Simultaneous Determination and Validation of Ramipril And Hydrochlorthiazide by RP-HPLC Method

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Abstract: RP-HPLC method in which simultaneous determination of Ramipril and Hydrochlorthiazide, in bulk and combined formulations was carried on a reverse phase C-18 column using a mobile phase consisting of Orthophosphate buffer: acetonitrile(60:40v/v) with a neutral pH. The mobile phase was pumped at a rate of 0.8ml/min and the UV detection was carried out at 294 nm. The linearity was found to be in the range of 50-150 µg/ml and 50-150µg/ml with regression coefficient 1, for Ramipril and Hydrochlorthiazide respectively. The peaks obtained were sharp having clear baseline separation with a retension time of 2.4 min and 1.4 min for Ramipril and Hydrochlorthiazide respectively.

Key words: Ramipril, Hydrochlorthiazide, RP-HPLC.

Introduction:

Ramipril is chemically(2s,3as,6as)-1-[(s)-N-[(s)-1-carboxy-3-phenyl Propyl] alanyl] octahydro cyclopenta [b]pyrrole-2- Carboxylic acid-1-ethyl ester 2,4,7-triamino,6-phenylpteridine has molecular formula C_{23}H_{32}N_{2}O_{5} and molecular weight416.511g/mol. Ramipril is an angiotensin-converting enzyme(ACE) inhibitors used to treat high blood pressure (hypertension), congestive heart failure and chronic renal failure. Ramipril inhibits angiotensin-converting enzyme (ACE) and reduces the angiotensin II levels which leads to the reduction of aldosterone secretion thus lowering of blood pressure.

Aldosterone is a hormone produced by the adrenal glands to help regulate the salt and water balance in your body.7-15

Hydrochlorthiazide is chemically 6-chloro-3,4-dihydro-2H-1,2,4, benzothiadiazine- 7-sulphonamide 1,1dioxide. It belong to thiazide diuretics, widely used in treatment of hypertension and edema associated with mild to moderate congestive heart failure.14-6. It increases the rate of urine excretion by the kidneys through decreased tubular reabsorption of sodium and chloride ions and by increasing osmotic transport of water to the renal tubules, which in turn lowers cardiac output and blood pressure. Ramipril shows an additive effect in reducing the systolic and diastolic blood pressure when combined with Hydrochlorthiazide. In monotherapy non responders, the combination improves response and blood pressure normalization rates compared with monotherapy. In addition to providing enhanced blood pressure reduction and control, the combination of Ramipril and Hydrochlorthiazide is safe well tolerated with the lower incident of adverse effects compared to that observed in the monotherapy.7-15

On literature survey it was found that Ramipril and Hydrochlorthiazide has been estimated independently and in combination with the other drugs by several Spectrophotometric and Bioanalytical methods. However no method could be found for simultaneous estimation of Ramipril and Hydrochlorthiazide in bulk and pharmaceutical formulation and also no method is available for such estimation.
in the Pharmacopoeias. A successful attempt has been made for simultaneous determination of RAM and HCZ in combined dosage form. Therefore, it was thought worthwhile to developed simple precise, accurate and reliable RP-HPLC method for simultaneous estimation of both the drug in combined dosage form.\textsuperscript{16-28}

**Experimental:**

Chemicals and Reagents: Ramipril and Hydrochlorthiazide was provided by Sun Pharmaceutical industries(Bengalore, India). The commercially available tablets formulation Altace HCT was used for quantitative determination. Potassium dihydrogen orthophosphate was of analytical grade. Acetonitrile was HPLC grade purchased from Merck.Chem.Ltd., Mumbai.

**Instrumentation:**

The HPLC WAS MODEL SHIMADZU lc-2010 composing quaternary pump, autosampler, mobile phase degaser, heated column thermostat, and variable uv detector. The mobile phase contained 0.01M potassium dihydrogen orthophosphate-acetonitrile 960:40) and flow rate was maintained at 0.8 ml/min. Chromatographic seperations were performed at ambient temperature on a c18 column and the injection volume was 10µl.

Standard stock solution: Standard stock solution containing Ramipril and Hydrochlorthiazide by weighing 10 mg each of standard Ramipril and Hydrochlorthiazide and transferred into two separate volumetric flasks. Both of drugs were dissolved in 50 ml of mobile phase with shaking and then volume was made up to the mark with mobile phase with shaking then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of each drug. These stock solutions were filtered through 0.45 µm Nylon 47mm membrane filter paper.

**Calibration curves:**

From stock solutions the different concentrations were prepared with appropriate dilutions in the range of 50-150 µg/ml for RAM and HCZ. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area vs the drug concentration. The areas exhibited linear responses with r²=1 for RAM and r²=1 for HCZ. The results were shown in table-2

**Table no. 2. System suitability parameters:**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RAM</th>
<th>HCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg/ml)</td>
<td>50-150</td>
<td>50-150</td>
</tr>
<tr>
<td>Slope</td>
<td>7885</td>
<td>14024</td>
</tr>
<tr>
<td>Intercept</td>
<td>1972</td>
<td>578.7</td>
</tr>
<tr>
<td>Regression coefficient (r²)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.825</td>
<td>0.037</td>
</tr>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>2.50</td>
<td>0.412</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>2.463</td>
<td>1.423</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Resolution factor</td>
<td>7.935</td>
<td></td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>4274</td>
<td>3668</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Accuracy</th>
<th>MEAN±Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>99.78±0.92</td>
</tr>
<tr>
<td>100%</td>
<td>99.51±0.89</td>
</tr>
<tr>
<td>120%</td>
<td>99.11±0.50</td>
</tr>
</tbody>
</table>
Analysis of Tablet formulation:

To determine the content of RAM and HCZ in tablets (Label claim 5mg RAM and 12.5 mg HCZ) the twenty tablets were weighed, their mean weight determined. And powered equivalent to 12.5 mg was transferred into a 100 ml volumetric flask and dissolved in sufficient quantity of mobilephase. The contents were ultrasonicated for 20 mins. And the volume was made up to the mark with mobile phase. Then the solution was then filtered through 0.2 µm Nylon 47 mm membrane filter. The solution was further diluted to get concentration 50 µg/ml of RAM and 125 µg/ml was subjected to proposed method, injected with applicable volume 10 µl, and amount of RAM and HCZ was determined. The assay procedure was repeated for five times. Chromatogram of tablet solution is shown in figure-1 and results are shown in table-1.

Table no.1 Result of RAM and HCZ in marketed formulation (n=6)

<table>
<thead>
<tr>
<th>Marketed Formulation</th>
<th>Drug</th>
<th>% Amount found±SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altace tablet</td>
<td>RAM</td>
<td>99±0.21</td>
<td>1.22</td>
</tr>
<tr>
<td>Altace tablet</td>
<td>HCZ</td>
<td>99±1.05</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Validation of HPLC method:

Specificity and Selectivity:

The specificity of the RP-HPLC method was determined by complete separation of Ramipril and Hydrochlorthiazide with parameters like retention time ($t_r$), resolution ($R_S$) and tailing factor ($T_I$). Here tailing
factor for peaks of Ramipril and Hydrochlorthiazide, was less than 2% and resolution was also more than 1%. The peaks obtained for Ramipril and Hydrochlorthiazide were sharp and have clear baseline separation

**Precision:**

Precision of the method was studied as intraday and interday precision, variations. Intra-day and Interday variation was determined by standard solutions of 50µg/ml Ramipril and 125 µg/ml Hydrochlorthiazide was analysed six times at different time intervals in the same day. In inter-day precision a set of five sample mixtures containing 50 µg/ml of Ramipril and 125 µg/ml of Hydrochlorthiazide were prepared and analyzed at same time on different days. The results are shown in Table-3

**Table no.3. Statistical Evaluation of precision of developed method.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAM</td>
<td>% Mean±SD</td>
<td>100±0.08</td>
</tr>
<tr>
<td>HCZ</td>
<td>99.99±0.03</td>
<td>99.99±0.03</td>
</tr>
</tbody>
</table>

**Accuracy:**

The accuracy of analytical method is the closeness of the test results obtained by that method to true value. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. Accuracy studies were performed by standard addition method at the 80,100 and 120% levels as stated in ICH guidelines. The results are shown in Table-2

**Robustness:**

The robustness study was done by making small changes in optimized method parameters like change in mobilephase ratio, change in flowrate and change in column temperature. There is no significant impact on retention time and tailing factor.

**Ruggedness:**

It should show the reliability of analysis with respect to deliberate variations in method parameters like different laboratories, different analysts. The solution containing 50µg/ml of RAM and125µg/ml of HCZ was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate and column temperature.

**Conclusion:**

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of RAM and HCZ in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than 2, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of RAM and HCZ in multicomponent pharmaceutical preparations.

**References:**

8. Parimoo P. In; Pharmaceutical Analysis, CBS publishers and distributors, New Delhi, 145.

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