DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DICLOFENAC POTASSIUM AND FEBUXOSTAT IN TABLET DOSAGE FORMS.


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ABSTRACT
In the present study deals the simultaneous estimation of Diclofenac potassium and Febuxostat in combined tablet dosage form have been developed using 0.1 N NaOH as a solvent. Two method is developed one is simultaneous equation method at 275 nm and 314.5 nm. The second is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 293 nm (isoabsorptive point) and at 314.5 nm the (maximum absorption of Febuxostat). Beer’s law obeyed in concentration range of 6-30 μg/mL and 3-15 μg/mL for Diclofenac potassium and Febuxostat respectively. The accuracy of the methods was assessed by recovery studies was found to be 99.60 ± 0.389 and 99.74 ± 0.213 for simultaneous equation method and 99.74 ± 0.166 and 99.72 ± 0.200 for Q analysis (absorption ratio) method for Diclofenac potassium and Febuxostat respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keywords: Diclofenac potassium, Febuxostat, Simultaneous equation method, Q-analysis spectrophotometric method.

INTRODUCTION
Diclofenac Potassium chemically it is -[(2, 6-dichlorophenyl) amino] benzene acetic acid monopotassium salt, with a molecular weight of 334.25 g/mol. The empirical formula is C_{14}H_{10}C_{12}KNO_{2}. It comes under the category of Non-steroidal anti-inflammatory agent and inhibits prostaglandin synthesis. It is official in British Pharmacopeia, United States Pharmacopeia and European Pharmacopeia[1-5]. Febuxostat is a xanthine oxidase inhibitor. The active ingredient in Febuxostat is 2-[3- cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole- 5-carboxylic acid, with a molecular weight of 316.38 g/mol. The empirical formula is C_{16}H_{16}N_{2}O_{3}S. It was recently approved by the European Medicines Agency on February 21, 2008 and USFDA on Feb 13, 2009[6-9]. Febuxostat is not official in any pharmacopoeia. The chemical structures of DKP & FEB shown.Fig.1&2. The combined dosage forms of DKP and FEB are available in the
market for the treatment of GOUT. Deep literature survey reveals that, not a single analytical method is reported for the determination of these drugs in combined dosage forms. The present manuscript describes simple, accurate, precise, rapid and economic spectrophotometric methods for simultaneous estimation of DKP and FEB in tablet dosage form using 0.1 N NaOH as a solvent.

![Chemical structure of Diclofenac Potassium (DKP)](image)

**FIG.1 Chemical structure of Diclofenac Potassium (DKP).**

![Chemical structure of Febuxostat (FEB)](image)

**FIG.2 Chemical structure of Febuxostat (FEB).**

**MATERIALS AND METHODS**

**Chemicals and Reagents**

DKP and FEB bulk powder was kindly gifted by A.P.M.C. Pharmacy College, Himatnagar, Gujarat, India and Intas Pharmaceuticals, Ahmedabad, Gujarat, India. Respectively the commercial fixed dose combination XANFEB DSR was procured from the local market. All other chemicals used were analytical grade. 0.1 N NaOH and calibrated glass wares were employed throughout the work.

**Apparatus**

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. A Reptech electronic weighing analytical balance based on EMFC technology and a Toshcon...
ultrasonic bath (Toshniwal process instrument pvt ltd.) was used in the study.

Preparation of standard stock solutions
An accurately weighed quantity of DKP (10 mg) and FEB (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved in 10 ml methanol and diluted to the mark with 0.1 N NaOH to obtain standard solution having concentration of DKP (100 g/ml) and FEB (100 g/ml).

Method 1:
The standard solutions of DKP (10 g/ml) and FEB (10 g/ml) were scanned separately in the UV range of 200-400 nm to determine λmax of both the drugs. The λmax of DKP and FEB were found to be 275 nm and 314.5 nm respectively (Fig.3). Five standard solutions having concentration 6, 12, 18, 24 and 30 μg/ml for DKP and 3, 6, 9, 12 and 15 g/ml for FEB were prepared in 0.1 N NaOH using the solutions having concentration 100 g/ml. The absorbance of resulting solutions was measured at 275 nm and 314.5 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of DKP and FEB in sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of DKP and FEB at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration$^{[25]}$.

\[ C_x = \frac{A_2 ay_1 - A_1 ay_2}{A_2 ay_1 - ax_1 ay_2} \] \ldots (1)

\[ C_y = \frac{A_1 ax_2 - A_2 ax_1}{A_2 ay_1 - ax_1 ay_2} \] \ldots (2)

Where,
A1, A2 -- absorbance of the mixture,
ax1, ax2 -- denotes absorptivities of the x at 275 nm and 314.5nm respectively,
ay1, ay2 -- denotes absorptivities of Y at 275nm and 314.5 nm respectively,
C_x = concentration of DKP.
C_y = concentration of FEB.
Fig. 3: Overlaid Spectra of Diclofenac potassium (DKP) and Febuxostat (FEB).

Method 2:
The standard solutions of DKP (10 g/ml) and FEB (10 g/ml) were scanned in the UV range of 200-400 nm to determine isoabsorptive point. The isoabsorptive point was found to be 293 nm (Figure 3). Five standard solutions having concentration 6, 12, 18, 24 and 30 g/ml for DKP and 3, 6, 9, 12 and 15 g/ml for FEB were prepared in 0.1 N NaOH using the solutions having concentration 100 g/ml. The absorbance of resulting solutions was measured at 293 nm (isoabsorptive point) and 314.5 nm (λmax of FEB) and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of DKP and FEB in sample solution was determined by solving the respective Q-analysis equations generated by using absorptivity coefficients and absorbance values of DKP and FEB at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration.25

For DKP

\[
C_x = \frac{Q_M - Q_y}{Q_x - Q_y} \times \frac{A1}{ax1} \]  

\ldots (3)

For FEB

\[
C_y = \frac{Q_M - Q_x}{Q_y - Q_x} \times \frac{A1}{ax1} \]  

\ldots (4)
Where,

\[ Q_M = \frac{\text{Absorbance of Sample at 314.5 nm}}{\text{Absorbance of sample at 293 nm}} \]

\[ Q_X = \frac{\text{Absorptivity of DKP at 314.5 nm}}{\text{Absorptivity of DKP at 293 nm}} \]

\[ Q_Y = \frac{\text{Absorptivity of FEB at 314.5 nm}}{\text{Absorptivity of FEB at 293 nm}} \]

A1 = Absorbance of sample at isoabsorptive point,
ax1 = Absorptivities of DKP at isoabsorptive point.

Validation of the proposed method:
The proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines\(^{[26]}\).

Linearity (Calibration curve)
The calibration curves were plotted over a concentration range of 6-30 g/ml and 3-15 g/ml for DKP and FEB respectively for Simultaneous equation and Q-analysis. Accurately measured standard solutions of DKP (6, 12, 18, 24 & 30 ml) and FEB (3, 6, 9, 12 and 15 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with 0.1 N NaOH for simultaneous equation Method. Accurately measured standard solutions of DKP (4, 6, 8, 10 & 12 ml) and FEB (3, 6, 9, 12 and 15 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with 0.1 N NaOH for Q-analysis Method. The absorbances of the solutions were measured at 275 and 314.5 nm against 0.1 N NaOH as blank for simultaneous equation Method. The absorbances of the solutions were measured at 293 and 314.5 nm against 0.1 N NaOH as blank for Q-analysis Method. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision
The intraday and interday precision of the proposed methods was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days.
different concentrations of standard solutions of DKP and FEB for both methods.

Accuracy (recovery study)
The accuracy of the method was determined by calculating recovery of DKP and FEB by the standard addition method. Known amounts of standard solutions of DKP and FEB were added at 80, 100 and 120 % level to prequantified sample solutions of DKP and FEB. (100 g/ml for DKP and 40 g/ml for FEB) The amounts of DKP and FEB were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times for both methods.

Limit of detection and Limit of quantification
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]
\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \( \sigma \) = the standard deviation of the Intercept of Calibration curve and \( S \) = slope of the calibration curve.

Analysis of DKP and FEB in combined Dosage Form (Tablet)
Twenty tablets were accurately weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg DKP & 40mg FEB was dissolved in 10 ml methanol and sonicated for 20 min and volume was made up to 100ml. The solution was filtered through Whatman filter paper No 41 and aliquot portion of filtrate was diluted to produce solution having concentration of 10 g/ml of DKP and 4 g/ml of FEB. The absorbance of sample solution was measured at selected wavelengths and the concentrations of the two drugs were estimated using equations (1) and (2) for simultaneous equation method and equations (3) and (4) for absorbance ratio method. The analysis procedure was repeated six times and the results are depicted in Table 2.

RESULTS AND DISCUSSION
TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER OF THE CALIBRATION CURVES

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Parameters</th>
<th>Method 1</th>
<th></th>
<th>Method 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DKP</td>
<td>FEB</td>
<td>DKP</td>
<td>FEB</td>
</tr>
<tr>
<td>1</td>
<td>Wavelength (nm)</td>
<td>275</td>
<td>314.5</td>
<td>275</td>
<td>314.5</td>
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<tr>
<td>2</td>
<td>Beer’s law limit (μg/ml)</td>
<td>6-30</td>
<td>6-30</td>
<td>3-15</td>
<td>3-15</td>
</tr>
<tr>
<td>3</td>
<td>Regression equation (y = mx + c)</td>
<td>y = 0.031x - 0.011</td>
<td>y = 0.003x - 0.004</td>
<td>y = 0.016x - 0.014</td>
<td>y = 0.063x - 0.031</td>
</tr>
<tr>
<td>4</td>
<td>Slope (m)</td>
<td>0.031</td>
<td>0.003</td>
<td>0.016</td>
<td>0.063</td>
</tr>
<tr>
<td>5</td>
<td>Intercept (c)</td>
<td>0.011</td>
<td>0.004</td>
<td>0.014</td>
<td>0.031</td>
</tr>
<tr>
<td>6</td>
<td>Correlation coefficient (r²)</td>
<td>0.9997</td>
<td>0.9985</td>
<td>0.9984</td>
<td>0.9991</td>
</tr>
<tr>
<td>7</td>
<td>LOD (μg/ml)</td>
<td>0.25</td>
<td>0.80</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>8</td>
<td>LOQ (μg/ml)</td>
<td>0.77</td>
<td>2.43</td>
<td>0.07</td>
<td>0.49</td>
</tr>
<tr>
<td>9</td>
<td>Precision (% RSD)</td>
<td>0.77</td>
<td>1.38</td>
<td>0.95</td>
<td>0.55</td>
</tr>
<tr>
<td>10</td>
<td>Interday (n=3)</td>
<td>0.88</td>
<td>1.69</td>
<td>1.42</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>Intrady (n=9)</td>
<td>0.88</td>
<td>1.69</td>
<td>1.42</td>
<td>1.04</td>
</tr>
</tbody>
</table>

TABLE 2: RESULTS OF RECOVERY STUDIES

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Amount of pure drug added (ml)</th>
<th>Simultaneous equation</th>
<th>Q-Absorbance method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DKP (100ug/ml)</td>
<td>FEB (100ug/ml)</td>
<td>DKP</td>
</tr>
<tr>
<td>80 %</td>
<td>18</td>
<td>7.2</td>
<td>99.915</td>
</tr>
<tr>
<td>100 %</td>
<td>20</td>
<td>8.0</td>
<td>99.169</td>
</tr>
<tr>
<td>120 %</td>
<td>22</td>
<td>8.8</td>
<td>99.739</td>
</tr>
<tr>
<td>Mean % recovery</td>
<td></td>
<td></td>
<td>99.60767</td>
</tr>
<tr>
<td>SD</td>
<td>0.389956</td>
<td>0.213294</td>
<td>0.166386</td>
</tr>
<tr>
<td>RSD</td>
<td>0.391492</td>
<td>0.213847</td>
<td>0.166815</td>
</tr>
</tbody>
</table>
The overlain spectra of DKP and FEB exhibit λ max of 275 nm and 314.5 nm for DKP and FEB respectively which are quite separated from each other. Additionally one isoabsorptive point was observed at 293nm. This wavelength was selected for simultaneous estimation of DKP and FEB for Q value analysis and it is assumed to be sensitive wavelength. The criteria for obtaining maximum precision by Simultaneous equation method were calculated and found to be out side the range 0.1-2 and for Q- analysis ratios of absorbances at 2 different Wavelengths were found to be constant. Standard calibration curves for DKP and FEB were linear with correlation coefficients (r) values in the range of 0.9984 – 0.9997 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.0015 – 0.0045. The calibration curves were repeated three times in a day and the average % RSD was found to be 0.86 for DKP and 0.70 for FEB; similarly the method was repeated for three different days and average % RSD was found to be for 1.26 DKP and 1.18. for FEB . The accuracy of the methods was confirmed by recovery studies.

CONCLUSION
The proposed methods for simultaneous estimation of DKP and FEB were found to be simple, accurate, economical and rapid. Due to accuracy, precision and sensitivity, the developed simultaneous equation method and Q-ratio method could be used for routine analysis of DKP and FBE in tablet dosage forms.

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REFERENCES:


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