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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF FEBUXOSTAT IN TABLET DOSAGE FORM ON A KANAK COLUMN

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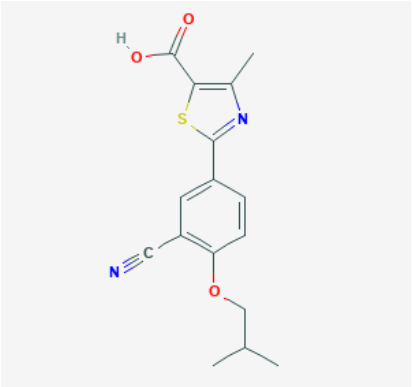
ABSTRACT

An accurate, precise, robust, sensitive and simple Reverse-Phase HPLC (RP-HPLC) isocratic method was developed and validated for analysis of Febuxostat Standard. The chromatographic conditions of Febuxostat (pure) standard was performed on Kanak C-18 (250 x 4.6mm, 5.0µm) column that was performed at 25°C temperature when10µl volume was injected into the system.The mobile phase used was Phosphate buffer (pH 2.5): Methanol, 20: 80 (v/v) at flow rate of 1.0ml/min and detection was carried out at 316nm. The retention time obtained was 5.6min (± 0.5min) that was found to be linear in the range of 10-50 ppm. Limit of Detection (LOD) and Limit of Quantification (LOQ) obtained was 0.024 and 0.078ppm respectively. The %RSD for reproducibility, precision and robustness parameters were within the specified limits according to ICH guidelines. The analytical investigation of Febuxostat drug on Inertsil column showed peak with wide fronting and tailing as compared to the peak shape obtained on Kanak™ column.

INTRODUCTION

Febuxostat is a white crystalline powder(Spandana R*, Pushpa Latha E 2015)which is chemically known as 2-(3-cyano4-isobutoxy phenyl)-4-methyl-1,3-thiazole-5-carboxylic acid (Ponnuveetil Gopi Sunitha; Kaliappan Ilango 2014; Derasari 2017; Ranjith *et al*. 2017; Ravisankar *et al*. 2015; Gide *et al*. 2014).It is non-hygroscopic in nature having melting point between 205°C to 208°C(Challa Sudheer*1, S. Alekhya1, P. Lavanya1, E. Mounika1, T. Mahalakshmi1, A. Sireesha1 2017).It is classified as a non-purine xanthine oxidase inhibitor (Anuradha. S* 2017; Reddy Musirike, Reddy K, and Mallu 2015; Derasari 2017) used in the treatment of hyperuricemia and chronic gout (Mukthinuthalapati *et al*. 2013; Derasari 2017; Challa Sudheer*1, S. Alekhya1, P. Lavanya1, E. Mounika1, T. Mahalakshmi1, A. Sireesha1 2017; Ravisankar *et al*. 2015; Gide *et al*. 2014). Febuxostat is administered to people who are intolerant of allopurinol (Younes, El-Kady, and Elzanfaly 2016). The drug is soluble in dimethylformamide; soluble in dimethyl sulfoxide; sparingly soluble in ethanol; slightly soluble in methanol and acetonitrile; and practically insoluble in water (Sudheer Subrahmanya Muvvala *et al*. 2012; Challa Sudheer*1, S. Alekhya1, P. Lavanya1, E. Mounika1, T. Mahalakshmi1, A. Sireesha1 2017; Mukthinuthalapati *et al*. 2013).

Structure: Febuxostat(PubChem CID: 134018)(Sudhir S. Muvvala, Ratnakaram, and Nadendla 2012; N and Sultana 2016; Anuradha. S* 2017)



Empirical formula: $C_{16}H_{16}N_2O_3S$. (PubChem CID: 134018)

Molecular weight: 316.38g/mol(R. S. Kumar and Kumar 2012)

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There are few methods reported for analysis of Febuxostat drug in the pure form using UV Spectrophotometer,(Pg and Ilango 2014) RP-HPLC, UPLC, LC/MS and MS/MS techniques(Vetrichelvan 2016) and HPTLC (Ch, Suthakaran, and B 2018; Anuradha. S* 2017). Febuxostat has been better determined in pharmaceutical dosage forms by reversed-phase HPLC (Harde, Wankhede, and Chaudhari 2014; Younes, El-Kady, and Elzanfaly 2016; Mukthinuthalapati *et al*. 2013).Thus, majority of the papers focus on method development from different samples or in combination with other drugs and in formulations (Jansen *et al*. 2010). However, several studies which are available focus on evaluation of the drug or determination of Febuxostat from urine and plasma samples. Literature review also shows that few HPLC methods focus on simultaneous determination of Febuxostat along with other drugs and in formulations.

Studies performed for this drug on HPLC have showed better results on BDS column as compared to ODS column. Inthe BDS column, residual silanols aredeacivated and silanol activity is reduced, it is end-capped column and it is good for basic compound (Ltd, n.d.). One of theBDS column named Kanak(Kanak column) has shown better results with all other factors in the range of its maximum limits(Anuradha. S* 2017)due to its high carbon load which aims at better resolution (Ltd, n.d.).Method development involves optimizing the sample in the given conditions and checking its multiple parameters with known standard. Thus, new method development on Kanak column for Febuxostat is proven better for validation. However, method validation of pure API form is also important with respect to the drug discovery and development stage.

The parameters used for method validation are Retention time, Peak Area, Theoretical plates, Relative Standard deviation, Linearity, Accuracy, Specificity, Precision, Repeatability, Carry over and Limit of detection (LOD) and Limit of quantification (LOQ)(Ravisankar *et al*. 2015).

MATERIALS AND METHOD

Reagents and chemicals

An analytically pure sample of Febuxostat API was procured from National Facility for Biopharmaceutical (Mumbai, India).The label claim was 80mg. Acetonitrile (HPLC grade), Methanol (HPLC grade), Orthophosphoric acid, Sodium Hydroxide (NaOH) and Water (HPLC grade) were purchased from Merck Life Science Pvt Ltd (India). All chemicals were of HPLC analytical grade and used as received.

Instrumentation

The analysis of drug is carried out on AGILENT 1260 Infinity II Quad pump series HPLC system equipped with an autosampler and Variable Wavelength Detector (VWD). A Kanak C-18 column (250x4.6mm, 5µm particle size) was used for separation at 25°C. The obtained data was compiled and computed using ChemStation software.

Chromatographic Conditions

The Reversed Phase High Pressure Liquid Chromatography (RP-HPLC) analysis was carried out using Isocratic mode. Separation was achieved at 25˚C on KanakC-18 column (250x4.6mm 5µm particle size) as stationary phase. Methanol and 0.01M Phosphate buffer (80:20 v/v) adjusted with NaOH (pH 2.5±0.2) was used as mobile phase. The flow rate was 1.0ml/min and the run time was 10 minutes. The injection volume was 10µl. The quantification of Febuxostat was recorded at 316 nm by VWD detector.

Preparation of Standard stock solution

Standard stock solution was prepared by accurately weighing 10mg of Febuxostat API in 100ml (100ppm) of diluent (mobile phase). For linearity, 100ppm of Febuxostat was diluted over a range of concentration from 10ppm to 50ppm. Specificity was also performed at concentration of 100ppm. The repeatability, inter-day and intra-day precision was carried out using 30 ppm of Febuxostat API (3 injections, n=3) and Carry Over was performed using blank solution containing the mobile phase and finally linearity was checked for 5 different concentrations. (10, 20, 30, 40 and 50 ppm)To validate the above method, the collected data was analyzed and compared with USP chromatographic criteria.

System suitability

The System Suitability parameters were checked by injecting three injections of 30 ppm into chromatographic system prepared from 100 ppm Standard Febuxostat stock solution. To ensure system suitability, a standard solution was injected on to the system. Tailing factor (T) and Column efficiency (N) were calculated for Febuxostat standard and its formulation.

Method Validation

Specificity: Specificity of the method was performed by diluting the sample and the standard with mobile phase and injecting the standard and sample at same concentration in triplicate to measure the analyte response.

Accuracy: The percentage recovery was calculated by injecting six individual series of diluted sample with standard solution to a level such that % RSD was not more than 5% at LOQ level.

Linearity: Linearity of the method was demonstrated over the concentration range of 10-50 ppm Febuxostat. Each concentration was prepared in triplicates, 10µl of each standard solution was injected at the optimized chromatographic conditions and the chromatograms were recorded. The retention time, average peak areas and the number of plates were recorded. Calibration curves were constructed by plotting peak area on Y-axis against concentration on X-axis and regression equation was calculated by the method of least squares. The correlation coefficient, y-intercept, slope of the regression line were submitted.

Precision: Precision of the method was reported by injecting six replicates of standard solution consecutively under the same analytical conditions. The %RSD of individual peaks was calculated.

Intra-day and Inter-day

For intra-day and inter-day, a total of three injections of 30ppm of sample Febuxostat were injected in triplicates on same day at different intervals and on 3 different consecutive daysin the chromatographic system.

Analysis of formulation: After setting the chromatographic conditions, the instrument was stabilized to obtain a steady baseline. Then, equal volumes of blank, standard preparation

and test preparation were injected thrice separately into the column and the chromatograms were recorded. Peak area response of analyte peak was measured.

Repeatability

The repeatability was carried out using 30 ppm of standard solution of Febuxostat (6 injections i.e. n =6)

Carry over

The carry over was performed using blank solution (mobile phase) in between the standard solution of Febuxostat.

Robustness and Ruggedness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by 1.0 ± 0.2 ml / min and change in the ratio of mobile phase (80± 2% absolute).(Vaibhav, Mohit, and Sadhana 2013) Ruggedness was determined by the varying the analyst, instrument and different column of varied grades. The relative standard deviation of the results obtained from different analyst's instruments is < 1.0 %. To ascertain the system suitability for the proposed method, a number of statistical values such as theoretical plates, peak asymmetry, have been calculated with the observed readings and the results complies within the specified limits.

RESULTS

The aim of the proposed study was to develop method for Febuxostat drug in its pure form and to validate the method. The wavelength of detection was selected at 316 nm as Febuxostat showed maximum absorbance at given wavelength. Resolution and peak symmetry were found to be as shown in figure 2 and results in table 1 on Kanak C18 column. The obtained results were calculated in %RSD form and are NMT 2% as concise in table 1. The parameters such as Specificity, Accuracy, Precision, Intraday and Interday, Repeatability, Robustness and Ruggedness resulted in NMT 2% of its maximum limit. The results are summarized in table 1 and 2.

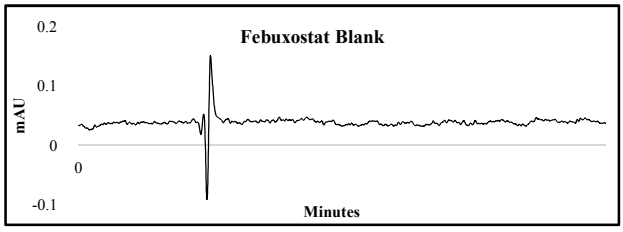

Figure 1 Chromatogram of Blank (Before Run)

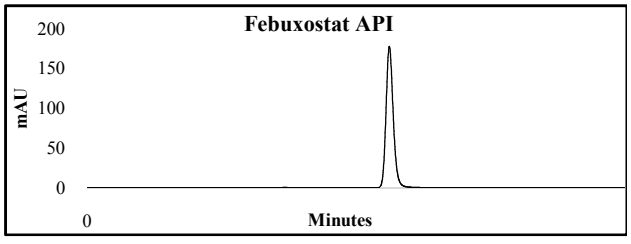

Figure 2 Chromatogram of Febuxostat API (100ppm) showing Specificity

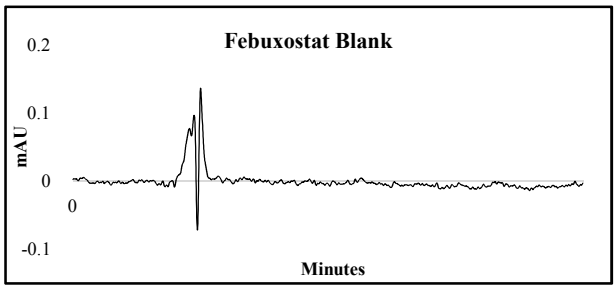

Figure 3 Chromatogram of Blank (After run)

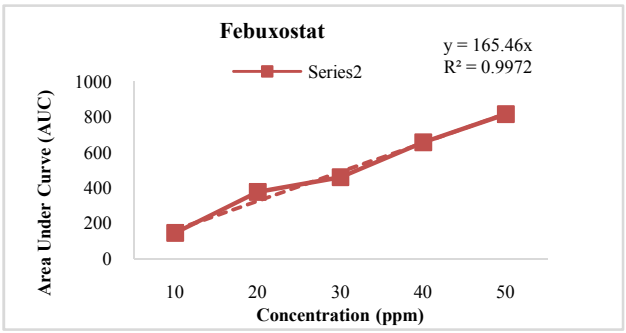

Figure 4 Linearity Chromatogram of Febuxostat

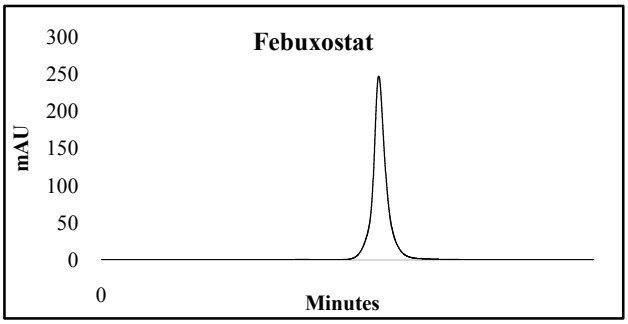

Figure 5 Chromatogram of Febuxostat on Inertsil Column (API at 100PPM)

Table 1 Results of reproducibility, Interday and Intraday and Robustness

Sr. No.	Validation Parameters	SD		%RSD	
		Peak Area	USP Plate Number	Peak Area	USP Plate Number
1	Specificity	1.665	324.378	0.1	0.89
2	Reproducibility	0.578	141.563	0.125	0.004
3	Interday	0.591	116.573	0.128	0.341
4	Intraday (Morning)	0.565	100.955	0.122	0.294
	Intraday (Evening)	4.193	262.298	0.85	0.77
5. Flow Rate	1.2ml/min	0.211	46.716	0.04	0.133
	1.0ml/min	1.057	421.357	0.212	1.216
	0.8ml/min	0.167	146.661	0.036	0.432
6. Mobile Phase 20:80 - (Buffer: MeOH) (v/v)	18:82 - (Buffer: MeOH) (v/v)	2.32	27.465	0.52	0.08
	20:80 - (Buffer: MeOH) (v/v)	1.08	49.153	2.132	0.144
	22:82 - (Buffer: MeOH) (v/v)	4.032	269.52	0.87	0.779
7. On Inertsil Column	Specificity	6.694	52.205	0.15	0.42

Table 2 Results of System suitability, LOD, LOQ and Linearity

Sr. No.	Parameters	Febuxostat
	System Suitability	
a.)	USP Tailing Factor(<2.0)	1.2
b.)	USP Plate Count (Avg.) (>3000)	34000
c.)	Peak Area (%RSD)	>5.0
	LOD and LOQ value (µg/mL)	
a.)	LOD value (3.3×SD/S)	0.024
b.)	LOQ value (10×SD/S)	0.078
	Linearity (10 - 50 µg/mL)	
a.)	Linearity	Y=165.46x
b.)	Linearity equation	0.00
c.)	Correlation coefficient (r)	0.997

DISCUSSION

Method Validation

The defined method was validated for Febuxostat drug and the validation parameters were assayed. As shown in Fig. 4.0, Linearity for Febuxostat drug was obtained between 10 ppm to 50 ppm which signifies the method is sensitive enough to perform the RP-HPLC based assay for detection and quantitation of Febuxostat from the matrix component. The acceptance criteria of correlation coefficient for the method to be linear is 0.99 and the linear regression value was obtained for Febuxostatwas 0.997 (G. V. Kumar and Shashikala 2016). The current method proves to be linear and was assessed by the least square regression method(Derasari 2017) even though the make of column packing material is variable.The comparative data showed that the column used in the study resulted in faster elution of Febuxostat drug with the developed method. Kanak^{TM} columns comprising of higher carbon loading (19%)and surface area (300m^2/g) provided better peak shape of Febuxostat as compared to the other columns used in this study (Ltd, n.d.).

The method was found to be specific for Febuxostat, showing %RSD as 0.89 for both standard as well as formulation which is NMT 1% as per ICH guidelines as given in figure 2.The accuracy and precision of the method was affirmed by standard and samples injected in replicate that showed in % RSD as 0.004. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve (Reddy 2015, 2018).The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 0.023 ppm and 0.078 ppm respectively by the described method(Rao 2013).The criteria was compared withthe guidelines which are falling within the specified limit(Vaibhav, Mohit, and Sadhana 2013).The following method was found to be precise for the Febuxostat drug and showed results in %RSD form that was found to be less than 2%.

The resultsturned out well for reproducibility that was ascribed in %RSD manner as Peak area, Tailing factor and Plate number were 0.125, 0.006 and 0.004 respectively (Vaibhav, Mohit, and Sadhana 2013).A blank was injected between the standard injection and it revealedno carryover on the chromatogram.To verify the system suitability parameters of HPLC grade, few variations were made inchromatographic conditions such as flow rate and mobile phase and %RSD results showed less than 5% of maximum limit. The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters. Ruggedness of the method was determined by carrying out the method on different columns of similar types, which concluded that the developed method was rugged(Rao 2013).

CONCLUSION

The proposed method of RP-HPLC for Febuxostat standard was found to be accurate, precise, robust, linear, sensitive and rapid for routine analysis of the drug in its pure form and can be further usedfor the study of the drug. The following method was reliable and can be adopted as a reference to study the Febuxostat standard as well as its formulations on Kanak^{TM}column. In spite of difference in silica of stationary phase, the obtained results were not exceeding beyond its thresholds limits. Being loaded with high carbon, Kanak^{TM}column prove to be beneficial for early elution of the molecule with no traces on column. Due to the rapid and better resolution of the peak obtained with appropriate peak height and resolution Kanak^{TM} column can be preferredmore as compared to other columns.

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