DEVELOPMENT AND VALIDATION OF RAMIPRIL ESTIMATION FROM CAPSULES USING VISIBLE SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, rapid, sensitive, extraction free and cost effective visible spectrophotometric method has been developed for the determination of Ramipril in bulk and capsule formulations. The proposed method is based on the formation of yellowish brown coloured species by the drug with Folin reagent and exhibits \( \lambda_{\text{max}} \) at 456.5 nm. The calibration graph is linear over the concentration range of 16-48 \( \mu \)g/ml with Molar absorptivity of 4.98783X10^3 \( \text{L/mol/cm} \) and Sandell’s sensitivity of 0.08351 \( \mu \)g/cm². 0.001 abs. unit. The proposed method is applied to commercial available capsules and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of the Ramipril in capsule formulations without the interference of excipients.

Keywords: ACE inhibitor, Beer’s Law, Estimation, Folin reagent, visible Spectrophotometry.

INTRODUCTION

Ramipril (RAM) (Fig.1) is highly lipophilic, long acting angiotensin-converting enzyme (ACE) inhibitor and chemically it is (2S, 3aS, 6aS)-1-[[5-N-[[5-[3-carboxyl-2-phenylpropyl]lalanly]] octahydro cyclopenta [b]pyrrole-2-carboxylic acid-1-ethyl ester]. It is used in the treatment of hypertension, congestive heart failure and diabetic nephropathy with microalbuminuria. Ramipril acts as a prodrug of diacid obtained after oral administration. RAM is official in USP 2 and BP 3 with microalbuminuria. Ramipril acts as a prodrug of diacid converting enzyme (ACE) inhibitor and chemically it is (2S, 3aS, 6aS)-1-[[5-N-[[5-[3-carboxyl-2-phenylpropyl]lalanly]] octahydro cyclopenta [b]pyrrole-2-carboxylic acid-1-ethyl ester]. It is used in the treatment of hypertension, congestive heart failure and diabetic nephropathy with microalbuminuria. Ramipril acts as a prodrug of diacid obtained after oral administration. RAM is official in USP 2 and BP 3 which describes HPLC and potentiometric titration method for its determination in bulk and pharmaceutical dosage forms.

All the chemical reagents used were of analytical grade. Folin reagent (NQS) was prepared by mixing 30 ml of potassium hydrogen phosphate (0.067M) and 970 ml of disodium hydrogen phosphate (0.067M) and the pH of the solution was adjusted to 8.0.

Standard solution: The standard stock solution (1mg/ml) of RAM was prepared by dissolving 100mg of RAM initially in 10 ml of methanol and then followed by dilution to 100ml with distilled water. The working standard solution of RAM (400 \( \mu \)g/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

RESULTS AND DISCUSSIONS

In developing this method, a systematic study 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, volume and strength of Folin reagent and pH buffer solution and solvent for final dilution of the colored species were studied and the optimum conditions were established. The optical characteristics such as Beer’s law, Sandell’s sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing 3/4th of the amount of the upper Beer’s law limits) were calculated and the results are summarized in Table-1. Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (Se), % range of error (0.05 and 0.01 confidence limits) were calculated using – MS Excel-2007. These results are shown in Table-1.
Fig. 2: Showing the absorption spectra of RAM – NQS

Fig. 3: Showing Beer’s law plot

Fig. 4: Showing the scheme
Commercial formulations containing RAM were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (reported UV in methanol λ max = 218 nm) 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 preanalyzed formulations at three different concentration levels (50%, 75% and 100%). These results are summarized in Table-2.

Chemistry of colored species

In the present investigation, the presence of aliphatic secondary amino group of RAM permits the development of visible spectrophotometric method for its determination through the nucleophilic substitution reaction with folin reagent. The formation of colored species with this reagent may be assigned through above analogy as shown in Scheme (Fig. 4).

CONCLUSIONS

The reagents utilized in the proposed method are cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. Moreover, the method is free from interference by common additives and excipients. The proposed visible spectrophotometric method for the estimation of RAM possesses reasonable precision, accuracy, simple, sensitive, and can be used as alternative method to the reported ones for the routine determination of RAM depending on the need and situation.

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REFERENCES


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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max (nm)</td>
<td>456.5</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>1.6 - 48</td>
</tr>
<tr>
<td>Saddle’s sensitivity</td>
<td>0.003507307</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>4987.827</td>
</tr>
<tr>
<td>Regression equation (Y)</td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.124</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.015</td>
</tr>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.998</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.6035</td>
</tr>
<tr>
<td>% Range of errors (95%)</td>
<td></td>
</tr>
<tr>
<td>Confidence limits</td>
<td></td>
</tr>
<tr>
<td>0.01 significance level</td>
<td>0.6335</td>
</tr>
<tr>
<td>significance level</td>
<td>0.9955</td>
</tr>
</tbody>
</table>

# Y = a+bx, where Y is the absorbance and x is the concentration of Ramipril in µg/ml.

Table 2: Analysis of ramipril by proposed and reference methods

<table>
<thead>
<tr>
<th>Method</th>
<th><em>Formulation</em></th>
<th>Labeled Amount (mg)</th>
<th>Found by Proposed Methods</th>
<th>Found by Reference Method ± SD</th>
<th>% Recovery by Proposed Method ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Amount found ± SD</strong> t f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NQS</td>
<td>Capsule-1</td>
<td>5</td>
<td>4.96 ± 0.038</td>
<td>1.816</td>
<td>4.878</td>
</tr>
<tr>
<td></td>
<td>Capsule-2</td>
<td>5</td>
<td>4.952 ± 0.033</td>
<td>2.596</td>
<td>4.876</td>
</tr>
</tbody>
</table>

# Recovery of 10 mg added to the pre analyzed sample (average of three determinations).

Reference method (reported UV method) using methanol (λ max = 218 nm).
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