

DEVELOPED AND VALIDATED REVERSE PHASE HPLC METHOD FOR THE DETERMINATION OF FEBUXOSTAT IN BULK AND FORMULATIONS

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ABSTRACT

The objective of the present work was to develop a simple and accurate RP-HPLC method for the estimation of febuxostat in bulk and pharmaceutical dosage forms, using C18 column 250 x 4.6 mm i.d, 5µm particle size in isocratic mode, with mobile phase comprising of Methanol and ortho - Phosphoric acid in the ratio of 90: 10 v/v. The flow rate was 1ml/min and the detection was monitored by UV detector at 316nm. The retention time for febuxostat was found to be 5.28 min. The proposed method has permitted the quantification of febuxostat over linearity in the range of 10-100 µg/ml and its percentage recovery was found to be 100.2%. The intraday and inter day precision were found 0.63% and 0.51%, respectively.

Keywords: RP-HPLC, Validation, Febuxostat. Gout, Recovery

INTRODUCTION

Gout is the most common form of inflammatory joint disease in men aged >40 years and is commonly characterized by hyperuricaemia and recurrent attacks of acute arthritis. Sustained hyperuricaemia is considered to be the precursor and the underlying cause of gout. The management of hyperuricaemia associated with gout is therefore one of the most important cornerstones in the management of gout. Management of hyperuricaemia is achieved by the use of drugs that increase uric acid total body clearance (e.g. uricosurics or uricase) or drugs that decrease uric acid production (e.g. xanthine oxidase inhibitors). 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 gout¹. Gout is the collective name for several disorders that are characterized by the formation and deposition of monosodium urate (MSUr) crystals. The condition is associated with recurrent episodes of acute joint pain due to the deposition of MSUr crystals in the synovial fluid. In addition to the effects observed in the joints, skin/subcutaneous tissue and kidneys may also be affected by tophaceous deposits, cellulitis, urate nephropathy, and/or kidney stones, respectively. The active substance is a new chemical entity designated as 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid. The structure of febuxostat is shown in Figure 1. It is a selective inhibitor of XO that has been developed for the treatment of hyperuricemia and gout, as it was found to have a potent inhibitory activity for XO/xanthine dehydrogenase (XDH) during evaluation of a range of newly synthesized molecules².

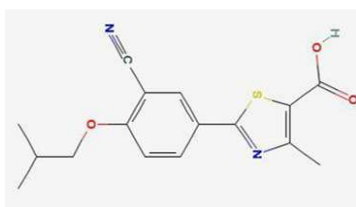


Fig. 1: Chemical structure of febuxostat

MATERIALS AND METHODS

Chemicals and Reagents

Drug was received as a gift from Ajanta Lab, Ltd, Mumbai, India. HPLC grade Methanol (Merck) were procured from the local market. All other chemicals and reagents employed were of analytical grade and were purchased from S.D. Fine Chemicals, India. Millipore Milli Q plus (Bangalore, India) purification system was used to prepare high pure water.

Instrumentation

A Gradient HPLC PU 2080 Plus (Make : JASCO) with UV- 2075 Plus detector and RP-C18 column was used. A Rheodyne injector with a 20µl loop was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing. A reversed-phase Hi-Q-Sil C-18 column (250 x 4.6 mm i.d. particle size 5µm) used for separation.

Chromatographic conditions

Hi-Q-Sil C-18 column (4.6 x 250mm, 5µm particle size) was used as stationary phase. Ortho phosphoric acid and Methanol in the ratio of 10:90 v/v was used as mobile phase and was filtered before use through 0.22µm membrane filter. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 316 nm. To ascertain the suitability of the proposed chromatographic conditions, system suitability tests were carried out and the results are shown in Table 1. Chromatogram of standard solution containing FBX is shown in Fig. 2.

Preparation of mobile phase and stock solution

Standard stock solution of FBX was prepared by transferring 25 mg in to 25 ml volumetric flask. Sufficient amount of mobile phase (Methanol and ortho-Phosphoric acid in the ratio of 90: 10 respectively) was added, sonicated and remaining volume was made up to the mark with mobile phase, which gave the concentration of 1000µg/ml of the drug. Aliquots of standard stock solution were appropriately diluted with mobile phase to obtain concentration 10 - 100µg/ml.

Method development

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Analysis of Tablet Formulation

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample, was studied by analyzing five concentrations of the drug, and process was repeated for five times each. It was done over the range of 300-1500ng/band³⁻⁵.

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability

of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, solutions of febuxostat at four different concentrations (4, 8, 12, and 16 µg/mL) were determined in triplicate⁶⁻⁸.

Reproducibility

The reproducibility of the method was checked by determining precision on the same instrument, but by a different analyst. For both intra-day and inter-day variation, solutions of febuxostat at three different concentrations (8, 12, and 16 µg/mL) were analyzed in triplicate⁹⁻¹².

Accuracy

Accuracy is the measure of how close the experimental value is to the true value. Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of febuxostat (12 µg/mL) were spiked with 50, 100, and 150% extra febuxostat standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%) and standard error of mean (SEM) were calculated for each concentration¹³⁻¹⁶.

Ruggedness

Ruggedness test was determined between two different days, analysts and instruments. The value of RSD was to be found 0.9 which is within acceptance criteria of below 2.0% showed ruggedness of developed HPLC method¹⁷.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of febuxostat. Robustness was determined by using reagents from two different lots and two different manufacturers¹⁸.

LOD and LOQ

LOD and LOQ were determined by the standard deviation ($S_{y/x}$) method. The LOD was determined by the analysis of samples with known concentrations of analyte and by establishing through visual evaluation the minimum level at which the analyte could be reliably detected. The LOQ was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precision. LOD and LOQ were determined from the slope, S , of the calibration plot and the standard deviation of the response for the blank sample, $S_{y/x}$, by use of the formulae $LOD = 3.3 \times S_{y/x}/S$ and $LOQ = 10 \times S_{y/x}/S$ ¹⁹.

Sample solution stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 72 h under laboratory bench conditions (25 ± 1 °C) and under refrigeration (8 ± 0.5 °C). An accurately weighed quantity of the pure drug was dissolved in Methanol and suitably diluted with mobile phase to get a final concentration of 12 µg/mL. The solution was subjected to HPLC analysis immediately and after a period of 24, 48 and 72 h²⁰.

System suitability

A system suitability test of the chromatography system was performed before each validation run. Six replicate injections of a system suitability/calibration standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the six suitability injections were determined²¹⁻²².

RESULT AND DISCUSSION

Several systematic trials were performed to optimize the Chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Febuxostat in pharmaceutical dosage forms. The proposed chromatographic developed system was suitable for effective separation and quantitation of Febuxostat. A typical Chromatogram obtained (Figure-1) by using the mobile phase. The mobile phase consisting of Methanol and ortho - Phosphoric acid in the ratio of 90:10(%v/v) was found to be an appropriate mobile phase allowing adequate elution of drug by using a Symmetry C18 column at a flow rate of 1.0 ml/min. The blank chromatogram showed no interference from the endogenous substances. The calibration curves for FBX were constructed by plotting the area of the peaks versus concentration. Linearity was observed in a concentration range from 10 to 100 µg/mL. This concentration range corresponds to 10 to 100% of the target concentration of 100 µg/mL. A linear regression by the least squares method was then applied. The value of the determination coefficient ($r^2 = 0.9999$) showed excellent linearity of the calibration curve for the method. The peak areas of the drugs were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. The sample recoveries from the formulation were in good agreement with their respective label claim indicating that there is no interference from the tablet excipients. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 5mins for eluting FBX. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, precision, repeatability of measurement of peak area as well as repeatability of sample application. Since this developed method can be used for routine analysis of one component in formulation. The results of robustness study also indicated that the method is robust and is unaffected by small deliberate variations in the method parameters. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of FBX.

CONCLUSION

The developed HPLC method is accurate, precise, reproducible and specific. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of febuxostat in bulk drugs and in pharmaceutical dosage forms.

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Table 1: Concentration Vs Mean Peak area of febuxostat

S. No.	Concentration (µg/ml)	Mean Peak Area*	%RSD
1	10	1352844	0.55
2	20	2427612	0.48
3	30	3768792	0.40
4	40	4874239	0.61
5	50	6250967	0.65
6	60	7458749	0.10
7	70	8773874	0.06
8	80	9989689	0.08
9	90	11108236	0.07
10	100	12296478	0.09

*Mean of five values, Regression equation

Table 2: Statistical data of calibration curves of febuxostat

S. No.	Parameters	Febuxostat
1	Linearity	10-100µg/ml
2	Regression equation	$y = 12344x + 37186$
3	Standard deviation of slope	0.011
4	Relative standard deviation of slope (%)	0.458
5	Standard deviation of intercept	0.256
6	Correlation coefficient (r^2)	0.999
7	Detection limit (µg/ml)	0.1587
8	Quantitation limit (µg/ml)	0.5487

Table 3: Result of Precision studies

Parameters	Component	% Estimation*	S.D.	C.V.
Intra-day	Feb	100.01	0.6325	0.6321
Inter-day	Feb	99.97	0.5130	0.5136

Table 4: Result of recovery studies

Level of Recovery	Component	Amt. Taken (mg)	Amt. of pure drug added (mg)	% recovery*	S.D.	C.V.
80 %	Feb	80	64	99.97	0.4425	0.4419
100 %	Feb	80	80	100.01	0.3963	0.3969
120 %	Feb	80	96	100.2	0.5809	0.5814



Fig. 1: Typical chromatogram of Febuxostat

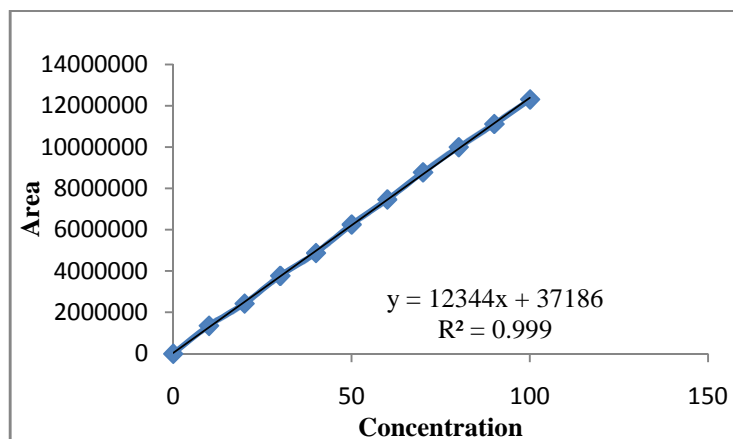


Fig. 2: Standard calibration curve Febuxostat

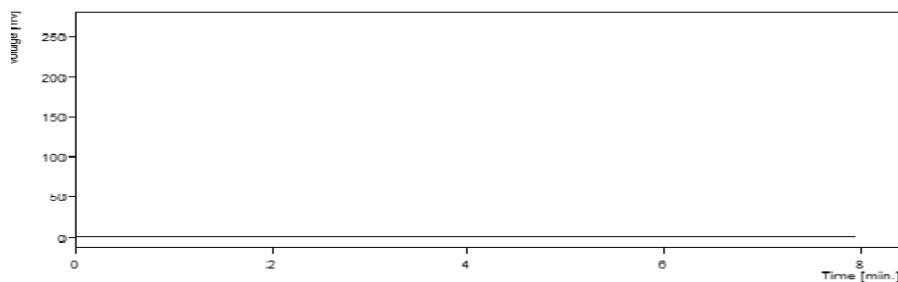


Fig. 3: Febuxostat blank

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