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Original Article

Development and Validation of a Novel Stability Indicating UV-Spectrophotometric Method for Estimation of Febuxostat in Bulk and Pharmaceutical Formulation (Tablets)

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ABSTRACT

Introduction: The present research work involves the development of a simple, economic, accurate, quick and reproducible UV spectrophotometric method for the estimation of Febuxostat in bulk as well as its pharmaceutical formulation i.e. tablets. **Materials and Methods:** Phosphate buffer pH 6.8 was used for the preparation of stock solution. Different solutions of drug were prepared by diluting the stock solution with the same buffer. **Results:** Febuxostat was estimated at UV maxima of 312 nm in pH 6.8 phosphate buffer using UV-Visible double beam spectrophotometer. The drug concentration was found to obey Beer's law over a concentration range of 1–10 µg/ml with line equation y = 0.078x+0.062 and correlation coefficient of 0.999. Results obtained were validated statistically and by recovery study method. **Conclusion:** The result of analysis was validated according to ICH guidelines and found that the proposed method can be used for quality control of pharmaceutical formulations and routine laboratory analysis.

Key words: Febuxostat, Uricosuric, UV-spectrophotometry, Calibration, Validation, ICH.

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INTRODUCTION

Gout is a rheumatic condition due to the deposition of monosodium urate crystals (tophi) in the joints or soft tissues and synovial fluid due to its saturation in blood. It is associated with increased serum uric acid levels (greater than 7 mg/dL). The diagnosis of gout is based on uric acid crystals found in the joints, tissues, or body fluids, as well as on gouty attacks or flares characterized by intense pain, swelling, redness, and heat. There are four clinical stages viz. asymptomatic hyperuricemia, acute gouty arthritis, inter-critical gout and chronic tophaceous gout.[1] Hyperuricemia is a condition in which plasma or serum urate concentration greater than 70 mg/l (>420 µ mol/l) and it is present in approximately 5% of the population in the world. Serum uric acid is the primary important risk factor for the development of gout. Sustained hyperuricemia is a risk factor for acute clinically progressive stages of gout-like gouty arthritis, tophaceous gout and uric acid nephrolithiasis. Most patients with hyperuricemia will never have an attack of gout and remain untreated. The available treatment option is uricosuric agent, increasing uric acid excretion and xanthine oxidoreductase inhibitor, which reducing the synthesis of uric acid. Allopurinol and Febuxostat are of two main xanthine oxidoreductase inhibitors.[2]

The Febuxostat chemically is 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5- carboxylic acid with a molecular weight of 316.38. The molecular formula is $C_{16}H_{16}N_2O_3S$. Tablets available in the local market for oral use contain the active ingredient, febuxostat in two dosage strengths; 40 mg and 80 mg.[3] Febuxostat is a novel nonpurine selective inhibitor of xanthine oxidase (NP-SIXO) which is currently under investigation for the management of hyperuricaemia in patients with gout.[1] It works by non-competitively blocking the molybdenum pterin centre which is the active site on xanthine oxidase. A few UV-spectrophotometric methods were reported for the determination of Febuxostat in bulk and formulation[3,4], however, no method is available for quantitative determination using simple buffer solution and without the use of reagents like methanol.

Literature review also revealed different bioanalytical methods like LC-MS/MS, UPLC tandem mass spectrometry, LC-MS and UPLC-MS for the determination of Febuxostat in biological samples like human and dog plasma.[5]

So the objective of the work was to develop simple, rapid, accurate and specific UV spectrophotometric method for the estimation of Febuxostat in bulk and pharmaceutical dosage forms using pH 6.8 phosphate buffer solution. The method was further validated for the parameters like precision, accuracy, sensitivity and linearity. The limit of detection (LOD) and limit of quantification (LOQ) were also determined. The results of analysis were validated statistically and by recovery studies.[6]

MATERIALS

Febuxostat was obtained as a gift sample from Ami Life Sciences Pvt. Ltd., Maharashtra. Febuxostat tablets were procured from local pharmacy. All the reagents used in study were of analytical grade. Double distilled water was used throughout the experiment. A double beam Systronics UV-Visible Spectrophotometer, model UV-2201 (India) with a spectral bandwidth of 1nm, wavelength accuracy of ±0.5nm and a pair of 1cm quartz cells were used to measure absorbance of the resulting solutions over a range of 200-400 nm.

METHODOLOGY

Preparation of Buffer

Phosphate buffer pH 6.8 was prepared according to the method prescribed in Indian pharmacopoeia.[7]

Preparation of standard stock solution of Febuxostat

An accurately weighed 100 mg of Febuxostat was dissolved in phosphate buffer pH 6.8 in a 100 ml volumetric flask and the volume was adjusted up to the mark with phosphate buffer to obtain a stock solution of concentration 1000 µg/ml. The solution was sonicated for 2 min.

Determination of λ_{max}

The stock solution was diluted appropriately to obtain a working solution with a concentration 10µg/ml. Aliquots of working solution were transferred to a series of 10 ml volumetric flasks and volume in each flask were adjusted to 10 ml with phosphate buffer to obtain a concentration range of 1 to 10 µg/ml. The solution was scanned in UV range of 200-400 nm using phosphate buffer pH 6.8 as a blank and absorbance maximum was found to be 312 nm. The absorbance of different solutions was measured at 312 nm against blank and calibration curve of Febuxostat was constructed.

Preparation of Sample

Twenty tablets of Febuxostat were weighed and finely powdered. Amount equivalent to 100 mg was transferred to 100 ml volumetric flask, dissolved in phosphate buffer pH 6.8 and made up the volume with phosphate buffer to obtain a concentration of 1000 µg/ml. The solution was sonicated for 2 min. The absorbance of sample solution was measured and amount of Febuxostat was determined by referring to the calibration curve.

VALIDATION OF DEVELOPED METHOD

Purpose

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result meeting its predetermined specifications and quality characteristics. The validity of the method was tested regarding linearity, specificity, accuracy, and precision according to ICH Q2B recommendations.[6]

Linearity

Absorbance and concentration relationship was described by Beer's law. It states that the monochromatic light's intensity decreases as the number of absorption molecules increases.[8] A linear correlation was found between absorbance at λ_{max} and various concentrations of Febuxostat. The linearity graph obeyed Beer's law in the range from 1 to 10μg/mL and it was described by regression equation ($y = mx + c$) and correlation coefficient (r^2) which were displayed on the graph.

Accuracy and Precision

Accuracy and precision of the method were evaluated with the help of percent recovery standard deviation (SD), and percent relative standard deviation (RSD) by using standard addition method. Three levels of standard drug (20%, 40%, and 60%) were spiked individually with the 10-mg equivalent of drug of Febuxostat analyzed in three replicates during the same day (intraday precision) and three consecutive days (intraday precision).

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of drug (10µg/mL). From the resulting absorbances the standard deviation and relative standard deviation were calculated.[9]

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The method for the determination of LOD and LOQ is based on residual standard deviation of regression line and slope. To determine LOD and LOQ, the specific calibration curve was studied using the sample containing analyte in the range of detection limit and quantization limit.

Limit of detection (LOD) and limit of quantification (LOQ) decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by Equations.

LOD (µg/ml) = 3.3 x SD / S

Where,

SD = the standard deviation of the response

S = the slope of the calibration curve (mean)

LOQ (µg/ml) = 10 x SD / S

Where,

SD = the standard deviation of the response

S = the slope of the calibration curve (mean).

Robustness

Robustness of the method was proved by analyzing the standard solutions 10 μg/mL of Febuxostat by two different wavelengths using the same experimental and environmental conditions. Robustness was tested by varying detection wavelength (±2 nm) of optimized conditions from the standard detection wavelength.

Ruggedness

Ruggedness of the method was determined by analyzing repeatedly for six times the standard solution having 10μg/ml of Febuxostat by two different analysts using the same experimental and environmental conditions.[10]

Forced Degradation Study

A 2-ml aliquot of standard stock solution of Febuxostat (1mg/mL) was taken in four replicates in a volumetric flask (100 ml) and mixed with 10 ml of 0.1N HCl (acid hydrolysis), 0.1N NaOH (alkaline hydrolysis), 5% H_2O_2 (oxidative degradation) and set aside for 1 h at room temperature. Solution was diluted up to mark with phosphate buffer. For thermal degradation, solid drug was kept in an oven at 100ºC for 24h. After cooling to room temperature, 10 μg/ml concentration drug solution was prepared as per above said method. Finally, absorbance of all the solutions resulted from acid and alkaline hydrolysis, and oxidative degradation and thermal degradation were measured at 312 nm against respective solvent as blank in each case.[11]

Analysis of pharmaceutical formulation

Twenty commercial tablets of Febuxostat were weighed, powdered and tablet powder equivalent to 10 mg of Febuxostat was dissolved in phosphate buffer 6.8 in a 10-ml volumetric flask. The solution was sonicated for 15 minutes, centrifuged at 100 rpm for 15 min and filtered through Whatmann filter paper No.41. From clear solution, further dilutions were made to get 1 mg/ml of Febuxostat. From the filtrate aliquots were taken and suitably diluted with phosphate buffer as per the requirement. The data obtained was substituted in the regression equations obtained and the percentage purity was determined.[9]

RESULTS AND DISCUSSION

The objective of the study was to develop the method of Febuxostat under the suitable spectral conditions. Estimation of Febuxostat by UV spectrophotometric method was carried out. Buffer solution of Febuxostat showed absorbance maximum (λ_{max}) at 312 nm and at this wavelength distilled water did not show any significant absorbance, therefore, further analysis was carried out at 312 nm.

Linearity

The linearity studies of the drug were performed by plotting different concentrations of standard solution against their respective absorbance. The calibration curve was drawn by taking concentration of the drug on x-axis and absorbance on y-axis and linear regression analysis was then applied as shown in Table 2. Following equation of calibration curve was obtained for Febuxostat:

$y = 0.078x + 0.062$

The drug was found to be linear in the concentration range of 1-10µg/ml. The Correlation co-efficient values were found to be 0.999, and the calibration curve showed that the drug obeyed Beer's law limit within the concentration range.

Precision

The intraday precision was determined by analyzing the drug at particular concentration for three times on the same day taking the time intervals of 2 h at 10:00 am, 12:00 pm and 2:00 pm respectively. The inter day precision was determined similarly, analyzing the samples daily, for three consecutive days. The results obtained from intraday and inter day precision are shown in Table 3.

Accuracy (Recovery studies)

To ensure the accuracy method, recovery studies were performed by standard addition method at 20%, 40% and 60% levels of drug concentration, to the pre-analyzed samples and percent recovery values were calculated. The results are shown in the Table 4.

Repeatability

The repeatability of the instrument was validated by taking the absorbance of six samples of the same concentration (8 µg/ml). The mean concentration was found to be 6.86 µg/ml. The results were obtained in Table 5.

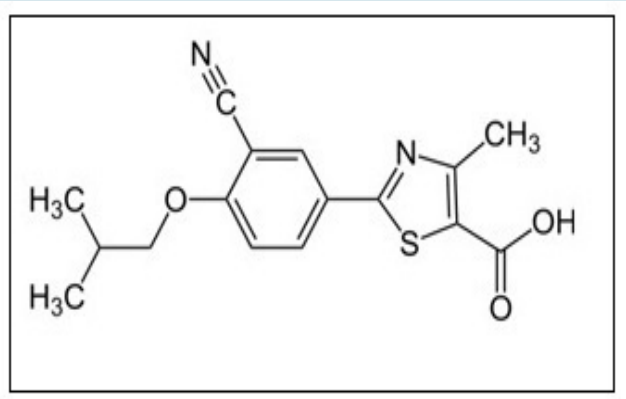

Figure 1: Chemical sturucture of Febuxostat.

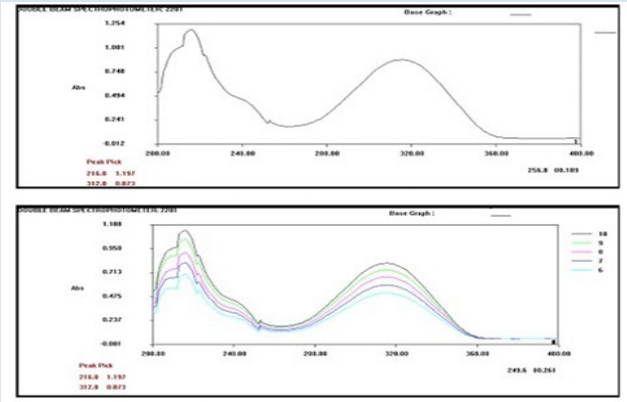

Figure 2a: UV Spectrum of Febuxostat;
Figure 2b: Overlay Spectra of Febuxostat.

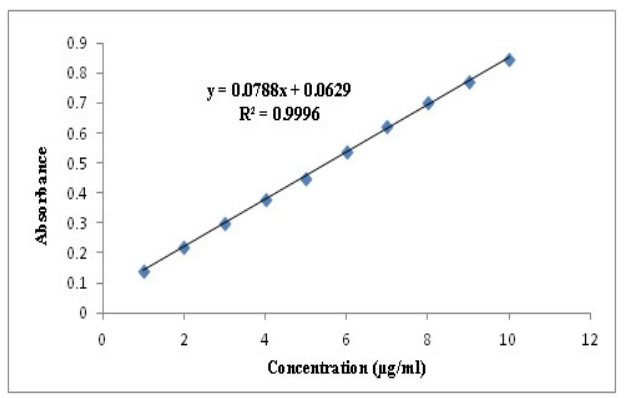

Figure 3: Calibration curve of Febuxostat in Phosphate buffer pH 6.8

Table 1: Validation parameters

Validation Parameter	Results
Absorption maxima (λ_{max})	312 nm
Linearity Range	1-10µg/ml
Regression equation	y = 0.078x+0.062
Slope	0.078
Y- intercept	0.062
Correlation Co-efficient (R2)	0.999
LOD	1.008
LOQ	3.502
Repeatability (%RSD)	0.145

Table 2: Spectrophotometric data for calibration curve

Concentration (µg/ml)	*Mean Absorbance ± SD
1	0.14±0.006
2	0.22±0.028
3	0.30±0.044
4	0.38±0.058
5	0.45±0.074
6	0.53±0.090
7	0.62±0.103
8	0.70±0.114
9	0.77±0.133
10	0.84±0.140

*Each value is an average of three determinations

Table 3: Results of Intra Day and Inter Day Precision

Concentration taken (µg/ml)	Concentration found (µg/ml) (*n=3)			Mean	SD	% RSD
	10:00 am	12:00 pm	02:00 pm			
10	8.987	9.008	9.144	9.04	0.08	0.88
9	7.701	7.781	7.882	7.788	0.09	1.15
8	7.701	7.504	7.601	7.60	0.09	1.1

Inter Day Precision

Concentration taken (µg/ml)	Concentration found (µg/ml) (*n=3)			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
10	9.324	9.356	9.435	9.371	0.05	0.5
9	8.288	8.358	8.358	8.337	0.04	0.4
8	6.487	6.559	6.61	6.552	0.06	0.9

*Each value is an average of three determinations.

Table 4: Results of accuracy studies

Recovery level	Initial conc. (µg/ml)	Added drug concentration *(n=3)	Amount recovered (µg/ml)	% Recovery	% RSD
20	10	2	11.83	98.58	0.225
40	10	4	14.01	100.07	0.625
60	10	6	15.92	99.5	0.250

*Each value is an average of three determinations.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

According to the values of the standard deviation and slope, limit of detection and limit of quantification were calculated. The results are shown in Table 1.

Robustness

Robustness studies assumed that the small variations in any of the variables did not significantly affect the results. The results obtained are given in the Table below.

Table 5: Results of repeatability studies

Concentration taken (µg/ml)	Concentration found (µg/ml)
8	6.87
8	6.87
8	6.87
8	6.85
8	6.87
8	6.85
Mean	6.86
SD	0.01
%RSD	0.145

Table 6: Results of robustness studies

Concentration taken (µg/ml)	Concentration found (µg/ml)	
	at 310 nm	at 314 nm
8	6.80	6.87
Mean	6.82	6.88
SD	0.008	0.006
%RSD	0.117	0.872

Table 7: Results of ruggedness studies

Concentration taken (µg/ml)	Concentration found (µg/ml)	
	Analyst 1	Analyst 2
8	6.82	6.81
8	6.82	6.80
8	6.82	6.81
8	6.82	6.80
8	6.80	6.80
8	6.80	6.80
Mean	6.81	6.80
SD	0.01	0.005
%RSD	0.14	0.07

Ruggedness

The results did not show any major statistical difference between operators suggesting that method developed was rugged. The results were shown in Table 7.

Degradation study

Stability indicating property of analyte was performed by forced degradation studies. Febuxostat was subjected to various stress conditions like acid, alkaline, hydrogen peroxide induced degradation, and thermal condition. Analysis was performed by measuring absorbance of Febuxostat (after subjecting it to stressed conditions) at λ_{max} of pure drug.

Percentage degradation was calculated by the formula.

% degradation = (expected concentration - actual concentration) × 100/ expected concentration, and percentage recovery also was calculated for each case. Results revealed no change in absorbance of drug solution at

Table 8: Degradation study parameters

Parameter Study	Concentration taken (µg/ml)	Concentration found (µg/ml) *(n=3)	% Degradation	% Recovery
Acid Hydrolysis	8	7.07	11.625	88.375
Alkaline Hydrolysis	8	7.23	9.5	90.403
Oxidative Hydrolysis	8	7.60	5.0	95.000
Thermal Degradation	8	7.12	10.99	89.01

*Each value is an average of three determinations

Table 9: Results of analysis of marketed formulation

Formulation	Label claim (mg)	Amount Obtained (mg)	% Label Claim±SD
FEBUTAZ	40	39.88	99.7±0.542

alkaline hydrolysis and oxidative hydrolysis. The percentage recovery was very close to or more than 90% in all cases which indicated the drug stability. The results of forced degradation study are shown in Table 8.

Analysis of marketed formulation

The wavelength was recorded at 312 nm of the marketed formulation. Analysis of tablet formulation was carried out and observations were recorded in Table 9.

DISCUSSION

Solution of Febuxostat in pH 6.8 buffer showed absorbance maximum (λ_{max}) at 312 nm (Figure 2), and at this wavelength distilled water did not show any significant absorbance. Therefore, further analysis was carried out at 312 nm. Least square regression equation of Febuxostat in buffer solution has shown that the r^2 value very close to 1 indicated high degree of correlation between two variables i.e. absorbance and concentration (Figure 3). Beer's law was obeyed over a concentration range of 1–10 μg/mL. LOD and LOQ values for the drug were found and all the parameters of calibration curve were displayed in Table 1.

To check accuracy and precision, assays were carried out for three times within a day (intraday precision) and in three consecutive days (inter day precision) by adding three different levels of analyte to the formulation. % RSD values were ≤1.15 (intraday) and ≤0.9 (inter day) indicating high precision of developed method. Accuracy of the method was ascertained as mean % recovery between measured actual concentration and taken concentration for Febuxostat. The values of % recovery was very close to 100%, which demonstrate high accuracy of the proposed method (Table 4). Robustness studies assumed that the small variations in any of the variables did not significantly affect the results (Table 6). This provided an indication for the reliability of the proposed method during routine analysis. Ruggedness studies (Table 7) didn't show any statistical difference between operator's observations suggesting that the method was rugged.

Stability indicating property of analyte was performed by forced degradation studies. Febuxostat was subjected to various stress conditions like acid, alkaline, hydrogen peroxide induced degradation, and thermal conditions. Results revealed the percentage recovery more than or close to 90% which indicates the drug stability. The analyte showed slight degradation with alkaline and oxidative stressed conditions. But there was significant change in absorbance after acid hydrolysis (% degradation of 11.62%) and thermal stressed conditions (% degradation of ~11%), confirming the susceptibility of drug to the said conditions (Table 8).

CONCLUSION

A method was developed and validated for quantification of Febuxostat. This analytical methodology was developed according to the guidelines laid down. The method was validated as per the guidelines laid by ICH. The results of the validated tests were found to be satisfactory and therefore the proposed spectrophotometric method was found to be simple, sensitive, accurate, precise, reproducible and economical which can be applied successfully for routine quality control analysis of Febuxostat in bulk form as well as pharmaceutical formulation (tablets).

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CONFLICT OF INTEREST

Authors have none to declare.

ABBREVIATION USED

LC-MS: Liquid chromatography; Mass spectroscopy; UPLC: Ultra Performance Liquid chromatography; LOD: Limit of Detection; LOQ: Limit of Quantification; NP-SIXO: Nonpurine Selective Inhibitor of Xanthine Oxidase; SD: Standard Deviation; RSD: Relative Standard Deviation.

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PICTORIAL ABSTRACT

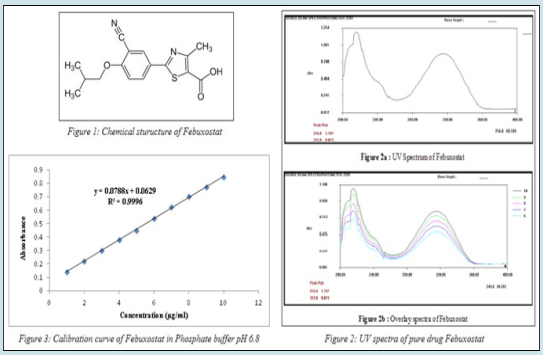

Figure 1: Chemical sturucture of Febuxostat

Figure 3: Calibration curve of Febuxostat in Phosphate buffer pH 6.8

Figure 2: UV spectra of pure drug Febuxostat

SUMMARY

- The present research work discusses the development and validation of a UV-spectrophotometric method for the estimation of Febuxostat.
- A simple, accurate, cost effective, and reproducible spectrophotometric method has been developed for the estimation of drug in bulk as well as in pharmaceutical dosage form i.e. tablets.

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