NEW VALIDATED UV SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF FEBUXOSTAT IN BULK AND FORMULATION

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ABSTRACT
Two simple, rapid zero and first order derivative UV spectrophotometric methods were developed for the estimation of febuxostat in formulation. In zero order spectrophotometry absorbance values were measured at 314nm. In first order derivative spectroscopy amplitude was measured from 293 (maxima) to 336nm (minima). The methods obeyed linearity in the range of 2 to 30µg/ml (zero order) and 1 to 30µg/ml (first order). The LOD and LOQ were found to be 0.103µg/ml, 0.313µg/ml for zero order and 0.270µg/ml, 0.818µg/ml for first order derivative respectively. The % RSD value is less than 2% and the % recovery was near 100% for both the methods. The developed methods have been validated for linearity, precision and accuracy and were found to be linear, precise and accurate according to ICH-guidelines.

Keywords: Febuxostat, Amplitude, UV Spectrophotometric Method, First Order Derivative Spectroscopy, ICH

INTRODUCTION
Febuxostat (Figure 1) is chemically 2-(3-cyano-4-isobutoxyphenyl)-4 methyl-1, 3-thiazole-5-carboxylic acid. The molecular mass of Febuxostat (C16H16N2O3S) is 316.374gm/mol. Febuxostat is a urate lowering drug, a non-competitive inhibitor of xanthine oxidase that is indicated for use in the treatment of hyperuricemia and chronic gout (Love et al., 2010). It works by non-competitively blocking the molybdenum pterin centre which is the active site on xanthine oxidase. Different UV spectroscopic methods (Lakade et al., 2011, Sheth et al., 2012 (Difference UV spectroscopy), Sudhir et al., 2013, Bagga et al., 2011, Rajyalakshmi et al., 2013, Raviteja et al., 2013) were reported for the determination of Febuxostat in bulk and formulation.

Figure 1: Structure of Febuxostat

Several RP-HPLC (Reddy et al., 2013, Cong et al., 2012, Nageshwara et al., 2012, Annapurna et al., 2012, Chandra et al., 2012, Rajyalakshmi et al., 2013, Sameer et al., 2012, Muvvala et al., 2012) and UPLC (Sahu et al., 2013) methods were also reported for the analysis of Febuxostat. Literature review
also revealed different bioanalytical methods like LC-MS/MS (Chandu et al., 2013), UPLC tandem mass spectrometry (Lukram et al., 2013), LC-MS (Wang et al., 2013) and UPLC-MS (Zhang et al., 2013) for the determination of Febuxostat in biological samples like human and dog plasma. An attempt had been made to develop simple and sensitive UV spectroscopic methods for the regular analysis of Febuxostat in bulk and formulation and validated as per ICH guidelines (ICH 2005).

MATERIALS AND METHODS

Instruments
A Shimadzu model 1800 double beam UV-Visible spectrophotometer with spectral width of 1nm, wavelength accuracy of ±0.3nm and a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (Ver.2.4.) All weights were taken on a Shimadzu electronic balance.

Chemicals and Reagents
Febuxostat standard (purity 99.0 %) was obtained as a gift sample from Therdose pharma private Ltd., Hyderabad. It is available in the local market with the brand name FEBUTAZ (Sun Pharmaceuticals Industries Ltd.) as 40 mg and 80 mg tablets. Analytical grade potassium di hydrogen phosphate (Merck) and sodium hydroxide (Qualigens) were purchased. Double distilled water was used.

Preparation of 0.05M Phosphate Buffer (pH 6.0): 50.0 ml of 0.2 M Potassium di hydrogen phosphate and5.6 ml of 0.2M sodium hydroxide were mixed in a 200 ml volumetric flask and made up to volume with distilled water.

Preparation of Stock, Working Standard and Sample Solutions: Stock solution was prepared by accurately dissolving 25.0 mg of Febuxostat in methanol in a 25 ml volumetric flask (1000µg/ml). Aliquot from the stock solution of Febuxostat was appropriately diluted with methanol to obtain working standard (100µg/mL). Further dilutions were made with 0.05M Phosphate buffer (pH 6.0) to obtain concentrations in the range of 1 to 30µg/ml.

Solutions of Febuxostat were scanned in the range of 200-400 nm. The drug showed absorption maximum at 314 nm in the zero order spectra. All the zero order spectra were derivitised using first order transformations and the amplitude was measured in the range of 293 (maxima) to 336nm (minima) in the first order derivative spectra. The zero order and first order derivative spectra are shown in Figure 2 & 3.

Validation
Linearity: A series of solutions (1-30µg/mL) were prepared in phosphate buffer, pH 6.0 and scanned. The linearity was evaluated by linear regression analysis for both the methods by analysing different concentrations of the standard solutions of Febuxostat. The calibration curves for zero order (conc. vs. absorbance) and first order derivative (conc. vs. derivative absorbance) spectrophotometry are shown in Figure 4 & 5. The results and optical characteristics are shown in Table 1.

Precision: The precision of the proposed methods was determined as per the ICH guidelines by analysing replicates of three different concentrations (5, 10, 15µg/mL) at different time intervals on the same day (intraday precision) and on different days (intraday precision). % relative standard deviation (RSD) was calculated and the results are shown in Table 2.

Accuracy: To ascertain the accuracy of the proposed method, recovery studies were carried out by standard addition method by adding 80%, 100%, and 120% of pure drug sample solution to the pre analysed formulation solution. The results are shown in Table 3.

LOD & LOQ: The LOD and LOQ were calculated from the equations, LOD=3.3σ/S and LOQ=10σ/S, where σ is the standard deviation of the response and S is the slope of the standard curve. The results are shown in Table 1.

Assay
Twenty commercial tablets of Febuxostat were weighed, powdered and tablet powder equivalent to 25 mg of Febuxostat was dissolved in methanol in a 25 mL volumetric flask. The solution was sonicated for 10 minutes to enhance the extraction of drug, filtered and from the filtrate aliquots were taken and suitably
diluted with phosphate buffer as per the requirement. The data obtained was substituted in the regression equations obtained in zero order and first derivative methods and the percentage purity was determined. The results are shown in Table 4.

RESULTS AND DISCUSSION
Febuxostat showed wavelength maxima at 314nm (zero order) and amplitude was measured from 293nm to 336nm (first order derivative) in 0.05M Phosphate buffer, pH 6.0. Beer Lambert’s law was obeyed in the range of 2-30 µg/mL and 1-30 µg/mL for zero order and first order derivative methods. The linear regression equations were found to be y = 0.0764x + 0.0052 (r² = 0.9996) and y = 0.004x + 0.0003 (r² = 0.999) for zero order and first order respectively. LOD and LOQ values were found to be 0.103 & 0.313 (zero order), 0.270 & 0.818 (first order derivative) respectively which indicates the sensitivity of the methods. % RSD values for intraday and interday precision studies were found to be in the range of 0.35 - 0.96 (zero order) and 0.0 –1.44 (first order derivative) which are within the acceptable limit (< 2.0%) indicating that the method is precise. The percentage recovery values in accuracy studies were found to be in the range of 99.1-100.7 (zero order) and 99.2-100.16 (first order derivative). % RSD values in recovery studies were also within the acceptable limit indicating that the method is accurate. The assay values for the marketed formulation analysed by both the methods were obtained as 99.03 and 100.08 respectively and were found to be satisfactory.

Figure 2: Over lay zero order spectrum of Febuxostat

Figure 3: Over lay first order derivative spectrum of Febuxostat
Table 1: Optical characteristics of Febuxostat

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Zero order</th>
<th>First order</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λ max / Maxima-Minima (nm)</td>
<td>314</td>
<td>293-336</td>
</tr>
<tr>
<td>2</td>
<td>Beers law limit (µg ml⁻¹)</td>
<td>2-30</td>
<td>1-30</td>
</tr>
<tr>
<td>3</td>
<td>Molar absorptivity (l mol⁻¹ cm⁻¹)</td>
<td>24570</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Sandell’s sensitivity (µg/cm²/0.001)</td>
<td>0.133</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Intercept (c)</td>
<td>0.005</td>
<td>0.0003</td>
</tr>
<tr>
<td>6</td>
<td>Slope (m)</td>
<td>0.076</td>
<td>0.004</td>
</tr>
<tr>
<td>7</td>
<td>Correlation coefficient (r²)</td>
<td>0.9996</td>
<td>0.999</td>
</tr>
<tr>
<td>8</td>
<td>LOD (µg/mL)</td>
<td>0.103</td>
<td>0.270</td>
</tr>
<tr>
<td>9</td>
<td>LOQ (µg/mL)</td>
<td>0.313</td>
<td>0.818</td>
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Table 2: Intraday and inter day precision study of Febuxostat

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>*Mean ± SD, %RSD</th>
<th>Interday</th>
<th>First order Interday</th>
<th>First order Interday</th>
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<tr>
<td>5</td>
<td>99.9±0.79, 0.79</td>
<td>99.86±0.55, 0.55</td>
<td>98.50±0.0</td>
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<tr>
<td>10</td>
<td>99.1±0.95, 0.96</td>
<td>99.36±0.42, 0.43</td>
<td>100.08±1.44, 1.44</td>
<td>100.08±1.44, 1.44</td>
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<tr>
<td>15</td>
<td>100.6±0.92, 0.92</td>
<td>100.33±0.35, 0.35</td>
<td>100.06±0.96, 0.96</td>
<td>100.06±0.96, 0.96</td>
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</table>

*Mean of three determinations at each level

Table 3: Recovery study of Febuxostat

<table>
<thead>
<tr>
<th>Level of recovery (%)</th>
<th>Drug in formulation (µg/mL)</th>
<th>Drug added (µg/mL)</th>
<th>*Drug recovered (µg/mL)</th>
<th>*Recovery ± SD, % RSD</th>
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<tbody>
<tr>
<td></td>
<td>Zero order</td>
<td>First order</td>
<td>Zero order</td>
<td>First order</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>8</td>
<td>17.83</td>
<td>17.86</td>
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<tr>
<td></td>
<td>99.1±0.40, 0.40</td>
<td></td>
<td>99.2±0.61, 0.62</td>
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<tr>
<td>100</td>
<td>10</td>
<td>10</td>
<td>19.95</td>
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<tr>
<td></td>
<td>99.7±0.82, 0.82</td>
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<td>99.48±0.98, 0.99</td>
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<tr>
<td>120</td>
<td>10</td>
<td>12</td>
<td>22.15</td>
<td>22.04</td>
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<tr>
<td></td>
<td>100.7±1.04, 1.04</td>
<td></td>
<td>100.16±1.14, 1.13</td>
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*Mean of three determinations at each level

Table 4: Assay of marketed formulation of Febuxostat

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Label claim (mg)</th>
<th>*Amount obtained (mg)</th>
<th>*% label claim ± SD</th>
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<tr>
<td>FEBUTAZ</td>
<td>40</td>
<td>39.42</td>
<td>40.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99.03±0.462</td>
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</table>

*Mean of three determinations

Conclusion
From the above results it is concluded that, the developed methods are simple, rapid, sensitive, precise and accurate. Hence, these methods can be applied for the routine quality control analysis of Febuxostat in bulk and formulations.

ACKNOWLEDGMENT
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REFERENCES
Research Article


