International Journal of Pharmaceutical Biological and Chemical Sciences

Research Article

ESTIMATION OF RELATED SUBSTANCES OF FEBUXOSTAT IN BULK & 40/80/120mg TABLETS BY RP-HPLC

M.NARESH CHANDRA REDDY*[1], K.B.CHANDRA SEKHAR[2]

*[1]Research Scholar, Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, India
[2] Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, India

ABSTRACT

A simple reverse phase liquid chromatographic method was developed for the determination of related substances in Febuxostat in bulk and Tablets. The related substances amide impurity, methyl ester impurity and ethyl ester impurity with Febuxostat separated on a Poroshell 120 E18C_{18} column (500 mm x 4.6 mm; 2.7µ) using a mobile phase mixture of phosphate buffer pH 3.0 and methanol in a gradient program at a flow rate of 1.0ml/min at a wavelength of 318 nm. The method was validated for Linearity, speficity, LOD, LOQ, accuracy, robustness, ruggedness, precision, Filter paper variation and solution stability. The retention time of Febuxostat was 3.6±0.1 min. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for the quantitative estimation of the impurities in drug and pharmaceutical dosage form.

KEYWORDS

Method development and validation, Febuxostat, Related substances, Poroshell C_{18} column, RP-HPLC, Tablets.

INTRODUCTION

Febuxostat, 2-[3-cyano-4- (2-methylpropoxy)phenyl)-4-methylthiazole-5-carboxylic acid is a non-purine, XO inhibitor [1]. Febuxostat is selective inhibitor of xanthine oxidase for the treatment of hyperuricemia and gout[2,3]. it was recently approved by the European Medicines Agency on April 21, 2008 and was USFDA on Feb 16, 2009. Febuxostat is not official in any pharmacopoeia. As per the literature survey Febuxostat Determination was UV spectrophotometric method[4]. RP-HPLC method[5]. HPLC and LC-MS/MS[6]. Bioequivalence and pharmacokinetics evaluation[7]. Since this drug is being marketed in domestic and international market the present investigation by the author describes a rapid, accurate and precise RP – HPLC method for the determination of related substances Amide impurity(2-(3-carbamoyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid), Methyl ester impurity (Methyl 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate) and Ethyl ester impurity (Ethyl 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylate) from Febuxostat bulk sample and pharmaceutical dosage form. The detector responses were linear in the concentration range of 0.625 – 5.0 µg/ml of drug and its related substances. The method was validated as per ICH guidelines.

EXPERIMENTAL

Chromatographic Conditions

Agilent 1200 series HPLC consisting pump, Auto sampler, VWD & photo diode array detector, thermostatted column compartment connected with Open lab and EZ Chrom software connected with a poroshell 120EC18C_{18} 50x4.6mm, 2.7µm.

Reagents

HPLC grade methanol, water were purchased from E. Merck Co; Mumbai, India, and potassium Dihydrogen orthophosphate, ortho phosphoric acid AR grade were purchased from SD Fine Chem. Mumbai, India were used in the study.

Mobile phase

Accurately weigh 2.72g of potassium Dihydrogen ortho phosphate in 1000ml of water, and mix, adjust pH 3.0 with dilute phosphoric acid. Filter the solution through 0.22µ nylon filter. The buffer and methanol 40:60 was used as mobile phase preparation A, methanol was used as mobile phase mobile preparation B, the solutions were filtered through 0.22µ membrane filter and were degassed and Febuxostat and its impurities were eluted in a gradient program given in Table 1. The mobile phase was sonicated by using Biotechnics India Sonicator, Mumbai; the flow rate of the mobile phase was maintained at 1.0ml/min. The column temperature was maintained at 30ºC and the detection of the drug

was carried out at 318nm with an injection volume of 5µl.

Diluent

Prepare a filtered and degassed mixture of methanol and water (90:10 v/v).

Preparation of standard stock solution

Weigh accurately about 25 mg of Febuxostat working standard and transfer into a100 mL volumetric flask, add 60 mL of diluent and sonicate to dissolve for about 5min. Further dilute 1 mL to 100mL with diluent makes up the volume with diluent.

Test preparation:

Weigh 20 Tablets and crush in to powder. Transfer the powder equivalent to 125 mg of Febuxostat into 250 mL volumetric flask, add 150 mL of diluent and sonicate to dissolve for About 15 min further make up the volume with diluent. Filter through 0.22µ Nylon filter.

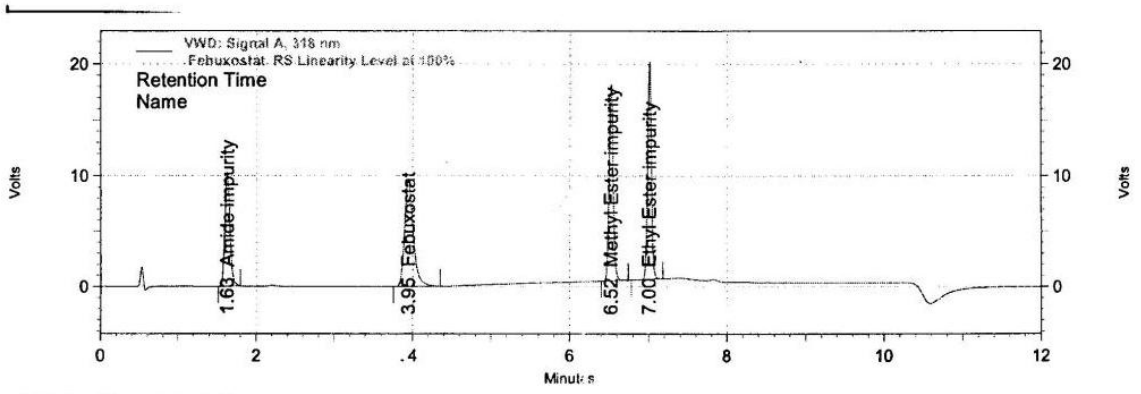
Placebo preparation:

Weigh the placebo powder equivalent 125 of Febuxostat and transfer into 250 mL Volumetric flask, add 150 mL of diluent and sonicate to dissolve for about 15min, further Make up the volume with diluent. Filter through 0.22µ Nylon filter.

LINEARITY AND CONSTRUCTION OF CALIBRATION CURVE

The quantitative determination of the drug was accomplished by a standard method. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working dilution of Febuxostat, Amide impurity, methyl ester impurity and ethyl ester impurity in the range of 0.625-5.0µg/ml, 0.628 to 5.025 µg/ml, 0.626 to 5.005 µg/ml and 0.627 to 5.015µg/ml respectively were prepared by taking suitable aliquots of working standard solution with diluent. 5µl quantity of the dilution was injected each time in to the column at a flow rate 1.0ml/min. Each dilution was injected 6 times in to the column. The drug elutes was monitored at 318 nm and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. The regression of the plot was completed by least squares regression method. This regression equation was later used to estimate the amount of Febuxostat and its impurities in pharmaceutical dosage form. A representative chromatogram for the separation of Febuxostat and its impurities is given in **Fig.1**

Fig 1 : Chromatogram of Febuxostat and impurities



VWD: Signal A, 318 nm

Results

Name	Retention Time	Area	Area %
Amide impurity	1.63	783093	18.65
Febuxostat	3.95	1231182	29.32
Methyl Ester impurity	6.52	1076486	25.64
Ethyl Ester impurity	7.00	1108168	26.39
Totals		4198929	100.00

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive precise and accurate HPLC method for the separation of Febuxostat in bulk drug and in pharmaceutical dosage form and forced degradation. In order to achieve optimum separation of the component peaks, mixtures of buffer with methanol and acetonitrile in different combinations were tested as mobile phase on a C_{18} stationary phase. A binary mixture of buffer and methanol in a gradient elution was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for Febuxostat was 3.6 ± 0.1, for Amide impurity was 1.53 ± 0.1, for methyl ester impurity was 6.6 ± 0.1min and for ethyl ester impurity was 7.0 ± 0.1mn. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of Febuxostat were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 1.0) was observed for Febuxostat and impurities, the regression characteristics are given in **Table 2**.

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The impurity content in capsules was quantified using the proposed analytical method are given in **Table 3**.

The percentage of individual and total impurities observed were deliberate changes in the method proves that the method is robust. The robustness study results are presented in **Table 4**. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The results are presented in **Table 5**. The difference between initial and bench top stability sample for % of individual impurities and total impurities were found within the acceptance criteria which indicates the solution were stable up to 48 hours. The results are presented in **Table 6**.

The precision was established by six replicate injections at LOQ level of the test preparation containing impurities of interest. The values of relative standard deviation were found to be within the acceptance limit, indicating the injection repeatability of the method. The results are presented in **Table 7**.

The specificity of the HPLC method was determined by the good resolution of impurities with Febuxostat. It was observed that there was no interference of blank and placebo at the retention time of analyte and impurity peaks. Peak purity of analyte and individual impurities should not be less than 0.99 the results of specificity data for degradation study are given in **Table 8**.

The intermediate precision (ruggedness) of the method was by carried out precision study in six preparations of a sample in a single batch sample by two different analysts, on two different columns and on two different instruments was found to be within the acceptance limit, which shows that the method is rugged. The results are presented in **Table 9**.

The filter paper variation of the method was carried out by injected filtered through different 0.22 µ membrane filters, the difference between % of individual and total impurities were found within the acceptance limit. The results are presented in **Table 10**.

Table 1: Gradient Programme

Time	Mobile Phase-A	Mobile Phase-B
0.00	90	10
3.00	90	10
6.00	20	80
9.00	20	80
9.10	90	10
12.00	90	10

Table 2: Linearity of Febuxostat and impurities

	Febuxostat	Amide impurity	Methyl ester impurity	Ethyl ester impurity
Correlation coefficient	1.0000	1.0000	1.0000	1.0000
Slope	495481	313775	432682	445279
Y-Intercept	5251.61	1119.33	1508.99	562.98
Residual sum square	3.6620×10^8	1.1097×10^8	2.3911×10^8	2.1764×10^8
Residual standard deviation	9568	5267	7732	7376

Table 3: Recovery of Febuxostat 40/80/120mg Tablets

Recovery of Amide impurity

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery	%RSD
LOQ level	0.009	0.008	91.9	93.8	1.76
LOQ level	0.009	0.008	94.9		
LOQ level	0.009	0.008	94.6		
50%	1.23	1.14	92.8	94.1	1.17
50%	1.23	1.16	94.6		
50%	1.23	1.16	94.8		
100%	2.46	2.25	91.5	91.4	0.25
100%	2.46	2.25	91.5		
100%	2.46	2.24	91.1		
150%	3.68	3.42	92.8	93.1	0.39
150%	3.68	3.44	93.5		
150%	3.68	3.42	93.0		
200%	4.91	4.56	92.9	92.7	0.22
200%	4.91	4.55	92.5		
200%	4.91	4.56	92.8		

Recovery of methyl ester impurity

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	%Mean Recovery	%RSD
LOQ level	0.006	0.006	102.4	100.1	2.84
LOQ level	0.006	0.006	100.9		
LOQ level	0.006	0.006	96.9		
50%	1.23	1.13	92.2	93.7	1.39
50%	1.23	1.17	94.7		
50%	1.23	1.16	94.1		
100%	2.46	2.32	94.1	93.5	0.54
100%	2.46	2.29	93.1		
100%	2.46	2.30	93.5		
150%	3.69	3.67	99.3	98.0	1.38
150%	3.69	3.57	96.6		
150%	3.69	3.62	98.0		
200%	4.92	4.73	96.1	95.4	0.67
200%	4.92	4.68	94.9		
200%	4.92	4.68	95.1		

Recovery of ethyl ester impurity

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery	%RSD
LOQ level	0.006	0.006	101.1	99.8	1.48
LOQ level	0.006	0.006	100.1		
LOQ level	0.006	0.006	98.2		
50%	1.23	1.14	93.1	92.4	0.72
50%	1.23	1.13	92.2		
50%	1.23	1.12	91.8		
100%	2.45	2.25	91.7	91.4	0.33
100%	2.45	2.24	91.5		
100%	2.45	2.23	91.1		
150%	3.68	3.43	93.4	93.8	0.38
150%	3.68	3.46	94.1		
150%	3.68	3.45	93.9		
200%	4.90	4.57	93.2	93.0	0.22
200%	4.90	4.55	92.8		
200%	4.90	4.56	93.0		

Table 4: Robustness study

Condition	% RSD
Normal Condition (i.e as such condition)	0.20
Flow changed to 0.9 mL/min	1.82
Flow changed to 1.1mL/min	1.45
Column Temperature changed to 25°C	1.77
Column Temperature changed to 35°C	1.20
pH changed to 2.8	0.20
pH changed to 3.2	0.92

Table 5: LOD Study

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration	Value(µg/ml)
1	Febuxostat	2.8	0.0003	0.3
2	Amide impurity	2.7	0.0005	0.5
3	Methyl ester impurity	2.3	0.0004	0.4
4	Ethyl ester impurity	2.2	0.0004	0.4

LOQ Study

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration	Value(µg/ml)
1	Febuxostat	9.8	0.0010	1.0
2	Amide impurity	10.3	0.0018	1.8
3	Methyl ester impurity	9.5	0.0012	1.2
4	Ethyl ester impurity	10.3	0.0012	1.2

Table 6: Solution stability study

Time (hours)	% of Amide imp	% of difference	% of Methyl ester imp	% of difference
Initial	0.10	NA	0.01	NA
After 24 hours	0.10	Nil	0.003	0.007
After 48 hours	0.11	0.01	0.00	0.01

Table 7: Precision study Feboxostat 40mg Tablets

S. No	Amide impurity	Methyl ester impurity	Ethyl ester impurity
1	714404	1011476	963952
2	702378	974846	946743
3	743571	1078442	1050308
4	700967	975956	946647
5	699643	968576	948152
6	693988	1014024	952716
Avg:	**709158.5**	**1003886.67**	**968086.33**
SD:	**18141**	**41447**	**40808**
% RSD:	**2.6**	**4.1**	**4.2**

Febuxostat 80mg Tablets

S. No	Amide impurity	Methyl ester impurity	Ethyl ester impurity
1	652971	959485	986207
2	645622	986720	979169
3	662094	987637	990815
4	667296	994306	994326
5	680631	1038910	1059349
6	675870	1000838	1015748
Avg:	**664080.67**	**994649.33**	**1004269**
SD:	**13352**	**25869**	**29671**
% RSD:	**2.0**	**2.6**	**3.0**

Febuxostat 120mg Tablets

S. No	Amide impurity	Methyl ester impurity	Ethyl ester impurity
1	721258	972457	974487
2	736971	974329	987399
3	737386	970470	988673
4	735673	969478	987623
5	739791	978799	995011
6	737368	973405	989630
Avg:	**734741.17**	**973156.33**	**987137.17**
SD:	**6738**	**3302**	**6796**
% RSD:	**0.9**	**0.3**	**0.7**

Table 8: Specificity study

S. No.	Compound Name	Peak Purity	RT (Individual)	RT (Spiked sample)
1	Febuxostat	1.00000	3.57	3.59
2	Amide impurity	1.00000	1.53	1.53
3	Methyl ester impurity	1.00000	6.59	6.57
4	Ethyl ester impurity	1.00000	7.06	7.05

Table 9 : Intermediate Precision (ruggedness) study Febuxostat 40mg Tablets

S. No	Amide impurity	Methyl ester impurity	Ethyl ester impurity
1	694844	522320	1456671
2	693892	520206	1438871
3	720979	543767	1486364
4	678173	512577	1437559
5	695263	525937	1448823
6	692478	523956	1463867
Avg:	**695938.17**	**524793.83**	**1455359.17**
SD:	**13860**	**10377**	**18258**
% RSD:	**2.0**	**2.0**	**1.3**

Febuxostat 80mg Tablets

S. No	Amide impurity	Methyl ester impurity	Ethyl ester impurity
1	659388	979479	995505
2	670122	993355	1019693
3	663980	981806	994942
4	665608	989079	1008723
5	689456	1038258	1081124
6	684060	1004144	1034185
Avg:	**672102.33**	**997686.83**	**1022362**
SD:	**11982**	**21745**	**32432**
% RSD:	**1.8**	**2.2**	**3.2**

Febuxostat 120mg Tablets

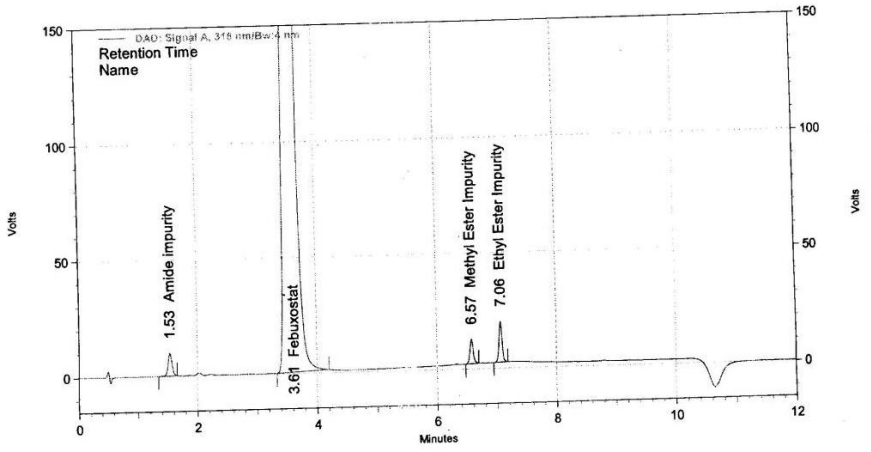
S. No	Amide impurity	Methyl ester impurity	Ethyl ester impurity
1	731399	947758	1004955
2	734346	958815	1017025
3	774081	1026780	1092666
4	718979	943829	1000096
5	741951	958834	1008155
6	729180	949130	999729
Avg:	**738322.67**	**964191**	**1020437.67**
SD:	**19044**	**31262**	**35949**
% RSD:	**2.6**	**3.2**	**3.5**

Table 10: Filter Variation Study

	Centrifuged	Nylon Filter	PVDF Filter
% of Amide impurity	0.10	0.10	0.10
% Difference	NA	Nil	Nil
% of Methy ester impurity	0.01	0.02	0.02
% Difference	NA	0.01	0.01
% of Ethyl ester impurity	0.10	0.10	0.10
% Difference	NA	Nil	Nil
% of Total impurities	0.30	0.30	0.30
% Difference	NA	Nil	Nil

The tablet preparations were carried out and the impurities were well separated with Febuxostat, a representative chromatogram given in **Fig 2**.

Fig 2. Chromatogram of febuxostat in tablets with impurities



DAD: Signal A, 318 nm/Bw:4 nm
Results

Name	Retention Time	Area	Area Percent	Peak purity
Amide impurity	1.53	94417	0.40	1.00000
Febuxostat	3.61	23142288	98.66	1.00000
Methyl Ester Impurity	6.57	85238	0.36	1.00000
Ethyl Ester Impurity	7.06	133555	0.57	1.00000
Totals		23455498	100.00	

Hence there is no systematic HPLC method has been developed for the estimation of related substances in Febuxostat and in pharmaceutical dosage form, the proposed method useful in regular quality control analysis in pharmaceutical formulation. The method was validated the parameters such as linearity, precision, accuracy, robustness, ruggedness, solution stability studies.

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***Corresponding author address:**
M.NARESH CHANDRA REDDY
Department of Chemistry,
Jawaharlal Nehru Technological University,
Anantapur, Andhra Pradesh, India,
Email: nareshchandrareddy@gmail.com