	6	6	11.90	99.16±0.179	0.180
150	6	9	15.16	101.1±0.10	0.098

**Average of six determinations, **percentage relative standard deviation.*

Table 5: Determination of Ruggedness results for Favipiravir at 361nm by Zero order spectroscopy.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.3916	0.3916
±Standard deviation*	0.0011	0.0012
%RSD	0.2810	0.3060

**Average of six determinations, **percentage relative standard deviation.*

FIGURES

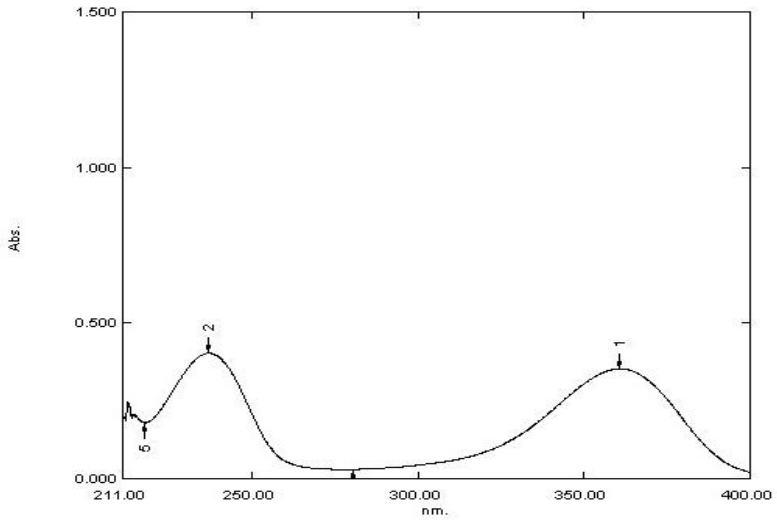


Fig. 2: Zero order spectrum of Favipiravir at 361nm.

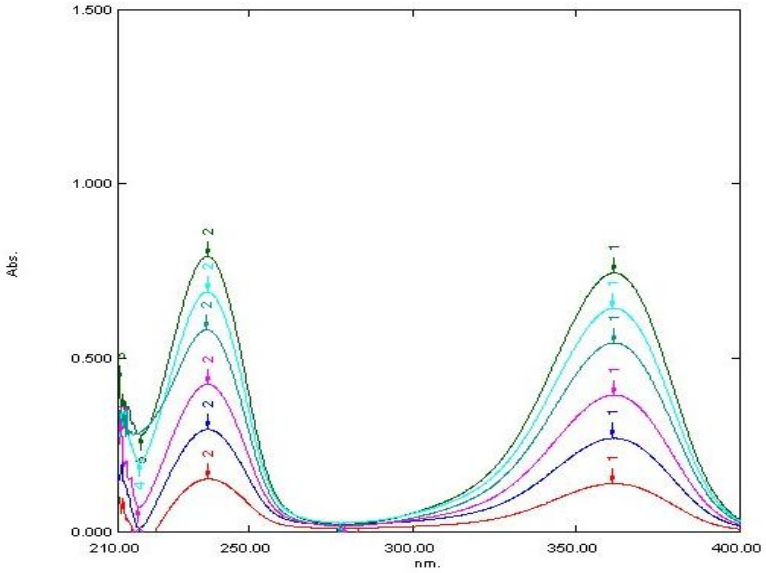


Fig.3: Zero order overlain spectra of Favipiravir showing absorbance at 361nm.

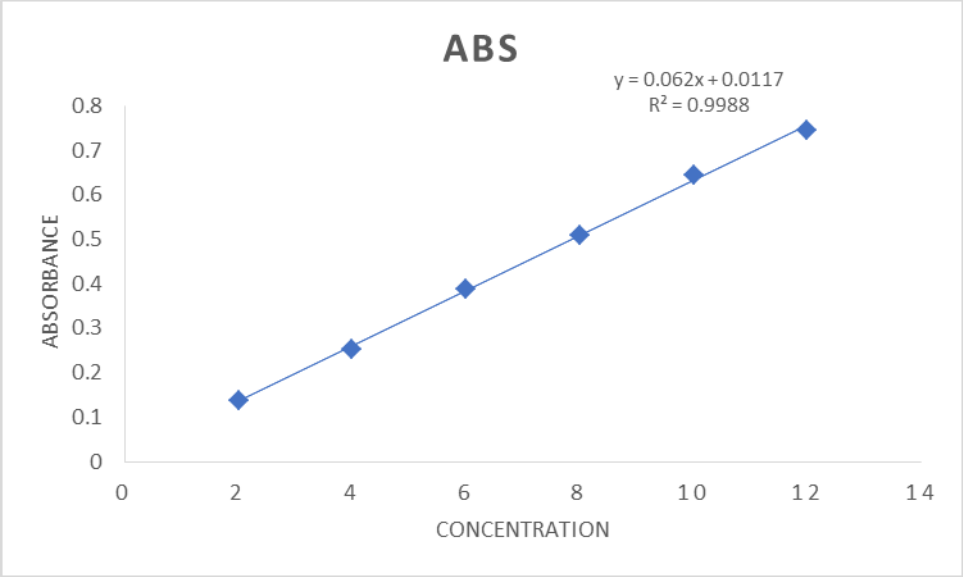


Fig.4: Calibration curve of Favipiravir by zero order spectroscopy.

CONCLUSION

As per ICH guidelines, the present analytical work was carried out and met the acceptance criteria. It was concluded that the developed analytical method was simple, specific, accurate, economical and sensitive and can be used for routine analysis of Favipiravir in bulk drug and in pharmaceutical dosage forms.

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