Spectrophotometric determination of lamotrigine using oxidative coupling reaction in Pharmaceutical dosage forms.

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ABSTRACT

A Simple, precise, accurate and sensitive extractive method has been developed for the quantitative estimation of lamotrigine in bulk drugs and pharmaceutical formulations. The method is based on reaction of oxidative coupling of lamotrigine with 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) to form green colored product. Lamotrigine at its λmax 663 nm shows linearity in the concentration range of 2-8µg/ml. The relative standard deviations of 0.404% were obtained. Linear relationships with good correlation coefficients 0.999 were found between absorbance and the corresponding concentrations of the drug. The reliability and performance of the proposed methods was validated statistically the percentage recovery ranged from 96.6 and 105% respectively. The results of analysis for this method have been validated statistically and by recovery studies.

KEYWORDS:
spectrophotometry oxidative coupling 3-Methyl-2-Benzthiazolinone hydrochloride lamotrigine ferric chloride.

1.INTRODUCTION

Lamotrigine is a member of the sodium channel blocking class of antiepileptic drugs. It is a triazine derivative that inhibits voltage-sensitive sodium channels, leading to stabilization of neuronal membranes. It also blocks L-, N-, and P-type calcium channels and has weak 5-hydroxytryptamine-3 (5-HT3) receptor inhibition. These actions are thought to inhibit release of glutamate at cortical projections in the ventral striatum and anti-glutamatergic effects have been pointed out as promising contributors to its mood stabilizing activity. Lamotrigine was determined with or without combination of several drugs by HPLC spectrophotometrically. Although spectrophotometric methods are the instrumental methods of choice commonly used in the industrial laboratories. No colorimetric method with oxidative coupling mechanism has been reported so far for the determination of lamotrigine.

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Therefore the need for a fast, low cost and selective method is mandatory especially for routine quality control analysis of pharmaceutical products containing lamotrigine. The spectrophotometric method was based on the oxidative coupling reaction of lamotrigine with the reagent 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH).

2.MATERIAL AND METHODS

Apparatus
A shimadzu UV Visible spectrophotometer model 1800 with 1cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

Reagents and solutions
All employed chemicals were of analytical grade and high purified water was used throughout the protocols. Lamotrigine was obtained as gift sample from cipla Mumbai, India. The formulation was purchased from the local market.

Standard solutions
Standard lamotrigine stock solution (1000 µg/ml) was prepared by dissolving 100mg of drug in 100ml of methanol. Working solutions of the drug were prepared by dilution of the stock solution. The marketed tablet form of lamotrigine used in the determination was EZEDOC10 with a labelled strength of 10 mg and manufactured by Lupin Ltd, Mumbai, India.
Reagents
3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) 0.5 % (w/v)
0.5 g of MBTH reagent was accurately weighed transferred into a 100 ml calibrated flask, dissolved in distilled water, and make up the volume up to the mark to obtain a solution of 0.5% (w/v).
Ferric chloride (1%): Freshly prepared was prepared by dissolving 1 g of ferric chloride in 100 ml of distilled water

General recommended procedures
Procedure for calibration graph
Standard solutions of lamotrigine in methanol, having final concentrations in the range of 3-7 µg/ml were transferred into a series of 10 ml volumetric flasks. To each 2 ml of MBTH, 2 ml of ferric chloride was added and the volume was made up to mark with methanol and allowed to stand for 20 minutes. The contents were diluted up to 10 ml with methanol. The absorbance of each solution was measured at 633 nm against the reagent blank. The colored species was stable for 2 h and the amount of drug in the sample was computed from its calibration curve and absorption spectra are represented in Figure 2 and Figure 3.

Procedure for pharmaceutical formulations
Ten tablets were weighed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the 10 mg of lamotrigine was weighed intoa100 ml volumetric flask containing about 50 ml of methanol. It was shaken thoroughly for about 5-10 minutes, filter thoroughly with whatman filter paper to remove insoluble matter and diluted to the mark with methanol to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with methanol to obtain a concentration of 5 µg/ml. Then to that solution 2 ml of 0.5% MBTH, 2 ml of 1% FeCl₃ is added. The mixture was then gently shaken and the appearance of green color occurs. The contents were diluted up to 10 ml with methanol.

3. RESULT & DISCUSSION
In the present method, the drug reacts with MBTH in the presence of FeCl₃ to give a green colored product. Actually, this is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The colored products were found to be stable for 4 hours, at room temperature. Reproducible results were obtained in the temperature range of 20-40 °C. The reagent blank has negligible absorbance in the range used for detection of the ezetimibe. Beer’s law is obeyed in the range of 2-8 µg/ml for ezetimibe.
In the present investigation, MBTH reagent forms colored complex with ezetimibe and the absorbances were measured at 633 nm respectively. An oxidative coupling reaction takes place with ezetimibe with MBTH reagent. Therefore, the present study was devoted to explore MBTH reagent as oxidative coupling reagent for the determination of ezetimibe in pure and pharmaceutical dosageforms. Optimization of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimized via a number of preliminary experiments. The optimum conditions for the reaction were carefully studied. Maximum absorption at 633 nm was obtained immediately upon using 2 ml of 1% FeCl₃ and 2 ml of 0.5% MBTH at ambient temperature and the product remained stable for 4 h.

Optimization of parameter
The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. Different concentrations and different volumes were tried for all the reagents, by varying the parameters at a time. In this method it was found that optimum concentration of MBTH reagent was 0.5% w/v and optimum concentration of FeCl₃ was 1% w/v. The optimum volume was found to be 2 ml for MBTH and that of FeCl₃ was 2ml.

Stability of the Chromogen
The reaction between lamotrigine and MBTH completed within 20 minutes. The green colour developed was found to be stable for long period and showed no change in the colour intensity with time. This allowed the method to be followed for the intra-daystudies.

Quantification
The limits of the Beer’s law, the molar absorptivity and the Sandell’s sensitivity values were evaluated and are given in Table 1. Regression analyses of the Beer’s law plots at their respective 2max values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, Y = bX + c (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>2max/ nm</td>
<td>633 nm</td>
</tr>
<tr>
<td>Beers law limits (µg/ml)</td>
<td>2.8</td>
</tr>
<tr>
<td>Molar absorptivity (1/lmol/cm)</td>
<td>409.43x10⁻³</td>
</tr>
<tr>
<td>Correlation coefficient (R)</td>
<td>0.999</td>
</tr>
<tr>
<td>Sandell’s sensitivity (ng cm⁻²)</td>
<td>0.0178</td>
</tr>
<tr>
<td>Regression equation (y)</td>
<td>y = 0.062x+0.003</td>
</tr>
<tr>
<td>Slope, b</td>
<td>0.062</td>
</tr>
<tr>
<td>Intercept, c</td>
<td>0.033</td>
</tr>
<tr>
<td>Relative standard deviation</td>
<td>0.404</td>
</tr>
<tr>
<td>Range of error (95% confidence limits)</td>
<td>0.165</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.093</td>
</tr>
<tr>
<td>Limit of quantification (µg/ml)</td>
<td>0.258</td>
</tr>
</tbody>
</table>
\[ Y = bX + c, \] where \( X \) is the concentration of drug in \( \mu g/ml \); Average of six determinations.

**Validation of the method**
The validity of the method for the assay of lamotrigine examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer’s law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure ezetimibe were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table 2. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

<table>
<thead>
<tr>
<th>S.N o</th>
<th>Standard lamotrigine (ml)</th>
<th>Standard lamotrigine (µg)</th>
<th>Sample lamotrigine (ml)</th>
<th>Sample lamotrigine (µg)</th>
<th>Absorbance at 633nm</th>
<th>Amount of lamotrigine from std.graph</th>
<th>Recovery of std (mg)</th>
<th>% Recovery</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>2</td>
<td>0.2</td>
<td>2</td>
<td>0.30</td>
<td>9</td>
<td>4.1</td>
<td>2.01</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>3</td>
<td>0.2</td>
<td>2</td>
<td>0.44</td>
<td>8</td>
<td>5.97</td>
<td>2.97</td>
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<tr>
<td>3</td>
<td>0.4</td>
<td>4</td>
<td>0.2</td>
<td>2</td>
<td>0.60</td>
<td>7</td>
<td>8.05</td>
<td>4.15</td>
</tr>
</tbody>
</table>

**Linearity**
To establish linearity of the proposed methods, a series of solutions of lamotrigine for 2-8 µg/ml, were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

**Precision**
The precision of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer’s range and finding out the absorbance by the proposed method.

**Accuracy**
The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations three serial dilutions were prepared from independent stock solutions and analyzed. Accuracy was assessed as the percentage relative error and mean % recovery (Table 3).

<table>
<thead>
<tr>
<th>Drug</th>
<th>S .N o</th>
<th>Label Claim (mg)</th>
<th>Amount found*</th>
<th>% Purity*</th>
<th>Average (%)</th>
<th>S.D</th>
<th>R.S.D*</th>
<th>RSD*</th>
<th>S.E.M</th>
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<tbody>
<tr>
<td>lamotrigine</td>
<td>1</td>
<td>10</td>
<td>9.98</td>
<td>99.8</td>
<td>99.31</td>
<td>0.0453</td>
<td>0.587</td>
<td>0.632</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>9.95</td>
<td>99.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>9.85</td>
<td>98.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>9.93</td>
<td>99.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>9.92</td>
<td>99.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>9.96</td>
<td>99.6</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 3: Evaluation of accuracy and precision

SD. Standard deviation; SEM. Standard error of mean; RSD. relative standard deviation; intraday precision, b. interdayprecision.
Ruggedness
To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drugs were carried out. The within-day RSD values were less than 1% and this indicate that the proposed method has reasonable ruggedness.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ for ezetimibe by the proposed method were determined using calibration standards.

LOD and LOQ were calculated as 3.3 $\sigma$/S and 10 $\sigma$/S, respectively, Where S is the slope of the calibration curve and $\sigma$ is the standard deviation of y-intercept of regression equation.

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CONFLICTS OF INTEREST
The authors do not have any conflict of interest.

REFERENCE
[3].Chaudhari BG, Patel NM, Shah PB, Stability-indicating reversed-phase liquid chromatographic