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DEVELOPMENT AND VALIDATION OF SECOND-ORDER DERIVATIVE UV SPECTROPHOTOMETRIC ESTIMATION OF FAVIPIRAVIR ANTIVIRAL DRUG

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ABSTRACT: Objectives: The present study aims to develop a simple, precise, accurate, and reproducible spectrophotometric Second order derivative method for estimating Favipiravir in bulk and pharmaceutical dosage form. **Method:** Acetonitrile was used as an organic solvent. The maximum absorption of the drug was found to be at 323nm. The developed method was validated and applied for the estimation of Favipiravir by using a spectrophotometric second-order derivative. **Results:** The linearity was established in the 5 to 25µg/ml concentration range for favipiravir with a correlation coefficient (r^2) of 0.9991, respectively. Percent recovery studies were obtained in the range of 99.55-101.25%. Both interday and intraday precision was found to be satisfactory. The limit of detection and quantitation were found to be 1.5µg/ml and 1.2µg/ml, respectively. The method was statistically validated according to ICH guidelines for linearity, accuracy, and precision. The relative standard deviation was found to be less than 2%. **Conclusion:** The proposed method is simple to use, affordable and cost-effective. It can be successfully applied to estimate favipiravir in bulk and pharmaceutical dosage form and used for routine analysis.

INTRODUCTION: The antiviral drug favipiravir was discovered by Japanese company Toyoma Chemical Co. Ltd. which has activity against influenza and was successfully identified by the screening. It is pyrazine analog as shown in **Fig. 1.** It is prodrug and (T-705) has a reduced molecular weight of 157.1 g/mol. In Japan, it was given the go-ahead for medical use in 2014 to treat newly emerging or reemerging pandemic influenza virus infections (Shiraki and Daikoku, 2020; Hayden and Shindo, 2019) [1].

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Favipiravir was also approved in China in February 2020 for the treatment of new influenza, and it is currently being researched in Chinese populations for the experimental treatment of the emerging COVID-19 [2]. Favipiravir is an extremely robust and selective influenza viral RNA polymerase inhibitor.

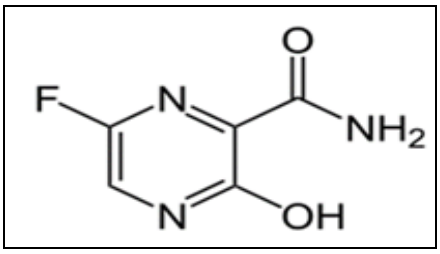

FIG. 1: CHEMICAL STRUCTURE OF FAVIPIRAVIR

It is effective against all influenza virus subspecies and strains, including those vulnerable to or

resistant to widely viable protease and M2 inhibitors. Other RNA viruses that Favipiravir was evaluated against exhibited antimicrobial activities [3]. There are 37 studies recorded in the PubMed database as of October 12th, 2020, to examine the appropriateness of this drug for the treatment of covid-19 [4]. A small chemical antiviral, favipiravir, prevents viral replication by preventing purine nucleosides from incorporating into the nascent viral RNA and blocking the function of viral RNA dependent RNA polymerase. Favipiravir has been the subject of numerous clinical trials in both an inpatient and outpatient context for COVID-19 due to its mechanism of action, effectiveness against SARS-CoV-2 *in-vitro*, and recognized safety profile for treating illnesses such as influenza. Included are initial findings from open-label studies carried out in China that showed improving patient recovery and viral elimination [5]. In 1,636 moderate and severe COVID-19 patients, a recent meta-analysis found no statistically significant difference between favipiravir treatment and the standard of care regarding mortality rates or need for mechanical ventilation [6]. The active form, favipiravir RTP, interacts with RNA-dependent RNA polymerase to inhibit viral genome replication in host cell. Furthermore, from another hypothesis, it is through that the active form can be incorporated into nascent RNA strand and prevent its elongation and viral proliferation and transcription [7]. The literature review found that three HPLC methods were developed and published to determine assay and impurity in active pharmaceutical ingredients [8-10]. No Second order derivative method is reported for determining favipiravir, which makes it simple and cost effective.

MATERIALS AND METHODS: Pharmaceutical gift sample of favipiravir was obtained from Abbott healthcare, Mumbai. favipiravir 400 mg per tablet was acquired from local market. Analytical-grade chemicals and reagents were used throughout obtained from RAP Analytical Lab Nashik, India.

The instrument used was UV-VIS Spectrophotometer (Model-UV2012, Software: UV-VIS Analyst) with a matched pair of 1cm quartz cell and sonication of sample solution was done by using ultrasonic cleaner (spectra lab, model: UCB-40, Mumbai, India).

Standard Stock Solution Preparation: Standard stock solution of favipiravir 1000 ppm concentration was prepared by dissolving 0.01 g of pure drug dissolved in 10 ml of acetonitrile. The drug's standard stock solution was diluted with acetonitrile to prepare the standard working solution.

Experimental Method:

Development of Method: The standard solution of Favipiravir was diluted with acetonitrile individually to achieve the concentration of 5ug/ml to 25ug/ml, and it was scanned in the UV range 800-200nm. Drugs were found to possess an A max of 323 nm.

Method Validation: Analytical method validation is proving that an analytical technique is adequate for its intended use. To ascertain the linearity, accuracy, precision, and assay of market formulation, the suggested method was validated by the International Conference on Harmonization Q2 R1 guidelines 16 for validation of analytical procedures.

Linearity: A mathematical relationship (function) is considered linear if it can be graphically represented as a straight line. Linearity and proportionality are concepts that go hand in hand. Different concentrations of the drug solution were further diluted with acetonitrile to get the final working standards of the concentration range of favipiravir as 5- 25µg/ml for linearity experiments. The linearity was validated using linear regression. Second-order derivative spectra were recorded, and absorbance was selected at 323nm, respectively.

Accuracy: Accuracy indicates how true or accurate the measured value. Accuracy can be improved by taking repeated readings to reduce the calculation error. The accuracy of the procedure was assessed using recovery trials utilizing the traditional addition method. The method was carried out at three levels (80%, 100% and 120%) for second order derivative.

Precision: Precision is determined by how closely two or more measurements coincide. Precision does not require accuracy. To measure the precision, standard samples of both drugs were produced in triplicates at three different concentration levels that covered the whole

linearity range. Intraday and interday data were used to calculate the precision, which was then represented as a percentage of RSD.

Detection Limit and Quantitation Limit: Several methods for determining the detection and quantitation limits are described in the ICH guideline. These include visual assessment, signal-to-noise ratio, response standard deviation, and calibration curve slope calculations. Based on the third approach, the LOD and LOQ in the current study were determined using the 3.3/S and 10/S criteria, respectively. Here, denotes the standard deviation of the regression lines' y-intercepts, and S denotes the slope of the calibration curve.

Assay of Marketed Formulation: Accurately weighed 4mg of 20 tablets powdered was added to a volumetric flask of 25 ml, diluted to the appropriate level with solvent, and then sonicated for 30 minutes. To obtain a solution with 4mg/ml, produced solution was transferred to a 10 ml volumetric flask and diluted with the mobile phase.

RESULTS AND DISCUSSION: Second-order derivative spectra were obtained at 323 nm using acetonitrile as the solvent, shown in **Fig. 2.**

The linear range of favipiravir was 5-25µg/ml. Linearity was evaluated by regression equation and was found to be 0.991. Recovery studies were conducted, and it was found that for favipiravir (shown in **Table 1**), the percentage recovery was 99.55% - 101.15%. Precision was expressed to reduce in percent RSD values both Intra and Inter days. It suggests that the procedures have a high degree of precision.

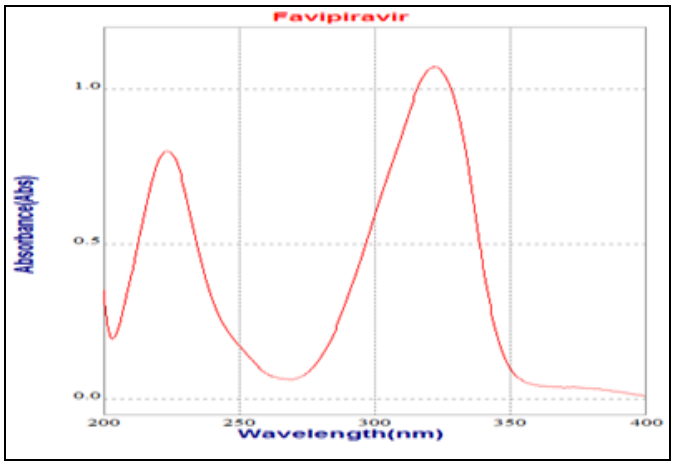

FIG. 2: SECOND ORDER DERIVATIVE OF FAVIPIRAVIR

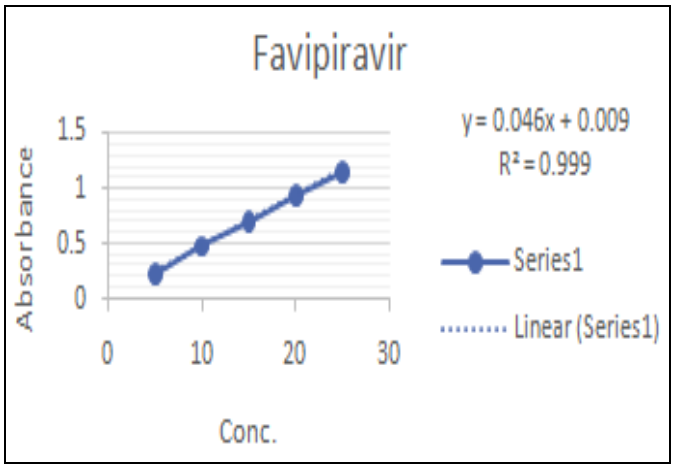

FIG. 3: CALIBRATION CURVE OF FAVIPIRAVIR

RSD was found to be satisfactory as shown in **Table 2** and **3** respectively. The assay of marketed formulation was found to be 98.26% for favipiravir as shown in **Table 4**. The summarized validation parameter was given in **Table 5.**

The aliquots of drugs used in linearity studies (5-25µg/ml) were converted to second derivative spectra and the derivative absorbance at 323nm for Favipiravir were measured. The calibration graphs of Favipiravir were plotted at 323nm. The following regression equation for Favipiravir were obtained **Fig. 3** as y = 0.046x + 0.009; R2 = 0.9991. Result of recovery studies are shown in **Table 2**.

The recovery study done by standard addition. Method has given satisfactory results at three concentration levels. Recovery studies found was 99.55-101.25%. Both interday and intraday precision was found to be <2% RSD as shown in **Table 3**.

The validation parameter was summarized in **Table 4**. The results of the analysis of formulation was found to be 99.23%. Hence the optimized method was evaluated and validated in terms of linearity, precision and accuracy. The proposed method was found to be accurate and specific for the analysis of favipiravir in bulk and tablet formulation.

TABLE 1: RECOVERY STUDIES OF FAVIPIRAVIR

Conc. (µg/ml)	Amount of drug added(µg/ml)	% Accuracy ± SD
5	5	99.55 ±0.35
5	10	99.87 ±0.69
5	15	101.25 ±0.98

TABLE 2: INTRADAY PRECISION RESULTS FOR FAVIPIRAVIR

Conc. (µg/ml)	Absorbance ± SD	%RSD
15	0.7083 ±0.0008	1.56
15	0.7086 ±0.0005	1.63
15	0.7081 ±0.0006	1.70

TABLE 3: INTERDAY PRECISION RESULTS FOR FAVIPIRAVIR

Conc. (µg/ml)	Absorbance ± SD	%RSD
15	0.7079 ±0.0009	1.69
15	0.7080 ±0.0009	1.71
15	0.7078 ±0.0008	1.75

TABLE 4: RESULTS OF ASSAY OF FAVIPIRAVIR

Tablet	Label claim(mg/tablet)	% label claim± SD
	400mg	98.26±0.45

TABLE 5: VALIDATION PARAMETER OF FAVIPIRAVIR

Parameter	Favipiravir
Linearity(µg/ml)	5-25
Co-relation coefficient(r^2)	0.9991
Slope	0.046
Intercept	0.009
LOD(µg/ml)	1.5
LOQ(µg/ml)	1.2

CONCLUSION: Using a second-order derivative UV spectrophotometric approach, a quick, easy, accurate, and exact method was obtained to estimate Favipiravir in bulk and pharmaceutical dosage form. The proposed method can be used to analyze commercial formulations.

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CONFLICTS OF INTEREST: Nil

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