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# VALIDATED VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT FOR THE ESTIMATION OF FAVIPIRAVIR BY OXIDATION TECHNIQUE

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**ABSTRACT**: A simple, precise, accurate and reproducible visible spectrophotometric method was developed and validated for the estimation of Favipiravir (FVP) in bulk and pharmaceutical formulation. Ferric chloride is an oxidizing agent. Favipiravir was reacts with ferric chloride and form reddish brown coloured complex which can be estimated spectrophotometrically at 445 nm. The developed method was validated as per ICH guidelines. Method validation parameters like linearity, precision, accuracy, LOD and LOQ, robustness, ruggedness and assay were assessed. Linearity range was 20 - 100µg/ml with correlation coefficient ($R^2$) of 0.9996. LOD and LOQ were found to be 6.43µg/ml and 19.48µg/ml respectively. The percentage relative standard deviation for intraday precision was 0.78% and for inter-day precision was 0.60%. Accuracy was within the limits. Percent relative standard deviation for change in wavelength and analyst variation was 0.60% and 0.52% respectively. The validated method was finally applied to pharmaceutical formulations.

**Key Words**: Favipiravir (FVP), International Conference on Harmonization (ICH).

**INTRODUCTION**: Favipiravir[1] (FVP) is an antiviral drug with molecular weight of 157.104 g/mol and molecular formula $C_5H_4FN_3O_2$. FVP is soluble in ethanol, DMSO and slightly soluble in water. Favipiravir is a member of the class of pyrazines. It is an anti-viral agent that inhibits RNA-dependent RNA polymerase of several RNA viruses and is approved for the treatment of influenza. Favipiravir[2] inhibited the replication of viral genome. Anti-viral activity of favipiravir was attenuated in the presence of purine nucleosides or purine bases, indicating competition of favipiravir with purine nucleosides.

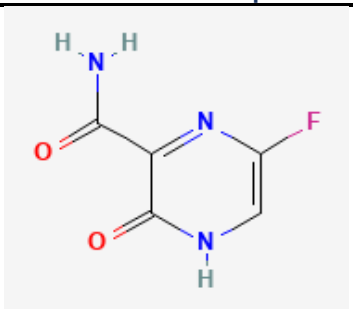


**Figure 1: Chemical Structure of Favipiravir**

**Materials and Methods:**

**Chemicals and reagents**

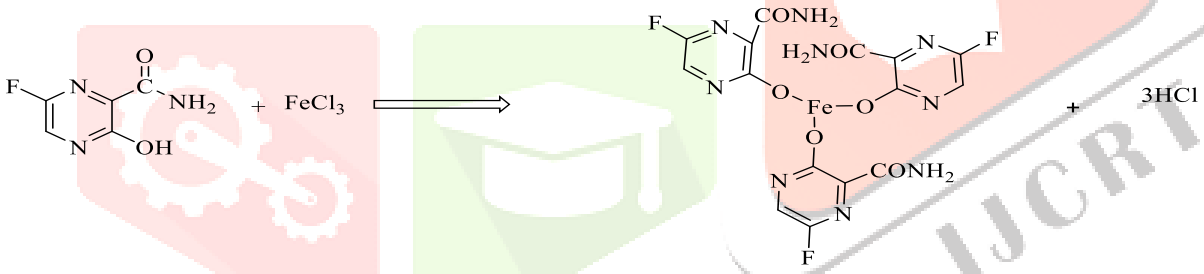
Favipiravir (pure drug) was obtained as a gift sample from Glenmark Dr. Reddy's Laboratories, ferric chloride ($FeCl_3$), Ethanol were purchased from Merk Ltd (Mumbai, India). All chemicals and reagents used were of analytical grade. Tablets containing drug Favipiravir (Fabiflu ® 400mg) were procured from local market.

**Instruments**: Electronic Precision balance (Infra Instruments Pvt. Ltd., Chennai), PC Based Double Beam Spectrophotometer 2202 (Systronics, India).

**Principle:**

Ferric chloride is an oxidizing agent. Favipiravir reacts with ferric chloride and form reddish brown coloured complex which can be estimated spectrophotometrically at 445 nm.

The schematic reaction is



Favipiravir Reddish brown complex

**Preparation of solutions:**

**Preparation of stock Solution:** Accurately 25mg of FVP pure drug was weighed in a 25 ml of volumetric flask, dissolved in 5ml of ethanol and made up to 25ml with distilled water to obtain a stock solution of 1000µg/ml.

**Preparation of 0.5% $FeCl_3$:** 500 mg of $Fecl_3$ was accurately weighed in a 100ml volumetric flask, dissolved in small quantity of distilled water and then made up to 100ml with distilled water.

**METHOD DEVELOPMENT:**

**Determination of λ max of Favipiravir:**

**Preparation of solution for the determination of absorption maxima**:

1ml of standard FVP drug solution (1mg/ml) was taken in a 10ml volumetric flask, to it 1ml of $FeCl_3$ (0.5%) was added. It was kept aside for 2-3 min for color development. Then it was made up to 10ml with distilled water.

**Preparation of blank solution**:

1ml of $FeCl_3$ was taken in a 10ml volumetric flask, to it 2-3 drops of ethanol was added and made up to 10ml with distilled water.

**Procedure:**

The absorbance of the drug solution was observed at different wavelengths (400 – 800nm) using blank solution as reference. The wavelength at which maximum absorbance was observed is $\lambda_{max}$.

**Sample Solution (Marketed Formulation Analysis):** For the preparation of the sample solution, twenty tablets were weighed and the average weight was determined. The tablets were crushed into a fine powder and tablet powder equivalent to 25 mg of Favipiravir (Label claim: 200 mg Favipiravir per tablet) was accurately weighed and transferred into a 25 ml volumetric flask and dissolved in 5ml of ethanol and made up to 25ml with distilled water, shaken well and filtered. Then 1 ml solution was pipette out and transferred into a 10 ml volumetric flask and make up volume up to mark with distilled water, from which 1 ml was further diluted to get the solution of 10 µg/ml.

**Optimization of 0.5% ferric chloride ($FeCl_3$) solution:**

**Preparation of solution for the optimization of 0.5% Ferric chloride:**

Different volumes of 0.5% Ferric chloride solutions were taken as reference and standard drug solution with different volumes of ferric chloride were taken and determined for absorbances.

Then the volume of 0.5% Ferric chloride solution was optimized.

**Validation of the developed method**

The validation of the developed analytical method was done according to the International Conference on Harmonisation (ICH) guidelines. The method is validated for linearity, recovery, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

## RESULTS AND DISCUSSION:

**Table 1: Determination of λmax for Favipiravir**

| S. No | Wavelength(nm) | Absorbance | S. No | Wavelength(nm) | Absorbance |
|---|---|---|---|---|---|
| 1 | 385 | 0.454 | 22 | 490 | 0.581 |
| 2 | 390 | 0.587 | 23 | 495 | 0.549 |
| 3 | 395 | 0.600 | 24 | 500 | 0.517 |
| 4 | 400 | 0.616 | 25 | 505 | 0.480 |
| 5 | 405 | 0.634 | 26 | 510 | 0.446 |
| 6 | 410 | 0.652 | 27 | 515 | 0.414 |
| 7 | 415 | 0.672 | 28 | 520 | 0.381 |
| 8 | 420 | 0.686 | 29 | 525 | 0.345 |
| 9 | 425 | 0.700 | 30 | 530 | 0.314 |
| 10 | 430 | 0.708 | 31 | 535 | 0.281 |
| 11 | 435 | 0.716 | 32 | 540 | 0.252 |
| 12 | 440 | 0.717 | 33 | 545 | 0.229 |
| 13 | 445 | 0.720 | 34 | 550 | 0.201 |
| 14 | 450 | 0.715 | 35 | 555 | 0.181 |
| 15 | 455 | 0.710 | 36 | 560 | 0.160 |
| 16 | 460 | 0.705 | 37 | 565 | 0.141 |
| 17 | 465 | 0.693 | 38 | 570 | 0.120 |
| 18 | 470 | 0.674 | 39 | 575 | 0.109 |
| 19 | 475 | 0.655 | 40 | 580 | 0.094 |
| 20 | 480 | 0.631 | 41 | 590 | 0.071 |
| 21 | 485 | 0.608 | 42 | 600 | 0.051 |

**Result**: The absorption maximum (λmax) of Favipiravir was found to be 445 nm

**Table 2: Optimization of 0.5% ferric chloride ($FeCl_3$) solution**

| S. No | Contents in the flask | | Absorbance |
|---|---|---|---|
| | Blank | Sample | |
| 1. | 0.5 ml of ferric chloride+ 2-3 drops of ethanol made up to 10ml with distilled water | 1ml stock solution+ 0.5 ml ferric chloride, allowed to stand for 2-3 minutes and then made up to 10ml with distilled water. | 0.594 |
| 2. | 1 ml of ferric chloride + 2-3 drops of ethanol made up to 10ml with distilled water | 1ml stock solution+ 1 ml ferric chloride, allowed to stand for 5 minutes and then made up to 10ml with distilled water. | 0.622 |
| 3. | 1.5 ml of ferric chloride+ 2-3 drops of ethanol made up to 10ml with distilled water | 1ml stock solution+ 1.5 ml ferric chloride, allowed to stand for 5 minutes and then made up to 10ml with distilled water. | 0.618 |
| 4. | 2 ml of ferric chloride+ 2-3 drops of ethanol made up to 10ml with distilled water | 1ml stock solution+ 2 ml ferric chloride, allowed to stand for 5 minutes and then made up to 10ml with distilled water. | 0.616 |
| 5. | 2.5 ml of ferric chloride+ 2-3 drops of ethanol made up to 10ml with distilled water | 1ml stock solution+ 2.5 ml ferric chloride, allowed to stand for 5 minutes and then made up to 10ml with distilled water. | 0.610 |

**Result:** 1 ml of 0.5% ferric chloride solution was optimized.

**Method Validation:**

**Linearity:**

**Table 3: Linearity data of Favipiravir**

| Concentration (mcg/ml) | Absorbance |
|---|---|
| 20 | 0.169 |
| 40 | 0.335 |
| 60 | 0.515 |
| 80 | 0.680 |
| 100 | 0.870 |

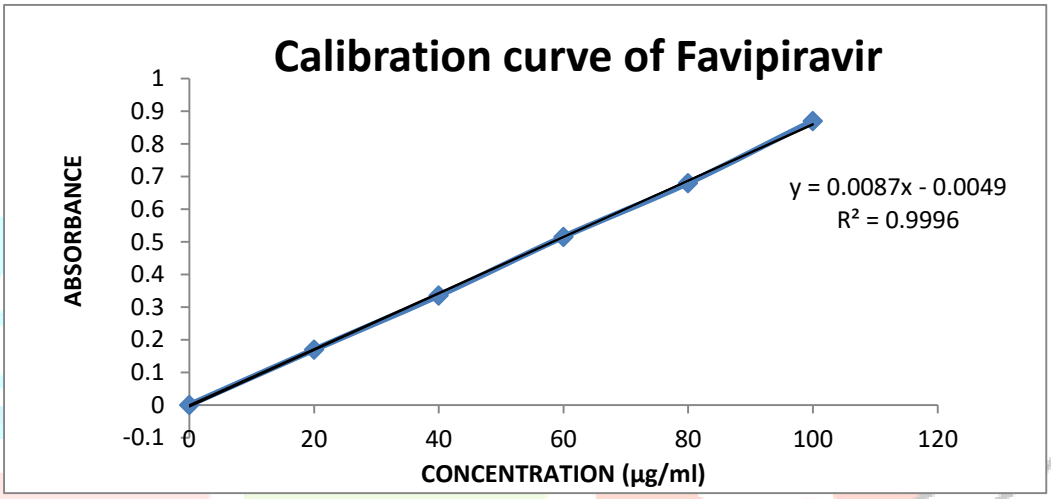



**Figure 2: Calibration curve of Favipiravir**

**Result:** The linearity range was 20µg/ml – 100µg/ml. The Regression equation (y = mx+c) obtained was 0.0087x-0.0049. Correlation coefficient ($R^2$) value obtained was 0.9996.

**Precision:**

i) **Intraday precision**: 6 determinations of 80 mcg/ml solution were done 3 times in a day and observed for absorbances.

**Table 4: Intraday precision data of Favipiravir**

| Morning | Afternoon | Evening |
|---|---|---|
| 0.685 | 0.698 | 0.682 |
| 0.680 | 0.678 | 0.678 |
| 0.690 | 0.676 | 0.685 |
| 0.682 | 0.682 | 0.685 |
| 0.678 | 0.685 | 0.688 |
| 0.679 | 0.681 | 0.681 |

**Result:** % RSD for intraday precision was found to be 0.78%

ii) **Interday precision:** 6 determinations of 80 mcg/ml solution were done for 3 consecutive days and observed for absorbances.

**Table 5: Interday precision data of Favipiravir**

| Day – 1 | Day – 2 | Day - 3 |
|---|---|---|
| 0.688 | 0.690 | 0.681 |
| 0.689 | 0.682 | 0.686 |
| 0.681 | 0.688 | 0.685 |
| 0.680 | 0.685 | 0.684 |
| 0.690 | 0.683 | 0.689 |
| 0.682 | 0.682 | 0.676 |

**Result:** % RSD for interday precision was found to be 0.60%.

**Robustness:** 6 determinations of 80mcg/ml solution were done and observed for absorbances at different wavelengths (444nm and 446nm) with respect to optimized wavelength (445nm).

**Table 6: Robustness data of Favipiravir**

| 444 nm | 445 nm | 446 nm |
|---|---|---|
| 0.676 | 0.678 | 0.679 |
| 0.682 | 0.685 | 0.685 |
| 0.685 | 0.690 | 0.682 |
| 0.678 | 0.688 | 0.684 |
| 0.688 | 0.684 | 0.690 |
| 0.680 | 0.686 | 0.686 |

**Result:** % RSD for change in wavelength was found to be 0.60%.

**Ruggedness:** 6 determinations of 80mcg/ml solution was done by two different analysts and observed for absorbances.

**Table 7: Ruggedness data of Favipiravir**

| Analyst - 1 | Analyst - 2 |
|---|---|
| 0.690 | 0.688 |
| 0.685 | 0.691 |
| 0.682 | 0.689 |
| 0.685 | 0.682 |
| 0.682 | 0.687 |

**Result:** % RSD for analyst variation was found to be 0.52%.

**Limit of detection and Limit of quantification:**

LOD and LOQ were determined from calibration curve. LOD and LOQ for Favipiravir were found to be 6.43µg/ml and 19.48µg/ml respectively.

**Accuracy:** Accuracy was performed at three different levels and percent recovery was found to be 100.59%.

**Assay**: Assay was performed for marketed formulation ( AVIGAN 200mg) and was found to be 99.85%.

## CONCLUSION:

From the literature review only very few colorimetric methods have been developed. The developed visible spectrophotometric method was found to be simple, sensitive, linear, precise, accurate and also robust. The developed method was validated as per ICH guidelines and satisfactory results were obtained. The method has an acceptable precision and accuracy. Hence, this method can be effectively applied for routine analysis in research institutions.

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