DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION FEBUXOSTAT RELATED SUBSTANCES IN BULK AND FORMULATIONS

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ABSTRACT

A novel simple RP-HPLC method has been developed for the estimation of Febuxostat related substances in bulk and formulations. The impurities observed in the Febuxostat API were amide impurity, Acid impurity, Secondary - butoxy acid impurity, Tertiary- butoxy acid impurity and ECI (Ethyl2-(3-cyano-4-isobutoxy phenyl) 4-methylthiazole-5-carboxylate) impurity. Ortho phosphoric acid (0.1%) as mobile phase – A, Methanol: Acetonitrile as (85: 15 v/v) mobile phase- B followed by gradient elution was confirmed by number of trials. The method was developed on kromosil C_{18} column (250mm$\times$5mm, 4.6μm). Febuxostat peak was eluted at 22 min and other impurities at their respective retention times. The developed method was validated as per ICH guidelines parameters like Accuracy, Linearity, LOD, LOQ and robustness were performed. The drug and all other impurities were linear at about 0.15-1.125 µg/ml range. The method was satisfied by all the validation parameters and suitable for the estimation Febuxostat related substances in routine analysis.

KEYWORDS: Febuxostat, related substances, method development, validation, Kromosil C_{18} column and RP-HPLC.

INTRODUCTION

Febuxostat is chemically 2-(3-cyano-4-isobutoxyphenyl) -4-methyl-1, 3-thiazole-5-carboxylic acid using for the treatment of gout and hyperuricemia. Febuxostat inhibits xanthine oxidase by blocking its molybdo pterinium site to controls the conversion of

hypoxanthine to xanthine leads to decreasing the uric acid production. The drug is not official in any Pharmacopoeia approved by the European medicines agency on April 21, 2008 and by the USFDA Feb 16, 2009. Impurities are degradation products in the active pharmaceuticals arise at any stage of drug development smaller concentration of impurities also affects the therapeutic efficacy of the drug. The observed impurities in the FEB drug were Impurity-A(Amide Impurity) 2-(3-carboxy-4-isobutoxypheny1)-4-methylthiazole-5-carboxylic acid, Impurity-B (Acid Impurity) 2-(3-carboxy-4-isobutoxypheny1)-4-methylthiazole-5-carboxylic acid, Impurity-C (Tertiary - butoxy acid Impurity) 2 - (4-tert- butoxy-3- cyanophenyl)-4-methylthiazole-5-carboxylic acid, Impurity-D (Secondary - butoxy acid Impurity) 2-(4-sec-butoxy-3-cyanophenyl)-4-methylthiazole 5-carboxylic acid and Impurity-E (ECI Impurity) Ethyl2-(3-cyano-4-isobutoxy phenyl) 4-methylthiazole-5-carboxylate. Structural formula of FEB $C_{16}H_{16}N_2O_3S$ and molecular weight is 316.378mg/mol. FEB is available in 40mg,80mg doses and it's a white crystalline powder soluble methanol and Acetonitrile. Apart from the literature survey reveals that there are several methods have been reported on estimation FEB in formulations by spectrophotometric, HPLC, LC/MS and MS/MS techniques. But there are no methods have been reported on the estimation of related substances in FEB. So the present investigation has been aimed to develop a simple and rapid RP-HPLC method on FEB related substances.

Present article reveals different impurities found in the API's, methods for identifying and the possible measures to deal with the interferences caused by them.

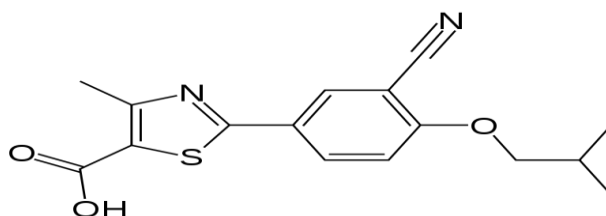Impurity profile is the description of identified and unidentified impurities present in new drug substances.

Fig no. 1: Structure of Febuxostat

MATERIALS AND METHODS

FEB and its related substances obtained as souvenir by MSN laboratories, Hyderabad, India. Tablets FEBUTAZ (40 mg) was obtained from local pharmacy. All Reagents and chemicals in this method were of analytical grade.

APPARATUS

The instruments used in this method were HPLC (2996) with auto sampler.

PREPARATION OF MOBILE PHASES

Mobile phase A

1ml of Ortho Phosphoric acid was accurately measured with 1ml micro pipette and transferred into 1000 ml of water and filtered through 0.22µm Nylon filter and degassed for 10 minutes.

Mobile phase B

Measured methanol and acetonitrile in the ratio of 85: 15 v/v into a 1000 ml measuring cylinder and filtered through 0.22µm nylon filter and degassed for 10 minutes

Standard Stock

Accurately weighed and transferred about 10mg each of FEB and its related substances working standard into a 10ml of volumetric flask. Added about 3ml of diluents and sonic ate to dissolve, made up to the volume with diluents and mixed well. Diluted 1mL of the above solution to 10ml with diluents and mixed well.

Standard Solution

Diluted 0.375ml of Standard stocks to 50ml with diluents and mixed well, that indicates0.75µg/ ml concentration of each substance. This solution was used for the evaluation of system suitability parameters.

Sample Solution

Accurately 160mg of sample(powdered tablet) was weighed and transferred into a 50ml Volumetric flask. Add about 30ml of diluents and sonicated for 10 minutes with intermittent shaking at room temperature, made up to the volume with diluents and mixed well.

Sample Solution

Weighed accurately about 20 tablets and powdered. Weighed 160mg of powdered tablet equivalent to 25 mg of FEB and transferred it into a 50 ml volumetric flask, added 30 ml of diluents, sonicated for 10 minutes with intermittent shaking and made up to the mark with diluents. This solution was used for the validation of the developed method and the following parameters were considered for the validation.

Chromatographic conditions

Mobile phase-A   : 0.1% OPA in water

Mobile phase-B   : Methanol: Acetonitrile ((85: 15) v/v.

Column   : Hypersil Gold (250 mm x 4.6 mm, 5.0 µm).

Detector   : UV detector.

Flow rate   : 1.0 mL/min.

Column temperature: 40°C.

Run time   : 45 min.

Wavelength   : 315 nm.

Diluent   : Mobile phase-B.

Injection volume   : 10 µL.

Program   : Gradient (gradient program was listed in Table. no 1).

METHOD VALIDATION

Linearity

The linearity was performed by external standard calibration method at the optimized chromatographic conditions, stock solutions of FEB and its related substances were prepared by using mobile phase-B as diluents. Various concentrations of FEB and its related substances were prepared in the range of 0.15–1.125 µg/mL. 10 µL of each solution was injected, the procedure was repeated for six times and the chromatograms were recorded at 315 nm. The chromatograms were shown in Figure 8. The calibration curve was plotted using concentration against peak area. The correlation coefficient values were 0.9996, 0.999, 0.9993, 0.9997, 0.9996 and 0.9997 for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E and FBX respectively. Correlation coefficient values of FEB and its related substances were within the limit. Results were listed in Table.2.

Accuracy, as Recovery

Accuracy of the method was confirmed by recovery studies. To the pre analyzed formulation (FEBUSTAT) a known quantities of raw materials of FEB and its related substances were added at different levels. The procedure was followed as per the analysis of formulations. The amount of each impurity recovered was calculated. This procedure was repeated for three times at each level. The SD and % RSD were calculated.

Robustness

The Robustness of an analytical method is a measure of its capacity of the method to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The sample solution was injected; the chromatograms were recorded at various conditions of flow and temperature. Robustness of the method was confirmed by change in flow in ± 0.1mL level and column temperature ± 5°C of the developed method, sample solution was injected and evaluated for the system suitability parameters and results were listed in Table. No 3.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD, LOQ of the method was confirmed by S/N ratio running the solutions of reference solution followed by the blank. Calculate the S/N ratio with respect to the blank and the results were calculated. And the results were listed in Table. No 4.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive and accurate RP-HPLC method for the separation of FEB related substances in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixtures of 0.1% ortho phosphoric acid 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 FEB was 23.170 ± 0.1, for Imp-A was 10.443 ± 0.1, for Imp-B was 13.568 ± 0.1min, for Imp-C was 20.281 ± 0.1mn, for Imp-D was 21.380 ± 0.1mn and for Imp-E was 34.640 ± 0.1mn. Each of the samples was injected six times and the sample retention times were observed in all cases.

Linearity

The peak areas of FEB and its related substances were reproducible as indicated by low coefficient of variation. A good linear relationship (r2 = 0.999) was observed for FEB and impurities, the regression characteristics are given in Table 2.

Accuracy (recovery)

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.

Robustness

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 and it was found that the method satisfying all system suitability parameters

LOD and LOQ

The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method LOD and LOQ were calculated based on S/N ratio. The results are presented in Table 5.
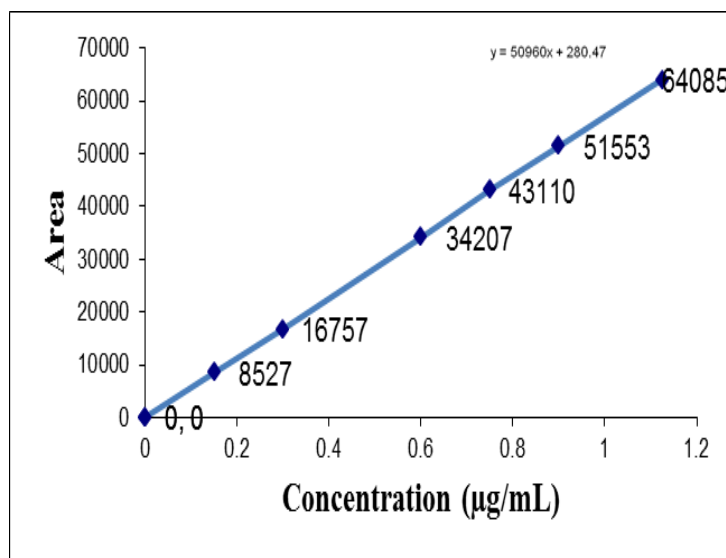

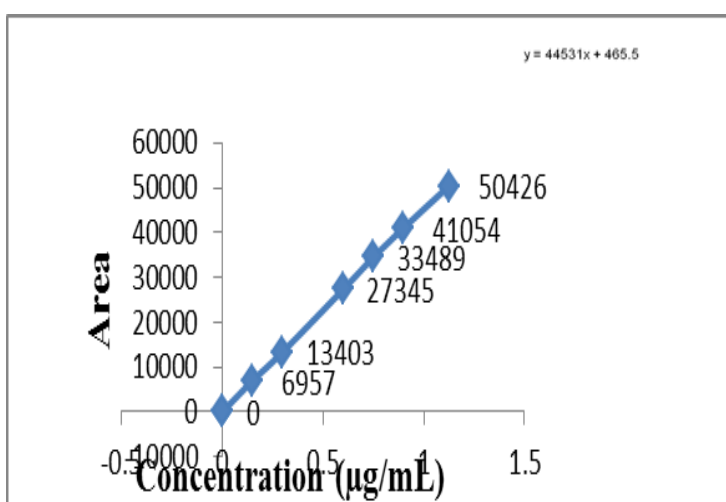**Figure-2 Calibration Curve of Impurity-A**
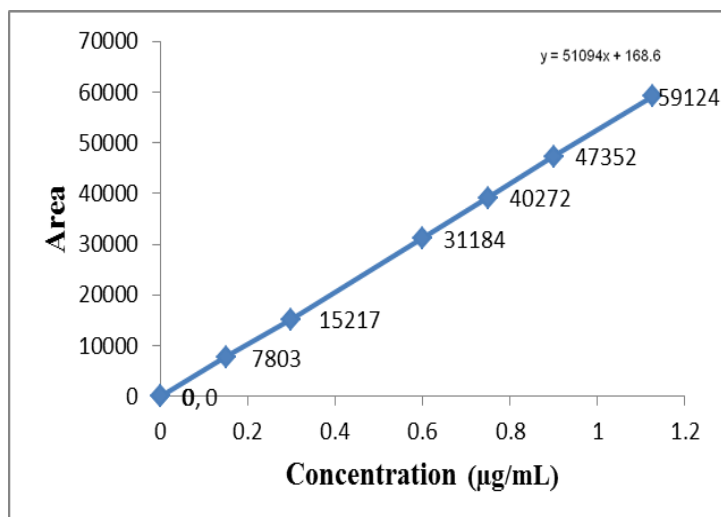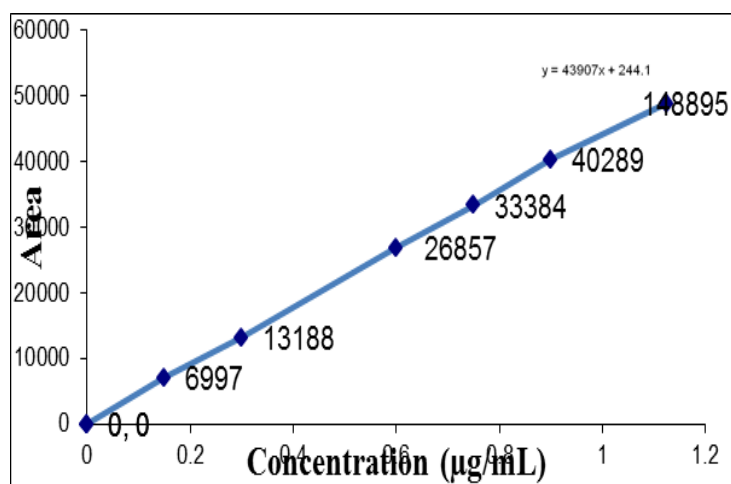

Figure-3 Calibration Curve of Impurity-B

Figure-4 Calibration Curve of Impurity-C

Figure-5 Calibration Curve of Impurity-D

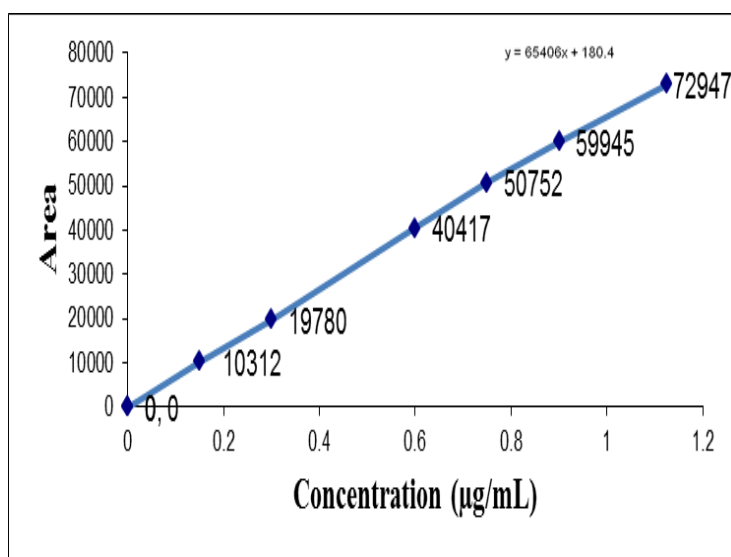**Figure-6 Calibration Curve of Impurity-E**

$y = 65406x + 180.4$
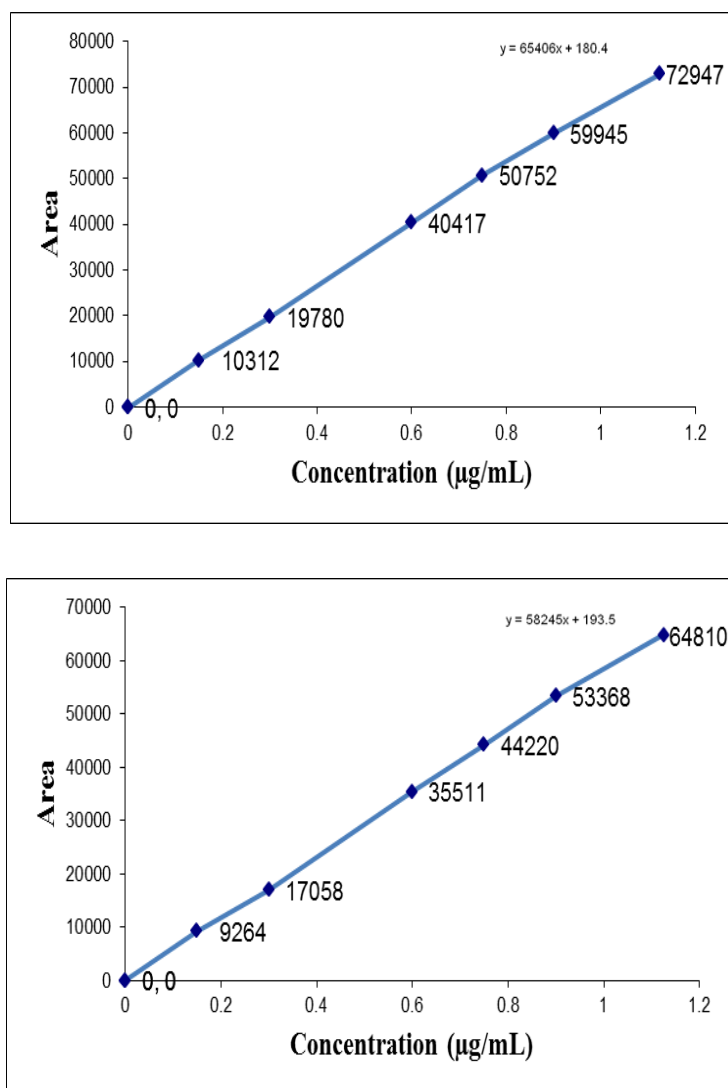

$y = 58245x + 193.5$

Figure-7 Calibration Curve of Impurity-Febuxostat

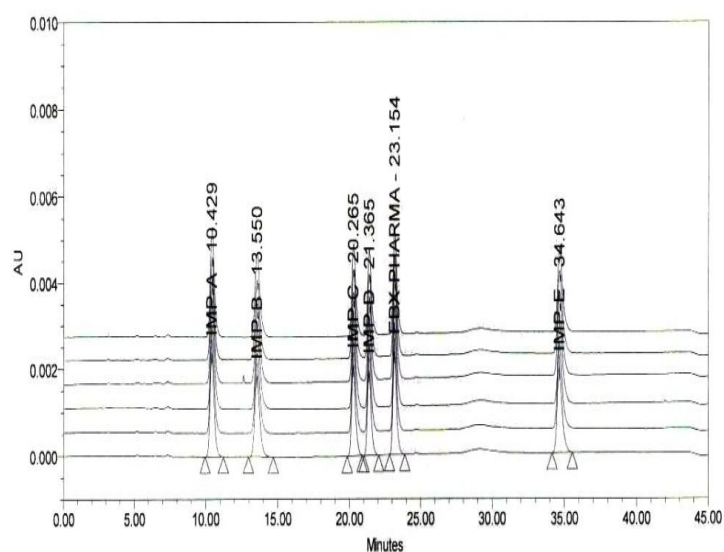**Fig-8 Optimized Chromatgram of Febuxostat and its related Substances**

Table-1 Gradient Programe

Time (min)	M.P-A (%)	M.P-B (%)
0	50	50
10	50	50
25	30	70
35	30	70
40	50	50
45	50	50

Table-2 Linear Regression Data for Febuxostat related Substances

PARAMETER	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Fbx
Detection wavelength	315nm					
Linearity range (µg/ml)	0.15-1.125	0.15-1.125	0.15-1.125	0.15-1.125	0.15-1.125	0.15-1.125
Slope	44530	50960	51094	43907	65406	58315
Intercept	465.5281	280.47	168.4714	244.1826	180.417	193.8
Correlation coefficient	0.9996	0.999	0.9993	0.9997	0.9996	0.9997
Retention time (±0.1min)	10.429	13.550	20.265	21.365	34.643	23.154

Table - 3 LOD & LOQ

Impurity Name	Concentration for LOD(µg/ml)	S/N ratio	Concentration for LOQ(µg/ml)	S/N ratio
Imp-A	0.01068	4.36	0.0356	11.53
Imp-B	0.01332	2.77	0.0444	9.56
Imp-C	0.0129	3.68	0.043	10.35
Imp-D	0.01491	3.48	0.0497	10.0
Imp-E	0.01248	3.13	0.0416	11.77
FBX	0.0117	4.16	0.039	10.52

Table-4 Robustness Study

CONDITION	%RSD
Actual flow(1.0ml)	1.29
Flow changed to 0.9ml(0.1ml)	1.42
Flow changed to 1.1ml(-0.1ml)	1.97
Actual temperature 40^0C	0.85
Temperature changed to 35^0C(-5^0C)	1.04
Temperature changed to 45^0C($+5^0$C)	1.28

CONCLUSION

The developed RP-HPLC technique is simple, accurate and cost effective. The result of analysis clearly indicates absence of interference from the excipients in the formulation. The statistical analysis proves that method is reproducible and selective for the analysis of Febuxostat related substances in bulk and tablet formulation.

ACKNOWLEDGEMENT

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