DETERMINATION OF RALOXIFENE HYDROCHLORIDE BY OXIDATIVE COUPLING REACTION IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT
A simple and sensitive visible spectrophotometric method for the determination of Raloxifene hydrochloride using 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) reagent has been developed in bulk and tablet dosage forms. It is based on the formation of intense blue colored products by treating the drug with MBTH reagent in the presence of ferric chloride with an absorbance maximum of 649.3 nm. The Regression analysis of Beer’s Law showed good correlation in a general concentration range of 10-30µg/ml. The proposed method is validated with respect to accuracy, precision, linearity and limit of detection. The results of analysis have been validated statistically by repetition and recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of raloxifene in tablet form without the interference of excipients.

Keywords: Beer’s Law, Ferric chloride, MBTH, Raloxifene, Visible Spectrophotometric method.

INTRODUCTION
Raloxifene hydrochloride (RLX) is a second generation selective estrogen receptor modulator (SERM) non steroidal compound that belongs to the benzothiopene class of compounds. The chemical designation is methanone, [6-hydroxy-2-(4-hydroxyphenyl) benz[d]thien-3-yl]-[4-[2-(l-piperidinyl)ethoxy]phenyl]-, hydrochloride.

RLX is used in the treatment and prevention of osteoporosis and is an estrogen agonist in bone, where it exerts an anti-resorptive effect. The drug is listed in Merck Index 1. Some analytical methods which include HPLC 2-10, LC-MS-MS 11-12, Capillary electrophoresis 13, Resonance Rayleigh Scattering (RRS) 14, UV 15-16 and visible spectrophotometric 17-21 have been reported in the literature for the determination of RLX in pharmaceutical preparations. The main purpose of the present study was to establish a relatively simple, sensitive and validated spectrophotometric method for the determination of RLX in pure form and in pharmaceutical dosage forms, since most of the previous methods involve critical reaction conditions or tedious sample preparations and less specificity. Honing and Fritsch 22 described oxidative coupling of MBTH with aromatic amines or phenols in the presence of an oxidant under acidic conditions to form an intense colored oxidative coupling products. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and MBTH-Fe (III) 23. The method can be extended for the routine assay of RLX formulations.

MATERIALS & METHODS (EXPERIMENTAL)
A Systronics UV/Visible spectrophotometer model -2203 with 10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. MBTH (Fluka, 0.2%, 8.56x10^-3M, solution prepared by dissolving 200mg of MBTH in 100ml distilled water), Ferric chloride (Qualigens, 0.5%, 1.65x10^-2M solution prepared by dissolving 500mg of ferric chloride hexahydrate in 100ml of 0.1N HCl) were prepared.

Preparation of Standard stock solution: 100mg RLX was dissolved initially in 10ml of methanol and then followed by dilution to 100 ml with distilled water to get 1mg/ml stock solution. This solution was further diluted stepwise with the same solvent to obtain working standard solution concentration of 100µg/ml.

Sample solution: About 20 tablets were weighted to get the average tablet weight and pulverized and the powder equivalent to 100mg of RLX was weighed, dispersed in 25ml of isopropyl alcohol (IPA), sonicated for 30minutes and filtered through whatman filter paper no.41. The filtrate was evaporated and the residue was used for the preparation of working sample solution in the same way as under working standard solution.

Determination of wavelength maximum (λmax): The 3.0 ml of working standard solution of RLX (100µg/ml) was taken in 10ml calibrated tube. To this, 1.5ml MBTH and 1.0ml of ferric chloride was added successively, kept for 10min at room temperature for complete color development. The volume was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-760 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Fig.2), it was concluded that 649.3 nm is the most appropriate wavelength for analyzing RLX with suitable sensitivity.

Preparation of calibration graph: Aliquots of working standard RLX solution (100µg/ml) such as 1.0, 1.5, 2.0, 2.5, 3.0 ml were taken separately in a series of 10ml graduated test tubes, to get a concentration of 10, 15, 20, 25 and 30 µg/ml respectively. A 1.5 ml portion of MBTH (8.56x10^-3M) solution was added to each test tube and allowed to stand for 2 minutes at room temperature. Then 1.0ml of ferric chloride (1.65x10^-2M) solution was added, kept for 10 minutes and diluted to the mark with distilled water. The absorbance was measured at 649.3 nm against a similar reagent blank within 30 min. The calibration graph was constructed by plotting the drug concentration versus absorbance (Fig.3).

RESULTS AND DISCUSSIONS
In the present investigation the reactive electrophilic intermediate formed in situ from MBTH upon treatment with an oxidant Fe (III), was found to oxidative couple with RLX which possesses p-substituted phenol group. Based on the analogy, the probable sequence of reactions is presented in scheme (Fig.4). In developing a method, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed. The effect of various parameters such as time, temperature, nature and concentration of oxidant, volume and strength of MBTH reagent, order of addition of reagents on color development and solvent for final dilution on the intensity and stability of the colored species were studied and the optimum conditions were established. Among the various oxidants (NaIO4,
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K₂Cr₂O₇, Chloramine-T, potassium hexacyanoferrate (III), Ce (IV) and Fe (III) tried in combination with MBTH for oxidative coupling reaction. Ce (IV) and Fe (III) were responded for color development with MBTH. But MBTH-Fe (III) was found to be the best by virtue of high €max values and stability considerations. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. So distilled water is selected as a solvent for final dilution of the colored species. The optical characteristics such as Beer’s law limit, Sandell’s sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing 3/4% of the amount of the upper Beer’s law limits), Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (Se) and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in Table-1.

Commercial formulations containing RLX were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (reported UV method in methanol λ max 289nm) for formulations were compared statistically by the t-and f-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in Table-2. The ingredients usually present in formulations of RLX did not interfere with the proposed analytical method.

Fig. 1: Showing Chemical structure of RLX

Fig. 2: Determination of maximum wavelength for the detection

Fig. 3: Calibration curve of RLX
Fig. 4: Scheme for the colored reaction of the RLX with MBTH

Table 1: Optical characteristics, precision and accuracy of proposed analytical method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>649.3nm</td>
</tr>
<tr>
<td>Beer's law limit (µg/ml)</td>
<td>10–30</td>
</tr>
<tr>
<td>Sandell's sensitivity (µg/cm²/0.001 abs. unit)</td>
<td>0.047169</td>
</tr>
<tr>
<td>Molar absorptivity (Litre/mole/cm)</td>
<td>$1.0813 \times 10^4$</td>
</tr>
<tr>
<td>Regression equation ($Y^*$)</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.134</td>
</tr>
<tr>
<td>Slope(b)</td>
<td>0.026</td>
</tr>
<tr>
<td>Correlation Coefficient ($R^2$)</td>
<td>0.998</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.6035</td>
</tr>
<tr>
<td>% Range of errors (95% Confidence limits)</td>
<td></td>
</tr>
<tr>
<td>0.05 significance level</td>
<td>0.634</td>
</tr>
<tr>
<td>0.01 significance level</td>
<td>0.993</td>
</tr>
</tbody>
</table>

*$Y = a + bx$, where $Y$ is the absorbance and $x$ is the concentration of raloxifene in µg/ml

Table 2: Analysis of Raloxifene Hydrochloride in Pharmaceutical formulations

<table>
<thead>
<tr>
<th>Method</th>
<th>*Formulations</th>
<th>Labeled Amount (mg)</th>
<th>Found by Proposed Methods</th>
<th>Found by Reference Method</th>
<th>% Recovery by Proposed Method ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLX-MBTH</td>
<td>Tablet-1</td>
<td>60</td>
<td>59.629 ± 0.159</td>
<td>0.457</td>
<td>1.265</td>
</tr>
<tr>
<td></td>
<td>Tablet-2</td>
<td>60</td>
<td>59.681 ± 0.136</td>
<td>0.960</td>
<td>1.972</td>
</tr>
</tbody>
</table>

**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits $t = 2.57$ and $f = 5.05$**
CONCLUSION
The reagents utilized in the proposed method are readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical method is validated as per ICH guide lines and possess reasonable precision, accuracy. The method offers the advantages of rapidity, simplicity, sensitivity and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents and can be used as an alternative method to the reported ones for the routine determination of RLX depending on the need and situation.

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