Research Article **CODEN: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FEBUXOSTAT IN DRUG SUBSTANCE BY RP-HPLC METHOD

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ABSTRACT

Analytical method was developed for the estimation of Febuxostat in drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (Symmetry C1875*4.6mm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in Water:ACN. The flow rate was 0.8 ml/ minute and ultra violet detector at 315nm. The average retention time for Febuxostat found to be 1.8 min the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 50-150µg/ml for Febuxostat.

KEYWORDS

Febuxostat, Isocratic, HPLC, Symmetry C18, Formic acid, Acetonitrile and Validation.

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INTRODUCTION

Febuxostat was used to treat chronic gout and hyperuricemia. More effective than standard doses of allopurinol, but not more effective than higher doses of allopurinol. Febuxostat works by decreasing the amount of uric acid that is made in the body. It is used to prevent gout attacks.

Febuxostat is a non-hygroscopic material, appearance was white crystalline powder that is freely soluble in dimethylformamide; soluble in dimethylsulfoxide; slightly soluble in methanol and acetonitrile; and practically insoluble in water. The melting range is 205°C to 208°C.

Febuxostat is chemically designated as 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methyl-1, 3-thiazole-5-carboxylic acid. Its molecular formula is $C_{16}H_{16}N_2O_3S$, and its molecular weight is 316.375g/mol.

EXPERIMENTAL[1-14]

Equipments

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Symmetry 5µ, 75mm × 4.6mm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Febuxostat were obtained as gift samples from Fortune pharma training institute, Sri Sai Nagar, KPHB and Hyderabad, India.

HPLC-grade Acetonitrile was from qualigens reagents Pvt Ltd. Formic acid (AR grade) was from SD fine chem.

Chromatographic conditions

The sample separation was achieved on a C18 (5 µ, 75 cm X 4.6 mm i. d.) SYMMETRY column, aided by mobile phase mixture of 0.1% v/v formic acid in Water:Acetonitrile. The flow rate was 0.8 ml/ minute and ultra violet detector at 315nm that was filtered and degassed prior to use, Injection volume is 5µl and ambient temperatures.

Preparation of mobile phase

Buffer Preparation

Take accurately 1ml of formic acid in 1000mL of water.

Mobile phase

Then add 20 volumes of buffer and 80 volumes of Acetonitrile mixed well and sonicated for 5min.

Preparation of standard stock solution

A 50mg of pure Febuxostat were weighed and transferred to 50 ml of volumetric flask and dissolved in ACN. The flask was shaken and volume was made up to mark with ACN to give a primary stock solution containing 1000µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with ACN to give a solution containing 100µg/ml of Febuxostat.

Preparation of sample solution

A 50mg of Febuxostat sample were weighed and transferred to 50 ml of volumetric flask and dissolved in ACN. The flask was shaken and volume was made up to mark with ACN to give a primary stock solution containing 1000µg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with ACN to give a solution containing 100µg/ml of Febuxostat.

RESULTS AND DISCUSSION

Determination of Working Wavelength (λmax)

10 mg of the Febuxostat standard drug is taken in a 10 ml volumetric flask and dissolved in ACN and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the ACN to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using ACN as blank. The λmax was found to be 315nm.

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: ACN (20:80). The flow rate was 0.8 ml/ minute brought sharp peaks. The chromatogram was shown in Figure No.1.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 50-150 µg/ml of Febuxostat. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.5 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 50µg/mL, 75µg/mL, 100µg/mL, 125µg/mL and 150 µg/mL of Febuxostat.

Curve established with concentration verses peak areas was plotted by injecting the prepared solutions

and the obtained data were subjected to regression analysis using the least squares method (Table No.1 and Figure No.2).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively (Table No.2).

LOD = 3.3 δ/S ……. (1)

LOQ =10 δ/S …….. (2)

Where,

σ = Standard deviation of the Response

The slope S may be estimated from the calibration curve of the analyte.

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100 µg/ml of FEBUXOSTAT without changing the parameter of the proposed chromatographic method (Table No.3).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Febuxostat by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Febuxostat. The percentage recovery results obtained are listed in Table No.4.

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Detection wavelength varied ±2nm and flow rate varied ±0.2 ml/min. The results were shown in (Table No.5).

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The % RSD (Relative Standard Deviation) of assay results between two analysts calculated i.e.

This indicates the method was rugged. The results were shown in Table No.6.

Table No.1: Linearity

S.No	Level	Concentration (mg/mL)	Peak area
1	50%	0.05	1426130
2	75%	0.075	2134202
3	100%	0.10	2868478
4	125%	0.125	3667499
5	150%	0.150	4394017

Table No.2: LOD and LOQ values Calculated from calibration curve

S.No		mg
1	LOD	0.003
2	LOQ	0.009

Table No.3: Summary of peak areas for method precision

S.No	Sample No	Retention time	Peak area	% Assay
1	1	1.797	2891238	100.9
2	2	1.796	2871372	100.2
3	3	1.796	2885066	100.7
4	4	1.796	2862985	99.5
5	5	1.792	2887878	101.0
6	6	1.794	2865894	100.0
7	Mean	1.795	2877406	100.4
8	% RSD	0.10	0.42	0.57

Table No.4: Recovery data

Level	S.No	% Recovery of Febuxostat	Average
50	1	99.4	99.5%
	2	99.3	
	3	99.6	
100	1	100.9	100.6%
	2	100.2	
	3	100.7	
150	1	100.9	100.8%
	2	100.7	
	3	100.8	

Table No.5: Results of Robustness study

S.No	parameter	Rt of Febuxostat	Theoretical plates	Asymmetry
1	Decreased flow rate (0.7ml/min)	2.036	3493	1.20
2	Increased flow rate (0.9ml/min)	1.595	2963	1.14
3	Wave Length 313nm	2.219	2107	1.30
4	317	2.219	2126	1.31

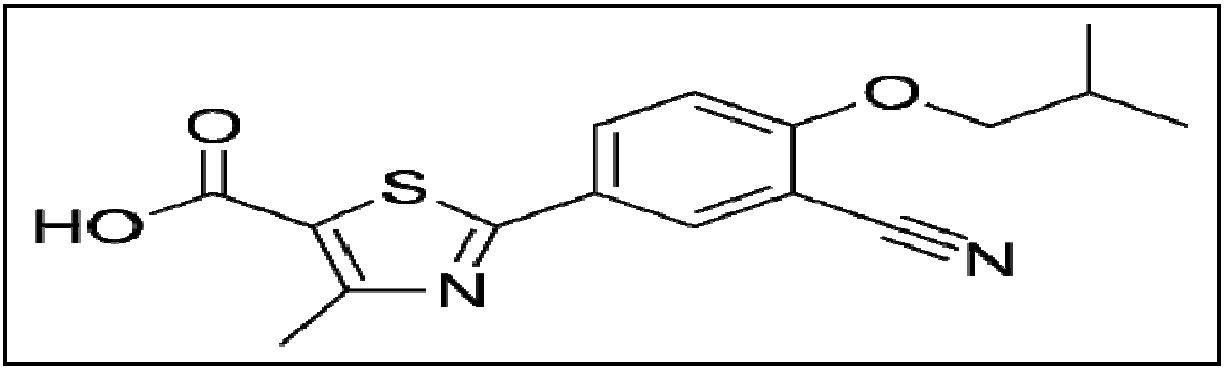
Table No.6: Results of Ruggedness

S.No			%Assay	%RSD
1	Analyst-1	Febuxostat	100.9	0.49%
2	Analyst-2	---	100.2	---

Table No.7: Validation parameters of evaluated method

S.No	Parameter	Limit	Value Obtained
1	Linearity concentrations Range (mg/mL) Correlation coefficient	NLT 0.990	0.05 to 0.15 mg\ml 0.9998
2	Method precision (Repeatability) (%RSD, n = 6)	98.0 to 102.0 %	99.5 to 101.0 %
3	Accuracy (% Recovery)	98-102%	99.85 to 100.8%
4	Robustness	It should be meet System suitability criteria	Complies
	Flow Variation(0.7mL to 0.9 mL/min)		
	Wavelength Variation (313nm to 317nm)		
5	Ruggedness (Intermediate Precision) (% RSD analyst to analyst variation)	NMT2%	0.49%

*RSD = Relative standard deviation



Structure of Febuxostat

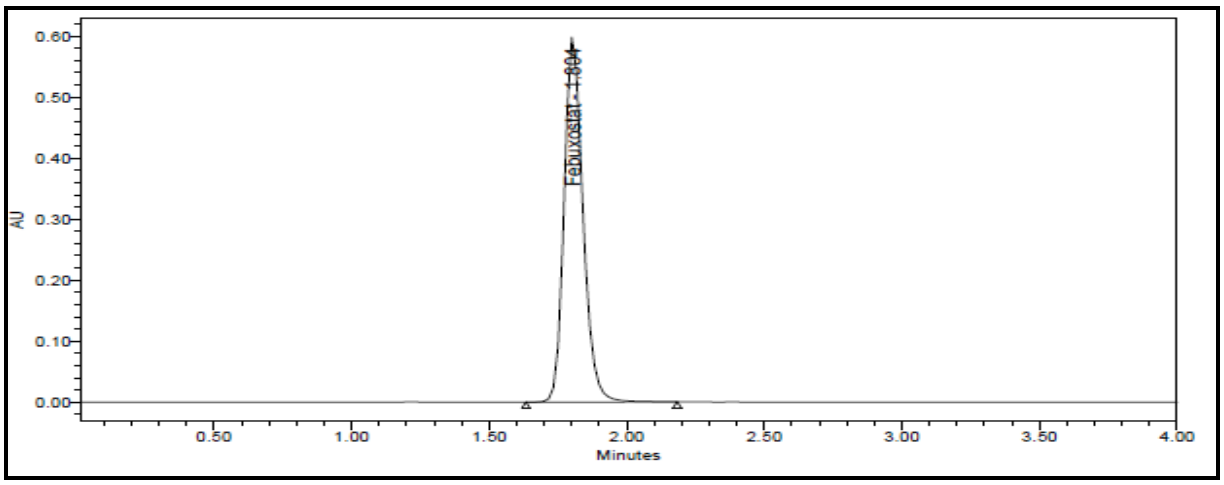


Figure No.1: Chromatogram of Febuxostat

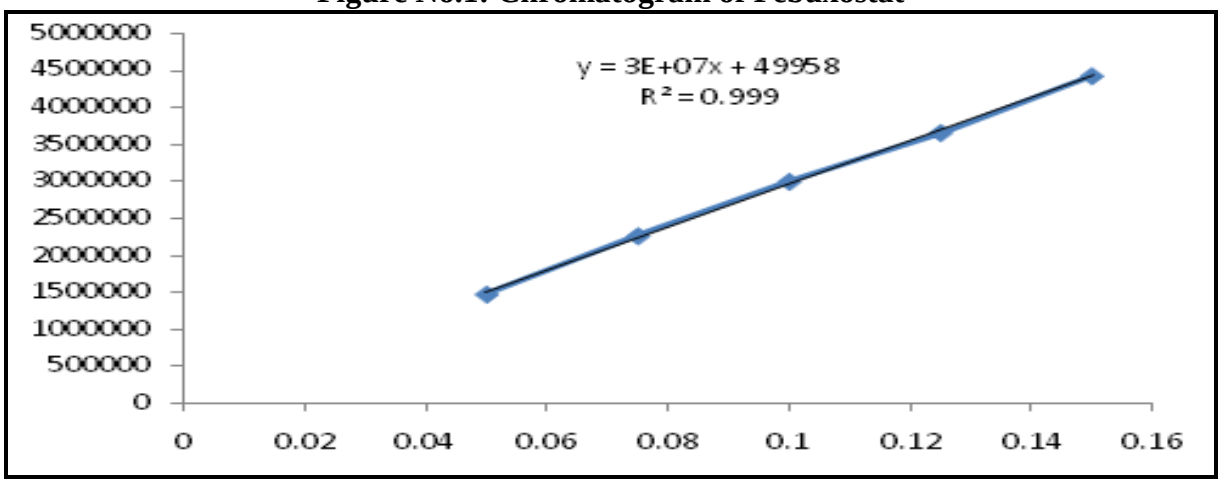


Figure No.2: Linearity (calibration) curve of Febuxostat

CONCLUSION

From the above results analytical method was concluded that, estimation of FEBUXOSTAT was found to be simple, precise, accurate and high resolution and shorter run time makes this method more acceptable and cost effective and it can be use for regular analysis in institutions, quality control department in industries, approved testing laboratories.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Chemistry, Vikas PG College, Vissannapeta, Krishna, Andhra Pradesh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Challa Sudheer *et al.* Method development and validation for the estimation of Febuxostatin drug substance by RP-HPLC method, *International Journal* of *Research in Pharmaceutical and Nano Sciences,* 6(2), 2017, 55-60.