RP-HPLC Method for the Simultaneous Determination of Ramipril and Hydrochlorothiazide in Tablet Dosage Form

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ABSTRACT: A simple, rapid, and precise reversed-phase high-performance liquid chromatographic method has been developed for simultaneous determination of Ramipril and Hydrochlorothiazide. The two drugs were separated on a 150 mm x 4.6 mm id. 5 µm particles, phenomenex C18 column. The mobile phase was Acetonitrile and 0.025 M sodium perchlorate monohydrate (46:54) pH adjusted to 2.8 with orthophosphoric acid, at a flow rate of 0.5 ml/min. UV detection was performed at 215 nm. The method was validated for linearity, accuracy, precision, and limit of quantitation. Linearity, accuracy, and precision were acceptable in the ranges (2.5-1.25 µg/ml) for Ramipril, and (12.5-62.5 µg/ml) for Hydrochlorothiazide. The average retention times for hydrochlorothiazide and Ramipril were 4.95 and 12.60 min, respectively. The calibration curves were linear (r > 0.999) in the range for each analyte. The % recovery for Ramipril and Hydrochlorothiazide is 99.47 and 99.74 respectively. No spectral or chromatographic interferences from the tablet excipients were found. This method which is rapid, simple and does not require any separation process has been successfully applied to the assay of commercial fixed dose formulations.

Key words: Ramipril, Hydrochlorothiazide, RP-HPLC

INTRODUCTION

Ramipril, (Figure 1) [2-[N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl)] - L-alanyl]-(1S, 3S, 5S)-2-azabicyclo [3-3-0] octane carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduce the degradation of bradykinin1. Literature survey reveals few analytical methods for the determination of ramipril in pharmaceutical preparations and biological fluids, viz. radioimmunoassay2, spectrophotometry3, potentiometry 4,5 GC, 6,7 and HPLC8,9.

Hydrochlorothiazide (Figure 2) (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide) is a thiazide-class diuretic used for treatment of hypertension.10 Hydrochlorothiazide inhibits the absorption of sodium and chloride at the beginning of Distal convoluted tubule. Many analytical procedures have been described for the individual determination of Hydrochlorothiazide, jointly with other pharmaceutical substances, including high performance liquid chromatography (HPLC)11-17, polarography18, capillary zone electrophoresis19, and spectrophotometry20-29 procedures.

A combination of 2.5 mg Ramipril, and 12.5 mg Hydrochlorothiazide is very widely used for treatment of hypertension but a literature search

Figure 1: Structure of Ramipril
revealed that no HPLC method is available for simultaneous HPLC determination of these two drugs in such pharmaceutical preparations. An HPLC method was, therefore, developed for analysis of Ramipril, and Hydrochlorothiazide in their combined dosage forms. The method described is simple, rapid, precise, and accurate.

EXPERIMENTAL

Chemicals and Reagents

Pure samples of Ramipril and Hydrochlorothiazide were obtained from Alembic limited, vadodara. HPLC grade Acetonitrile, sodium perchlorate monohydrate, orthophosphoric acid procured from Merck and methanol from Rankem. Highly pure water was prepared by using Millipore system. The pharmaceutical preparations of combination of Ramipril and Hydrochlorothiazide are CARDACEH (Aventis Pharma Ltd., India) and ECATOR-H (Torrent Ltd., India). The commercial formulation of Ramipril and Hydrochlorothiazide is available in the ratio of 1:5 (2.5/12.5 mg).

Instrumentation

A High performance liquid chromatography system Adept series CECIL CE 4201 with UV/Visible detector was used for analysis. The data was recorded by using the software Power stream. The column used for separation was octadecyl silane (C18) with length 250mm and internal diameter 4.6mm (Phenomenex) as well as particle size 5µ.

Solubility Determination

Solubility of Ramipril and Hydrochlorothiazide was determined in different solvents. Both the drugs were found to be soluble in Acetonitrile and Methanol.

Wavelength Selection

The λmax for Ramipril is 210nm and for Hydrochlorothiazide it is 225 nm. The isobestic point for both the drugs was found to be 215 nm so it is selected as detection wavelength (Figure 3).

Preparation of Standard Stock Solution

**Preparation of Ramipril Standard Stock Solution:** 10.0 mg of Ramipril working standard was weighed and transferred in 10 ml volumetric flask. About 2 ml methanol was added and sonicated to dissolve it. Finally volume was made up to 10 ml with methanol. (1000µg/ml, stock solution A).

From stock solution A 2.5 ml was taken, transferred in 25 ml volumetric flask and volume was made up to 25 ml. (100µg/ml, stock solution B).

From stock solution B 2.5 ml was taken, transferred in 25 ml volumetric flask and volume was made up to 25 ml. (10µg/ml, stock solution C).

Finally five sub dilutions were made from stock solution C in the concentration range of 2.5-12.5µg/ml.

**Preparation of Hydrochlorothiazide Standard Stock Solution:** 10.0 mg of Hydrochlorothiazide working standard was weighed and transferred in 10 ml volumetric flask. About 2 ml methanol was added and sonicated to dissolve it. Finally volume was made up to 10 ml with methanol. (1000µg/ml, stock solution A).
From stock solution A, 2.5 ml was taken, transferred in 25 ml volumetric flask and volume was made up to 25 ml. (100µg/ml, stock solution B).

Five sub dilutions were made from stock solution B in the concentration range of 12.5-62.5µg/ml.

Calibration Curve
To establish the linearity of analytical method, a series of dilution ranging from 2.5-12.5µg/ml for Ramipril and 12.5-62.5µg/ml for Hydrochlorothiazide was prepared. All the solution were filtered through 0.22 mm membrane filter and injected, the chromatograms were recorded and it was repeated for six times. A calibration graph was plotted between the mean peak area vs. respective concentration and the regression equation was derived. The correlation coefficient for Ramipril was 0.9994 and for Hydrochlorothiazide 0.9997.

Preparation of Mix Standard
From the stock solutions C (for Ramipril) and B (for Hydrochlorothiazide), dilutions of different concentration were prepared in the ratio of 1:5 for Ramipril and Hydrochlorothiazide respectively and chromatogram was recorded after injecting the mix standard (Fig 4).

RESULT AND DISCUSSION
To develop a precise, accurate and suitable RP-HPLC method for the simultaneous estimation of Ramipril and Hydrochlorothiazide, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination (Table 1). The results obtained by the assay of marketed formulation are summarized in Table 2. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table 3.

Validation: The proposed HPLC method was validated as per ICH guidelines.

Linearity
Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Ramipril and Hydrochlorothiazide were found to be 2.5-12.5 µg/ml and 12.5-62.5 µg/ml, respectively. The regression equation for Ramipril and Hydrochlorothiazide were found to be y = 308745x - 6522 and y = 105141x +118621 with coefficient of correlation, (r) 0.9994 and 0.9997 respectively.

Accuracy
Recovery studies were performed to validate the accuracy of developed method. To the preanalysed sample solution, a definite concentration of standard drug was added and then its recovery was analyzed. The percent recovery for Ramipril
was found to be 99.47 % and for Hydrochlorothiazide it was 99.74% (Table 4).

Table 2
Result of Ramipril and Hydrochlorothiazide in Marketed Formulation (n=6)

<table>
<thead>
<tr>
<th>Marketed Drug</th>
<th>% Amount found</th>
<th>% RSD ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARDACEH</td>
<td>100.09 ± 1.88</td>
<td>1.87</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>99.84 ± 1.77</td>
<td>1.78</td>
</tr>
<tr>
<td>ECATOR-H</td>
<td>100.42 ± 1.99</td>
<td>1.98</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>100.48 ± 1.13</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Table 3
System Suitability Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ramipril</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>2.5 – 12.5</td>
<td>12.5 – 62.5</td>
</tr>
<tr>
<td>Correlation Coefficient (r²)</td>
<td>0.9994</td>
<td>0.9997</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>308745</td>
<td>105141</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.22</td>
<td>1.93</td>
</tr>
<tr>
<td>No. of theoretical plates</td>
<td>4567</td>
<td>7315</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>12.60</td>
<td>4.95</td>
</tr>
</tbody>
</table>

Precision

A. Repeatability

Dilutions of different concentrations were prepared and triplicates of each dilution were analyzed in same day for repeatability and the results were subjected to statistical analysis. The %RSD for Ramipril was 0.29 and for Hydrochlorothiazide it was 0.12 which is according to ICH norms.

B. Intermediate Precision:

In this triplicate of each dilution was analyzed in different days and by different analysts. In all the condition %RSD was less than 1 which shows method is precise (Table 5).

Table 4
Statistical Data for Accuracy

<table>
<thead>
<tr>
<th>Statistical data</th>
<th>Ramipril</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Mean</td>
<td>99.47</td>
<td>99.74</td>
</tr>
<tr>
<td>SD</td>
<td>1.67</td>
<td>0.33</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>1.68</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table 5
Statistical Data for Precision

<table>
<thead>
<tr>
<th>Statistical parameter</th>
<th>Ramipril</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.29</td>
<td>0.12</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>0.295</td>
<td>0.124</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Day to day</td>
<td>0.37</td>
<td>0.09</td>
</tr>
<tr>
<td>b. Analyst to Analyst</td>
<td>0.47</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase or flow rate were made to check the method’s capacity to remain unaffected. The method found to be unaffected by changing the pH from 2.8 to 3 and 2.5. It also does not show any changes due to alteration of mobile phase ratio from Acetonitrile and 0.025 M sodium perchlorate monohydrate (46:54) to (40:60) and (50:50) (Table 6).

Table 6
Statistical Data for Robustness

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ramipril</th>
<th>Hydrochlorothiazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in pH of Mobile Phase</td>
<td>1.01</td>
<td>0.14</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>1.01</td>
<td>0.144</td>
</tr>
<tr>
<td>Change in Ratio of Mobile Phase</td>
<td>0.65</td>
<td>0.16</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>0.64</td>
<td>0.15</td>
</tr>
</tbody>
</table>

CONCLUSION

The proposed method gives a good resolution between two drugs Ramipril and Hydrochlorothiazide. It can be concluded that the
method is sufficiently specific and reproducible in the analysis of three drugs with good resolution. The correlation coefficient for each drug is not less than 0.9994 which shows the good regression for linearity. All the parameters for these drugs met the criteria of ICH guidelines for method validation. Maximum recovery obtained by this developed method. The % recovery for Ramipril and Hydrochlorothiazide is 99.47 and 99.74 respectively. As the precision, accuracy and robustness are concern the maximum %RSD found is 1.68 %.

The developed method may therefore be recommended for routine quality control analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination of Ramipril and Hydrochlorothiazide in pharmaceutical tablet dosage form.

References