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Cleaning method development and validation by UV method for quantitative assessment of favipiravir residue in manufacturing area

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Abstract

Favipiravir is a potent antiviral substance and has one of the drugs of choice in treating patients affected with COVID-19. A well-prepared cleaning validation master plan was proposed and executed for ensuring the low level of carryover of favipiravir into the next drug product. The current study aimed at developing a precise analytical method using a UV spectrophotometer and validating the same for verification of residues of favipiravir in the manufacturing equipment. A stratified swab sampling method was employed for collecting the residues on a stainless steel 316 sheet (4 × 4 × 2 mm). Swabs were streaked ten times bi-directionally all along the SS plate horizontally, vertically, and diagonally as an approach to collect the residue. UV detection was made at λmax 367 nm using methanol as diluent. The calibration curve was found to be linear in the range of 1 to 10 µg/ml. With a regression value of R^2 = 0.999. Method precision and intermediate precision were carried out and RSD was found to be 0.06 and 0.058%. Accuracy at three different concentrations was performed and found to be 98.8% to 102% in (50,100,150%). %recovery factor was found to be 99.09% (usually it should be >80%). All the validation parameters were performed and they were within the limits. The simplicity of the developed spectrophotometric method can help analyze the favipiravir on a routine basis.

1. Introduction

In the pharmaceutical industry, there is an incredible need for cleaning equipment and handling regions. Inappropriate cleaning can prompt defilement and cross-contamination (Agrawal *et al.*, 2020). A drug product can be tainted by different residue materials like residues of recently utilized dynamic drug fixings, a natural substance, cleaning specialists and residue particles (Baokar *et al.*, 2013). The primary goal of GMP comprises the anticipation of contamination and cross-contamination of materials. The purpose of cleaning validationis to check the adequacy of the cleaning strategies for the evacuation of deposits of the past item, additives, cleaning specialists and microbial pollutants. Cleaning validation satisfies the necessity of administrative bodies and keeps up with item quality and security of the purchasers (Forsyth and Haynes, 1998).

Favipiravir is a type of antiviral drug. Favipiravir was approved in Japan in 2014 to treat cases of influenza during a pandemic condition (Bharti mittu and Chauhan, 2015). It is most typically used to treat patients with COVID-19. 6-fluoro-3-hydroxy-2-pyrazine carboxamide is its biochemical name, and it is sold under the brand names ; avigan, fabiflu and favipil. A pyrazine derivative, favipiravir is an antiviral drug (Blessy *et al.*, 2014). The method involves the inhibition of the RNA-dependent RNA polymerase molecule, which is required for viral genome transcription and replication. Toyama Chemical Co., Ltd developed the drug in Japan for the treatment of influenza A and B, and it is only approved for use in Japan (Ibrahim Bulduk, 2021).

Jyothi and Kavya (2021) established a single UV approach for favipiravir and concluded that the supplied spectrophotometric method should be used to estimate the novel antiviral repurposing medicine favipiravir because no simple UV spectrophotometric method for estimation has been disclosed. Favipiravir was developed and validated according to ICH principles since the medicine has a wide range of formulations that can be generated to treat different virus.

Dikma Technologies devised a simple HPLC-UV technique for simultaneous measurement of ivermectin, molnupiravir, remdesivir, favipiravir and ritonavir diamonsil® Plus C18 column (Kathiresan *et al.*, 2003; Megahed *et al.*, 2021; Shiraki and Daikoku, 2020).

The proposed approach was effectively implemented for the commercial formulations of favipiravir tablets, according to Nadendla and Patchala, who developed the HPLC method with a PDA detector (Nadendla and Abhinandana, 2021). Furthermore, the proposed method's key features are that it is cost-effective and environmen-tally friendly, with a retention time of nearly 4.622 min (Zhu *et al.*, 2019; Saber, 2020; Sohrabi, 2019).

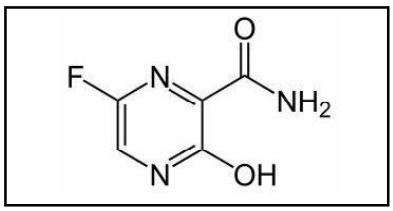

Figure 1: The structure of favipiravir.

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According to a literature review, no work on cleaning validation of favipiravir has been published. The current research focuses on the development and validation of cleaning methods for quantitative estimation of favipiravir residue in the manufacturing area.

2. Materials and Methods

2.1 Instruments

Double beam UV Spectrophotometer Elico model with spectral treats SL-210 software is used.

2.1.2 Chemicals and reagents

Favipiravira gift from Hetro Labs Hyderabad., Swabs with polypropylene sticks were obtained from Himedia Lab. Ltd. Mumbai, India. Hi clean swab PW1160-100 No. Stainless steel (316) plate with a size: of 4 × 4 × 2 mm thickness was used in this experimental work. Methanol HPLC grade from Rankem Chemicals Ltd was used throughout the study.

2.2 Preparation of solutions for standard stock

2.2.1 Standard stock solution

5 mg of favipiravir was weighed and placed in a 5 ml volumetric flask, which was then diluted to the desired concentration with methanol to get 1000 µg/ml.

2.2.2 Standard stock solutions

Pipette 1 ml of the aforesaid standard stock solution into a 10 ml volumetric flask and top up with methanol to the mark. The concentration of the resultant solution is 100 µg/ml. Various serial dilutions of this standard stock solution-II were prepared to attain concentrations of 1,2,3,4,5,6,7,8,9,10 µg/ml.

2.2.3 Preparation of test solution

Ten favipiravir tablets were precisely weighed and taken to a dry and clean mortar, where they were ground into a powder form, weighed tablet powder equivalent to 10 mg (11.54 mg), and transferred to a 10 ml volumetric flask. 5 ml diluent was added and sonicated for 15 min to dissolve it, then it was built up to the mark (1000 µg/ml).

2.2.4 Preparation test solution

When 1 ml of the aforesaid test solution-I was transferred to a 10 ml flask and diluted with methanol, 100 µg/ml was obtained. To create 10 µg/ml, 1 ml of the aforementioned solution was added to 10 ml of the flask.

2.2.5 Selection of a wavelength for favipiravir analysis

1 ml of the standard stock solution-II was transferred to a 10 ml volumetric flask and diluted with methanol to a concentration of 10 µg/ml. The UV range (200-400 nm) was used to scan the final solution.

2.3 Cleaning method

In the cleaning process, swabs and stainless steel plates are employed. On a stainless steel plate, a 1 ml volume of 10 µg/ml favipiravir concentration was pipette and evaporated to dryness. The heads of swabs were soaked in methanol and the whole surface of the plates was cleaned with swabs dampened with the appropriate solvent, starting from the outside and working inwards, horizontally, and then vertically. The heads of the swabs were placed in a 10 ml volumetric flask and diluted with 5 ml of the solvent squeezed over the flask's walls. The volumetric flasks were sonicated for 15 min after being capped. Methanol can be used to make up the volume. Ultraviolet-visible spectroscopy was used to test the solution's absorbance at 367 nm.

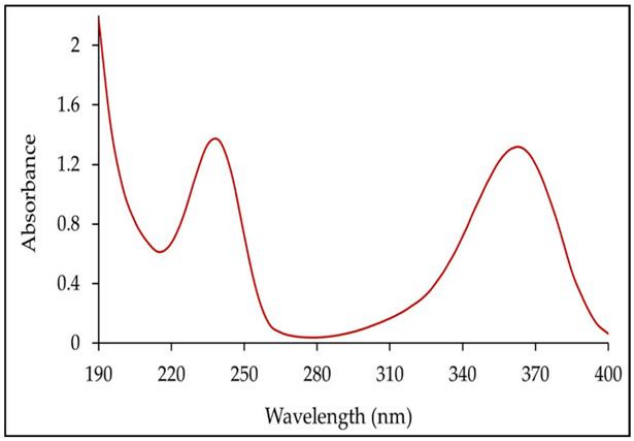

Figure 2: Spectra showing maximum wavelength of favipiravir in methanol.

Figure 3: Stainless steel plate.

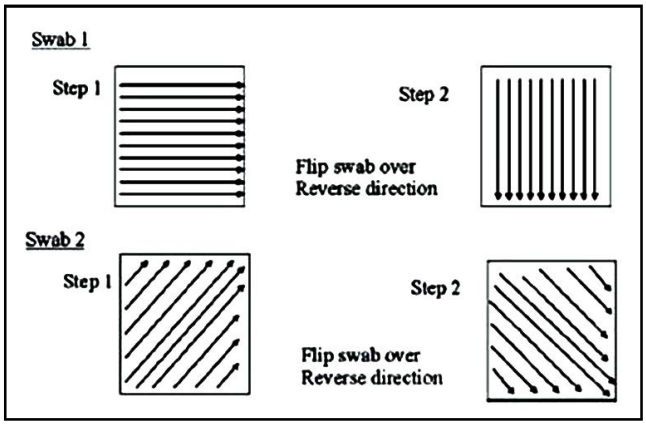

Figure 4: Swabbing.

2.4 Analytical method validation by UV spectroscopy method

2.4.1 Linearity

To test the linearity of the analytical method, a series of dilutions ranging from 1 to 10 µg/ml were made. The absorbance readings were measured at 367 nm. The linearity curve was plotted against

the concentration verse absorbance. Beer's compliance with Lambert's law (linearity) was identified and stated to be within concentration range.

2.4.2 Precision

Precision: Precision was considered at two levels

2.4.2.1 Repeatability (intra-day repeatability)

Six repetitions of 10 µg/ml concentrations were used to test intra-day precision. For intra-day precision, all replications were prepared on the same day and analytical validation was done.The data is shown as % relative standard deviation and values are acceptable, indicating that the approach is precise.

2.4.2.2 Intermediate precision

To attain intermediate precision, favipiravir was taken at a concentration of 10 µg/ml in six formulations. The intermediate precision investigation took several days to complete. The data is shown as %relative standard deviation and values are acceptable, indicating that the approach is precise.

2.4.3 Accuracy

Measurement of recovery for three concentrations that cover the method's range was used to determine the method's accuracy (50, 100 and 150 per cent of the test solution concentration). Three sets of absorbances were generated for each concentration, and the results were recorded. The favipiravir medication concentrations were then calculated.

Pipetted 0.2 ml, 0.4 ml and 0.6 ml of favipiravir at respective concentrations of 2,4,6 µg/ml onto a stainless steel plate with a predefined 10 cm^2 surface area and leave to evaporate. The heads of adsorbent swabs were saturated with methanol. Starting from the outside to the inside, the entire surface of the plates was wiped down, first horizontally, then vertically and diagonally fashioned. The heads of the swabs were placed in a 10 ml volumetric flask with 5 ml solvent. After being capped, these volumetric flasks were sonicated for 15 min and diluent to make up the difference in volume. From these concentrations, 1 ml of each µg/ml concentration was spiked with 4 µg/ml of test solution from these concentrations. A UV-vis spectrophotometer was used to determine the solution's absorbance using methanol as a referenceat λ max 367nm.

2.4.4 limit of detection (LOD) and Limit of quantitation (LOQ)

Limits of detection (LOD) and quantitation (LOQ) were used to determine the sensitivity of the estimate procedure.

The following are the measurement formulae:

3.3 x SD/slope is the LOD

10 x SD/slope is the LOQ

where SD is the precision standard deviation of 10 µg/ml.

S is the calibration curve's slope.

2.4.5 Robustness

Robustness should be utilized as a parameter to characterize the method's stability in the face of changes in the method's internal factors.

2.4.6 Ruggedness

When external elements such as the analyst, laboratory, equipment, reagents and days change, ruggedness should be utilized as a criterion for evaluating the consistency of the results.

2.5 %assay method for favipiravir tablet

Ten favipiravir tablets were weighed exactly and taken to a dry and clean mortar, where they were ground into a fine powder, weighed tablet powder equivalent to 10 mg (11.54 mg) and placed in a 10 ml volumetric flask. 5 ml diluent was added and sonicated for 15 min to dissolve it, after which it was gradually increased to the desired concentration (1000 µg/ml). The solution was then transferred to a 10 ml flask and diluted with methanol to achieve a concentration of 100 µg/ml. 1 ml of the above-mentioned solution was added to 10 ml of the flask to make 10 µg/ml.

% assay = (absorbanceof sample/absorbance of standard) x (concentration of standard/concentration of sample) x 100

= (0.9332/0.9458) x (10/9.86) x 100

= 99.58%

3. Results

3.1 Linearity

As shown in Figure 5, the calibration plot of absorbance versus concentration was determined to be linear over the concentration range of 1 to 10 g/ml. The correlation coefficient (r^2) was 0.9999 (Table 1). As a result, test results were directly proportional to analyte concentration (amount) in the sample.

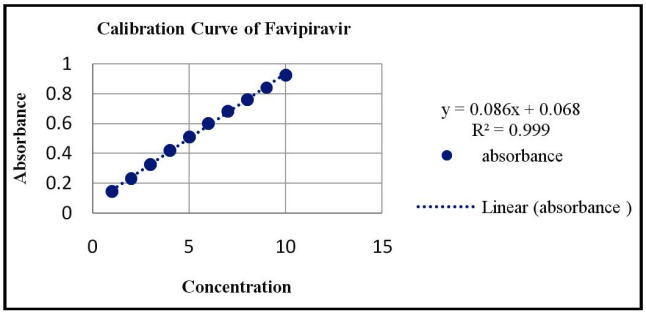

Figure 5: Calibration curve of favipiravir by UV method.

Table 1: Results of linearity studies

Concentration (µg/ml)	Absorbance
1	0.1464
2	0.2335
3	0.3286
4	0.4213
5	0.5123
6	0.6012
7	0.6852
8	0.763
9	0.8427
10	0.9458

3.2 Precision

Table 2 shows the computed per cent RSD of intra-day and intermediate precision tests. The per cent RSD of six replicates should not exceed 2 for a precise analytical procedure. The intra-day percent RSD of six replicates was 0.062 per cent and the inter-day per cent RSD was 0.058 per cent. The procedure was good and exact, as seen by the low per cent RSD value.

Table 2: Results of precision data

S.No	Concentration (µg/ml)	Intra-day precision	Intermediate precision
1	10	0.9458	0.9458
2	10	0.9461	0.9460
3	10	0.9464	0.9465
4	10	0.9468	0.9468
5	10	0.9470	0.9470
6	10	0.9474	0.9472
Mean		0.9465	0.94655
SD		0.00059	0.00055
%RSD		0.0628	0.05895

Table 3: Results of accuracy data

%conc	Sample amount (µg/ml)	Amount added (µg/ml)	% recovery	%recovery means	SD	%RSD
50%	4	2	100	100.2	0.213	0.2
	4	2	100.2			
	4	2	100.3			
100%	4	4	100	100.6	0.8165	0.81
	4	4	100.4			
	4	4	101.5			
150%	4	6	100	100.1	0.115	0.114
	4	6	100.11			
	4	6	100.23			

3.3 Accuracy

The goal of this experiment was to demonstrate that the assay findings obtained by the proposed approach were accurate. Table 3 shows the results of the recovery investigations. With lower RSD values, mean recovery was found to be 100.2%, 100.6%, 100.1%.

3.4 Limit of detection (LOD) and limit of quantification (LOQ)

The standard deviation of the response (σ) and slope of the calibration curve (S) was obtained using the calibration curve and calculations were performed using equations (3.3 σ)/S for LOD and (10 σ)/S for LOQ. The LOD was determined to be 0.022 g/ml, while the LOQ was 0.0678 g/ml. Table 4 summarises the findings.

3.5 Robustness

The robustness of the presented technique was tested using changes in the solvents and lambda max. The % RSD was found to be within the acceptable limit. The findings are summarised in Table 5.

Table 4: Results of LOD and LOQ

Parameters	Observed values
Slope of regression	0.0869
Standard deviation	0.0869
LOD	0.022 µg/ml
LOQ	0.0678 µg/ml

Table 5: Robustness data results

S.No.	Concentration (µg/ml)	Wave length (nm)	Absorbance	Calculations
1	10	367 nm	0.9555 0.9560 0.9614	Mean=0.9576 SD=0.003272 %RSD=0.34
2	10	368 nm	0.9481 0.9490 0.9401	Mean=0.945733 SD=0.004899 %RSD=0.52

3.6 Ruggedness

The ruggedness of the suggested approach was investigated by varying the environmental conditions, such as employing different instruments, analysts and equipment. The percent RSD was found to be within acceptable bounds.

Table 6: Results of ruggedness data

S. No.	Concentration (µg/ml)	Absorbance analyst-1	Absorbance analyst-2
1	10	0.9458	0.9457
2	10	0.9460	0.9463
3	10	0.9463	0.9467
4	10	0.9465	0.9471
Mean		0.94615	0.9464
SD		0.0031	0.0056
%RSD		0.032	0.058

3.7 % recovery factor

Favipiravir concentrations of 10 µg/ml were pipette onto a stainless steel plate in a 1ml volume and allowed to evaporate. The head of adsorbent swabs was saturated with methanol and the complete surface of the plates was progressively wiped in a horizontal and then vertical direction, starting from the outside towards the center. The swabs' heads were placed in a 10 ml volumetric flask containing 5 ml of the solvent, which was then capped and sonicated for 15 min. Squeezed around the container's walls and filled with methanol to make up the volume. A UV-vis spectrophotometer was used to test the solution's absorbance at 367 nm.

$$\%\text{recovery factor} = (\text{swab sample absorbance}/10\ \text{ppm absorbance}) \times 100$$

$$= 0.9271/0.9356 \times 100$$

$$= 99.09\%$$

Table 7: Summary of results

Validation parameters	Results
Absorbance maximum (nm)	367 nm
Linearity range	1-10 µg/ml
Accuracy of % recovery	98.88%-102% (50, 100, 150%)
% RSD (precision)	0.06% and 0.058%
Correlation coefficient (r^2)	0.999%
%assay	99.58%
LOD	0.022 µg/ml
LOQ	0.0678 µg/ml

4. Discussion

From this study we measure the concentration of left over residual substance with linear Correlation coefficient 0.999.A stratified swab sampling method was employed for collecting the residues on a stainless steel 316 sheet (4 × 4 × 2 mm). Swabs were streaked ten times bi-directionally all along the SS plate horizontally, vertically, and diagonally as an approach to collect the residue.

UV detection was made at λmax 367 nm using methanol as diluent. The calibration curve was found to be linear in the range of 1 to 10 µg/ml. Method precision and intermediate precision were carried out and RSD was found to be 0.06 and 0.058%. Accuracy at three different concentrations was performed and found to be 98.8% to 102% in (50, 100, 150%). % recovery factor was found to be 99.09% (usually it should be >80%). All the validation parameters were performed and they were within the limits.

5. Conclusion

Cleaning validation tests were conducted by the cleaning validation protocol. The results of the investigation showed that swabs recovered well (99.5%). In terms of linearity, precision, accuracy and repeatability investigations, this proposed UV spectroscopic method was validated according to ICH requirements. All of the validation parameters were confirmed to be within the acceptable range. The devised method was effectively implemented in the manufacturing area to estimate favipiravir residue. The developed spectrophotometric method's simplicity will make it easier to analyze favipiravir regularly.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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