REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC
ESTIMATION OF ANTI-GOUT IN PHARMACEUTICAL DOSAGE FORM

Mahesh Nasare, S. Harshini*, A. Manikanta Kumar and V. Prakash Diwan

Department of Pharmaceutical Analysis and Quality assurance,
School of Pharmacy, Anurag group of Institutions, Venkatapur (V),
Ghatkesar (M), Rangareddy, Hyderabad, Andhra Pradesh, India.

ABSTRACT
A simple, specific, accurate, precise, selective, economic and rapid reverse phase high performance liquid chromatography (RP-HPLC) method for the estimation of febuxostat in bulk and pharmaceutical dosage forms has been developed and validated. The chromatographic separation for febuxostat was achieved on a reverse phase Phenomenex® (Luna 5μ C18 (2) 100A (250 × 4.60 mm) i.d column in isocratic mode at ambient temperature by using mobile phase consisting of water : acetonitrile (30:70% v/v) delivered at a flow rate of 0.7 mL min⁻¹. The retention time of febuxostat and internal standard (tinidazole) were found to be 2.495 ±0.177 and 3.915 ± 0.005 minutes respectively. The analyte molecule was monitored at 314 nm by UV detector. The developed method gave good resolution between febuxostat and internal standard. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and ruggedness were determined according to ICH guidelines. The calibration curve was linear over the concentration range of 1-7μg mL⁻¹. The mean recoveries obtained for febuxostat were found to be 99.02 to 102 %, so the proposed method is accurate. Limit of detection and limit of quantification for febuxostat were 0.01 and 1μg mL⁻¹ respectively, therefore the method employed as a more convenient for the analysis of febuxostat and its related compounds in drug substance and formulations and can be used for routine quality control analysis.

Keywords: Febuxostat; Method development; Validation, HPLC.

1. INTRODUCTION
Febuxostat is a non-purine selective inhibitor of xanthine oxidase Fig:1 (a)¹. The IUPAC name for febuxostat is (2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid). 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. It is used in the treatment of gout²,³.

Tinidazole is an anti-parasitic drug used against protozoan infections Fig:1 (b). The IUPAC name for tinidazole is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitroimidazole).

Literature survey reveals that there is only one method for the estimation of febuxostat in the bulk drugs and in the formulation by using isocratic RP-HPLC method⁴,⁵. Therefore, there is aim to develop and validate a new, simple, precise and economical RP-HPLC method for the estimation of febuxostat in bulk and pharmaceutical dosage forms.
2. MATERIALS AND METHODS

2.1. Drugs and solvents
Febuxostat and tinidazole pure drugs were supplied as gratis samples by Mankind Pharmaceutical limited (Hyderabad, India). Acetonitrile (HPLC grade), methanol (HPLC grade) were obtained from Merck specialities private limited (Mumbai, India). Water (HPLC grade) was obtained from Qualigen Fine chemicals (Mumbai, India).

2.2. APPARATUS
HPLC method development and validation were done on SHIMADZU (Japan) liquid chromatograph equipped with LC-20AD pump, LC 20A UV/Vis detector and Rhodyne 7725 injection with a 20 µL loop. The chromatographic separation was performed using reverse phase phenomenex® Luna 5µ C18 (2) 100A (250 × 4.60 mm) column. The output signal was monitored and processed Using LC solutions software. Other instruments used are SHIMADZU electronic balance BL-220H (SHIMADZU corporation, Japan), Value 1 stage vacuum pump Model: VE115, Fast clean ultrasonic cleaner.

2.3. Pharmaceutical formulation
Febuxor-40mg manufactured by Mankind Pharmaceutical Limited is used for analysis.

2.4. Chromatographic conditions
The method was developed by using SHIMADZU HPLC containing reverse phase phenomenex® Luna 5µ C18 (2) 100A (250 × 4.60 mm) i.d column using Water and acetonitrile (30:70 % v/v) as mobile phase at a flow rate of 0.7 mL min⁻¹ in isocratic mode at detection wavelength 314nm. The mobile phase was filtered through a 0.45 µm membrane filter using value 1 stage vacuum pump and de-aerated in ultra sonic bath sonicator. The room temperature was maintained. The sample injected volume was 20 µL.

2.5. Preparation of Standard solutions
10mg of febuxostat were dissolved in 100ml of mobile phase to obtain standard stock solutions of 100 µg mL⁻¹. Working standard solutions of febuxostat were prepared from stock solutions in the concentration ranges of 1-100 µg mL⁻¹ by suitable dilution of the stock solutions with mobile phase. Samples in triplicates were made for each concentration and peak areas plotted against the corresponding concentration to obtain the calibration graph.

2.6. Preparation of Sample solution for assay
Twenty Febuxor-40mg each containing 40mg of febuxostat were weighed, average weight was calculated and powdered. A quantity equivalent to 10mg of febuxostat was weighed and transferred into 100 ml volumetric flask. It is extracted with mobile phase. The volumetric flask was sonicated for 20 minutes and the solution was made up to the volume with mobile phase and filtered. Suitable aliquots of formulation solution were prepared and injected to HPLC to obtain concentration in the linearity range.

3. RESULTS AND DISCUSSION

3.1. HPLC method development and optimization
For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. Symmetrical peaks with good separation were obtained as shown in fig.2 with reverse phase phenomenex® Luna 5µ C18(2) 100A (250 × 4.60 mm) i.d column using mobile phase containing water and acetonitrile (30:70 % v/v) at a flow rate of 0.7 mL min⁻¹ at detection wavelength 314nm at which good detector response was obtained for the drug. There is no interference from the diluents and excipients present in the pharmaceutical formulation.

3.2. Validation of the method
The proposed method was validated as per International Conference on Harmonisation (ICH) guidelines.

3.2.1. Linearity and Range
A series of solutions of febuxostat were prepared from the standard stock solutions
in the concentrations ranging from 1 to 100 µg mL\(^{-1}\) and injected into the HPLC system (Fig:2). The linearity of the drug was established by plotting their concentration versus their peak area individually and the slope, Y-intercept and the correlation coefficient were calculated and reported as required by ICH guidelines. The correlation coefficient of febuxostat were found to be 0.997. Table 1 summarizes the values obtained for the linearity studies.

### 3.2.2. Precision

The Precision of the method was determined by repeatability (intra day) and intermediate precision (inter day). Intra-day precision was determined by injecting six samples on the same day. The %RSD obtained for febuxostat were found to be 0.429. Inter-day precision was determined by injecting the same samples over six consecutive days. The % RSD obtained for febuxostat were found to be 0.513. Table 2 summarizes the values obtained for the precision studies.

### 3.2.3. Accuracy

The accuracy of the method was evaluated by determination of recovery of febuxostat at three levels of concentrations that is 50%, 100%, and 150%. This is done by applying the method to drug sample to which known amount of standard has been added. The mean recovery results for the drug ranges from 99.2 - 102%. Table 3 summarizes the recovery studies.

### 3.2.4. Specificity

The method specificity was assessed by comparing the chromatograms obtained from the drug and excipients mixture with those obtained from blank (excipients solution in water without drug). If the analytes should have no interference from other extraneous components and well resolved from them then the method is specific (Fig 3).

### 3.2.5. LOD and LOQ

The LOD is the smallest concentration of the analyte that gives a measurable response and LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOD and LOQ were calculated by the equations given in the ICH guide lines. LOD with signal/noise ratio of 3:1 was found to be 0.01µg mL\(^{-1}\) and LOQ with signal/noise ratio of 10:1 was found to be 1µg mL\(^{-1}\) for febuxostat. Table 4 summarizes LOD and LOQ values for febuxostat.

### 3.2.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust. Table 5 summarizes robustness values.

### 3.2.7. Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for febuxostat that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method. Table 6 summarizes ruggedness values.

### 3.3. Analysis of marketed formulation

The proposed RP-HPLC method was applied to the estimation of febuxostat in febuxor-40mg and drug content in each sample were calculated by comparison with the appropriate standard solution of the drug. No interference due to excipients was detected in the chromatograms produced. Table 7 summarizes the analysis values.

### 4. CONCLUSION

The RP-HPLC method is considered simple, reliable, selective providing satisfactory accuracy, precision with lower limits of detection and quantification with more specific and sensitive without any interference from the excipients. More over
the shorter duration of analysis for febuxostat makes the reported method suitable for routine analysis in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

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Fig. 1: Structures of (a) Febuxostat, (b) Tinidazole

Fig. 2: A typical chromatogram of standard febuxostat (1μg mL⁻¹), tinidazole (1 μg mL⁻¹) measured at 314 nm
Table 1: Linear Regression data for calibration curves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Febuxostat</th>
</tr>
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<tbody>
<tr>
<td>Linearity range (μg mL⁻¹)</td>
<td>1-7</td>
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<tr>
<td>Correlation coefficient</td>
<td>0.997</td>
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<tr>
<td>Slope</td>
<td>1.453</td>
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<td>Intercept</td>
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Table 2: Precision Studies

<table>
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<tr>
<th>Drug</th>
<th>Intra-day precision %</th>
<th>Inter-day precision %</th>
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<tr>
<td></td>
<td>% RSD</td>
<td>% RSD</td>
</tr>
<tr>
<td>Febuxostat</td>
<td>0.429</td>
<td>0.513</td>
</tr>
</tbody>
</table>

*mean of six observations

Table 3: Accuracy Studies

| Drugs     | Amount taken μg mL⁻¹ | Amount added μg mL⁻¹ | Total amount found μg mL⁻¹ | % Recovery | % RSD |
|-----------|----------------------|----------------------|---------------------------|------------|
| Febuxostat| 40                   | 20                   | 58.9                      | 98.16%     | 1.128 |
|           | 40                   | 79.1                 | 98.87%                    | 0.983      |       |
|           | 60                   | 99.98                | 99.98%                    | 0.245      |       |

*mean of six observations

Table 4: LOD and LOQ Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Limit of detection (LOD) μg mL⁻¹</th>
<th>Limit of Quantification (LOQ) μg mL⁻¹</th>
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<td>Febuxostat</td>
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Table 5: Robustness Studies

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<th>Drug</th>
<th>Mobile phase ratio (water: acetonitrile)</th>
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<tr>
<td>Febuxostat</td>
<td>75:25</td>
<td>2.495</td>
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<td>85:15</td>
<td>2.388</td>
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<td></td>
<td>90:10</td>
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Table 6: Ruggedness Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Peak area (%)</th>
<th>% *RSD</th>
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<tbody>
<tr>
<td></td>
<td>Analyst 1</td>
<td>Analyst 2</td>
</tr>
<tr>
<td>Febuxostat</td>
<td>82980</td>
<td>89381</td>
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</table>

Table 7: Analysis of Marketed Formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labeled amount, mg tablet</th>
<th>Amount found, mg tablet</th>
<th>% Estimated</th>
<th>% *RSD</th>
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<tbody>
<tr>
<td>Febuxostat</td>
<td>40</td>
<td>39.26</td>
<td>98.15%</td>
<td>0.5953</td>
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*mean of six observations

REFERENCES