Enzymatic method for the determination of Perindopril erbumine and Repaglinide in bulk and dosage forms

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ABSTRACT

Two simple and sensitive enzymatic methods were developed for the determination of Perindopril erbumine (method A) and Repaglinide (method B) either in raw material or in pharmaceutical formulations. Both methods A and B are based on oxidative coupling reaction between drug and 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of Hydrogen peroxide and enzyme horseradish peroxidase to produce colored product, which is measured spectrophotometrically at 425nm and 440nm respectively. Beer’s law is obeyed in the concentration range of 10-50 µg/ml and 8-40 µg/ml for method A and method B respectively. The proposed methods were successfully applied to the assay of perindopril erbumine and repaglinide in tablet preparations with recoveries varying from 99.50 to 100.50% and 99.75 to 100.15%, with standard deviation of 0.0015 for method A and 0.00118 for method B. The results were statistically compared with those of the reference method. No significant interference was observed from the excipients commonly used as pharmaceutical aids with the assay procedure.

Key words: Perindopril erbumine, Repaglinide, Sandell’s sensitivity, Beer’s Law.

INTRODUCTION

Perindopril erbumine¹, (2S,3(infinity)S,7(infinity)S)-1-[(S)-N-[(S)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), belongs to a group called angiotensin converting enzyme (ACE) inhibitors. The effect of this drug is, it drops down the blood pressure and decreases the workload of the heart. Literature survey revealed that few analytical methods have been reported for the estimation of Perindopril erbumine; spectrophotometric²⁴, HPLC⁵⁶, LC–MS/MS⁷⁸, include visible spectrophotometric¹¹,¹², HPLC¹³ and electrochemical¹⁴ methods.

To the best of our knowledge, there is no work in the literature reported about the enzymatic method for the analysis of perindopril erbumine and repaglinide in either biological fluids or pharmaceutical formulations. Hence the author has made an attempt to develop two simple, sensitive and rapid spectrophotometric methods for the estimation of perindopril erbumine and repaglinide in bulk drugs and in pharmaceutical formulations.

EXPERIMENTAL

Apparatus

¹ An ELICO Model SL-159 double beam, UV-VIS spectrophotometer (Elico India Ltd., India) with 1.0 cm matched quartz cells was used for absorbance measurements.

Systronic digital pH meter was used to
adjust and determine the hydrogen ion concentration (pH) of the solutions.
- Remi desktop centrifuge with 24,000 rpm for the extraction of horseradish peroxidase (HRP).
- Homogenizer with a high speed blender 3-4 x 15 sec. for homogenization of Horseradish root.

Materials and Reagents

All materials and reagents were of analytical grade and double distilled water was used.
- Perindopril erbumine and Repaglinide bulk samples (gift sample from local Pharmaceutical industry)
- Aqueous solutions (0.2%) of MBTH.
- Hydrogen peroxide (0.01M): Prepared by dissolving 0.10 ml of 30% H₂O₂ in 200 ml of reagent grade distilled water just prior to experiments.
- Phosphate buffer (0.1M, pH-7.0): Potassium dihydrogen phosphate-di sodium hydrogen phosphate buffer was prepared as follows.

Stock Solutions for buffer
- 0.5 M KH₂PO₄ solution: 68.04g of KH₂PO₄ is dissolved in 1 liter of reagent grade distilled water.
- 0.5 M Na₂HPO₄ solution: 71g of Na₂HPO₄ is dissolved in 1 liter of reagent grade distilled water.

39 ml of 0.5 M KH₂PO₄ + 53.6 ml of 0.5 M Na₂HPO₄ were diluted to 1000 ml at 25°C.

Standard and Sample solution of Perindopril erbumine and Repaglinide:

Method A
Accurately weighed 100mg of Perindopril erbumine was dissolved in 100ml of distilled water to give a concentration of 1mg/ml. The final concentration was brought to 200 μg/ml.

Method B
Accurately weighed 100mg of repaglinide was dissolved in 100ml of methanol to give a concentration of 1mg/ml. The final concentration was brought to 400 μg/ml.

Extraction of the enzyme (Horseradish Peroxidase)
A turnip (Horseradish root) weighing 40 g was Peeled, washed, and cut into 1” cubes. The sliced pieces were homogenized in 200 ml of buffer in a blender at high speed for 15 minutes. The extract is clarified by centrifugation (10-15,000 rpm/ 10 min.) and filtered through Whatman No. 1 filter paper. The extract for stability was stored in toluene for at least a week at 4°C. The extract was suitably diluted for further experimental analysis.

Assay Procedure

Method A
Into a series of 25ml calibrated test tubes, 15ml buffer (pH 7.0) solution, 2 ml of reagent (MBTH), 1 ml of hydrogen peroxide (0.01M) and 1 ml horse radish root solution (1:1diluted) and aliquots of perindopril erbumine solution, were added and made up to the mark with distilled water. The tubes were incubated at room temperature for 15 minutes. The absorbance was measured after complete color formation at lₘₐₓ of 425 nm against reagent blank. The amount of the drug in the sample was computed from corresponding calibration graph.

Method B
Into a series of 25ml calibrated test tubes, 15ml buffer (pH 7.0) solution, 2 ml of reagent (MBTH), 1 ml of hydrogen peroxide (0.01M) and 1 ml horse radish root solution (1:1diluted) and aliquots of repaglinide solution, were added and made up to the mark with distilled water. The tubes were incubated at room temperature for 15 minutes. The absorbance was measured after complete color formation at λₘₐₓ of 440 nm against reagent blank. The amount of the drug in a given sample was computed from the corresponding calibration graph.

RESULTS AND DISCUSSION

The methods A and B are based on the oxidative coupling reaction of the drugs, perindopril erbumine and repaglinide, with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of hydrogen peroxide and horseradish peroxidase enzyme to give a colored product. Actually, this is an enzyme catalyzed oxidative coupling reaction of MBTH with the drugs.
the reaction conditions, on oxidation by the enzyme in the presence of hydrogen peroxide, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with perindopril erbumine and repaglinide to form the colored product which shows $\lambda_{\text{max}}$ at 425nm and 440nm respectively. The colored products were found to be stable for 5 hours (method A) and 4 hours (method B) at room temperature.

### Investigation of Assay Parameters

#### Order of addition of reactants

The suitable order or addition of reactants in the determination of perindopril erbumine (method A) and repaglinide (method B) for attaining maximum color and stability was buffer-MBTH-hydrogen peroxide- peroxidase enzyme-drug.

#### Effect of variation of temperature

All experiments and absorbance measurements were carried out at laboratory temperature ($28^\circ\pm3^\circ$). At low temperatures ($20^\circ$C) the time required for attaining maximum color is more. At high temperatures ($35^\circ$C) the stability of the colored species is less. So laboratory temperature is preferred for both the methds.

### Table 1: Optical characteristics, precision and accuracy of proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A (Perindopril)</th>
<th>Method B (Repaglinide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>425</td>
<td>440</td>
</tr>
<tr>
<td>Beer's law limit (ig/ml)</td>
<td>10 - 50</td>
<td>8 – 40</td>
</tr>
<tr>
<td>Sandell's Sensitivity (ig/cm$^2$/0.001 abs. unit)</td>
<td>0.0806</td>
<td>0.0373</td>
</tr>
<tr>
<td>Molar absorptivity (Litre.mole$^{-1}$.cm$^{-1}$)</td>
<td>$2.547 \times 10^4$</td>
<td>$1.210 \times 10^4$</td>
</tr>
<tr>
<td>Stability of Color (hours)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Regression equation (Y)*</td>
<td>0.009</td>
<td>-0.0106</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0011</td>
<td>0.0027</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>1.2</td>
<td>0.555</td>
</tr>
<tr>
<td>% RSD$^\dagger$</td>
<td>1.2</td>
<td>0.555</td>
</tr>
<tr>
<td>% Range of errors (95% confidence limits):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 significance level</td>
<td>1.01</td>
<td>0.464</td>
</tr>
<tr>
<td>0.01 significance level</td>
<td>1.48</td>
<td>0.686</td>
</tr>
<tr>
<td>Correlation coefficient®</td>
<td>0.9998</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

* $Y = a + bx$, where $Y$ is the absorbance and $x$ is the concentration of drug in $\mu$g/ml
$^\dagger$ For six replicates

### Table 2: Assay and recovery of perindopril and repaglinide in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Labelled amount(mg)</th>
<th>Recovery by reference method (%)$^*$</th>
<th>Recovery by Proposed methods (%)$^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perindopril</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tablet I</td>
<td>4</td>
<td>99.5</td>
<td>100.5</td>
</tr>
<tr>
<td>Tablet I</td>
<td>4</td>
<td>100.25</td>
<td>99.5</td>
</tr>
<tr>
<td>Repaglinide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tablet I</td>
<td>0.5</td>
<td>98.5</td>
<td>99.75</td>
</tr>
<tr>
<td>Tablet I</td>
<td>0.5</td>
<td>99.4</td>
<td>100.15</td>
</tr>
</tbody>
</table>

* Reference method was UV method developed in the laboratory.
** Recovery amount was the average of six determinants
**Effect of pH**

Different phosphate buffers with pH range of 5-8 were tried and pH 7 was the pH of choice for getting maximum absorbance.

**Volume of buffer**

15 ml of buffer was needed to bring the suitable pH in 25 ml of solution.

**Optical Characteristics and Validation of the Methods:**

Optical characteristics for both the methods, such as Beer’s law limits, molar absorptivity and Sandell’s sensitivity, are given in Table -1. The linearity, slope and the intercepts were calculated using the regression equation. Precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure and commercial samples of the drug, whose concentration was within Beer’s law range. Values of relative standard deviation (RSD) and range of error at 95% confidence level were calculated for all the methods and are shown in Table 1.

**Analysis of pharmaceutical preparations**

Application of the proposed methods to the determination of perindopril erbumine and repaglinide in its dosage forms was successfully made; the results are presented in Table-2. The excellent recoveries obtained indicated the absence of any interference from the excipients.

**CONCLUSION**

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing perindopril erbumine and repaglinide showed no interference from the common excipients. Hence, these methods could be considered for the determination of perindopril and repaglinide in the quality control laboratories.

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