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A stability indicating RP-HPLC method development for determination of Febuxostat in tablet dosage form

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ABSTRACT

A reliable and sensitive isocratic stability indicating RP-HPLC method has been developed and validated for assay of Febuxostat in tablets and for determination of content uniformity. An isocratic separation of Febuxostat was achieved on Zodiac C18 column (250 X 4.6 mm, 5µ) particle size columns with a flow rate of 1.1 ml/min and using a UV detector to monitor the eluate at 218nm. The mobile phase consisted of Acetonitrile : Methanol (85:15 v/v).The drug was subjected to oxidation, hydrolysis, photolysis and thermal degradation. All degradation products in an overall analytical run time of approximately 10min with the parent compound Febuxostat eluting at approximately 4.87min. Response was a linear function of drug concentration in the range of 40-100µg/ml (r 2 = 0.9991). Accuracy (recovery) was between 98.00- 100.86. Degradation products resulting from the stress studies did not interfere with the detection of Febuxostat and the assay is thus stability-indicating.

Introduction:

Febuxostat is in a class of medications called xanthine oxidase inhibitors, use in the treatment of hyperuricemia and chronic gout [1]. Gout is a type of arthritis in which uric acid, a naturally occurring substance in the body, builds up in the joints and causes sudden attacks of redness, swelling, pain, and heat in one or more joints. It works by decreasing the amount of uric acid that is made in the body. Febuxostat is used to prevent gout attacks, but not to treat them once they occur.

Febuxostat received marketing approval by the European Medicines Agency on April 21, 2008[2] and was approved by the U.S. Food and Drug Administration on February 16, 2009[3]. A committee of the British National Institute for Health and Clinical Excellence concluded that it is more effective than fixed-dose (300 mg) allopurinol in lowering serum uric acid concentration and committee recommended febuxostat for people who are intolerant of all opurinol[4].

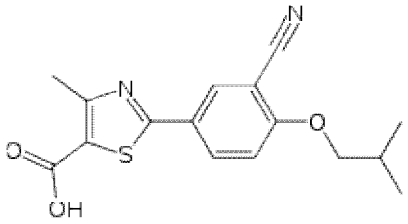


Figure. A: Structure of Febuxostat

Febuxostat is a non-purine selective inhibitor of xanthine oxidase. It works by non-competitively blocking the molybdenum pterin center which is the active site on xanthine oxidase. Xanthine oxidase is needed to successively oxidize both hypoxanthine and xanthine to uric acid. Hence, febuxostat inhibits xanthine oxidase, therefore reducing production of uric acid. Febuxostat inhibits both oxidized as well as reduced form of xanthine oxidase because of which febuxostat cannot be easily displaced from the molybdenum pterin site. Many long and short-term clinical trials have proved the efficacy of Febuxostat in the treatment of gout and lowering uric acid levels. In these studies Febuxostat was found to be superior to Allopurinol in reducing the serum uric acid levels [5-9]. The adverse effects associated with febuxostat therapy include nausea, diarrhea, arthralgia, headache, increased hepatic serum enzyme levels and rash[10,11]. It may cause you to have high levels of liver enzymes in blood.

Experimental:

Materials and Methods:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Febuxostat isocratic PEAK-HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. TECHCOMP UV/Visible spectrophotometer with HITACHI software used for wavelength scanning.

Chemicals and Solvents:

Acetonitrile, Methanol, Water, used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

The mobile phase:

A mixture of Acetonitrile: Methanol in the ratio of 85:15v/v was prepared and used as mobile phase.

Standard solution of the drug:

For analysis we 1000µg/ml standard Febuxostat solution was prepared by dissolving 10mg of drug in to 10 ml of Acetonitrile and dissolved for 5minutes with sonicator then filter with vacuume filtration kit through 0.45µ Millipore filter paper. And required concentrations were obtained from 1000µg/ml solution by proper dilution.

Preparation of sample solution for tablets assay

Twenty ULORIC tablets were weighed, crushed and mixed in a mortar and pestle for 20 min. A portion of powder equivalent to the weight of 10mg was accurately weighed into 100 ml volumetric flasks and 20 ml of HPLC-grade Acetonitrile was added to flask. The volumetric flask was sonicated for 20 min to effect complete dissolution of drug and the solutions were then made up to volume with HPLC Acetonitrile. Aliquots of the solution were filtered through a 0.45 µm nylon filter and 1 ml of the filtered solution was transferred to a 10 ml A-grade volumetric flask and made up to volume with mobile phase. Likewise sample was further diluted to get required concentration.

Detection wavelength

The spectrum of diluted solutions of the Febuxostat methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the Febuxostat were showed that a wavelength was found to be 218nm.

Forced degradation studies for API and tablets of Febuxostat:

To determine whether the method was stability indicating, Febuxostat active pharmaceutical ingredient (API) Powder Were Stressed under Different Conditions to Promote Degradation. According to ICH guidelines the development and validation of stability-indicating potency assays. The methanol was used as solvent and diluents in all forced degradation studies. All solutions were prepared to use in forced degradation studies were prepared by dissolving Febuxostat in small volume Acetinitrile and later diluted with aqueous 0.1N hydrochloric acid, aqueous 0.1N sodium hydroxide and 3% aqueous hydrogen peroxide. To study the effect of thermal stress API powder were exposed to dry heat (80^0C) in a convection oven for 48 h. The sample was then removed from the oven, and amounts of powder equivalent to the 100 mg were analyzed as per the proposed method. To study photo stability of Febuxostat, the solutions of Febuxostat were exposed to UV light at 100-280nm for 48h. Approximately 50mg API was spread on glass dish in a layer less 2mm thick. After removal from the light cabinet, sample was analyzed. After removal from the light cabinet, sample was analyzed. With the objective of evaluating stability of Febuxostat, the drug at a concentration of 100µg/ml was used and subjected to forced degradation for the detection of febuxostat. After the degradation these solutions was diluted to methanol to get starting concentration of 60µg/ml. All the stressed samples of Febuxostat were analyzed and peak purities were checked using photodiode array detector (PAD).Febuxostat was well resolved from its degradation products, indicating the stability indicating assay more specific.

Light (Normal and UV light) –

To demonstrate the degradation of the sample, it was kept in open Petri dish at Lab light and UV light. After 48 hours exposure at Lab light and UV light sample solutions prepared and inject once in the chromatographic conditions.

Thermal:

Sample taken in to Petri dish and keep in oven at 40°C up to 48 hours. After expose of the samples and prepare sample solution and inject once. Evaluate the degradedness in chromatogram and compare to Initial values

Acid:

To prepare acid hydrolyzed sample at zero hours and after 48 hours. About 300 mg sample in taken to 20 mL of 0.1 N Hydrochloric Acid. After 48 hours 5 ml of acid hydrolyzed sample solution taken in to 25 mL volumetric flask and neutralize with 5 mL of 0.1 N sodium hydroxide solutions and make up with diluents. The above solutions injected once after system suitability solution and evaluate the degradants in chromatogram and compare with standard values

Base:

To prepare base hydrolyzed sample 48 hours, about 300 mg sample taken in to 20 mL of 0.1 N Sodium Hydroxide solutions. After 48 hours 5 ml of Base hydrolyzed sample solution taken in to 25 mL volumetric flask and neutralize with 5 mL of 0.1 N Hydrochloric acid solutions and make up with diluents. The above solutions injected once after system suitability solution and evaluate the degradants in chromatogram and compare with without base hydrolysis values.

Hydrogen Peroxide:

To prepare oxidized sample at zero hours and after 48 hours. About 300 mg sample in to 20 mL of 3% Hydrogen Peroxide. After 48 hours 5 ml of oxidized sample solution taken in to 25 mL volumetric flask and make up with diluents. The above solutions injected once after system suitability solution and evaluate the degradants in chromatogram and compare with without oxidized (Initial) values.

Aqueous:

About 300 mg sample taken in to 20 mL of aqueous solution. After 48 hours 5 ml of sample solution taken in to 25 mL volumetric flask and make up with diluents. The above solutions inject once after system suitability solution and evaluate the degradants in chromatogram and compare with without aqueous values.

Results and discussion:

HPLC Method development:

Simple, rapid, economic stability-indicating RP-HPLC Method has been developed for determination of Febuxostat in the presence of its degradation products. The method was optimized to provide a good separation of the components (acceptable theoretical plates and resolution between peaks) with a sufficient sensitivity and suitable peak symmetry (peak tailing factor >2) in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, floe rate, and detector wave length were studied. The use of hydrophobic stationary phase normally provides adequate retention of organic non polar molecules. The chromatographic separation was achieved using as RP C18 Column because it was suitable to resolve the degradation products from Febuxostat with adequate resolution and give symmetrical peak shapes. For Febuxostat Acetonitrile was used as a diluents and it is a well known solvent for various pharmaceutical compounds. Our experiments and data reported in the literature showed that both the methanol and acetonitrile could be used an organic modifier in the mobile phase. The use of acetonitrile could be used an organic modifier resulted in the better sensitivity compare to methanol. Tests involving the use of mixtures of acetonitrile and different buffer solutions (eg, potassium phosphate or ammonium acetate buffer) were made to optimize the mobile phase with different pH values, Finally Acetonitrile and methanol in the ratio of 85:15(v/v) was selected as mobile phase whose combination gives good peak symmetry, sensitivity, and shorter retention time. Our experiment revealed that isocratic elution with simple mobile phase. The method has many advantages like simplicity, isocratic conditions, shorter run time, low injection volume, smaller particle size, and less flow rate, inexpensive mobile phases than the method reported in the literature. Under these conditions the retention time of Febuxostat was 4.87 min, with a good peak shape (peak symmetry), and chromatography analysis time was 10min. the proposed method conditions were shown in table 1.

Method validation:

The method was validated as per the ICH guidelines for validation of analytical procedure for different validation parameters. The method was varied for its specificity, robustness, ruggedness, LOD and LOQ. A system suitability test was also carried out to evaluate the reproducibility of the analytical system using five replicate injections of a reference solution.

System suitability:

To know the reproducibility of the method system stability test was employed to establish the parameters such as tailing factor, theoretical plates, resolution, asymmetric factor, limit of detection, and limit of quantification the values are shown in table.2.

Accuracy:

To ensure the reliability and accuracy of the, the recovery studies were carried out by adding a known quantity of drug with pre analyzed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated at three different concentrations 50%, 100%, 150% to the active ingredients by adding a known amount of Febuxostat standard to a sample known concentration and calculating the recovery of Febuxostat with RSD (%) and % recovery for each concentration. The mean % recoveries were in between 98.95 to 99.5 and were shown in Table 3. There was a high recovery 100.86 of Febuxostat indicating that the proposed method for the determination of Febuxostat in the tablet in the tablet dosage forms was highly accurate.

Precision:

The precision of the method was determined by repeatability and intermediate precision studies. Repeatability was evaluated by performing six determinations (n=6) at the same concentration, during the same day, under the same experimental conditions. Intermediate precision was evaluated by comparing the assays on three different days using different analyst. The result revealed the precision with % RSD for intraday and interday was 0.40, 0.78 respectively. The results were shown in Table 4.

Linearity:

The linearity was evaluated by linear regression analysis by the least-square regression method, which was used to calculate the r-value, y-intercept, and slope of the regression line. The analytical curves were constructed by plotting peak areas against the respective concentrations. From the stock reference solution of Febuxostat; six concentrations were prepared in the mobile phase in the range of 40-100µg/ml. It was found to be linear with a correlation coefficient r^2 of 0.998, the corresponding linear regression equation being y=2601x+12526. Linearity results and calibration graph were shown in table 5 and graph 1 respectively.

Robustness of the method:

To determine the robustness of the developed method experimental conditions was deliberately changed. To study the effect of eluent flow rate, and mobile phase ratio 85:15 to 90:10(v/v) and to 80:20(v/v).In all the above varied conditions, the proposed method indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. The results were shown in table 6.

Selectivity:

The results of stress testing studies indicated a high degree of selectivity of this method for Febuxostat. The degradation of Febuxostat was found to be similar for both the tablets and API powder.

LOD&LOQ:

Limit of Detection (LOD) and Limit of Quantification (LOQ), the limits of detection and quantitation were calculated by the method based on the standard deviation and the slope of the calibration plot, using the formula. The Limit of Detection and Limit of Quantification for Febuxostat is 0.5µg/ml and 1.5µg/ml.

Forced degradation studies:

Febuxostat was exposed to different stress conditions and the degradation products were well separated with greater resolution. The conditions of degradation were shown in table 8.

Formulation assay:

The validated method was applied to the determination of Febuxostat in commercially available ULORIC tablets. Fig. 3. illustrates typical HPLC chromatogram obtained following the assay of ULORIC tablets. The result of the assay undertaken yielded 98.86 of label claim for AT Febuxostat. The results of the assay indicate that the method is selective for the analysis of Febuxostat without interference from the excipients used to formulate and produce these tablets.

Conclusions

A simple, rapid, accurate and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of Febuxostat in API and tablet dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The proposed method has the ability to separate the drug from their degradation products, related substances, excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

Table 1: Optimized chromatographic conditions for estimation of Febuxostat

PARAMETER	CONDITION

Mobile phase	Acetonitrile : Methanol: 85:15(v/v)
Pump mode	Isocratic
Ph	5.1
Diluents	Methanol
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	218nm
Injection Volume	20 µl
Flow rate	1.1m/min
Run time	10minutes
Retention Time	4.87minits

Table: 2 System suitability parameters for Febuxostat

S.no	Parameters	Value
1	Retention time	4.87minits
2	Theoretical plates	8142
3	Tailing factor	1.39
4	Limit of Detection	0.5µg/ml
5	Limit of Quantification	1.5µg/ml

Table: 3 Accuracy of the method:

S.NO	Recovery	Target conc.*	Spiked conc.	Final conc.	Recovered Mean ± SD#	%RSD	%Recovery Mean ± SD	RSD of Recovery
1	50%	40	20	60	59.37±0.18	0.30	98.95±0.31	0.31
2	100%	40	40	80	79.60±0.40	0.50	99.5±0.50	0.50
3	150%	40	60	100	99.09±1.54	1.55	99.09±1.54	1.55

Table: 4 Intraday and interday precisions:

S.NO	Intraday (60µg/ml)	Interday (60µg/ml)	Ruggedness (60µg/ml)
1	168898	168898	171190
2	169608	169608	168641
3	169830	169830	168679
4	168209	168209	168299
5	168434	168434	168341
6	169935	169935	171353
RSD:	0.36	0.40	0.78

Table: 5 Linearity results

Level	Concentration of Febuxostat In µg/ml	peak area
Level – 1	40	117097
Level – 2	50	140988
Level – 3	60	167042
Level – 4	70	197552
Level – 5	80	220293
Level – 6	90	250193
Level – 7	100	269373
Range:40-100µg/ml	Slope:2601 Intercept:12526 Correlation Coefficient :0.998	

Graph 1: Calibration curve of Febuxostat

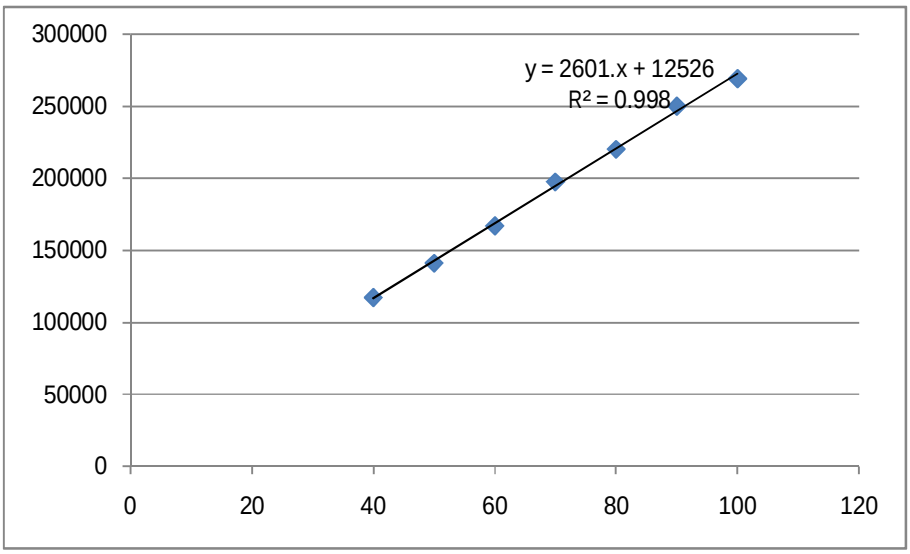


Table: 6 Robustness of the method

Condition	Mean area	% difference
Unaltered	167042	……
Mp change-1 90:10 Mp change-2 80:20	168695 169359	0.99 1.39
Wl change-1 223nm Wl change-2 213nm	537986 546553	-0.24 1.34
pH-1 5.0	167956	0.55
pH-1 5.2	166923	0.07

Table: 7 Forced degradation studies

Condition	Report
Base	Main peak split into 2 peaks
Light	Main peak split into 4 peaks
Peroxide	Main peak split into 3 peaks
Uv light	Main peak split into 3 peaks
Thermal	Main peak split into 3 peaks
Acidic	Main peak split into 3 peaks
Aqueous	Main peak split into 2 peaks

Table: 8 Stability of Febuxostat

Time in min	Area found	% Assay
0	89496	100
1	88851	99.28
2	89501	100.01
4	89229	99.70
6	88472	98.85
12	89627	100.15
18	89738	100.27
24	90989	101.67
36	88669	99.07

Formulation assay

S.NO	Brand Name	Concentration	Amount found	%Assay
1	ULORIC	60 µg/ml	59.31 µg/ml	98.86

Figure.2: standard chromatogram of Febuxostat

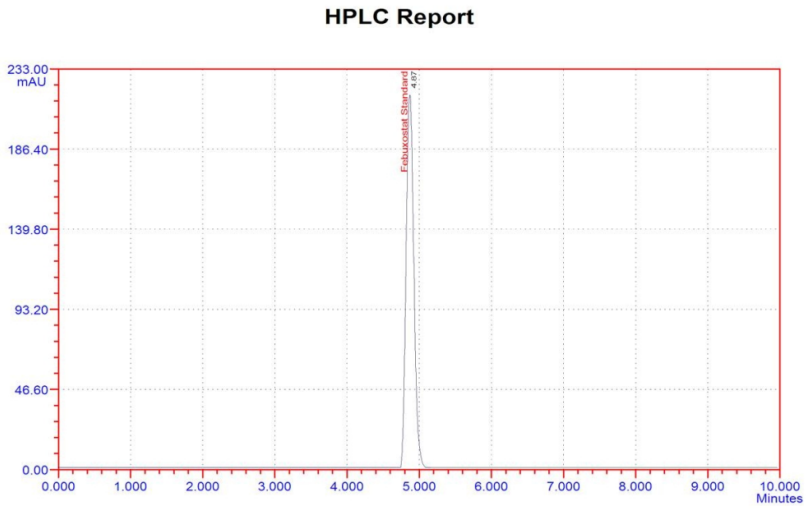
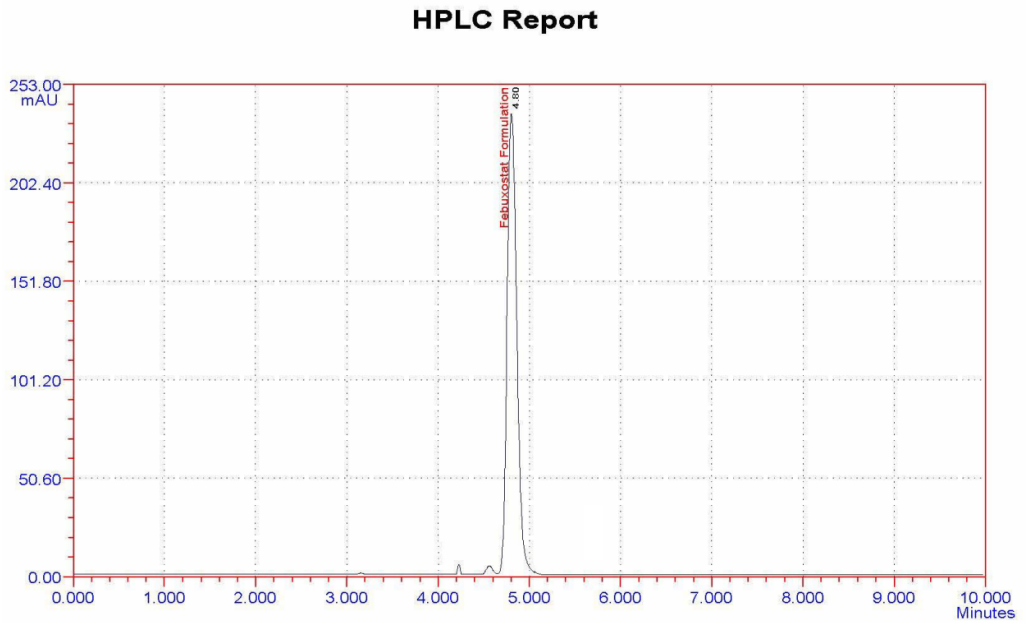


Figure.3: sample chromatogram of Febuxostat



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