Estimation of Febuxostat drug present in formulation by RP-HPLC

Ashwini Gunda1,*, N.Arvindkumar2, Karanaker Reddy3, T, Anand Kumar4
1. University College of Pharmaceutical Sciences, Osmania University, Hyderabad, India.
2. University of Swinburne, Victoria, Australia.
3. Bharat Institute of Technology, Ibrahim patnam, Hyderabad, India.
4. Pulla reddy college of pharmacy, Hyderabad, India

Received on: 10-11-2011; Revised on: 15-12-2011; Accepted on: 12-01-2012

ABSTRACT

A novel reverse phase liquid chromatographic technique was developed for the analysis of Febuxostat. Developed method was simple, accurate and precise. This method is capable to spate and analyse the drug in the presence of all other potential components. The method was developed by using the Symmetry C18 (250×4.6 mm, packed with 5 µm) column. Methanol and Phosphate buffer with pH 3 (60:40% v/v) used as mobile phase. The analyte molecule was monitored at 318 nm by UV detector. The retention of the drug was found to be 11.20 min more or less 0.5 min. The method was validated according to the ICH guidelines. Validation report shows that the method was specific, accurate, precise and robusted for the estimation of Febuxostat.

Key words: Febuxostat, ICH guidelines, Method development and Validation.

INTRODUCTION

Febuxostat is chemically 2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid. It is available in the form non hygroscopic crystalline nature and freely soluble in dimethylformamide, soluble in dimethylsulphide, sparingly soluble in ethanol, slightly soluble in methanol and acetoniitrile, and practically insoluble in water1-3. Febuxostat is orally administered a non-purine, selective inhibitor of xanthine oxidase being developed for the management of hyperuricemia in patients with gout. Febuxostat is not expected to inhibit other enzymes involved in purine and pyrimidine synthesis and metabolism at therapeutic concentrations4-6. There are several analytical method are reported on the analysis of Febuxostat. HPLC method for analysis of metabolites present in the blood serum7. Simultaneous analysis of substrates, products, and inhibitors of xanthine oxidase by RP-HPLC and gas chromatography were reported8. Several other techniques such UV method for dissolution studies of Febuxostat tablets, TLC, HPLC method for the analysis of Febuxostat and its related substance present in the bulk drug were noticed from the literature9,10. The developed analytical method is simple, specific, accurate and precise for the routine analysis of Febuxostat drug in the formulation. Validation of method according to ICH guidelines11,12.

Fig. Structure of Febuxostat [2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid]

Experimental:

Chemical sand reagents: Febuxostat marketed tablets and standard Febuxostat drug were supplied by Alembic chemicals, India, the HPLC grade methanol purchased from RANKEM chemicals, New Delhi, India, potassium dihydrogen ortho phosphate and orthophosphoric acid purchased from standard chemicals, Mumbai, India. High purity water was prepared by using Millipore MilliQ Plus water purification system (Millipore, Milford, MA, USA).

Equipment: The Agilent 1200 series HPLC consist of Quaternary pump with UV detector system (Agilent technologies, New Delhi, India). Symmetry C18 250×4.6mm, 5µm. The output signal was monitored and processed using empower-2 software. Other instruments like pH meter (Thermo scientific), Balance (Sartorius) and Sonicators (Ultrasonic Sonicators).

Chromatographic condition: the method was developed by using Symmetry C18 250×4.6mm, 5µm. Methanol and Phosphate buffer with pH 3 (60:40% v/v) used as mobile phase. The mobile phase was filtered through a nylon membrane (pore size0.45µm) filter. The flow rate of the mobile is 1.0ml/min. The column temperature was maintained at 25 ºC and the wavelength was monitored at 318 nm. The injection volume was 10µL.

Preparation of Mobile phase:

Buffer Preparation: Dissolve 2.72g of potassium dihydrogen ortho phosphate in 1000 mL of water and mix. Adjust the pH of this solution to 3.0 ± (0.05) with dilute ortho phosphoric acid. Filter the solution through 0.45µ Nylon filter.

Mobile Phase: Phosphate Buffer: Methanol (40:60 %v/v)

Diluent: Methanol: Water (90:10% v/v)

Standard Preparation:

Weigh accurately about 10mg Febuxostat working standard and transfer into a 100 mL volumetric flask, add 60 mL of diluent and sonicate to dissolve for about 5 min, further make up the volume with diluent.

Sample Preparation:

Weigh 20 tablets and crush into powder. Weigh powder equivalent to 125 mg of the Febuxostat and transfer into a 250 mL volumetric flask, add 120 mL of diluents and sonicate for 15 min, further make up the volume with diluent. Filter through 0.45µ Nylon filter. Further dilute the filtrate 5 mL to 25 mL with diluent.

Validation:

The proposed method validated according to ICH guidelines.

Specificity:

a) Febuxostat Identification

Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system. Chromatogram of standard and sample should be identical with near Retention time.
Chromatograms:

Blank chromatogram

Standard chromatogram

Sample chromatogram
Optimized chromatographic characteristics of Febuxostat by RP-HPLC

System Suitability of Febuxostat by RP-HPLC

There is no interference due to blank at the retention time of analyte. The blank should not show any peak at the retention time of analyte peak.

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure. Chromatogram of standard solution should be not more than 2.0%.

System suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0%.

The number of theoretical plates (N) for the Febuxostat peaks should be not less than 3000.

Parameters Observed values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear concentration</td>
<td>50 – 150 ppm</td>
</tr>
<tr>
<td>tₚ</td>
<td>318 min</td>
</tr>
<tr>
<td>Slope</td>
<td>8302.4</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9984</td>
</tr>
</tbody>
</table>

System Suitability of Febuxostat by RP-HPLC

Parameter cameo Observed value Acceptance criteria

USP tailing 1.21 Not more than 2.0
USP theoretical plates 9720 Not less than 3000

Accuracy of Febuxostat

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Concentration</th>
<th>Percentage Recovery</th>
<th>Mean percentage recovery</th>
<th>Standard deviation</th>
<th>Relative standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50%</td>
<td>101.05</td>
<td>101.05</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>50%</td>
<td>101.09</td>
<td>101.09</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>50%</td>
<td>100.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100%</td>
<td>100.25</td>
<td>100.25</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>100%</td>
<td>100.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100%</td>
<td>100.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>150%</td>
<td>98.38</td>
<td>98.6</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>8</td>
<td>150%</td>
<td>98.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>150%</td>
<td>98.74</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Linearity of Febuxostat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observed value</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td></td>
<td>Not more than 2.0</td>
</tr>
<tr>
<td>tₚ</td>
<td></td>
<td>Not less than 3000</td>
</tr>
<tr>
<td>%RSD of assay results</td>
<td></td>
<td>Not more than 2.0%</td>
</tr>
</tbody>
</table>

Linearity:

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 50 ppm to 150 ppm of Febuxostat. Plot a graph to concentration versus peak area. Correlation Coefficient (r²) should be not less than 0.999.

Precision:

Precision performed at two levels repeatability and intermediate precision.

a. Repeatability:

Determine the precision of test method by assaying 6 samples prepared from the same batch at different times as per test method and calculate %RSD of assay results. %RSD of 6 replicate injections should be not more than 2.0%.

b. Intermediate precision (Analyst to analyst variation):

To demonstrate Intermediate precision of assay method conduct Analyst to analyst variation by 2 different analysts. Analyst to analyst variability study can be performed individually or along with system to system or column to column variability. %RSD for area of assay results should be NMT 2.0%.

Accuracy:

The accuracy of the method was evaluated by determination of recovery of Febuxostat at three levels of concentrations. The sample solutions were spiked with Febuxostat standard solutions corresponding to 50%, 100%, and 150% of nominal analytical concentrations. (50µg/ml, 100µg/ml and 150µg/ml). The results showed good recovery within limits (98% – 101%).

Robustness:

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate and mobile phase composition which may differ but the responses were still within the specified limits of the assay.

a) Effect of variation of mobile phase composition

A study was conducted to determine the effect of variation in mobile phase ratio by Changing the ratio of mobile phase i.e. Buffer: Methanol from

<table>
<thead>
<tr>
<th>Time</th>
<th>Standard factor</th>
<th>% Assay Test 1</th>
<th>Test 2</th>
<th>% Difference Test 1</th>
<th>Test 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>NA</td>
<td>100.0</td>
<td>101.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>24hr</td>
<td>1.0095</td>
<td>100.9</td>
<td>100.8</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>48hr</td>
<td>1.0116</td>
<td>100.6</td>
<td>100.6</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Solution stability for Febuxostat standard and sample solutions

1. Effect of variation of mobile phase composition

A study was conducted to determine the effect of variation in mobile phase ratio by Changing the ratio of mobile phase i.e. Buffer: Methanol from...
Effect of variation in Buffer pH
A study was conducted to determine the effect of variation in Buffer pH. Standard solution was prepared and injected into the HPLC system by maintaining pH 2.8 and 3.2. The effect of variation in Buffer pH was evaluated.

effect of variation in temperature
A study was conducted to determine the effect of variation in temperature. Standard solution was prepared and injected into the HPLC system by keeping temperature 20°C and 25°C and 30°C. The effect of variation in temperature was evaluated.

effect of variation in flow rate
A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping flow rates 0.8 ml/min and 1.2 ml/min. The effect of variation of flow rate was evaluated.

effect of variation in filter:
To demonstrate the robustness, check the filter validation by using different filters namely 0.45µ PVDF hydrophilic membrane filter and 0.45µ Nylon membrane filter. The effect of variation in filter was evaluated.

Ruggedness:
Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments. And hence the proposed method was found to be rugged.

Conclusion
The proposed analytical method is simple, economical, rapid, sensitive, reproducible and accurate for the estimation of Febuxostat. A newer RP-HPLC method was developed for the pharmaceutical dosage forms. The proposed method gives reliable assay results with mobile phase Phosphate buffer: Methanol (40:60). The method does not suffer from any interference due to common excipients. Thus it shows that proposed method could be successfully applied to estimate commercial pharmaceutical products containing Febuxostat. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

Acknowledgement:
The authors are thankful to Karnaker Reddy. T. Author thankful Head of the department pharmaceutical analysis, Osmania University, Hyderabad, India his support during this work. We are also thankful to T. Karnaker Reddy for his moral support and guidance.

Reference:
4. R. Khosravan PhD, K. Erdman BS, L. Vernillet PharmD, PhD, J. T. Wu PhD, N. Joseph-Ridge MD, and D. Mulford PhD, Effect of febuxostat on pharmacokinetics of desipramine, a CYP2D6 substrate, in healthy subjects, Clinical Pharmacology & Therapeutics (2005) 77, P43-P43

Source of support: Nil, Conflict of interest: None Declared