# STATISTICAL VALIDATION OF THREE UV SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF FAVIPIRAVIR IN TABLETS

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**ABSTRACT**

In the present study, the three different UV spectrophotometric techniques were evolved and validated for determination of favipiravir (FAVI) in tablets. Single point method (1st method) includes the absorption maxima wavelength (λ) at 362.0 nm, area under curve (2nd method) includes λ choice between 357.0 – 367.0 nm and first order derivative (3rd method) includes 341.5 nm (maxima) and 379.5 nm (minima) as absorption λ for FAVI estimation. All the three methods were obeying the Beer's law with 5-30 μg $mL^{-1}$ concentration range in 0.1 N NaOH. The correlation coefficient were found to be 0.9991 (single point method), 0.9994 (area under curve) and 0.9998 (first order derivative method). The accuracy, LOD, LOQ and precision were determined for validation study as per ICH guideline. The present methods were statistically compared using single factor ANOVA test. Statistical results shows that there is no difference between these methods used for analysis. Hence, the suggested UV techniques have been effectively used to estimate FAVI in tablets for routine analysis.

**Keywords**

UV spectrophotometer, favipiravir, area under curve, first order derivative, validation

## 1. **Introduction**

An antiviral medication, favipiravir (FAVI) is used to treat influenza and may also be effective against other viral infections. Life-threatening diseases including the COVID19 virus, Lassa and Ebola viruses have all been treated with FAVI[1,2]. Chemically, FAVI (Fig. 1) is 6-fluoro-3-hydroxypyrazine-2-carboxamide, molecular weight is 157.10 g $mol^{-1}$ and molecular formula is $C_5H_4FN_3O_2$. The FAVI is a powder that is whitish, pale yellow in hue. It is only weakly soluble in 99.5% ethanol and just slightly soluble in acetonitrile, methanol and water[3]. A comparison between UV spectrophotometric and HPLC methods for quantifying FAVI was published by Bulduk et al[4]. A review of the literature found that different methods of analysis have been published for FAVI in API and formulations. These

techniques include RP-HPLC methods[5,6], stability-indicating RP-HPLC[7-10], LC-MS/MS[11] and UPLC-MS/MS[12]. Menthol assisted liquid-liquid micro-extraction of HPLC/UV measurement for FAVI in human plasma was reported by Abdallah et al[13]. Hailat *et. al.* and Duse et. al. described validation of FAVI in spiked human plasma[14,15]. In the reported literature UV method[16], ethanol and water solvent were used and all analysis was performed at 234 nm.

Literature survey reveals that no other methods for UV spectrophotometric analysis were reported. Hence, it was thought to estimate FAVI by using three different methods of analysis for improving analytical profile and linearity range of the drug. This present paper included single point, area under curve and first order derivative methods were developed and validated. The developed techniques for determination of FAVI and its tablets were simple, straightforward, accurate, repeatable, quick, affordable and precise.

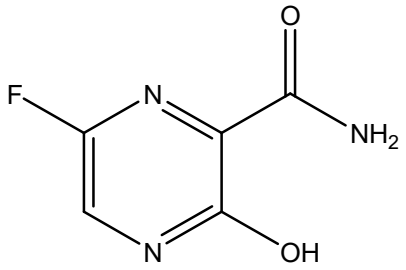



**Fig. 1: Structure of favipiravir**

## 2. Materials And Methods

### 2.1 Instruments and chemicals

All spectrophotometric estimations were performed using a Shimadzu UV-1800 double beam spectrophotometer with a 1 cm path length and Shimadzu UV-Probe software (version 2.35). FTIR Brukar, Mumbai, India and Shimadzu balance (AUX 220) with OPUS software were employed. Distilled water and sodium hydroxide (AR grade) was used as a solvent reagents. FAVI was obtained from Glenmark Life Sciences Ltd., Daund, Pune, Maharashtra, India. FAVI tablets were procured from local market. The brand name is Flumyc 400 from Ipca Laboratories Ltd., Mumbai, India was used.

### 2.2 FAVI standard solution

To generate the standard FAVI solution, 100 mg of FAVI was dissolved in enough 0.1 N NaOH solvent to yield 100 mL of solution in a volumetric flask, containing 1000 µg $mL^{-1}$ of pure FAVI solution. A working standard solution containing 100 µg $mL^{-1}$ was prepared by pipetting out an aliquot solution and diluting it with 0.1 N NaOH.

### 2.3 Analysis of FAVI tablet

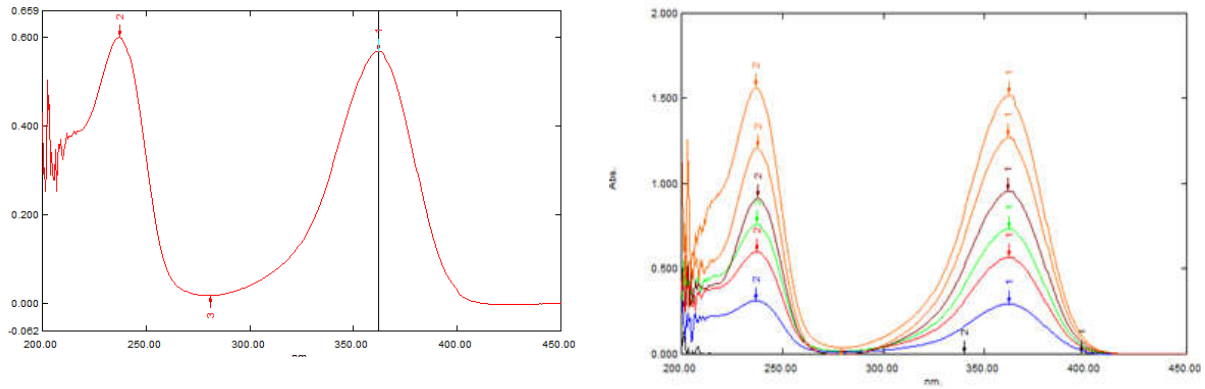
Accurately weighed and pulverized twenty tablets of FAVI. After weighing, transferring FAVI tablet powder euivalent 100 mg of FAVI into a 100 mL volumetric flask, 25 mL of 0.1 N NaOH was added to dissolve the tablet powder. After 20 min of sonication, the mixture was filtered through whatman filter paper 41. A final volume of 100 mL was make up using 0.1 N NaOH in order to achieve a concentration of 1000 µg $mL^{-1}$. A suitable dilution of 0.1 N NaOH was obtained by pipetting off an aliquot solution, yielding a concentration of 20 µg $mL^{-1}$. The tablet solution was prepared in different dilutions, examined five times and the concentration was determined using the calibration curve.

### 2.4 Procedure for UV methods of analysis

#### 2.4.1 Single point method

The wavelength maximum available with the instrument was used for this method. The absorbance maxima for FAVI was found to be 362 nm.

This mode scans FAVI solutions in the 400 - 200 nm wavelength region before providing the spectral data for sample solutions. To achieve this, standard drug solutions comprising 5 to 30 µg $mL^{-1}$ of FAVI were prepared. The multicomponent mode delivers a collected spectral data of all these solutions after scanning all of them. Solutions of FAVI were serially diluted with 0.1 N NaOH for linearity testing in order to acquire varying concentrations in the range of 5 to 30 µg $mL^{-1}$. UV Spectra and overlain of FAVI were shown in Fig. 2.




(A) (B)

**Fig. 2: (A) UV spectra in 10 µg mL$^{-1}$ and (B) overlain spectra 5 to 30 µg mL$^{-1}$ of FAVI**

### 2.4.2 Area under curve (AUC) method

To achieve a concentration of 20 µg mL$^{-1}$ for FAVI, the standard solutions were diluted with 0.1 N NaOH and scanned in the spectrum mode between 400 and 200 nm. Area under curve (AUC) in the 357- 367 nm region was chosen from the drug's spectra for study, as indicated in Fig. 3. At their respective AUC range of 357-367 nm, the calibration curve was produced in the concentration range of 5 to 30 µg mL$^{-1}$. The concentration of the sample solution can be estimated by utilizing the calibration curve.

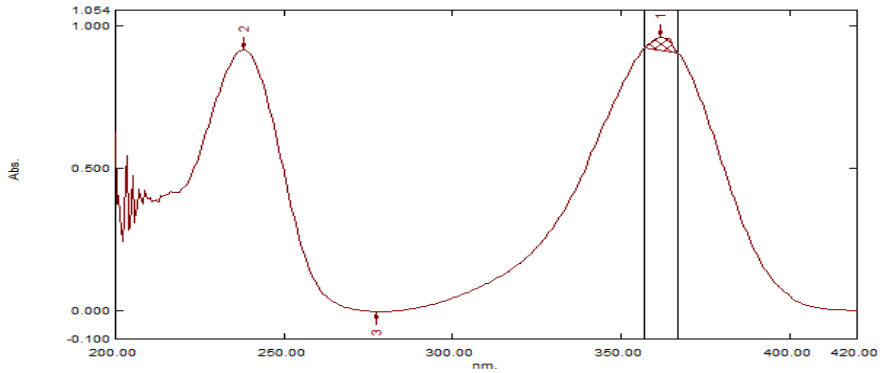
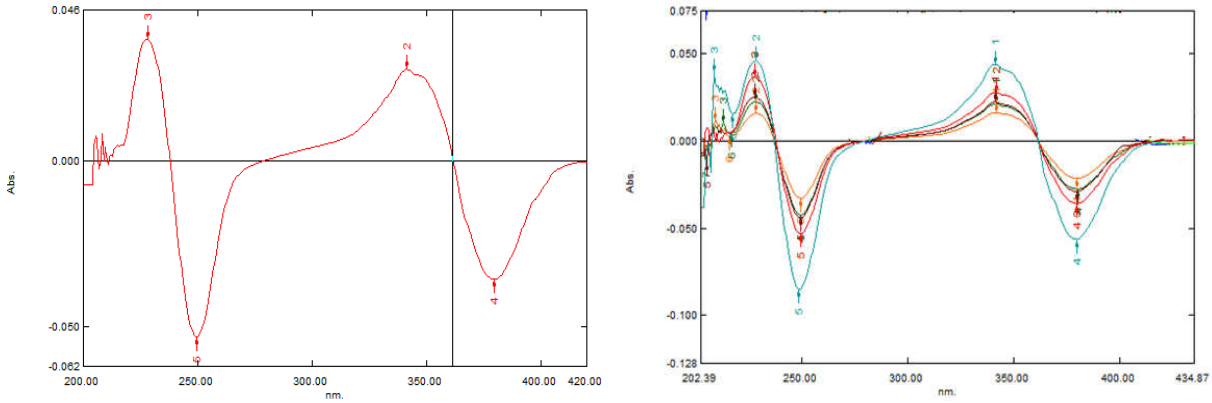



**Fig. 3: UV spectrum of FAVI for area under curve method (357 nm-367 nm)**

### 2.4.3 First order derivative (dA/dλ) method

The standard solutions was performed to get a 20 µg mL$^{-1}$ FAVI concentration. Each of these solutions was scanned independently between 400-200 nm in the UV spectrophotometer.

After that, the spectral data was processed to produce a first-order derivative spectrum. FAVI showed a zero crossing at 292.5 nm and 362.0 nm showed a significant dA/dλ. The wavelengths 341.5 nm and 379.5 nm were chosen as analytical wavelengths for the determination of FAVI maxima and minima, respectively, as shown in Fig. 4. A calibration curve was computed for each of the standard solutions. Individual FAVI standard solutions were serially diluted with 0.1 N NaOH for linearity testing in order to generate varying concentrations in the range of 5 to 30 µg $mL^{-1}$.




(A) (B)

**Fig. 4: (A) First order derivative spectra in 20 µg $mL^{-1}$ and (B) overlain spectra in 5 to 30 µg $mL^{-1}$ of FAVI**

## 3. Results and Discussion

### 3.1 Single point method

FAVI solution of 10 µg $mL^{-1}$ was obtained by appropriately diluting standard stock solution, and it was scanned in the spectrum mode between 400-200 nm in order to determine the analytical wavelength. The wavelength of 362 nm was chosen for investigation based on the FAVI λmax spectrum. At 362 nm, the concentration range of 5 to 30 µg $mL^{-1}$ was used to produce the calibration curve. Calibration graphs, as shown in Fig. 5, were produced by applying linear least-square regression analysis to the absorbance against drug concentration data.

### 3.2 AUC method

For selection of analytical wavelength, 20 µg $mL^{-1}$ solution of FAVI was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400-200 nm. From the spectra of drug, AUC in the range of 357-367 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 5 to 30 µg $mL^{-1}$ at their respective AUC range. By using calibration curve, the concentration of the sample solution can be determined. The data of absorbance verses drug concentration were treated by linear least-square regression analysis to obtain calibration graphs as shown in Fig. 5.

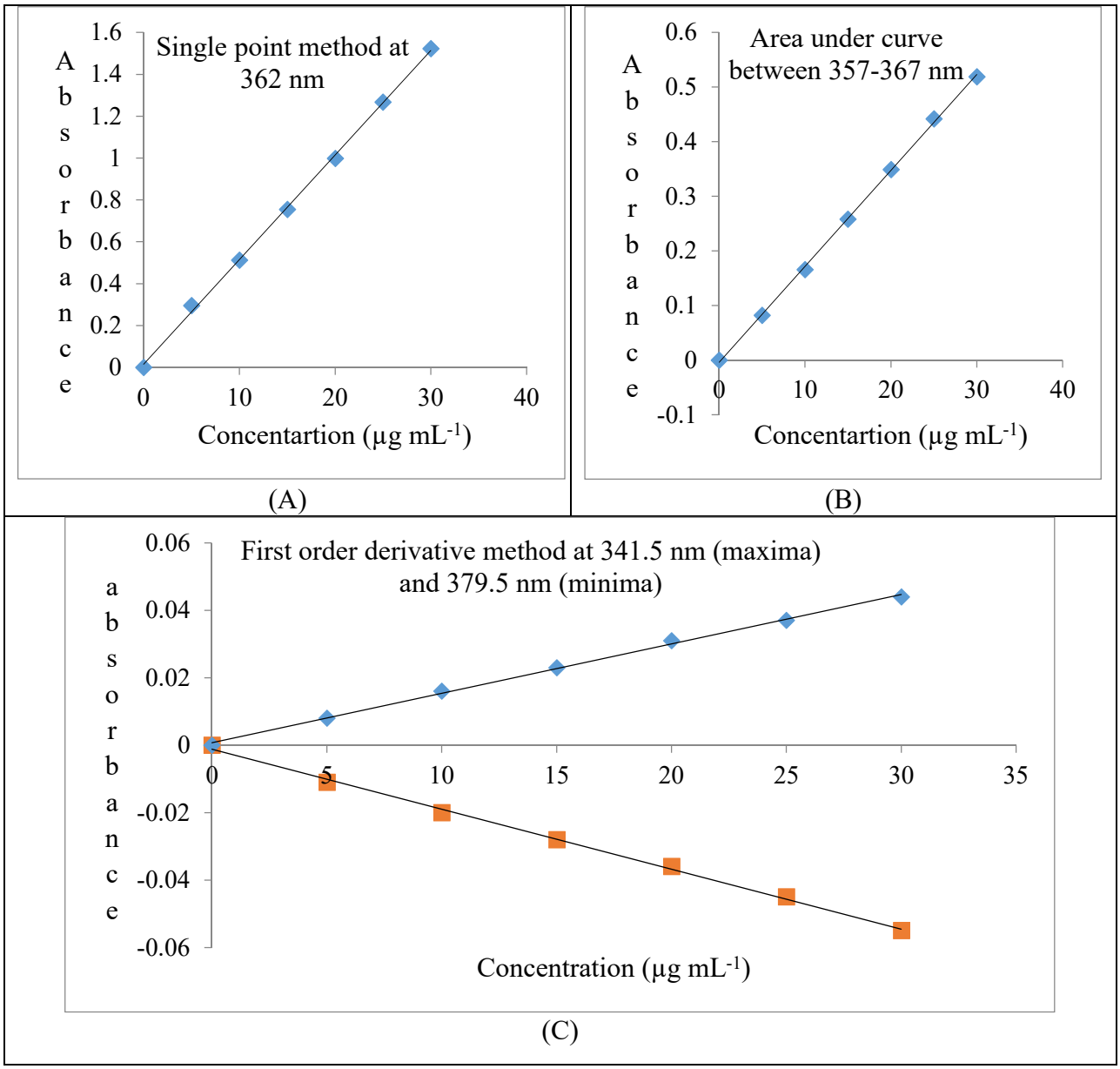



**Fig. 5: Calibration curve of (a) single point method, (b) area under curve method and (c) first order derivative method for FAVI**

### 3.3 First order derivative method

By appropriately diluting standard stock solution, a 20 µg $mL^{-1}$ solution of FAVI was obtained using this procedure and it was scanned in the spectrum mode between 400-200 nm. First order derivative spectra were obtained by derivating the acquired absorption spectra. Maxima and minima are showned in the first order derivative spectra at 341.5 nm and 379.5 nm, respectively. It is calculated to find the absorption difference, which is directly proportional to the standard solution concentration. First order derivative spectra were scanned to plot the calibration curve for FAVI in the concentration range of 5 to 30 µg $mL^{-1}$. The d1A/dλ1 calibration curve demonstrated linearity when plotted against drug concentration. To create calibration graphs, linear least-square regression analysis was applied to the absorbance versus drug concentration data as depicted in Fig. 5.

### 3.4 Method validation

#### 3.4.1 Linearity and range

For preparation of concentrations range of 5 to 30 µg $mL^{-1}$ for FAVI, amount of 0.5, 1, 1.5, 2, 2.5 and 3 mL were transferred from the standard stock solution of 1000 µg $mL^{-1}$ of FAVI into a series of 100 mL volumetric flasks. The concentrations obtained 5, 10, 15, 20, 25 and 30 µg $mL^{-1}$ were used for linearirt. Beer Lambert's Law was adhered for all three UV techniques (Table 1).

#### 3.4.2 Accuracy and precision

For accuracy, a known quantity of a standard FAVI to the preanalyzed FAVI tablet formulation was added. Recovery experiments were used to evaluate the accuracy at three different levels 80%, 100% and 120%. Three determinations were made at each level and the mean was noted. Table 2 displays the standard deviation and recovery study values, which were satisfactory. Using tablet powder equivalent to 100 mg of the FAVI label claim, the method precision was assessed. By performing the assay on five replicates of a single

concentration three times in the same day, the RSD value was used to determine the method repeatability. The test of five duplicates of a single concentration of FAVI over the course of three days was used to evaluate intermediate precision. Results of interday and intraday precision were shown in Table 3.

**Table 1: Results of linearity and range for FAVI**

| Parameter | Single point method | Area under curve method | First order derivetive method |
|---|---|---|---|
| Beer's Law range | 5-30 µg $mL^{-1}$ | 5-30 µg $mL^{-1}$ | 5-30 µg $mL^{-1}$ |
| Coefficient of corelation ($r^2$) | 0.9991 | 0.9994 | 0.9998 |
| Slope (m) | 0.0500 | 0.0177 | 0.014 |
| Intercept (c) | 0.0143 | 0.0077 | 0.014 |
| LOD | 0.1980 | 0.2125 | 0.2097 |
| LOQ | 1.0838 | 0.6442 | 0.6389 |

### 3.4.3 LOD and LOQ

The recommended spectrophotometric methods found that the detection limit and quantitation limit were satisfactory. LOD and LOQ was calculated for the lower limit of detection and minimum quantity of analyte tested.

### 3.4.4 Ruggudness

The ruggudenss were studies by using some small delibrat changes in the method. All the three methods were studied using ±0.5 nm change in wavelength and absorbance were noted. The results shows the RSD not more than 2.

**Table 2: Results of accuracy data for FAVI**

| Methods | Levels | Amount taken[a] (µg mL$^{-1}$) | Amount added[a] (µg mL$^{-1}$) | Amount recovered[a] (µg mL$^{-1}$) | % estimated[a] ± SD | % RSD |
|---|---|---|---|---|---|---|
| Single point method | 80 % | 10 | 8 | 8.08 | 100.45±0.5885 | 0.5858 |
| | 100 % | 10 | 10 | 10.19 | 100.97±0.6951 | 0.6884 |
| | 120 % | 10 | 12 | 12.35 | 101.59±19480 | 1.9174 |
| Area under curve | 80 % | 10 | 8 | 8.12 | 100.97±0.3986 | 0.3958 |
| | 100 % | 10 | 10 | 10.28 | 101.41±0.4030 | 0.3974 |
| | 120 % | 10 | 12 | 12.16 | 100.74±1.2590 | 1.2496 |
| First order derivative | 80 % | 10 | 8 | 8.15 | 100.88±0.8798 | 0.8721 |
| | 100 % | 10 | 10 | 10.21 | 101.09±1.9168 | 0.9069 |
| | 120 % | 10 | 12 | 12.39 | 101.79±1.9951 | 1.9598 |

[a]Average of three replicates

**Table 3: Results of precision data for FAVI**

| Methods | Parameters | Amount taken[a] (µg mL$^{-1}$) | Amount Added[a] (µg mL$^{-1}$) | % estimated[a] ±S.D. | % RSD | SEM |
|---|---|---|---|---|---|---|
| Single point method | Interday | 20 | 20.11 | 100.59±0.8851 | 1.8739 | 0.8431 |
| | Intraday | 20 | 20.18 | 100.91±0.9658 | 0.9570 | 0.4319 |
| Area under curve | Interday | 20 | 20.05 | 100.28±103067 | 1.3031 | 0.5844 |
| | Intraday | 20 | 20.28 | 101.43±0.8353 | 0.8235 | 0.3736 |
| First order derivative | Interday | 20 | 20.12 | 100.64±0.6034 | 0.5996 | 0.2699 |
| | Intraday | 20 | 20.19 | 100.96±0.5100 | 0.5051 | 0.2281 |

[a]Average of three replicates

**Table 4: Results of statistical data for FAVI by single factor ANOVA test**

| Methods Comparison | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Single point method Vs area under curve method | 0.0102 | 1 | 0.0102 | 0.0478 | 0.8324 | 5.3176 |
| Area under curve Vs first order derivative method | 0.0280 | 1 | 0.0280 | 0.1332 | 0.7245 | 5.3176 |
| Single point method Vs first order derivative | 0.0722 | 1 | 0.0722 | 0.3773 | 0.5560 | 5.3176 |

### 3.4.5 Statistical evaluation

A single factor ANOVA test was used to compare statistically the produced techniques and the results indicated that there was no significant difference between the three UV methods. The sum square (SS) quantifies the variability between the two groups. Degree of freedom (df), mean square (MS) was calculated. F test shows the one way ANOVA which is compare with the F critical value (F crit). The p value found to be greater than 0.05 shows the probability that null hypothesis is true which means that there is no difference between these two methods compared for analysis as shown in Table 4. Therefore, routine analysis of FAVI in bulk drug and tablets can used from these three developed methods.

## 4. Conclusion

UV- spectrophotometric methods were developed for FAVI using 0.1 N NaOH solution at $\lambda_{max}$ of 362 nm (Single point method), 357-367 nm (Area under curve) and 341.5 nm maxima and 379.5 nm minima (First order derivative). Linearity and range for three methods were found to be 5 to 30 µg $mL^{-1}$. The % RSD for accuracy, precision and ruggedness were found

to be satisfactory. The statistical data for compression shows no difference in the two consecutive methods. All the three methods were useful for the routine analysis of FAVI in bulk and tablets without any interference of excipients. Therefore, it has been proposed that the developed techniques can be employed in routine quality control of the FAVI and its formulations.

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