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Research Article

A RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FEBUXOSTAT IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Raul Saroj Kumar[1]*, B.V.V Ravi kumar[2], Patnaik Ajaya Kumar[3]

[1]M .R. College of Pharmacy, Phool-Baugh, Vizianagaram, A.P, India

[2]Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India

[3]Department of Chemistry, Ravenshaw University, Cuttack, Orissa, India

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*E-mail: saroj.raul@rediffmail.com

ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Febuxostat in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL min $^{-1}$ was employed on a Hypersil C_{18} column at ambient temperature. The mobile phase consisted of acetonitrile: phosphate buffer 60:40 (v/v) and the detection wavelength was at 320 nm. Linearity was observed in concentration range of 5-30 µg/mL. The retention time for Febuxostat was 3.4 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Febuxostat in pharmaceutical dosage forms.

KEY WORDS: Dosage forms, Estimation, Method development, Febuxostat, RP-HPLC, Validation.

INTRODUCTION

Febuxostat is a novel xanthine oxidase inhibitor indicated for use in the treatment of hyperuricemia and chronic gout [1-2]. Chemically it is 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid (Figure 1) with molecular weight 316.38. It achieves its therapeutic effect by decreasing serum uric acid. It inhibits both oxidized and reduced forms of xanthine oxidase and has very less effects on other enzymes of purine and pyrimidine metabolism [3,4].

Literature survey reveals that few spectrophotometric methods [5-7,] HPLC method [8-11], LC-MS method [12] has been reported for the estimation of Febuxostat. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Febuxostat in pharmaceutical dosage form as per ICH guidelines[13].

MATERIALS AND METHOD

Instrumental and analytical conditions

The HPLC analysis was carried out on Waters HPLC system (2695 module) equipped with 2487 dual lambda detector with auto Sampler and running on Waters Empower software. The column used is Hypersil C_{18} (150 × 4.6 mm, packed with 3 µm) and detection was performed at 320 nm. The injection volume of sample was 20 µL and the run time was 6 minutes. An isocratic mobile phase containing acetonitrile and 0.02 M phosphate buffer at 60: 40 (v/v) at the pH 3.5 was carried with the flow rate at 1.0mL min^{-1}. The mobile phase was filtered through 0.4µm membrane filter and degassed before use.

Reagents and chemicals

Febuxostat working standard was kindly gifted by pharmatrain, Hyderabad. Tablets were purchased from local pharmacy manufactured by Sun Pharmaceutical Ltd (Febutaz). Ultra pure water was obtained from a millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade. The optimum chromatographic conditions were summarized in table 7.

Preparation of mobile phase

Dissolved 2.7218 g of Potassium Di hydrogen orthophosphate in 1000 mL of water and mixed, pH adjusted to 3.5, sonicated to degas the buffer. Transferred 600 volumes of acetonitrile and 400 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of Standard Solution

Accurately weighed about 10 mg of Febuxostat and transferred into a 10mL volumetric flask and 7 mL of diluent was added and sonicate to dissolve it completely and the volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. Then 0.2 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with mobile phase and filter through 0.45 µm filters, which gives a solution of strength 20 µg/mL.

Preparation of sample solution

Weigh 20 Febuxostat tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Febuxostat into a 50 ml volumetric flask. Add about 25ml of diluent, 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 0.2 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.

METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity

From the standard stock solution, the various dilutions of Febuxostat in the concentration of 5, 10, 15, 20, 25 and 30 µg/mL were prepared. The solutions were injected using 20 µL injection volumes in to the chromatographic system at the flow rate of 1.0 mLmin^{-1} and the effluents were monitored at 320nm, chromatograms were recorded. Calibration curve of

Febuxostat was obtained by plotting the peak area ratio versus the applied concentrations of Febuxostat, given in table 1.The linear correlation coefficient was found to be 0.999, shown in figure2.

Precision

Repeatability of the method was checked by injecting replicate injections of 20 µg/mL of the solution for six times on the same day as intraday precision study of Febuxostat and the % RSD was found to be 0.58, given in table 2.

Accuracy

Febuxostat reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50%, 100% and 150%). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in table 3.

Specificity

Spectral purities of Febuxostat chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness

To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in table 4.

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Detection and quantitation limits

According to the determined signal-to-noise ratio, Febuxostat presented limits of detection of 0.1 µg/mL and limits of quantitation of 0.5µg/mL, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Febuxostat so that the values obtained should be considered as the limit of method sensitivity.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of Febuxostat and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in table 5.

Assay of Febuxostat tablet

Three different batches of Febutaz were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Febuxostat was transferred to a 50 ml volumetric flask followed by the addition of 25 ml of mobile phase. The solution was sonicated for 3 minutes and volume adjusted with the mobile phase then filtered through 0.45 µm membrane filter. Further dilutions were made to get the final concentration equivalent to 20 µg/mL of Febuxostat. The mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in table 6.

All the analyzed batches presented Febuxostat were very close to the labeled amount. The Febuxostat content in the tablets samples varied from 99.6 to 100.2%.

Table 1: **Linearity of Febuxostat**

Concentration (µg/mL)	Average area
5	687588
10	1334141
15	2050917
20	2694747
25	3318211
30	4057638

Table 2: Precision of Febuxostat

Injections	Area
1	2640273
2	2653438
3	2667036
4	2666592
5	2686770
6	2662546
Mean	2662776
SD	15505.21
% RSD	0.58

Table 3: **Accuracy of Febuxostat**

% Conc	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.98	99.6%	99.9%
100%	10.0	9.96	99.6 %	
150%	15.0	15.1	100.6 %	

Table 4: Robustness of Febuxostat

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate as per method 1.0mL/min	0.8 mL/min	2816250	3.441	5918.6	0.21
	As it is	2651365	3.449	6923.5	0.26
	1.2ml/min	2709341	3.442	8895.2	0.33
Mobile phase composition Acetonitrile: Buffer (60:40)	Acetonitrile: Buffer (55:45)	2698521	3.440	9952.6	0.36
	As it is	2676352	3.445	6787.8	0.25
	Acetonitrile: Buffer (65:35)	2712314	3.443	10215.8	0.38

Table 5: System Suitability of Febuxostat

Concentration	Injection	Area	R_t
20 µg/mL	Inj-1	2667516	3.441
	Inj-2	2657495	3.445
	Inj-3	2648771	3.442
	Inj-4	2659983	3.443
	Inj-5	2700125	3.441
	Inj-6	2654455	3.440
Statistical Analysis	Mean	2664724	3.442
	SD	18415.82	0.0017 89
	% RSD	0.69	0.05
	Tailing Factor	1.4	
	Plate Count	4224.3	

Table 6: Contents of Febuxostat in tablets (n=6)

Sample tablet	Batch	Label Claim (mg)	Amount found (mg)±SD	%Amount found
Febutaz(40mg)	1	10	9.98±0.14	99.8
	2	10	9.96±0.05	99.6
	3	10	10.02±0.06	100.2

S.D=Standard Deviation

Table7: Developed Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Hypersil C_{18} (150 × 4.6 mm, packed with 3 µm)
Mobile Phase	60:40 (Acetonitrile : Phosphate Buffer)
pH	3.5 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	6.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	320
Drugs Rt (min)	3.4

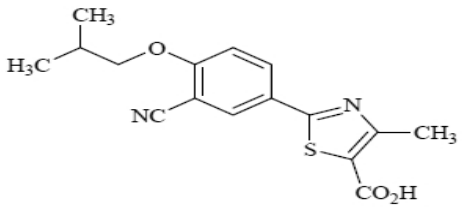

Figure 1: **Chemical structure of Febuxostat**

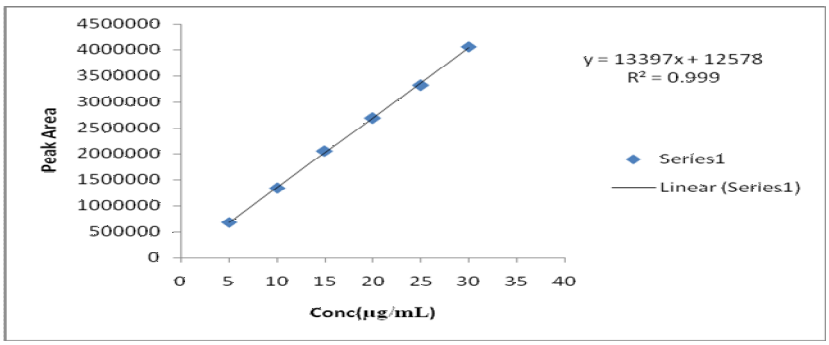

Figure 2: Linearity curve of Febuxostat

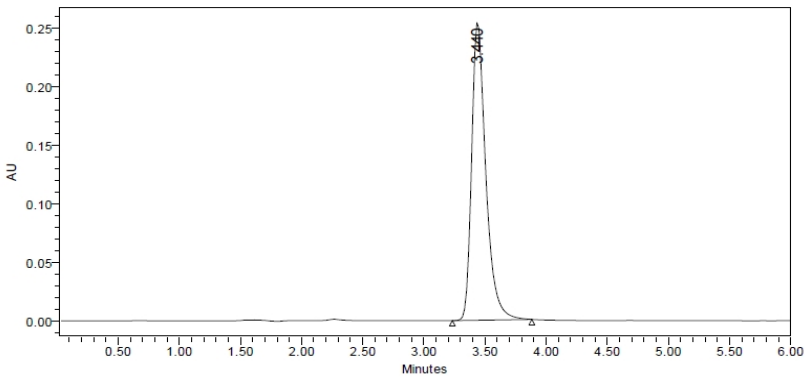

Figure 3: Standard Chromatogram of Febuxostat.

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Febuxostat was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the acetonitrile to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 60:40 (v/v).The retention time of Febuxostat was found to be 3.4 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Febuxostat in tablet formulation, given in table 7. A typical chromatogram showing the separation of Febuxostat is shown in figure 3.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Febuxostat in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Febuxostat in pharmaceutical dosage form.

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