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MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF FLUCONAZOLE IN PHARMACEUTICALS DOSAGE FORM

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ABSTRACT

In this research, a simple, accurate and validated Ultra-violet spectrophotometric method for determination of Fluconazole using multivariate regression was developed. The calibration process implemented here is based upon equations obtained from linear regression using absorbance data collected at 5 equidistant wavelengths to establish the correlation of concentration and observed spectral characteristics. The maximum absorbance of Fluconazole was found to be at 261 nm. Results were tested for statistical significance. In a range of concentration between 100-300 µg mL^{-1}, a linear plot with the regression coefficient of 0.9992 was acquired. The assay was performed and found to be 99.75% - 100.04% w/w.

Keywords: Fluconazole, UV spectrophotometry, Multivariate calibration, ICH guidelines

INTRODUCTION

Fluconazole (FLZ), which has a molecular weight of 306.271 g mol^{-1} and in terms of chemical composition, 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl) -2-propanol is a man-made substance that is used to prevent the growth of fungi & it is derived from triazole **[1]**. This drug is recommended for the prevention and treatment of deep organ candidiasis and disseminated candidiasis **[2]**. The substance possesses a wide range of effectiveness against many types of fungi. Through a comprehensive review of literature, multiple techniques for determining FLZ

concentrations in biological fluids and pharmaceutical formulations have been identified. These techniques include IR spectroscopy, UV spectrophotometry, and microbiological methods **[3]**.

FLZ is a crystalline powder that readily absorbs moisture and can dissolve freely in methanol, but only to a limited extent in water. FLZ occurred in polymorphic form with the melting points of Form I, Form II, and Form III are 135-136°C, 138-140°C, and 137-138°C, respectively. It acts as fungistatic agent by inhibiting fungal cytochrome P450 enzyme14α- demythylase. Demethylase activity in mammals is far less vulnerable to the effects of FLZ compared to fungal demethylase. Fungal resistance to azole-class medicines typically develops gradually over long-term medication treatment, leading to clinical failure in patients with impaired immune-systems **[4]**.

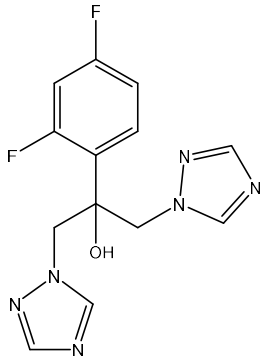

Figure 1: Structure of FLZ

The UV spectrophotometric examine is a straightforward, expeditious, cost-effective technique that provides reliable determination of chemicals. From the literature it was found that the various methods available for determination of FLZ are complex and not simplified as required. The current MVC method provides a well-developed ultra violet spectroscopic method with proper validation parameters in simplest form. The developed UV method is accurate and simple as it does not involve complicated procedures **[5]**.

FLZ is classified as a BCS class-I chemical due to its high solubility and permeability **[6]**.

According to a survey of the literature, many methods for finding FLZ in biological or pharmacological formulations have been documented. Few hyphenated methods for Fluconazole were described, including LC-MS/MS **[7]**, UPLC **[8]**, few chromatographic techniques, including HPLC **[3]**, HPLC-UV(1), spectrophotometry **[5]**, and spectrofluorimetry **[9]**.

For this sample of FLZ, no Multivariate calibration technique (MVC) utilizing UV spectrophotometry was described. Hence, the main goal of the present method is to design a UV spectrophotometric MVC for precisely determining the exact amount of FLZ. Applied analytical technique offers powerful, rapid, sensitivity, and low-cost quantitative analysis of investing admixtures under optimized conditions.

For each selected wavelength, such as 257, 259, 261, 263, and 265nm, equations can be derived to calculate the wavelength of

absorption of the sample (x) when recorded at those wavelengths (λ).

$A_{\lambda257}$= a X C_X + k1 ……. (1)

$A_{\lambda259}$ = b X C_X + k2…… (2)

$A_{\lambda261}$ = c X C_X + k3 …….. (3)

$A_{\lambda263}$ = d X C_X + k4 …… (4)

$A_{\lambda265}$ = e X C_X + k5 …… (5)

Since,

- A_λ= The sample absorbance;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_X = Sample concentration

The 5 equations mentioned before can be arranged as follows:

A_T = a x C_X + b x C_X + c x C_X + d x C_X + e x C_X + K_T…… (6)

The aforementioned equation may be reduced even more to

A_T = C_X (a+b+c+d+e) + K_T……. (7)

whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

To determine the amount of analyte X in a solution, use the formula.

$$Cx = \frac{A_T - K_T}{(a+Ab+c+d+e)} \quad \text{……(8)}$$

MATERIALS AND METHODS:

Chemicals and solvents employed:

- Methanol
- FLUMED ® TABLETS – (Label claim – 150 mg of FLZ), manufactured by ZYDUS Healthcare Limited. The medication formulations that were marketed were procured on a regional basis.

Solubility:

- Freely soluble in Methanol, Phosphate buffer PH 7.4, Soluble in organic solvents like ethanol, DMSO, and dimethyl formamide

Instrumentation:

- UV-VIS double beam Spectrophotometer (Lab India UV-3092).
- Electronic balance (SHIMADZU AY-220H).
- Soni-clean sonicator (model 160T, The Barton-Australia).

METHOD DEVELOPMENT

Solvent selection

The solvent used in the analysis to dissolve the drug, Methanol, was determined to be freely soluble.

Standard solution preparation

Accurately 100 mg of the drug component were diluted in 100 mL of Methanol to create the FLZ standard stock solution. We adjusted the concentration of this solution (100-300µgmL^{-1}) and utilized it for further investigation.

Preparation of sample solution

Measure the weight and grind into a fine powder. There are 10 tablets. Precisely measure a quantity of the tablet powder that is approximately equal to 25 mg of FLZ. Dilute this powder with 25 mL of Methanol and subject the mixture to sonication for a period of 10 minutes. Add an adequate amount of Methanol and make-up the volume to 50 mL. The previously produced solution is subjected to filtration and dilution using Methanol in order to achieve a concentration of 100 µg mL^{-1} of FLZ. The concentration of FLZ is determined by measuring the absorbance of the resultant solution at a wavelength of 261 nm.

λ_{max} determination & wavelength selected for Multivariate calibration

Using Methanol as the blank solution, the FLZ working standard solutions were scanned within the 200 to 400 nm. Methanol shows the maximum absorption at 261 nm. The MVC technique was positioned precisely between the absorption maxima at wavelengths of 257, 259, 261, 263, and 265 nm.

METHOD VALIDATION

The proposed method's linearity, accuracy, and precision were verified in accordance with ICH recommendations **[10]**.

Linearity

Enough dilution of the stock solution using methane allowed concentrations from 100 to 300 µg mL^{-1}. These concentrations then were used to evaluate FLZ's spectrum and linearity. The MVC approach helped us to determine and evaluate the absorbance of linear solution at the specific wavelength.

Limit of Quantification (LOD) & Detection

The Limits of Detection (LOD) and Limits of Quantification (LOQ) for FLZ were found by examining the slope of the calibration curve and the standard deviations of responses at a given wavelength.

$$LOD = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}} \quad \text{..... (9)}$$

$$LOQ = \frac{10 \times \text{Standard deviation}}{\text{Slope}} \quad \text{..... (10)}$$

Precision

Repeatability of the precision determined using intraday & interday precision. A standard FLZ solution with a concentration of 100 µg mL^{-1} was used to evaluate various degrees of accuracy. Five solutions were examined at five different wavelengths to assess repeatability. The prepared solutions were subjected to three measurements of absorbance at varying intervals on the same day for the intervariation scenario. Three more days of using the absorbance were employed to account for intravariation.

Accuracy

While the % of recovery results were calculated, the precision of the FLZ methodology was evaluated at 50, 100, and

150 percent of the concentrations of the previously investigated samples.

Assay

Measure the weight and grind into a fine powder. There are 10 tablets. Precisely measure a quantity of the tablet powder that is approximately equal to 25 mg of FLZ. Dilute this powder with 25 mL of Methanol and subject the mixture to sonication for a period of 10 minutes. Add an adequate amount of Methanol and make-up the volume to 50 mL. The previously produced solution is subjected to filtration and dilution using Methanol in order to achieve a concentration of 100 µg mL^{-1} of FLZ. The concentration of FLZ is determined by measuring the absorbance of the resultant solution at a wavelength of 261 nm.

RESULTS & DISCUSSION

FLZ standard solution was originally scanned between 200 and 400 nm. The maximum spectrum of FLZ has a wavelength of 261 nm. The UV spectrum of FLZ standards and samples was measured using Methanol as a blank solution, with a wavelength of 261nm selected for the maximum value of the MVC. **Figure 2** depicts the typical spectra of FLZ at a concentration of 100 µg mL^{-1}.

The linearity of the devised technique for FLZ was determined within the concentration range, which ranging from 50 to 150 percent for 100 µg mL^{-1} (100 to 300 µg mL^{-1}), in accordance with ICH Q2 R1 requirements. In **Figure 3,** the linearity spectrum of Fluconazole is depicted. Measuring the absorbance of Sample solutions diluted at five specific wavelengths—257, 259, 261, 263, 265 nm—generated the calibration curve. **Table 1** presents the found results in a tabular style. Within the selected concentration range, every one of the standard curves was found to be linear. The calibration graphs and regression analysis are shown respectively in **Figure 4-8** and **Table 2**.

Limit of Detection and Quantification

The linearity slope was employed for calculating the LOD and LOQ for FLZ, and many sample studies have supported this method. The average of all the absorbance was used to compute the LOD for FLZ, which was found to be 9.89 µg mL^{-1}. The mean absorbance was utilized to calculate LOQ for FLZ, resulting in a value of 29.98 µg mL^{-1}.

Precision

Figure 9 shows the system precision spectra for FLZ. **Figure 10** depicts the FLZ interday precision spectra. **Figure 11** for FLZ depicts the intraday precision spectra. For FLZ, the percentage RSD of the system's intraday and interday precision was calculated. It was discovered to be less than 2%, demonstrating the precision of the approach method. Comparing the results acquired

from other accuracy approaches, the suggested method exhibits good precision.

Accuracy

Figures 12 display Accuracy of the overlay spectra for FLZ was verified at 50, 100, and 150%. The FLZ findings are displayed in **Table 3**, and it was determined that the results were within acceptable bounds.

Assay of marketed formulations:

The spectrophotometric technique was applied to examine the amount of FLZ present in the tablet's composition. The Ultraviolet absorption spectra of a commercially available medicine were tested on three distinct occasions. Throughout the process of extraction and filtering, the pharmaceutical formulation maintained consistent and high analytical recovery values. The findings are presented in **Table 4**.

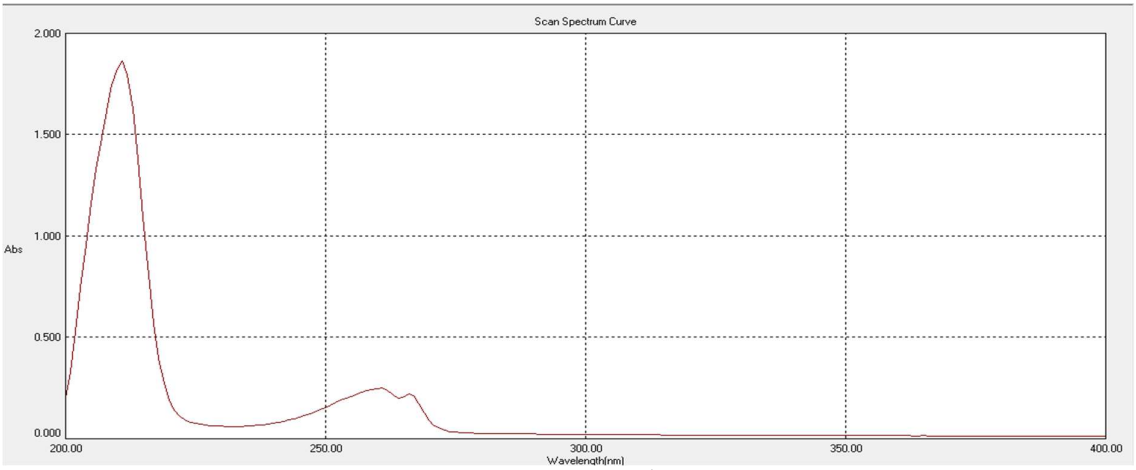

Figure 2: UV spectrum of standard FLZ (100 µg mL^{-1}) using Methanol as blank Linearity

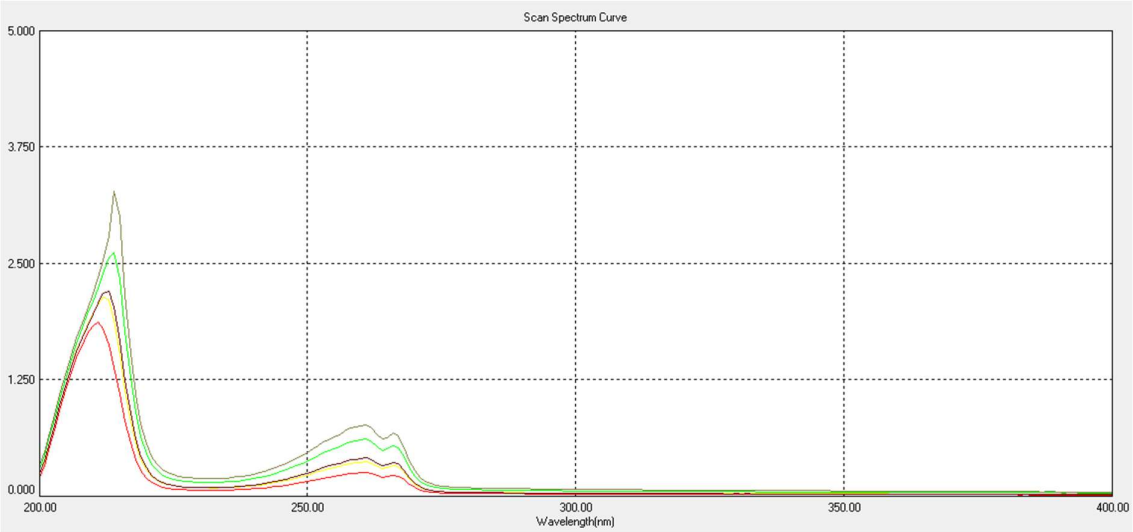

Figure 3: Linearity spectrum of FLZ (100-300 µg mL^{-1}) using Methanol as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

Concentration (µg mL^{-1})	257 nm	259 nm	261 nm	263 nm	265 nm
100	0.228	0.242	0.250	0.203	0.204
150	0.334	0.355	0.366	0.315	0.300
200	0.460	0.472	0.503	0.412	0.416
250	0.571	0.594	0.621	0.512	0.510
300	0.698	0.723	0.763	0.623	0.626

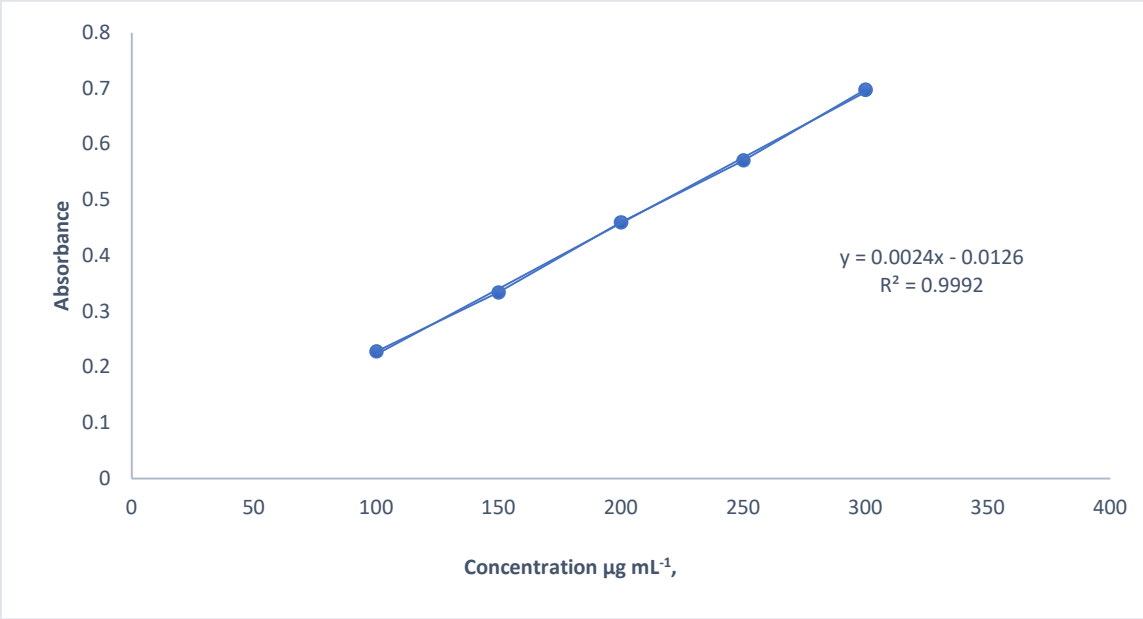

Figure 4: Calibration curve at 257nm

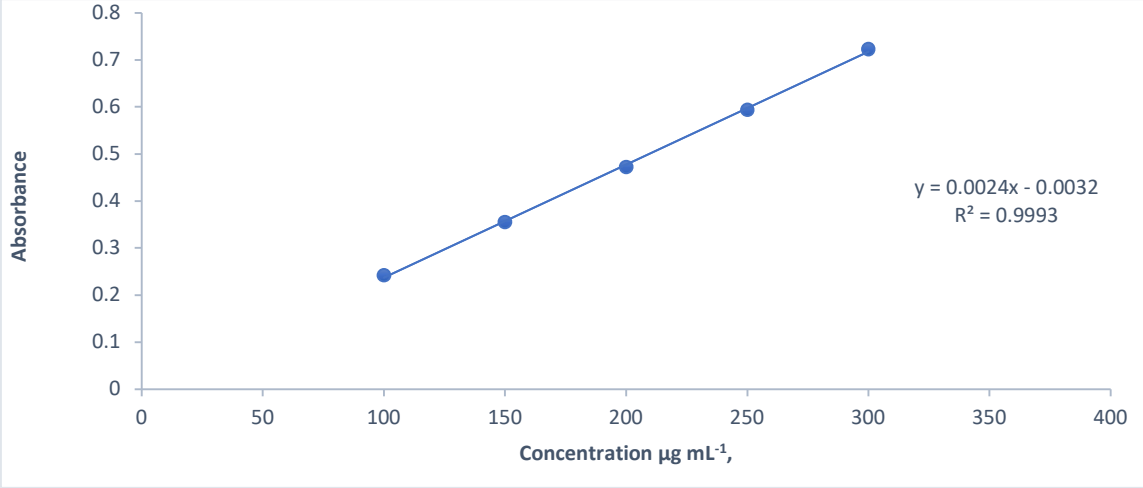

Figure 5: Calibration curve at 259nm

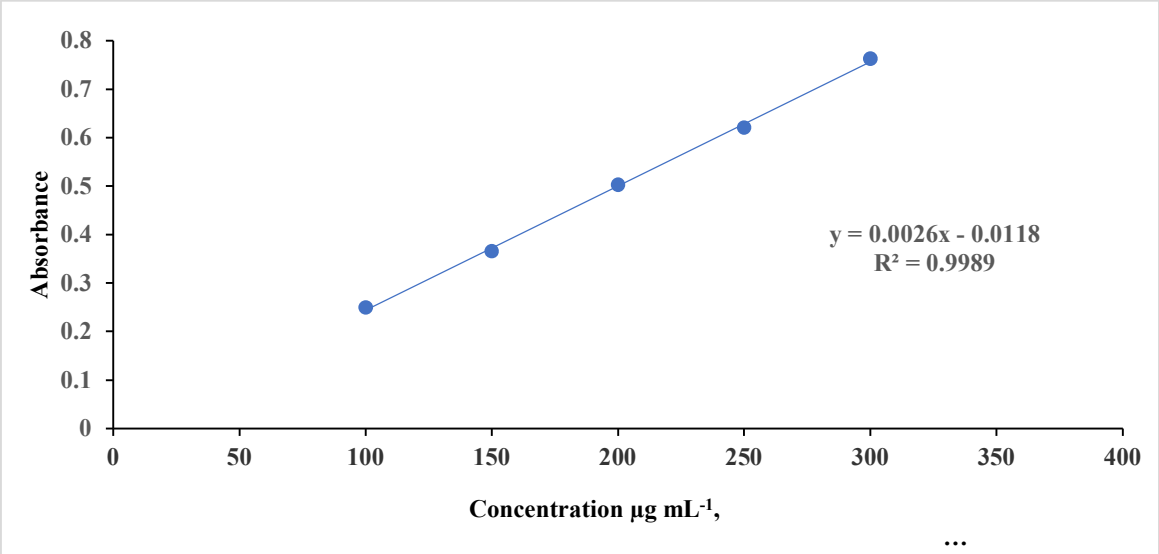

Figure 6: Calibration curve at 261 nm

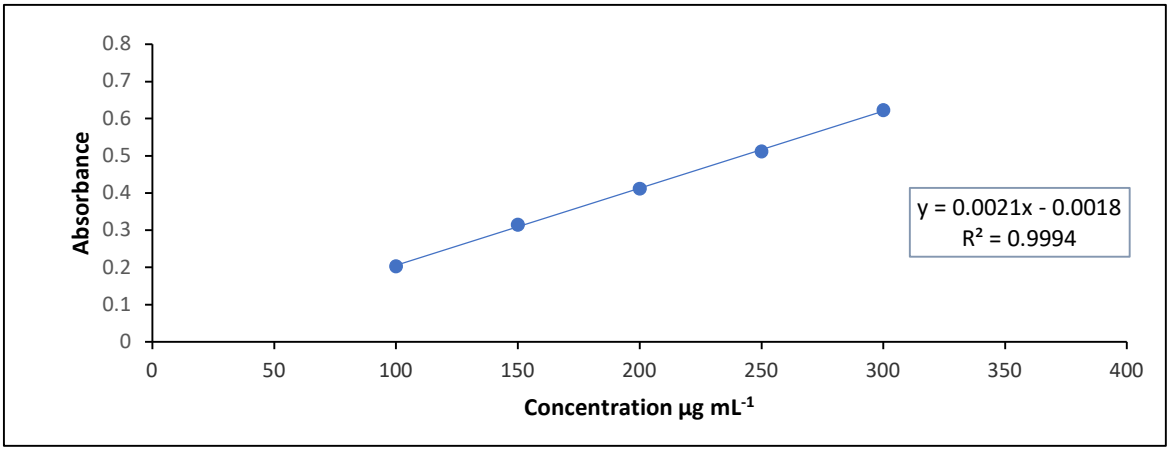

Figure 7: Calibration curve at 263 nm

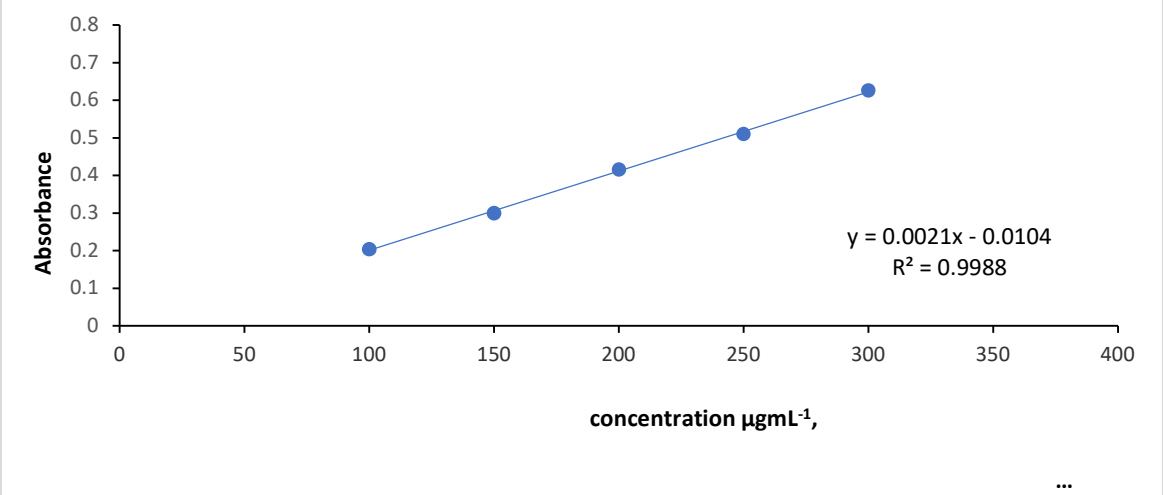

Figure 8: Calibration curve at 265 nm

Table 2: Linearity data shows statistical parameters at the selected wavelengths

Wavelength(nm)	Regression equation	Slope	Intercept	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
257	y = 0.0024x - 0.0126	0.0024	0.0126	0.9992	8.7134	26.4042
259	y = 0.0024x - 0.0032	0.0024	0.0032	0.9999	7.8802	23.8794
261	y = 0.0026x - 0.0118	0.0026	0.0118	0.9989	9.8965	29.9895
263	y = 0.0021x - 0.0018	0.0021	0.0018	0.9994	7.6363	23.1403
265	y = 0.0021x - 0.0104	0.0021	0.0104	0.9988	10.2733	31.1314

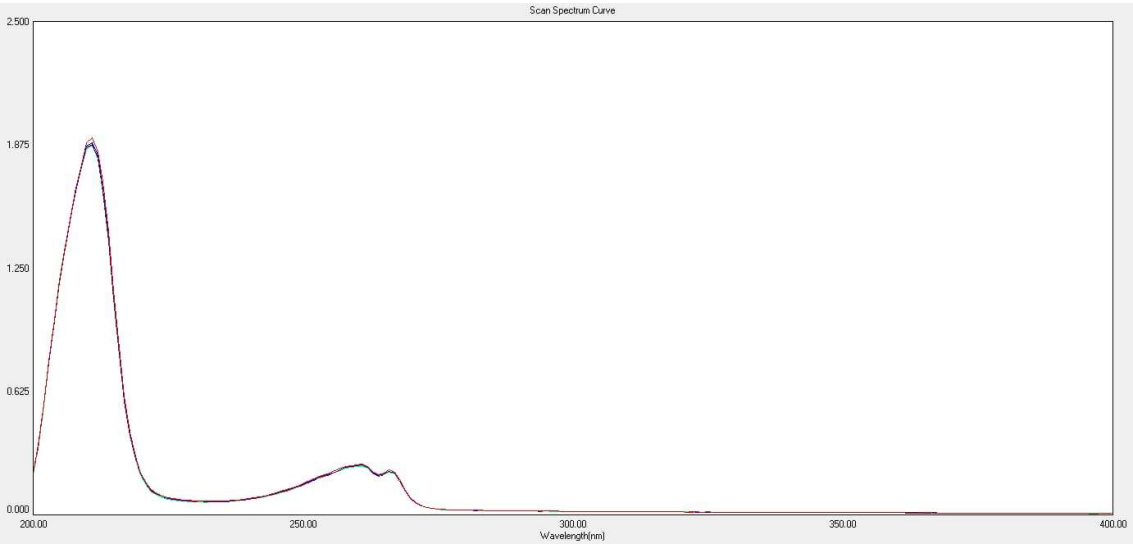

Figure 9: System precision overlay spectra of FLZ

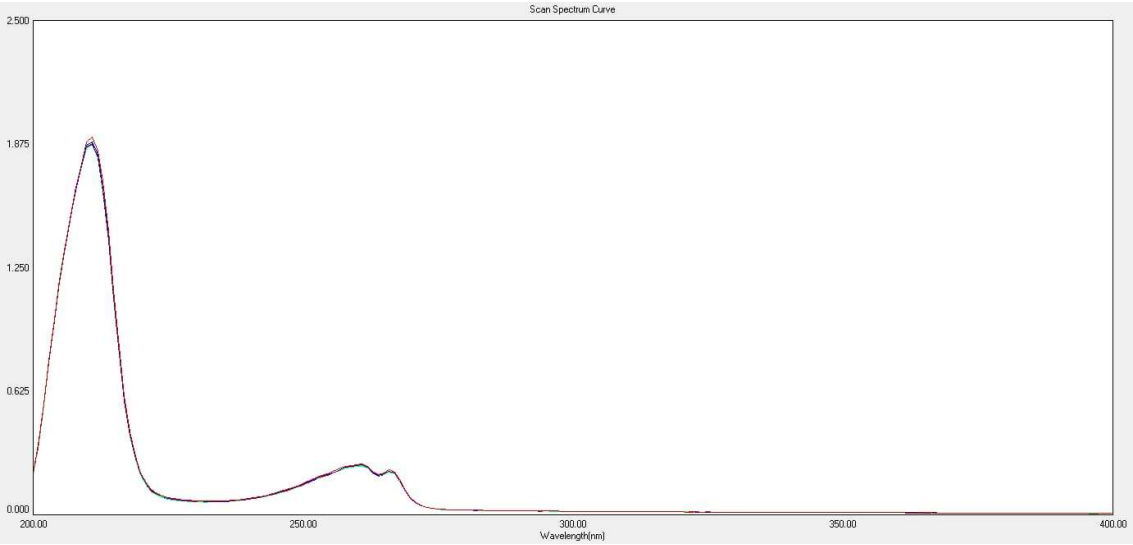

Figure 10: Interday precision overlay spectra of FLZ.

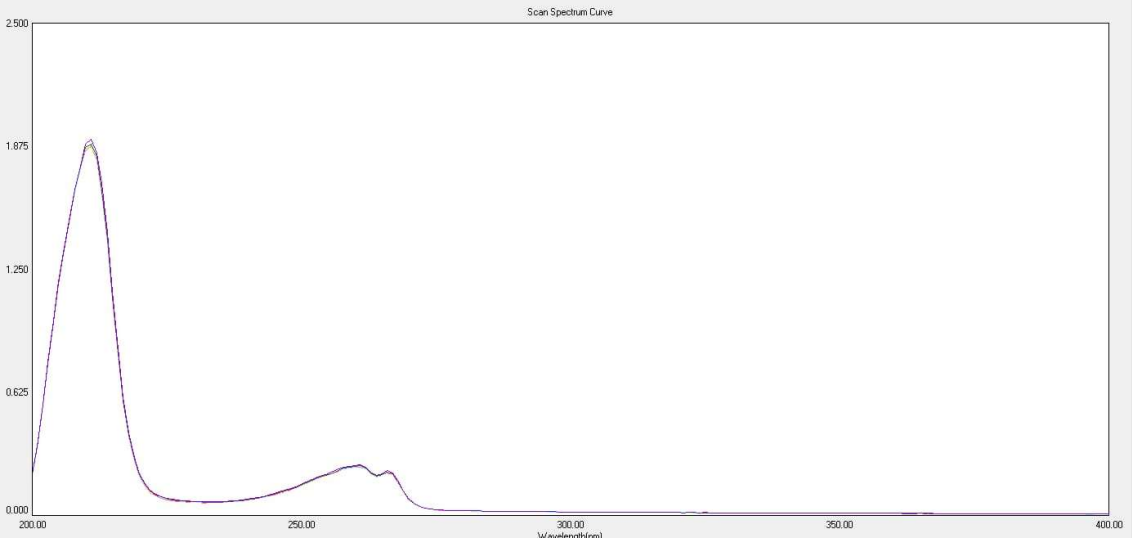

Figure 11: Intraday precision overlay spectra of FLZ.

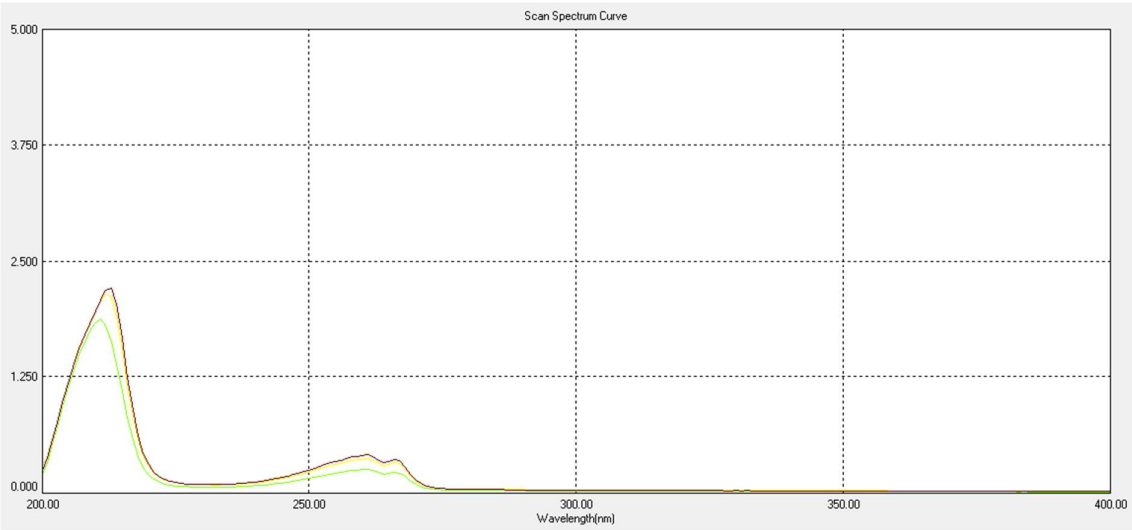

Figure 12: Overlay spectra of accuracy of FLZ 50, 100, 150 %

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery
257	80	20	99.87	99.87
		120	198.97	99.485
		220	299.01	99.67
259	80	20	99.98	99.98
		120	199.97	99.98
		220	301.23	100.41
261	80	20	99.50	99.50
		120	201.03	100.50
		220	298.97	99.65
263	80	20	99.75	99.75
		120	202.05	101.02
		220	299.35	99.78
265	80	20	99.25	99.25
		120	199.68	99.84
		220	298.99	99.66

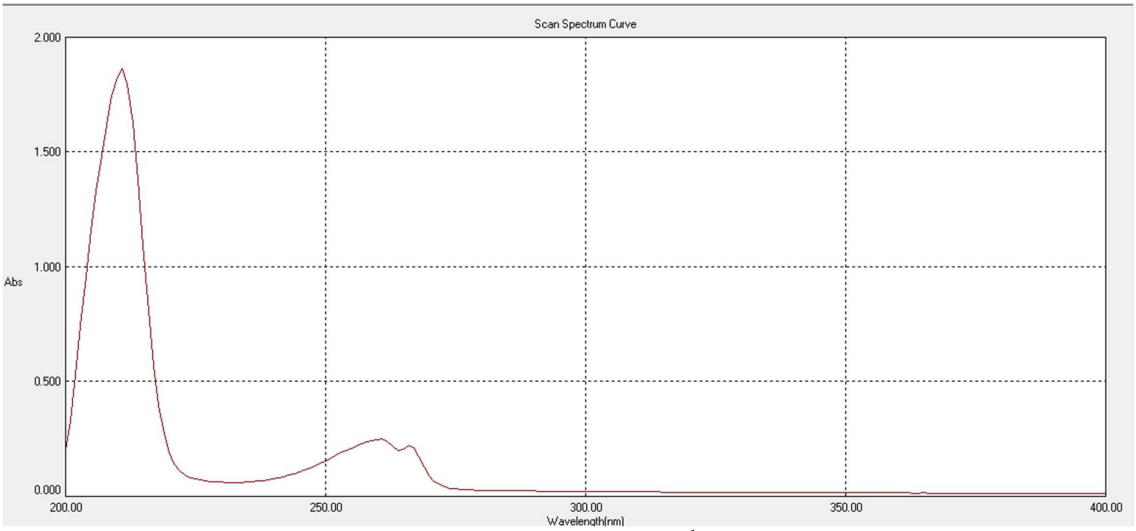

Figure 13: UV spectrum of standard FLZ (100µg mL^{-1}) using Methanol as a blank

Table 4: Assay of FLZ

Label claim (mg)	Amount estimated (mg)	% Assay
150	149.62	99.75
150	150.06	100.04
150	149.98	99.99
Average	149.89	99.92
SD		0.1563
% RSD		0.1564

CONCLUSION

Following ICH recommendations, the newly developed spectrophotometric approach for measuring FLZ was found to be within acceptable ranges after it was verified by evaluating a number of validation criteria. The methods described in this research showcased the sensitivity, accuracy, precision, and repeatability of measuring FLZ in tablet form. Due to its higher accuracy compared to existing UV spectrophotometric methods and its inclusion of a straightforward mathematical approach, we highly recommend using the offered methodology for regular study on FLZ in pharmaceutical formulations.

STATEMENT OF ETHICS

There are no animal or human participants used in this study's trials.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

There are no financial interests that could be at odds with this content.

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No financing has been reported.

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