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A PECULIAR, RESPONSIVE AND VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FAVIPIRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The main target is to improve and authenticate a easy, sensitive, convenient, worth-while and particular method for the determination of Favipiravir and bulk and formulation using 0.1N NaOH as solvent. The method developed obeys Beer's law at λ max of 238nm and the concentration ranges from 1-15 µg/ml. The correlation coefficient is R^2=0.9984 follows the regression equation as y=0.0544x-0.0039. According to ICH guidelines the method developed was validated for linearity, robustness, ruggedness, precision, LOD, LOQ and accuracy. As per the outcomes of the experiment, the process established is up to date, valid and diplomatic and be used for the approximation of Favipiravir in bulk and commercial dosage form.

Keywords: Favipiravir, Validation, UV Spectrophotometer, Linearity, Accuracy

INTRODUCTION

Favipiravir with IUPAC name as 6-fluoro-3-hydroxypyrazine-2-carboxamide is an anti-viral (anti-influenza). Chemical formula is given as $C_5H_4FN_3O_2$ and average weight is 157.104. It shows it's mechanism by preventing the viral genome replication by inhibiting RNA polymerase **[1]**.

Upon literature review it was found that there was HPLC-UV methods for quantification of Favipiravir so far **[2-3]**. In this process the author developed a UV method for the quantification of Favipiravir in bulk as well as pharmaceutical dosage form which is unheard of.And method

validated according to the ICH guidelines **[4-6]**.

Favipiravir structure is what that is placed below **[7]**.

Figure 1: Structure of Favipiravir

MATERIALS AND METHODS

INSTRUMENTS AND MATERIALS

A premium sample of Favipiravir which is 99.6% pure was obtained from Hyderabad, spectrum labs. And analytical category of chemicals and reagents are used for this purpose. Instruments include LAB INDIA (T60) DOUBLE BEAM UV/VISIBLE SPECTROPHOTIOMETER [8] and ELITE analytical balance. Favipiravir of 200mg possessing the hallmark as FABIFLU was acquired from community pharmacy.

Figure 2: Double beam UV Spectrophotometer

STANDARD STOCK SOLUTION CONSTRUCTION

Favipiravir standard stock solution was made by dissolving accurately weighed amount of 100mg of Favipiravir in 100ml of 0.1N NaOH in 100ml volumetric flask (consisting mark up to which is to be filled). This gives us a 1000 µg/ml concentration of Favipiravir **[4]**.

WORKING STANDARD SOLUTION CONSTRUCTION

10ml of the prepared standard stock solution is taken and transferred to volumetric flask of 100ml and made up to

the mark with 0.1N NaOH, which gives 100 µg/ml concentration of Favipiravir **[4]**.

0.1 N NaOH (Sodium Hydroxide) CONSTRUCTION

Weigh 4 grams of sodium hydroxide and transfer to 1 litre volumetric flask, now make up to the mark using distilled water to get 0.1N NaOH **[4]**.

ASCERTAINMENT OF λmax OF FAVIPIRAVIR

The working standard solution (100 µg/ml) of Favipiravir is examined using UV Spectrophotometry in the span of 200-400nm using 0.1N NaOH solution as blank. The λ max i.e. the absorbance was set up highest at 238nm.

ESTABLISHMENT OF CALIBRATION CURVE

The solutions whose concentration varies from 1-15 µg/ml was made from the working standard solution (100 µg/ml) using 0.1 N NaOH as solvent. These solutions are scanned at 238nm (it is the λmax which is already found) and the following absorbance values are noted down. Using the obtained values **[Table 1]** a graph is plotted to get a calibration curve.

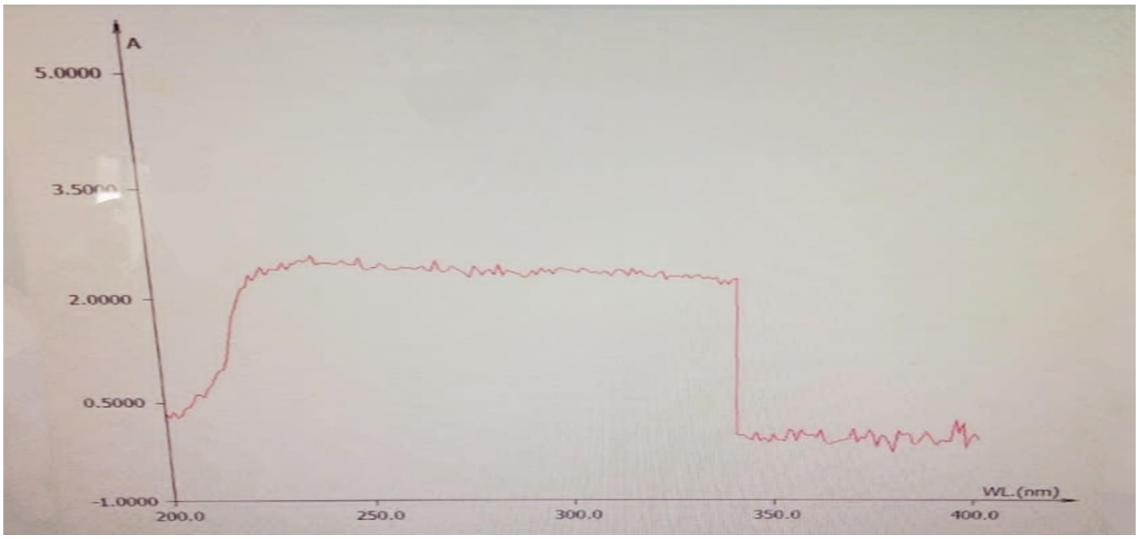

Figure 3: Determination of λmax of Favipiravir

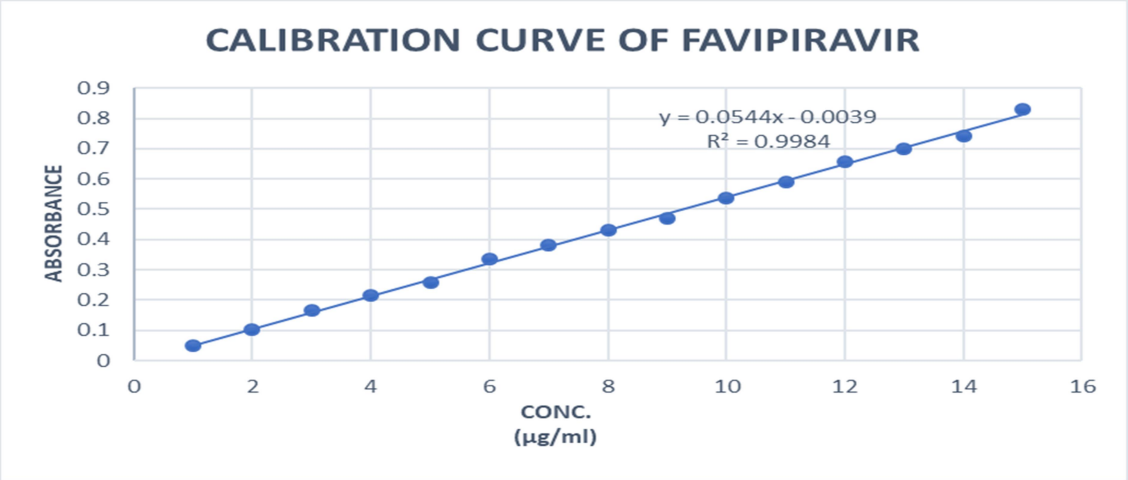

Figure 4: Standard Calibration Curve of Favipiravir

ASSAY SOLUTION CONSTRUCTION

The developed method was used for the analysis of marketed Favipiravir tablets (FABIFLU 200 mg). 10 tablets were taken and weighed individually and finally crushed to get the powder. Now the powder that is equivalent to 100mg of Favipiravir was taken into 100ml volumetric flask and dissolved with little amount of 0.1N NaOH same and this dissolved solution is then sonicated for 15 minutes and finally it was made up to the mark using 0.1 N NaOH. This solution was filtered using a #44 grade Whatman filter paper. And from this, a solution with 8µg/ml concentration was made by dilution and analysed using a double beam UV Spectrophotometer with 0.1 N NaOH as blank in the range of 200-400nm and the spectrum was recorded at 238nm. The Favipiravir drug concentration was determined using the linear regression analysis.

METHOD VALIDATION

Validation is defined as the procedure for the establishment of documented evidence, which provides high degree of assurance that a specific process will continuously provide desired results or a product meeting it's predetermined specifications and quality attributes. The method developed is validated according to the ICH guidelines for range of parameters like Linearity, Range, Precision, Accuracy, Robustness, Ruggedness, LOD, LOQ and Sensitivity.

RANGE

Range is defined as interim between the higher and lower analyte concentration in the sample for which it has been determined that the analytical process has a acceptable level of linearity, precision and accuracy.

LINEARITY

The potential of an analytical procedure is to reproduce test outcomes that are directly proportional to the concentration of an analyte. Linearity should be estimated by

visual examination of a plot of data as a function of analyte concentration. For determination of linearity at least 5 concentrations are required.

ACCURACY

Accuracy is defined as the pronouncement of proximity of agreement between the conventional true value i.e. accepted reference true value and the determined or found value. Accuracy is found by using 9 determinations covering at least of 3 concentrations.

PRECISION

The closeness of agreement between the drawn values by analysing the same sample multiple times under prescribed test conditions. There are three levels of repeatability, intermediate precision, and reproducibility.

Repeatability is a measure of the accurateness under the similar working conditions greater than a short interval of time, under customary working conditions of the scientific process with the identical hardware it is also known as intra-day precision.

Reproducibility also termed as inter-day precision.

Precision is demonstrated in terms of % Relative Standard Deviation.

% RSD= (standard deviation)/Meanx100

Standard Deviation is given as-

$SD=\sqrt{((\sum [(x-\bar{x})]^2)/(n-1))}$

Where n= no. of entries

RUGGEDNESS

The ruggedness of an analytical technique is the level of reproducibility of test results by analysing the similar sample under various conditions like laboratories, instruments, analysts, reagents, etc.

ROBUSTNESS

Robustness of an analytical process is the capacity to remain consistent by slight but deliberate changes in parameters.

SENSITIVITY

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug was calculated by using equations according to ICH guidelines.

Limit of Detection- It is the lowest concentration of analyte in the sample that can be determined, but not necessarily quantitated and it is given by LOD= (3.3Xσ)/S

Limit of Quantification – It is the concentration of analyte that can be quantified with a specified limit of accuracy and precision and it is given by LOQ= (10Xσ)/S

Where S= Slope and σ= Standard deviation

LINEARITY

1-15 µg/ml ranging concentrations of solutions are prepared from the working standard solution (100µg/ml). These prepared solutions are scanned in the UV range of 200-400nm using a double beam UV Spectrophotometer where the blank used is 0.1N NaOH solution and the

respective absorbance valuesare obtained at 238nm. From this data a calibration curve is plotted by taking concentration on x-axis and absorbance on y-axis.

PRECISION

Precision of the developed method is expressed using the intra-day and inter-day precision studies. In intra-day studies 6 variety solutions of the similar concentration i.e. 8µg/ml were made and analysed three times a day i.e. morning, afternoon and evening and absorbance values are recorded **[Table 3]**. Where as in inter-day precision the same 8µg/ml concentration solutions are prepared and analysed 6 times for 5 successive days and the respective absorbance values are tabulated **[Table 4]**. From the data obtained the % Relative Standard Deviation (RSD) was found and recorded.

ACCURACY

The various concentrations of solutions whose percentages are 80%, 100%, 120% are prepared for obtaining the accuracy data. In this process the amount of pharmaceutical formulation of the FABIFLU is kept persistent and the amount of the bulk drug was diversified. Triplicate solutions were prepared for each percentage and analysed. The % recovery was obtained from the data and reported which indicates the accuracy **[Table 5]**.

ROBUSTNESS

The robustness is determined by analysing the solutions of concentration 8µg/ml at +1 nm and -1nm wavelength with respective to the wavelength of the λmax. For example, if the λmax wavelength is 238nm then the solutions are scanned at ±1nm i.e. 237nm and 239nm along with 238nm. The %RSD values are calculated and recorded **[Table 6]**.

RUGGEDNESS

Ruggedness data is obtained by recording the absorbance values of the sample solutions by two different analysts. And the %RSD is calculated and reported **[Table 7]**.

SENSITIVITY

The limit of detection (LOD) and limit of quantification is calculated using the following below mentioned formulas as per ICH guidelines and recorded **[Table 8]**.

Limit of Detection: LOD= (3.3Xσ)/S

Limit of Quantification: LOQ= (10Xσ)/S

Where σ= standard deviation

S= slope

Table 1: Linearity data

Concentration (µg/ml)	Absorbance
1	0.0487
2	0.1037
3	0.1660
4	0.2162
5	0.2579
6	0.3359
7	0.3818
8	0.4327
9	0.4692
10	0.5377
11	0.5904
12	0.6566
13	0.6995
14	0.7436
15	0.8316

Table 2: Repeatability data

Concentration (µg/ml)	Absorbance	Statistical Analysis
8	0.4424	Mean=0.4324
8	0.4418	% RSD=0.11%
8	0.4427	
8	0.4419	
8	0.4420	
8	0.4421	

Table 3: Intra-day precision

Concentration (µg/ml)	% RSD			Average % RSD
	1	2	3	
8	0.10	0.10	0.11	0.10

Table 4: Inter-day precision

Concentration (µg/ml)	% RSD			Average % RSD
	1	2	3	
8	0.12	0.11	0.11	0.11

Table 5: Accuracy data

Level of addition (%)	% Recovery	%Mean recovery ±SD
80%	99.3%	99.66%±0.51
100%	99.7%	
120%	100.0%	

Table 6: Robustness data

Concentration (µg/ml)	Absorbance		
	Change in wavelength		
	237nm	238nm	239nm
1	0.4310	0.4427	0.3841
2	0.4298	0.4418	0.3847
3	0.4312	0.4419	0.3839
4	0.4308	0.4421	0.3844
5	0.4299	0.4424	0.3840
6	0.4311	0.4420	0.3846
%RSD	0.14%	0.11%	0.08%

Table 7: Ruggedness data

Concentration (µg/ml)	Analyst 1		Analyst 2	
8	0.4327	Mean=0.4322	0.4346	Mean=0.4348
8	0.4318	%RSD=0.08%	0.4352	%RSD=0.05%
8	0.4321		0.4348	
8	0.4324		0.4345	
8	0.4320		0.4348	
8	0.4326		0.4347	

Table 8: LOD and LOQ data

Limit of Detection (LOD)	Limit of Quantification (LOQ)
0.0086 µg/ml	0.0260 µg/ml

RESULTS AND DISCUSSION

ICH guidelines are taken as the basis for this method development and validation. The proposed method was validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The concentration range which was chosen i.e. 1-15µg/ml complied with Beer's Law. The regression equation and correlation coefficient were found to be y=0.0544x-0.0039 and R^2=0.9984 using the regression methodology. The precision data where the %RSD values are less than 2% at each level clearly indicate that the validated method is precise and can be used for Favipiravir analysis. Recovery studies are used to study the accuracy. The high recovery values show how much accurate is the method developed. The procedure is said to be sufficient robust and rugged from the above data analysis. LOD and LOQ values indicate the system is sensitive. The method is specific as there is no interference observed with the excipients of the formulation. The assay value obtained from analysis of Favipiravir tablets (FABIFLU 200 mg) proved that the determined value was near to the mentioned label amount. All the validated parameters are with in the limits i.e. %RSD values are less than 2% and are acceptable. List of validated parameters are presented in **[TABLE 9]**.

Table 9: Data of validated parameters

Parameters	Results
Absorption maxima (nm)	238nm
Linearity (µg/ml)	1-15 µg/ml
Regression equation	Y=0.0544x-0.0039
Slope	0.0544
Intercept	0.0039
Correlation coefficient (R^2)	0.9984
LOD (µg/ml)	0.0086 µg/ml
LOQ (µg/ml)	0.0260 µg/ml
Accuracy (%Mean recovery ±SD)	99.66%±0.51
% Assay	99.4%
Precision	0.11%
Intra-day precision (%RSD)	0.10%
Inter-day precision (%RSD)	0.11%

CONCLUSION

UV Spectrophotometric method was expanded and validated for the evaluation of Favipiravir in pure as well as commercial dosage form. The developed method is found to be straightforward, error-free, precise, complete, accurate, specific, linear, robust and reproducible. The put forward analytical method can be used for regular analysis of Favipiravir in bulk come pharmaceutical dosage form.

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