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

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ABSTRACT

The current study focuses on creating a unique methodology and testing it for the quantification of Febuxostat (FBX) in formulations and bulk. The primary objective of this research work was to develop a facile, precise, responsive, and validated UV spectrophotometric assay for Febuxostat utilizing the multivariate regression approach. This multivariate calibration technique relied upon equations generated from a linear regression model based on the mathematical relationship between absorbance and concentration at five equidistant wavelengths. Febuxostat exhibited a maximum absorption at a wavelength of 314 nm. The findings underwent statistical analysis to determine their significance. A linear plot exhibiting a regression coefficient of 0.999 was obtained for concentrations ranging from 4 to 6 µg mL^{-1}. The assay reported to be 99% to 101.13% w/w.

Keywords: Febuxostat, Anti- gout agent, Multivariate calibration, UV spectrophotometry, Assay, Pharmaceutical formulations, ICH guidelines

INTRODUCTION

A rheumatic disorder, known as gout is caused by development and accumulation of crystals of monosodium urate (MSUr) within the joints, soft tissues, and synovial fluids as a result of its elevation in blood concentration. The illness is associated with

repeated and recurring episodes of acute joint pain caused by the buildup of MSUr crystals in synovial fluid. The condition mostly impacts the joints and the skin/subcutaneous tissues, although its consequences have also been documented in the kidneys through conditions such as cellulitis, urate nephropathy, and kidney stones **[1]**. Gout is detected through the formation of urate crystals inside the joints, tissues, and bodily fluids. This condition includes gout attacks or flares, which are marked by intense pain, swelling and redness. When plasma or serum urate levels rise above 70 mg l^{-1} (or 420 µmol L^{-1}), the condition is known as hyperuricemia. It affects about 5% of the global population. The available therapy options include uricosuric agents, which increases the elimination of uric acid, and xanthine oxidoreductase inhibitors, thereby reduces uric acid synthesis. Allopurinol and Febuxostat are the key inhibitors of xanthineoxidoreductase **[2]**. Febuxostat offers innovative therapy options for gout that is resistant to other treatments or for individuals who cannot tolerate allopurinol. This helps to address the widespread occurrence of the ailment and the limited availability of effective drugs to reduce excessive levels of uric acid worldwide **[1]**.

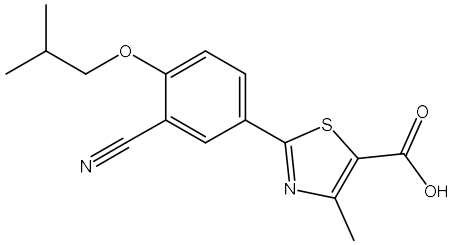

Figure 1: Structure of FBX

Febuxostat has a molecular weight of 316.38 and is chemically known as 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid. The chemical formula of the drug is given as $C_{16}H_{16}N_2O_3S$. The local market offers oral tablets containing febuxostat in two label claims: 40 mg and 80 mg. It is a non-purine, selective inhibitor of both xanthine oxidase and dehydrogenase. It inhibits xanthine oxidase activity by non-competitively inhibiting the molybdenum pterin center **[2]**. With a rapid absorption rate, Febuxostat reaches Cmax in around 60 minutes. Febuxostat can be administered regardless of meal or antacid consumption **[3]**. Febuxostat exhibits a high affinity (98%) for human plasma proteins, specifically at the diazepam binding site. Febuxostat has a half-life of approximately 12 hours on average **[1]**. Febuxostat is a biopharmaceutical categorization system (BCS) class II drug that has low solubility in aqueous solutions and high permeability. Its solubility in water is fairly poor, which is the fundamental barrier to its formulation development **[4], [5]**.

Literature review has revealed various analytical methods like UV spectrophotometry **[6]**, RP-HPLC **[7]**, LC-MS/MS **[8]** have been reported. So, the objective of the research work is the development of a facile, accurate, sensitive UV spectrophotometric technique with MVC in focus.

For each chosen wavelength, corresponding equations can be derived by measuring the absorbance of a sample (Febuxostat) at different wavelengths: 304 nm, 309 nm, 314 nm, 319 nm, and 324 nm.

$A_{\lambda 248} = aX\ C_X + k1$ …… (1)

$A_{\lambda 250} = bX\ C_X + k2$…… (2)

$A_{\lambda 252} = cX\ C_X + k3$ …….. (3)

$A_{\lambda 254} = dX\ C_X + k4$ …… (4)

$A_{\lambda 256} = eX\ C_X + k5$ …… (5)

Whereas,

- A_λ= Absorbance of sample;
- a, b, c, d, e = Slope of the linear regression functions for a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_X = Concentration of sample

The equations mentioned previously are arranged as follows:

$A_T = a \times C_X + b \times C_X + c \times C_X + d \times C_X + e \times C_X + K_T$…… (6)

The preceeding equation can further be simplified as,

$A_T = C_X (a+b+c+d+e) + K_T$……. (7)

Wherein,

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

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

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

MATERIALS AND METHODS

Chemicals and solvents employed

- Methanol (MeOH)
- UBEXA® TABLETS – (Label claim – 40 mg of FBX), manufactured by Lupin Ltd., The medication formulations that were marketed were procured on a regional basis.

Solubility

Methanol and N, N-dimethylformamide are free soluble, while DMSO and ethanol are slightly soluble. Water is weakly soluble.

Instrumentation

- UV-Vis double beam Spectrophotometer (Lab India UV-3092).
- Electronic balance (SHIMADZU AY-220H).
- Soniclean sonicator (model 160T, Thebarton-Australia).

ANALYTICAL METHOD DEVELOPMENT

Selection of solvent

The solvent of analysis was chosen to be Methanol due to its ability to be freely soluble and its suitability for dilutions.

Preparation of the standard solution

100 mg of the drug FBX was dissolved in methanol (100 ml) to prepare the standard stock solution. The solution's concentration was adjusted (4-6 µg mL^{-1}) and utilized for further investigation.

Preparation of sample solution

After precisely weighing 40 milligrams of FBX, it was added to a 100 ml volumetric flask. After adding 30 mL of methanol, the solution was sonicated for 10 minutes. To create a stock solution 400 µg mL^{-1} concentration, methanol was added to the desired volume. For analysis, solutions ranging from 4 to 6 µg mL^{-1} were generated from the stock solution.

λ_{max} determination and selection of wavelength for multivariate calibration

The working standard of FBX was observed over a wavelength range of 200-400nm against methanol as blank solution with an absorption maxima of 314 nm. The wavelength of the MVC technique was therefore located between these absorption maxima, at 304, 309, 314, 319 and 324nm.

METHOD VALIDATION

The proposed method's validation parameters were validated in accordance with ICH recommendations **[9]**.

Linearity

The stock solution was made up with methanol to produce solutions with levels ranging from 4 to 6 µg mL^{-1}. These were used to assess FBX's linearity and spectral area. The absorbance was measured at the appropriate wavelengths and analyzed using the MVC method.

Quantification limit and Detection limit

The subsequent calculations were performed to estimate the detection limits (LOD) and quantification limits (LOQ) for FBX, taking into account the slope of the calibration curve and the SD of results for a specific wavelength.

$$LOD = \frac{3.3 \text{ x standard deviation}}{\text{Slope}} \quad \text{..... (10)}$$

$$LOQ = \frac{10 \text{ x standard deviation}}{\text{Slope}} \quad \text{..... (11)}$$

Precision

The precision was assessed by evaluating both intraday and interday variations. Accuracy levels were determined using a 5 µg mL^{-1} standard FBX solution. To assess repeatability, five solutions were analyzed at five distinct wavelengths. For intraday precision, the absorbance of solutions previously prepared was repeated 3 times at different periods in a single day. For interday

precision, the absorbance measurements were repeated over three consecutive days.

Accuracy

FBX's accuracy has been examined by evaluating sample solutions at concentrations that correspond to 50%, 100%, and 150% of the previously evaluated concentrations. The recovery percentages were then used to calculate the method's precision.

Assay

Weigh ten tablets and finely powder them. Accurately weigh a quantity of FBX tablet powder approximately equivalent to 40mg and dissolve it in 30 ml of methanol in a volumetric flask and sonicate for 10mins. Add adequate amount of methanol to produce 100mL solution. Dilute the resultant solution with methanol to attain a level of 5 µg mL^{-1} of FBX. Measure the absorbance of this solution at 314 nm to determine the concentration of FBX.

RESULTS AND DISCUSSION

The standard FBX solution was primarily observed in the wavelength range (200–400 nm). The maximum absorption was detected at 314 nm. The UV spectra of both standard and sample FBX solutions were recorded, with methanol serving as a blank, and observations conducted at 314 nm for multivariate calibration.

Figure 2 displays the usual spectra of FBX at 5 µg mL^{-1}

Linearity

The developed FBX method's linearity was examined within the concentration range of 50% to 150% of 5 µg mL^{-1} (4 to 6 µg mL^{-1}), in conformance with ICH Q2 R1 criteria. **Figure 3** depicts the linearity spectrum of the FBX. A calibration curve was created by measuring the absorbance of reference solutions at five different wavelengths (304, 309, 314, 319, and 324 nm).

Table 1 displays the results, which show that all standard curves within the prescribed concentration range were linear. The calibration graphs and regression analysis are illustrated in **Figures 4-8 and Table 2**.

Detection limit and Quantification limit

Linearity slope was applied to calculate the LOD and LOQ for FBX, and numerous sample investigations have validated this approach. The LOD for FBX was 0.0428 µg mL^{-1}, calculated by taking the average of all absorbance values. The LOQ for FBX was calculated as 0.9279 µg mL^{-1}, based on the average of all absorbance values.

Precision

Figure 9 depicts the system precision spectra for the FBX. **Figure 10** illustrates the FBX's interday precision spectrum. **Figure 11** displays the intraday precision spectra of the

FBX. The percentage RSD of FBX's intraday and interday precision was computed. It was found to be less than 2%, proving the accuracy of the approach method. When compared to the findings obtained from other accuracy methodologies, the suggested method demonstrates good precision.

Accuracy

Figure 12 depicts the combined spectra for the FBX, which were validated for correctness at 50, 100, and 150%. The FBX findings are displayed in **Table 3**, and it was determined that the results were within acceptable bounds.

Assay of commercially available formulations:

The developed spectrophotometric technique was utilized to measure FBX levels in marketed tablet formulations. The UV absorption spectra of a commercial drug has been determined three times. During extraction and filtration, the pharmaceutical formulation's excellent analytical value for recovery were maintained. **Table 4** summarizes the findings of the investigation.

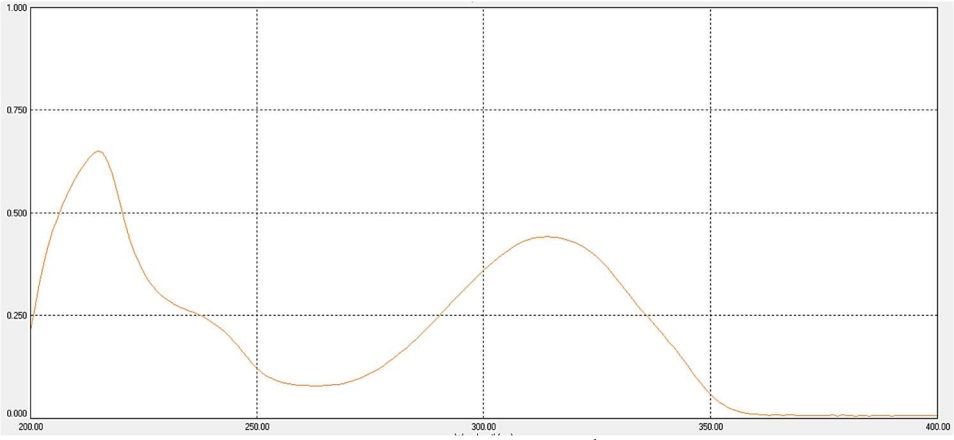

Figure 2: UV spectra of standard FBX (5 µg mL^{-1}) using methanol as blank

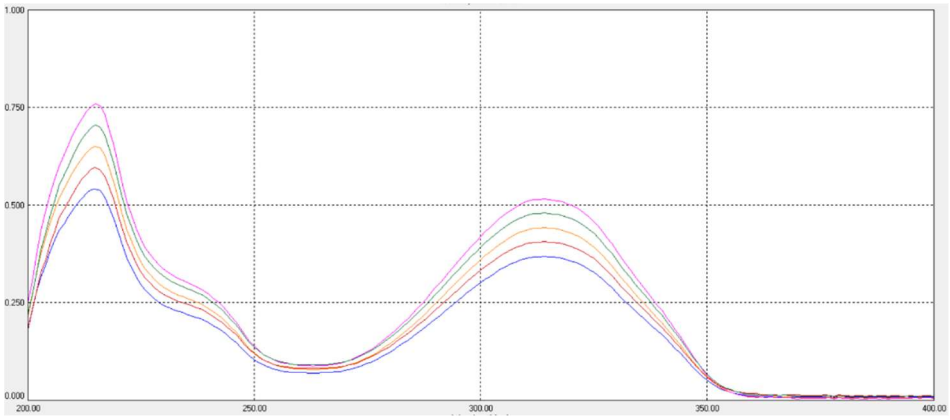

Figure 3: Linearity spectra of FBX (4-6 µg mL^{-1}) using methanol as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

Concentration (μgmL^{-1})	304nm	309nm	314nm	319nm	324nm
4	0.332	0.359	0.369	0.360	0.335
4.5	0.367	0.396	0.406	0.398	0.370
5	0.398	0.431	0.442	0.432	0.402
5.5	0.432	0.468	0.480	0.469	0.437
6	0.463	0.503	0.516	0.504	0.469

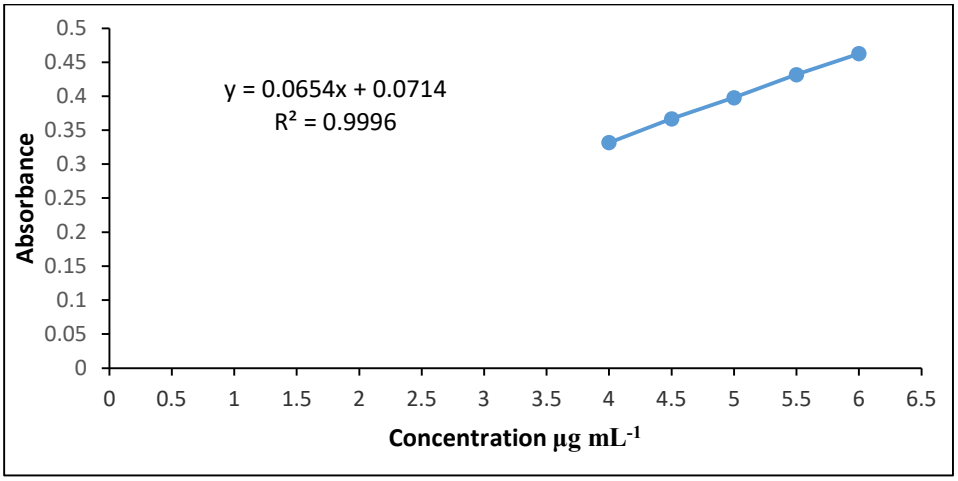

Figure 4: Calibration curve at 304nm

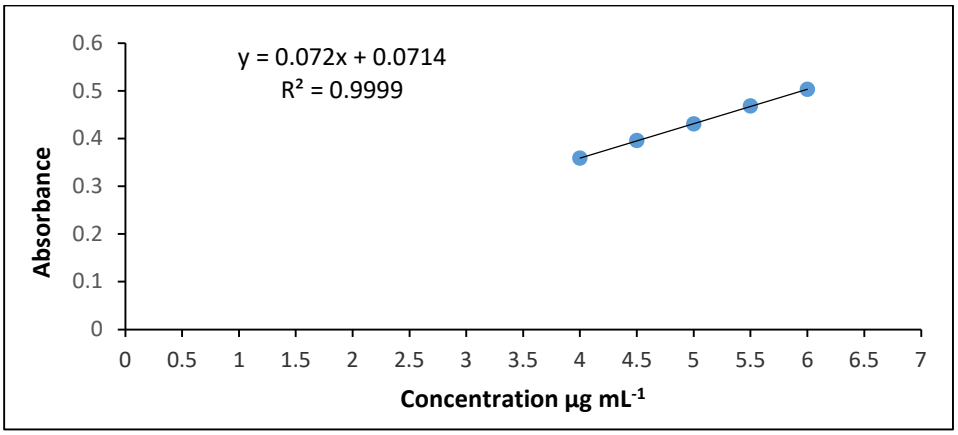

Figure 5: Calibration curve at 309nm

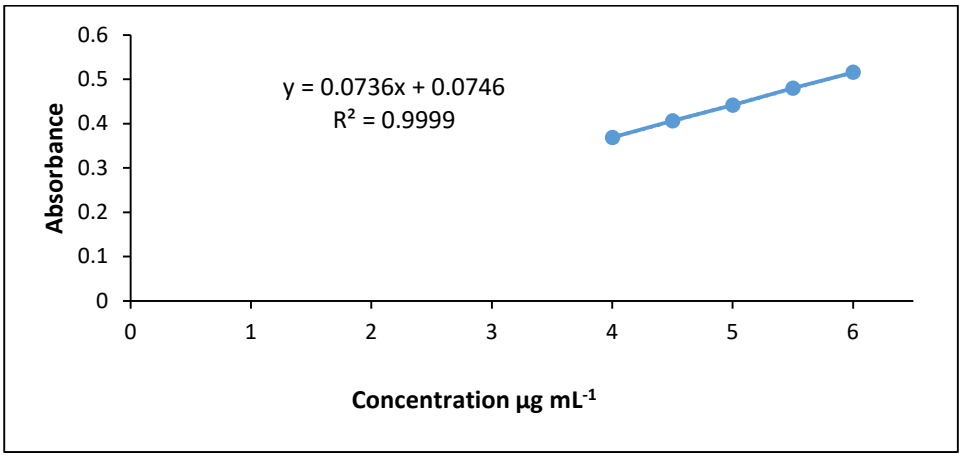

Figure 6: Calibration curve at 314nm

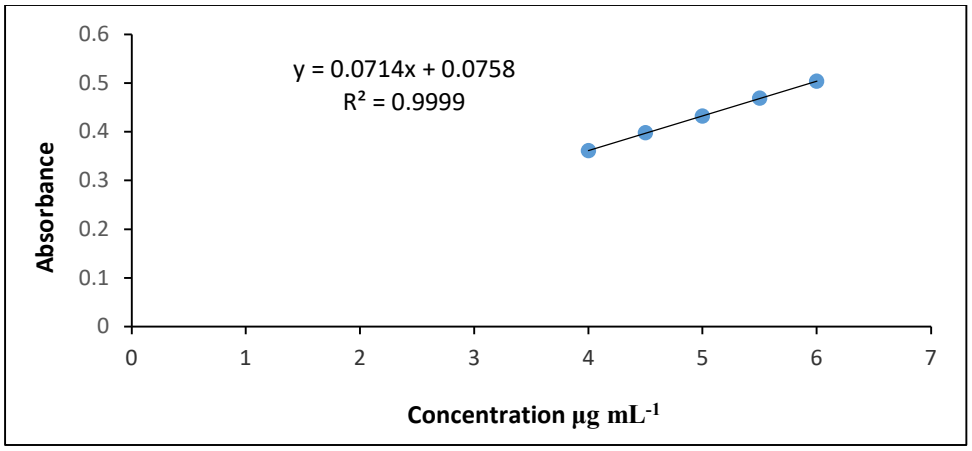

Figure 7: Calibration curve at 319nm

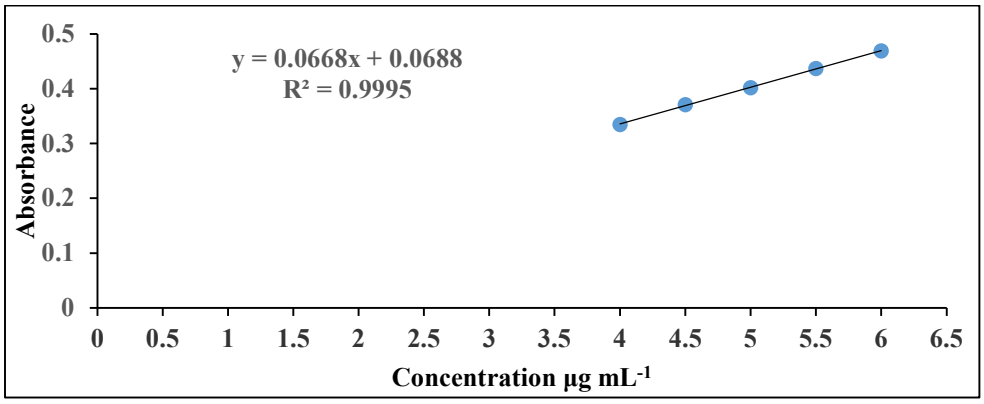

Figure 8: Calibration curve at 324nm

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})
304	y = 0.0654x + 0.0714	0.00654	0.0714	0.9996	0.0604	4.1733
309	y = 0.072x + 0.0714	0.0072	0.0714	0.9999	0.0289	0.0878
314	y = 0.0736x + 0.0746	0.00736	0.0746	0.9999	0.0231	0.0701
319	y = 0.0714x + 0.0758	0.00714	0.0758	0.9999	0.0367	0.1114
324	y = 0.0668x + 0.0688	0.00668	0.0688	0.9995	0.0650	0.1970

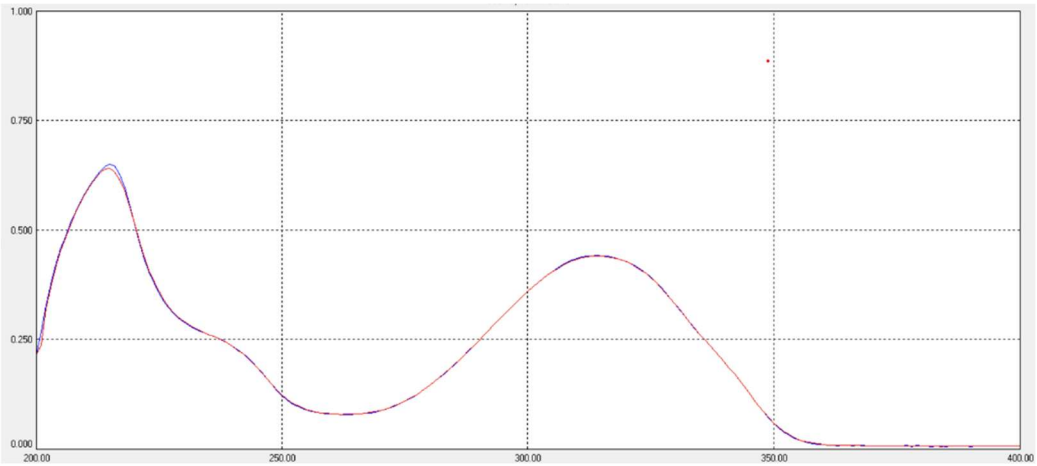

Figure 9: System precision overlay spectra of FBX

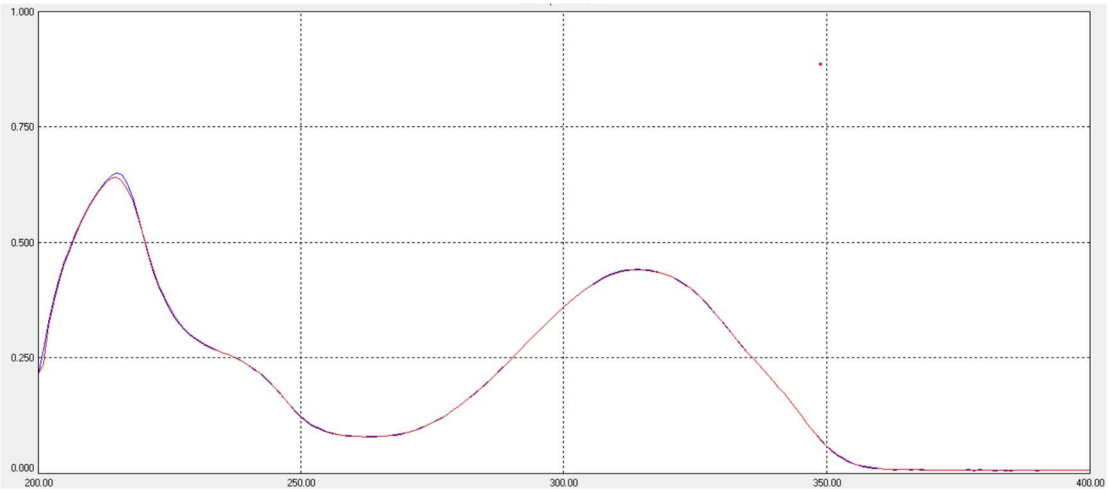

Figure 10: Interday precision overlay spectra of FBX.

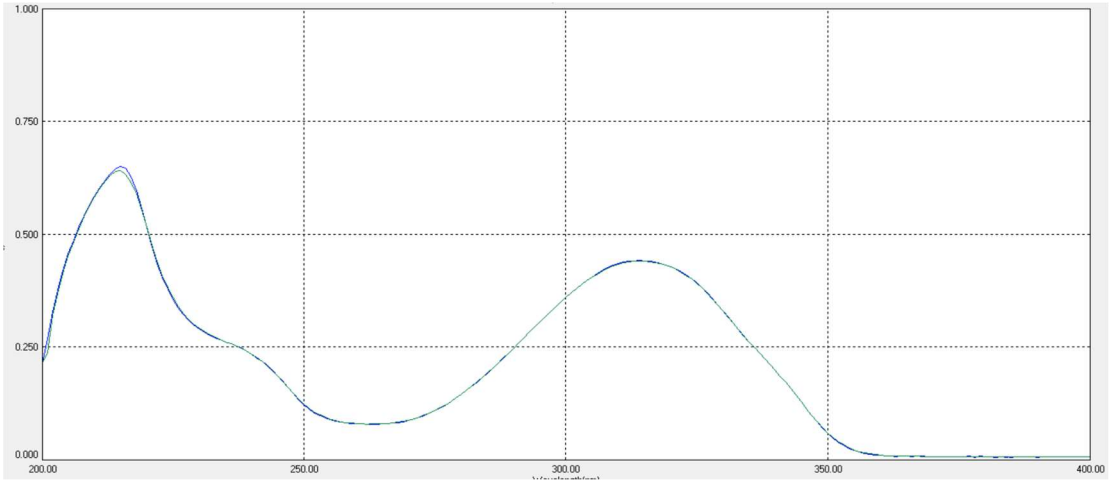

Figure 11: Intraday precision overlay spectra of FBX

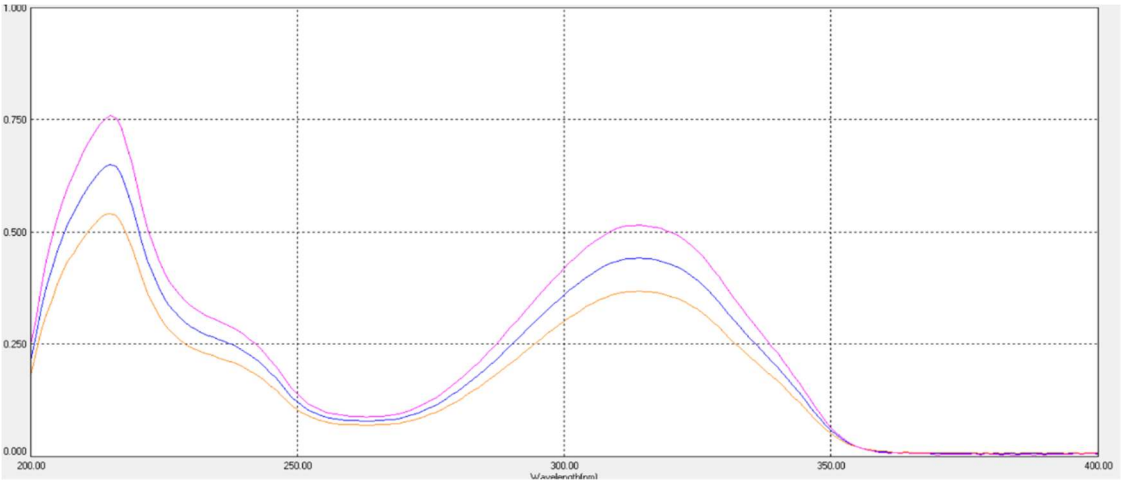

Figure 12: Overlay spectra of accuracy of FBX at 50, 100, and 150 %

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	% Recovery
304	3	1	3.97	99.25
		2	5.01	100.2
		3	5.98	99.66
309	3	1	3.96	99.0
		2	5.08	101.6
		3	5.97	99.5
314	3	1	3.98	99.50
		2	4.97	99.4
		3	5.92	98.66
319	3	1	3.99	99.75
		2	5.01	100.2
		3	5.98	99.66
324	3	1	3.97	99.25
		2	4.96	99.2
		3	5.99	99.83

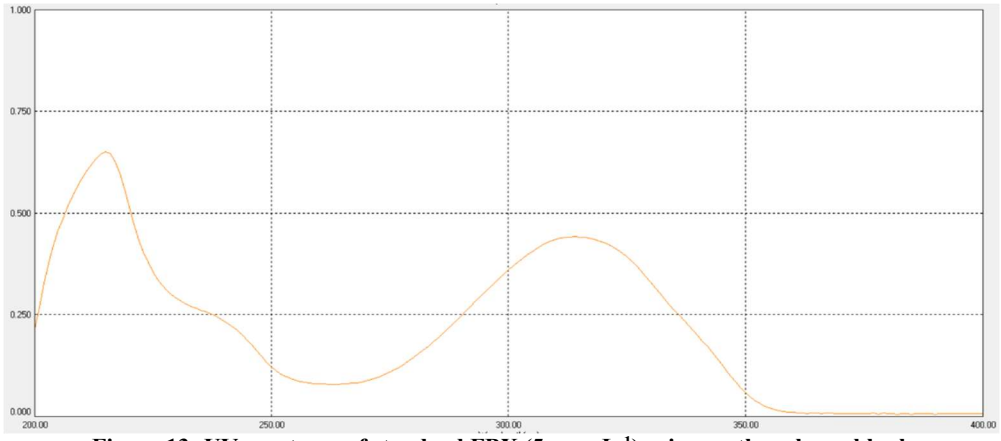

Figure 13: UV spectrum of standard FBX (5 µg mL^{-1}) using methanol as a blank

Table 4: Assay of FBX

Label claim (mg)	Amount estimated (mg)	% Assay
40	39.6	99.0
40	40.45	101.13
40	39.8	99.50
Average	39.95	99.88
SD		1.1110
% RSD		1.1124

CONCLUSION

In conclusion, the newly developed spectrophotometric technique for measuring FBX has been validated and meets the acceptable criteria outlined in ICH guidelines. This method has proven to be a facile, precise, responsive method for the tablet formulation of FBX. Given its superior accuracy compared to existing UV spectrophotometric methods and its straightforward mathematical approach, it is strongly recommended to use this methodology for routine research on FBX in pharmaceutical formulations. The results demonstrated that the method is effective for

analyzing FBX in pharmaceutical formulations, with consistent linearity and precision across tested concentrations. The technique showed reliable performance in quantifying FBX levels in marketed tablet formulations, highlighting its practical application for quality control in the pharmaceutical industry.

STATEMENT OF ETHICS

There is no involvement of animal or human participants in this study's trials.

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DISPUTE 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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