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Development and Validation of UV Spectrophotometric Method of Febuxostat in Bulk and Tablet Formulations

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Abstract
The present research works discuss the development of a UV estimation method for febuxostat. Simple, fast, accurate and cost efficient and reproducible. Spectrophotometric method has been developed for the estimation of febuxost at in bulk and tablet formulations. The wave length (λ max) selected for the febuxost at was 315 nm. The linearity for this drug at the selected wavelength is lies between 0.2 to 1µg/ml.Beer's law obeyed in this concentration range with correlation coefficient of 0.9999. The limit of detection and limit of quantification was found to be 1.0585 & 3.2077 µg/ml respectively. The validity of the described procedure was assessed. The proposed method was successfully applied to the determination of febuxostat in pharmaceutical formulations without any interference from common excipients.
Keywords: Febuxostat, Absorbance, Validation, Detection Limit

Introduction
Febuxostat chemically is 2- [3-cyano -4- (2- methlypropoxy) phenyl] - 4- methlythiazole-5-carboxylic acid (**Figure 1**). It is a non purine selective inhibitor of xanthine oxidase that is indicated for use in the treatment of hyperuricemia and gout[1]. In contrast to allopurinol, febuxostat inhibits both oxidized and reduced forms of xanthine oxidase [2, 3] and has minimal effect s on other enzymes of purine and pyrimidine metabolism[3, 4]. A study comparing febuxostat to allopurinol found that more individuals treated with febuxostat had decreased levels of uric acid, but there was no difference in the amount of initial gout flares or the surface area of gout tophi [5]. Literature survey revealed only one hplc method for the estimation of febuxostat and its related substances6. The aim of the study is to develop a simple , sensitive , accurate and precise method for determination of febuxostat in pharmaceutical formulations and bulk drugs using UV spectrophotometer.

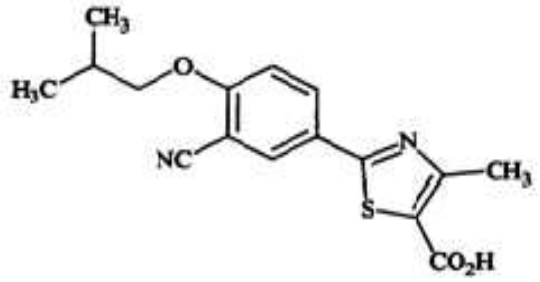
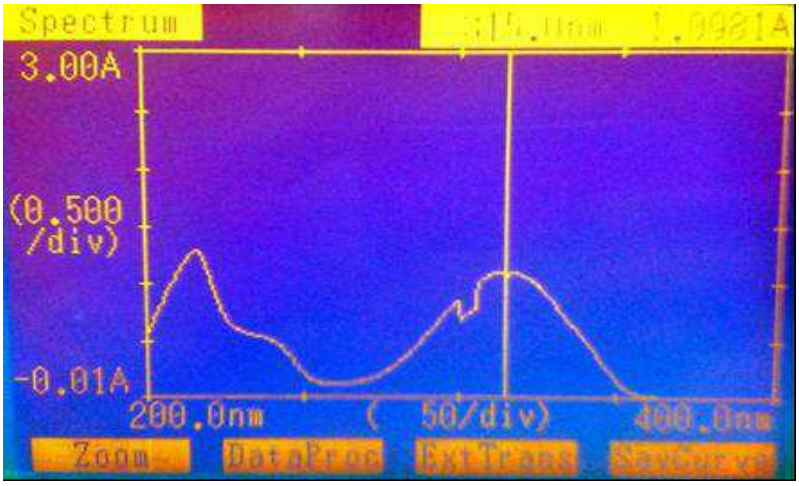


Fig. 1: Structure of Febuxostat

Experimental
Instruments: An analytical UV- Visible Spectrophotometer (UV-2080) with a matched pair of 10 mm quartz cells were used for experimental purpose.
Materials: Febuxostat was obtained as a gift sample from Lupin pharma limited; Methanol AR was procured from MERCK Limited, Mumbai. The commercially available marketed tablet brand containing Febuxostat, 40 mg in each tablet have been used for estimation.
Scanning and determination of maximum wavelength (λmax): In order to ascertain the wavelength of maximum absorption (λ max) of the drug, qualitative solution of the drug was prepared in methanol and scanned using UV spectrophotometer within the wavelength region of 200-400 nm against methanol as blank. The resulting spectra are

shown below (**Fig. 2**) and the absorption curve showed characteristic absorption maxima at 315 nm for Febuxostat.

Spectrum of Febuxostat (200-400nm):



Preparation of Standard Stock Solutions: Standard stock solution (primary) was prepared by dissolving 10 mg of febuxostat in 10 ml of methanol to get concentration of 1mg/ml (1000µg/ml). Secondary stock solution was prepared daily by diluting 1ml of the primary stock solution to final volume of 10 ml using methanol to get concentration of 0.1mg/ml (100µg/ml).

Preparation of calibration standard solutions: The calibration standard solutions were prepared daily by diluting secondary stock solution with methanol to get calibration standard solutions of 0.2, 0.4, 0.6, 0.8 and 1 µg/ml of febuxostat to construct Beer's law plot for pure drug, the absorbance was measured at λmax 315 nm, against methanol as blank.

Procedure for formulations:

Twenty tablets of febuxostat were accurately weighed, finely powdered and mixed. A portion of the powder equivalent to 10mg of febuxostat was transferred into a 10 ml volumetric flask and 10ml of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with methanol to give final concentration (10µg/ml). The absorbance of these solutions was measured at 315 nm. The amount of febuxostat per tablet was calculated using the calibration curve.

Validation: Validation is one of the most important steps in method development for analytical determinations. The main validation parameters 6 such as linearity and range, and precision, limit of detection (LOD), limit of quantitation (LOQ), recovery and ruggedness were evaluated in developed method.

Linearity andRange:

Under the experimental conditions, the calibration graphs of the absorbance versus concentration were found to be linear over the range of 0.2-1 µg/ ml for proposed method. The statistical analysis of data obtained for the estimation of febuxostat in pure solution indicated high level of accuracy for the proposed methods as evidenced by the low values of standard deviation and coefficient of variation (**Table 1**). The linear regression equation obtained was Y = 0.1035X+0 where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution (**Figure 3**). Linearity of the regression equation and negligible scatter of points for the two drugs by the proposed methods were demonstrated from the highly significant ($p > 0.05$) correlation coefficient value. The reported slope values without intercept on the ordinate at 95% confidence limits, suggested that the calibration lines of febuxostat solutions in methanol did not deviate from the origin as the above-obtained values fall within the confidence limits. (**Table 2**).

Table1: Linearity Table of Febuxostat

S.No	Conc(µG/ML)	Absorbance
1	0	0.0000
2	0.2	0.0214
3	0.4	0.0421
4	0.6	0.0634
5	0.8	0.0841
6	1.0	0.1055

Linearity Curve of Febuxostat

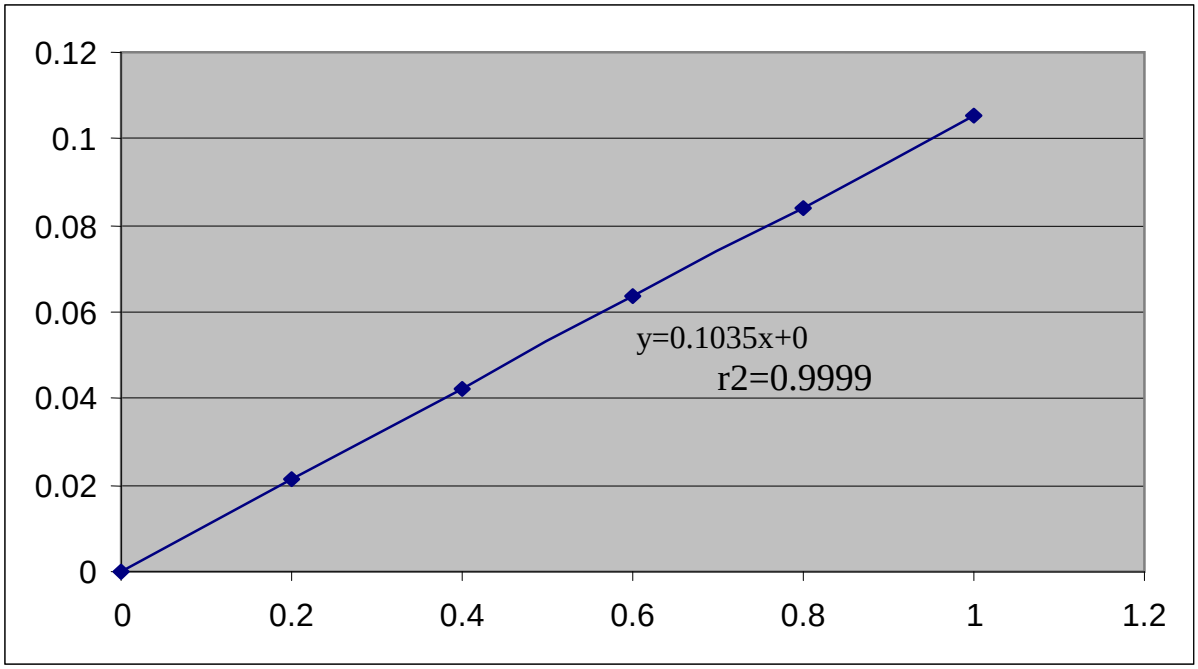


Table 2: Regression Analysis of Data for the Estimation of Febuxostat from Standard Solution

Statistical Parameters	febuxostat
Regression equation	Y=0.1035X+0
Correlation coefficient	0.9999
Slope with out intercept	0.0805X

Precision:

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of Febuxostat in the linear range (0.3, 0.6 and 0.9 µg/ml) were analyzed in 5 independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). The Results are shown in table 3.

Table.3 Intra Day & Inter Day Precision Readings

S.No	Conc (µG/ML)	Intra Day	Inter Day
1	0.3	0.0298	0.0345
2	0.6	0.0642	0.0629
3	0.9	0.0915	0.0968
4	Mean	0.0620	0.0650

Detection Limit & Quantitation Limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by using the relation 3.3σ/S and 10 σ/S respectively, where σ is the standard error of estimate and S is the slope. Calculated values of limit of detection (LOD) and quantitation (LOQ) for Febuxostat were found to be 1.0585 and 3.2077 µg/ml respectively.

Ruggedness:

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10 µg/ml of febuxost at using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts. Thus, the proposed methods could be considered rugged. The Results are shown in table 4

Table.4 Ruggedness Data At 10(µG/ML) By Two Analysts at Different Days

Test Conc(µg/ml)	Analyst--1	Analyst—2
10(µg/ml)	0.9505	0.9515
10(µg/ml)	0.9479	0.9495
10(µg/ml)	0.9515	0.9499
10(µg/ml)	0.9499	0.9512
10(µg/ml)	0.9510	0.9502
MEAN	0.9502	0.9505
S.D	0.0014	0.0008
% RSD	0.1473	0.0841

Recovery:

The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard, which has not been subjected to sample pre-treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample. It is best established by comparing the responses of extracted samples at low, medium and high concentrations in replicates of at least 6 with those non-extracted standards, which represent 100% recovery.

$$\text{Absolute recovery} = \frac{\text{Response of an analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

If an internal standard is used, its recovery should be determined independently at the concentration levels used in the method. The Results are shown in table 5.

Table-5

S.No	Amount Added (µg/ml)	Amount found (µg/ml)	% Recovery
0%	10	10.22	102.2
80%	18	18.16	100.88
100%	20	20.35	101.75
120%	22	22.34	101.54

Analysis of pharmaceutical formulations:

The optimized spectrophotometric method was applied to the direct determination of Febuxostat in tablet using calibration curve method. From the absorbance value, the drug content per tablet (on an average basis) was calculated. The results are shown in below table-7

Table-6: analysis of pharmaceutical formulation

Formulation	Labeled amount(mg)	Amount recover (mg)	Percentage drug recovered	%RSD
Furic* tablets	40mg	39.8084	99.521	1.032

Conclusion

In this study a simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of Febuxostat in bulk drug and pharmaceutical formulations. This method was applied directly to the analysis of pharmaceutical dosage forms without the need of separation such as extraction steps prior to the drug analysis. As this proposed method has the low LOD value and wider linear range is more sensitive method. From the results obtained, we concluded that the suggested method showed high sensitivity and precision. Moreover, this method is simple and in expensive and it can be employed for the routine quality control of Febuxostat formulations.

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