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Spectrophotometric Determination of Febuxostat from Bulk and Tablet Dosage Form by Area Under Curve Method

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Abstract : The present research work discusses the determination of Febuxostat from bulk and tablet dosage form by UV Spectrophotometric Area under Curve method. A new, simple, specific, accurate and cost effective spectroscopic method has been developed for the estimation of Febuxostat in bulk as well as formulation. The optimum conditions for the analysis of the drug were established. Methanol was used as a solvent to prepare standard as well as sample solutions. The maximum wavelength (λmax) was found to be 315nm. For quantitative determination of Febuxostat, values were measured at 305.00 nm-325.00 nm. The validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. Calibration curve was observed with concentrations 2-10 µg/ml (R^2 = 0.9998). The % assay in commercial formulation was found to be 99.47 %. The recovery of proposed method was found to be 99.42%. The results of all validation parameters were found to be within acceptable limit. The developed method can be used for routine estimation of Febuxostat in bulk and tablet dosage forms.

Keywords : Febuxostat, Area under Curve**,** UV Spectrophotometric, ICH guidelines.

Introduction

Febuxostat (fig no. 1) is antigout agent. Chemically it is 2-[3-cyano-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid. The molecular formula of Febuxostat is $C_{16}H_{16}N_2O_3S$ and its molecular weight is 316.375 g/mol. Febuxostat is a non-hygroscopic, white crystalline powder that is freely soluble in dimethylformamide; soluble in dimethylsulfoxide; sparingly soluble in ethanol; slightly soluble in methanol and acetonitrile; and practically insoluble in water. The melting range is 205°C to 208°C. Febuxostat is a xanthine oxidase inhibitor. It works by blocking an enzyme in the body (xanthine oxidase), which lowers levels of uric acid in the blood. This helps to prevent gout flare-ups.[1, 2]Literature survey showed that few HPLC methods are established for determination of Febuxostat in pharmaceutical dosage forms. [3, 5, 18]

Experimental:

Materials and Methods:

The Febuxostat was kindly supplied as gift sample by Emcure Pharmaceuticals Ltd,Pune, India. Methanol used was of HPLC Grade, Febuxostat 40 mg tablets were purchased from local market. A double-beam UV-Visible spectrophotometer, model UV-1800 (Shimadzu, Japan) having two matched cells with1cm light path. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose.

Sonication of the solutions was carried out using an Ultrasonication (Spectralab UCB 40, India). Calibrated volumetric glasswares (Borosil) were used to perform study.

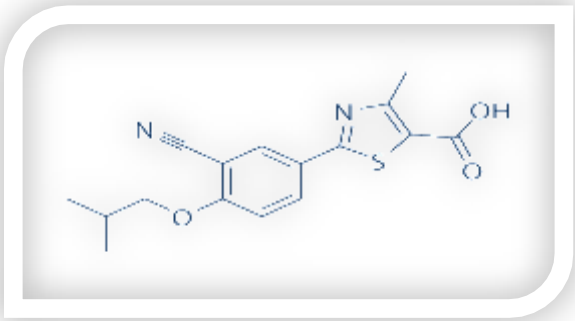

Figure 1: Chemical structure of Febuxostat

Method Development:

Preparation of Standard Solution:

Standard solution of Febuxostat was prepared by dissolving accurately weighed 100 mg of drug in 70 ml of methanol in 100 ml volumetric flask. The solution was allowed to sonicate for 10 min in order to get a clear solution. Then volume was made up to 100 ml with methanol to get solution of 1000 µg/ml concentration. From this solution 10 ml of solution was pipetted out and diluted up to 100 ml with methanol to obtain standard stock solution of 100 µg/ml concentration. Again the further dilutions were made to get desired concentrations of working standard solutions in the range of 2-10 µg/ml.

Selection of Wavelength:

Febuxostat 10 µg/ml working standard solution scanned between 400.00 nm – 200.00 nm in UV spectrophotometer by using methanol as blank after baseline correction. 315.00 nm wavelength was selected for further analysis.

Area Under Curve (AUC):

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing itemcalculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$\text{Area calculation}(\alpha + \beta) = \frac{\lambda 1}{\lambda 2} A d\lambda$$

Whereas, α is area of portion bounded by curved at a and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, $\lambda 1$ and $\lambda 2$ are wavelengths representing start and end point of curve region. In this study area was integrated between wavelength ranges from 305.00nm - 315.00nm.

Preparation of Calibration Curve:

Working solutions of Febuxostat were prepared of concentrations 2, 4, 6, 8, and 10 µg/ml respectively from 100 µg/ml standard stock solution using methanol as an solvent. These solutions were scanned from 400.00 nm – 200.00nm and Area under curve was integrated in the range of 305.00nm – 325.00 nm. Calibration curvewas plottedagainst concentration.(Figure 2)

Assay of Febuxostat (40 mg) Tablets:

Twenty tablets were taken and weighed. Average weight of these tablets was calculated. These tablets were crushed in mortal pestle. The tablet powder equivalent to 10 mg was accurately weighed, transferred in 100 ml volumetric flask and dissolved in methanol to obtain solution of 100 µg/ml concentration. Resulting solution was filtered by 0.45µ syringe filter after discarding first 5 ml of solution. The solution was further diluted with methanol to obtain working standard solutions which were prepared in triplicate and scanned at 315 nm.

Analytical Method Validation:

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.

Linearity and Range:

Linearity was determined by using working standard solutions between 2-10 µg/ml. The spectrums of these solutions were recorded and area under curve was integrated in wavelength range 305-325 nm. Calibration curve of Area under curve vs. Concentration was plotted after suitable calculation and simple linear regression was performed (Figure 3). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

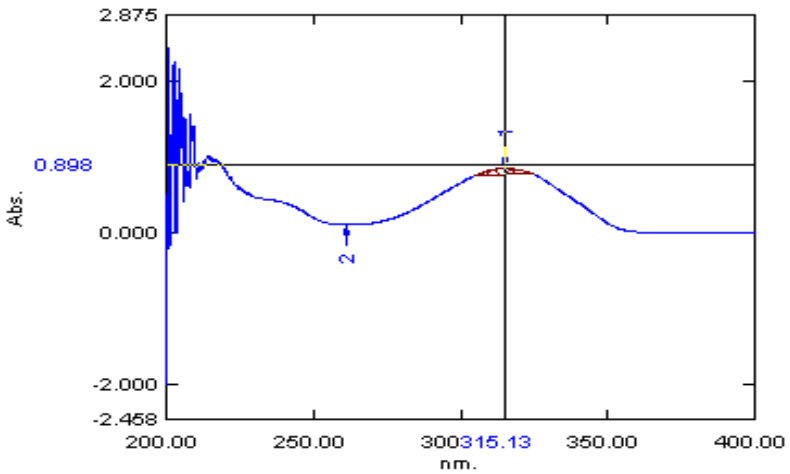

Figure 2: Area under curve ofFebuxostat solution

Table No. 1: Assay of Marketed Tablets of Febuxostat

Method	Label claim	Amount taken	Amount found (mg/tab)	% Assay
AUC	40 mg	10 mg	9.947 mg	99.47 %

Table No. 2: Febuxostat Calibration Data

Concentration (µg/ml)	Absorbance: Method(Area Under curve)
2	0.235
4	0.457
6	0.688
8	0.911
10	1.152

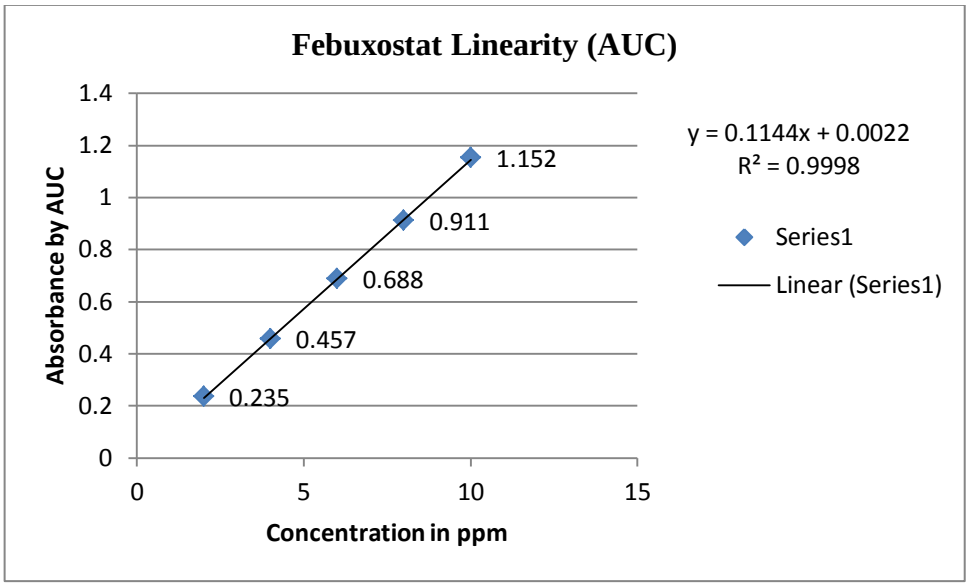

Figure 3: Calibration curve of Febuxostat

Method Precision:

Repeatability:

The precision of the method was checked by repeatedly injecting (n = 6) standard solutions of Febuxostat (7 µg/mL). Area under curve of each of these solutions was measured in the range of 305-325 nm. Percentage relative standard deviation (RSD) was calculated (Table 3).

Table No.3: Repeatability Results of Febuxostat

Drug	Concentration of drug (µg/ml)	%RSD
Febuxostat	7	0.171

Intermediate Precision (Reproducibility):

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of standard solutions of Febuxostat (6, 7 and 8 µg/mL). The results were reported in terms of relative standard deviation (RSD). The results were tabulated in Table4.

Table No. 4: Precision Results of Febuxostat

Drug	Concentration of drug (µg/ml)	%RSD	
		Intraday	Interday
Febuxostat	6	1.10	0.77
	7	0.70	0.72
	8	0.91	0.86

Accuracy:

The accuracy for the analytical procedure was determined at 80 %, 100 % and 120 % levels of standard solution. Area under curve was measured in the range of 305-325 nm and results were expressed in terms of % recoveries. Three determinations at each level were performed and % RSD was calculated. The results were tabulated in Table 5.

Table No.5: Accuracy Results of Febuxostat

Accuracy Level	Amount added	%Recovery	Mean %Recovery	%RSD	Mean %RSD
80%	5.4	99.46	99.42	0.83	1.08
100%	6	99.42		1.62	
120%	6.6	99.39		0.80	

Limit of Detection(LOD)andLimit of Quantitation(LOQ):

Six setsof knownconcentrations2 µg/ml–10 µg/ml were prepared six times and calibration curves were determined for eachset. The values ofLOD and LOQ were calculated by using following formula:

$$LOD = 3.3 \times \frac{SD}{S}$$

$$LOQ = 10 \times \frac{SD}{S}$$

Where,SD is standard deviation of y-intercept of the calibration curves, S is mean slope of six calibration curves.

Table No.6: LODand LOQ Results of Febuxostat

Drug	LOD (µg/ml)	LOQ (µg/ml)
Febuxostat	0.43	1.31

Result and Discussion:

An attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of Febuxostat in bulk and tablet dosage form.The obtained regression equation was $\int_{305}^{325} Ad\lambda$0.1144x + 0.0022 ($R^2$ = 0.9998)

Where, $\int_{305}^{325} Ad'\lambda$ is area under curve between 305.00nm – 325.00nm,x is concentration and R^2 is correlation coefficient. The R^2 value as 0.9998 indicates that developed method is linear. The praposed method was found to be precise as %RSD for intraday and interday precision were found to be within limits.The drug at each of the 80 %, 100 %and 120 % levels showed good recoveries (99.39% to 99.46%). Hence, it can be said that thismethod was accurate. The LOD and LOQ values found to be 0.43 µg/ml and 1.31 µg/ml, respectively. Assay was found to 99.47% for a pharmaceutical tablet dosage form which is consistent with the label claim. From all overstudiesitwas concluded that the method can be used for the routine analysis of the Febuxostat in tablet dosage form. The results of validation parameters are summarized in Table 7.

Table No.7: Summary of Validation Parameters

Sr. No.	Validation Parameter	Results
1	Rang	305.00 nm – 325.00 nm
2	Linearity range	2-10 µg/ml
3	Regression equation ($y = mx + c$)	y = 0.1144x + 0.0022
4	Slope (m)	0.1144
5	Intercept (c)	0.0022
6	Correlation coefficient (R^2)	0.9998
7	Repeatability (% RSD)	0.171
8	Intraday (% RSD)	0.903
9	Interday (% RSD)	0.783
10	Accuracy (Mean % Recovery)	99.42
11	LOD (µg/ml)	0.43
12	LOQ (µg/ml)	1.31

Conclusion:

From the results obtained, it can be concluded that that the proposed method was accurate, precise and consistent for the determination of Febuxostat in bulk and tablet dosage form. This method was validated as per ICH guidelines. The proposed method can be used for routine estimation of Febuxostat in bulk and pharmaceutical dosage forms.

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