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Development and Validation of Rp-HPLC Method for Determination of Febuxostat in Bulk and Pharmaceutical Dosage Formulations

Pragati R. Kamble[*1], Nikita V. Pandagale[2],Omkar D. Ghatage [3] ,Pramod B. Patil[4], Dr. S. V. Patil[5]

[1,2,3]Department of Pharmaceutical Quality Assurance, Ashokrao Mane College of Pharmacy, Pethvadgaon Kolhapur-416112, Maharashtra, India

[4]Department of Pharmaceutical Chemistry, Ashokrao Mane College of Pharmacy, Pethvadgaon, Kolhapur-416112, Maharashtra, India

[5]Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Pethvadgaon Kolhapur-416112, Maharashtra, India

Abstract:

A new accurate, specific, precise, high performance liquid chromatographic (HPLC) method has been developed and validated for the determination of Febuxostat in bulk and in its pharmaceutical dosage forms of tablet. Acetonitrile: Methanol (70:30) was used as the mobile phase at a flow rate of 1.0 ml/min using a Symmetry C18 column Finepack SIL C18T-5 (250x4.6 mm, 5m). The effluent was spectroscopically monitored at 314 nm. The intraday and inter-day precisions showed coefficients of variation ranged from 0.62% to 2.78% at different levels of concentrations. The calibration curve was linear with a correlation coefficient of 0.9947. The averages of the absolute and relative recoveries were found to be 98.67% to 98.96%. The proposed HPLC method was successfully applied to quantify the amount of Febuxostat in bulk and dosage forms in quality control.

Keywords: Febuxostat, HPLC, RP-HPLC, Regression, Spectroscopically.

Introduction:

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need Analytical Method Development and Validation by combining the therapeutic effects of two or more drugs in one product[1-2]. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

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Identification and quantification of impurities is a crucial task in pharmaceutical process development for quality and safety[3-4]. Related components are the impurities in pharmaceuticals which are unwanted chemicals that remain with the active pharmaceutical

ingredients (APIs), or develop during stability testing, or develop during formulation or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Various analytical methodologies are employed for the determination of related components in pharmaceuticals. There isa great need for development of new analytical methods for quality evaluation of new emerging drugs.

Febuxostat is chemically 2-(3-cyano-4-isobutoxyphenyl) -4-methyl-1,3-thiazole-5-carboxylic acid. The structure of febuxostat is shown in Fig.1.Febuxostat is a novel, orally administered non - purine drug that is indicated for use in the treatment of chronic gout and hyperuricemia in patients with gout and chronic tophaceous gout[5-6]. It is a non-purine selective inhibitor of xanthine oxidase works by noncompetitively blocking the molybdenum protein center which is the active site on xanthine oxidase (XO)[7-8]. Thus lowering urate concentrations in the body. It has minimal effects on other enzymes involved in purine and pyrimidine metabolism, and is metabolized primarily by glucuronide formation and oxidation in the liver[9-10].

Material And Method:

Material:

Standard API as Febuxostat was obtained as a gift sample from FDC Laboratory Limited, Aurangabad. Different Analytical grade Chemicals & Reagents like Methanol, Acetonitrile, Ortho-Phosphoric Acid, Hydrochloric acid (HCl), Sodium hydroxide (NaOH) was purchased from Merck Chemicals and SD fine chemical Ltd. Mumbai, Maharashtra.

Method

Chromatographic condition:

Finepack SIL C18T-5 was used as stationary phase. Acetonitrile (ACN): Methanol used as mobile phase. A constant flow of 1.0 ml/min was maintained throughout the analysis and the detection was monitored by UV detector at 314 nm.

Preparation of Febuxostat Standard and Sample

Standard and sample stock solutions (500 µg/ml) were prepared by weighing 5 mg of drug into 10ml volumetric flask, respectively. Drug was dissolved into small volume of diluents, Acetonitrile: Methanol (1:1) and sonicated for 1min. Then volume was made up to 10 ml with diluents. From the each stock solution, 1ml solution were transferred to volumetric flask and volume was made up to 10 ml to became 50µg/ml of final standard and sample solution, respectively.

Preparation of Stock Solutions and Calibration Samples of Std.:

Stock solution was prepared by dissolving accurate amount of reference standards in methanol at a concentration of 1 mg/ml for Febuxostat. A series of working standard solutions were obtained by further diluting the stock solution in methanol. Calibration standards were prepared by spiking the appropriate amounts of the standard solutions to yield final concentrations of 10, 20, 40, 60, 80 and 100µg/ml. The quality control (QC) samples were similarly prepared at concentrations of 20, 60 and 100 µl/ml for low, medium and high concentration QC samples, respectively. All solutions were kept at room temperature before use.

Preparation of Stock Solutions and Calibration Samples of Formulation:

ULORIC (Containg 80mg Febuxostat) was procured from chemist. 20 tablets were accurately weighed and content was crushed to fine powder. Powder equivalent of 50 mg of Febuxostat was weighed and transferred to 50 ml volumetric flask containing 25-30 ml methanol and content was shaken for 15 minutes. Volume was adjusted to 50 ml with methanol and filtered through Whatman's filter paper No.41 and first few ml was rejected. 25 ml solution was further diluted in 50 ml volumetric flask with optimized mobile phase to prepared stock solution (500µg/ml).

A series of working standard solutions Calibration standards were prepared by spiking the appropriate amounts of the standard solutions to yield final concentrations of 10, 20, 40, 60, 80 and 100µg/ml. The quality control (QC) samples were similarly prepared at concentrations of 20, 60 & 100 µl/ml for low, medium and high concentration QC samples, respectively. All solutions were kept at room temperature before use.

Method development using HPLC:

After finalizing the mobile phase, the chromatographic separation was performed using Jasco HPLC (UV-4100) with Finepack SILC18T-5 using ChromNav software. The mobile phase system was optimized to give good resolution by mixture of solvents ACN and methanol in ratio of 70:30 v/v for 10 minutes at a flow rate of 1 ml/min. Each calibration curve was analyzed individually by using least square weight linear regression. Intra and inter day precisions were within limits (R.S.D _15%) and accuracy was in between the range 85 to 115%. Further validation was done according to US Food and Drug Administration (FDA) guidelines for stability, recovery and matrix effect.

Method Validation:

The developed HPLC method was validated as per the FDA guidelines for linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and specificity.

Linearity: The linear regression data for the calibration curve (n=3) showed good linear relationship over the concentration range of 10-100 µg for Febuxostat. Value of the significance and correlation coefficient confirm the linearity in the concentration range.

LOD and LOQ: The limit of detection and quantification was calculated for Febuxostat based on the equation, LOD=3.3(SD)/S and LOQ= 10(SD)/S according to the guideline. Where, SD= standard deviation of the response S= slope of the regression equation.

Precision: The precision of the method was verified by inter-day and intra-day precision. It was done by three replicate analysis of the composite sample. The % RSD calculated was found to be below 4 for Inter-day and Intra-day for both Febuxostat, following FDA guideline which recommends % RSD should below 15.

Repeatability: Repeatability of the method was checked by giving the standard solution of Febuxostat concentration 20µg/ml, 60µg/ml and 100µg/ml to the HPLC system. The % RSD was within an acceptable range that is <20%. The results are depicted in the table.

Recovery: The recovery study of Febuxostat were carried out by standard addition method in which three different concentration of the API was spiked additionally to get a solution of three different concentrations of 80%, 100% and 120% for three concentrations of Febuxostat drug determined.

Stability: Freeze thaw stability and bench top stability was performed. Freezed thaw stability was done for three concentrations by keeping in deep freeze condition for 24 hours and at room temperature for another 24 hr followed by again in deep freeze for next 24 hrs. Significant changes were measured and reported.

Result and Discussion:

Method Development:

Various trials were performed using solvents of varying concentrations which includes methanol and ACN prior to finalize the optimized mobile phase. Few of the trials are listed below in table and chromatograms are given below.

Table No 1: Trials for estimation of Febuxostat

Trial No.	Mobile Phase Composition	Result
1	Methanol: ACN (50:50)	Fronting (Fig. No 1)
2	Methanol: ACN (40:60)	Tailing (Fig. No 2)
3	Methanol: ACN by gradient elution	Broadening (Fig. No 3)
4	Methanol: ACN (30:70)	Optimize (Fig. No 4)

Chromatogram:

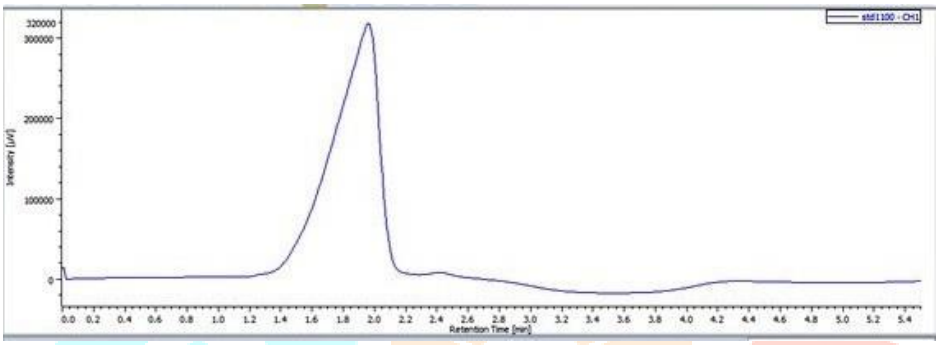

Figure No 1: Methanol: ACN (50:50) Fronting

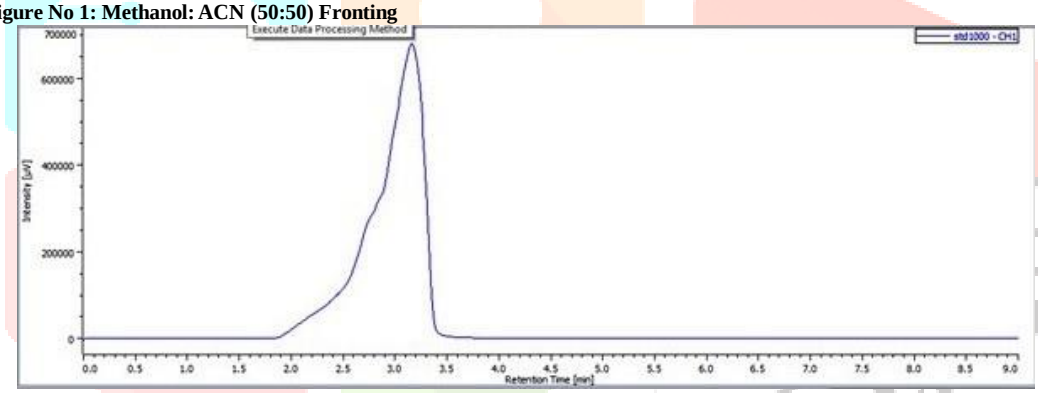

Figure No 2: Methanol: ACN (40:60) Tailing (Fig.2 b)

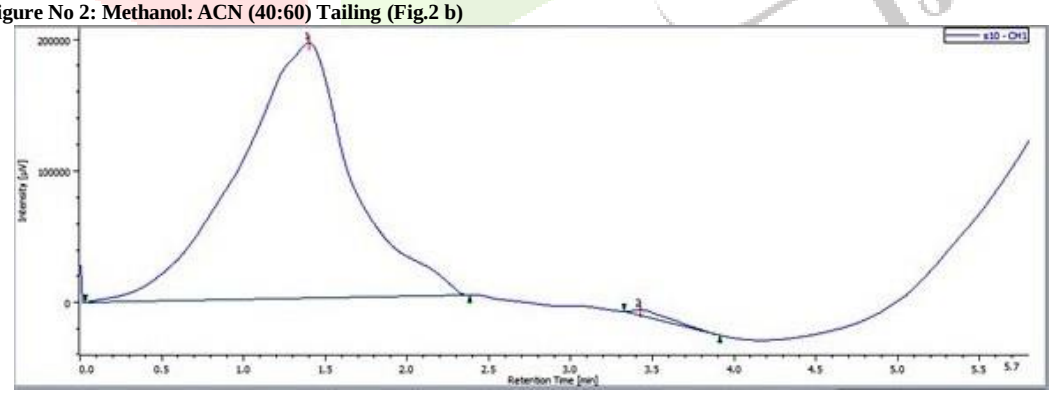

Figure No 3: Broadening by Methanol: ACN in gradient elution

The developed method shows good resolution for Febuxostat in mobile phase of Methanol: ACN (30:70 v/v) at Rt of 2.9 min.

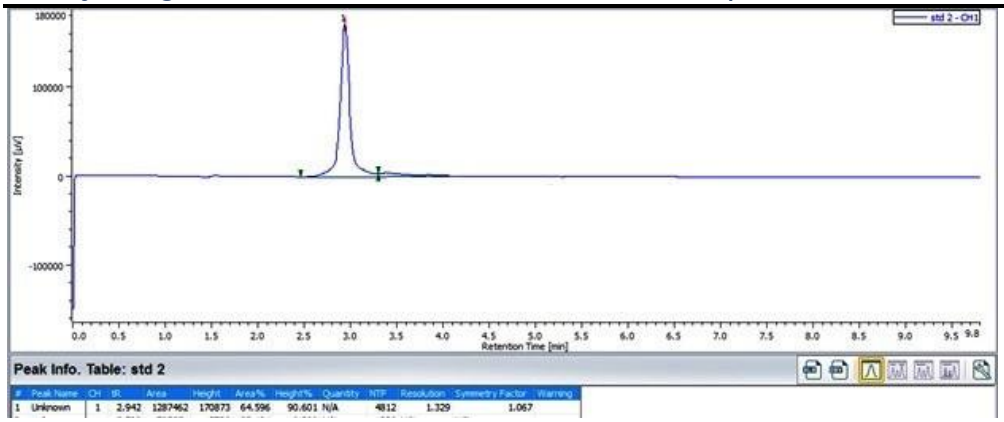

Figure No 4: Optimize chromatogram at Methanol: ACN (30:70)

Method Validation:

Linearity

Linear regression data for the calibration curve (n=3) showed good linear relationship over the concentration range of 10-100µg/ml for Febuxostat (correlation coefficient r^2=0.99 for std. and r^2=0.999 for formulation) the data were subjected to regression analysis. Value of the significance and correlation co-efficient confirm the linearity in the concentration range.

Table No 2: Linearity of Febuxostat standard for conc. range 10-100 µg/ml

Std. Conc. (µg/ml)	Area Under Curve				Standard Deviation
	Replicate1	Replicate2	Replicate3	Mean	
10	21024	22014	21168	21402	0.41
20	39177	38422	38594	38731	0.48
30	55433	56019	56017	55823	0.25
40	85025	84957	84622	84868	0.20
50	99412	100456	99844	99904	0.18
60	121004	122743	122466	122071	0.29
70	150896	151450	151788	151378	0.59
80	179855	180044	181295	180398	0.44
90	195365	196042	195996	195801	0.65
100	219003	218665	218000	218556	0.82

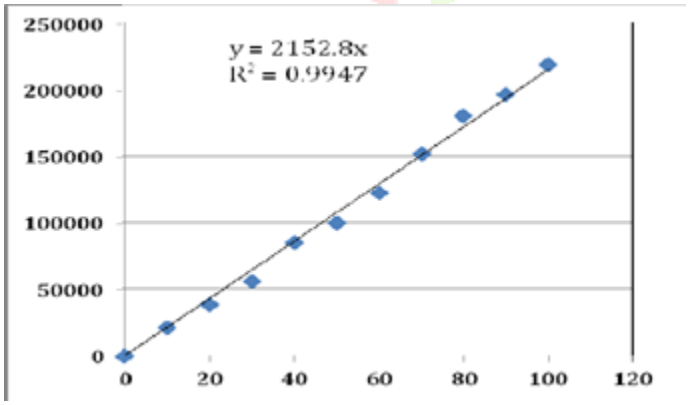

Fig No 5: Linearity of Febuxostat standard

Table No 3: Linearity of formulation of Febuxostat for conc. range 10-100 µg/ml

Std. Conc. (µg/ml)	Area Under Curve				Standard Deviation
	Replicate1	Replicate2	Replicate3	Mean	
10	21587	20884	20655	21042	0.25
20	38674	39647	38991	39104	0.30
30	55934	56025	56245	56068	0.14
40	84002	83799	84058	83953	0.20
50	99853	99584	100263	99900	0.10
60	122610	123054	122850	122838	0.25
70	151380	150321	150045	150582	0.20
80	180361	181005	180977	180781	0.50
90	196234	195984	196100	196106	0.40
100	210698	209661	210118	210159	0.60

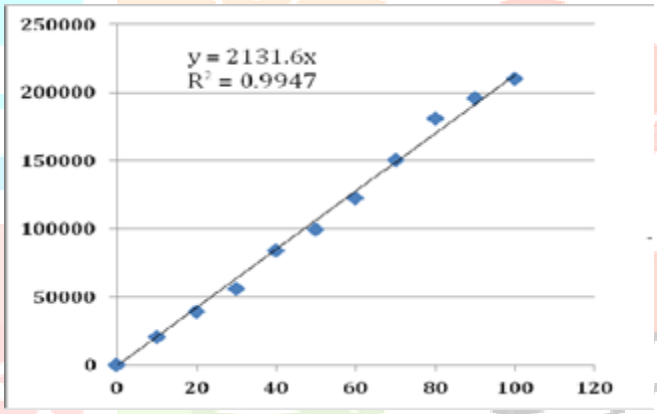

Fig No 6: Linearity of formulation of Febuxosta

Precision:

The Precision of the method was verified by interday and intraday precision withthree replicate analysis of Febuxostat for three different concentrations. The developed method was found to be precise as the RSD values for intraday and inter-day precision studies were <20% as recommended by USFDA guidelines and given in table.

Inter-day & Intra-day Precision:

For Inter-day precision (n=3) %RSD were found to be 0.6%-2.78% for std. and 5.6%-1.3% for formulation while for Intra-day precision (n=3) %RSD were found to be 0.84%-2.69% for std. and 3.5%-1.3% for formulation. %RSD was <15% which is in the acceptable range and results were given in the table No 4.

Table No 4: Inter Day Precision and Intra Day Precision of Febuxostat

Sr. No.	Conc. µg/ml	%RSD			
		Inter Day Precision		Intra Day Precision	
		Std	Formulation	Std	Formulation
1.	20	2.78	4.4	2.69	2.29
2.	60	0.68	5.6	1.04	1.28
3.	100	0.62	1.38	0.84	3.49

LOD and LOQ

The limit of detection and quantification were calculated found to be 0.31µg/ml and 0.965 µg/ml for Febuxostat and 0.93 and 4.05 µg/ml for formulation respectively. MQC was found to be 4µg/ml and HQC was found to be 100 µg/ml.

Repeatability

Repeatability of the method was checked by analyzing the standard solution of Febuxostat at concentration 20, 60 and 100 µg/ml, %RSD was found to be <20% whichis in the acceptable range as given in table no 5.

Table No 5: Repeatability of Febuxostat

Sr. No.	Conc. µg/ml	%RSD	
		Std	Formulation
1.	20	0.71	0.98
2.	60	0.08	0.24
3.	100	0.39	1.47

Recovery:

The recovery study was carried out by standard addition method for Febuxostat std. and formulation in which three different concentration of the API was spiked 80%, 100% and 120% and the total amount of the drug was determined. The % recovery was found as shown in Table No 6.

Table No 6: Recovery of Febuxostat

Febuxostat Standard				
Concⁿ (µg/ml)	% Drug added	Concentration (µg/ml)		Percentage found (%)
		Expected	Found	
20	80%	36	35.72	98.73
	100%	40	39.55	98.87
	120%	44	43.46	98.47
60	80%	108	107.2	99.32
	100%	120	118.9	99.03
	120%	132	131.3	99.3
100	80%	180	179.33	99.14
	100%	200	197.47	98.73
	120%	220	219.29	99.06
Marketed formulation of Febuxostat				
Concⁿ (µg/ml)	% Drug added	Concentration (µg/ml)		Percentage found (%)
		Expected	Found	
20	80%	36	36.02	100.04
	100%	40	38.26	95.65
	120%	44	43.59	99.02
60	80%	108	106.98	98.71
	100%	120	118.27	98.55
	120%	132	131.35	99.22
100	80%	180	179.65	99.49
	100%	200	199.8	99.9
	120%	220	220.23	100.1

Stability:

Freeze thaw stability was performed for three concentrations (n=3) 20, 60 and 100µg/ml and % RSD was found to be 6.1, 2.4, 0.4 for Febuxostat Std. and 0.38, 1.34, 0.18 for Febuxostat formulation. Bench top stability was performed for three conc. And % RSD was found to be 2.87-3.90, 3.26-1.65 and 2.563.41 for Febuxostat Std. and formulation respectively. Both values are <15% and are in the acceptable range as per US-FDA guidelines.

Conclusion:

The analytical result by the proposed method suggested that the method is simple, reliable, accurate and precise. The most striking feature of this method is its simplicity, sensitivity, and reproducibility and suitable for routine analysis of Febuxostat.

There is no other analytical method suitable for routine analysis of Febuxostat in oral formulation. The results obtained from developed analytical method for estimation of Febuxostat indicate that the method is simple, accurate and precise hence can be used for routine analysis of commercially available formulations.

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