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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF FEBUXOSTAT IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

An accurate and precise HPLC method was developed for the estimation of Febuxostat. Separation of the drug was achieved on a reverse phase C_8 column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 40:60 v/v. The flow rate was 1.0 ml/min and the detection wavelength was 320 nm. The linearity was observed in the range of 5-60 µg/ml with a correlation coefficient of 0.999. The retention time of Febuxostat was 3.145 min. The proposed method was validated as per the ICH guidelines for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Febuxostat in tablet dosage forms.

Keywords: Febuxostat, Estimation, RP-HPLC, Validation, Tablets.

INTRODUCTION

Febuxostat is a novel xanthine oxidase inhibitor indicated for the chronic management of hyperuricemia in patients with gout[1]. It achieves its therapeutic effect by decreasing serum uric acid. Chemically it is 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid (Figure 1)[2]. Febuxostat is a non purine analogue inhibitor of both the oxidized and reduced forms of xanthine oxidase. It was found to be more than 10-30 times potent than allopurinol in animal studies[3]. A few spectrophotometric[4-5], HPLC[6-9], LC-MS[10-11], TLC[12] and GC[13] methods were reported earlier for the determination of Febuxostat in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of Febuxostat in bulk samples and in tablet dosage forms.

MATERIALS AND METHODS

Chromatographic Conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Symmetry YMC ODS C_8 column (150 x 4.6 mm; 3.0 µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and Solvents

The working standard of Febuxostat was provided as gift sample from Sumages Pharma Pvt. Ltd., Bhimavaram, India. Febuxostat tablets were purchased from local market. HPLC grade acetonitrile were purchased from E. Merck (India) Ltd., Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC grade water obtained from Milli Q water purification system was used throughout the study.

Preparation of phosphate buffer
2.5 grams of potassium di hydrogen phosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents
400 ml of the phosphate buffer was mixed with 600 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard solution
Accurately weigh and transfer 10 mg of Febuxostat working standard into a 10 ml volumetric flask, add about 7 ml of diluents, sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Preparation of sample solution
Weigh 20 Febuxostat tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Febuxostat into a 10 ml volumetric flask. Add about 7 ml of diluents, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Calibration Plot
About 10 mg of Febuxostat was weighed accurately, transferred into a 10 ml volumetric flask and dissolved in 7 ml of a 40:60 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 1000 µg/ml solution. From this, a working standard solution of the drug (40 µg/ml) was prepared by diluting with the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 5-60 µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area (Figure 2) was found to be linear in the concentration range of 5-60 µg/ml of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Febuxostat in tablet dosage forms.

Procedure
A mixture of phosphate buffer and acetonitrile in the ratio of 40:60 v/v was found to be the most suitable mobile phase for ideal separation of Febuxostat. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. Inject 20 µl of the standard, sample solutions into the chromatographic system and measure the area for the Febuxostat peak. The detection of the drug was monitored at 320 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.145 min. A typical chromatogram showing the separation of the drug is given in Figure 3.

Validation of the proposed method
The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines for the estimation of Febuxostat[14]. Solution containing 40 µg/ml solution of Febuxostat was subjected to the proposed HPLC analysis to check method precision and intermediate precision of the method and the results are furnished in Table-2. The accuracy of the HPLC method was assessed by analyzing solutions of Febuxostat at 50, 100 and 150% concentration levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Estimation of Febuxostat in tablet dosage forms
Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Febuxostat in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of Febuxostat was transferred into a 10 ml

volumetric flask and dissolved in 5 ml of a 40:60 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 3 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. This solution containing 40 µg/ml of Febuxostat was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Febuxostat was found to be 3.145 min. Quantification was linear in the concentration range of 5-60 µg/ml. The regression equation of the linearity plot of concentration of Febuxostat over its peak area was found to be y=712853.348+44148.3x (r^2=0.999), where x is the concentration of Febuxostat (µg/ml) and y is the corresponding peak area. The number of theoretical plates calculated was 2981, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.018 µg/ml and 0.060 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Febuxostat by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Febuxostat and can be reliably adopted for routine quality control analysis of Febuxostat in its tablet dosage forms.

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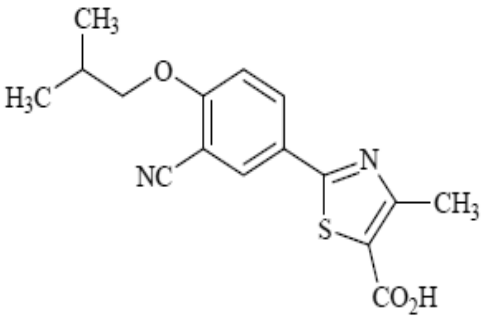

Fig. 1: Chemical structure of Febuxostat

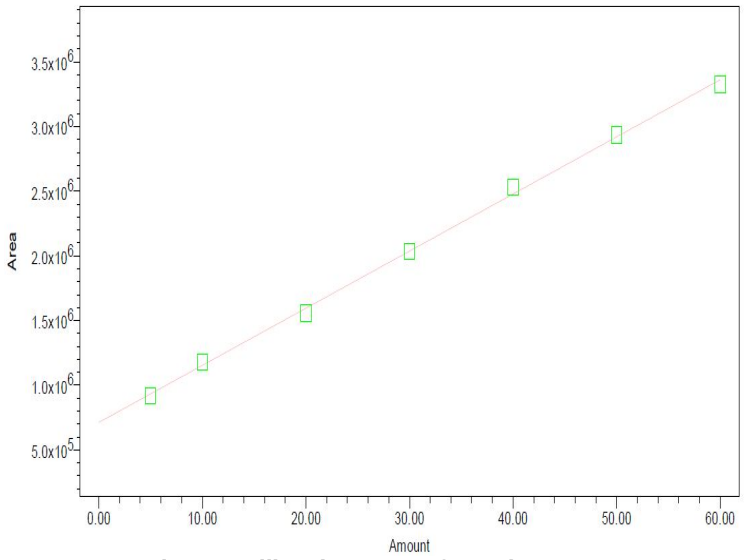

Fig. 2: Calibration curve for Febuxostat

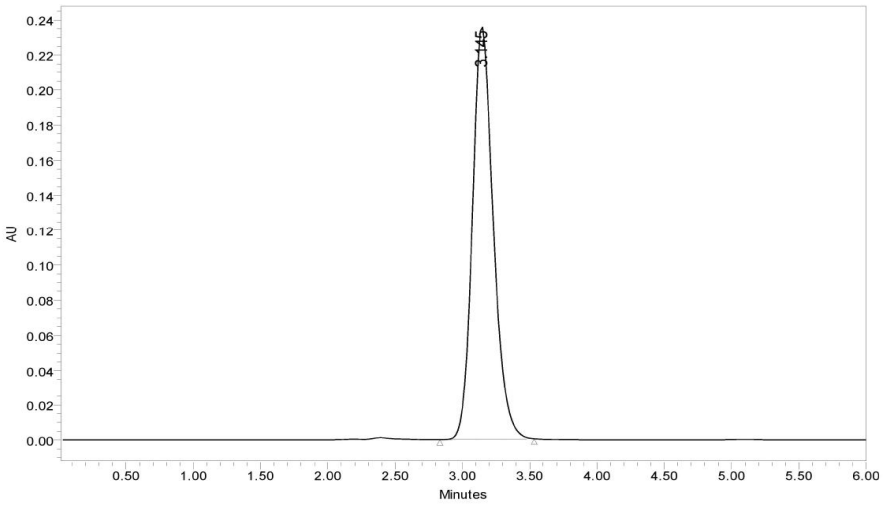

Fig. 3: Typical chromatogram of Febuxostat

Table 1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=7)
5	916802
10	1179605
20	1559210
30	2035388
40	2531097
50	2933843
60	3325913

Table 2: Precision of the proposed HPLC method

Concentration of Febuxostat (40µg/ml)	Peak area	
	Method precision	Intermediate precision
Injection-1	2518407	2559753
Injection-2	2523339	2565169
Injection-3	2492385	2566650
Injection-4	2533304	2565082
Injection-5	2526496	2567109
Injection-6	2536436	2566095
Average	2521727.8	2564976.3
Standard Deviation	15796.82	2934.0
%RSD	0.62	0.11

Table 3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	5.0	4.93	98.7%	98.9%
100%	10.0	9.98	99.8%	
150%	15.0	14.7	98.2%	

Table 4: System suitability parameters

Parameter	Result
Linearity ((μg/ml)	5-60
Correlation coefficient	0.999
Theoretical plates (N)	2981
Tailing factor	1.2
LOD (μg/ml)	0.018
LOQ (μg/ml)	0.060

Table 5: Assay studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
FABULAS	10	9.99	99.9

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